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Pseudomonas Aeruginosa AmpR Transcriptional Regulatory Network

Deepak Balasubramanian

Florida International University, deeps66@gmail.com

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

PSEUDOMONAS AERUGINOSA AMPR
TRANSCRIPTIONAL REGULATORY NETWORK

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Deepak Balasubramanian

2013

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Deepak Balasubramanian, and entitled *Pseudomonas aeruginosa* AmpR transcriptional regulatory network, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Stephen Lory

John Makemson

Giri Narasimhan

Fernando Noriega

Kalai Mathee, Major Professor

Date of Defense: March 08, 2013

The dissertation of Deepak Balasubramanian is approved.

Dean Kenneth G. Furton
College of Arts and Sciences

Dean Lakshmi Reddi
University Graduate School

Florida International University, 2013

DEDICATION

To appa, amma and anna for the unconditional love and support

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This dissertation is the result of the support of many people. It is difficult to overstate my gratitude to my mentor, Dr. Kalai Mathee. With her enthusiasm, her inspiration, she helped to make science even more fun than it already is. I'm forever in debt! Throughout my stay here, she provided encouragement, good teaching, great company, and lots of ideas. I would have been lost without her. Thanks Kalai!

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ABSTRACT OF THE DISSERTATION
PSEUDOMONAS AERUGINOSA AMPR
TRANSCRIPTIONAL REGULATORY NETWORK

by

Deepak Balasubramanian

Florida International University, 2013

Miami, Florida

Professor Kalai Mathee, Major Professor

In *Enterobacteriaceae*, the transcriptional regulator AmpR, a member of the LysR family, regulates the expression of a chromosomal β -lactamase AmpC. The regulatory repertoire of AmpR is broader in *Pseudomonas aeruginosa*, an opportunistic pathogen responsible for numerous acute and chronic infections including cystic fibrosis. Previous studies showed that in addition to regulating *ampC*, *P. aeruginosa* AmpR regulates the sigma factor AlgT/U and production of some quorum sensing (QS)-regulated virulence factors. In order to better understand the *ampR* regulon, the transcriptional profiles generated using DNA microarrays and RNA-Seq of the prototypic *P. aeruginosa* PAO1 strain with its isogenic *ampR* deletion mutant, PAO Δ *ampR* were analyzed. Transcriptome analysis demonstrates that the AmpR regulon is much more extensive than previously thought influencing the differential expression of over 500 genes. In addition to regulating resistance to β -lactam antibiotics via AmpC, AmpR also regulates non- β -lactam antibiotic resistance by modulating the MexEF-OprN efflux pump. Virulence mechanisms including biofilm formation, QS-regulated acute virulence, and diverse physiological processes such as oxidative stress response, heat-

shock response and iron uptake are AmpR-regulated. Real-time PCR and phenotypic assays confirmed the transcriptome data. Further, *Caenorhabditis elegans* model demonstrates that a functional AmpR is required for full pathogenicity of *P. aeruginosa*. AmpR, a member of the core genome, also regulates genes in the regions of genome plasticity that are acquired by horizontal gene transfer. The extensive AmpR regulon included other transcriptional regulators and sigma factors, accounting for the extensive AmpR regulon. Gene expression studies demonstrate AmpR-dependent expression of the QS master regulator LasR that controls expression of many virulence factors. Using a chromosomally tagged AmpR, ChIP-Seq studies show direct AmpR binding to the *lasR* promoter. The data demonstrates that AmpR functions as a global regulator in *P. aeruginosa* and is a positive regulator of acute virulence while negatively regulating chronic infection phenotypes. In summary, my dissertation sheds light on the complex regulatory circuit in *P. aeruginosa* to provide a better understanding of the bacterial response to antibiotics and how the organism coordinately regulates a myriad of virulence factors.

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LIST OF SYMBOLS

SYMBOL	TERM/ UNIT
β	Beta
$^{\circ}\text{C}$	Degree celsius
Δ	Delta (deletion)
<i>et al.</i>	<i>et alia</i> (Latin: and others)
μg	Microgram
μl	Microliter
%	Percentage

LIST OF ACRONYMS AND ABBREVIATIONS

ACRONYM/ ABBREVIATION	TERM
ADP	Adenosine diphosphate
AHL	N-acyl homoserine lactone
AQ	2-alkyl-4 quinolone
ATP	Adenosine triphosphate
bp	Base pairs
cDNA	Complementary deoxyribonucleic acid
CFU	Colony forming unit
ChIP	Chromatin immunoprecipitation
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
DNA	Deoxyribonucleic acid
ECF	Extracytoplasmic function
<i>E. coli</i>	<i>Escherichia coli</i>
FDR	False discovery rate
GC	Guanine-cytosine
GlcNAc	N-acetyl glucosamine
GOID	Gene ontology identification
HLH	Helix-loop-helix
HTH	Helix-turn-helix

IclR	Isocitrate lyase regulator
LB	Luria Bertani
LPS	Lipopolysaccharide
LTTR	LysR-type transcriptional regulator
MB	Mega base pairs
MDR	Multi drug resistant
MFS	Major facilitator superfamily
MIC	Minimum inhibitory concentration
ml	Milliliter
mm	Millimeter
mM	Millimolar
MurNAc	N-acetyl muramic acid
NaCl	Sodium chloride
NADH	Nicotiamide adenine dinucleotide
NaOCl	Sodium hypochloride
OM	Outer membrane
ORF	Open reading frame
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PBP	Penicillin binding protein

PCP	Penta chlorophenol
PCR	Polymerase chain reaction
PFAM	Protein family
PQS	<i>Pseudomonas</i> quinolone signaling
qPCR	Quantitative real-time polymerase chain reaction
QS	Quorum sensing
RGP	Regions of genome plasticity
RNA	Ribonucleic acid
RND	Resistance nodulation division
<i>rrn</i>	Ribosomal RNA locus
SAGE	Serial analysis of gene expression
SSM	Synthetic succinate medium
T3SS	Type III secretion system
T6SS	Type VI secretion system
TCS	Two component system
TFP	Type IV pili
wHTH	Winged helix-turn-helix
XDR	Extremely drug resistant

CHAPTER 1

Introduction

The model bacterium, *Pseudomonas aeruginosa*

The genus *Pseudomonas* encompasses metabolically versatile bacteria that can utilize a wide range of organic and inorganic substrates. It comes as no surprise then that members of this genus are found in a variety of habitats in soil and water and are also important pathogens of plants, animals and humans (1). Walter Migula of the Karlsruhe Institute in Germany named the genus *Pseudomonas* in the 1894. He defined the genus based on cell morphology as,

‘Cells with polar organs of motility. Endospore formation of spores occurs in some species but infrequently (for instance, Pseudomonas violacea)’ (2).

The definition was brief and somewhat vague. We now know that none of the pseudomonads produce spores and what Migula observed were probably refractile granules. In Greek, *Pseudes* means false, *Monas* means units, and the genus was described as such in the Bergey’s Manual of Determinative Bacteriology and later in the Bergey’s Manual of Systematic Bacteriology (3,4). However, it has been hypothesized that Migula was actually referring to the nanoflagellate *Monas* whose cells bear a close resemblance to *Pseudomonas* cells and did not define the genus as ‘false units’ (5). Pseudomonads have now been studied extensively and the genus is now described as follows in the Bergey’s Manual of Systematic Bacteriology:

Straight or slightly curved rods but not helical, 0.5-1.0 μm in diameter by 1.5-5.0 μm in length. Most of the species do not accumulate granules of poly- β -hydroxybutyrate, but the accumulation of poly-hydroxyalkanoates of monomer lengths higher than C4 may occur when growing on alkanes or gluconate. Do not produce prosthecae and are not surrounded by sheaths. No resting stages are known. Gram-negative. Motile by one or

several polar flagella; rarely non-motile. In some species, lateral flagella of short wavelength may also be formed. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor; in some cases, nitrate can be used as an alternate electron acceptor, allowing growth to occur anaerobically. Xanthomonadins are not produced. Most, if not all, species fail to grow under acid conditions (pH 4.5 or lower). Most species do not require organic growth factors. Oxidase positive or negative. Catalase positive. Chemoorganotrophic. Strains of the species include in their composition the hydroxylated fatty acids 3-OH 10:0 and 12:0, and 2-OH 12:0, and ubiquinone Q-9. Widely distributed in nature. Some species are pathogenic for humans, animals or plants. The mol% G+C content of the DNA is 58-69.

Type species: Pseudomonas aeruginosa (Schroeter 1872) Migula 1900

Classification of strains into this genus now requires extensive polyphasic data, including molecular and biochemical characterization. Many strains produce pili and fimbriae. The cells are non-capsulated though they produce at least three different extracellular polysaccharides (alginate, Pel and Psl) under some growth conditions. The cells are aerobic but can grow anaerobically using nitrate as the terminal electron acceptor. This makes some species function as denitrifiers in the soil environment, though all species can use nitrate as a carbon source (6). Metabolic versatility is a hallmark of this genus and can utilize a wide range of organic and inorganic substrates as carbon source.

The type species of this genus is *P. aeruginosa*. The species name *aeruginosa* is Latin for *verdigris* meaning copper rust, referring to the blue-green pigment seen in lab cultures. The coloration is due to production of two extracellular pigments, pyocyanin and pyoverdine. *P. aeruginosa* can switch lifestyles from being an environmental isolate, found

in the soil and water, to a human pathogen (7). It is an epitome of opportunistic human pathogens, being implicated in a wide range of infections of the pulmonary tract (8,9), urinary tract (10-13), burns (14-16), wounds (17,18), lung cancer patients (19), chronic obstructive pulmonary disease (20), bronchiectasis (21) and in pediatric and adult AIDS patients (22). It causes persistent nosocomial infections (23,24) and is also a major pathogen in cystic fibrosis patients. One of the major weapons in its arsenal is its resistance to antibiotics. It is both inherently resistant and can also acquire resistance genes from other bacteria making it a notorious pathogen. Understandably, numerous studies over the years have focused on elucidating the mechanisms of pathogenesis and antibiotic resistance of *P. aeruginosa*. Since the sequencing of the genome (25), the emphasis of these studies has shifted from conventional pathogenesis research, where the focus was on individual virulence determinants, to a much larger scale involving whole genome analyses.

The prototypic reference strain of *P. aeruginosa* is PAO1, isolated from a burn wound (26), and was the first *P. aeruginosa* strain to be sequenced (25). The most prominent feature of the PAO1 genome is its size. It has one of the largest bacterial genomes with 6.26 million base pairs comprising of almost 90% protein coding genes (25,27). The genome complexity of *P. aeruginosa* explains its versatile nature to adapt and thrive in a wide range of environments. The genome has 5,570 open reading frames (ORFs), a number that is close to simple eukaryotes like *Saccharomyces cerevisiae*. Other features of the PAO1 genome include the presence of four ribosomal RNA loci (*rrn*) that are the regions of the longest repeats and a high GC ratio at 66.6% (25). Following the sequencing of the genome, high-density DNA microarrays were developed for *P.*

aeruginosa (28) that offered us a chance to take snapshots of the dynamic responses of this versatile genome to various stimuli. An important study in this context, which analyzed the genomes of both environmental and clinical isolates of *P. aeruginosa*, suggested the presence of a core genome in different strains and expression of different sets of genes to facilitate growth in particular environments (29). This calls for a robust control over gene expression mediated by transcriptional regulators. About 10% of the PAO1 genome is comprised of transcriptional regulators suggesting a tight control over the way genes are expressed in *P. aeruginosa* (25).

Subsequent to PAO1, other *P. aeruginosa* strains such as PA14 (30), PA2192 (a CF isolate) (31), C3719 (the Manchester epidemic strain) (32), and PACS2 (33), have been sequenced. Comparative genome analysis of the five sequenced *P. aeruginosa* strains has led to the identification of a set of core shared genes that all the genomes contain, the majority of which are housekeeping genes (33). In addition, there are accessory genomes comprising of mostly hypothetical ORFs or those related to transposons and bacteriophages, and genes involved in adaptation specific to each of the five strains (33). The accessory gene set also contains transcriptional regulators that may control expression of ORFs in the core genome. In order to better manage *P. aeruginosa* infections, it is imperative for us to understand the workings of the bacterium, what are the environmental signals it recognizes, how are these signals translated into a transcriptional response and how can we interfere with any of these processes.

Antibiotics, antibiotic resistance and AmpR

Antibiotics are antimicrobials that are produced by certain soil bacteria and actinomycetes as secondary metabolites. They kill other bacteria at low concentrations

and have been used to treat bacterial infections, in human and veterinary medicine, and in agriculture. The antibiotic era was ushered in by the serendipitous discovery of penicillin by Alexander Fleming. Subsequently, the ‘father of antibiotics’ Selman Waksman, identified over 20 different antibiotics that have revolutionized the way we manage bacterial infections. The mode of action of antibiotics varies but fall into two main categories: bactericidal and bacteriostatic. Bactericidal antibiotics kill bacteria while bacteriostatics only inhibit growth.

Antibiotics are also classified based on their chemical structure and mode of action. For example, DNA synthesis is inhibited directly by fluoroquinolones (nalidixic acid and ciprofloxacin) by affecting the topoisomerases, and indirectly by trimethoprim-sulfamethaxazole by inhibiting tetrahydrofolic acid synthesis. Rifamycins (rifamycin, rifampicin, rifapentine) inhibit RNA synthesis by acting on the DNA-dependent RNA polymerase. Protein synthesis inhibitors (aminoglycosides, macrolides, tetracyclines, ketolides, phenicols) act primarily by interfering with the activity of the 30S and 50S ribosomes affecting translation. Cell wall synthesis inhibitors include β -lactams (penicillins, cephalosporins, carbapenems), glycopeptides (vancomycin, teicoplanin) and lipopeptides (daptomycin, polymixin B) that inhibit various stages of the peptidoglycan synthesis process.

Antibiotics affect disparate cellular functions and four groups of genes have been identified based on their expression response to antibiotic treatment: genes that are directly affected by target inhibition (group 1); genes affected indirectly by antibiotic action (group 2); genes affected due to secondary effects of antibiotics (group 3); those that are differentially regulated in response to antibiotics but are not related to the drug

target (group 4) (34). Determining the transcriptional response of novel antimicrobials and comparing it to the transcriptional profiles of known antibiotics can potentially also provide information on the mechanism of action (35).

The role of antibiotics in nature is much debated. The contention is that if the initial intent of antibiotics production by bacteria was to wipe out the competition, one should find antibiotics at inhibitory concentrations in, for example, soil but this is not the case. Moreover, antibiotics are produced as secondary metabolites, during the late-log to stationary phase. If the primary role of antibiotics was to provide a growth advantage by wiping out competition, they should be produced in the log phase when the producer cell needs the most nutrients. Recent studies have shown that antibiotics at low concentration can in fact enhance biofilm growth and motility (36). It has therefore been proposed that antibiotics could function as signaling molecules in the environment (37). In *Streptomyces* spp., antibiotic production is under the control of a cell-cell communication mechanism called quorum sensing (QS) (38). In *P. aeruginosa*, PQS, the signaling molecule of one of the three QS systems, possess antibacterial activity at very high concentrations (39,40). In addition, some antibiotics have immunomodulatory effects; macrolides have anti-inflammatory properties and are used in CF patients to exploit this property rather than as an antibacterial (41). Rifampicin, on the other hand has pro-inflammatory activity (42).

Antibiotic resistance

β -Lactamases, enzymes that degrade β -lactam antibiotics, are presumed to have evolved around 2 million years ago (43). However, the race to evolve resistance to the newer antimicrobials has been accelerated in the recent decades with the widespread use

of antibiotics. Bacteria have quickly become resistant to almost all natural, synthetic and semi-synthetic antibiotics used to eradicate them (44). In fact, microbes seem to have the upper hand in the arms race. In spite of their evolutionary simplicity, bacteria possess extreme adaptability that has helped them survive over millions of years.

Bacteria resist antibiotics in a variety of mechanisms that include reduced membrane permeability, target alteration, efflux pumps, and hydrolytic enzymes (Figure 1.1). Some bacteria, such as *P. aeruginosa*, are intrinsically resistant to antibiotics while most of them can acquire antibiotic resistance genes from other organisms via horizontal gene transfer. Transfer can occur on mobile elements such as transposons, uptake of free DNA, be mediated by bacteriophages or by conjugation between the donor and recipient bacteria. Acquisition of such genes can lead to enhanced resistance to antimicrobials for the recipient bacteria. On the other hand, some mutations can reduce resistance, though typically the reduction in levels of resistance is not dramatic. Such low level increases in resistance are important in the clinical setting (45).

Development of resistance to an antibiotic is seen to follow soon after its introduction. Fleming noted this soon after he discovered penicillin. Penicillin had a MIC of 0.04 µg/ml when first introduced in 1940 against *Streptococcus pneumonia* (46), increased to 0.12-1 µg/ml in 1980 (47) and by the 1990s, the strains became completely resistant prompting discontinued usage. With other bacteria, the development of resistance is even more drastic. Quinolones, such as ciprofloxacin, were first used to treat *P. aeruginosa* in 1990 when the MIC was <0.5 µg/ml (48). In less than 8 years, clinical isolates started displaying intermediate resistance (8 µg/ml in 1998) (49) and in 2009, the resistance has increased to 32-64 µg/ml (50). The same trend is also seen with the

aminoglycoside, tobramycin for *P. aeruginosa*. The clinical isolates started displaying resistance (<2 µg/ml in 1980, 8 µg/ml in 1987 and ≥ 16 µg/ml in 2000) in a very short time span (51-53).

Antibiotic resistance in *P. aeruginosa*

P. aeruginosa displays a high level of resistance to many of the clinically relevant antibiotics. Resistance is a cumulative measure of three main types mechanisms: intrinsic, acquired and adaptive. Whereas intrinsic and acquired resistances are stable and heritable, adaptive resistance is dependent on the environment and is inducible. The antibiotic resistance mechanisms of *P. aeruginosa* are summarized in Figure 1.1.

Intrinsic resistance

Intrinsic resistance is a characteristic feature of *P. aeruginosa* and is principally mediated by low outer membrane permeability. The outer membrane in Gram-negative bacteria act as molecular sieves, and uptake of hydrophilic molecules is by diffusion through water-filled porins. The major porin in *P. aeruginosa* is OprF and most OprF channels are small similar to OprD and OprB.

Figure 1.1: Antibiotic resistance mechanisms in *P. aeruginosa*. Schematic representation of the multiple mechanisms of antibiotic resistance in *P. aeruginosa*. Cellular appendages and intracellular components are not shown for simplicity.

The *P. aeruginosa* outer membrane is thus 12 to 100 times less permeable compared to that of *E. coli* (54). Antibiotics enter *P. aeruginosa* through the porins (for example, imipenem through OprD), self-promoted uptake (polycationic antibiotics) or by diffusion through the membrane (hydrophobic molecules). Hydrophilic molecules, however, still equilibrate across the outer membrane quickly and this is where the other mechanisms of intrinsic resistance play a role. Efflux pumps such as MexAB-OprM, MexXY-OprM (55-57) and induction of the β -lactamase AmpC (58) effectively deal with the antibiotics by either pumping them out of the cell thereby reducing cytoplasmic retention time or by hydrolyzing them, respectively. These secondary resistance mechanisms rely on the reduced permeability for enhanced effectiveness. In addition, recent studies have identified many mechanisms that contribute to β -lactam resistance in *P. aeruginosa* (59-61). *P. aeruginosa* adaptively regulates resistance mechanisms in response to antibiotics contributing to the resistance profile seen with this pathogen

(34,62). Intrinsic resistance is thus multifactorial and a major contributing factor to the high level of clinical resistance displayed by *P. aeruginosa*.

Acquired Resistance

Acquired resistance of *P. aeruginosa* to antibiotics is achieved by horizontal gene transfer or by mutations. Resistance genes can be acquired on plasmids, pathogenicity islands, prophages, transposons or integrons, sometimes harboring resistance against multiple antibiotics. Typically, horizontal gene transfer affects resistance to aminoglycosides and β -lactams (63,64). Many plasmids that harbor genes encoding resistance to extended spectrum β -lactams and metallo β -lactams have now been identified (65).

Mutations can also lead to acquired resistance. Natural resistance frequencies for individual antibiotics vary between 10^{-6} to 10^{-9} , which can increase under conditions such as biofilm growth, DNA damage or in hypermutator strains (66-68). Mutations in the porin (69) or in their regulators (70) can lead to reduced uptake of antibiotics. Enhanced expression/activity of efflux pumps can also occur due to regulator mutations (71,72). Mutations can also lead to overexpression of hydrolytic enzymes that inactivate antibiotics conferring resistance to specific classes of antibiotics such as β -lactamases (73-76). Strains develop resistance to fluoroquinolones due to target alterations, a consequence of mutations (77-79). Recent work has also defined the phenomenon of 'creeping baselines', where resistance is acquired in seemingly non-significant increases in MIC but the cumulative resistance is clinically significant (35).

Adaptive Resistance

In response to specific environmental cues, cells display enhanced resistance to one or more antibiotics. When the stressor/signal is removed, the cells lose the resistance phenotype, though 'normal' levels of resistance are typically not restored (80). This phenomenon is termed 'adaptive resistance'. Though the phenomenon was observed in the 1960s (81), it has not been studied in detail. The four main defining features of adaptive resistance are (i) it is transient, (ii) not heritable, (iii) generally reverts upon removal of the inducing signal and (iv) is dependent on the environment (35). In addition to sub-MIC antibiotics, other identified triggers of adaptive resistance are environmental cues (anaerobiosis, ions and ionic binding, pH, carbon sources, polyamines), biofilm formation and swarming motility (35). Two-component systems such as ParRS and CprRS sense cationic peptides in the environment and trigger adaptive resistance (72,82).

In many cases, *P. aeruginosa* infections can be effectively dealt with. However, when *P. aeruginosa* forms biofilms, for example in the cystic fibrosis lung, eradication of infection becomes very difficult due to antibiotic resistance. The presence of persister cells further compounds this problem (83). This calls for urgent steps to not only find new treatment options but also to devise measures for more prudent use of antibiotics.

β -Lactam resistance in *P. aeruginosa*

β -Lactam antibiotics, in combination with aminoglycosides, are part of the current treatment regimen for *P. aeruginosa* infections (84). However, the phenomenon of β -lactam resistance is a major issue with *P. aeruginosa* acute and chronic infections. Antibiotic resistant isolates of *P. aeruginosa* are selectively favored *in vivo* in CF patients (85,86). A major mechanism of β -lactam resistance in *P. aeruginosa* is the

overproduction of the chromosomal β -lactamase, AmpC. Hyperexpression and presence of β -lactamases in the biofilm matrix, either free or in membrane vesicles, has been linked to the reduced effectiveness of β -lactam treatment (87-89). In the 90s, many *P. aeruginosa* CF isolates were found to either express high inducible or constitutively overexpress β -lactamase (90,91). One of the molecular mechanisms of overproduction of β -lactamases was attributed to insertional inactivation of the *ampD* gene in *P. aeruginosa* (73,92). Studying the regulation of *amp* genes will aid in understanding the molecular mechanisms of β -lactamase overexpression in *P. aeruginosa*.

Transcriptional regulation of the β -lactamase *ampC*

The LysR-type transcriptional regulator AmpR regulates inducible *ampC* β -lactamase expression. The genes *ampR* and *ampC* have been found in many organisms (93-97). In systems with inducible *ampC*, the *ampR* gene is always present adjacent to *ampC* but is divergently transcribed (98-100). The *ampR-ampC* arrangement in *P. aeruginosa* is similar to that seen in other organisms including *Citrobacter freundii* and *Enterobacter cloacae*. AmpR of *C. freundii* and *E. cloacae* can cross-complement each other (101) and sequence analysis revealed that *P. aeruginosa* AmpR is similar to that found in *C. freundii* (58%) and *E. cloacae* (62%) (102). In *C. freundii*, AmpR recognizes a 15-bp sequence 5' TCTGCTGCTAAATTT 3' in P_{ampC} (98,102). There is a putative AmpR binding site 5' TCTGCTCCAAATTT 3' in the *ampR-ampC* intergenic region in *P. aeruginosa*. AmpR has a helix-turn-helix (HTH) motif that is typical of DNA binding proteins (102). *C. freundii* AmpR has been shown to bind DNA through its HTH motif (98). In *P. aeruginosa*, the third helix of the AmpR HTH motif is critical for DNA

binding and amino acid residues important for DNA binding have been identified (O. Caille, unpublished data).

AmpR belongs to the LysR family of transcriptional regulators that typically autorepress their own expression (103,104). This has been suggested to be the case with *C. freundii* (98). The *ampR-ampC* intergenic region in *P. aeruginosa* is only 149 bp suggesting that *ampR* expression is autoregulated (102). Previous studies from our lab using P_{ampR} -*lacZ* fusion have shown that there is no difference in the expression from the *ampR* promoter between the wild-type and *ampR* mutant strains suggesting that *ampR* is not autoregulated in *P. aeruginosa* (105).

***P. aeruginosa* AmpR regulates other genes**

The regulatory role of AmpR was determined by comparing the properties of *P. aeruginosa* PAO1 with its isogenic *ampR* insertion mutant, PAO*ampR::aacC1*, henceforth referred to as PAO*ampR* (105). PAO*ampR* expressed high basal levels of β -lactamase even in the absence of antibiotic stress (105). Inducibility was restored by transcomplementation of *ampR*. There was no expression from P_{ampC} in PAO*ampR* (105). These data indicate that AmpR positively regulates *ampC* similar to data from other bacterial genera.

Interestingly, loss of *ampC* reduced but did not completely abolish β -lactamase activity. This, and previous observations with P_{ampC} -*lacZ*, led to the discovery of a second chromosomal β -lactamase, an oxacillinase PoxB (106). The *poxB* gene is part of a two-gene operon (*poxAB*). Introduction of this operon on a plasmid into *E. coli*, in the presence and absence of *P. aeruginosa ampR*, resulted in higher levels of β -lactamase activity only in the absence of *ampR*. Reduction of activity in the presence of *ampR*

suggested that AmpR negatively regulates *poxB* (106). Ongoing work in the Mathee lab is aimed at better understanding AmpR-regulation of *poxB*.

The PAO*ampR* strain also produced higher levels of pigments when cultured on plates or in broth suggesting that *ampR* may be involved in regulating pigment production (105). *Pseudomonas* produces green pigments that act as siderophores, chelating iron from the environment, offering the bacteria a survival advantage and enhancing virulence (107,108). Pyocyanin production was significantly higher in PAO*ampR* compared to PAO1, suggesting that AmpR negatively regulates pyocyanin production (105).

Pyocyanin production in *P. aeruginosa* is under the regulation of the *las* quorum-sensing (QS) system that along with the *rhl* system controls expression of many virulence genes in *P. aeruginosa* (109). Thus, AmpR dependence of two other virulence factors under QS control, LasA protease and LasB elastase were analyzed (105). LasA is a zinc metalloprotease of the β -lytic endopeptidase family (110). PAO*ampR* produced more LasA compared to PAO1, and the levels were restored on *ampR* complementation suggesting that AmpR negatively regulates LasA (105). This was also shown with P_{lasA}-*lacZ* fusions in PAO1 and PAO*ampR*. LasB elastase is a zinc metalloprotease that can inactivate a wide range of biological tissues and immunological agents (111). In PAO*ampR*, there was a two-fold induction in LasB production that was β -lactam-independent. The inducible phenotype was restored on complementing *ampR* in trans (105). This suggests that AmpR positively regulates LasB. P_{lasB}-*lacZ* fusions however, did not show a significant change in *lacZ* expression between PAO1 and PAO*ampR* indicating that AmpR does not regulate P_{lasB} directly. Expression of *lasI* and *lasR*, two

other QS genes, were shown to be higher in PAO*ampR* than in PAO1 (105) that demonstrates negative regulation of LasI and LasR by AmpR. These studies suggest that AmpR plays a central role in a regulon.

Role of AmpR in peptidoglycan recycling

The purported ‘natural’ role of AmpR in the cell is in the recycling of peptidoglycan. The peptidoglycan layer provides rigidity and shape to the bacterial cell. Not only is the peptidoglycan layer strong enough to withstand the internal pressure of the cell (~25 atm), it is also flexible and porous enough to allow diffusion of large molecules (112). The bacterial peptidoglycan is a complex polysaccharide of N-acetyl muramic acid (MurNAc) and N-acetyl glucosamine (GlcNAc) linked by β -1,4 glycosidic linkages (113). Multiple GlcNAc-MurNAc polymer chains are crosslinked by pentapeptide bridges (L-alanine-D-glutamine-*meso* diaminopimelic acid-D-alanine-D-alanine) forming a complex network of peptidoglycan (113,114).

Most of the peptidoglycan required for the growing cell is synthesized *de novo* (115). Biosynthesis of peptidoglycan is a complex process involving over 20 steps that take place in the cytoplasm, and on the inner and outer sides of the cytoplasmic membrane (115-117). However, not all of the peptidoglycan incorporated in the growing cell wall is newly synthesized. Both Gram-positive and Gram-negative bacteria are known to recycle peptidoglycan during cell division (118,119).

In *P. aeruginosa* and other bacteria, this recycling process is tied in with β -lactam resistance. The continuous breakdown and regeneration of peptidoglycan as part of remodeling in a growing cell is called turnover (120). The turnover process releases the disaccharide GlcNAc-anhydrous MurNAc (GlcNAc-AnhMurNAc) still attached to the

peptide chain. The chain may be three, four or five peptides long (GlcNAc-AnhMurNAc tri-, tetra-, and pentapeptides, respectively). These are transported into the cytoplasm by the AmpG permease (121). In *E. coli*, the acetylglucosaminidase NagZ acts on these products to cleave off GlcNAc resulting in tri-, tetra- and penta-peptides of AnhMurNAc (122). The NagZ homolog in *P. aeruginosa* (PA3005) has not been functionally demonstrated to be a glucosaminidase but its role in β -lactam resistance is established (123,124). Following NagZ, the amidase AmpD breaks the amide bond between the sugar and amino acid (121). The AmpD amidase was identified as being important in β -lactamase induction in *Citrobacter freundii* and *Enterobacter cloacae* (101,125). In addition to AmpD, *P. aeruginosa* has two other homologs, AmpDh2 and AmpDh3, and all three play a role in β -lactam resistance (73).

The recycled peptides and sugars then enter the *de novo* synthesis pathway where uridyl diphosphate (UDP)-GlcNAc is first converted to UDP-MurNAc by the enzymes MurA and MurB followed by addition of the pentapeptide side chain, forming UDP-MurNAc pentapeptide (115). The enzyme MraY then links UDP-MurNAc pentapeptide to an undecaprenyl attached on the inner side of the cytoplasmic membrane forming lipid I (116). Addition of a molecule of GlcNAc to lipid I results in the formation of lipid II (116). A flippase enzyme, presumably MurJ in *E. coli*, then flips lipid II to the periplasmic side of the cytoplasmic membrane (126). Penicillin binding proteins (PBPs) then take over the process of incorporating the newly synthesized sugar-peptide complex to the growing peptidoglycan chain (117).

Studies show that though this system is similar in *P. aeruginosa*, there are important differences. Details of peptidoglycan recycling and their ties with β -lactam

resistance have been studied in Enterobacteriaceae members and due to the homology of the corresponding proteins in *P. aeruginosa*, the proteins are presumed to be orthologous. The role of many players including AmpR, AmpC, AmpD and AmpG has been demonstrated in *P. aeruginosa* and are similar to their Enterobacteriaceae counterparts. In addition, new players have been identified in *P. aeruginosa*, such as the AmpD homologs AmpDh2 and AmpDh3 (73), and AmpP (127) adding to the complexity of the process. Further, studies in the Mathee lab have demonstrated that AmpR is an inner membrane protein (Caille *et al.*, personal communication), which leads to unique questions in how the effector molecules are transported to their binding sites on AmpR. In Figure 1.2, we propose a model that links cell wall recycling and β -lactam resistance in *P. aeruginosa* based on our current understanding of this process.

β -Lactam antibiotics target the PBPs, thus interfering with cell wall synthesis. When there are no β -lactams in the milieu, the concentration of the GlcNAc-UDP-MurNAc pentapeptide is high and this binds to the effector binding sites in AmpR, allowing AmpR to repress *ampC* β -lactamase expression. In the face of β -lactam attack, however, inhibition of PBP activity by the antibiotics leads to accumulation of the peptidoglycan degradation products (GlcNAc-AnhMurNAc tri-, tetra peptides), which serve as effectors by binding AmpR and activating *ampC* expression. Enhanced production of AmpC results in degradation of the antibiotics inactivating them, thus providing resistance. Recently, a second permease AmpP has been shown to be important in β -lactamase induction in *P. aeruginosa* (127). Further, AmpR has been demonstrated to be a membrane-bound regulator, with the HTH domain in the cytoplasm and effector-binding domain in the periplasm (Caille *et al.*, manuscript in preparation). We

hypothesize that AmpP siphons off some of the UDP-MurNAc pentapeptide from the cytoplasm for it to bind to the effector-binding domain of AmpR in the periplasm, repressing *ampC* expression.

Fig 1.2. Components of the bacterial peptidoglycan function as effectors in modulating AmpR activity. As part of the cell wall recycling process, the GlcNAc-anhyd-MurNAc-tri and tetrapeptides released are transported into the cytoplasm by AmpG. NagZ activity results in anhyd-MurNAc-peptides (potential positive regulators). They are recycled to UDP-MurNAc-pentapeptide (a negative regulator of AmpR activity) that subsequently forms lipid I. MurG adds GlcNAc to this molecule to form lipid II. Lipid II is transported to the periplasm by flippase activity utilizing energy from UDP hydrolysis and transglycosylases add it to the growing peptidoglycan chain. In our model, AmpR is an integral membrane protein and we propose a second transport protein, AmpP, transports the negative regulator (UDP-MurNAc-pentapeptide) to the periplasm to affect AmpR regulation. In the presence of β -lactam antibiotics, a different positive regulator (GlcNAc-anhyd-MurNAc pentapeptide) is formed due to antibiotic action on transglycosylases and this has been proposed to positively regulate AmpR action, leading to production of β -lactamases.

Transcriptional regulation: How does it work?

All cells, prokaryotes and eukaryotes, contain hundreds of thousands of genes that define and dictate everything about the organism. However, not the entire complement of the genome is expressed at any given time. Gene expression is both condition- and time-specific. Synthesis of proteins, the workhorses of the cell, is regulated at several stages and regulation of transcription is the first. One of the first steps in transcription is the binding of transcription factors to DNA. There are specific features in both DNA and proteins that are critical to this process. This section deals with the basics of protein-DNA interaction.

Features on the DNA

The outer surface of the DNA double helix is studded with sequence information that proteins recognize. On the helix, the edges of the base pairs are exposed and have hydrogen bond donors (H attached to N), hydrogen bond acceptors (N, O) and hydrophobic patches (methyl group on thymidine). The pattern of arrangement of these features is distinct for different bases only in the major groove of the DNA. In addition to these, transcriptional regulators also recognize the ‘distortions’ that are generated by the specific arrangement of nucleotides that alter the geometry of the double helix. For example, the repeat 5’ AAAANNN 3’ at 10 nucleotide intervals causes a slight local bending in the DNA. Such natural curves (bends) help form a tight fit between transcriptional regulators and DNA.

Features on the protein

DNA-binding proteins recognize specific sequences on the DNA because the surface features of the protein and the DNA they bind to bear extensive complementarity. DNA-protein interactions involve hydrogen bonds, ionic bonds and hydrophobic interactions. Though these binds are individually weak, there are typically about 20 interactions per recognition site, which is strong enough to bring about binding. The weakness of the individual interactions is also critical for reversal of the process, allowing control of regulation. Features on both DNA and protein determine binding specificity. Specific amino acids bond with specific bases on the DNA. Asparagine, for example, binds with adenine and arginine binds with guanine. Given that there are only four types of nucleotide bases while there are 20 amino acids, some amino acids can bind multiple bases. In addition to arginine, guanine can also bind with serine, lysine and histidine; adenine binds serine and asparagine; thymine interacts with alanine and serine while cytosine binds aspartic acid. Most regulatory proteins bind DNA as dimers or multimers because it is a simple way to achieve strong specific binding.

DNA-binding motifs in proteins

The regions of the protein that bind DNA can be classified on a structural basis into different types.

Helix-turn-helix (HTH) motif: HTH was the first DNA-binding motif to be discovered. It is the most common motif found in DNA-binding proteins and is prevalent in both prokaryotes and eukaryotes. The HTH motif is composed of two α -helices connected by a short extended chain of amino acids, which form the turn. The helices are held at a fixed angle of 120° by interactions between the helices. Typically, the helix at the C-terminal

end of the protein (the second helix) is the recognition helix that interacts with the major groove of the DNA. The way that this helix is presented to the exposed bases in the major groove depends on the structure of the rest of the protein and how it contacts the DNA. The side chain amino acids vary between proteins and confer specificity. While the HTH motif is more or less conserved, barring the variation that confers specificity, the proteins can differ enormously outside of the HTH motif. The regions outside the HTH also, sometimes, interact with the DNA to fine tune recognition. Typically, HTH motif-containing proteins bind DNA as symmetrical dimers to two similar/identical half-sites.

Homeodomains are a special class of HTH motifs that are present in eukaryotes. An important difference between regulators that contain HTH motif or homeodomain is that the latter can bind DNA as a monomer whereas the former binds DNA as a dimer or multimer.

Another variation of the HTH motif is the helix-loop-helix (HLH) motif. HLH motifs consist of a short α -helix connected by a loop to a longer α -helix. The loop connecting the two helices is flexible such that one helix folds back and packs against the other. The loop is also the site of dimerization (homo or heterodimers). Many HLH proteins lack a α -helical extension needed to bind DNA. When these truncated proteins form homo or heterodimers with other full-length proteins, they form partially active dimers that make only half of the necessary contacts. This is one way of keeping such regulatory proteins in check.

Zinc-finger motifs: These motifs are composed of amino acids and zinc atoms as part of the structure. All groups of zinc-finger motif-containing proteins have zinc as a structural

element and bind DNA via a α -helix. Several groups have been identified based on structural studies.

In the first type, a zinc molecule holds together a α -helix and a β -sheet. This type of arrangement is typically found in a cluster with other zinc fingers in tandem. Each α -helix contacts the major groove of the DNA. The specificity and strength of the interactions are enhanced by the presence of tandem repeats of α -helices. A second type of a zinc-finger motif is where two α -helices are packed together with zinc atoms in an arrangement that is similar to a HTH motif. They form dimers, which allows one of the two α -helices to bind DNA.

Leucine zipper coiled-coil: Originally identified as a dimerization motif, leucine zippers are α -helical segments. Leucine zippers mediate DNA binding and protein dimerization. Secondary α -helices, one from each monomer, join to form a short coiled-coil. The helices are held together by interactions between hydrophobic side chains, often at a leucine. The two helices separate just beyond the dimerization interface to form a Y-shaped structure, thus allowing the side chains to interact with the DNA.

β -ribbon proteins: β -ribbons can also bind DNA. The major groove of the DNA is recognized by a two-stranded β -sheet with side chains of amino acids extending from the sheet toward the DNA. DNA recognition depends on the amino acid sequence, like with α -helices.

Dissertation overview

P. aeruginosa is a bacterium that has fascinated researchers for decades and has been extensively studied. Numerous studies have addressed different regulatory aspects

of metabolism, physiology and pathogenesis. This has led to a great deal of understanding of the molecular mechanisms of gene regulatory processes in *P. aeruginosa*. However, with the advent of high-throughput genomics, our traditional understanding of regulatory repertoires is changing.

With this in mind, my study has two main objectives:

To better understand gene regulation in *P. aeruginosa*: This was achieved by performing global analyses of published data, specifically metaanalysis of transcriptome studies, and analyses of putative and established regulatory networks in *P. aeruginosa*. This objective is the basis of chapters 2 and 3 of this dissertation.

To exemplify the regulatory complexity in *P. aeruginosa* using AmpR: Previous studies in the lab have established that AmpR regulates some non- β -lactam resistance genes, in addition to regulating its primary target, *ampC* that encodes a β -lactamase. I explore the regulatory repertoire of the β -lactamase regulator AmpR adopting global approaches such as microarrays, RNA-Seq and ChIP-Seq. Chapters 4 and 5 of this dissertation address this objective.

CHAPTER 2

Comparative transcriptomic analyses of *Pseudomonas aeruginosa*

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Abstract

One of the hallmarks of bacterial survival is their ability to adapt rapidly to changing environmental conditions. Niche adaptation is a response to the signals received that are relayed, often to regulators that modulate gene expression. In the post-genomic era, DNA microarrays are used to study dynamics of gene expression on a global scale. Numerous studies have used *Pseudomonas aeruginosa*, a Gram-negative environmental and opportunistic human pathogenic bacterium, as the model organism in whole genome transcriptome analysis. Here, we review the transcriptome studies that have led to immense advances in our understanding of the biology of this intractable human pathogen. Comparative analysis of 23 *P. aeruginosa* transcriptome studies has led to the identification of a unique set of genes that are signal-specific and a core set that was differentially regulated. The 303 genes in the core set are involved in bacterial homeostasis making them attractive therapeutic targets.

Introduction

Bacteria rapidly adapt to changing conditions by altering gene expression. The process of bacterial adaptation has fascinated researchers as it provides important insights into their physiology. This information is especially useful for human pathogens because it can be applied to better combat infections. This premise has led to studies that examine gene expression on a global scale. An entire set of RNA transcripts produced by a population of cells at any given time during gene expression is referred to as a transcriptome (128). Variations in the external environment or genome perturbations, such as mutations, can alter the transcriptome of a cell. Transcriptome studies thus provide us with a snapshot of the organisms' response based on the genes that are expressed. Though there is not always a linear relationship between the expressed genes and the proteins synthesized, such studies give us a reasonable insight into the state of the cell.

In the pre-genomic era, transcriptome analysis relied on techniques such as differential display and RNA arbitrary primed PCR (129-131), cDNA-amplified restriction fragment length polymorphism (132-134), random expressed sequence tag sequencing (135), serial analysis of gene expression [SAGE; (136)], LongSAGE (137), SuperSAGE (138,139), and massively parallel signature sequencing (140). Prior sequence information is not required for these methods but they are both labor and time-intensive. Bacterial studies entered the genomic era with the sequencing of the *Hemophilus influenzae* genome in 1995 (141). Since then, the genomes of over 1000 bacteria have been fully sequenced. Large-scale sequencing has ushered the development of high throughput genome-wide expression techniques such as DNA microarrays. This

powerful technology has been harnessed in numerous studies, under diverse conditions to elucidate whole genome expression in both prokaryotes and eukaryotes. One such prokaryote is *Pseudomonas aeruginosa*, where DNA microarrays have been of immense help in understanding its complex biology.

P. aeruginosa

P. aeruginosa is a Gram-negative human pathogen of the family *Pseudomonadaceae* and can switch from being an environmental isolate, found in the soil and water, to a human pathogen (7). It is an epitome of opportunistic human pathogens, being implicated in a wide range of infections of the pulmonary tract (8,9), urinary tract (10,11,13), burns (14-16) and wounds (17,18) among others. It causes persistent nosocomial infections (23,24) and is also a major pathogen in cystic fibrosis patients. One of the major weapons in its arsenal is its resistance to antibiotics. It is both inherently resistant and can also acquire resistance genes from other bacteria making it a notorious pathogen. Understandably, numerous studies over the years have focused on elucidating the mechanisms of pathogenesis and antibiotic resistance of *P. aeruginosa*. Since the sequencing of the genome (25), the emphasis of these studies has shifted from conventional pathogenesis research, where the focus was on individual virulence determinants, to a much larger scale involving whole genome analyses.

The prototypic reference strain of *P. aeruginosa* is PAO1, isolated from a burn wound, and was the first *P. aeruginosa* strain to be sequenced (25). The most prominent feature of the PAO1 genome is its size. It has one of the largest bacterial genomes with 6.26 million base pairs comprising of almost 90% protein coding genes. The genome complexity of *P. aeruginosa* explains its versatile nature to adapt and thrive in a wide

range of environments. The genome has 5,570 open reading frames (ORFs), a number that is close to simple eukaryotes like *Saccharomyces cerevisiae*. Other features of the PAO1 genome include the presence of four ribosomal RNA loci (*rrn*) that are the regions of the longest repeats and a high GC ratio at 66.6% (25). Following the sequencing of the genome, high-density DNA microarrays were developed for *P. aeruginosa* (28) that offered us a chance to take snapshots of the dynamic responses of this versatile genome to various stimuli. An important study in this context (29), which analyzed the genomes of both environmental and clinical isolates of *P. aeruginosa*, suggested the presence of a core genome in different strains and expression of different sets of genes to facilitate growth in particular environments. This calls for a robust control over transcription mediated by transcriptional regulators. Analysis revealed that about 10% of the PAO1 genome is comprised of transcriptional regulators (25). This suggests the existence of a tight control over the way genes are expressed in *P. aeruginosa*.

Subsequent to PAO1, other *P. aeruginosa* strains such as PA14 (142), PA2192 [a CF isolate, (31)], C3719 [the Manchester epidemic strain, (32)] and PACS2 (33), have been sequenced. Comparative genome analysis of the five sequenced *P. aeruginosa* strains has led to the identification of a set of core shared genes that all the genomes contain, the majority of which are housekeeping genes (33). In addition, there is an accessory genome comprising of mostly hypothetical ORFs or those related to transposons and bacteriophages, and genes involved in niche adaptation specific to each of the five strains (33). The accessory set also contains transcriptional regulators that may control expression of ORFs in the core genome. Since the currently available microarray chips are based on the PAO1 genome, performing microarray studies with non-PAO1

strains would seriously underestimate the transcriptional profile. Though GeneChip (Affymetrix) contains 117 probe sets for ORFs from non-PAO1 *P. aeruginosa*, it is not sufficient to cover the accessory genomes from all the other sequenced strains. Analysis of five *P. aeruginosa* genomes revealed that 5021 genes are conserved between the strains (core gene set) and that the genome of each strain has anywhere between 79 to 507 unique genes that are part of the accessory genome for that strain (33). There is thus a gross under-representation of non-PAO1 genes on the Affymetrix GeneChip that will undoubtedly affect data interpretation. Thus, the next major advance in *P. aeruginosa* microarrays would be the development of a pangenome array, comprised of probe sets for the core and accessory genomes from all sequenced strains. This will greatly advance our understanding of the system by allowing researchers to study non-PAO1 strains and help appreciate better the biology of this versatile bacterium.

***P. aeruginosa* transcriptome studies**

***P. aeruginosa* and iron limitation**

Iron is an important cofactor that plays a critical role in many cellular processes. Low iron availability in host tissues is a major impediment that the bacteria have to overcome in establishing an infection. Bacteria have evolved an elaborate system of acquiring iron from the surroundings. *P. aeruginosa* secretes siderophores (pyochelin and pyoverdine) that chelate iron and bring it back into the cell via specific cell-surface receptors (108). Fur is a major repressor that tightly regulates this uptake process by controlling expression of the regulators, PvdS and PchR (143).

In one of the initial transcriptome studies of the response of *P. aeruginosa* to iron (144), stationary phase *P. aeruginosa* cells were grown in the presence and absence of

iron at 32 °C followed by gene expression analysis. Results indicated that 137 genes were upregulated on iron depletion (144). A more systematic study was performed by Palma *et al.*, which addressed how the transcriptome changes when iron-starved *P. aeruginosa* cells are fed with iron (145). Differential regulation was seen in mRNA levels of 177 genes. Most of the genes were down regulated, especially genes involved in transport of small molecules, transcriptional regulators and hypothetical proteins. It was interesting to note that genes involved in virulence were upregulated, some of which probably help the cells in overcoming the host innate immune system (145). An important finding was that 38 genes, including those coding for siderophore receptors, iron transport, regulators, sensors, sigma factors were identified whose transcription decreased on iron treatment. These 38 genes were proposed to be part of a regulon involved in iron response in *P. aeruginosa*. In another study, Zheng and coworkers identified a hypothetical protein, PA2384, that regulates over 70 genes involved in the iron uptake process in a Fur-independent manner (146). Moreover, deleting PA2384 also led to differential expression of many genes involved in cellular physiology, like translation, metabolism and electron transfer indicating a broader role for this protein.

***P. aeruginosa* and oxidative stress**

For bacteria to successfully infect a human host, they should be able to overcome many barriers. One primary defense mechanism is production of reactive oxygen species

(ROS) by the host cells that leads to oxidative stress in bacteria. Palma *et al.*, studied the response of *P. aeruginosa* to a natural oxidant in human systems, hydrogen peroxide, as an indication of the global transcriptional status of the cell's early response (147). The study confirmed that some of the genes that were earlier found to be important in a cell's response to oxidants were upregulated in addition to identifying 520 genes with a significant (>5-fold) change in mRNA levels (147). Of these, 216 genes were upregulated, seven of which were upregulated more than 50-fold, primarily hypothetical proteins of unknown function. Further, upregulated genes included cytotoxin genes such as exoenzymes S and T, and PvdS that control PrpL proteinase and exotoxin A expression and could potentially inhibit the cells of the innate immune system. Most of the genes that were down regulated are involved in metabolic pathways and ribosomal synthesis, which makes sense, for the cell under oxidative stress would not want to actively metabolize or grow but deal with the stress first (147).

In another study looking at response to hydrogen peroxide, stationary phase cells of three strains belonging to two clonal types were tested (148). Two of the strains, TB and 892 were cystic fibrosis (CF) isolates and their response was compared with PAO1, which belongs to a different clonal type. The expression profiles of the CF isolates were more similar to each other than to PAO1, sharing more than 80% of the differentially expressed genes with each other. This study highlighted the importance of clone-specific transcriptome profiles, variations seen in both intraclonal (between the two CF isolates) and interclonal (between CF isolates and PAO1) strains.

Genome-wide transcriptomes of the three strains, *P. aeruginosa* PAO1 and the two CF isolates TB and 892 were also analyzed for their response to superoxide-

generating paraquat (149). Less than 30 genes were differentially expressed in each of the three strains, of which only six were upregulated in all three. Upregulated genes belonged to oxidative stress response, transport and acetyl-CoA and acetoin metabolism. Four genes of a single operon coding for the superoxide dismutase SodB, catalase KatA and alkylhydroperoxide reductase AhpC were upregulated in three strains, highlighting their importance in *P. aeruginosa* superoxide stress response. Energy metabolism genes were downregulated to counter the superoxide stress (149).

Two recent papers by Small *et al.*, looked at the oxidative stress response of *P. aeruginosa*. Both of these articles focused on the effect of oxidizing agents routinely used in hospitals for surface sterilization and their effect on *P. aeruginosa*. The first is a transcriptome analysis of sodium hypochlorite (NaOCl)-induced oxidative stress (150). In this study, Luria-Bertani (LB) broth grown mid-log phase *P. aeruginosa* PAO1 cells were treated for 20 minutes with sub-lethal concentrations of bleach. RNA extraction and microarray analysis followed. Results were compared with similarly grown untreated cells. About 20% of the 5570 genes of *P. aeruginosa* were differentially expressed in response to the treatment, approximately 10% each being up- and downregulated. Responses similar to that seen with hydrogen peroxide were generated, with upregulation of catalase, alkyl hydroperoxide reductase, and glutathione peroxide/reductase genes indicating that the mode of action of bleach is similar to that of hydrogen peroxide in inducing the oxidant defense system genes. Genes involved in transport of sulfur compounds were highly upregulated. A possible explanation for this observation is that the free electrons in the sulfhydryl groups in the biological molecules react with the hypochlorous acid formed by exposure to NaOCl, a known strong oxidizer, countering its

effect. This would increase chances for cell survival (150). Genes that are repressed are primarily the ones involved in oxidative phosphorylation and electron transport indicating that the cells under NaOCl-induced stress would have lowered energy due to impaired metabolic processes.

Another study compared and analyzed the transcription patterns of three different oxidative damage-inducing agents: NaOCl, peracetic acid and hydrogen peroxide on *P. aeruginosa* (151). Over 550, 300 and 200 genes were differentially regulated as a result of NaOCl, peracetic acid and hydrogen peroxide, respectively. Of these, 63 genes were common to all three agents, 40 upregulated and 23 downregulated. About 25% of the common upregulated genes were 'related to phage, transposon or plasmid' and eight were to membrane protein. The primary downregulated genes were those involved in transport of small molecules. Other prominent gene categories included membrane proteins and putative enzymes. It is surprising to note that membrane protein genes were both up and downregulated. NaOCl resulted in the most downregulation of genes involved in energy metabolism suggesting that NaOCl may be more effective in limiting energy production than the other two agents. Peracetic acid exposure resulted in stimulating expression of genes involved in adaptation and protection and downregulation of transcriptional regulators. Several protective mechanisms were triggered after exposure of cells to hydrogen peroxide. A significant finding of this study was positive expression of the pyocin genes of the F-, R- and S- types after hydrogen peroxide treatment but was downregulated with the other two treatments. This has been linked to DNA damage and RecA activation that has been known to induce pyocins. RecA has been demonstrated to cleave single stranded DNA with the help of hydroxyl radicals that are formed by

exposure of cells to hydrogen peroxide. The induction of pyocin genes in response to hydrogen peroxide treatment, published separately (152), demonstrates that NaOCl exposure leads to significant differences in the transcription pattern of *P. aeruginosa* and is more lethal to the cell compared to either hydrogen peroxide or peracetic acid (151).

Nde *et al.*, tested the response of *P. aeruginosa* to ortho-phenylphenol (OPP) (153), a widely used disinfectant and antimicrobial agent whose mode of action on bacteria has not yet been elucidated. Using two different exposure times (15 minutes and 60 minutes), they identified genes coding for virulence, membrane and ribosomal proteins upregulated at both time points. Forty-five genes of the translation, post-translation modification and degradation were upregulated, primarily initiation, elongation and release factors (153). Genes encoding components of the SecY system, involved in export of various proteins, toxins and enzymes, showed enhanced expression as were some membrane associated proteins involved in small molecule transport, possibly indicating that the OPP-treated bacteria were transporting proteins across the cell membrane as part of an adaptive process. Also upregulated were genes of type IV pili assembly proteins, fatty acid biosynthesis and energy metabolism, all of which indicate adaptation (153).

***P. aeruginosa* and pentachlorophenol stress**

Response of *P. aeruginosa* to pentachlorophenol (PCP) was carried out recently (154). PCP is thought to embed in the membrane where it acts as a proton shuttle

uncoupling oxidative phosphorylation (155). In addition, since RpoS is the stationary phase sigma factor thought to be an important factor in the cell's stress response, *rpoS* mutants were also examined under the same conditions. Sixty genes showed two-fold or greater increase in transcription. RpoS did not play a protective role in the cell's response to PCP-induced stress. The highlight of this study is the induction of multiple operons involved in multidrug efflux, the first anthropogenic induction of the MexAB-OprM system with chemicals. Notably, two operons of the resistance-nodulation-cell division (RND) pumps were upregulated in addition to one operon that positively regulates MexAB-OprM. These are involved in antibiotic and solvent efflux. Putative major facilitator superfamily (MFS) proteins were also upregulated in response to PCP-induced stress. Selective downregulation of genes involved in central metabolism, fatty acid metabolism, energy metabolism and adaptation/ protection was observed (154).

***P. aeruginosa* response to copper**

Copper is one of the essential micronutrients playing an important role in cellular respiration, as part of electron transport and many biological reactions (156). Bacteria have to tightly control cellular levels of copper to prevent toxicity or starvation. Two studies looked at how *P. aeruginosa* responds to varying levels of copper.

Tietzal *et al.*, identified 41 genes that form a core response of *P. aeruginosa* to copper, comparing transcriptomes of cells exposed to high levels of copper either for a short time (copper-shocked, 405 genes) to those grown in copper [copper-adapted, 331 genes; (157)]. Genes involved in active efflux of copper included transporters, copper

oxidase, periplasmic thiol-disulfide interchange proteins, lyases, and transcriptional regulators.

Copper starvation of *P. aeruginosa* under aerobic conditions (158) led to downregulation of 132 genes while only 10 genes were upregulated. The most strongly downregulated genes were the iron acquisition genes, mostly those repressed by Fur, the iron-uptake regulator. One of the downregulated transcriptional regulators, PA3410, encodes a probable extracellular sigma factor (157). The upregulated genes included mostly hypothetical genes, and a bacterioferritin involved in iron storage. In fact, copper starvation and iron depletion (144,145) led to the expression of similar sets of genes but have opposite effects on them.

***P. aeruginosa* and sulfate starvation**

The CF lung mucin is highly sulfated, probably a defense mechanism to prevent degradation by bacterial enzymes (159,160). The metabolically versatile *P. aeruginosa* can utilize a variety of organic and inorganic compounds for growth. Under limiting conditions of inorganic sulfate, the cells can uptake organosulfur compounds for growth without affecting the growth rate (161). Global response to sulfate starvation on a non-mucoid CF isolate of *P. aeruginosa* E601 and PAO1 was addressed (162). The study was aimed at determining the genes regulated by sulfur supply and whether this was altered during CF infection, a condition simulated by exposure to mucin. Three groups of responses were observed: response to sulfur starvation, to mucin as a sulfur source and to mucin exposure (162). Functional analysis of differentially expressed genes revealed that both strains showed similar responses in the different functional classes, the maximally

regulated genes belonging to hypothetical proteins, transport of small molecules and putative enzymes. Unlike PAO1, the CF isolate E601 was shown to utilize mucin as a sulfur source. Moreover, several genes involved in the type III secretion system and exoenzyme S were induced, suggesting that cells recognize mucin not only as a sulfur source but also as a host component: a dual response (162).

***P. aeruginosa* and osmotic stress**

P. aeruginosa, being a versatile pathogen, can survive in a wide range of environments, some of which can be osmotically stressful. Transcriptional profiling of osmotically-stressed *P. aeruginosa* was studied (163). *P. aeruginosa* PA14 cells, grown in glucose minimal media, were exposed to osmotic stress, either under steady-state conditions (0.3 M NaCl and 0.7 M sucrose) or osmotically up-shocked (0.3 M NaCl for a short time). A major difference in the expression pattern between these two conditions is the number of genes that are differentially regulated. While 431 genes were differentially regulated on osmotic up shocking, only about 100 genes were more than 3-fold regulated under either condition of steady-state osmotic shock. These studies led to the identification of a steady-state osmotic shock regulon comprised of 66 genes. Forty of these are virulence-associated, primarily genes of the type III secretion system, two type III exotoxins and two chaperones. In contrast, the osmotically up-shocked cells showed repression of the type III secretion system genes that the authors hypothesize to be an energy-saving step. In addition, the sigma factor AlgT/U, previously known to be induced in varied stress conditions, and genes known to be involved in osmoprotection,

including hydrophilins whose exact role in osmoprotection has not yet been conclusively proven in bacteria (164) were induced in all the conditions of osmotic shocking (163).

***P. aeruginosa* and quorum sensing**

P. aeruginosa senses the cell density for coordinated expression of virulence factors using a global regulatory system called quorum sensing (QS). Sensing is achieved using small diffusible molecules that in *P. aeruginosa* and in other Gram-negative bacteria are the N-acylated homoserine lactones (165). *P. aeruginosa* has two N-acylated homoserine lactone systems, *las* and *rhl* (166). Transcriptome analyses of *lasI* *rhlI* mutants were performed by three groups (167-169). Two of these groups (167,168) identified about 300 genes that were induced by quorum sensing while the other group got only 163 differentially expressed genes as a result of use of more stringent criteria. The three studies had approximately 100 common genes and most belonged to the functional class of secreted factors. This group included toxins and extracellular enzymes. In addition, above average expression changes were seen in genes involved in metabolism, biosynthesis of cofactors and fatty acid metabolism, implying that QS signals mediated by N-acylated homoserine lactones were involved in regulation of many genes other than those involved in virulence. The third group, Hentzer *et al.*, targeted the QS system in an attempt to mitigate *P. aeruginosa* virulence (169) using a furanone isolated from the Australian seaweed *Delisea pulchra*. The authors elegantly show using transcriptome analyses that about 80% of the genes that were downregulated by the furanone were also repressed in an N-acylated homoserine lactones mutant. The furanone compound also affected biofilm formation and persistence of infection in a

mouse lung model. The three studies together led to the identification of a core set of 77 genes of the QS regulon that are positively regulated (169). Schuster and Greenberg (170) review many other studies of regulators that further elucidate the complex QS system and the intricate interconnections. They propose a complex network that involves 13 different regulators that interact with LasI and RhlI and form the QS regulon.

***P. aeruginosa* biofilms**

Quorum sensing has been implicated to be an important step in the formation of ordered, antibiotic-resistant bacterial communities called biofilms (171,172). Planktonic cells, in response to external stimuli, form biofilms in varied habitats, like in the CF lung, surgical implants and in various environmental situations exhibiting drastically altered physiology that lead to the hypothesis that the gene expression between these two states of cells are very different. One of the earliest studies that employed microarrays to understand differences in gene expression patterns between planktonic and biofilm cells, in addition to antibiotic resistance mechanisms in biofilms, was by Whiteley *et al* (173). Using chemostats, it was found that a surprisingly small number (1%) of genes were differentially regulated between planktonic and biofilm cells. The most highly upregulated genes were those of the Pfl-like prophage with nine of the 11 genes on the chromosome induced over 100-1000 fold in the biofilm cells. This could imply higher rates of gene transfer in biofilms via transduction. Genes involved in bacterial motility were repressed, as was *rpoS*, the stationary phase sigma factor. Strains harboring a mutant *rpoS* were shown to form larger more drug-resistant biofilms. Exposure of the biofilm to the antibiotic tobramycin followed by transcriptional analysis showed *tolA* was

upregulated, which can serve as a barrier to aminoglycoside uptake. Cytochrome C oxidase genes were repressed, probably slowing growth, negating antibiotic effect. In addition, antibiotic exposure also induced two probable efflux systems and many hypothetical genes.

Effect of the β -lactam imipenem on gene expression in planktonic and biofilm cells of *P. aeruginosa* was examined by Bagge *et al* (174). Microarray analysis of imipenem-exposed planktonic and biofilm cells revealed that the chromosomal β -lactamase *ampC* was overexpressed on imipenem exposure in biofilms and planktonic cells. Thirty-four genes were more than five-fold differentially regulated and included genes involved alginate production, flagella biosynthesis, cell division, peptidoglycan-related and type 4 pili biogenesis. In addition, biofilm cells also showed enhanced production of alginate, unlike in the Whiteley study with tobramycin. Bagge *et al* could not ascribe higher antibiotic resistance of biofilm cells to a specific set of genes due to similar expression patterns in planktonic and biofilm cells (174).

A more recent study makes a comprehensive analysis of transcriptomes of planktonic cultures (in log and stationary phase) and biofilms at 8, 14, 24 and 48 hours (microcolony and mature biofilms) using a static biofilm model (173). This study shows a similar gene expression pattern between developing biofilms and log phase planktonic cultures while mature biofilms have expression patterns similar to stationary phase cells. The most highly upregulated genes in biofilm cells were those of a Pfl-related temperate bacteriophage suggesting an important role for phage-mediated gene transfer in biofilm cells. Downregulated genes include those coding for pili that suggests that though pili are required for establishment of biofilms, they are not essential in mature biofilms. Also,

expression is relatively stable in developed biofilms with only 1% of the genes differentially expressed. This was the first study to look at global gene expression for two planktonic and two biofilm states of the cells and the authors have generated a comprehensive dataset for reference that compares transcriptomes of planktonic, developing and mature biofilms.

Transcriptomics of alginate production in *P. aeruginosa*

Alginate gene upregulation in *P. aeruginosa* is one of the primary modes of survival in the cystic fibrosis lung and converts the non-mucoid cells to a mucoid phenotype (175) as a result of over-production of the extracellular polysaccharide alginate. Production of alginate is part of a stress response in *P. aeruginosa* and is mediated by the alternate sigma factor AlgT/U, a sigma E homolog. AlgT/U is held sequestered by the anti-sigma factor MucA. Release of AlgT/U leads to alginate production and is part of a much larger response to help the cells adapt (176). Transcriptome analysis of the *mucA*, *algT/U*- *mucA* mutants and the wild type PAO1 showed that alginate was upregulated in the *mucA* mutant where there was no control on AlgT/U production. This control was lost in the double mutant as expected (177). What was exciting was that many genes responsible for toxin production, enzymes and transport were also upregulated indicating that AlgT/U was part of a regulon that coordinated expression of many genes, ultimately enhancing *P. aeruginosa* survival in the harsh CF lung environment.

P. aeruginosa, like other bacteria, produces flagella only when needed, for example in biofilm formation and has been implicated in establishing pulmonary

infections (178). AlgT/U was shown to negatively regulate flagella production in *P. aeruginosa* by downregulating an essential flagella regulator, FleQ (179). There are many regulators in flagella biosynthesis and a systematic study of several regulator mutants was performed by Dasgupta *et al* (180). Using transcriptome analysis, they found that flagella are assembled in a four-step process that is controlled by the sequential expression of different regulators.

Lipopolysaccharides and lipoproteins as virulence determinants

Lipopolysaccharides play a major role in *P. aeruginosa* virulence (181). The bacteria are known to modify their lipopolysaccharide layer in response to the milieu. Lipid A, a major constituent of the bacterial liposaccharide is hepta- or hexa-acetylated in strains causing acute infections while it is penta-acetylated in chronic infections like in the cystic fibrosis lung (182). Lipopolysaccharide changes with alginate production (183) and production of both of these important virulence factors are influenced by oxygen concentration (184,185,186). Though microarray studies that compare lipopolysaccharide mutants have not been published yet, Firoved *et al.*, have demonstrated that lipoprotein genes are the most highly upregulated when *P. aeruginosa* switches over to the mucoid phenotype by comparing the expression patterns of a mucoid (PAO578II) and the isogenic non-mucoid parent [PAO6865; (187)]. Eight of the nine genes that were upregulated over 30-fold encoded lipoproteins, which can have toxic effects in environs like the cystic fibrosis lung by disrupting host Toll-like receptor 2 signaling and are also antigenic, activating host defense mechanisms. Citing these reasons, some of these lipoproteins have been termed lipotoxins (187). Elucidating mechanisms of coregulation

of LPS production/ modification and conversion of the strains to mucoidy would help us better understand the adaptation/ pathogenesis of this versatile bacterium in chronic infections, like in the cystic fibrosis lung.

Transcriptome of a post transcriptional regulator

Burrowes *et al.*, studied the role of a small posttranscriptional regulatory protein RsmA (188). RsmB, a small untranslated regulatory RNA, binds RsmA limiting its activity (189). RsmA controls expression of many virulence genes and QS signaling molecule synthesis (189,190) and thus plays an important role in *P. aeruginosa* pathogenesis. Though some of the targets of RsmA were known this study focused on determining the direct and indirect regulatory control exerted by RsmA in *P. aeruginosa* (188). Transcriptional analysis of PAO1 and the *rsmA* mutant revealed that of the 506 genes that had significantly altered expression, genes involved in iron acquisition and antibiotic resistance, especially genes involved in the MexEF-OprN pump were upregulated and genes involved in *Pseudomonas* quinolone signal biosynthesis, and motility were downregulated. The data obtained suggests that RsmA is a global regulator (188).

***P. aeruginosa* interactions with human cells**

When a bacterium comes into contact with a host cell for the first time, there is a drastic change in the lifestyle of the bacteria that aid in colonization. The host, on the other hand, recognizes this threat and mounts an immune response. As one can predict, this race between the pathogen and host to outdo each other will undoubtedly be accompanied by changes in the gene expression profile in both organisms. Numerous

studies have looked at the response of the human immune system to *P. aeruginosa* exposure and we have a fairly good idea of the immune signals and molecules involved. One of the first studies to employ transcriptomics to investigate host responses to initial contact with *P. aeruginosa* was carried out by Ichikawa and Lory (191). Using a time course microarray experiment with lung epithelial cells exposed to *P. aeruginosa* and custom made cDNA microarrays containing about 1500 human clones with both characterized and uncharacterized genes, they determined that about 25% of the human genes represented on the array were differentially regulated. Of interest was a lipopolysaccharide-independent two-fold activation of the interferon regulatory factor-1 gene with no activation of other downstream genes that are normally interferon-activated. This indicated that *P. aeruginosa* activation of interferon regulatory factor-1 is different from the normal mechanism of interferon regulatory factor-1 activation mediated by cognate receptor binding (191). Using a type IV pili mutant, they demonstrate that PilA is the ligand responsible for LPS-independent activation of interferon regulatory factor-1. This study demonstrates the power of microarrays in understanding host-pathogen interactions by elucidating potentially new signaling pathways and by characterizing roles of different virulence factors involved in pathogen recognition and mounting an immune response.

Two other studies have employed DNA microarrays to study the interactions of *P. aeruginosa* to human epithelial cells but the results are contradictory with respect to the regulation of iron-responsive genes. In the first study by Frisk *et al* (192), *P. aeruginosa* was exposed to primary human airway epithelial cells for 4 and 12 hours *in vitro* and analyzed transcription post-exposure. Two major groups of genes were differentially

regulated: phosphate uptake genes were upregulated whereas iron acquisition genes were downregulated. This was contrary to the notion that iron is a very important limiting factor in the human host and one would have expected upregulation of the iron acquisition genes. The authors concluded that *P. aeruginosa* was able to acquire enough iron for infection that led to deregulation of those genes. The reverse was true for phosphate.

In an attempt to better understand the initial adaptation processes of *P. aeruginosa*, especially on interaction with human epithelia, a second study from the Greenberg lab (193) compared transcriptomes of *P. aeruginosa* PAO1 wild-type with a double mutant that was deficient in Type III secretion system and rhamnolipid biosynthesis. These two bacterial systems are key factors contributing to human airway epithelial cell damage. Strain-specific responses were seen. Whereas virulence genes like PA-1 galactophilic lectin were induced in the wild-type strain, alginate regulatory genes were induced in the mutant along with *gacA* (193), which is known to be upregulated in chronic *P. aeruginosa* infections (28). Also, Vfr, a known upregulated regulator in acute infections was downregulated indicating early stages of a chronic infection.

In contrast to the results obtained by Frisk *et al* (192), the Chugani study showed that iron starvation genes were positively regulated indicating that the infecting cells had little iron to utilize. This is in concordance with the belief that infecting bacterial cells have little access to iron in the human body. One possible explanation for these discordant results, as noted by the authors, is that the epithelial cell integrity in the previous study could have been compromised leading to leakage of intracellular iron resulting in iron-replete conditions. This would explain the increased expression of iron

dependent genes over time seen in the Frisk study. Epithelial cell integrity was monitored in the Chugani study by monitoring the transepithelial electrical resistance (Rt). An interesting finding by Chugani *et al.* is that the general pattern of gene expression is similar to that seen in CF isolates from chronic human infections.

Many bacteria use Type III secretion systems to inject proteins directly into host cells. An interesting whole genome array study by Wolfgang *et al* (29) looked at the conditions and regulatory mechanisms that control expression of the Type III secretion systems. They utilized the observation that Type III secretion system is induced by either growth in low-calcium medium or by cell contact and found eleven calcium dependent regulators that were involved in the process by growing cells in low calcium. Analysis of these proteins led to the discovery that a membrane-associated adenylate cyclase initiates a signaling pathway that controls expression of Type III secretion system genes (29).

The transcriptome studies of *P. aeruginosa* with human cells indicate the presence of as yet unidentified signals, from both the host and the bacteria during interactions. Unraveling these signals could provide us with valuable information to effectively counter infections by *P. aeruginosa* and microarrays will undoubtedly prove vital in this endeavor.

Comparative analysis of all transcriptomes

In order to better understand the response of *P. aeruginosa* to the different stress conditions, we compared significantly differentially regulated genes from the studies within each stress condition. This analysis was performed only for those conditions that had multiple studies and had publicly available gene lists. Our comparative analysis led

to the identification of common sets of genes that are regulated in response to each particular stress condition across the different studies. Additionally, we also identified unique genes that are specifically regulated only in individual studies. This data has been summarized in Table 2.1.

Table 2.1: Comparative analysis of significantly differentially regulated genes from various studies within each condition.

Stress condition	# of studies	Differentially regulated genes	
		Common	Unique
Iron	3	69	220
Quorum sensing	3	121	862
Copper	2	26	209
Oxidative stress	7	83	386
Biofilms	3	55	650

Analysis of significantly differentially regulated genes from all of the above studies led to the identification of 303 genes that form a core set and are differentially expressed in all the different conditions (Appendix; Supplementary information for Chapter 2, Table 1). This suggests that this set of core genes is important for maintaining homeostasis in *P. aeruginosa*. Functional categorization of the core set genes (Appendix; Supplementary information for Chapter 2, Figure 1) reveals that apart from the hypothetical genes (27%), the most highly differentially regulated genes encode membrane proteins (15%) followed by genes involved in transport of small molecules (11%), adaptation and protection (7%) and transcriptional regulators (6%) clearly indicating an adaptive response of *P. aeruginosa* to the changing environment. We then

compared this microarray core set of 303 genes with the core genome of *P. aeruginosa* that contains genes found in all *P. aeruginosa* sequenced strains (33). Most of these genes (90%) belong to the *P. aeruginosa* core genome, but we identified 29 genes belonging to the PAO1 accessory genome (Appendix; Supplementary information for Chapter 2, Table 1). This set of 29 genes contains two type 4 pili-related genes, genes involved in adaptation and protection, membrane proteins, transport of small molecules, metabolism and hypothetical genes, among others. A caveat to this analysis is that unlike PAO1, the other sequenced *P. aeruginosa* genomes do not have 100% coverage.

After subtracting the core set, we compiled sets of unique genes that are specifically differentially regulated in each of the conditions examined. Functional categorization of all the genes in the unique gene sets (Appendix; Supplementary information for Chapter 2, Table 2) revealed that overall, as expected, hypothetical genes were the most differentially regulated. In addition, genes involved in adaptation (membrane proteins, transport of small molecules, putative enzymes, transcriptional regulators and energy metabolism), showed the highest percentage of differential regulation.

Table 3 (Appendix; Supplementary information for Chapter2) lists all the unique genes identified under the various conditions used in these studies. In the iron studies, for example, genes of the secreted factors group (toxins, enzymes, alginate) constitute 7% of the differentially regulated genes. This supports the view that *P. aeruginosa* upregulates virulence genes in response to iron limitation to help survival in the host system (145). Analysis of the data from response of *P. aeruginosa* to copper reveals that 33% of the differentially regulated genes are membrane proteins, much more than the

small molecule transport genes (19%) or hypothetical genes (14%) suggesting that copper stress primarily affects the membrane. By far, the most interesting observation was from the osmotic shock study. A stark contrast to the other studies, there were only a very small percentage of hypothetical genes (3%) that were differentially regulated. Transcriptional regulators (37%), protein secretion/ export apparatus (33%) and two-component regulatory systems (10%) form over 70% of the genes differentially regulated under osmotic stress. The osmotic study was performed using *P. aeruginosa* PA14, which is known to be more virulent than *P. aeruginosa* PAO1 in a wide range of hosts (194-196). Since the chips used were designed for PAO1 that has 5571 ORFs as compared to 5905 ORFs in PA14, there could be an underrepresentation of the genes that are differentially regulated.

Conclusions

The transcriptome analysis studies that have been reviewed here provide evidence of the immense power of the DNA microarray. Microarray profiling generates an enormous amount of data that adds to our knowledge database. Genes that are differentially regulated by transcription factors, by host environment, by changing physiological conditions including response to external stimuli that could range from nutrients to other factors affecting growth, interactions with host cells all have been studied by microarrays. Analysis of transcriptome data generated in *P. aeruginosa* has been performed previously by Goodman and Lory (28), who show that about a quarter of the *P. aeruginosa* genome is differentially regulated in response to various stimuli. Our comparative analysis of the significantly differentially regulated genes from all these various studies show that about 35% of the *P. aeruginosa* genome (1932 genes) is

regulated overall. To arrive at more comprehensive understanding on how bacteria react to the ever-changing environment, we would need to recognize the importance of physiological experiments before the analysis of data, protocol standardization and appropriate controls.

The next major advances in DNA microarrays would be to develop technologies to analyze the changing transcriptome of the host-pathogen interaction process *in vivo* that would provide us with vital clues to better understanding the organism, and the development of pangenome arrays that would give us insights into the workings of this versatile bacterium. Together, these will help devise ways to better counter *P. aeruginosa* infections.

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CHAPTER 3

Transcriptional regulatory network in *Pseudomonas aeruginosa*

This chapter has been published:

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Abstract

Pseudomonas aeruginosa is found in a wide range of habitats, primarily in soil and water and is the epitome of opportunistic human pathogens. A myriad of virulence factors produced by the bacterium ensure its success as a pathogen. *P. aeruginosa* has one of the largest genomes among eubacteria and transcriptional regulators comprise about 8% of the genome. Sequence analysis of the regulators belonging to different families shows clustering while network analysis shows extensive crosstalk, and reveals empirically identified and novel interactions between regulators. Gene expression in *P. aeruginosa* is an intricately interlinked process and is exemplified in the regulation of virulence factor expression. Major regulatory processes such as quorum sensing involving multiple regulators translate external signals perceived by the bacterium into gene expression/repression via regulatory cascades. Many global regulators have been identified that serve to link different virulence systems. Understanding the role of the as yet uncharacterized transcriptional regulatory proteins will provide important insights into the physiology of this important human pathogen and has potential therapeutic implications.

Introduction

Pseudomonas aeruginosa is an environmental saprophyte that can cause a number of opportunistic infections in humans including infections of the pulmonary and urinary tracts, and skin (9,11,13). It is notably known for its ability to cause acute (15,18,24,197) and chronic (198-200) infections. *P. aeruginosa* is inherently resistant to antibiotics and can also acquire resistance genes by horizontal gene transfer (201). The ability of *P. aeruginosa* to infect animals, nematodes, plants and insects is due to the expression of a wide range of virulence factors that are either cell-associated or secreted. Cell-associated factors are involved primarily in adherence and colonization (pili, LPS, flagella) and motility (type IV pili, flagella). In addition, some components have multiple functions. The LPS, for example induces the immune system and has been linked to dysregulation of the host response and sepsis (202). In chronic infections, however, LPS modifications have been identified that make the bacteria less 'visible' to the immune system (203). In addition, *P. aeruginosa* secretes a plethora of extracellular virulence factors; a majority of them (LasA, LasB, protease IV, exotoxinA, lipases, phospholipase C) secreted via the Xcp Type II secretion system (204,205). *P. aeruginosa* can also specifically deliver toxins directly into the host cell using the sophisticated type III and type VI secretion systems [T3SS and T6SS, respectively; (206,207)].

Pathogenesis of *P. aeruginosa* is thus due to the concerted action of multiple virulence mechanisms and is typically not due to the specific action of a single virulence factor. An intricate network of regulation involving multiple transcriptional regulators (transcriptional control), and small RNA-binding proteins and regulatory RNA (post-transcriptional control) orchestrate the timed co-expression of multiple virulence

mechanisms. At the heart of the *P. aeruginosa* pathogenesis network is quorum sensing (QS), a system of interconnected sensors and regulators that control expression of virulence and other bacterial traits in a cell-density dependent manner (208). Key to the QS regulatory network are autoinducers (also known as quorumones): small diffusible chemical signals that help bacteria sense self and non-self population densities and coordinate expression of virulence factors under high cell numbers thus increasing the chances of a successful infection (208). To further enhance survival *P. aeruginosa*, like other bacteria, has evolved to combine signal recognition with transcriptional regulation for a quick and efficient response to changes in the environment. Critical to this process are the two-component systems (TCS) comprised of an inner membrane-bound sensor kinase that transduces the signal to a cytoplasmic response regulator, either directly or via a phosphorelay. In some cases, the two components are part of the same protein, the so-called hybrid sensor kinases that are known to be critical for *P. aeruginosa* pathogenesis (209-212). Auxiliary regulators connect histidine kinases with regulatory processes forming three-component regulatory signaling systems (213).

If genome sizes are any indication of complexity, *P. aeruginosa* is indeed a highly complex bacterium. With strains harboring 6-7 MB chromosomes (33,214), *P. aeruginosa* has one of the largest genomes in bacteria closely approaching that of lower eukaryotes such as *Saccharomyces*. In bacteria like *P. aeruginosa*, where the genome repertoire far exceeds many others, regulatory genes are acquired through horizontal gene transfer or evolution of paralogs that have new specificities for new sets of target genes. This is underscored by the comparative analyses of transcription factors from 175 prokaryotic genomes showing reduced conservation as compared to their target genes

(215). However, such extensive analysis still precludes our ability to predict the new lineage-specific transcription factors and the new interactions that continue to evolve as the organisms fight for their survival in a myriad of environments. Thus it is not surprising that a single species, such as *P. aeruginosa* with its ability to thrive in diverse environments, has a mosaic genome consisting of a conserved core component interrupted in each strain by combinations of specific blocks of genes (33). These strain-specific segments of the genome are found in limited chromosomal locations, referred to as regions of genomic plasticity (RGP), and favors survival by enhancing the bacteria's metabolic and/or virulence capabilities (33).

Transcriptional regulation in *P. aeruginosa* is a complex and intricately interlinked process. Many 'global' regulators have been identified (Vfr, MvfR, AmpR, Rsm, LasR, RhlR) that have a profound effect on the gene expression pattern. Though many transcriptomic studies have looked at global scale expression of genes, understanding the role of transcription factors, structure-function and interaction analyses and the associated networks in *P. aeruginosa* still lags behind *Escherichia coli*. However, certain networks such as QS and alginate regulation are much better characterized in *P. aeruginosa* than in others.

Figure 3.1: *P. aeruginosa* transcriptional regulatory protein families. A total of 434 amino acid sequences of transcription factors (Appendix; Supplementary information for Chapter 3, Table 1) were aligned using ClustalW (Version 2.1.10) (216). The first tree out of 31 most parsimonious trees (length = 69259) is shown. The consistency index is 0.155474 (0.147175), the retention index is 0.298198 (0.218198), and the composite index is 0.046362 (0.043887) for all the sites and parsimony-informative sites (in parentheses). The maximum parsimony tree was obtained using the Close-Neighbor-Interchange algorithm (217) with search level 2 (217,218) in which the initial trees were obtained with the random addition of sequences (10 replicates). All alignment gaps were treated as missing data. There were a total of 1137 positions in the final dataset, out of which 743 were parsimony informative. Phylogenetic analyses were conducted in MEGA4 (219). The different families that cluster together are colored. Members of families that do not form tight clusters (IclR, LacI/GalR, MerR, Cro-C1, CRP, ArsR, H-NS, OmpC, RpiR, and Dks/TraR) are colored black.

The key to understanding *P. aeruginosa* pathogenesis is to have a unified view of how the different regulators and regulatory networks are linked. This review will focus on the genome composition of the transcription factors in *P. aeruginosa* and attempt to glean meaningful interactions.

Families of transcriptional regulators in *P. aeruginosa*

Transcriptional regulators are grouped into numerous families based on their functional domains, particularly DNA and effector binding domains. Even though many regulators have been studied in *P. aeruginosa* and *in silico* analyses group them into different families (Figure 1), experimental evidence assigning function for most of the 434 regulators is still lacking. Phylogenetic analysis shows that not all members fall into tight groups indicating that sequence homology is not sufficient to cluster all the members (Figure 3.1). Some families, like MerR and Cro-cI (labeled in black font in Figure 3.1), are scattered all over the tree. Others, like the GntR family, form multiple clusters, while some members of well-formed clusters fall in other clusters. For example, PA5261 of the LysR family is grouped with one of the GntR clusters (Figure 3.1). Interestingly, the unclassified proteins, which do not have a known signature sequence,

cluster together (Figure 3.1). Experimental data such as structure analysis will help classify these proteins as well as the outliers into families.

This section deals with the major transcriptional regulator families in *P. aeruginosa* PAO1 and their prominent members. The characteristics of the major families are listed in Table 3.1.

LysR Family

LysR-type transcriptional regulators (LTTRs) are the largest family of prokaryotic transcriptional regulators (103,104). Many LTTRs are global activators/repressors of target gene expression. The targets are either divergently transcribed from them or are part of unlinked regulons at different locations on the chromosome (220,221). Divergent transcription from the target gene allows for autoregulation, though there are exceptions (104,105). This family is the namesake for the prototypic member LysR that activates *lysA* transcription involved in lysine biosynthesis in *E. coli* (222). LTTR members typically have a N-terminal winged helix-turn-helix (wHTH)-DNA binding domain (residues 1-65), C-terminus effector-binding domain (residues 100-173 and 196-206) and a domain that is needed for both functions (residues 227-253) (103). DNA binding, either as dimers or tetramers, occurs at the target promoters even in the absence of the effector signals. In most cases, co-inducer binding causes a change in footprint resulting in DNA bending and transcription activation (104).

Table 3.1: Characteristics of the major families of transcriptional regulators in *P. aeruginosa*

Family	# in <i>P.aeruginosa</i>	# in <i>E.coli</i>	DNA binding motif	Other domains	Regulation	Oligomerization	Prototype
LysR	127	45	N-term wHTH	C-term effector-binding; a DNA/effector-binding	Activator/repressor	Dimer/tetramer	<i>E. coli</i> LysR
AraC/XylS	61	21	C-term HTH	N-term dimerization/sugar-binding	Activator	Dimer	<i>E. coli</i> AraC
TetR	39	11	N-term HTH	C-term effector-binding/oligomerization	Repressor	Dimer/octamer	Enterobacterial TetR
LuxR	32	17	C-term HTH	N-term regulatory domain	Activator	Mutimer	<i>V. fischeri</i> LuxR
GntR	31	3	N-term HTH	C-term effector-binding/oligomerization	Repressor/activator	Homodimer	<i>B. subtilis</i> GntR
RpoN-binding	22	2	C-term HTH	Central RpoN-interacting; N-term effector-binding	Activator	Dimer/multimer	<i>E. coli</i> NtrC
Cro-C1	17	3	N-term HTH	C-term oligomerization	Repressor	Homodimer	Phage 434 (Cro); Phage lambda (C1)
IclR	9	8	N-term wHTH	C-term effector-binding/oligomerization	Repressor/activator	Dimer/tetramer	<i>E. coli</i> IclR

Family	# in <i>P.aeruginosa</i>	# in <i>E.coli</i>	DNA binding motif	Other domains	Regulation	Oligomerization	Prototype
AsnC	9	3	N-term HTH	C-term effector- binding/oligomerization	Repressor/ activator	Multimer	<i>E. coli</i> AsnC
LacI	7	15	N-term HTH	C-term effector- binding/oligomerization	Repressor	Dimer/tetramer	<i>E. coli</i> LacI
MerR	7	5	N-term HTH	C-term effector-binding	Activator/ repressor	Dimer	<i>E. coli</i> MerR
ArsR	4	2	N-term HTH	C-term effector- binding/dimerization	Repressor	Homodimer	<i>E. coli</i> ArsR

P. aeruginosa PAO1 has 127 LTTR members that despite controlling diverse functions (Appendix; Supplementary information for Chapter 3, Table 1) group together on amino acid sequence analysis (Figure 3.1). Though the functions of many members remain unknown, the few that are known are regulators of critical processes including virulence in *P. aeruginosa*. MvfR (PA1003), for example regulates QS in a LasIR/RhlIR-independent manner by modulating PqsE (PA1000), the quinolone response signal protein (223,224). The chromosomal β -lactamase regulator AmpR (PA4109) is a global regulator of virulence in *P. aeruginosa*. In addition to regulating β -lactam and non- β -lactam resistance, AmpR also positively modulates expression of many acute virulence factors and negatively regulates chronic infection phenotypes (105,225,226). OxyR (PA5344) is an example of a regulator controlling expression of multiple related phenotypes. Under oxidative stress, OxyR enhances expression of antioxidant genes like *katB*, *ahpCF* and *ahpB* (227). In addition, OxyR also plays a role in virulence (228), cytotoxicity (229), pyoverdine utilization, and pyocyanin and rhamnolipid production (230,231). Turner *et al.* recently identified the bistable regulon of a LysR-type regulator BexR (PA2432) that regulates virulence factors like AprA (232). Other characterized LysR-type members include an efflux pump regulator, MexT (PA2492) that has been recently shown to regulate other genes (233) and MetR (PA3587), a regulator of genes involved in methionine synthesis (234). Except for PA0207 (RGP1), PA1223 (RGP11), PA2054 (RGP20), PA2056 (RGP20), and PA2220 (RGP53), all LysR members are part of the core genome (Appendix; Supplementary information for Chapter 3, Table 1).

IclR Family

The isocitrate lyase regulator (IclR) family controls gene expression in prokaryotes in a signal-dependent manner (235). IclR members are distributed in over 46 species of eubacteria and archaea and typically regulate genes and operons involved in carbon metabolism or virulence. In the absence of specific substrates, IclR regulators typically repress specific catabolic genes, and in the presence of excess signaling molecules, de-repress expression (236). Two domains in the protein bring about regulation: a winged HTH domain at the N-terminus that binds DNA, and an effector-binding domain at the C-terminus. IclR-like regulators form dimers or tetramers and recognize palindromic sequences on the DNA (235). Some of the well-studied members of this family include the family namesake in *E. coli* IclR, a glyoxalate shunt repressor of the *aceBAK* operon involved in acetate utilization, the glycerol catabolism pathway repressor GlyR in *Streptomyces coelicolor* and KdgR, the pectin degradation pathway repressor in *Erwinia chrysanthemi*. A *Thermatoga maritima* IclR member TM0065, involved in xylulose metabolism was the first member of this family to be crystallized (237).

P. aeruginosa PAO1 has nine IclR homologs and all of them are yet to be characterized. A PA0155 (PcaR) homolog in *P. putida* has been demonstrated to regulate the *pca* operon involved in p-hydroxybenzoate degradation by binding both the -35 and -10 regions in the promoter (238). PA1015 is 42% similar to GlyR, a glycerol catabolic operon (*gylABX*) regulatory protein in *S. coelicolor* and is present immediately upstream of the *gylABX* promoter (239). In *P. aeruginosa* however, there is no *gylABX* operon in the immediate vicinity and the role of PA1015 remains unclear. All IclR members are

part of the core genome with the exception of PA3508 (RGP34) (Appendix; Supplementary information for Chapter 3, Table 1).

LacI/GalR Family

The LacI/GalR family members are metabolic regulators in prokaryotes that recognize sugar inducers. The prototypic members are the *E. coli* lactose repressor LacI and the galactose operon repressor GalR (240). They are typically localized in the cytoplasm and possess a N-terminal HTH motif of about 50-60 residues. The C-terminus contains regions that are involved in oligomerization and signal molecule recognition (241). LacI members function as either dimers or tetramers (242). Well-studied proteins with a LacI-type HTH include the *Bacillus subtilis* CcpA and CcpB involved in repression of several operons (243), the *Hemophilus influenzae* galactose repressor GalR (244), the *E. coli* lactose operon repressor LacI (245), and the *Salmonella typhimurium* fructose repressor FruR (246).

Five of the seven LacI homologs in *P. aeruginosa*, all of which are part of the core genome, are involved in carbohydrate transport/metabolism (Appendix; Supplementary information for Chapter 3, Table 1). A fructose transport repressor (FruR) homolog is found in *P. aeruginosa* PAO1 (PA3563). However, others are involved in virulence. NfxB (PA4600), a regulator of the MexCD-OprJ efflux pump has also been shown to affect motility, probably through its effect on the motility regulator MorA (PA4601), and QS-dependent phenotypes by inappropriate MexCD-OprJ efflux expression (247). Interestingly, there is another LacI-like regulator in the same locus,

PA4596, upstream of the MexCD-OprJ operon that is 74% similar to NfxB. Whether this is also involved in regulating MexCD-OprJ remains to be determined.

PtxS (PA2259) is an example of a transcriptional regulator controlling two very different pathways in *P. aeruginosa*. It is a repressor of the exotoxin A regulator PtxR (PA2258) (248) not by direct binding at P_{ptxR} from which it is divergently transcribed but through an unknown mechanism (249). PtxS is also part of a 5-gene *kgu* operon (PA2259-PA2263) involved in 2-ketoglutarate utilization, which it regulates by binding to two different operator sequences (250).

GntR Family

The prototypic member of this family is *Bacillus subtilis* GntR, a repressor of the gluconate operon (251,252). Many of the members of this family have been shown experimentally to be autoregulatory, enabling the prediction of operator sites and the discovery of *cis/trans* relationships (253). This family is further subdivided into four major (FadR, HutC, PlmA, MocR) and two minor (YtrA, AraR) subfamilies, which regulate various biological processes and important bacterial metabolic pathways (254). DNA binding by GntR members is inducer-independent. For example, GntR binds to the promoter of the operon in the absence of gluconate but not in the presence (255). This family of proteins has a wHTH domain about 60-70 residues at the N-terminus whereas the C-terminus contains a subfamily-specific effector-binding domain and/or an oligomerization domain (252). The GntR family of regulators binds as homodimers to two-fold symmetric DNA elements (256,257).

P. aeruginosa PAO1 genome harbors 31 regulators belonging to the GntR family, of which many are grouped together by sequence analysis (Figure 1) and only two are part of RGPs (PA2032 and PA2100 in RGP20 and RGP21, respectively). Based on sequence analysis, six of the transcriptional regulators are presumed to be involved in glycolate (PA5356), lactate (PA4769), gluconate (PA2320), histidine (PA5105), phosphate (PA3381) and N-acetyl-D-glucosamine (PA3757) catabolism. Of the 31 only the role of PA5499 (Np20) has been experimentally elucidated. Expression of *np20* is inducible *in vitro* by respiratory mucus, *in vivo* during infection of mice and was shown to be important in bacterial virulence in a neutropenic-mouse infection model (258). However, no structure-function studies are available.

LuxR Family

The LuxR-type transcription regulator superfamily derives its name from the *Vibrio fischeri* LuxR protein, which alters transcription in response to QS to produce bioluminescence (259-262). LuxR proteins contain two functional domains: a C-terminus HTH DNA binding domain and an N-terminal regulatory domain (263). The DNA binding domain consists of a four-helix bundle. The LuxR proteins bind promoters as multimers (264). Based upon the N-terminus, the LuxR family may be further subdivided into at least four groups: the FixJ group (265-268), the LuxR group [reviewed in (263)], the large ATP-binding regulators of the LuxR superfamily (LAL) group (269,270), and the autonomous effector domain LuxR-type regulators (264,271). Many proteins that have been classified as LuxR superfamily members do not belong to any of these groups. Moreover, new groups are still being identified (272).

P. aeruginosa PAO1 harbors 32 members of the LuxR superfamily (Appendix; Supplementary information for Chapter 3, Table 1) that group together in neighboring trees (Figure 3.1). Half of these appear to belong to the FixJ group. Of these, six have been demonstrated to function as TCS response regulators and 10 are predicted to be response regulators based upon PFAM analysis (Appendix; Supplementary information for Chapter 3, Table 1). A LuxR member, NarL (PA3879), acting in concert with the sensor kinase NarX (PA3878), represses expression of proteins involved in the arginine fermentation pathway for ATP generation. In addition, NarL also activates proteins in the nitrate reductase operon (NarH, PA3874), the porin OprE (PA0291) and the class II ribonucleotide reductase components NrdJa (PA5497) and NrdJb [PA5496, (273)]. RocA1 (PA3948), and its cognate sensor kinase RocS1 (PA3946), were identified as regulators of fimbrial cup genes [*cupB* and *cupC*, (274)]. Other studied members of this family include BfiR [PA4196, (275,276)], ErdR [PA3604, (277)], GacA [PA2586, (278)], and the QS regulators LasR (PA1430), RhlR (PA3477), QscR (PA1898), and VqsR (PA2591) [see section on QS below; (279-282)]. All LuxR superfamily members are encoded by the *P. aeruginosa* core genome (Appendix; Supplementary information for Chapter 3, Table 1).

RpoN-Binding Family

This family of transcriptional regulators activates the expression of genes from promoters recognized by core RNA polymerase associated with the alternative sigma-54 factor (RpoN). RpoN enhancer binding proteins share a conserved domain of about 230 residues involved in the ATP-dependent interaction with RpoN (283). The RpoN-binding

domain, which has ATPase activity, contains an atypical ATP-binding motif A (P-loop), and a motif B (284). These proteins are part of the ATPases associated with diverse cellular activities clan: proteins that perform chaperone-like functions and assist in the assembly, operation, or disassembly of protein complexes (285).

In *P. aeruginosa*, there are 22 RpoN-binding proteins, encoded by the core genome, which are involved in the regulation of metabolism, production of the exopolysaccharide alginate, virulence as well as biofilm formation (Appendix; Supplementary information for Chapter 3, Table 1). Nine of the 22 RpoN interacting proteins belong to signal transduction TCS and contain an N-terminal domain that can be phosphorylated by a sensor-kinase protein. Almost all of these proteins possess a HTH DNA binding domain in their C-terminus. At least six are NtrC-like RpoN enhancer binding proteins (PilR-PA4547, CbrB-PA4726, NtrC-PA5125, AlgB-PA5483, MifR-PA5511 and FleQ-PA1097). NtrC transcriptional activators, first described in the regulation of nitrogen metabolism of enteric bacteria, possess three primary domains: a RpoN-interacting conserved central domain, a C-terminal domain which contains the HTH DNA binding motif and a homologous receiver N-terminal domain (286).

The transcriptional regulator CbrB is part of the TCS CbrAB (PA4725-PA4726), involved in regulating the carbon-nitrogen balance in *P. aeruginosa*. Phosphorylated CbrB activates the expression of the *aot-argR* operon (arginine catabolism) and the *hiu* operon (histidine catabolism) (287). Two biofilm regulators, MifR (PA5511), part of a signal transduction network in microcolony formation (275), and RocR of the RocSAR (SadARS) three-component signaling system involved in biofilm maturation (288), have been defined. RocSAR consists of the histidine kinase RocS1 and two response regulators,

RocA1 (PA3948) and RocR (PA3947). The RocSAR system controls bacterial biofilm formation and virulence gene expression by regulating the transcription of various genes, including the *cup* fimbrial-gene clusters and Type III secretion system genes (288). AlgB (PA5483), also a part of TCS, activates alginate production in mucoid *P. aeruginosa*. Alginate has been implicated in the pathogenesis of *P. aeruginosa* including resistance to phagocytosis and adherence mechanisms (289).

MerR Family

The classical MerR proteins are dimeric transcriptional regulators of Gram-negative mercury resistance operons present in transposons Tn21 and Tn50 (290). Like many of the regulators already discussed, these regulators consist of two domains, a 70-amino acid N-terminal DNA-binding domain and a C-terminal effector-binding domain. The carboxy-terminus contains specific effector binding regions to respond to environmental stimuli such as heavy metals, oxidative stress, antibiotics or metal ions (290,291). The MerR proteins differ from many other classes of regulators in that they function as both an activator and repressor of the same gene. They achieve this by binding to the same *cis* element between -10 and -35 promoter regions containing a critical 19 bp region essential for MerR-dependent activation (290). MerR acts as a weak repressor in the absence of ligand (Hg(II)) and as an activator when bound to Hg(II) (292).

P. aeruginosa contains seven MerR regulators, two of which, SoxR (PA2273) and CueR (PA4778) are characterized. Like its *E. coli* homologue, the *P. aeruginosa* SoxR contains a [2Fe-2S] center (293). In *E. coli*, SoxR regulates genes involved in the

oxidative stress response to superoxide anions or nitric oxide by activating an AraC-type regulator SoxS (294,295), which is not conserved in *P. aeruginosa*. SoxR, however, binds weakly and activates transcription of the promoter from its divergently transcribed neighbor, *PA2274*, encoding a hypothetical protein (293). CueR controls a copper resistance regulon consisting of five operons and 11 genes (296), and is under regulation of LasR (*PA1430*), a LuxR member (296). *PA3689* is similar to CadR, a MerR family member from *P. putida* involved in cadmium resistance (297). All MerR proteins are encoded as part of the *P. aeruginosa* core genome (Appendix; Supplementary information for Chapter 3, Table 1).

TetR Family

The TetR family is named after the enterobacterial TetR repressor protein that confers resistance to tetracycline (298) and subsequently a family profile was developed (299). TetR members are typically repressors that contain a 60 residue N-terminal HTH DNA binding domain and a C-terminus effector-binding/oligomerization domain that bind DNA either as dimers or octamers (300-302). TetR, a well-studied example, is a repressor of the enterobacterial tetracycline exporter, TetA and binds to the operator sites in *P_{tetA}*. Binding of the tetracycline-Mg complex to DNA-bound TetR leads to depression of *tetA* and confer tetracycline resistance (303). TetR members primarily control multidrug efflux pumps, biosynthesis of antibiotics, catabolic pathways and virulence in both Gram-positive and Gram-negative bacteria.

There are 39 TetR members in *P. aeruginosa* PA01, all of which are encoded by the core genome (Appendix; Supplementary information for Chapter 3, Table 1). The

expression of the MexAB-OprM efflux pump (PA0425-PA0427) that confers resistance to β -lactams, in addition to diverse substrates, is under the control of two TetR members NalC (PA3721) and NalD (PA3574), of which NalC is thought to act via the primary repressor MexR (PA0424) (304-306). PsrA (PA3006) has been demonstrated to control expression of the *P. aeruginosa* T3SS (307), probably through its regulatory effect on RpoS (308). A TetR member, CifR (PA2931) represses expression of a toxin, CFTR inhibitory factor (Cif, PA2934) (309). Cif has been demonstrated to reduce expression of the CFTR protein in cell lines and proposed to play a role in aiding initial colonization by *P. aeruginosa* (310). CifR regulation of Cif thus aids in the later stages on infection when Cif function is no longer required (309). Other characterized *P. aeruginosa* TetR members (AguR-PA0294, AtuR-PA2885, DesT-PA4890, BetI-PA5374) are primarily metabolic repressors [Appendix; Supplementary information for Chapter 3, Table 1; (311-314)].

AraC Family

The *E. coli* L-arabinose operon activator, AraC exemplifies this group of transcriptional regulators with which members share a 99-residue C-terminus homology (315-317). AraC proteins harbor a C-terminus HTH motif, a N-terminus dimerization/sugar-binding domain and primarily activate expression of genes involved in sugar metabolism, stress response and virulence (299,318). Most AraC members, together with cAMP-receptor protein (CRP), activate promoters, either as monomers or dimers (319,320).

P. aeruginosa PAO1 has 61 AraC members (Appendix; Supplementary information for Chapter 3, Table 1). PchR (PA4227), an AraC member, is a regulator of the biosynthesis of an iron uptake protein, pyochelin, and acts both as a positive and negative regulator of *fptA* (PA4221) and *pchR* expression (321). PchR binds to a 32 bp PchR box in the promoter of genes that it regulates (322). ExsA (PA1713), a *Yersinia enterocolitica* VirF homolog, was initially identified as part of a trans-regulatory locus involved in the expression of the T3SS effector exoenzyme S (323,324) and has since been shown to regulate expression of the T3SS genes (reviewed in (325)). MmsR (PA3571) regulates the *mmsAB* operon (PA3569-PA3570) that encodes enzymes involved in valine metabolism (326). Other than PA1380 (RGP15) and PA2047 (RGP20), AraC protein-encoding genes belong to the core genome of *P. aeruginosa* (Appendix; Supplementary information for Chapter 3, Table 1).

Cro-C1 Family

This superfamily is named after the Cro and C1 repressors of the lysogenic phages 434 and lambda, respectively, where they are part of a binary switch that regulates lytic/lysogenic growth of the phage by differential binding to the operator sites (327,328). They generally have a 50-60 residue HTH domain at the N-terminus of the protein while oligomerization occurs at the C-terminus. The lambda Cro repressor is a 66 amino acid protein and binds DNA as a homodimer (329).

P. aeruginosa has 17 regulators belonging to this family, all of which are encoded by the core genome (Appendix; Supplementary information for Chapter 3, Table 1). Pyocin production in *P. aeruginosa* is under the tight regulation of PrtR (PA0611). As

part of the SOS response, RecA (PA3617) degrades PrtR, which inhibits transcription of the pyocin gene activator, PrtN [PA0610, (330)]. PrtR also modulates activity of a Dks/TraR member PtrB (PA0612), a repressor of the T3SS (331), linking secretion and pyocin synthesis in response to DNA damage. Two other hypothetical Cro/C1 members, PA0906 and PA4077 are 66% similar to PrtR, and PA4077 is co-transcribed with a hypothetical inner membrane protein PA4076 (332). Whether these are also involved in pyocin regulation is unclear. PA1359, a hypothetical regulator, bears 49% similarity to *E. coli* HipB, a known regulator of persister formation by modulation of HipA activity (333-335). Interestingly, PA1359 is part of a two-gene operon (332), similar to the *hipBA* operon in *E. coli*. None of the other members of this family have been characterized in PAO1.

AsnC Family

This family of transcriptional regulators is typified by asparagine synthase C of *E. coli* and is part of the feast/famine regulatory proteins (FFRP) that includes Lrp and Asn (336). Members of this family are distributed in both archaea and eubacteria. They typically contain a HTH DNA binding domain at the N-terminus and a dimerization/effector-binding domain at the C-terminus, which also plays a role in assembly of the protein. FFRP members bind DNA as dimers, tetramers, octamers or hexamers (337,338) and regulate genes in response to diverse signals. Identification of FFRPs is often complicated by the fact that the amino acid homologies between members are often only about 30% (336).

P. aeruginosa has nine AsnC members (Appendix; Supplementary information for Chapter 3, Table 1), all of which except PA2028 (RGP20) are encoded by the core genome. PA0513, an as yet uncharacterized regulator, is part of an 11-gene *nir* operon that is involved in the denitrification pathway (339). PA0515 is also part of the same operon and is listed as a probable AsnC-type transcriptional regulator in the *Pseudomonas* database (332) but has been excluded from our list (Appendix; Supplementary information for Chapter 3, Table 1) because it lacked a DNA binding motif in our analysis. BkdR (PA2246) is divergently transcribed from the *bkd* operon that is involved in valine, leucine, isoleucine degradation and has been characterized in *P. putida* (340). The *P. aeruginosa* homolog of Lrp (PA5308), which regulates the global leucine response in *E. coli*, regulates the *dadA* (PA5304) and *dadX* (PA5302) genes that are required for L-alanine utilization (341).

ArsR Family

The *E. coli* ArsR that represses the *ars* operon in response to arsenic is the prototype for this family of transcriptional repressors (342). This family of proteins binds the promoter region of genes that they regulate (primarily metal transport and detoxification) and dissociate upon effector binding due to allosteric changes, allowing transcription by derepression (343). The metal-binding site is at the C-terminus of the protein whereas the DNA binding domain is at the N-terminus. ArsR members typically function as homodimers (344).

There are four members in *P. aeruginosa* encoded by the core genome (Appendix; Supplementary information for Chapter 3, Table 1). *arsR* (PA2277) is part of

a four-gene cluster that has been demonstrated to confer resistance to arsenic and antimony when expressed in a heterologous *E. coli* *ars* mutant (345). PA0547 is an uncharacterized ArsR-type regulator that is part of a two-gene operon with the S-adenosylmethionine synthetase gene, *metK* (PA0546). The other two ArsR members in *P. aeruginosa* are also uncharacterized (Appendix; Supplementary information for Chapter 3, Table 1).

Other Families

Other *P. aeruginosa* regulatory families include the Dks/TraR family (PtrB (PA0612) and DksA (PA4723)), H-NS silencing proteins (MvaT (PA4315) and MvaU (PA2667)), CRP family (Vfr- PA0652 and Anr- PA1544), OmpC family (AmgR- PA5200), OmpR-PhoP class (PA4381) and the RpiR family (PA5506).

Unclassified Regulators

In addition to the families listed above, 37 regulators do not contain the signature motif for any family but still are significant contributors to the regulatory processes in *P. aeruginosa* (Appendix 2; Supplementary Table 1). Of these, 27 have been characterized. CreB (PA0463), the regulator of the CreBC TCS operon, along with CreD (PA0465), encoded immediately downstream, are involved in colicin resistance in *E. coli* (346). The CreBC TCS is involved in regulating β -lactam resistance in *Aeromonas* spp. (347) while in *P. aeruginosa* it contributes to resistance only in a $\Delta PBP4$ background (348). LexA (PA3007) is a repressor of RecA and is induced upon DNA damage, as part of the SOS response (349). IrlR (PA4885) is part of a two-component system, a homolog of which has been shown to be involved in heavy metal homeostasis in *Alcaligenes eutrophus*

(350). A few other members [KdpE (PA1637), DauR (PA3864), GltR (PA3192)] are metabolic regulators.

***P. aeruginosa* transcriptional regulatory network**

Studying regulatory networks aid in elucidating interactions between the different transcriptional regulators and provide important insights into processes and pathways. To better understand regulatory networks and identify network motifs, analysis of network structure has proved useful. Two major network motifs that aid in bacterial response to environmental signals have been described: feed-forward motifs, and single-input and multiple-input motifs (215). Using published data on bacterial regulatory networks (215) and STRING analysis (351) of *P. aeruginosa* PAO1 regulators, a transcriptional regulatory network was obtained (Figure 3.2a) consisting of 288 regulators and 593 target genes. As seen in regulatory networks in other bacteria (215), the *P. aeruginosa* network also consists of feed-forward motifs, and single- and multiple-input motifs (Figure 3.2a). Vfr (PA0652), the *P. aeruginosa* homolog of *E. coli* cyclic AMP receptor protein and a regulator of virulence and QS, has the highest number of interacting partners, followed by the anaerobic growth and cyanide synthesis regulator Anr, an *E. coli* Fnr homolog (Figure 3.2a). Interestingly, both these regulators belong to the diverse Crp family of transcriptional regulators that have a N-terminal nucleotide-binding domain similar to CRP, in addition to a C-terminus HTH (352).

Figure 3.2a: Transcriptional regulators and interacting partners. Previously inferred regulators of *P. aeruginosa* (215) and those obtained by text mining using STRING (351) analysis, were used to construct the transcriptional regulatory network using Cytoscape (Version 2.7.0) (353). The network consists of 288 regulators and 593 target genes resulting in 1095 interactions. The transcriptional regulators with the highest interactions are shown in the figure (See also Appendix; Supplementary information for Chapter 3, Table 2).

Figure 3.2b: Interactions among transcriptional regulators. VisANT [Version 3.86; (354)] was used to visualize a total of 63 interactions between the regulators from the 1095 interactions of Fig 2a. Vfr emerges as the key regulator. Other interactions such as the pyocin induction network and an uncharacterized network appear isolated from these interactions.

Conclusions about hierarchy among the regulators are difficult to draw from this network but we used the data to identify interactions between the regulators alone without their corresponding target genes. The Vfr regulatory hub stood out in this analysis (Figure 3.2b). Vfr has been demonstrated experimentally to regulate multiple virulence mechanisms in *P. aeruginosa*. Network analysis shows that Vfr is linked to various other critical pathogenesis networks including alginate production, efflux systems, quorum sensing, iron uptake, flagella, T3SS, and metabolic regulons (Figure 3.2b). While some of these links have already been established (355-357), it would be interesting to see whether the other interactions pan out. Our Cytoscape analysis also revealed the established interactions between PrtR, PrtN and PtrB to regulate pyocin production and T3SS in *P. aeruginosa* [Figure 3.2b; (330,331)]. Moreover, PA5157, an uncharacterized regulator that bears 60% similarity to the *E. coli* MarR protein involved in regulating multiple antibiotic resistance and oxidative stress (358), interacts with three other hypothetical regulators PA1619, PA3898 and PA2028 (Figure 3.2b). Whether these genes are part of a network contributing to the antibiotic resistance profile of *P. aeruginosa* is not known.

Virulence regulatory systems in *P. aeruginosa*

Network modeling, while providing us with a framework to study, has its limitations in requiring confirmation. The absolute proof of a regulatory interaction comes from empirical data, which serves to either confirm or negate findings from the bioinformatics analyses. We chose the QS system in *P. aeruginosa*, to highlight the similarities in complexity between modeled and established regulatory networks.

Quorum sensing (QS)

QS, a cell population density-dependent signaling system important in host-pathogen interaction, is involved in regulating virulence factor production, swarming motility, biofilm maturation, and efflux pump expression (reviewed in (208)). Two types of QS signals are produced by *P. aeruginosa*, *N*-acylhomoserine lactones and 4-quinolones (359-361). Central to *N*-acylhomoserine lactone QS signaling are LasR (PA1430) and RhlR (PA3477), two LuxR-type regulators that respectively bind *N*-(3-oxododecanoyl)homoserine lactone (3-oxo-C12-HSL) and *N*-butanoylhomoserine lactone (C4-HSL) (359,360). These HSLs are synthesized by the cognate synthetases, LasI and RhlI (279,362). Expression of *lasI* and *rhlI* is regulated by positive feedback loops mediated by 3-oxo-C12-HSL:LasR and C4-HSL:RhlR. LasR is higher up in the *P. aeruginosa* QS hierarchy and 3-oxo-C12-HSL:LasR activates *rhlR* and *rhlI* expression (363,364). The binding site for LasR and RhlR has been termed the *las-rhl* box [CT-N₁₂-AG; (365,366)] and LasR binding sites have been identified in the promoters of many genes including known and putative regulators such as MvfR (PA1003), RsaL (PA1431), VqsR (PA2591), RhlR (PA3477), PvdS (PA2426), AmrZ (PA3385), PA2588, PA4778,

and PA1760 (365). In addition, three microarray studies identified a common set of 102 genes in the LasR/RhlR regulons (167-170,367).

Most QS-regulated genes are expressed during stationary phase and are dependent on the stationary phase sigma factor RpoS (PA3622, (366,368)). Several additional regulators also play a role in QS (Figure 3.3). Vfr binds and activates transcription of P_{lasR} (369) and P_{rhlR} (370), and represses the 4-hydroxy-2-quinolone-dependent QS pathway (371). Transcriptome analysis indicates that Vfr affects the expression of more than 100 genes in *P. aeruginosa* including T3SS genes (29). RsaL (PA1431), which is divergently transcribed from the *lasI* gene, encodes an autoregulating repressor that inhibits not only *lasI* transcription, but also genes involved in pyocyanin and cyanide synthesis (372,373). Interestingly, RsaL repression is dominant over 3-oxo-C12-HSL:LasR activation of *lasI* to counteract the positive feedback loop (372). RsaL binds as a dimer (373) and regulates expression of 130 genes, including those involved in virulence, independent of its QS effect (373). Another transcription factor that appears to co-regulate QS genes with LasR and RhlR is the anaerobic regulator Anr (PA1544). Together with LasR and RhlR, Anr directly activates the hydrogen cyanide biosynthetic *hcnABC* genes [*PA2193-PA2195*, (374)]. Bioinformatic analysis identified putative Anr consensus binding sites, the Fnr/Anr box, in about 25% of QS regulated genes, suggesting that Anr may regulate these genes under oxygen limiting conditions (170). Interestingly, studies suggest that the growth conditions affect the *las-rhl* hierarchy (375,376).

Figure 3.3: Association between QS regulators in *P. aeruginosa*. Interactions between the different transcriptional regulators (blue boxes) and sigma factors (yellow boxes) to control QS gene expression are depicted. The regulation is positive (green lines), negative (red lines), or both (black lines) and can be direct or indirect. PQS refers to the *Pseudomonas* quinolone signaling molecule. See text for details and references of individual interactions.

P. aeruginosa synthesizes over 50 4-quinolones, however, two, 2-heptyl-4-quinolone (HHQ) and 2-heptyl-3-hydroxy-4-quinolone (PQS) have been shown to act as QS signals (361,377,378). Synthesis of these 4-quinolones is dependent upon the *pqsABCDE* (PA0996-PA1000) and *phnAB* (PA1001-PA1002) operons (377). The *pqsE* gene is dispensable for 4-quinolone synthesis but is required for virulence (379,380). A LTTR member, MvfR (also known as PqsR; PA1003), regulates the *pqs* operon (381). MvfR expression is positively regulated by LasR:3-oxo-C12-HSL, and negatively regulated by RhIR (382) and the PqsR-mediated PQS regulator, PmpR (PA0964, (383)). Transcriptome analysis revealed an extensive MvfR regulon that includes RsaL (PA1431), AlgT/U (PA0762), RsmA (PA0905), and LasR (PA1430) (224). Promoter binding of MvfR is enhanced by either HHQ or PQS (381). HHQ is converted to PQS by PqsH (PA2587) whose expression is LasR:3-oxo-C12-HSL-dependent (377,380). PQS differs from HHQ in that it can sequester iron and induce an iron starvation response by activating genes involved in siderophore biosynthesis and iron scavenging (378,384). Thus PQS affects gene expression in multiple ways: *mvfR*- and *pqsE*-dependent expression, *mvfR*-dependent and *pqsE*-independent expression, and through iron sequestration in a *mvfR*- and *pqsE*-independent manner (378).

QscR (PA1898) and VqsR (PA2591) are two additional LuxR-type QS regulators in *P. aeruginosa* (Figure 3.3). QscR closely resembles the HSL-responsive LuxR

homologues and has been proposed to control the timing of QS-regulated genes by repressing *lasI* expression (280). Transcriptome analysis identified 400 genes as being part of the QscR regulon (385). Additional experiments suggest that QscR can also act by mediating a response to 3-oxo-C10-HSL produced by other bacteria or form heterodimers with LasR or RhlR (386). The *vqsR* promoter contains a *las-rhl* box and *in vivo* occupancy of LasR has been demonstrated (282,365). Consistent with this finding, *vqsR* mutants failed to produce C4-HSL or 3-oxo-C12-HSL, showed reduced pyocyanin and protease production, and reduced virulence in a *C. elegans* model system due to a negative impact on expression of the QS regulon (282). VqsR functions downstream of VqsM (PA2227), an AraC type regulator (282,387). Mutation in *vqsM* reduced expression of *rhlR*, *rsaL*, *vqsR*, *myfR*, *pprB*, *rpoS*, *lasI* and *rhII* (387).

Other regulators have also been implicated in QS signaling. Mutations in the gene encoding the FIS regulator, *PA1196*, downregulate expression of *rhII* and *rhlR* in addition to *pqsA*, which encodes the enzyme that modifies anthranilate for entry into the *Pseudomonas* quinolone signal (PQS) biosynthetic pathway (388). Transposon insertional mutants of *PA5499* (*np20*) reduce *rhII* expression as well as phenazine and cyanide production (380). Mutations in a LTTR AmpR have also been shown to modulate QS activity, linking QS with β -lactam resistance in *P. aeruginosa* (105,225,226).

QS is also regulated post-transcriptionally through the action of the small regulatory RNAs, *rsmZ* (PA3621.1) and *rsmY* (PA0527.1) (389,390). *rsmYZ* antagonize the RNA-binding protein RsmA (PA0905), which negatively regulates QS genes. The GacSA two-component system is activated at high cell densities and most of the effects

of the Gac/Rsm pathway on QS can be attributed to increased C4-HSL levels (389,391). QteE (PA2593), though not a transcriptional regulator, controls the QS threshold by lowering LasR protein stability without affecting *lasR* transcription or translation, and by reducing RhlR levels (392). The stringent response also affects the QS regulon. Under starvation conditions, RelA (PA0934) enhances production of guanosine tetraphosphate (ppGpp) (393). Overexpression of RelA, also caused by decreased membrane fluidity (394), resulted in premature expression of *rpoS*, *lasR* and *rhlR* and premature synthesis of 3-oxo-C12-HSL and C4-HSL (395). Both expression and stringent response of expression of 3-oxo-C12-HSL is induced by a serine amino acid analogue (395).

Although there have been many advances, much remains unknown in our understanding of QS regulation in *P. aeruginosa*. Recent studies by Chugani and Greenberg showed that a *lasR*, *rhlR*, and *qscR* triple mutant retained the ability to regulate 37 genes, including those in the kynurenine pathway, in response to acylhomoserine lactones (396). The kynurenine pathway serves as the source of anthranilate for the synthesis of the PQS (396,397).

Conclusions and perspectives

Computational analyses and mathematical models have greatly advanced our understanding of regulatory networks (215,398,399), some of which have been confirmed by empirical data. Our enhanced understanding of gene regulation in bacteria has come a far way from transcriptional regulators alone, as traditionally thought, to include post-transcriptional and post-translational control, including regulatory RNAs, RNA-binding proteins, sigma factors, DNA supercoiling, proteolysis and others. In *P. aeruginosa*, as with numerous other bacteria, a majority of the transcriptional regulators and open

reading frames have yet to be characterized in terms of gene function and mode of regulation, which in itself undermines our efforts in predicting gene networks. Nevertheless, such models provide us with insight into potential interactions and give us a framework to design experiments to elucidate complex networks, and further reinforce empirical data. In addition, they also help identify paralogs and orthologs between genera. The wealth of data that we now possess from numerous *P. aeruginosa* whole genome transcriptome analyses [reviewed in (28,400)], though critical in our understanding of the system and in identifying potential therapeutic targets, further expose the gap in our knowledge in accurately determining links between signals recognized by the regulators and how they are transduced to their target genes. Analysis of regulator-mutant transcriptomes helps fill in the gap, but warrant further studies, especially in linking metabolic regulation with virulence. It is exciting to know that complementing data from traditional molecular biological experiments with data from high-throughput, whole genome analyses such as microarray, RNA deep sequencing, and network modeling will undoubtedly advance our understanding of how bacteria regulate gene expression, as individuals and as communities in response to environmental signals.

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CHAPTER 4

The regulatory repertoire of *Pseudomonas aeruginosa* AmpC β -lactamase regulator AmpR includes virulence genes

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Abstract

In *Enterobacteriaceae*, the transcriptional regulator AmpR, a member of the LysR family, regulates the expression of a chromosomal β -lactamase AmpC. The regulatory repertoire of AmpR is broader in *Pseudomonas aeruginosa*, an opportunistic pathogen responsible for numerous acute and chronic infections including cystic fibrosis. In addition to regulating *ampC*, *P. aeruginosa* AmpR regulates the sigma factor AlgT/U and production of some quorum sensing (QS)-regulated virulence factors. In order to better understand the *ampR* regulon, we compared the transcriptional profile generated using DNA microarrays of the prototypic *P. aeruginosa* PAO1 strain with its isogenic *ampR* deletion mutant, PAO Δ *ampR*. Transcriptome analysis demonstrates that the AmpR regulon is much more extensive than previously thought, with the deletion of *ampR* influencing the differential expression of over 500 genes. In addition to regulating resistance to β -lactam antibiotics via AmpC, AmpR also regulates non- β -lactam antibiotic resistance by modulating the MexEF-OprN efflux pump. Other virulence mechanisms including biofilm formation and QS-regulated acute virulence factors are AmpR-regulated. Real-time PCR and phenotypic assays confirmed the microarray data. Further, using a *Caenorhabditis elegans* model, we demonstrate that a functional AmpR is required for *P. aeruginosa* pathogenicity. AmpR, a member of the core genome, also regulates genes in the regions of genome plasticity that are acquired by horizontal gene transfer. Further, we show differential regulation of other transcriptional regulators and sigma factors by AmpR, accounting for the extensive AmpR regulon. The data demonstrates that AmpR functions as a global regulator in *P. aeruginosa* and is a positive regulator of acute virulence while negatively regulating biofilm formation, a

chronic infection phenotype. Unraveling this complex regulatory circuit will provide a better understanding of the bacterial response to antibiotics and how the organism coordinately regulates a myriad of virulence factors in response to antibiotic exposure.

Introduction

Pseudomonas aeruginosa is one of the leading opportunistic Gram-negative nosocomial pathogens. This is particularly true in critically ill patients, where multi-drug resistant *P. aeruginosa* is a severe problem. It is the leading pathogen in ventilator-associated pneumonia with a mortality rate of 40-60% (401). *P. aeruginosa* is also a primary cause of urinary tract infections in the US and Europe (402), wound infections leading to bacteremia with one-third to two-thirds mortality rate (403,404), pulmonary infections including cystic fibrosis (CF) (405), lung cancer patients (19) and in pediatric and adult AIDS patients (22). Inability to eradicate the infection is partly due to intrinsic and acquired antibiotic resistance of *P. aeruginosa*. Antibiotic resistant isolates of *P. aeruginosa* are selectively favored *in vivo* in CF patients (85,86). Resistance of *P. aeruginosa* to the β -lactam class of antibiotics, currently used to treat *P. aeruginosa* infections, is partly mediated by a group of genes belonging to the *amp* system.

The *amp* genes were first discovered in *Enterobacter cloacae* to confer resistance to β -lactams (93) and later in other members of *Enterobacteriaceae* (94-97). The products of *amp* genes in *E. cloacae* and other organisms include the AmpC β -lactamase, the AmpG permease, a putative AmpE permease, the AmpD cytoplasmic amidase, and the transcriptional regulator AmpR (93-97). Recent studies have identified another permease, AmpP that is required for β -lactamase induction in *P. aeruginosa* (406). Expression of *ampC* is regulated by AmpR. The *ampR* gene is located adjacent to *ampC* and is divergently transcribed in *C. freundii* and *E. cloacae*, as well as in *P. aeruginosa* (98-100). AmpR of *C. freundii* and *E. cloacae* can cross-complement each other (101) and *P. aeruginosa* AmpR is similar to that found in *C. freundii* (58%) and *E. cloacae*

(62%) (102). In *C. freundii*, AmpR binds to a 15 bp sequence 5' TCTGCTGCAAATTT 3' (98,102) and there is a nearly identical putative AmpR binding site (5' TCTGCTCCAAATTT 3') in the *ampR-ampC* intergenic region in *P. aeruginosa* (105). AmpR has a helix-turn-helix motif that is typical of DNA-binding proteins and the *C. freundii* AmpR binds DNA using this motif (98). The AmpR-AmpC system is also conserved in many other pathogens including *Burkholderia cenocepacia* (407), *Yersinia enterocolitica* (408), and *Stenotrophomonas maltophilia* (409).

AmpR belongs to the LysR family of transcriptional regulators that typically autorepress their own expression (103) which has been demonstrated in *C. freundii* (98). In *P. aeruginosa*, however, there is no evidence of autoregulation (105). It has been postulated that the signals mediating *ampC* regulation by AmpR are peptidoglycan degradation products that function as effector molecules and are brought into the cell cytoplasm from their point of origin in the periplasm via the AmpG permease (79). *In vitro* studies have demonstrated that *C. freundii* AmpR can both activate and repress *ampC* expression depending on its interaction with specific peptidoglycan degradation products (410). Thus the levels of these cell wall intermediates dictate AmpR regulation of *ampC* and the known regulatory repertoire of AmpR in Enterobacteriaceae have been limited to regulating *ampC* expression (79,95,410). Previous studies comparing the properties of *P. aeruginosa* PAO1 with its isogenic *ampR* insertion mutant, PAO*ampR::aacCI*, have shown that AmpR regulates *ampC* as well as some quorum sensing (QS) genes (105). This led us to hypothesize that the regulatory role of *P. aeruginosa* AmpR is more extensive than previously thought.

To test the hypothesis that AmpR regulates different pathways in *P. aeruginosa* and to identify the AmpR regulon, we compared the expression profile of wild-type PAO1 and that of an in-frame *ampR* deletion mutant, PAO Δ *ampR*, with and without sub-MIC β -lactam stress. Our data suggests that *P. aeruginosa* AmpR is a master regulator affecting the expression of over 500 genes. Functional analyses demonstrate the negative regulatory role of AmpR of multiple virulence mechanisms including biofilm formation and the MexEF-OprN multidrug efflux pump. Further, we demonstrate that AmpR positively regulates multiple acute virulence factors. Using a *C. elegans* model, we demonstrate that AmpR is required for pathogenesis in *P. aeruginosa*. This study establishes the critical regulatory role that AmpR plays in antibiotic resistance, virulence and general metabolism in *P. aeruginosa*.

Results

Deletion of *ampR* reduces β -lactam resistance of PAO1

In contrast to previous studies that looked at the role of *P. aeruginosa* AmpR using an insertion mutant, this study employed PAO Δ *ampR*, an in-frame deletion mutant in the prototypic *P. aeruginosa* PAO1. The presence of the *ampR* deletion was confirmed by PCR and restriction digestion of the amplicons (data not shown). AmpR is a known positive regulator of the chromosomal AmpC β -lactamase in different bacterial species (96,102,411). Consequently, after constructing PAO Δ *ampR*, the strains were tested for altered production of β -lactamase. The resistance profile of β -lactam antibiotics for PAO1, PAO Δ *ampR* and PAO Δ *ampR* (pAmpR) shows that, as expected, loss of *ampR* enhances strain sensitivity to β -lactams and expressing *ampR* in *trans* on a low-copy plasmid can restore this defect with multiple β -lactam antibiotics (Fig 4.1a). Loss of

ampR seems to have a stronger effect on penicillins (ampicillin, amoxicillin and piperacillin, with and without β -lactamase inhibitors), imipenem and tazobactam than the cephalosporins tested. This finding is interesting because AmpC is a cephalosporinase. Overexpression of *ampC* under P_{tac} control, however, results in enhanced resistance to the cephalosporin ceftazidime (D. Zincke, personal communication). β -Lactamase quantification showed that PAO Δ *ampR* produced significantly lower amounts in response to β -lactam stress compared to PAO1 (PAO1: 11.27 mU vs. PAO Δ *ampR*: 6.5 mU, p value 0.0003; Fig 4.1b), which is in agreement with the E-test data. The loss of induction was recovered by expressing *ampR* from a low-copy plasmid (PAO Δ *ampR*: 6.5 mU vs. PAO Δ *ampR* (pAmpR): 11.35 mU, p value 0.004; Fig 4.1b). These data clearly reiterate the role of AmpR in β -lactam resistance in *P. aeruginosa* as previously described (105). The PAO Δ *ampR* strain was used for all further assays.

A

Class	Antibiotic	MIC ($\mu\text{g/ml}$)*		
		PAO1 (vector [§])	PAO Δ ampR (vector [§])	PAO Δ ampR (pAmpR)
Penicillins	Ampicillin	>256	12	>256
	Ampicillin/sulbactam	96	6	64
	Amoxicillin	>256	12	>256
	Amoxicillin/clavulanic acid	>256	6	>256
	Oxacillin	>256	>256	>256
	Piperacillin	3	1.5	3
	Piperacillin/tazobactam	2	1	1.5
Cephalosporins	Cefaclor	>256	>256	>256
	Ceftazidime	1.5	1	1
	Cefepime	2	1	1
Penems	Imipenem	2	0.38	1
	Meropenem	1	1.5	1.5
Monobactam	Aztreonam	3	0.75	2

* MIC was determined by the E-test, as described in the methods section

[§] Vector- pMMB67EH-Gm

Figure 4.1. Antibiotic resistance profile of PAO Δ *ampR*. A clean in-frame deletion of *ampR* was generated in *P. aeruginosa* PAO1 as described in the methods section to generate PAO Δ *ampR*. Fig 4.1a shows the resistance profile of the strains to the four major classes of β -lactam antibiotics. Representative data from three different biological replicate trials are shown. The amount of β -lactamase produced was quantified (Fig 4.1b) in the presence (+) and absence (-) of sub-MIC concentration of a β -lactam inducer.

Loss of *ampR* affects ability of PAO1 to kill *C. elegans*

The importance of *ampR* in virulence was determined in a *C. elegans* model, as reported previously (412,413). Using the fast killing (paralytic) assay, we monitored the ability of PAO1 and its isogenic *ampR* mutant, PAO Δ *ampR* to kill *C. elegans* over eight hours. PAO Δ *ampR* showed reduced pathogenicity, killing only 15% of the nematodes compared to the 38% killed by the wild-type PAO1 at the end of the study period (p value < 0.05 at all time points; Fig 4.2). The results indicate that a functional AmpR is required for full pathogenicity of *P. aeruginosa* in the nematode model. To characterize the full extent of AmpR-mediated regulation of *P. aeruginosa* pathogenesis, we analyzed PAO Δ *ampR* further.

Figure 4.2. Effect of *ampR* deletion on pathogenicity to *C. elegans*. The fast killing assay was used to test the effect of loss of *ampR* on the *C. elegans* killing ability of PAO1. *p*-value < 0.05 at all time points.

AmpR regulates numerous genes in *P. aeruginosa*

Using DNA microarrays, we compared the expression profiles of PAO1 and PAO Δ *ampR*, without (uninduced) and with (induced) sub-MIC β -lactam stress to identify genes that are regulated under the different conditions. Pair-wise comparisons of the datasets of significantly differentially regulated genes (p value ≤ 0.01 , $\geq$ two-fold) either with or without sub-MIC β -lactam stress led to the identification of 32 genes (PAO1 uninduced vs. PAO1 induced; Condition A), 258 genes (PAO Δ *ampR* uninduced vs. PAO Δ *ampR* induced; Condition B), 345 genes (PAO1 uninduced vs. PAO Δ *ampR* uninduced; Condition C) and 338 genes (PAO1 induced vs. PAO Δ *ampR* induced; Condition D) (Fig 4.3). As seen in Fig 4.3, the expression of 345 genes is altered in the absence of AmpR (Condition C), clearly indicating that AmpR influences the expression of numerous genes in *P. aeruginosa*.

Quantitative real-time PCR (qPCR) was used to verify the microarray results. Genes for the verification analysis were selected across the spectrum of regulation, based on the raw microarray reads after normalization, including both up and downregulated genes. Twelve genes were selected for initial qPCR analysis, six each from the up and downregulated sets, using *clpX* (*PA1802*) as the reference control gene since *clpX* expression did not change in our microarray data between the strains and conditions tested. qPCR data showed the same trend of either up- or downregulation of the genes as in the microarray, validating our microarray observations, notwithstanding the variations expected due to differences in the sensitivity of the two assays (Table 4.1).

Figure 4.3. Scatter plots of significantly regulated genes. Only genes that showed significant ($p \leq 0.01$) differential regulation under the various conditions are depicted as colored squares. The colors represent the extent of gene expression from low (blue) to high (red) in either condition, as depicted in the color scale. The two outer green diagonal lines in each plot represent the two-fold cutoff. Each sub-plot depicts the differential gene expression between two strains/conditions (shown along the plot axes): Condition A- PAO1 uninduced vs. PAO1 induced; Condition B- PAO Δ ampR uninduced vs. PAO Δ ampR induced; Condition C- PAO1 uninduced vs. PAO Δ ampR uninduced; Condition D- PAO1 induced vs. PAO Δ ampR induced.

Table 4.1: Microarray vs. qPCR. Confirmation of microarray data was performed by qPCR, using RNA isolated from cells as explained in the text. Data shown is gene expression in penicillin non-treated PAO Δ *ampR* cells, normalized to expression in PAO1.

Locus ID/ Gene	Fold change	
	Microarray	qPCR
PA0610/ <i>priN</i>	12	6
PA0762/ <i>algU</i>	5	3
PA3602	5	10
PA2493/ <i>mexE</i>	108	8089
PA4121	7	2
PA1708/ <i>popB</i>	-8	-4
PA2193/ <i>hcnA</i>	-7	-3
PA1078/ <i>flgC</i>	-5	-2
PA2069	-38	-93
PA2331	-7	-40

AmpR regulates genes both in the absence and presence of β -lactam stress

Subsets of genes that are differentially regulated either due to loss of *ampR* or due to β -lactam antibiotic exposure or both (Fig 4.3) could potentially be regulated under more than one condition and this overlap would be misinterpreted in the total number of genes regulated in each condition. To address this issue, the 973 differentially regulated genes ($p \leq 0.01$, $FC \geq 2$ -fold) in the four pair-wise comparisons (Conditions A-D in Fig 4.3) were plotted in 4-way Venn diagrams and separated into upregulated (Fig 4.4a) and downregulated (Fig 4.4b) genes.

From these two Venn diagrams, genes that were dependent exclusively on either AmpR or β -lactams or on both were identified. Comparison of Conditions A and B yield genes unique to each condition (U1, D1 and U2, D2 respectively in Fig 4.4a-4.4b). There are 18 upregulated genes (U1 in Fig 4.4a) that are unique to Condition A (response of PAO1 to β -lactam stress). This includes AmpR-dependent genes (there is a functional AmpR in these strains that helps in the response to β -lactams) and AmpR-independent genes (some of these genes may be regulated in an AmpR-independent manner). In contrast, in Condition B (response of *PAO Δ ampR* to β -lactam stress), 61 genes are upregulated in response to antibiotics (U2 in Fig 4.4a). AmpR may negatively regulate these genes, since their expression is enhanced in the absence of AmpR, or their expression is AmpR-independent. The observation that loss of *ampR* leads altered gene expression in PAO1 in response to sub-MIC β -lactam stress (similar to that seen in the absence of β -lactam stress) further hints at a global regulatory role for AmpR in *P. aeruginosa*.

Figure 4.4. Venn diagram of the differentially regulated genes. Distribution of significantly ($p \leq 0.01$) differentially regulated (>2 -fold) genes in PAO1 and PAOΔ*ampR* with (induced) and without (uninduced) β -lactam treatment showing upregulated (Fig 4.4a) and downregulated (Fig 4.4b) genes. The transcriptional regulators, sigma factors and small RNAs in each group are identified either by their gene names or PA numbers. Annotations are from the *Pseudomonas* Genome database (332).

Of the 18 upregulated genes in U1 (PAO1 induced), there could also be a subset of genes that are AmpR- and β -lactam dependent due to a functional AmpR in PAO1. In order to identify and separate exclusively β -lactam regulated genes, we compared these 18 upregulated genes to see if they were downregulated in Condition B (in D2) and identified *PA0465* (*creD*), *PA0466* (hypothetical) and *PA3889* (hypothetical). So, of the 18 genes in U1, only these three genes are specifically AmpR-regulated. Using a similar logic (for key, see (Appendix; Supplementary information for Chapter 4, Table S1), we identified β -lactam dependent genes (Appendix; Supplementary information for Chapter 4, Tables S2A, S2B) to fall in regions U6, D6, U7, D7, U8 and D8 in addition to U1 and D1 of the Venn diagram (Fig 4.4a, 4.4b). The genes that were regulated exclusively in response to β -lactam antibiotics include *mrcB*, a penicillin-binding protein 1 (PBP1) that is involved in peptidoglycan synthesis and is upregulated 3.5-fold (corrected *p*-value 3.71E-03). Genes of putative RND efflux and ABC transporters are also upregulated (Appendix; Supplementary information for Chapter 4, Table S2A), as are genes involved in flagellar biosynthesis and the *sox* operon (*PA5416-PA5419*; involved in amino acid metabolism). Genes that are downregulated in response to β -lactam exposure include 10 membrane proteins suggesting adaptation to stress, in addition to genes of the *phz2* phenazine biosynthetic operon.

The genes that are AmpR-dependent (U2, D2, U4 and D4: 387 genes) and AmpR- β -lactam dependent (U3, D3, U5 and D5: 282 genes) (Fig 4a, 4b) are of interest to us, since AmpR influences both these gene sets. To further eliminate β -lactam specific genes, we compared the AmpR-dependent and AmpR- β -lactam dependent gene lists between themselves as well as to the 206 β -lactam-specific genes (Appendix; Supplementary

information for Chapter 4, Table S2A). This led to the identification of 520 genes whose expression is influenced by AmpR, of which 313 are AmpR-dependent (Appendix; Supplementary information for Chapter 4, Table S3A, Table S3B) and a further 207 that are AmpR- β -lactam dependent (Appendix; Supplementary information for Chapter 4, Table S4A, Table S4B).

Functional categorization of AmpR-regulated genes

Functional categorization of the 520 AmpR-regulated genes was then performed and compared to the distribution in the *P. aeruginosa* PAO1 genome from the *Pseudomonas* Genome database (332) to look for enrichment of specific classes of genes in the different conditions (Fig 4.5). Category N genes (secreted factors, toxins, enzymes, alginate) were found to be downregulated in an AmpR-dependent manner and this correlated with decreased production of some secreted virulence factors (elaborated in the following sections). Similarly, genes in category G (cell wall, LPS, capsule) were downregulated, indicating that AmpR positively regulates these genes. Moreover, metabolic genes (amino acid biosynthesis - category E; central intermediary metabolism - category Q) show marginal downregulation in the *ampR* mutant. Genes involved in carbon compound catabolism (category S), adaptation and protection (category K), and those related to phage/transposon/plasmid (category V) were upregulated in the *PAO Δ ampR*, indicating negative AmpR regulation in an AmpR-dependent manner (Fig 4.5). Interestingly, genes encoding transcriptional regulators (category L) show differential regulation indicating that AmpR could potentially regulate other genes indirectly via intermediate transcriptional regulators.

Figure 4.5. Enrichment of functional categories. Functional categorization of the AmpR-dependent and AmpR- β -lactam dependent genes was performed to identify enrichment of specific classes of genes relative to their distribution in PAO1. ‘+’ and ‘-’ refers to upregulation and downregulation of genes, respectively in either an AmpR-dependent or AmpR- β -lactam-dependent manner. The most differentially regulated categories are labeled in the figure using the corresponding code, mentioned in the figure table. Functional categories, and their codes and percentages in PAO1 are from the *Pseudomonas* Genome database (332).

In the presence of β -lactam stress, loss of *ampR* leads to downregulation of membrane proteins (category D), putative enzymes (category O) and those involved in translation (category F), while motility and attachment genes (category T) were upregulated (Fig 4.5). In addition to membrane proteins (category D), the most differential regulation across all conditions was of the hypothetical proteins (category C), but until the functions of these proteins are determined, no significant conclusions can be drawn.

AmpR binding site analysis

In an attempt to identify the genes that are directly regulated by AmpR, a bioinformatics approach was adopted. IEM and RSA analyses were performed to identify, refine and scan the *P. aeruginosa* genomes as well as the AmpR-regulated genes from the microarray data for potential AmpR-binding sites. The consensus sequences derived from the AmpR- and AmpR- β -lactam dependent genes are similar, yet distinct (Fig 4.6 A, C). Using these consensus sequences to search the upstream regulatory regions of the PAO1 genome led to the identification of the AmpR-dependent element in the upstream region of 244 genes and the AmpR- β -lactam-dependent element in the upstream region of 207 genes. The motifs derived from the IEM and RSA analysis for both the AmpR-dependent and AmpR- β -lactam-dependent genes are almost identical (Fig 6). Of the genes identified in the microarray analysis, only 11.9% of AmpR-dependent and 14.5% of AmpR- β -

lactam-dependent upstream regulatory regions were identified by IEM or RSA analysis as having an AmpR binding site. This suggests that perhaps AmpR is exerting its effect by either directly or indirectly altering expression of a global transcription regulator. Studies aimed at identifying the direct targets of AmpR are needed to identify genes that are directly regulated by AmpR.

Regulation of the *amp* genes by AmpR

β -Lactam resistance in *P. aeruginosa* is mediated, in part, by the *amp* genes that are also tied in with the cell wall recycling process (414). The genes involved in this process include the regulator AmpR (105,415), the chromosomal β -lactamase AmpC (95,96,411,416), the permeases AmpG and AmpP (406,417) and the amidases AmpD, AmpDh2 and AmpDh3 (73,418). In addition, the hydrolase NagZ also plays a role in β -lactam resistance (419,420). Since AmpR is known to positively regulate AmpC expression, we hypothesized that AmpR also regulates the other *amp* genes. qPCR analysis revealed downregulation of the *amp* genes in PAO Δ *ampR*, normalized to expression in PAO1 (Fig 4.7), indicating a positive regulatory role for AmpR in the expression of *ampG* (RQ - uninduced: 0.59 ± 0.01 , *p*-value 0.006; induced: 0.63 ± 0.01 , *p*-value 0.04), *ampP* (RQ - uninduced: 0.1 ± 0.01 , *p*-value 0.004; induced- 0.86 ± 0.01 , *p*-value NS) , *ampD* (RQ - uninduced: 0.82 ± 0.01 , *p*-value NS; induced 0.67 ± 0.01 , *p*-value 0.04), *ampDh2* (RQ - uninduced: 1.01 ± 0.05 , *p*-value NS; induced- 0.56 ± 0.01 , *p*-value 0.002), *ampDh3* (RQ - uninduced: 0.98 ± 0.007 , *p*-value NS; induced: 0.66 ± 0.04 , *p*-value 0.02) and *nagZ* (RQ - uninduced: 0.27 ± 0.06 , *p*-value 0.002; induced: 0.51 ± 0.01 , *p*-value 0.0002).

Figure 4.6: AmpR binding site analysis. The putative AmpR binding site was refined using IEM algorithm and used to search the upstream promoter elements of AmpR-regulated and AmpR- β -lactam regulated genes (listed in Appendix, Supplementary information for Chapter 4, Tables S3, S4) in PAO1 and other *P. aeruginosa* strains. DOOR database (421,422) was used to identify the operons. The RSAT tool (423) was used to identify PAO1 promoter sequences containing the identified AmpR- and AmpR- β -lactam-regulated genes. The output is represented using WebLogo (424) for the AmpR-dependent genes from IEM (A) and RSA (B), and AmpR- β -lactam dependent genes from IEM (C) and RSA (D)

Figure 4.7. Expression of the *amp* genes in PAOΔ*ampR*. RNA was isolated from cells exposed to β-lactam antibiotic, reverse transcribed to cDNA and tested in triplicate by qPCR with gene-specific primers, as described in the text. The expression of *ampC* (β-lactamase), *ampD*, *ampDh2*, and *ampDh3* (amidases), *ampG* and *ampP* (permeases), and *nagZ* (glycoside hydrolase) were tested in the *ampR* mutant relative to PAO1. Values have been normalized to expression in PAO1 under the same conditions (log₁₀ RQ=1) and bars above or below the line represent up- and down-regulation, respectively. * *p* value <0.05

Specifically, when the cells are exposed to β-lactams, they need amidase activity to help in the peptidoglycan recycling process. This is reflected in upregulation of the amidases, (AmpD, AmpDh2, and AmpDh3) and AmpC only when the cells are exposed to β-lactams. Simultaneously, AmpG, which functions to transport the degraded peptidoglycan material into the cytoplasm (406) is upregulated by AmpR (downregulated in PAOΔ*ampR*) in an inducer-independent manner. This shows that AmpR upregulates the amidases and AmpC β-lactamase in response to β-lactams while upregulating AmpG (Fig 4.7), and agrees with the proposed model for peptidoglycan recycling in *P. aeruginosa* (406). The data, thus, demonstrates the central role of AmpR in influencing expression of the cell wall recycling/AmpC-mediated β-lactam resistance machinery in *P. aeruginosa*.

AmpR regulates the expression of antibiotic resistance and virulence systems

It has previously been shown that AmpR regulates the expression of genes related to QS and protease production (425). Microarray analyses from this study show that AmpR affects the expression of multiple virulence systems in *P. aeruginosa*, as explained further in this section.

Resistance-Nodulation-Division (RND) efflux systems

RND transporters are tripartite pumps present in Gram-negative bacteria that are involved in the efflux of antibiotics and several other compounds decreasing cytoplasmic retention and thus conferring resistance (426). All of the *P. aeruginosa* strains sequenced so far carry 12 known and putative RND efflux pumps, suggesting that the efflux pumps are an integral part of the *P. aeruginosa* genome (25,33). The MexEF-OprN efflux system that is primarily concerned with resistance to fluoroquinolones, chloramphenicol and trimethoprim (427) was significantly upregulated in PAO Δ *ampR*. The genes *mexE* (seven-fold), *mexF* (89-fold) and *oprN* (103-fold) are overexpressed in the *ampR* mutant in the absence of antibiotic stress in microarray studies (Appendix; Supplementary information for Chapter 4, Table S3A) and overexpression of the first gene of the operon, *mexE*, was confirmed by qPCR (Table 4.1). MexT, an activator of this efflux system (427,428), is not significantly differentially regulated (1-fold) in the microarray studies, but is upregulated in an inducer-independent manner when tested by qPCR (RQ: uninduced 7.5 ± 0.11 , *p*-value 0.02; induced 7.0 ± 0.25 , *p*-value 0.02). However, the negative regulator MexS shows no differential regulation either in microarray or qPCR analysis (data not shown). Carbapenems use the outer membrane porin OprD to gain

entry into the cell (429) and MexT negatively regulates this porin both at the transcriptional and post-transcriptional level (79,430,431). Indeed, qPCR analysis shows that with upregulation of *mexT*, there is a downregulation of *oprD* expression in the *ampR* mutant compared to PAO1 (RQ: uninduced 0.31 ± 0.004 , *p*-value 0.001; induced 0.08 ± 0.000002 , *p*-value <0.0001). Surprisingly, OprD downregulation did not lead to increased resistance to imipenem and meropenem (Fig 1a). In addition, we did not see differential regulation of the other known MexT regulator, MvaT (432) in our microarray analysis.

To further investigate whether upregulation of this pump translates into a resistance phenotype, we determined the MICs for MexEF-OprN substrate antibiotics by the standard broth microdilution method (433). *PAOΔampR* showed enhanced resistance to four of the antibiotics tested when compared to the resistance profile of wild-type PAO1, correlating microarray and qPCR data with the phenotype (Table 4.2). This suggests that AmpR is involved in resistance to β -lactam antibiotics by regulating AmpC as shown earlier, and non- β -lactam antibiotics via the MexEF-OprN efflux pump. Analysis of the promoter regions of the genes of this pump and their regulators (MexS and MexT) using the putative AmpR-binding site (434) as a query sequence did not reveal signs of AmpR binding, suggesting indirect regulation by AmpR.

MexAB-OprM was the first RND-type efflux pump to be reported in *P. aeruginosa* and has very broad substrate specificity including β -lactam antibiotics and non-antibiotic substrates (435). In fact, it has been implicated to play a more significant role in resistance to β -lactam antibiotics than β -lactamases (58,436). Using the putative *P. aeruginosa* AmpR binding site (434), we identified a potential AmpR binding site

Table 4.2: *ampR* deletion affects susceptibility to MexEF-OprN substrates. The minimum inhibitory concentrations (in µg/ml) of MexEF-OprN substrate antibiotics to PAO1 and PAOΔ*ampR* were determined by broth microdilution method (see text for details).

Antibiotic	MIC (µg/ml)	
	PAO1	PAOΔ <i>ampR</i>
Ofloxacin	4	32
Ciprofloxacin	0.25	2
Chloramphenicol	128	512
Trimethoprim	200	>200

upstream of the MexR repressor of this pump in the MexR-MexA intergenic region (5' AAGCCTGCAAATGT 3') indicating possible regulation of this pump by AmpR. qPCR analysis of *mexR* expression revealed downregulation of this gene in PAOΔ*ampR* compared to PAO1 (RQ: uninduced 0.46 ± 0.006 , *p*-value 0.002; induced 0.4 ± 0.007 , *p*-value 0.01). It is thus interesting to note that AmpR not only positively regulates AmpC β-lactamase but potentially also MexAB-OprM, two different mechanisms of resistance to β-lactams. The MexAB-OprM efflux can also pump out fluoroquinolones (79) and the enhanced quinolone resistance of PAOΔ*ampR* seen in the MIC studies is potentially due to a combined effect of upregulation of the MexEF-OprN and the MexAB-OprM efflux pumps. In addition, AmpR negatively regulates a two-gene putative RND efflux operon *PAI435-PAI436* (nine-fold and four-fold respectively in microarray) that codes for a

membrane fusion protein and efflux transporter, respectively. Potential AmpR regulation of the MexGHI-OpmD efflux pump is discussed in the QS section.

QS-regulated virulence factors

Many of *P. aeruginosa* virulence factors are QS-regulated and form a critical component of pathogenesis (208,437). In our previous meta-analysis of published *P. aeruginosa* transcriptomes, we identified differentially regulated sets of system-specific and condition-specific genes, including QS-regulated genes (400). Using this as our knowledge base, the *ampR* microarray profile was compared to identify differentially regulated QS-specific genes. The microarray data shows that AmpR influences expression of many QS-regulated genes (Appendix; Supplementary information for Chapter 4, Table S5).

To further verify AmpR-mediated regulation of QS virulence factors, we quantified the production of pyocyanin, LasA protease, and LasB elastase. Pyocyanin is a redox active exotoxin pigment that contributes to lung pathophysiology of chronic *P. aeruginosa* infections (438) and interferes with multiple host cellular functions (439). Genes in the locus of the *phzI* operon that is involved in QS-regulated phenazine synthesis, including *phzA1* (*PA4210*, 4-fold down), *phzB1* (*PA4211*, 21-fold down), *phzS* (*PA4217*, 28-fold down), *phzM* (*PA4209*, 4-fold down), and the MexGHI-OpmD efflux pump (*PA4205-PA4208*, 11-30-fold down) that plays a role in pumping out the pigments (440), show decreased expression in *PAOΔampR*. In agreement with this data, there was a statistically significant (*p*-value <0.0001) reduction in pyocyanin production by the *ampR* mutant, compared to PAO1 and this effect was independent of β-lactam stress on

the cells (Table 4.3). The data indicates that AmpR influences pyocyanin production which is in agreement with the *C. elegans* killing data (Fig 4.2), since phenazines are major players in *C. elegans* mortality in the fast-killing assay (413).

Elastases (pseudolysins) are highly toxic zinc metalloproteases that play a critical role in immunomodulation (441,442), host tissue damage aiding invasion (443) and biofilm formation (444). The LasB elastase production was also severely affected due to the loss of *ampR* ($p\text{-value} \leq 0.02$) in an inducer-independent manner (Table 4.3). Along with LasB, a zinc metalloendopeptidase, LasA plays a major role in *P. aeruginosa*-induced keratitis (445). Reduction in LasA protease production, however, was significantly lower ($p\text{-value} < 0.05$) in PAO Δ *ampR* only when the strains were exposed to sub-MIC β -lactam stress (Table 4.3), and this is in agreement with data from microarray (8-fold downregulated) and qPCR (RQ: uninduced- NS; induced 0.21 ± 0.07 , $p\text{-value} < 0.0001$) analysis.

Microarray data shows that loss of *ampR* also affects other QS-regulated virulence genes, such as the *hcn* operon *PA2193-PA2195* (5- to 7-fold downregulated) that is involved in the production of hydrogen cyanide. Cyanide toxicity is the primary mode of fast killing of *C. elegans* by *P. aeruginosa* PAO1 (446). The downregulation of the *hcn* operon concurs with reduced killing by PAO Δ *ampR*.

Table 4.3: Effect of deletion of *ampR* on QS-regulated virulence phenotypes

Strain	Pyocyanin production ^a		LasA activity ^b		LasB activity ^c	
	UI	IN ^d	UI	IN ^d	UI	IN ^d
PAO1	22.31 ± 0.18	24.30 ± 1.10	0.37 ± 0.005	0.39 ± 0.004	1.65 ± 0.25	1.54 ± 0.15
PAOΔ<i>ampR</i>	2.29 ± 0.11 ^e	5.74 ± 0.56 ^f	0.26 ± 0.002	0.19 ± 0.002 ^g	0.5 ± 0.03 ^h	0.57 ± 0.08 ⁱ

^aPyocyanin concentrations were expressed as µg pyocyanin produced per µg protein.

^bLasA activity was expressed as change in OD600 per hour per µg of protein.

^cLasB elastase activity was expressed as change in OD495 per µg of protein.

^dInduction was carried out with 100 µg/ml penicillinG.

^{e,f}*p*-value<0.0001 when comparing PAO1 and PAOΔ*ampR*

^{g,h}*p*-value<0.02 when comparing PAO1 and PAOΔ*ampR*

ⁱ*p*-value<0.01 when comparing PAO1 and PAOΔ*ampR*

The expression of the galactophilic lectin *lecA* (PA2570) is also downregulated in PAO Δ *ampR* (RQ: uninduced- 0.22 ± 0.0005 , *p*-value 0.0001; induced: 0.16 ± 0.003 , *p*-value 0.0005) indicating positive AmpR regulation. LecA facilitates bacterial entry into host cells by aiding in adhesion to endothelia and epithelia (447) and is involved in biofilm formation (448). It has previously been shown that *lecA* expression is regulated by the sigma factor RpoS (449) and by the QS regulator RhlR (449). Thus, the effect of AmpR on *lecA* expression could be mediated indirectly via RpoS and/or RhlR. As predicted, RpoS was downregulated (RQ: uninduced- 0.55 ± 0.08 , *p*-value 0.01; induced 0.51 ± 0.02 , *p*-value 0.0003), indicating AmpR positive regulation in a β -lactam-independent manner. Also, since we see downregulation of multiple QS phenotypes, RhlR is also potentially involved in AmpR-mediated *lecA* regulation.

QS activates the operon *PA2327-PA2331* (168) coding for a probable ABC transporter. Wolfgang *et al.* found this operon to be repressed when *P. aeruginosa* was grown in CF respiratory liquid containing media (450). Genes of the operon are significantly upregulated (6- to 18-fold) in PAO Δ *ampR* indicating that AmpR negatively regulates this operon and further connects AmpR to QS regulation, adding another regulatory player in this complex regulatory network.

Biofilms

Successful biofilm formation is dependent on nutrient availability, motility and QS (451). Comparison of gene expression profiles from this study with the biofilm-specific gene list generated from our previous analysis of *P. aeruginosa* transcriptomes (400) revealed the differential regulation of many biofilm genes in PAO Δ *ampR*

(Appendix; Supplementary information for Chapter 4, Table S6). This suggests a role for AmpR in biofilm regulation, either directly or indirectly. Testing the tube biofilm-forming ability of the strains revealed that PAO Δ *ampR* formed better biofilms, compared to PAO1 (Fig 4.8). The difference was significant at all time points tested (p -value ≤ 0.03) over a period of 72 hours indicating that AmpR is a negative regulator of biofilm formation.

Microarray data also revealed upregulation of *PA4651* (encoding a probable pili assembly protein; 11.6-fold), and PA4306 (coding for Flp, Type IVb pilin, 36-fold) in PAO Δ *ampR*, in the absence of antibiotics. Since type IV pili-mediated twitching and flagella-mediated swimming motilities are known proponents for biofilm formation at different stages (452), we tested for these phenotypes with PAO1 and PAO Δ *ampR*. In the absence of β -lactam antibiotic stress, the PAO Δ *ampR* strain demonstrated enhanced twitching ability (PAO Δ *ampR*: 15.1 mm $\pm$ 1.1 mm; PAO1: 2 mm $\pm$ 0.1 mm; p value < 0.0001) potentially explaining its ability to form better biofilms. Moreover, unlike in PAO1, under sub-MIC β -lactam stress, PAO Δ *ampR* showed a marginal but statistically significant increase in twitching zones (uninduced: 15.1 mm $\pm$ 1.1 mm; induced: 16.5 mm $\pm$ 0.8 mm; p -value 0.04). This observation is in agreement with the enrichment of the gene set for the ‘motility and attachment’ functional category under β -lactam stress in PAO Δ *ampR* (see section above). There was, however, no difference in the swimming motility of the strains (data not shown), although *fleR* of the FleSR two-component system that is involved in the flagella biosynthesis regulatory pathway (180) was differentially regulated in the *ampR* mutant (RQ: uninduced- 0.45 $\pm$ 0.1, p -value 0.0007; induced- 0.32 $\pm$ 0.01, p -value 0.0006).

Figure 4.8. AmpR affects biofilm formation. AmpR regulates many genes involved in biofilm formation (Appendix, Supplementary information for Chapter 4, Table S6) that is reflected in the tube biofilm assay monitored for 72 hours. The strains tested were wild type PAO1, PAOΔampR, PAOΔretS (positive control), and PAOΔpelΔpslΔalgD (negative control). The inset, taken at 72 hours, demonstrates the superior biofilm formation capacity of PAOΔampR compared to PAO1. *p*-values comparing PAOΔampR with PAO1: 24 hrs- 0.002; 48 hrs- 0.03; 72 hrs- 0.007.

The Pel polysaccharide is a glucose-rich exopolymer, encoded by the *pel* operon (*PA3058-PA3064*) that along with the mannose-rich Psl polysaccharide plays a major role in pellicle formation (453,454). PAO Δ *ampR* forms darker red colonies on Tryptone-Congo red agar plates compared to PAO1 (data not shown) signifying higher Pel production (453). This is consistent with the observation that the *ampR* mutant produces better biofilms. Collectively, these data suggest that AmpR negatively influences biofilm formation in *P. aeruginosa* either directly or indirectly.

Recently, a novel efflux pump that confers antibiotic resistance in *P. aeruginosa* biofilms has been identified (455). Deletion of the operon (*PA1874-1877*) encoding this pump in PAO1 enhances sensitivity to gentamycin, tobramycin and ciprofloxacin, especially in biofilm cells. Genes in this operon are downregulated four-fold in the *ampR* mutant strain. This observation, though not verified separately, suggests that AmpR positively regulates this operon, thus possibly contributing to non- β -lactam antibiotic resistance in biofilms.

Secretory systems

The type III secretion system (T3SS) is essential for *P. aeruginosa* not only for contact-dependent toxin delivery to host cells but also for phagocyte evasion (206,456). Using a burn mouse model, it was shown that loss of T3SS results in reduced virulence (457). The genes encoding the regulatory and structural components of the T3SS in *P. aeruginosa* are concentrated in one locus (*PA1690-PA1725*) whereas the effectors and their chaperones are scattered in the genome (332). Deletion of *ampR* from PAO1 led to the downregulation of a few T3SS genes in the regulatory-structural gene cluster (Fig

4.9) indicating positive AmpR-regulation. These include the genes encoding the regulators PcrH (involved in regulating ExoS synthesis) and ExsE (a secreted regulator of the ExsCED regulatory cascade), and the translocator proteins PopB and PopD. qPCR confirmation of the microarray findings for the T3SS genes was done using RNA isolated from Ca^{2+} depleted inducing media (MinS-NTA), since Ca^{2+} is a known inhibitor of T3SS (325). Since *pcrH*, *popB* and *popD* are the last three genes of a 12-gene operon, we tested expression of only *pcrH* by qPCR, which showed a downregulation in $\text{PAO}\Delta\text{ampR}$ compared to PAO1 (RQ: uninduced- 0.71 ± 0.06 , *p*-value 0.02; induced- 0.88 ± 0.03 , *p*-value 0.003). Similarly, *exsE*, the second gene of the *exsCEB* operon was also downregulated in $\text{PAO}\Delta\text{ampR}$ (RQ: uninduced- 0.57 ± 0.01 , *p*-value 0.001; induced- 0.69 ± 0.18 , *p*-value NS) indicating positive regulation of these genes by AmpR.

Transcriptional regulation of T3SS in *P. aeruginosa* is a complex process and involves multiple tiers of regulation (325). One of the mechanisms of control involves a small RNA-binding protein RsmA and non-coding small RNAs (sRNA), *rsmY* and *rsmZ*. Sequestration of RsmA by the sRNAs inhibits its activity. RsmA has an extensive virulence regulon that is tied in with the GacSA two-component system regulatory cascade (390,458). In $\text{PAO}\Delta\text{ampR}$, *rsmZ* is downregulated (RQ: uninduced- 0.21 ± 0.03 , *p*-value 0.01; induced- 0.78 ± 0.09 , *p*-value NS) and corresponds with an upregulation of RsmA (in microarray, uninduced - 2.3 fold; corrected *p*-value 0.009; induced- NS). Some of the T3SS genes are, however, downregulated in the *ampR* mutant (Fig 4.9). RsmA is a positive regulator of T3SS. This suggests that the effect of AmpR on T3SS is probably not via RsmA, or involves an additional step of post-transcriptional regulation. PtrB

Figure 4.9. Regulation of secretion genes by AmpR. Fold changes (FC) as determined by microarray experiments in the absence of β -lactam stress of the Type VI secretion (▲, HSI-I) and Type III secretion (■) genes in the *ampR* mutant strain, normalized to expression in the wild-type strain PAO1. Gene annotations are from the *Pseudomonas* Genome database (332).

represses T3SS gene expression (331) and since AmpR regulates PtrB expression (see section below), AmpR regulation of T3SS is potentially via PtrB.

P. aeruginosa T6SS is involved in chronic CF infections (459). RsmA also negatively regulates genes of the Type 6 secretion system (T6SS), particularly of the HSI-I system (458). Thus, downregulation of genes of the T6SS in PAO Δ *ampR* (Fig 4.9) is possibly through an indirect effect of AmpR on RsmA.

AmpR regulates genes found in regions of genome plasticity

Comparative analysis of five *P. aeruginosa* chromosomes identified RGPs that are strain-specific (33). These are genome segments that can be acquired by horizontal gene transfer, bacteriophages or transposons (33). We wanted to determine whether an endogenous transcriptional regulator, such as AmpR, could regulate expression of genes acquired by the strain, such as RGP genes. Microarray analysis revealed that in the PAO Δ *ampR* mutant, 31 RGP genes are regulated in an AmpR-dependent manner and an additional eight RGP genes under sub-MIC β -lactam stress (Appendix; Supplementary information for Chapter 4, Table S7).

Most of the RGP03 (*PA0612-PA0628*) and RGP04 (*PA0641-PA0648*) ORFs belong to functional category V (related to phage, transposon and plasmid) and code for the two classes of high molecular weight pyocins, types R and F. Pyocins R and F are related to bacteriophage tails and kill susceptible cells thus conferring a survival advantage on the producing strain (460). Thirty-two percent of the RGP genes regulated in an AmpR-dependent manner are clustered and localized to RGP03. The expression of 10 RGP03 genes and two RGP04 genes (59% and 20% of genes in RGP03 and RGP04,

respectively) are significantly upregulated (6-8-fold) in *PAOΔampR* as seen in microarray data (Appendix; Supplementary information for Chapter 4, Table S7). This suggests that AmpR is involved in negatively regulating these genes, either directly or indirectly.

PrtN (PA0610), and the product of the first gene in RGP03, *ptrB* (PA0612), is a positive regulator of pyocin production, both of which are repressed by PrtR (PA0611) (330). Upon DNA damage, RecA (PA3617) degrades PrtR, thus inducing pyocin production (330). In *PAOΔampR* subjected to sub-MIC β -lactam stress compared to PAO1, *prtN* (12-fold), *recA* (3-fold) and *ptrB* (20-fold) are upregulated, while the negative regulator PrtR is downregulated (qPCR relative quantity: uninduced- 0.88 ± 0.4 , p -value 0.004; induced- 0.76 ± 0.109 p -value 0.0002) implying that AmpR is a negative regulator of pyocin production.

AmpR regulates other transcriptional regulators

The AmpR-regulation of hundreds of genes (Fig 4.4a, 4.4b) could be by direct binding to the promoters or in a subset of genes, indirectly through intermediate transcriptional regulators, sigma factors or regulatory RNAs. In a preliminary attempt to further elucidate the AmpR regulon, we looked at the transcriptional regulators and sigma factors that are AmpR-regulated. Of the 430 transcriptional regulators in *P. aeruginosa* PAO1 (332), 19 met the selection criteria in our microarray analysis (see materials and methods) and are over 2-fold significantly differentially regulated in the *ampR* mutant (Table 4.4). This suggests that AmpR regulates a proportion of the genes through intermediate transcriptional regulators.

Table 4.4: Transcriptional regulators and sigma factors regulated by AmpR

Transcriptional regulators				
Locus Tag	Gene Name	Product Name	Corrected <i>p</i>-value	Fold change
PA0463	<i>creB</i>	two-component response regulator CreB	8.39E-03	-2.1
PA0479		probable transcriptional regulator	9.90E-03	-2.1
PA0610	<i>prtN</i>	transcriptional regulator PrtN	3.81E-03	11.6
PA0611	<i>prtR</i>	transcriptional regulator PrtR	6.10E-03	-3.9
PA0612	<i>ptrB</i>	repressor, PtrB	3.44E-03	19.5
PA1196		probable transcriptional regulator	9.89E-03	2.6
PA1663		probable transcriptional regulator	5.13E-03	-3.9
PA1707	<i>pcrH</i>	regulatory protein PcrH	3.98E-03	-3.8
PA2281		probable transcriptional regulator	9.89E-03	-2.1
PA2588		probable transcriptional regulator	3.77E-03	-27.9
PA3508		probable transcriptional regulator	1.60E-03	3.5
PA3604		probable two-component response regulator	6.96E-03	-2.2
PA4296	<i>pprB</i>	two-component response regulator, PprB	6.01E-03	7.4
PA4781		probable two-component response regulator	9.10E-03	2.8
PA4853	<i>fis</i>	DNA-binding protein Fis	6.65E-03	-3.3
PA5059		probable transcriptional regulator	6.33E-03	4.0
PA5105	<i>hutC</i>	histidine utilization repressor	8.27E-03	-3.1
PA5380	<i>gbdR</i>	GbdR	5.75E-03	2.6
PA5483	<i>algB</i>	two-component response regulator AlgB	7.83E-03	2.4

Sigma factors

Locus Tag	Gene Name	Product Name	Corrected <i>p</i> -value	Fold change
PA0762	<i>algT</i>	sigma factor AlgU	3.35E-03	5.4
PA1300		probable sigma-70 factor, ECF subfamily	9.59E-03	2.5
PA3622	<i>rpoS</i>	sigma factor RpoS	6.65E-03	2.2

Three of the 24-known/putative sigma factors (461) are also over 2-fold differentially regulated, including RpoS and AlgT/U (Table 4.4). The stationary sigma factor, RpoS controls virulence in different bacteria including *P. aeruginosa* (462-464). Since *rpoS* expression is upregulated in the stationary phase (461,463) and the RNA used for microarray analysis was harvested two hours post- β -lactam induction (at OD $\sim$ 3.0), some of the phenotypic changes seen in PAO Δ *ampR* is likely to be RpoS-mediated.

To investigate this possibility, RNA was harvested 40 minutes post-induction (OD₆₀₀ of $\sim$ 1.0) and the expression of known RpoS-dependent and RpoS-independent genes was compared between PAO1 and PAO Δ *ampR*. As expected, RpoS expression was significantly higher at 2 hours than at 40 minutes in PAO Δ *ampR* compared to PAO1 (*p*-value: uninduced 0.0049, induced 0.0023; Fig 4.10), and this increase in RpoS expression corresponded with a growth phase-dependent increase in the expression of two of the RpoS-dependent genes, *lecA* (*p*-value: uninduced 0.0061; induced 0.0043) and *lecB* (*p*-value: uninduced NS; induced 0.0002) (Fig 4.10). However, expression of the MexEF-OprN activator, MexT, which is regulated in an RpoS-independent manner, did not change at the different time points tested (Fig 4.10). This suggests that AmpR, via RpoS,

regulates genes of the RpoS regulon in *P. aeruginosa* in a growth phase- and stress-dependent manner, which is in agreement with previous studies (463,464). Moreover, the MexT data suggests that harvesting the cells either after 40 minutes or 2 hours of β -lactam treatment does not affect AmpR-mediated gene expression for non-RpoS-dependent genes (Fig 10).

The sigma factor AlgT/U is a master regulator of alginate biosynthesis (465,466) and we have shown previously with an *ampR* insertion mutant that there is crosstalk between AmpR and AlgT/U (425). Our microarray data shows upregulation of AlgT/U in PAO Δ *ampR* (5.4-fold, corrected *p*-value 3.35E-03), indicating negative AmpR regulation (Table 4.4) in agreement with previous findings (425).

Figure 4.10. Gene expression in PAO Δ *ampR* at 40 minutes and 2 hrs post- β -lactam exposure. RNA was isolated from PAO1 and PAO Δ *ampR* cells exposed to β -lactams for either 40 minutes or 2 hours and reverse transcribed to cDNA. The expression of the sigma factor *rpoS*, *lecA* and *lecB* (galactophilic lectin genes known to be RpoS-regulated), and *mexT* (MexEF-OprN efflux pump regulator that is not RpoS-regulated) were tested by qPCR with gene-specific primers, as described in the text. Values have been normalized to expression in PAO1 under the respective conditions. ** *p*-value <0.006; *** *p*-value 0.0002 as determined by unpaired *t* test.

Subtractive transcriptomics of the AmpR regulon

Meta analysis of 18 condition-specific *P. aeruginosa* transcriptomes led to the identification of an expression core gene set of 303 genes that are significantly differentially regulated under all the different conditions analyzed and were proposed to be involved in maintaining cell homeostasis (400). Comparing the 520 genes whose regulation is AmpR-dependent to the core led to the identification of 57 genes (of the 520) that were part of the expression core genes (Fig 4.11). Further, we wanted to identify genes that are specifically under AmpR-regulation and those that are not involved in other pathways. To derive this list, we compared the 463 AmpR-dependent genes (520 minus 57 core genes) with the 1726 genes that are regulated in other conditions/by other regulators [1598 condition-specific genes (400), and 128 genes that are either RpoS (368) or AlgT/U-regulated (467)]. This comparison reduced the number of AmpR-dependent genes to 133 (from 313) and the AmpR- β -lactam-dependent genes to 86 (from 207) (Fig 4.11; Appendix; Supplementary information for Chapter 4, Table S8).

Figure 4.11. Comparison of the AmpR transcriptome with other transcriptomes. AmpR-dependent and AmpR- β -lactam-dependent genes were compared with the 303 genes of the expression core and the 1726 condition-specific genes identified previously as part of a meta-analysis of 18 *P. aeruginosa* transcriptomes (400).

The exclusively AmpR-dependent genes include the O antigen chain regulator *wzz*, the *pca* genes involved in carbon compound catabolism and *creB*, which codes for the response regulator of the CreBC TCS (Table S8). The CreBC TCS has been demonstrated to be involved in β -lactam resistance in *Aeromonas* spp. (468). CreBC positively regulates expression of an inner membrane protein CreD in *E. coli* (469) and in *P. aeruginosa* the CreBCD system contributes to β -lactam resistance only in a Δ BPB4 background (348). Microarray analysis of PAO Δ *ampR* in the presence β -lactam stress shows reduced *creB* expression (-2.1, corrected *p*-value 8.4E-03). Expression of *creD*, however, is significantly increased in PAO Δ *ampR* compared to PAO1 under β -lactam stress as determined by qPCR (RQ: induced 11.04 ± 0.0001 , *p*-value < 0.0001). This is in agreement with a previous report that also showed *creD* overexpression in cefoxitin-treated PAO Δ *ampR* cells (348). AmpR thus positively regulates *creB* while negatively

regulating *creD* expression suggesting potential AmpR regulation of *creD* in a CreB-independent manner.

Phenotypic microarray analysis of PAO Δ *ampR*

Since loss of *ampR* led to dysregulation of over 500 genes as seen in DNA microarrays, we decided to characterize its metabolic effect using phenotypic microarrays. Biolog analysis was performed with PAO1 and PAO Δ *ampR* in the absence of antibiotic stress. In all, seven phenotypes were gained and 47 phenotypes were lost (Appendix; Supplementary information for Chapter 4, Table S9). PAO Δ *ampR* grew marginally better in media containing nutritional supplements including citrulline, histidine, shikimic acid, leucine, serine, spermidine and pyridoxal. This indicates that AmpR is a negative regulator of utilization of these agents. Of the 47 observed phenotypes that were lost in PAO Δ *ampR*, 45 belong to the sensitivity panel. Fourteen of the 45 observed phenotypes were associated with antibiotics, further supporting the role of AmpR as a major regulator of antibiotic resistance in *P. aeruginosa*.

Discussion

P. aeruginosa AmpR, a LysR-type transcriptional regulator and a positive regulator of the chromosomal *ampC* β -lactamase expression, has been shown previously to play a role in regulating a few QS-dependent phenotypes and the alginate master regulator, AlgT/U (105,425). In this study, we determined the whole genome expression profiles of a clean in-frame deletion mutant of *ampR* in *P. aeruginosa* PAO1 under normal conditions and under sub-MIC β -lactam stress, using DNA microarrays. The results demonstrate that AmpR influences the expression of 313 genes in the absence of β -lactam stress and an additional 207 genes when exposed to sub-MIC β -lactam stress. The AmpR regulon is thus much more extensive than previously thought including virulence, antibiotic-resistance and metabolic genes.

Multi-drug resistant *P. aeruginosa* isolates are a frequent occurrence in many acute and chronic infections (201). β -lactamases and efflux pumps are major mediators of antibiotic resistance in *P. aeruginosa* (79). We show that in addition to positively regulating the *ampC* and potentially the MexAB-OprM efflux pump by modulating expression of the MexR repressor, AmpR also mediates non- β -lactam resistance by negatively regulating the MexEF-OprN efflux pump. The PAO1 strain used for constructing PAO Δ *ampR* was the strain used in the genome-sequencing project (25), which has an 8 bp insertion in the MexEF-OprN activator *mexT* leading to premature *mexT* termination (470) and consequent non-inducibility of the MexEF efflux pump. Strains with a *nfxC* mutation, however, have different ways to overcome this, including secondary mutations and deletion of the 8 bp insertion (428,470), leading to activation of the MexEF-OprN efflux. This was also observed in *nfxC* mutants isolated in a mouse

model (471). However, there was no differential expression of the MexT activator in both these studies (470,471). With PAO Δ *ampR*, even though *mexT* expression is upregulated in a β -lactam-independent manner, this will still not be able to overcome the effect of the 8 bp deletion. It has, however been suggested that there is a putative LacI-like repressor binding site in the *mexT-mexE* intergenic region (472) and that there is a second repressor that binds this site regulating expression of *mexEF-oprN* (473). This suggests that the LTTR AmpR potentially regulates this LacI-type repressor, leading to induction of the MexEF-OprN pump in a MexT-independent manner. The outer membrane porin OprD serves as a conduit for the entry of carbapenems into the cell (429). Although we see decreased expression of *oprD*, the strain is still sensitive to imipenem and meropenem, which is contrary to expectation. However, our finding is in agreement with a previous observation where *mexEF-oprN* overexpressing strains showed no altered imipenem susceptibility (474), the associated mechanism remains to be elucidated. Previous studies have also demonstrated an inverse correlation between β -lactam resistance and biofilm formation, both *in vitro* and in CF isolates (475-477). Our data supports these findings as far as β -lactam antibiotics are concerned, since AmpR positively regulates production of AmpC β -lactamase while negatively regulating biofilm formation. However, negative regulation of the MexEF-OprN efflux (providing resistance to fluoroquinolones, chloramphenicol and trimethoprim) by AmpR (Table 4.2, Fig 4.12) suggests that the antagonistic regulation of antibiotic resistance and biofilm formation is dependent on the class of antibiotics. The physiological advantage to the bacteria in this context is unclear. Co-regulation of β -lactam and fluoroquinolone resistance by AmpR is significant in itself, since this puts AmpR among one of the few proteins that regulates resistance to multiple

classes of antibiotic (478). In addition, since fluoroquinolones are part of the current systemic antibiotic treatment regimen for *P. aeruginosa* infections (201), this finding could potentially have important therapeutic implications.

QS is at the heart of the virulence regulatory network in *P. aeruginosa* with multiple regulators feeding into the regulation process (170). We have shown previously that AmpR is also part of the QS regulatory process and regulates production of proteases and pyocyanin (105,425), but the determined extent of the regulation was limited due to the experimental approaches adopted. Using whole genome transcriptome, we show here that the AmpR-influenced QS regulon is much more extensive than previously thought (Appendix; Supplementary information for Chapter 4, Table S5). QS regulated phenotypes, such as pyocyanin and protease production are positively regulated by AmpR in the current study (Fig 4.12), which is in contrast to that seen in our previous analyses (105). We believe that this difference stems from the fact that in our previous studies, we used an insertion mutant (*aacCI* cassette inserted into the *PstI* site of *ampR*) whereas in this study, we have used a clean in-frame deletion mutant of *ampR*. One potential reason for the discrepancy may be that the gentamycin cassette insertion at the *PstI* site (554 bases into the *ampR* coding region) (105) did not disrupt the N-terminus HTH motif of AmpR. Since LysR members are known to bind DNA even in the absence of inducer signals (104), this intact HTH motif might have somehow interfered with the regulatory process. The *P. aeruginosa* PAO1 insertion *ampR* mutant in the previous study produced more pyocyanin compared to the wild-type PAO1 (105). In contrast, in this study, we find that PAO Δ *ampR* produces significantly lower amounts of phenazines as compared to PAO1 (Table 4.3). The differences in phenazine production were also

translated into differential susceptibilities in the *C. elegans* paralytic assays [Fig 4.2; (425)] since phenazines are one of the major contributors to *C. elegans* toxicity in this assay (195) explaining the reduced killing of *C. elegans* with this strain (Fig 4.2). Furthermore, with PAO Δ *ampR*, the microarray, qPCR and phenotypic data concur, and support our current findings.

The MexGHI-OpmD (PA4205-PA4208) efflux pump is involved in the efflux of acriflavin, ethidium bromide, novobiocin, rhodamine, and vanadium, and in maintaining QS homeostasis (440). Mutants of *mexI* and *opmD* have been demonstrated to be impaired in QS-related phenotypes including swarming motility, production of elastase, rhamnolipids, pyocyanin and pyoverdine (440). This pump is downregulated in PAO Δ *ampR* indicating positive AmpR regulation and correlates with some of the observed phenotypes, such as decreased production of elastase and pyocyanin. Furthermore, SoxR is known to regulate this pump (479), but is not differentially regulated in the microarray data.

AlgR regulates the *hcnABC* operon genes (PA2193-PA2195) involved in hydrogen cyanide synthesis in *P. aeruginosa* (480). In the *ampR* mutant, these genes are downregulated five- to seven-fold without a corresponding significant differential regulation of *algR* (< two-fold). AmpR, however, negatively regulates AlgT/U (5.4-fold upregulated in PAO Δ *ampR* in the absence of antibiotics) in agreement with previous findings (425), and AlgT/U regulates *algR* (481,482). Thus the regulation of the *hcnABC* operon in PAO Δ *ampR* is potentially through AlgT/U-mediated regulation of AlgR (Fig 4.12). Moreover, RpoS, in conjunction with the Gac-Rsm regulatory system, has been shown to regulate oxidative stress-mediated resistance in *P. fluorescens* (483). In our

study, both RpoS and the sRNA, *rsmZ* are regulated in an AmpR- β -lactam-dependent and AmpR-dependent manner, respectively. This could also be a potential mode of regulation of the *hcn* operon in PAO Δ *ampR*, provided the regulatory mechanism is conserved in *P. aeruginosa*. AmpR also negatively influences expression of the galactophilic lectin LecA (PA2570) (Fig 4.10) that is RpoS and QS-regulated. LecA has been shown to be critical to the gut pathogenicity of *P. aeruginosa* and to enhance cytotoxic effects of exotoxins by inducing a permeability defect (484). These results further support the hypothesis that AmpR regulates virulence in *P. aeruginosa*.

P. aeruginosa produces bacteriocins called pyocins that kill susceptible cells through either pore-formation and subsequent leakage of cytoplasmic contents, or by endonuclease activity (460). The two high molecular weight pyocins, types R and F, are proposed to be remnants of lysogenic phages and resemble phage tails. Like lysogenic phages, they are induced in response to DNA damage, linked to the RecA-mediated SOS response (485) and other stress conditions such as exposure to hydrogen peroxide (152) or ciprofloxacin (486). AmpR influences the expression of many of these genes including the regulators PtrR, PtrN, PtrB, and the SOS response mediator RecA (Table 4.4; section in results). Specific and significant differential regulation of these genes under β -lactam stress in PAO Δ *ampR* implies a role for AmpR in influencing expression of these genes under stress conditions. Moreover, pyocin production confers a survival advantage by killing neighboring susceptible cells and 97% of the *P. aeruginosa* CF isolates tested showed bacteriocin-like killing activity (487).

Figure 4.12. AmpR is a master regulator of gene expression in *P. aeruginosa* PAO1. AmpR positively regulates resistance to β -lactam antibiotics by upregulating expression of the *amp* genes, *nagZ* and downregulating *creD*. In addition, AmpR affects fluoroquinolone resistance by negatively regulating expression of *mexT*, the positive regulator of the MexEF-OprN efflux pump. Expression of the virulence and stress response sigma factor, RpoS and QS-regulated acute virulence factors is downregulated in PAO Δ *ampR*, indicating positive AmpR regulation. AmpR also negatively regulates biofilm formation via an unknown mechanism. AmpR modulates levels of the small RNA *rsmZ*, whose levels are lower in PAO Δ *ampR* with a corresponding enhanced expression of RsmA. Downregulation of some of the T3SS genes in the *ampR* mutant is possibly by regulating *ptrB* expression, via PrtR. Further, two major regulators of the alginate biosynthetic pathway, AlgT/U and AlgB are negatively regulated by AmpR, thereby potentially also regulating alginate production. Whether these AmpR interactions are direct or indirect needs to be investigated.

The pyocin genes are located in RGP03 and RGP04 and are acquired by *P. aeruginosa* (33). Though some RGP loci contain regulators that control expression of RGP genes (488), it is interesting to note that an endogenous regulator such as AmpR is able to regulate acquired genes and highlights the transcriptional versatility of *P. aeruginosa*.

Negative regulation of the genes involved in biofilm formation and pyocin biosynthesis by AmpR (Fig 4.12) fits with the profile of AmpR as a negative regulator of chronic infection phenotypes and positive regulator of acute infection, as seen with the QS-regulated phenotypes. RsmA, a small RNA-binding protein, is a global regulator of virulence in *P. aeruginosa* and is intricately tied in with the GacSA TCS (390,458). Two small RNAs, *rsmZ* and *rsmY*, which bind and sequester RsmA, keep RsmA activity in check in *Pseudomonas* and other bacteria (389,458,489-491). In *P. aeruginosa*, RsmA positively regulates T3SS and negatively regulates biofilm formation (492,493). AmpR positively regulates *rsmZ* (downregulated in PAO Δ *ampR*), resulting in downregulation of RsmA (upregulated in PAO Δ *ampR*). PAO Δ *ampR* forms enhanced biofilms (Fig 4.8) and shows decreased production of QS regulated phenotypes compared to PAO1 (Table 4.3).

These data suggest that regulation of some of the phenotypes seen in an *ampR* mutant is not via modulating the activity of *rsmZ* and consequently RsmA. However, analysis of the *rsmZ* promoter for potential AmpR binding sites using a previously identified putative AmpR binding site (434) revealed a weak AmpR consensus (5' CCCGCGCCTTTTGT 3'). The possibility of direct AmpR regulation of *rsmZ* remains to be elucidated.

In conclusion, unlike AmpR found in other Enterobacteriaceae members, the targets of *P. aeruginosa* AmpR are widely distributed in the genome and consist of over 500 genes. Since the genes regulated include transcriptional regulators, sigma factors and non-coding RNAs, a subset of these genes are possibly regulated indirectly by AmpR via intermediate regulators. It is also interesting to note that AmpR regulates genes that have been acquired by horizontal gene transfer, which reflects on the transcriptional versatility of *P. aeruginosa*. Although the exact AmpR regulatory mechanisms are as yet unclear, this study highlights the complexity and multi-tiered regulatory processes by which *P. aeruginosa* controls expression of genes of diverse functions. Teasing apart the AmpR regulatory network will involve differentiating direct and indirect AmpR-regulated genes that will advance our understanding of how this bacterium regulates multiple different pathogenesis and metabolic mechanisms. This understanding will potentially lead to identifying mechanistic targets that can help in dealing with intractable *P. aeruginosa* infections, and other bacterial pathogens that harbor similar systems.

Materials and Methods

Bacterial strains, nematodes, media and primers

All experiments were performed with either the wild type or derivatives of *P. aeruginosa* PAO1 (25) and *Escherichia coli* DH5 α cultivated in Luria Bertani (LB) broth (Difco, USA) incubated at 37 °C unless specified otherwise. The strains and plasmids used in this study are listed in Table 4.5. Nematode assays were performed with *Caenorhabditis elegans* strain N2 (Bristol) and *E. coli* OP50 obtained from the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). *E. coli* OP50 was grown in nematode growth medium (1.7 % agar, 0.35 % peptone, 0.34 % K₂HPO₃, 0.3 % NaCl, 0.012 % MgSO₄, 0.011 % CaCl₂, 0.0005 % cholesterol) whereas the *P. aeruginosa* strains used in the nematode assays were grown on brain-heart infusion agar (Difco, USA). Biofilm assays were performed in T-broth (tryptone 10.0 g/L, NaCl 5.0 g/L). T-agar plates were prepared by addition of 1% agar to T-broth. MinS-NTA minimal media (494) was used for RNA isolation to determine expression of T3SS genes. Cation-adjusted Mueller Hinton broth and agar (Difco, USA) was used in MIC assays. For pyocyanin production, strains were cultivated in King A medium (Difco, USA). Antibiotics were supplemented, when needed, at the following concentrations for *E. coli*: ampicillin (Ap) 100 μ g/ml, gentamycin (Gm) 15 μ g/ml, tetracycline (Tc) 15 μ g/ml; for *P. aeruginosa*: Gm 75 μ g/ml, Tc 60 μ g/ml. Primers used in this study are listed in Appendix; Supplementary information for Chapter 4, Table S10.

Table 4.5: Strains and plasmids used in this study

Strain/ plasmid	Relevant characteristics	Source
Strains		
<i>Escherichia coli</i>		
DH5 α	General purpose cloning strain; $\Delta(lacZ)M15$	New England Biolabs
OP50	Food source for culturing <i>C. elegans</i>	<i>C. elegans</i> Genetics Center
DBS7	DH5 α harboring pEXG2 carrying the 1520 bp $\Delta ampR$ fragment (pDBS7)	This study
DBS220	DH5 α harboring the 944 bp promoterless <i>ampR</i> ORF cloned into pCR2.1 TOPO (pDBS220)	This study
DBS271	DH5 α containing the 944 bp <i>ampR</i> ORF subcloned as a <i>EcoRI-SacI</i> fragment from pDBS220 into pMMB67EHGm	This study
Strain/ plasmid		
Relevant characteristics		
Source		
<i>Pseudomonas aeruginosa</i>		
PAO1	Wild-type	(25)
PKM314	PAO1 with pMMB67EH-Gm (empty vector)	This study
PKM315	PAO $\Delta ampR$; In-frame deletion of <i>ampR</i> (PA4109)	This study
PKM316	PAO $\Delta ampR$ with pMMB67EH-Gm (empty vector)	This study
PKM317	PAO $\Delta ampR$ (pAmpR); <i>ampR</i> ORF on pMMB67EH-Gm (pDBS271) moved into PAO $\Delta ampR$; IPTG-inducible; Gm ^R ; <i>ampR</i> complementing clone	This study
PAOretS::aacCI	Gm insertion mutant of <i>retS</i> ; hyper biofilm-former	S. Lory
PAO $\Delta pel \Delta psl \Delta algD$	In-frame triple deletion of the <i>algD</i> ORF and <i>pel</i> and <i>psl</i> operons; biofilm non-former	S. Lory
<i>Staphylococcus aureus</i>		
DBS116	Wild type <i>S. aureus</i> used for LasA assays	Lab collection
Plasmids		
pCR2.1 TOPO	TA cloning vector for PCR products; Ap ^R , Km ^R ; ColE1 fl ori lacZ α	Invitrogen
pRK600	Helper plasmid for conjugation	[133]

pEXG2	Allelic exchange vector; Gm ^R , <i>colE1 ori</i> , <i>oriT</i> , <i>sacB</i>	(495)
pMMB67EH-Gm	Gm ^R ; IncQ, RSF1010, lacI _q P _{tac} expression vector	(496)
pDBS7	pEXG2 with the $\Delta ampR$ fragment, flanked by <i>EcoRI</i> and <i>BamHI</i> sites	This study
pDBS220	pCR2.1 TOPO containing the 944 bp <i>ampR</i> ORF, PCR amplified from the PAO1 genome using DBS_ <i>ampRF2</i> and DBS_ <i>ampRR2</i>	This study
pDBS271	pMMB67EH-Gm containing the <i>ampR</i> ORF, subcloned as an <i>EcoRI</i> - <i>SacI</i> fragment from pDBS220	This study

Construction of deletion mutants, complementation clones

An unmarked *ampR* null mutant of *P. aeruginosa* was generated by gene splicing (497) using primers KMampRUF1 and KMampRUR1 (to generate the upstream product P1, flanked by *EcoRI* and *NheI* sites), and primers KMampRDF1 and KMampRDR2 (to generate the downstream product P2, flanked by *NheI* and *BamHI* sites). After sequencing to ensure absence of mutations, P1 and P2 were spliced together to obtain a 1520 bp deletion fragment of *ampR* containing stop codons in all three reading frames at their junction (inserted as part of the *NheI* sites in the primer). This was then sequenced and subcloned into a *P. aeruginosa* non-replicative plasmid pEXG2 (495) as an *EcoRI*-*BamHI* fragment and moved into PAO1 by allelic exchange (498) using pRK600 (499) as the helper plasmid. Double crossover mutants were selected for the loss of plasmid (gentamycin-sensitive, sucrose counter-selection). The presence of deletion in PAO Δ *ampR* was confirmed using standard molecular methods (PCR and restriction analysis of amplicons) and biological assays (antibiotic sensitivity pattern and β -lactamase assays). Complementation of the *ampR* deletion was achieved by amplifying the *ampR* ORF along with the *ampR*-*ampC* intergenic region using primers DBS_*ampRF*2 and DBS_*ampRR*2 and cloning into pCR2.1 TOPO using TA cloning technique. After confirming absence of mutations by sequencing, the 944 bp *ampR* ORF was moved into pMMB67EH-Gm (496) as an *EcoRI*-*SacI* fragment. The plasmid was then moved into PAO Δ *ampR* by electroporation (500), selecting for gentamycin-resistant colonies.

***C. elegans* virulence assay**

The *P. aeruginosa* - *C. elegans* standard paralysis assay was modified from previous protocols (446). Overnight bacterial cultures were diluted 1:1000 and plated onto brain heart infusion agar plates. The plates were incubated for 18-24 hours at 37 °C for the formation of bacterial lawns. Meanwhile, a synchronized culture of L4 stage larvae hermaphrodite Bristol N2 *C. elegans* was washed off *E. coli* OP50-seeded nematode growth medium plates using M9 media. The nematodes were centrifuged at 1300 x g for two minutes and washed twice with M9 medium to remove residual *E. coli* bacteria. Thirty to 35 nematodes were then added to each of the *P. aeruginosa* bacterial lawns. Both live and paralyzed nematodes were scored at two-hour intervals for eight hours via microscopic observation. Nematodes were considered dead when they did not respond to physical stimuli. Each strain was tested in triplicate.

RNA isolation, generation of cDNA probes, microarray experiments and data analysis

P. aeruginosa strains PAO1 and PAO Δ ampR, with and without β -lactam antibiotic treatment were used for RNA extraction. The cells were subcultured at 37°C, 300 rpm from overnight cultures grown in LB broth to an OD600 of 0.01. When the cells reached an OD600 of 0.5-0.6, they were split into two flasks. One of the flasks was treated with sub-MIC concentration of penicillin (100 μ g/ml penicillin G Potassium salt, Fisher Bioreagents) for two hours. The RNA was stabilized by addition of phenol-ethanol mixture (390) prior to extraction (RNeasy Mini Kit, Qiagen) following manufacturer protocols. Residual DNA was removed using RQ1 RNase-free DNase (Promega) and the

RNA repurified using the kit. After quantification by measuring absorbance at 260nm (Beckman DU640, Beckman Coulter), and preliminary quality check on a denaturing agarose gel (NorthernMax Gly, Ambion), the RNA integrity was analyzed using RNA Nano chips in an Agilent Bioanalyzer 2100. cDNA was then synthesized by annealing NS5 random primers to total purified RNA, spiked with *Bacillus subtilis* internal control RNAs and subsequent extension carried out using SuperScript III reverse transcriptase (Invitrogen). Fragmentation of cDNA was performed using RQ1 DNase (Promega), and the fragments were biotin labeled. Hybridization with GeneChip *P. aeruginosa* genome array (Affymetrix) and scanning were performed according to manufacturer specifications.

Data analyses from three biological replicates for each of the conditions tested were performed after normalizing and summarizing probe level measurements using Guanine Cytosine - Robust Multiarray Average (GC-RMA). Only genes that fit stringent criteria (expression cutoff: 50-100% stringency; p -value ≤ 0.01 of one-way ANOVA data corrected by Benjamini Hochberg FDR; fold-change ≥ 2.0) were selected for further analysis. All microarray data analysis was performed using GeneSpring GX 10.0 Expression Analysis software (Silicon Genetics). The microarray data is MIAME compliant and has been deposited in NCBI GEO (accession number GSE33188).

Bioinformatics analysis of the AmpR-binding site

The promoters of AmpR-dependent and AmpR- β -lactam-dependent genes (listed in Appendix; Supplementary information for Chapter 4, Tables S3 and S4) were used to refine the AmpR binding motif using the Iterative Enhancement of Motifs (IEM)

algorithm (434). The AmpR binding site in the promoter of *ampC* (5'-TCTGCTCCAAATTT-3') was used to search the AmpR-dependent or AmpR- β -lactam-dependent promoters from *P. aeruginosa* PAO1 and their orthologs from *P. aeruginosa* strains PA14, PA2192, C3719 and PACS2. The output of IEM was a motif matrix. WebLogo (424) was used to graphically represent the multiple sequence alignment of the output. The Regulatory Sequence Analysis Matrix Scan Tool (RSA) (423) was then used to identify PAO1 promoter sequences containing the identified AmpR-dependent or AmpR- β -lactam-dependent motifs in the respective gene sets. The RSA output was then used to generate a WebLogo for the gene sets.

Quantitative real-time PCR

Specific genes that were significantly, over two-fold up or down regulated between *P. aeruginosa* PAO1 and PAO Δ *ampR* as seen in microarray experiments were verified by qPCR. Total RNA isolation and reverse transcription into cDNA was as described for the microarrays without addition of spike transcripts, fragmentation or labeling. For qPCR, the ABI Step One (Applied Biosystems) cyclers were used with PowerSYBR Green PCR MasterMix with ROX (Applied Biosystems). Expression was normalized to *clpX* (*PA1802*), whose expression was determined to remain constant between the samples and conditions tested. Assays were performed at least in biological triplicate, each with technical triplicates, for every gene analyzed. Melt curves were determined to ensure primer specificity. The cycling conditions used were 95 °C/ 2 minutes (holding); 40 cycles of 95 °C/ 15 sec, 60 °C/ 1 min (cycling); 95 °C/ 15 sec, 60 °C/ 1 min, 95 °C/ 15 sec (melt curve). Differential regulation of the T3SS genes,

including *rsmZ* was determined using RNA isolated from cells grown in MinS-NTA inducing media (494) from PAO1 and PAO Δ *ampR*, essentially as described above.

Gene expression in PAO Δ *ampR* were normalized to the corresponding PAO1 values, for both the β -lactam uninduced and induced conditions and is presented as relative expression in PAO Δ *ampR* $\pm$ standard error.

Determination of minimum inhibitory concentration (MIC)

MICs were determined one of two ways. For testing the MIC of β -lactams, E-test was used following manufacturer protocols (BioMerieux). The MICs of the MexEF-OprN efflux pump substrates (ofloxacin, chloramphenicol, ciprofloxacin, and trimethoprim) were determined by standard broth microdilution method (433). Briefly, serial two-fold dilutions of the antibiotics were incubated at 37 °C for 16-18 hrs with 5×10^5 CFU/ml of bacteria in a total volume of 200 μ l in 96-well flat bottom polystyrene plates. The highest dilution of antibiotic that prevented bacterial growth was considered as the MIC. The assays were performed at least in triplicate, each with technical triplicate, for each antibiotic in cation-adjusted Mueller Hinton broth.

Quantifying β -lactamase activity

β -lactamase activity was quantified as described previously (105). Briefly, cells in LB broth at an OD600 of 0.5-0.6 were treated with 100 μ g/ml Penicillin G for two hours at 37 °C. The cells were then harvested, OD normalized, and lysed with BugBuster Protein Extraction Reagent (Novagen) and r-Lysozyme (Novagen), and treated with Benzonase nuclease (Novagen). The amount of β -lactamase was quantified in the soluble fraction by determining hydrolyzing activity on nitrocefin (Oxoid, England). Protein

concentrations in the samples were determined by Bradford assay. Enzyme activity was expressed as milliunits of β -lactamase (nanomoles of nitrocefin hydrolyzed per minute per microgram of protein).

Biofilm assays

Time course biofilm assays were performed in 12x75mm round-bottom glass culture tubes (VWR) as described previously (453). Briefly, fresh overnight T-agar plate cultures of the test strains were scraped into 1 ml T-broth and diluted to a final OD₆₀₀ of 0.0025. Aliquots of 1 ml per tube were made at time zero and incubated static at room temperature for 24, 48, 72 and 96 hours. To assay for pellicle formation, the tubes were washed with running tap water five times, after discarding the cultures and stained with 1% crystal violet for 20 minutes. After pouring off the dye, the tubes were washed thoroughly with running tap water 10 to 15 times. Quantification of the attached and stained cells was done at 590 nm after solubilization of the dye with absolute ethanol.

Motility assays

Twitching and swimming assays were performed on 1% and 0.3% agar plates, respectively, as described previously (501).

Protease assays

LasA protease activity was measured by the ability of the strain supernatant to lyse boiled *Staphylococcus aureus* cells as described (502). Overnight culture supernatants of the test strains (100 μ l) were mixed with 900 μ l of a Tris-HCl (pH 8.5) suspension of

boiled *S. aureus* culture diluted to a final OD600 of 0.8. The lysis was monitored over an hour and LasA activity was expressed as the change in OD600 per hour per μg protein.

LasB elastolytic assay was performed with an elastin-congo red (ECR, Sigma) conjugate (503). The overnight culture supernatants (100 μl) were mixed with 900 μl of ECR buffer (100 mM Tris, 1 mM CaCl_2 , pH 7.5) containing 20 μg of ECR. Tubes were incubated shaking for one hour at 37 °C, and the supernatant was read at 495 nm. LasB activity was expressed as change in A495 per μg protein compared to an LB control.

Pyocyanin production

The amount of pyocyanin produced was determined by extracting the pigment from overnight King A culture supernatants. A 5 ml 18-hour supernatant was mixed with 3 ml of chloroform to extract pyocyanin into the chloroform phase. Pyocyanin was then extracted with 0.2 N HCl, the absorbance measured at 520 nm, and the pyocyanin concentration expressed as μg pyocyanin produced per μg of protein (504).

Phenotypic Microarray (PM)

PM profiles of *PAO Δ ampR* were compared to that of wild-type PAO1 in the absence of antibiotic stress to test the effect of *ampR* deletion. PM arrays (Biolog Inc., Hayward, CA, USA) comprise of about 2000 tests spanning 20 96-well plates and include ~800 tests for carbon, nitrogen, phosphorous and sulfur utilization, ~100 tests each for pH growth range and osmotic sensitivity, and ~1000 tests for chemical sensitivity. Suspensions of control and test strains, in duplicate, were prepared in inoculating fluid containing 0.01% tetrazolium violet and transferred to the PM plates. After incubation for 24 hours, growth differences between the strains were determined

from the kinetic response curves obtained by measuring changes in the color of the redox dye in each well in the OmniLog® incubator reader. Better growth of PAOΔ*ampR* in the presence of a specific test compound compared to PAO1 indicates gain of the phenotype (AmpR negatively regulates the phenotype) whereas poorer growth of the mutant compared to the wild type in a specific well indicates loss of phenotype due to *ampR* deletion (AmpR positively regulates the phenotype). OmniLog® PM software was used for data analysis.

Statistical analysis

All data were analyzed for statistical significance using *t*-test on GraphPad statistical analysis software, except for the microarray data, which was performed on GeneSpring GX 10.0 as mentioned earlier.

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Chapter 5

Deep Sequencing Expands the *Pseudomonas aeruginosa*

AmpR Regulon to Include Regulatory RNAs

Abstract

A Gram-negative opportunistic bacterium, *Pseudomonas aeruginosa* is a major human pathogen implicated in a number of acute and chronic infections. Of particular concern is the wide prevalence of *P. aeruginosa* in hospitals. Multiple virulence factors determine pathogenicity in *P. aeruginosa*. Virulence factor expression is a tightly regulated process and about 8-10% of the *P. aeruginosa* genome encodes transcriptional regulators. Some regulators control the expression of different pathogenic determinants in response to largely unidentified signals. Previously, using DNA microarrays we identified that AmpR, a transcriptional regulator of the LysR family and a positive regulator of the AmpC β -lactamase, also regulates other virulence phenotypes in *P. aeruginosa*. Specifically, AmpR turns on acute virulence factor production and represses chronic ones. Using RNA-Seq, this study further expands the regulatory role of *P. aeruginosa* AmpR. Further, quantitative real-time PCRs and phenotypic assays establish the role of AmpR in regulating diverse processes such as oxidative stress response, heat-shock response and iron uptake. The Gac-Rsm pathway is a major regulatory switch in determining expression of acute versus chronic virulence factors in *P. aeruginosa*. Gene expression studies demonstrate AmpR-dependent expression of the QS master regulator, LasR. ChIP-Seq studies with a chromosomally tagged AmpR show direct AmpR binding to the *lasR* promoter. Our studies establish the global regulatory role of *P. aeruginosa* AmpR in pathogenesis by contributing to regulation of quorum sensing and the quorum-sensing pathway. This is the first study that analyzes the response of *P. aeruginosa* to antibiotics using RNA-Seq and ChIP-Seq.

Introduction

Pseudomonas aeruginosa is a ubiquitous bacterium and can be isolated from diverse sources, including plants, animals and humans. A high degree of nutritional versatility and adaptability ensures that *P. aeruginosa* is able to colonize a wide range of natural and man-made habitats. In humans, *P. aeruginosa* is seldom part of the microbial flora and is found in less than 2% - 6% of individuals in the skin, throat and nasal mucosa (505). An opportunistic, nosocomial pathogen, *P. aeruginosa* colonization rates in hospitalized patients, however, can be over 50% and especially so in cases of mucosal or cutaneous breach, or in immunocompromised individuals (506). *P. aeruginosa* is also the leading cause of mortality in cystic fibrosis patients (84).

The pathogenic potential of *P. aeruginosa* is multifactorial and can be broadly classified into cell-associated and secreted virulence factors. The cell-associated virulence factors are typically structural components of the cell, such as the lipopolysaccharide (LPS), pili and flagella (507-509). The process of quorum sensing (QS) regulates expression of many of the major secreted virulence factors. QS is a mechanism of coordinating gene expression based on the population density, employed by both non-pathogenic and pathogenic bacteria (165). Quoromones (acyl homoserine lactones) are small diffusible molecules that mediate QS communication between cells to synchronize virulence gene expression (208). Precise signaling is ensured by the species-specific nature of quoromones, although crosstalk between related bacteria is known to occur (510). *P. aeruginosa* employs three interdependent mechanisms of QS namely the Las, Rhl and *Pseudomonas* quinolone system (PQS). The Las system is at the top of the regulatory hierarchy, above the Rhl system, while PQS interacts with both Las and Rhl

[reviewed in (511,512)]. In *P. aeruginosa*, the QS process controls production of secreted enzymes and toxins such as LasA, LasB and ToxA, redox-active compounds such as phenazines (512), and in the case of chronic infections, the formation of bacterial communities called biofilms (451). In addition, some efflux pumps, such as MexGHI-OpmD, which play a role in pumping out quorumones from the cytoplasm to the cell exterior, are also QS-regulated (440,513). A majority of the 12 putative and established RND efflux pumps in *P. aeruginosa*, however, are involved in antibiotic resistance (79,514).

Antibiotic resistance is a major problem in dealing with *P. aeruginosa* infections. The current treatment regimen for *P. aeruginosa* is typically a combination therapy of β -lactams, aminoglycosides and quinolones (84,515). However, a six-year survey by the National Nosocomial Infections Surveillance System of the CDC revealed that *P. aeruginosa* isolates were resistant to many commonly used antibiotics in both ICU and non-ICU patients (516). The infection rates with antibiotic-resistant *P. aeruginosa* were as high as 36% (516).

P. aeruginosa has multiple mechanisms of antibiotic resistance (79). Resistance to the β -lactam class of antibiotics is primarily conferred by the chromosomally encoded β -lactamase AmpC, whereas the MexEF-OprN efflux pump mediates quinolone resistance (79,427). Our recent study demonstrated that the LysR-type transcriptional regulator AmpR that positively regulates *ampC* expression, also regulates the MexEF-OprN efflux pump (225). In addition, *P. aeruginosa* AmpR has a global regulatory effect on gene regulation and is a regulator of many virulence determinants and transcriptional regulators (225,425). Using DNA microarrays and complementary assays, we have

demonstrated that the AmpR regulon consists of over 500 genes, involved in virulence and metabolism (225). AmpR thus activates expression of many acute virulence phenotypes while chronic infection phenotypes were repressed. Interestingly, the AmpR regulon included the small regulatory RNA rgRsmZ (225). Given the extensive nature of the AmpR regulon, we hypothesized that other small regulatory RNAs could also be AmpR-regulated. The microarray platform, however, is not designed to detect small RNAs effectively. Moreover, given the limited sensitivity of microarrays, genes that were not identified in the study could also potentially be AmpR-regulated. In addition, proteomic analyses identified targets that were not identified in the microarray studies (unpublished data). This prompted use of RNA-Seq and ChIP-Seq to identify some non-coding RNAs to be part of the AmpR regulon and determine direct targets of AmpR regulation. Furthermore, we assign a role for AmpR in previously unidentified critical cellular processes such as iron uptake, and in response to oxidative stress and heat shock. This study establishes AmpR as a critical regulator of *P. aeruginosa* virulence and physiology.

Results

RNA-Seq analysis expands the AmpR regulon

In order to study the function of AmpR in *P. aeruginosa*, a clean in-frame deletion strain (PAO Δ *ampR*) was constructed in PAO1 in a previous study (225). PAO1 and its isogenic *ampR* mutant, PAO Δ *ampR* were used for gene expression profiling using RNA-Seq. In order to be able to compare results from the two studies, the experimental conditions for RNA-Seq was similar to the microarray experiments that were performed

earlier (225). PAO1 and PAO Δ *ampR* were subjected to sub-MIC β -lactam stress before RNA isolation and cDNA synthesis as described in the methods section.

Using RNA-Seq, the transcription profiles of PAO1 and PAO Δ *ampR* were compared in the presence (induced) and absence (uninduced) of sub-MIC β -lactam stress. After data normalization between the replicates under each condition, the expression values across the entire genome for PAO Δ *ampR* was normalized to PAO1. The PAO Δ *ampR* data in the uninduced (Panel A, Figure 5.1) and induced (Panel B, Figure 5.1) were plotted. The expression profiles were markedly different in PAO Δ *ampR* compared to PAO1, both in the absence and presence of antibiotic exposure, attesting to the global regulatory role of AmpR in *P. aeruginosa*. As expected, the β -lactamase *ampC*, which is under positive AmpR regulation (105,225), is significantly downregulated under β -lactam stress in PAO Δ *ampR* (Panel B, Figure 5.1). Expression of genes identified in a previous study to be positively regulated by AmpR such as the elastase *lasB* (225) were also differentially regulated here (Panels A, B; Figure 5.1). The MexEF-OprN efflux pump that provides resistance against fluoroquinolones and chloramphenicol is negatively regulated by AmpR [Panels A, B; Figure 5.1, (225)]. We had demonstrated AmpR to be a positive regulator of the QS system and in agreement, genes such as *pqsA* and *rhlA*, which are key players in QS-mediated gene regulation, are downregulated in the *ampR* mutant (Panel A, Figure 5.1). These findings, in addition to others discussed in the following sections, add credence to the current study.

In addition to the genes that were identified in the microarray, the normalized data identified two transcriptional regulators (PA1015 and PA2588) and one ECF sigma factor

Figure 5.1: Gene expression in *PAOΔampR*. Relative gene expression in *PAOΔampR* compared to *PAO1* (normalized to one), based on RNA-Seq data, is shown in the absence (top panel) and presence (bottom panel) of sub-MIC β -lactam stress. Some significantly regulated genes are named. Gene annotations are from the *Pseudomonas* Genome Database (27).

(FemI, PA1912) to be differentially regulated (Panel A, Figure 5.1). PA2588 is a putative transcriptional regulator of the AraC family (27). Interestingly, it is located downstream of *pqsH*, which is an important component of the PQS system (380). The role of AmpR in regulating the PQS system is discussed in a later section. The ECF sigma factor FemI, upregulated in PAO Δ *ampR*, is part of a two-gene operon with *femR* (PA1911). FemR, along with FemA (PA1910), are involved in uptake of the mycobacterial siderophore mycobactin (517). Differential regulation of *femI*, along with the gene encoding the pyochelin receptor *fptA* (Panel B in Figure 5.1), suggests a role for AmpR in iron uptake regulation and is further explored in the later sections of this study. In addition, non-coding RNAs such as *P36*, *PA2744.1* and *PA3133.1* are also differentially regulated (Figure 5.1).

Identification of AmpR- and AmpR- β -lactam-dependent gene sets

The RNA-Seq data was normalized and pairwise comparisons were performed to determine differential gene expression (fold change ≥ 2.0 , Bonferroni correction of *p*-value ≤ 0.05). The four pairwise comparisons performed were PAO1 uninduced vs. PAO1 induced (Condition I), PAO Δ *ampR* uninduced vs. PAO Δ *ampR* induced (Condition II), PAO1 uninduced vs. PAO Δ *ampR* uninduced (Condition III), and PAO1 induced vs. PAO Δ *ampR* induced (Condition IV). This led to the identification of a total of 2568 genes that were differentially expressed across all four pair-wise comparisons: Condition I- 384 genes, Condition II- 672 genes, Condition III- 532 genes, and Condition IV- 980 genes. Although these numbers are indicative, they are not a true measure of the AmpR

Figure 5.2: Venn diagram of differentially regulated genes. Distribution of significantly (p -value ≤ 0.01) regulated genes (≥ 2.0-fold) in PAO1 and PAO Δ ampR without (uninduced) and with (induced) sub-MIC β -lactam stress.

regulatory repertoire due to potential overlaps between the conditions, i.e., individual genes could be differentially expressed under more than one condition. In order to address this issue, the pair-wise comparison data was plotted using a four-way Venn diagram (Figure 5.2). Of the 2568 genes that were identified in the original pair-wise analysis under all four conditions, 865 genes were found to be redundant. From Figure 5.2, 1703 non-redundant genes were identified across the four conditions, representing 31% of the PAO1 genome. Further, genes that were dependent on AmpR, AmpR- β -lactam, and β -lactam were identified in accordance with the expression criteria listed in the Appendix (Supplementary information for Chapter 5, Table 1). For example, genes in category B (Figure 5.2) are regulated in an AmpR-dependent manner, independent of β -lactam stress.

Twenty-four genes (category O) were omitted from further analysis since they were differentially regulated under all four pair-wise analysis conditions, irrespective of AmpR and/or antibiotic stress. A further 56 genes could not be assigned unambiguously for analysis [category H (44 genes), category K (1 gene), category M (4 genes), category N (7 genes)] and were omitted. Thus, of the remaining 1623 genes, we identified a total of 654 AmpR-dependent genes (Appendix; Supplementary information for Chapter 5, Table 2), 483 genes that were AmpR-dependent only under β -lactam stress (Appendix; Supplementary information for Chapter 5, Table 3), and 486 genes that are differentially expressed in response to β -lactam stress independent of AmpR (Appendix; Supplementary information for Chapter 5, Table 4).

AmpR-regulated genes are enriched in specific functional categories

The positively and negatively AmpR-regulated genes, both in the presence and absence of sub-MIC β -lactam exposure, were functionally categorized based on the *Pseudomonas* Genome Database [(27), Figure 5.3]. The AmpR-regulated genes were expressed as a percentage of the distribution of each functional category in the PAO1 genome (taken as 100%). This revealed a gross upregulation of genes related to phage, transposon or plasmid (45% genes of category 'v' genes in AmpR-positive regulated gene set; Figure 5.3). Functional class 'v' was shown to be positively regulated by AmpR in a previous transcriptome study as well (225). Similarly, genes of functional class 'n' (secreted factors- toxins, enzymes, alginate) were also shown to be under AmpR positive regulation (46% of genes of the category 'n' genes in the AmpR-positive regulated gene set; Figure 5.3). We have previously demonstrated that *ampR* mutants are impaired in production of extracellular enzymes such as LasA and LasB, and some of the type III secretion genes (225). Similarly, genes belonging to functional categories 'k' (adaptation and protection; 19%), 'zA' (antibiotic resistance and susceptibility; 10%) and 'q' (central intermediary metabolism; 11%) were also positively regulated by AmpR (Figure 5.3). This finding agrees with our previous observations from DNA microarrays and phenotypic microarrays that AmpR is an important regulator of antibiotic resistance, cell wall recycling enzymes and metabolism in *P. aeruginosa* (225).

Figure 5.3: Functional categorization of AmpR-regulated genes. AmpR differentially regulated genes are functionally categorized and expressed as a percentage of the respective category in the PAO1 genome (27). The functional categories are (a) DNA replication, recombination, modification and repair; (b) fatty acid and phospholipid metabolism; (c) hypothetical; (d) membrane proteins; (e) amino acid biosynthesis, metabolism; (f) translation, post-translational modification, degradation; (g) cell wall/LPS/capsule; (h) transport of small molecules; (i) energy metabolism; (j) biosynthesis of cofactors, prosthetic groups, carriers; (k) adaptation, protection; (l) transcriptional regulators; (m) two-component regulatory systems; (n) secreted factors- toxins, enzymes, alginate; (o) putative enzymes; (p) chaperones, heat-shock proteins; (q) central intermediary metabolism; (r) nucleotide biosynthesis and metabolism; (s) carbon compound catabolism; (t) motility and attachment; (u) chemotaxis; (v) related to phage, transposon, plasmid; (w) non-coding RNA genes; (x) protein secretion, export apparatus; (y) cell division; (zA) antibiotic resistance, susceptibility; (zB) transcription, RNA processing, degradation.

The AmpR negatively regulated genes are involved in translation (category f - 17%), energy metabolism (category i - 12%), nucleotide metabolism (category r - 11%), chaperones and heat shock (category p - 13%), and cell division (category y - 13%). Genes involved in motility and attachment (category t - 10%) are positively regulated by AmpR under β -lactam stress. Similarly, AmpR negatively regulates genes involved in chemotaxis under β -lactam stress (Figure 5.3).

To determine whether the functional categorization in the different gene sets is significant, enrichment analysis was performed using GOEAST (518). Primarily, gene ontology identifications (GOIDs) belonging to biological processes and cellular components were enriched in the AmpR positively regulated and negatively regulated gene sets, respectively (Appendix; Supplementary information for Chapter 5, Table 5). GOIDs belonging to ribosomal protein biosynthesis and oxidative phosphorylation were statistically significantly enriched in the AmpR-dependent negatively regulated gene set [log odd ratio >1.5; p -value <0.05; (Appendix; Supplementary information for Chapter 5, Table 5)]. There was no enrichment in the AmpR- β -lactam-dependent gene sets.

In the AmpR-positively regulated gene set, many genes involved in pyoverdine biosynthesis were significantly enriched [log odd ratio >2.5, *p*-value <0.03; (Appendix; Supplementary information for Chapter 5, Table 5)]. Also enriched in the same gene set are the genes encoding the major catalase KatA, and the superoxide dismutase SodA. The physiological effects of enrichment of these genes on iron acquisition and oxidative stress response, and the role of AmpR in their regulation are discussed in later sections. Furthermore, genes such as the QS regulator *rhlR*, the stress phase sigma factor *rpoS*, which were identified to be AmpR-regulated previously (225), also showed significant enrichment (Appendix; Supplementary information for Chapter 5, Table 5).

Regulation of small RNAs (sRNAs) by AmpR

RNA-Seq allows detection of expression profiles of sRNAs. Previous microarray studies with PAOΔ*ampR* showed dysregulation of the small regulatory RNA rgRsmZ (225). This led us to hypothesize that other sRNAs may also be AmpR-regulated but were not detected in the microarray studies due to technical limitations. RNA-Seq analysis of the *ampR* mutant revealed dysregulation of many non-coding RNAs (ncRNAs), both in the absence and presence of sub-MIC β-lactam stress (Appendix; Supplementary information for Chapter 5, Tables 2, 3). Some of these were tRNAs, which is expected given their abundance. Downregulated ncRNAs in PAOΔ*ampR* (AmpR positive regulation) include *PA1030.1* (*P8*; uninduced -30-fold, *p*-value 2.14E-06; induced -92-fold, *p*-value 1.03E-14), and *prfF1* (*PA4704.1*; uninduced -4-fold, *p*-value 0.00E+00; induced NS), whereas expression of *P7* (*PA0887.1*; uninduced 6.3-fold, *p*-value 0.00E+00; induced 3.1-fold, *p*-value 5.35E-14) and *amiL* (*PA3366.1*; uninduced

2.5-fold, p -value 1.82E-13; induced NS) were upregulated (AmpR negative regulation). Interestingly, rgRsmZ, which was detected in the microarray analysis, was not detected in RNA-Seq but dysregulation was confirmed by qPCR (discussed in the following sections).

AmpR mediated regulation of some of the sRNAs was determined by qPCR. AmpR was found to positively regulate expression of *PA5181.1* (*P34*; RQ: uninduced 0.39 ± 0.06 , p -value 0.0003; induced 0.38 ± 0.016 , p -value 0.002). Expression of *P34* requires RpoS (519). Since AmpR positively regulates RpoS (225), AmpR regulation of *P34* is likely via RpoS. Positive regulation of *P32* (*PA4758.1*) by AmpR and its physiological effects on the AmpR mutant strain are discussed in the section on heat-shock response. Further, AmpR was also found to positively regulate the antisense RNA asPrrF1 (Appendix; Supplementary information for Chapter 5, Table 2) and is discussed further in the following section.

AmpR regulates iron uptake positively

Iron is critical in many biological reactions across kingdoms and *P. aeruginosa* is no exception. However, freely available iron is in a poorly soluble and biologically unusable ferric (Fe^{3+}) form at neutral pH in aerobic conditions (107). In order to circumvent this issue, *P. aeruginosa* has evolved high affinity iron uptake systems mediated by siderophores. Siderophores are iron chelators that bind extracellular iron and transport it to receptors on the cell surface [reviewed in (107)]. *P. aeruginosa* can produce and take up heme- or iron-siderophore complexes (520). In addition, *P. aeruginosa* also synthesizes outer membrane receptors for siderophores produced by

other bacteria including pyoverdines produced by other pseudomonads (521), aerobactin (522) and enterobactin (522,523). *P. aeruginosa* produces two main types of siderophores, pyoverdine and pyochelin (107). Pyoverdine is the green-yellow fluorescent pigment that is produced typically under conditions of iron limitation (524). The *pvd* genes encode pyoverdine and are clustered (*PA2385-PA2426*) on the PAO1 genome (27). RNA-Seq analysis of the *ampR* mutant revealed downregulation (3-fold to 103-fold) of many *pvd* genes (Appendix; Supplementary information for Chapter 5, Table 2). This includes *pvdS* (*PA2426*), the ECF sigma factor (uninduced -7.6, *p*-value 3.49E-09, induced -38.2, *p*-value 1.93E-08) that is known to regulate expression of the *pvd* genes (525). Genes encoding the second siderophore system, pyochelin, are part of a gene cluster (*PA4220-PA4231*) and consist of three operons (*PA4220-PA4221*, *PA4222-PA4226*, *PA4228-PA4231*). Genes of all these operons (*PA4224-PA4226*, *PA4228-PA4231*) are significantly downregulated (14-fold to 193-fold) in *PAOΔampR* in a β-lactam-independent manner (Appendix; Supplementary information for Chapter 5, Table 2). This is also reflected in the GOEAST analysis of the AmpR-positively regulated gene set revealing a significant enrichment (log odds ratio ≥ 1.0 , *p*-value ≤ 0.05) of the *pvd* genes (Appendix; Supplementary information for Chapter 5, Table 5). These findings suggest that AmpR is potentially involved in iron uptake.

Further, comparison of AmpR-regulated genes from this study with the iron-related genes identified as part of a previous transcriptome metaanalysis study (400) revealed overlaps (Figure 5.4A). Genes involved in pyochelin biosynthesis are part of the 19 genes that are shared between the iron-regulated gene set and AmpR-positively regulated genes (Figure 5.4A). The overlapping genes also include *prpL*, a PvdS-

regulated protease that has been implicated in virulence (526) and *pfeR* encoding the transcriptional regulator of the PfeSR two-component system that positively regulates the enterobactin outer membrane receptor [PfeA; (527)]. Moreover, asPrrF1, which is a positive regulator of iron uptake, is also downregulated in PAO Δ *ampR* in the RNA-Seq (uninduced: 4-fold, *p*-value <0.001; induced: NS) and qPCR (uninduced: 0.47 ± 0.04 , *p*-value 0.004; induced: NS) assays. However, the master repressor of iron uptake Fur (143) is not significantly differentially regulated in the *ampR* RNA-Seq analyses. Downregulation of genes involved in siderophore biosynthesis, and comparison with previous metaanalysis studies led us to hypothesize that AmpR plays a positive regulatory role in iron uptake.

To further study the function of AmpR in iron uptake, growth curves were performed in iron-limited synthetic succinate media [SSM, (528)]. Deletion of AmpR resulted in impaired growth in SSM compared to PAO1 (orange lines; Figure 5.4B). Maximum growth difference between the strains was seen in the log and early stationary phases (*p*-value <0.0001 at all time points between 4 and 14 hours) of growth (orange lines; Figure 5.4B). The reduced growth of PAO Δ *ampR* in iron-deficient media can be a result of impairment in either uptake or utilization of iron. To address this question, growth curves were performed with exogenously added FeCl₃, making the media iron-replete (blue lines; Figure 5.4B). Addition of iron to the media enhances the growth rate of both PAO1 and PAO Δ *ampR* (blue lines; Figure 5.4B), compared to growth in SSM without exogenously added iron (orange lines; Figure 5.4B). Moreover, under iron-replete conditions, growth of the two strains is very similar till about 10 hours after which the *ampR* mutant again shows significantly reduced growth (blue lines; Figure 5.4B).

Figure 5.4: AmpR regulates iron uptake. (A) Comparing the AmpR positively and negatively regulated genes against the iron-regulated gene set shows overlaps between the datasets. (B) Growth in iron-limited media of PAO1 (solid lines) and PAO Δ *ampR* (dashed lines) in the absence (orange lines) and presence (blue lines) of exogenously added FeCl₃.

Thus, in the presence of excess iron in the log phase, PAO Δ *ampR* shows no growth deficiency.

This observation, and the transcriptome data, strongly suggests that AmpR plays a role in iron uptake, and not iron utilization. The growth impairment seen under iron-replete conditions with PAO Δ *ampR* between 10 and 16 hours is significant (blue lines, Figure 5.4B; p -value ≤ 0.0003 at all points). This is possibly due to the fact that PAO Δ *ampR* uses up all the freely available iron to maintain growth rates similar to PAO1 for the first 10 hours of the experiment. When conditions start to become iron limiting (after 10 hours, Figure 5.4B), continued growth of PAO Δ *ampR* is hampered due to impaired iron uptake. Moreover, under iron-replete conditions, neither strain produced pyoverdine, seen visually as a lack of yellow-green color of the cultures (data not shown), suggesting pyoverdine-independent iron uptake. *P. aeruginosa* also has an uncharacterized low affinity iron uptake system that functions under iron replete conditions (P. Cornelis, personal communication) and a citrate-mediated iron uptake system (529), potentially explaining growth.

In addition to the siderophore-mediated uptake, expression of the heme acquisition protein HasAp (PA3407) is downregulated in an AmpR- β -lactam-dependent manner [34.9-fold downregulated, p -value 8.5E-08; (Appendix; Supplementary information for Chapter 5, Table 3)]. This further attests to the role of AmpR in iron uptake in *P. aeruginosa*. The pH of the media is known to influence growth in SSM (528) but there was no difference in the pH of the media between the strains (data not shown). Thus, the gene expression and phenotypic data clearly indicate a positive regulatory role for AmpR in iron uptake in *P. aeruginosa*.

***P. aeruginosa* AmpR regulates heat-shock response by modulating rgP32 expression**

RNA-Seq analysis revealed that AmpR positively regulates the small RNA rgP32 [20.8-fold downregulated in *PAOΔampR*, *p*-value 7.77E-14; (Appendix; Supplementary information for Chapter 5, Table 2)]. P32 is the last gene of a three-gene operon with *dnaJ* and *dapB* (27). DnaJ is part of the Hsp70 heat-shock response system (530). The DnaJ-DnaK-GrpE (PA4760-PA4762) chaperone Hsp70 system prevents premature folding of nascent polypeptides and, along with the GroEL (Hsp60) system, helps in the heat-shock response in bacteria (530,531). DnaK is the *P. aeruginosa* homolog of *E. coli* Hsp70 (27). Conversion between the ATP- or ADP-bound forms of DnaK are controlled by DnaJ and GrpE, which function as a co-chaperone and a nucleotide exchange factor, respectively (532). Given that the small RNA is part of the operon encoding for the Hsp70 system, we hypothesized differential regulation of the Hsp70 heat shock system in *PAOΔampR*. qPCR analysis showed that the expression of *grpE* (RQ: uninduced 0.51 ± 0.004 , *p*-value 0.0017; induced 0.38 ± 0.003 , *p*-value 0.0003), *dnaJ* (RQ: uninduced 0.65 ± 0.045 , *p*-value 0.015; induced 0.6 ± 0.005 , *p*-value 0.0004), and *dnaK* (RQ: uninduced 0.45 ± 0.02 , *p*-value 0.0019; induced 0.62 ± 0.007 , *p*-value 0.0028) are downregulated in *PAOΔampR* (Figure 5.5A). The positive regulation is independent of sub-MIC β -lactam in the system (Figure 5.5A). This suggests that the cell wall intermediate products, shown previously to be probable AmpR effector molecules in inducing β -lactam resistance (121,410), play no role in AmpR-mediated regulation of Hsp70-encoding genes.

Downregulation of the Hsp70 heat-shock system genes in the *ampR* mutant led us to hypothesize that, compared to PAO1, *PAOΔampR* would behave differently at higher temperatures. However, growth curves of the *ampR* mutant were not different from

PAO1 at 30°C, 37°C or 43°C (data not shown). The heat tolerance of the two strains was then examined by enumerating CFUs after exposure of both log- and stationary-phase cells to 50°C, inducing the cellular heat-shock response. There was no significant difference between the input cells and the control cells (grown at 30°C) for either the log or stationary phases. However, PAO Δ *ampR* log-phase cells were more susceptible compared to PAO1, with >90% of cells killed after three hours of 50°C exposure (*p*-value 0.0018; Figure 5.5B). The stationary phase cells also followed a similar trend as the log phase cells. Loss of *ampR* led to a 99.9% loss in cell viability compared to PAO1 when stationary phase cells were exposed to 50°C for one hour (*p*-value 0.0014; Figure 5.5B).

The stationary phase cells are more sensitive to the elevated temperature compared to the log phase cells, and show a 2-log greater drop in CFU even after a brief exposure (Figure 5.5 B). This is counterintuitive since stationary phase cells are thought to be more resistant to changing conditions compared to log phase cells. Downregulation of the stationary phase sigma factor RpoS in PAO Δ *ampR* (225) potentially plays a role in the enhanced sensitivity of PAO Δ *ampR* cells. Regulation of the heat-shock sigma factor RpoH was also not significantly different between the strains in the RNA-Seq analysis (data not shown). This, however, is not surprising since expression of transcriptional regulators are typically not highly differentially regulated to achieve effective function. Dysregulation of the *hsp70* genes, confirmed by reduced temperature tolerance of PAO Δ *ampR*, suggests a positive regulatory role for AmpR in the heat-shock response of *P. aeruginosa*.

Figure 5.5: Regulation of heat-shock response by AmpR. (A) qPCR of *hsp70* genes: RNA was isolated from PAO1 and PAOΔampR cells, without and with sub-MIC β-lactam stress, reverse transcribed to cDNA, and tested by qPCR with gene-specific primers, as described in the text. Relative gene expression in PAOΔampR is shown without (grey bars) and with (black bars) sub-MIC β-lactam exposure. Values have been normalized to expression in PAO1 under the same conditions (log10 RQ=1) and bars above and below the threshold represent up- and down-regulation, respectively. (B) CFU counts of heat-shock exposed and unexposed cells in the log and stationary growth phases: Cells grown at 30°C were OD600 normalized, split into two aliquots and maintained at 30°C and 50°C for varying periods of time (3 hours for log phase, 1 hour for stationary phase) before enumeration. Data of PAO1 (grey bars) and PAOΔampR (black bars) are represented. *p*-values * <0.02, ** < 0.003, *** <0.0005.

AmpR positively regulates *P. aeruginosa* oxidative stress response

Hydrogen peroxide (H₂O₂) is a byproduct of O₂ metabolism whose deleterious effects on cells include altered membrane potential (533) and DNA mutation caused by single-stranded nicks (534). Intracellular H₂O₂ detoxification is achieved by the enzyme catalase and *P. aeruginosa* has four homologs: KatA (PA4236), KatB (PA4613), KatE (PA2147) and KatN [PA2185; (535)]. Of these, KatA is the major catalase and is expressed in all stages of cell growth but is produced more in the stationary phase (535). RNA-Seq analysis of PAOΔ*ampR* revealed downregulation of *katA* expression (-2.1-fold) compared to PAO1 in the absence of antibiotic stress, suggesting AmpR-dependent expression (Appendix; Supplementary information for Chapter 5, Table 2). Differential expression of *katA* was validated using qPCR (RQ uninduced: 0.12 ± 0.01 , *p*-value 0.0012). The small RNA rgRgsA (*PA2958.1*), which requires GacA and RpoS for its expression, contributes to hydrogen peroxide resistance (536). Expression of rgRgsA is downregulated over 2-fold in PAOΔ*ampR* (Appendix; Supplementary information for Chapter 5, Table 2), indicating positive AmpR regulation.

Previous meta-analysis studies of *P. aeruginosa* transcriptomes led to the identification of genes that were specifically differentially regulated under oxidative stress conditions (400). The differential regulation of *katA* and rgRgsA in PAOΔ*ampR* prompted comparison of the AmpR-regulated genes with the oxidative stress gene set. Seventy genes were shared between the two conditions, 48 and 22 of which are positively and negatively regulated by AmpR, respectively (Figure 5.6A). The 48 positively regulated genes include the major *P. aeruginosa* catalase *katA*, and four genes involved in PQS signal biogenesis [*pqsA* (*PA0996*), *pqsE* (*PA1000*), *phnA* (*PA1001*) and *phnB*

(*PA1002*)]. Interestingly, 22 of the 48 genes are clustered in a single locus on the genome that is involved in the production of R- and F-type pyocins (460,537), and are located in RGP03 and RGP04 (33). These genes were also identified in a previous transcriptome study to be AmpR-regulated (225). Most of these 22 AmpR-downregulated genes that are shared with the oxidative stress gene set are involved in metabolism including six of the *nuo* genes, which synthesize components of NADH dehydrogenase I (538). Another member of the LTTR family of transcriptional regulators, OxyR regulates *katA* expression in response to oxidative stress (539). Although *ampR* deletion in PAO1 did not affect *oxyR* expression in the RNA-Seq analysis (data not shown), qPCR analysis revealed that AmpR positively regulates *oxyR* expression (RQ uninduced: 0.31 ± 0.012 , *p*-value 0.001).

To determine whether reduced expression of *katA* and other oxidative stress-response genes translates into an observable phenotype, the H₂O₂ sensitivity of PAO1 and PAO Δ *ampR* was compared using the gradient plate method (540). PAO Δ *ampR* demonstrates a concentration-dependent reduced growth compared to PAO1 on the H₂O₂ gradient (Figure 5.6B), suggesting an impaired resistance to oxidative stress. This finding is in agreement with downregulation of oxidative stress response genes in PAO Δ *ampR*.

Thus, the transcriptomic and phenotypic data demonstrate a role for AmpR in positively regulating oxidative stress response in *P. aeruginosa*.

Figure 5.6: AmpR regulates resistance to oxidative stress. (A) Comparing the AmpR positively and negatively regulated genes against the oxidative stress gene set shows overlaps between the datasets. Overlapping genes include those identified previously to play a role in the oxidative stress response. (B) Gradient plates demonstrate decreased resistance of PAO Δ *ampR* to H₂O₂, compared to the control plate without H₂O₂. Representative data from four independent experiments is shown.

AmpR regulates phenazine production by modulating expression of *phzA1-G1* and *phzA2-G2* operons

The PAO Δ *ampR* strain is impaired in producing pyocyanin (225). *P. aeruginosa* PAO1 and PA14 have two redundant operons *phzA1-G1* (PA4210-PA4216; *phz1* operon) and *phzA2-G2* (PA1899-PA1905; *phz2* operon) that are involved in biosynthesis of the phenazine precursor, phenazine-1-carboxylic acid (27). This precursor is then sequentially modified by a methyltransferase (PhzM, PA4209) and a monooxygenase (PhzS, PA4217) to form pyocyanin (541). All the genes involved in pyocyanin biosynthesis and export (*phz1* and *phz2* operons, *phzM*, *phzS*, *mexGHI-opmD*) was significantly downregulated in the *ampR* mutant (positive AmpR regulation) in the RNA-Seq analysis [Figure 5.7A; (Appendix; Supplementary information for Chapter 5, Table 2)]. Positive AmpR regulation of *phzA1*, *phzA2* (the first genes of *phz* operons), *phzH* and *phzM* was also confirmed by qPCR (Figure 5.7B). The GOIDs under which *phzM* and *phzS* are classified (antibiotic metabolic processes, antibiotic biosynthetic processes, drug metabolic processes) also showed functional enrichment [log-odd ratio 3.46, *p*-value 0.03; (Appendix; Supplementary information for Chapter 5, Table 5)]. Recently, it has been demonstrated that, in *P. aeruginosa* PA14, the *phz1* operon is expressed in liquid cultures whereas the *phz2* operon is responsible for phenazine production in solid cultures (541). It is therefore interesting to note that in PAO Δ *ampR*, both operons are downregulated in liquid cultures, indicating positive AmpR regulation. The findings are possibly attributable to strain differences but are worth further investigation.

Figure 5.7: Regulation of phenazine genes by AmpR. (A) Genes involved in phenazine biosynthesis are significantly downregulated in PAOΔ*ampR* as seen in RNA-Seq analysis. (B) Differential regulation of the first genes of the phenazine biosynthetic operons *phzA1* and *phzA2*, and the modifying enzymes *phzH* and *phzM* were validated by qPCR.

The *phz1* operon is known to be QS regulated while the regulators of the *phz2* operon are not known (388,541). Downregulation of the *phz2* operon in PAO Δ *ampR* suggests a potential positive regulatory role for AmpR of this operon and is a novel finding.

The PQS system is also positively regulated by AmpR

Of the 654 AmpR-dependent genes, 313 and 341 genes are positively and negatively regulated, respectively. Genes that are positively regulated include the QS-regulated genes *lasA*, *lasB*, *rhlAB* and *rhlR* (Appendix; Supplementary information for Chapter 5, Table 2). In addition, AmpR also positively regulates the *hcnABC* operon, which is QS-regulated and is responsible for hydrogen cyanide biosynthesis (373). These concur with our previous findings that AmpR is a positive regulator of some QS phenotypes (225) and positive regulation of *lasR* and *rhlR* expression by AmpR was confirmed by qPCR (Figure 5.8). Furthermore, even under sub-MIC β -lactam stress, AmpR positively regulated expression of *lasR* (RQ induced: 0.84 ± 0.03 , *p*-value 0.001) and *rhlR* (RQ induced: 0.71 ± 0.007 , *p*-value 0.0001).

The PQS system is a critical part of QS signaling in *P. aeruginosa*, and complements the *las* and *rhl* systems (361,542). Genes of the two operons *pqsABCDE* (PA0996-PA1000) and *phnAB* (PA1001-PA1002), and *pqsH* (PA2587) are involved in PQS biosynthesis (543). PhnAB converts the PQS precursor chorismate to anthralinate, which is further converted to the signaling molecule PQS by PqsA-D and PqsH (380,543). The RNA-Seq data shows that AmpR positively regulates all these genes except for *pqsH* (Appendix; Supplementary information for Chapter 5, Table 2).

Figure 5.8: AmpR positively regulates critical QS regulators of the Las, Rhl and PQS systems. Expression of the genes was determined by qPCR. The expression levels in *PAOΔampR* are shown, normalized to expression in *PAO1*. *p*-values * <0.02, ** < 0.003, *** <0.0005.

We validated the RNA-Seq data using qPCR and demonstrate positive regulation of the first gene of the *pqs* operon (*pqsA*), in addition to *pqsH* (Figure 5.8). MvfR (PA1003) lies downstream of the *pqs* genes and positively regulates genes in this cluster (224,544). qPCR analysis reveals that AmpR positively regulates *mvfR* expression (Figure 5.8). Thus, it is likely that AmpR-mediated regulation of the PQS system in *P. aeruginosa* is via MvfR.

In addition, QscR (PA1898), which is a QS regulator in *P. aeruginosa* (280) and is in the same locus as and upstream of the *phzA2* operon, was also downregulated in the *ampR* mutant, as determined by qPCR (RQ: uninduced- 0.38 ± 0.17 , *p*-value 0.0057). This further supports the role of AmpR as a QS regulator.

V5-tagged AmpR is functional *in vivo*

The previous and current study suggests that the AmpR regulon in *P. aeruginosa* is extensive (225,425). However, it is highly unlikely that all the genes are under direct AmpR regulation. AmpR possibly indirectly controls a subset of genes. Accordingly, we identified transcriptional regulators in the AmpR regulon that could be potential targets of AmpR direct regulation [Appendix; Supplementary information for Chapter 5, Tables 2, 3, (225)]. Moreover, using the putative AmpR binding site (434), *in silico* analysis of the *P. aeruginosa* PAO1 genome identified potential genes that have an AmpR-binding site in their promoter (225). Some of these targets were confirmed to be differentially regulated using DNA microarrays (225). However, direct AmpR targets have not been demonstrated as yet.

In order to identify the direct targets of AmpR, ChIP-Seq studies were performed using a 3x-V5-tagged AmpR integrated as a single copy on the chromosome. Since AmpR has a positive regulatory role in β -lactam resistance, the functionality of the tagged AmpR was verified by determining the MIC. Amoxicillin had an MIC of 4 μ g/ml for PAO Δ *ampR* whereas the wild-type PAO1 is resistant (>256 μ g/ml). The MIC of amoxicillin on PAO Δ *ampR*::*ampR*-V5 was >256 μ g/ml, similar to PAO1 indicating that tagging AmpR at the C-terminus did not inhibit function. Chromatin immunoprecipitation was then performed both in the presence and absence of sub-MIC β -lactam stress. Before proceeding with the high-throughput sequencing, validity of the pulldown was tested by qPCR for P_{ampC} . AmpR occupancy data for the *ampC* promoter revealed that compared to the input DNA, the ChIP DNA showed a 17.1 ± 1.2 -fold and 21.5 ± 4.3 -fold higher occupancy, in the absence and presence of β -lactam stress, respectively. This demonstrated that the tagged AmpR protein was able to effectively bind the *ampC* promoter *in vivo*. Thus, the MIC and ChIP-qPCR studies confirmed *in vivo* functionality of the V5-tagged AmpR protein.

Identifying direct AmpR targets by ChIP-Seq

After confirming functionality of the tagged AmpR protein *in vivo*, ChIP and input DNA samples were processed on the Helicos sequencer. Data analysis was performed on the CLC Genomics Workbench as described in the methods section. The target regions on the PAO1 genome that AmpR binds to are shown in Table 1. All the loci in the table have Wilcoxon *p*-values < 3.0E-05 and, thus, are significant. In agreement with the ChIP-qPCR data (previous section), ChIP-Seq data showed that

Table 5.1: AmpR ChIP peaks. ChIP-Seq studies were performed on *P. aeruginosa* PAOΔ*ampR* strain harboring V5-tagged AmpR in the absence (uninduced) and presence (induced) of sub-MIC β-lactam stress. Regions on the chromosome that were enriched in the ChIP DNA samples compared to the input DNA are shown. All readings have a Wilcoxon Filter *p*-value $\leq 3.0\text{E-}05$.

Uninduced

Chromosomal Locus	Average Read Length	% Reads mapping to locus	Strand	FDR (%)	Flanking Genes	
					5'	3'
586888-586930	42	88	+	2.0E-01	<i>rsmY</i>	<i>PA0528</i>
797294-797374	80	53	+	7.5E-01	<i>PA0728</i>	<i>PA0729</i>
901840-901853	13	95	-	1.9E-01	<i>PA0826</i>	<i>ssrA</i>
1668963-1669003	40	95	+	1.0E+00	<i>ffs</i>	<i>PA1531</i>
1921430-1921543	113	95	+	1.2E+00	<i>oprF</i>	<i>cobA</i>
3123393-3123411	18	83	-	5.3E-02	<i>PA2763</i>	<i>PA2764</i>
4057616-4057641	25	89	-	1.6E-09	<i>fdxA</i>	<i>rsmZ</i>
4592895-4593005	110	97	-	1.3E-04	<i>PA4108</i>	<i>ampR</i>
4362457-4362649	192	88	+	1.6E+00	<i>PA4140</i>	<i>PA4141</i>
4782725-4783036	311	89	-	3.4E+00	<i>rplA</i>	<i>rplK</i>
4956459-4956671	212	91	-	1.9E+00	<i>rnpB</i>	<i>PA4422</i>
5387789-5387840	51	95	-	6.8E-02	<i>PA4802</i>	<i>PA4802.1</i>
5884393-5884467	74	94	+	1.3E+00	<i>ssrS</i>	<i>PA5228</i>
5986032- 5986133	101	90	-	1.7E+00	<i>rpmG</i>	<i>rpmB</i>
6183549-6183590	41	88	-	2.6E+00	<i>PA5492</i>	<i>polA</i>

Induced

Chromosomal Locus	Average Read Length	% Reads mapping to locus	Strand	FDR (%)	Flanking Genes	
					5'	3'
586884-586929	45	96	+	2.3E-03	<i>rsmY</i>	<i>PA0528</i>
901794-901852	58	92	-	3.1E-03	<i>PA0826</i>	<i>ssrA</i>
1668962-1669008	46	98	+	6.3E-02	<i>ffs</i>	<i>PA1531</i>
3206872-3207029	157	96	+	1.0E-02	<i>PA2852.1</i>	<i>oprI</i>
4057616-4057641	25	88	-	3.2E-05	<i>fdxA</i>	<i>rsmZ</i>
4592896-4593007	111	73	-	2.5E-03	<i>PA4108</i>	<i>ampR</i>
4956491-4956668	177	91	-	1.9E-03	<i>rnpB</i>	<i>PA4422</i>
5308608-5308852	244	93	+	3.8E-02	<i>crcZ</i>	<i>PA4726.2</i>
5387787-5387840	53	94	-	7.2E-02	<i>PA4802</i>	<i>PA4802.1</i>
5884382-5884467	85	93	+	3.7E-01	<i>ssrS</i>	<i>PA5228</i>
6183549-6183593	44	90	-	1.3E+00	<i>PA5492</i>	<i>polA</i>

AmpR binds to promoter DNA upstream of *ampC* under both induced and uninduced conditions (Table 1). This is typical of LysR-type transcriptional regulators, which bind their target sequences irrespective of effector binding (103,104) and is also seen in the *P_{ampC}* ChIP-qPCR (previous section).

The region with the least % false discovery rate (FDR) value, irrespective of inducer presence, is within *rgRsmZ* (*PA3621.1*). The locus that was pulled down in ChIP is on the negative strand (Table 5.1) and corresponds to a 25 bp region within the *rsmZ* gene. AmpR-dependent regulation of the *rsmZ* gene was also seen previously in transcriptome studies using microarrays (225) and in the current RNA-Seq analysis (Appendix; Supplementary information for Chapter 5, Table 2). The rgRNAs *rsmY* and *rsmZ* are thought to be functionally redundant (545) and play a major role in the acute to chronic lifestyle transition of *P. aeruginosa* (209,210,390). Transcription of *rsmY* and *rsmZ* is repressed by NarL [PA3879, (546)], CafA [PA4477, (547)], and MvaT/U [PA4315, (390)] and is activated by GacA [PA2586, (548,549)].

In an attempt to understand the role of AmpR this process, qPCR assays were performed with the other players of this well-established regulatory cascade (Figure 5.9). Two hybrid sensor kinases LadS (PA3974) and RetS (PA4856) have a positive and negative effect, respectively on the sensor kinase GacS (209,210). GacS activates transcription of *rgRsmZ* and *rgRsmY* indirectly through GacA (390). The rgRNAs *rsmY* and *rsmZ* sequester the RNA-binding protein RsmA (PA0905), which plays a key role in regulating over 500 genes, controlling the acute versus chronic lifestyle transition (458). qPCR analysis revealed that in the *ampR* mutant, expression of *ladS* (RQ: uninduced 0.35 ± 0.018 , p -value <0.0001 ; induced 0.42 ± 0.012 , p -value <0.0001), and *rgRsmZ* (RQ:

Figure 5.9: Quantitative PCR analysis of Gac-Rsm pathway genes. Relative expression of genes of the RetS-LadS-GacSA-Rsm pathway in PAOΔ*ampR* compared to PAO1 was analyzed in the absence (grey bars) and presence (black bars) of sub-MIC b-lactam stress. Gene expression in the *ampR* mutant has been normalized to the corresponding condition in the wild-type strain and expressed as relative numbers of gene-specific transcripts. *p*-values: * ≤ 0.02 , *** < 0.0001 .

uninduced 0.43 ± 0.059 , *p*-value = 0.01; induced 0.71 ± 0.006 , *p*-value = 0.02) was downregulated, indicating that AmpR is required for their expression (Figure 5.9). No dysregulation of *retS*, *gacS* or *gacA* was observed (Figure 5.9). Our previous microarray studies (225), and our current RNA-Seq, qPCR and ChIP-Seq studies show that AmpR activates transcription of rgRsmZ. Furthermore, *P. aeruginosa* AmpR is a positive regulator of acute virulence factors, many of which were QS-regulated, while negatively regulating chronic infection phenotypes such as biofilm formation (225). The current ChIP-Seq data seems to suggest that this regulation by AmpR is mediated by the regulatory RNA rgRsmZ.

Binding site analysis

The ChIP data was used to identify the AmpR binding site. A region of about 200 bp upstream of the genes that were identified to be AmpR-bound (Table 5.1) was used as input for regulatory sequence analysis tools [RSAT; rsat.ulb.ac.be; (423)]. These regions were queried using the putative AmpR-binding site identified earlier (434) and the matrix derived as part of genome-wide analysis of AmpR-binding (225). The AmpR binding sites that were identified in the promoter regions of the AmpR-regulated genes were then used as input to generate a WebLogo under both uninduced and induced conditions (Figure 5.10). The DNA motif that AmpR seems to bind, both in the presence and absence of effectors, is almost identical except for minor changes at positions 1, 2, 3 and 5 (Panels A and B, Figure 5.10). The AmpR motif, like the LTTR box (104), is AT-rich and the bases that are critical for AmpR-binding seems to be A's and T's (at positions 1, 6, 9, 10, 13 and 14; Figure 5.10).

Figure 5.10: ChIP-Seq data based AmpR binding site analysis. Promoters of the genes that were identified by ChIP-Seq to be AmpR-regulated, either in the absence or presence of β -lactam stress, were scanned for the presence of the putative AmpR-binding motif using RSAT. The binding sites upstream of each gene were then used as input to generate a WebLogo for the uninduced (top panel) and induced (bottom panel). p -value 0.001

LasR is a direct target of AmpR

The regulator LasR is at the top of the QS regulatory hierarchy in *P. aeruginosa* (363,364,370). Other regulators, in addition to LasR, regulate the Rhl and PQS systems [reviewed in (511)]. Our findings have identified that AmpR is one of these regulators [Figure 5.8; (225)]. One of these regulators is AmpR, which has been demonstrated previously to positively regulate the Las and Rhl systems transcriptionally (PQS section above) and phenotypically (225). ChIP-Seq data suggested that AmpR regulates the QS master regulator LasR directly (Table 5.1), and the presence of a putative AmpR binding motif upstream of the LasR ORF strengthens the finding. In order to validate the ChIP-Seq data, ChIP-qPCR was performed for selected targets using the V5-tagged AmpR strain.

As expected, the *ampC* promoter showed 79-fold enrichment in the ChIP DNA compared to the control DNA, demonstrating strong AmpR binding. AmpR pulldown of P_{ampC} was also confirmed using a VSVG-tagged AmpR (data not shown), and agrees with previous observations that AmpR is a positive regulator of *ampC* expression by direct binding to P_{ampC} (550). ChIP-qPCR data also showed that the *lasR* promoter showed 3-fold enrichment compared to the input DNA indicating direct binding of AmpR to P_{lasR} . This binding, though not as strong as AmpR binding at P_{ampC} , is significant. Transcriptome and phenotypic data show that even at this reduced binding AmpR is able to bring about significant changes in the QS system [Figure 5.8, (225)].

Comparison of microarray, RNA-Seq and proteome data

In order to get a comprehensive picture of AmpR regulation in *P. aeruginosa*, we have performed transcriptomics studies, using DNA microarrays (225) and RNA-Seq (this study), and proteomics analysis (unpublished data). The transcriptomics and proteomics studies were performed using the same two strains (PAO1 and PAO Δ *ampR*), under identical conditions (without and with sub-MIC β -lactam stress). Comparing the microarray and RNA-Seq datasets revealed little overlap in the number of genes regulated (Figure 5.11). This seems contrary to previous findings that demonstrate a better overlap between data from the two techniques (551-553). However, several genes that were identified in the microarray studies to be AmpR-regulated (225) were also identified in the RNA-Seq study. Examples have been discussed in the previous sections and include both positively regulated (numerous QS-regulated genes, *ampC*) and negatively regulated (*mexEF-oprN*, alginate regulators) genes. Thus, though the total number of genes showed poor overlap between the transcriptome assays, the phenotypes regulated are the same. Further, similar to previous studies with other systems (554,555), there is a poor overlap between the AmpR transcriptome and proteome studies (Figure 5.11). This can be attributed to a variety of reasons that may be technical (sample preparation, sensitivities of the techniques) or functional (post-transcriptional and/or post-translational regulation). For example, in *Desulfovibrio vulgaricans*, variations between mRNA and protein levels were attributed to differences in expression (34-44%), stability of protein (5%) and mRNA (2%) (556). Codon usage, amino acid composition, and the Shine-Dalgarno sequence contributed to an additional 15-26% variation (555).

Figure 5.11: Comparative analyses of AmpR microarray, RNA-Seq and proteome datasets. The AmpR positive (A), AmpR-negative (B), AmpR- β -lactam positive (C) and AmpR- β -lactam negative (D) gene sets from our previous microarray (225) and the proteome (unpublished) studies were compared with the RNA-Seq data from the current study.

With the AmpR-positively regulated gene set (Figure 5.11A), genes of the type VI secretion system (*PA1657*, *PA1662*, *PA1664*), the hydrogen cyanide biosynthetic operon *hcnABC* (*PA2193-PA2195*), and the membrane fusion component of the MexGHI-OpmD efflux pump (MexH, PA4206) are positively regulated in both the microarray and RNA-Seq datasets. In agreement with our previous phenotypic assays (225), LasA (PA1871), a QS-regulated protease is also differentially expressed at the protein level (Figure 5.11A). PopD (PA1709), which along with PopB (PA1708) forms the translocation pore on the eukaryotic cell surface for injection of T3SS effectors (557), is AmpR-positively regulated in both the microarray and proteome analyses.

Using an insertion mutant of *ampR* and transcriptional fusions, we have previously demonstrated crosstalk between AmpR and the alginate master regulator AlgT/U, where AmpR negatively regulates AlgT/U, which in turn positively regulates *ampR* expression (425). In addition, AmpR also negatively regulates the anti-anti-sigma factor MucB (PA0764), as seen in both microarray and RNA-Seq analyses (Figure 5.11 B). The genes encoding AlgT/U and MucB are part of the same operon (*algT/U-mucA-mucB-mucC-mucD*) and the apparent contradiction in AmpR-mediated regulation of *algT/U* and *mucB* is most likely resolved at the posttranscriptional level. Genes of the *mexEF-oprN* efflux pump are also downregulated in both the transcriptome analyses (Figure 5.11B), and concurs with data from MIC studies (225). The positive regulator of pyocin production PrtN (PA0610) was identified in both the microarray (AmpR negatively regulated) and proteome studies (AmpR negatively regulated under β -lactam stress; Panels B, D, Figure 5.11). This concurs with the upregulation of about 39 genes of the pyocin cluster PAO Δ *ampR* in the microarray analysis (225).

AmpR negatively regulates PA4378 in the microarray, and RNA-Seq analyses. PA4378 is annotated as InaA that has a 61% similarity to the pH-inducible protein InaA in *E. coli* (27). It is part of a three-gene operon (PA4377-PA4379) and both the array and RNA-Seq data show that in addition to PA4378, PA4379 is also upregulated (AmpR negative regulation). In *P. aeruginosa* PA7, the gene PA4378 is annotated as a lipopolysaccharide kinase (27). BLASTP analysis reveals that the PA4378 has homologs in other species of *Pseudomonas* in addition to strains of *P. aeruginosa* (data not shown) and the operon is part of the core genome in *P. aeruginosa* strains (33). InaA has also been identified previously in *Erwinia ananas* to be involved in ice nucleation (558). In *E. coli*, *inaA* expression is regulated by SoxRS, the superoxide stress response system, and *marRAB* the multiple antibiotic resistance (Mar) operon (559). Downregulation of *inaA* expression by AmpR in both transcriptome studies, along with positive regulation of oxidative stress (Figure 5.6), suggests that AmpR might regulate *inaA* and oxidative stress via SoxRS. However, there was no differential regulation of *soxRS* in the transcriptome studies.

Discussion

In the opportunistic human pathogen *P. aeruginosa*, gene expression is a tightly controlled process and many regulators act in concert to control virulence traits (511,512). *In silico* analyses and empirical evidence have identified critical regulators such as Vfr, the *P. aeruginosa* homolog of the *E. coli* cAMP receptor protein (CRP), to be central to *P. aeruginosa* pathogenicity (355,560-562). This study establishes the role of *P. aeruginosa* AmpR as not only a regulator of virulence factors but also important physiological processes such as response to oxidative stress and heat shock. AmpR positively regulates

the stress sigma factor RpoS and a growth phase-dependent expression of some RpoS-regulated virulence factors has been demonstrated in *PAOΔampR* (225). So, a subset of the AmpR-regulated genes is possibly regulated via RpoS. In addition, subtractive transcriptomics of the AmpR regulon identified AmpR-regulated genes that were not shared with other transcriptomes (225). RNA-Seq has a better coverage than microarrays in detecting transcript levels (563). Expression of some genes that were not identified in the microarrays was differentially regulated in the AmpR RNA-Seq study. In this study, we identified some sRNAs to be targets of AmpR regulation. Selected sRNAs were pursued further to establish a phenotypic difference arising due to AmpR-mediated regulation.

Iron acquisition is a critical determinant of *P. aeruginosa* pathogenicity and has been proposed as a potential target to counter infections (564). In the healthy lung, iron is typically bound by ferritin and transferrin, and free iron is available only in very low quantities (565). In the CF lung, however, higher iron concentration is a potential reason that allows bacteria such as *P. aeruginosa* to colonize (565). The primary mode of sequestration of extracellular iron in *P. aeruginosa* is by use of the siderophores pyoverdine and pyochelin produced under iron-limiting conditions (566,567). AmpR positively regulates iron uptake, as evidenced by a reduced expression of both siderophore gene expression and PQS in *PAOΔampR*, and growth impairment under iron-limited conditions (Figure 5.4). However, since iron is not a limiting factor in the CF lung, as evidenced by iron-rich CF sputa (565,568), AmpR is likely not important in iron uptake in this setting.

In the CF lung, *P. aeruginosa* turns mucoid by overproducing the extracellular polysaccharide alginate in response to the H₂O₂ released by polymorphonuclear leukocytes (569). It has also been established that the ECF sigma factor AlgT/U is the master regulator of alginate production by turning on expression of the *algD* operon (481). Thus, the enhanced sensitivity of PAOΔ*ampR* to H₂O₂ is interesting in the light of the fact that AmpR negatively regulates *algT/U* expression in response to unidentified signals (425). Taken together, these studies suggest that, on one hand, AmpR negatively regulates alginate production by repressing *algT/U* transcription, while on the other hand, is required for resistance against H₂O₂, which turns on alginate production. Thus, when the bacterium is in the CF lung, the role of AmpR in establishing chronic infections seems conflicting. One possible explanation for this seemingly contradictory data is the role of other regulators. Even though loss of AmpR leads to enhanced *algT/U* transcription, posttranslational control by the anti-sigma factor MucA prevents AlgT/U-mediated *algD* transcription (570). Moreover, gene regulation is a complex interlinked process in *P. aeruginosa* and multiple tiers of regulation for critical pathways is common (511,560,571).

Positive regulation of iron uptake, PQS and oxidative stress by AmpR seem to be disparate phenotypes but they are, in fact, interlinked [Figure 5.12, (511)]. The relationship between iron uptake and QS is complex and some transcriptional regulators involved in QS regulation also modulate iron response (168,572,573). MvfR is one such

Figure 5.12: AmpR-mediated regulation of virulence and physiological processes in *P. aeruginosa*. AmpR affects expression of QS genes by directly binding to P_{lasR} and modulating *lasR* expression. AmpR also positively regulates the PQS system by modulating levels of the antisense RNA *asPrrF1*, thus also affecting iron uptake. By regulating expression of the stationary phase sigma factor RpoS (225), *oxyR* and the small RNA *rgRgsA*, AmpR positively regulates the oxidative stress response. The genes encoding the Hsp70 heat-shock response system are also positively regulated by AmpR. This, and previous findings (225) demonstrate that AmpR is a major regulator of virulence and physiological processes in *P. aeruginosa*.

example. The iron-responsive sigma factor PvdS (PA2426) turns on *mvfR* transcription in response to iron starvation (144,574). Our data demonstrates that AmpR positively regulates *mvfR* expression (Figure 5.8). Furthermore, the antisense RNAs asPrrF1 and asPrrF2 positively regulate production of the signaling molecule PQS (575), which helps in iron chelation, facilitating the activity of pyoverdine and pyochelin (378,384,576). We show here that AmpR positively regulates asPrrF1, but not asPrrF2, in addition to the *pvd* and *pch* genes, thus affecting iron uptake and PQS synthesis. AmpR also positively regulates the stationary phase sigma factor RpoS and some RpoS-regulated genes such as the galactophilic lectins LecA and LecB (225). Since the link between QS and RpoS is established (366,368), AmpR regulation of both these processes is not surprising (Figure 5.12). RpoS also positively regulates expression of the sRNA *rgsA* (PA2958.1), which is also regulated by GacA, and contributes to hydrogen peroxide resistance in *P. aeruginosa* (536). AmpR also plays a role in this process, as demonstrated here (Figure 5.12). PAO Δ *ampR* shows dysregulation of not only *rpoS* and the sRNA *rgsA* but also OxyR, the major regulator of the catalase KatA that ultimately affects resistance to hydrogen peroxide (Figure 5.12).

CF lung isolates are known to be heterogeneous genotypically and phenotypically [reviewed in (577)]. Higher mutation rates are the driving force for the *P. aeruginosa* population heterogeneity in the CF lung (578-580). Specifically, mutations in *mucA* and *lasR* arise early in the colonization process, followed by mutations in anti-mutator genes such as *mutS*, *mutT*, *mutY* and *mutM* (581). MucA mutations trigger the regulated intramembrane proteolytic cascade, freeing AlgT/U and allowing for overexpression of alginate [reviewed in (582)]. Mutations in *lasR* would abolish expression of QS-regulated

acute virulence factors [reviewed in (583)]. In the CF lung, *P. aeruginosa* loses the ability to produce acute virulence phenotypes after initial colonization and starts to overexpress chronic infection traits (584). AmpR negatively regulates *algT/U* expression, which is a chronic infection phenotype (425). AmpR mutant strains display some characteristics reminiscent of CF isolates, including acquisition of fluoroquinolone resistance, reduced production of QS-regulated virulence factors such as proteases and pyocyanin, and enhanced biofilm formation [this study, (225)]. Furthermore, PAO Δ *ampR* RNA-Seq studies show upregulation of the genes *mutY* (PA5147; 2.4-fold, *p*-value 9.25E-06) and *mutM* (PA0357; 2.6-fold, *p*-value 5.28E-06) under β -lactam stress (Appendix; Supplementary information for Chapter 5, Table 3), indicating negative AmpR regulation. So, one would expect the mutation frequencies of PAO Δ *ampR* to be lower than PAO1, given that some anti-mutators are overexpressed. However, the mutation frequencies for rifampicin and streptomycin were not significantly different between PAO1 and PAO Δ *ampR* (data not shown). This seeming contradiction is not surprising since *mutY* and *mutM* are known to be weak anti-mutators in *P. aeruginosa* and potentially have a lower effect than strong anti-mutators such as MutS (581). The data suggests that inactivating *ampR* in the CF lung, in addition to other mutations, will help *P. aeruginosa* colonize better. However, the occurrence and frequency of *ampR* mutations in CF isolates needs to be determined.

Our previous and current analyses showed that AmpR positively regulates QS by modulating expression of major QS regulators such as LasR [Figure 5.8, (225)]. ChIP-Seq and complementary data demonstrate that AmpR directly binds to and activates *lasR* expression, thus modulating QS phenotypes. AmpR binding site analysis using the ChIP-

Seq data revealed a motif that was very similar to that identified in our previous studies using microarray studies (225). Comparatively however, the motif in Figure 5.10 appears to be more refined and is likely to resemble the AmpR binding site closely because it is based on the promoters of genes identified in the ChIP study. Minor motif variations from the consensus sequence are expected. For this reason, identifying consensus sequences for transcription factor binding motifs using data from multiple promoters is likely to be more accurate compared to footprinting studies that look at individual promoters.

In conclusion, the data presented here, and previously by our lab, demonstrate that *P. aeruginosa* AmpR is a critical component of not only virulence and metabolic regulation but also physiological processes. The clinical significance of AmpR is highlighted in a recent study, which demonstrated that constitutive AmpR-activating mutations were responsible for AmpC overproduction in extremely drug-resistant high-risk *P. aeruginosa* clinical isolates (585). Moreover, given the global effect that AmpR has on the regulatory repertoire of *P. aeruginosa*, it is likely that other virulence traits exhibited by high-risk clinical isolates (585) are also AmpR-mediated. Small molecule inhibitors targeting specific proteins such as *P. aeruginosa* NagZ (123) and *Vibrio cholera* LuxO (586) show therapeutic promise. Inhibitors of AmpR function will render the strain sensitive to β -lactam antibiotics and reduce the production of acute virulence factors. Thus, combination therapies using AmpR inhibitors and antibiotics will potentially provide us with means to counter *P. aeruginosa* infections, and warrants further investigation.

Materials and methods

Strains, media and culture conditions

The strains used in this study are listed in Table 5.2. The wild-type *P. aeruginosa* PAO1 and its isogenic in-frame *ampR* deletion strain, PAO Δ *ampR* used in this study are described earlier (25,225). These strains were used for the RNA-Seq study, with and without sub-MIC β -lactam stress, essentially as described previously (225).

For ChIP-Seq studies, AmpR was tagged at the 3' end with a 3x-V5 epitope tag. Briefly, the 3x-V5 epitope was PCR amplified from plasmid ZM474 (587) using primers DBS_V5F and DBS_V5R containing *KpnI* and *NheI* sites, respectively. The *NheI* site also contains termination codons in all three reading frames to prevent runoff transcription into the plasmid. The 3x-V5 amplicon was cloned into pCR2.1 TOPO (Invitrogen) to generate plasmid pDBS206 and sequenced to ensure absence of mutations. The *ampR* ORF with the native promoter but without the stop codon was PCR amplified using primers DBS_*ampR*F1 (with a *KpnI* site) and DBS_*ampR*R (with a *SacI* site), cloned into pCR 2.1 TOPO (pDBS215) and sequenced. The 1112-bp *ampR* ORF with the native promoter was then moved as a *KpnI*-*SacI* fragment from pDBS215 upstream of and in-frame with the 3x-V5 tag in pDBS206 to obtain plasmid pDBS222. The 3x V5-tagged *ampR* was then moved into mini-CTX2 [pDBS227; (588)] as a *KpnI*-*NheI* fragment, generating plasmid pDBS234. After confirmation by PCR and restriction digestion, the plasmid pDBS234 was moved into PAO Δ *ampR* by electroporation (500). This resulted in strain DBS248 that was used for the ChIP-Seq studies. Functionality of the tagged AmpR in DBS248 was verified by determining the MIC of the β -lactams ampicillin-sulbactam and amoxicillin, and by ChIP-qPCR.

Table 5.2: Strains and plasmids used in this study

Strain/ plasmid	Relevant characteristics	Source
Strains		
<i>Escherichia coli</i>		
DH5 α	General purpose cloning strain; $\Delta(lacZ)M15$	New England Biolabs
DBS206	<i>E. coli</i> DH5 α harboring 3x-V5 tag on pCR2.1 TOPO	This study
DBS215	DH5 α with <i>ampR</i> ORF and the <i>ampR-ampC</i> intergenic region PCR cloned into pCR2.1 TOPO	This study
DBS222	DH5 α harboring <i>ampR</i> ORF tagged with 3x V5-tag on pCR2.1 TOPO	This study
DBS234	DH5 α with mini CTX2, containing 3x V5-tagged <i>ampR</i>	This study
<i>Pseudomonas aeruginosa</i>		
PAO1	Wild-type	(25)
PKM315	PAO $\Delta ampR$; In-frame deletion of <i>ampR</i> (<i>PA4109</i>)	(225)
DBS248	PAO $\Delta ampR::ampR$ -V5; 3x-V5-tagged <i>ampR</i> cloned onto mini-CTX2 and moved into PKM315	This study
Plasmids		
pCR2.1 TOPO	TA cloning vector for PCR products; Ap ^R , Km ^R ; ColE1 fl ori <i>lacZ</i> α	Invitrogen
ZM747-V5	3x V5 tag C-term and N-term of yeast URA-3 in pBlueScript; pDBS193	(587)
Mini CTX2	Plasmid for single-copy gene integration in <i>P. aeruginosa</i> ; Tc ^R	(588)
pDBS206	3x V5 tag PCR amplified from ZM747-V5 and cloned into pCR2.1 TOPO	This study
pDBS215	1112 bp <i>ampR</i> ORF along with the <i>ampR-ampC</i> intergenic region, PCR amplified with primers DBS_ <i>ampR</i> F1 and DBS <i>ampR</i> ; cloned into pCR2.1 TOPO	This study
pDBS222	<i>ampR</i> ORF from pDBS215 subcloned as <i>KpnI-SacI</i> upstream of and inframe with 3x V5 tag in pDBS206	This study
pDBS234	3x V5-tagged <i>ampR</i> ORF from pDBS222 subcloned as a <i>KpnI-NotI</i> fragment into mini-CTX2	This study

All strains were grown in standard LB media with aeration, unless otherwise specified. Synthetic succinate medium (SSM) was used as the iron-limited media (528) and contained (g/l) K_2HPO_4 6.0, KH_2PO_4 3.0, $(NH_4)_2SO_4$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.2, sodium succinate 4.0, pH 7.0. Antibiotics were used at the following concentrations: for *E. coli* - gentamycin 15 µg/ml, tetracycline 15 µg/ml, ampicillin 100 µg/ml; for *P. aeruginosa* - gentamycin 75 µg/ml, tetracycline 60 µg/ml, carbenicillin 150 µg/ml.

Library preparation for RNA-Seq analysis

Total RNA was isolated from PAO1 and $PAO\Delta ampR$ with and without sub-MIC β -lactam stress as described previously (225) using the hot-phenol extraction protocol (589). RNA quality was analyzed on the Agilent Bioanalyzer and those with RNA Integrity Numbers of 8.0 or above was used for rRNA depletion using the MICROBExpress Kit (Ambion). After rRNA depletion, cDNA synthesis was performed using the SuperScript III First-Strand Synthesis System (Invitrogen) as per manufacturer protocols. Terminal transferase (New England Biolabs) and dATP (New England Biolabs) were used to poly-A tail the cDNA samples to obtain tail lengths of 100-300 bases following manufacturer instructions. The 3' ends were then blocked with biotinylated ddATP (Perkin Elmer) using terminal transferase (New England Biolabs), followed by cleanup with the MinElute cleanup kit (Qiagen). Tailed and blocked samples were then processed on the HeliScope Single Molecule Sequencer at the Molecular Biology Core Facility, Dana Farber Cancer Institute, Boston, MA.

ChIP-Seq sample preparation

For ChIP-Seq studies, the 3x-V5-tagged AmpR containing strain (DBS248) was grown and exposed to sub-MIC β -lactam stress as described previously (225). Protein-DNA interactions were then crosslinked *in vivo* with formaldehyde (final concentration of 1%) at room temperature for 20 minutes and quenched for 15 minutes with 0.25 M glycine. After three washes with 1X PBS, the cells were resuspended in 1 ml lysis buffer (10 mM Tris pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% deoxycholic acid and 0.5% N-laurylsarcosine) containing a protease inhibitor cocktail (Roche). After chilling on ice, the cells were sonicated to shear the DNA to a size range of 0.5-1 kb. Cellular debris was removed by centrifugation and a 3 μ l aliquot of the supernatant was checked on an agarose gel. A 50 μ l aliquot of the supernatant was stored as the input DNA and the rest was immunoprecipitated using DynaBeads Protein G (Life Technologies), which was previously equilibrated and bound with anti-V5 monoclonal antibody (Sigma) as per manufacturer instructions. After immunoprecipitation overnight, the beads were washed 5x with RIPA buffer (50 mM HEPES pH 7.5, 500 mM LiCl, 1 mM EDTA, 1% NP40, 0.7% deoxycholic acid, 50 mM NaCl in 1xTE). The beads were resuspended in 100 μ l elution buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS) at 65°C for 30 minutes, centrifuged to remove residual beads and incubated at 65°C overnight to reverse the crosslink. TE buffer was then added (100 μ l) and the samples were treated with RNase (37°C for 2 hours). The immunoprecipitated proteins were removed with proteinase K (55°C for 2 hours), and the DNA cleanup performed using the Qiagen Mini Reaction Cleanup kit. RNase and proteinase K treatment was also performed for the input DNA. DNA concentrations were determined using Quant-iT PicoGreen dsDNA Kit (Life

Technologies). Before proceeding further, AmpR occupancy of the *ampC* promoter was determined using qPCR as described previously (225).

The DNA samples were then poly-A tailed, blocked with biotinylated ddATP, purified and processed on the Helicos sequencer as described in the RNA-Seq section.

Data analysis for RNA-Seq and ChIP-Seq

The raw data files for both RNA-Seq and ChIP-Seq studies from the Helicos sequencing runs were converted to the FASTA format using Helisphere Open Source project. The sequence reads were then mapped to the *P. aeruginosa* PAO1 genome (NCBI reference sequence NC_002516.2) using CLC Genomics Workbench, version 5 (CLC Bio). For RNA-Seq analysis, replicates of each sample were merged and pairwise comparisons were performed to obtain comparative expression. The total number of reads per gene between samples was normalized using RPKM ([reads/kb gene]/[million reads aligning to the genome]). Bonferroni correction was performed and FDR *p*-values were calculated. For RNA-Seq, only genes with a proportions fold-change of ≥ 2.0 and with Bonferroni corrected *p*-value of ≤ 0.05 were considered for further analysis. For ChIP-Seq, in addition to calculating FDR, peaks were shifted based on read length and filtered using a maximum Wilcoxon *p*-value of $1.0E-04$.

Enrichment of functional categories

The gene sets that were positively and negatively regulated by AmpR without and with β -lactam stress were functionally categorized based on the *Pseudomonas* Genome Database (27). Gene distribution under individual categories in PAO1 was considered as 100% and the relative distributions in each of the four gene sets were plotted. Enrichment

of specific functional categories in the individual datasets were determined using GOEAST (518). The Log odds-ratio was calculated for the GOEAST enrichment. The bigger the value of the ratio, the higher the relative abundance of the GO term is compared to a random condition.

Quantitative real-time PCR (qPCR) assays

Genes that were differentially regulated in the RNA-Seq analysis, and those that had not been tested in our previous microarray analysis (225) were selected for qPCR confirmation. RNA isolation and cDNA synthesis were performed as described previously (225). Ten nanograms of cDNA were used per reaction well in the qPCR assays. Expression of the test genes was normalized to *clpX* (*PA1802*).

Hydrogen peroxide sensitivity

To determine differences in H₂O₂ sensitivities of PAO1 and PAOΔ*ampR*, gradient-plate assay was used (540). Briefly, 37 ml of LB agar held at 50°C was supplemented with 4-8 μM H₂O₂ and poured onto tilted 90 mm petri dishes. After solidification, 37 ml of LB agar without H₂O₂ was poured onto the plates on a flat surface to generate a gradient. OD600 normalized overnight LB cultures of the strains were then used to form a 75 mm streak across the gradient using a cotton swab. H₂O₂ sensitivity was scored as the extent of growth into the gradient, compared to the control (on LB plates without H₂O₂). The assay was performed in triplicate and representative results are shown.

Growth in iron-limited media

The differential growth abilities of the *ampR* mutant vis-à-vis the wild-type strain were determined on SSM (528). Input cells for the growth curve assays were grown overnight in SSM from fresh LB plates. For the assay, the OD₆₀₀ of the SSM cultures were normalized to 0.02 in SSM and the OD 600 of 200 µl aliquots in 96-well flat bottom tissue culture plates (Nunc) was monitored for 17 hours at 37 °C. Conditions were made iron-replete conditions by supplementing SSM with 100 µM FeCl₃ (528).

Temperature sensitivity assays

The ability of PAO1 and PAOΔ*ampR* to resist elevated temperatures was examined. Briefly, cells grown in LB broth to either the log (OD₆₀₀ 0.6-0.8) or stationary (OD₆₀₀ >2.0) phase at 30°C were exposed to 50°C for 1-3 hours. Control aliquots were maintained at 30°C as controls. Cell counts were determined for test and control samples before and after exposure by plating for CFUs.

Statistical analyses

All the data from qPCR and phenotypic assays were examined for statistical significance using the unpaired two-tailed *t*-test on GraphPad analysis software (www.graphpad.com). RNA-Seq and ChIP-Seq data were analyzed for significance on CLC Genomics Workbench as described above.

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CHAPTER 6

Discussion

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Metabolic versatility, intrinsic and acquired antibiotic resistance, biofilm formation, and production of multiple virulence factors make *P. aeruginosa* a formidable pathogen. The virulence machinery of *P. aeruginosa* is comprised of both cell-associated determinants (such as lipopolysaccharides, pili, flagella) and numerous secreted factors (such as elastases, proteases, exotoxins, pyocyanin, extracellular polysaccharides; Figure 6.1). One of the mechanisms by which *P. aeruginosa* senses external signals is using sensor proteins that, via phosphotransfer or phosphorelay, activate specific transcriptional regulators. These sensor-regulator protein pairs are called two-component systems (TCS). The *P. aeruginosa* PAO1 genome encodes about 127 TCS members, compared to 60 in *Escherichia coli* (590) and 70 in *Bacillus subtilis* (591), reflecting the adaptability of *P. aeruginosa*. TCS and their modifications also feed into major regulatory pathways and play a critical role in allowing cells to modulate gene expression in response to environmental conditions (213,592). Many of the secreted virulence factors and phenotypes such as biofilm formation are under the control of a cell density recognition mechanism called quorum sensing (QS) that aids in the coordinated expression of genes (208,451). QS is key to virulence gene expression in many bacteria and serves as an attractive target for antibacterial chemotherapy (593).

In humans, acute *P. aeruginosa* infections in specific sites such as the CF lung eventually lead to chronic infections. This is caused by adaptive modifications in the infecting clonal type resulting in diverse morphotypes (594). The transition to a chronic

Fig 6.1: *P. aeruginosa* virulence determinants. The major cell-associated and secreted virulence factors of *P. aeruginosa* are shown.

infection phase is the result of numerous changes in cellular physiology in response to external stimuli. The changes include downregulation of acute virulence genes with a concomitant upregulation of chronic infection phenotypes and antibiotic resistance, facilitating recalcitrant infections [reviewed in (595)]. Successful establishment of chronic infection (host invasion, establishment of acute infection and the subsequent transition to the chronic phase) involves tightly regulated expression of many genes associated with metabolism, virulence and antibiotic resistance. Several key players in these transition processes have been identified and include transcriptional and post-transcriptional regulators (209,210,225).

Gene regulation in *P. aeruginosa* is a complex process involving numerous transcriptional regulators, regulatory RNAs (rgRNA) and sigma factors. The *P. aeruginosa* genome is over 6 MB (25), approaching that of lower eukaryotes. The genome is plastic and has acquired genes and undergone extensive rearrangements in order to adapt to specific niches (33). The large genome of *P. aeruginosa* supports a multitude of regulatory networks with approximately 8% of the total genome dedicated to the regulatory proteins (27). *P. aeruginosa* PAO1 encodes 434 putative and identified transcriptional regulators, 24 putative sigma factors and 34 predicted small RNAs (25,519,560,596). Moreover, predicted regulatory networks indicate that there is an extensive crosstalk between the different transcriptional regulators (560,597). These networks, however, are based in part on *in silico* analyses and their validity needs to be established. Understanding the virulence regulatory networks in *P. aeruginosa* will provide a framework for future studies to better understand pathogenic processes in *P. aeruginosa* and in related bacteria.

***P. aeruginosa* AmpR is a major regulator of antibiotic resistance**

Antibiotic resistance is a major concern when dealing with *P. aeruginosa* infections in the hospitals. The current treatment regimen for *P. aeruginosa* infections is a combination therapy of aminoglycosides, quinolones and β -lactam antibiotics (84). The major chromosomally encoded β -lactamase that determines β -lactam resistance in *P. aeruginosa* is AmpC. Previous studies have established that mutations leading to loss of AmpR function render the strain sensitive to β -lactam antibiotics (105). Also, transcriptional fusions and mutation studies of P_{ampC} in *ampR* knockout strains have been used to demonstrate the positive regulatory role of AmpR at the *ampC* promoter [O.

Caille, unpublished data, (105)]. AmpR, a member of the LysR family of transcriptional regulators, is thus a critical player in determining resistance to the β -lactam class of antibiotics. AmpR also positively regulates MexR (Chapter 4), a positive regulator of the MexAB-OprM efflux pump, which contributes to β -lactam resistance. The CreBCD system is known to be involved in regulating β -lactam resistance (124,348) and AmpR regulates CreD in a CreB-independent manner (Chapter 4). Thus, AmpR regulates β -lactam resistance in more ways than one.

Furthermore, AmpR functions as a negative regulator of a second chromosomal β -lactamase, an oxacillinase termed PoxB, whose activity spectrum is limited primarily to carbapenems [D. Zincke, unpublished data; (106)]. The physiological significance of negative regulation of *poxB* β -lactamase expression by AmpR is not yet clear. EMSA studies suggest indirect regulation of *poxB* by AmpR (O. Caille, unpublished data), although it is possible that recognition and binding of P_{pox} by AmpR requires as yet unidentified signals. Interestingly, although PoxB is classified as an oxacillinase based on sequence homology with other oxacillinases, it demonstrates no significant activity against oxacillin (D. Zincke, personal communication).

In addition to regulating β -lactam resistance positively, AmpR also regulates quinolone resistance (Chapter 4). Gene expression and phenotypic assays indicate that AmpR negatively regulates transcription and ultimately function of the *mexEF-oprF* efflux system, by modulating expression of the positive regulator MexT (Chapter 4). Repression of *mexT* expression by AmpR is β -lactam independent (Chapter 4). It is interesting that AmpR positively and negatively regulates resistance to β -lactams and quinolones, respectively. Furthermore, phenotypic microarray data suggests that AmpR

influences resistance to many multiple antibiotics (Chapter 4), and is thus among the very few identified transcriptional regulators in *P. aeruginosa* that regulate resistance to different classes of antibiotics.

AmpR regulates quorum sensing in *P. aeruginosa*

Quorum sensing (QS) is a mechanism that bacterial cells use to gauge population densities that allow them to coordinate gene expression (165). Intercellular communication is particularly useful in the case of opportunistic pathogens to coordinate expression of virulence factors, and when the cells are part of microbial communities called biofilms (208). In *P. aeruginosa*, expression of many of the acute virulence factors is under QS regulation [reviewed in (598)]. Also, the decision to form biofilms in planktonic cultures is QS-influenced (599). Previous studies using an insertion mutant of *ampR* indicated that AmpR is a negative regulator of some QS-regulated virulence factors, such as the production of pyocyanin, and extracellular proteases such as LasA and LasB (105). However, using a clean in-frame deletion strain, gene expression and phenotypic studies, this dissertation shows that AmpR is really a positive regulator of QS-regulated acute virulence factors that are under Las-Rhl regulation (Chapter 4). The discrepancy between the insertion and deletion mutants is probably because in the insertion mutant, the AmpR HTH domain remains unaffected [Chapter 4; (106)]. Furthermore, RNA-Seq data also shows that AmpR positively regulates the PQS signaling system (Chapter 5). Thus, AmpR positively regulates all three the QS systems, and consequently, the associated virulence mechanisms in *P. aeruginosa*.

Clinical significance of AmpR

The infecting clonal types of *P. aeruginosa* undergo many changes at the site of infection in order to adapt and colonize, a process driven by mutations (577,600,601). Several recent studies have identified genes that are mutated in either clinical isolates of *P. aeruginosa* or in strains that have been subjected to CF-like growth conditions (600,602-604). Mechanisms that contribute to β -lactam resistance are also affected in the adaptation process including overexpression of efflux pumps, downregulation of the carbapenem porin OprD, and hyperproduction of AmpC (76,348,605). Some strains that have been implicated in outbreaks harbor more than one mutation resulting in multidrug-resistant (MDR) and extensively drug-resistant (XDR) clones (606,607). The incidence of MDR and XDR clones of *P. aeruginosa* in patients is on the rise and undermines treatment strategies (608,609). Some MDR/XDR clones that disseminate to multiple hospitals worldwide posing a grave threat to human health have been termed 'high-risk clones' (610). Three such clones ST235, ST111 and ST175 are more prevalent than the rest. A recent study analyzing 190 bloodstream isolates from 10 hospitals determined that about 57% of them were antibiotic-resistant, of which 33% of isolates were MDR/XDR (585). Genetic analysis of the molecular mechanisms contributing to enhanced resistance of the XDR clones revealed combinations of resistance to β -lactams (AmpC overproduction, inactivation of OprD), fluoroquinolone resistance (point mutations in GyrA), resistance to gentamycin and tobramycin (*aadB* gene acquired on a class I integron), and upregulation of aminoglycoside resistance (mutation in the *mexZ* repressor of the MexXY-OprM efflux pump) (585).

Specifically, in a majority of the most-prevalent ST175 high-risk XDR/MDR isolates analyzed, a novel mutation in AmpR (Glycine 154 Arginine) was the reason for constitutive activation of *ampC* expression (585). The G154R mutation was found only in strains that overproduce AmpC. In the sporadic XDR/MDR and moderately resistant strains, other *ampR* mutations were detected (E114A, G283E, M288R, A51T) but these polymorphisms are also found in wild-type strains such as PA14 (27,585). ComPLEMENTING an *ampR* deletion strain in *trans* with a plasmid harboring *ampR*-G154R enhanced *ampC* expression and resistance to ceftazidime (585). In the light of studies in dissertation that demonstrates AmpR to be a positive regulator of acute virulence factors and antibiotic resistance (Chapters 4, 5), it is very possible that AmpR overexpression contributes to the success of high-risk XDR clones such as ST175. This, however, remains to be examined.

In *C freundii*, AmpR becomes a constitutive activator of *ampC* expression due to amino acid substitutions R86C, G102E and D135N (611,612), of which only the D135N mutation has been found in a clinical isolate (92). Studies in our lab have demonstrated that mutating the aspartic acid residue at position 135 to asparagine (D135N) in *P. aeruginosa* AmpR locks it in the constitutively active conformation (O. Caille, unpublished data). However, the G102E mutation in *P. aeruginosa* AmpR seems to affect AmpR stability more than activity (O. Caille, unpublished data). These studies from the Mathee lab and elsewhere demonstrate that AmpR plays an important role in regulating antibiotic resistance in *P. aeruginosa*. Moreover, as demonstrated in Chapters 4 and 5, AmpR is also a critical regulator of *P. aeruginosa* virulence and metabolism.

AmpR is one of 434 transcriptional regulators identified in the PAO1 genome, many of which remain uncharacterized. With such a large complement of regulatory proteins and accessory metabolic genes, it is no surprise that *P. aeruginosa* is able to adapt and thrive in a wide range of habitats. Understanding how AmpR and other regulators orchestrate the virulence and metabolic processes in *P. aeruginosa* in response to external signals is critical to dealing with infections caused by this successful opportunistic pathogen.

Major virulence regulatory systems in *P. aeruginosa*

The experimentally established virulence regulatory network in *P. aeruginosa* is depicted in Figure 6.2. Our lab and others have previously performed *in silico* analyses of the *P. aeruginosa* transcriptional regulatory network (560,597,613). Comparing those networks with the network depicted in Figure 6.2 clearly demonstrates the gap in knowledge between predicted networks and established ones. An important contributing factor to this discrepancy is the fact that the functions of the majority of the genes in the PAO1 genome remain unknown. Deep sequencing, transcriptome metaanalysis (28,400), and complementary studies will aid in assigning functions to the hypothetical genes and undoubtedly narrow this knowledge gap.

Cis regulatory elements (CREs) form a critical part of transcription. CREs are non-coding DNA sequences present in or near a gene, and often contain binding sites for transcription factors and/or other regulators of transcription (614). The two major CREs are promoters and enhancers (615,616). The promoters contain the binding sites for transcription factors and other regulatory molecules such as sigma factors and regulatory RNAs (617-619). Enhancers, once thought to be part of only eukaryotes, are found

widely in prokaryotes also and function in conjunction with the σ^{54} -RNA polymerase (620-622). This section will focus on the transcriptional and post-transcriptional regulation of critical pathways that determine *P. aeruginosa* pathogenesis.

Quorum Sensing

QS is a signaling mechanism that bacteria use to regulate gene expression in a population density-dependent manner and was first demonstrated in *Vibrio fischeri* (623). In QS, the bacteria produce and secrete small molecules called autoinducers or quoromones. When these molecules reach a concentration threshold, they diffuse back into the cell to elicit a coordinated response promoting group survival (624). *P. aeruginosa* uses QS to regulate production of various virulence determinants such as extracellular proteases, iron chelators, efflux pump expression, biofilm formation, motility, and the response to host immune signals (625). This is achieved using two types of autoinducers, *N*-acyl-homoserine lactones (AHLs) and 2-alkyl-4 quinolones (AQs) (512).

AHL-mediated QS

P. aeruginosa has two canonical AHL QS signaling pathways, the *las* and *rhl* systems. Together, these pathways affect expression of approximately 10% of the *P. aeruginosa* transcriptome (170). The *lasI* (PA1432) and *rhlI* (PA3476) genes encode the *N*-3-oxododecanoylhomoserine lactone (3-oxo-C12-AHL) synthetase (359,626) and *N*-butyrylhomoserine lactone (C4-AHL) synthetase, respectively (360,362,627,628). The resulting AHLs then bind and activate their cognate LuxR family regulators, LasR

(PA1430) (359) or RhlR (PA3477) (362). LasR and RhlR multimerize in the presence of their cognate AHL (629,630). In *in vitro* studies, LasR-DNA interaction is cooperative and non-cooperative in the presence or absence of a dyad symmetry in the binding sites, respectively (631). Rhl-regulated promoters have binding sites with a dyad symmetry (366).

AQ-mediated QS

P. aeruginosa synthesizes two AQ QS signals, 2-heptyl-3-hydroxy-4-quinolone (PQS) and its precursor, 2-heptyl-4-quinolone (HHQ) (378). Both PQS and HHQ enhance *in vitro* binding of the LysR-type transcription regulator, MvfR (also known as PqsR, PA1003), to the promoter of the *pqsABCDE* operon (PA0996-PA1000), suggesting that they function as MvfR effectors (381). Microarray analysis identified 141 genes differentially expressed in a *mvfR* mutant strain, including *lasR*, *algT/U* (PA0762), *rsmA* (PA0905), and *rsaL* (PA1431) (224). PQS also acts independently of MvfR to induce expression of the Fur regulon through its ability to bind iron (378,384) and membrane vesicle formation by inducing membrane curvature (576,632). PmpR (PA0964), a YebC member, negatively regulates MvfR (Figure 6.2) (383).

QS regulation

The *las*, *rhl*, and PQS/HHQ/MvfR systems all exhibit positive feed-forward autoregulation (544,633). In addition, the *P. aeruginosa* AHL systems function in a hierarchical manner, as the 3-oxo-C12-AHL-LasR complex positively regulates *rhlI*, *rhlR* and *mvfR* expression as well as *lasI* (363,364,634). Exceptions to this have been noted.

RhlR has been shown to regulate LasR-dependent genes in strains lacking *lasR* (375), and timing of *lasI*, *lasR*, *rhlI* and *rhlR* expression can vary drastically depending upon growth conditions (376).

Many global regulators have been shown to modulate QS-dependent genes. RpoS (PA3622), the stationary phase sigma factor affects approximately 40% of the QS regulon (366,368). RpoS binding sites have been identified in several of the QS-dependent promoters. RpoS also affects *lasR* and *rhlR* expression and LasR binding sites have been identified in promoters of other transcriptional regulators in the QS regulon, including *PA2588*, *PA4778*, *pvdS* (*PA2426*), *vqsR* (*PA2591*) and *rsaL* (365). Chromatin immunoprecipitation studies have shown occupancy by histone-like silencers MvaT (PA4315) and MvaU (PA2667) on *lasI*, *lasR*, *mvfR*, *rpoS* and *rsaL* (635). RsaL plays an important role in *las* signaling homeostasis, by binding to the *lasI* promoter and preventing LasR-mediated activation (373). In addition to affecting gene expression through *las* regulation, microarray analyses indicate that RsaL affects expression of 130 genes, including direct regulation of pyocyanin and hydrogen cyanide genes (373). RsaL also appears to be important in regulating the transition from planktonic to a sessile state as *rsaL* mutants exhibit increased swarming motility and fail to form biofilms (636). RsaL expression is under the control of LysR-type regulator OxyR (PA5344) (637). The *lasI* promoter region has also been shown to be bound by CzcR (PA2523), which is required for expression of *rhlI* and *rhlR* in addition to *lasI* (Figure 6.2) (638). CzcR is part of the CzcRS TCS, shown to be involved in carbapenem and heavy metal resistance (639).

VqsR (PA2591), which is induced by H₂O₂ or human serum (282) and is under LasR regulation (640), regulates QS through inhibition of the LuxR-type regulator, QscR (PA1898, Figure 6.2) (641). Though QscR binds 3-oxo-C12-AHL, its specificity is not as stringent as LasR (642). The QscR regulon partially overlaps that ascribed to the *las* and *rhl* systems, but also has unique targets (385). In the absence of AHL, QscR can multimerize and form heterodimers with LasR and RhlR (643). QscR also plays a role in LasI homeostasis as mutations in *qscR* result in premature *lasI* expression (280). An AraC family member VqsM (PA2227) regulates VqsR in addition to numerous genes involved in QS including RsaL, PprB (PA4296), MvfR, RpoS as well as AlgT/U and MexR (PA0424) (644).

Additionally, *pqsH* (PA2587), which encodes the enzyme responsible for oxidation of HHQ to form PQS, is positively regulated by the *las* system (377,380) and negatively regulated by the *rhl* system (544). PQS is derived from anthranilate, which is synthesized by the kynurenine pathway (645). Kynurenine pathway anthranilate is also required for *N*-decanoyl-homoserine lactone (C10-AHL) dependent signaling that is independent of *las*, *rhl*, and *qscR* (645). The receptor for this signaling is yet to be identified (396). Besides potential heterodimerization with QscR, additional post-transcriptional regulation of QS has been described. In one such mechanism, QteE (PA2593) destabilizes LasR and RhlR and in the absence of *qteE* the quorum threshold-requirement for activation of QS-dependent genes is lost (646). RsmA negatively regulates *rhl* and *las* signaling, resulting in reduced AHL levels (190). Moreover, it has been shown the RNA chaperone Hfq (PA4944) positively regulates *rhlI* translation via *rsmY* and RsmA (647).

Recently, our lab has established a role for the β -lactamase regulator AmpR (PA4109) in activating QS-regulated genes [Chapter 4, (225)]. The production of QS-regulated secreted virulence factors such as LasA (PA1871) and LasB (PA3724) proteases, and pyocyanin production is significantly impaired in AmpR-deficient strains. Further, loss of *ampR* reduced virulence in the *Caenorhabditis elegans* toxicity assay [Chapter 4, (225)]. In addition, AmpR regulates non- β -lactam resistance by repressing activity of the MexEF-OprN (PA2493-PA2495) efflux pump, the alginate master regulator AlgT/U (226) and biofilm formation [Chapter 4, (225)], suggesting that it plays a role in maintaining the acute mode of infection.

Two component regulatory systems (TCSs)

TCS are sophisticated signaling mechanisms marked by a highly modular design that have been adapted and integrated into a wide variety of cellular signaling circuits. The archetypical TCS is composed of a membrane integrated sensory histidine kinase (HK) and a cytoplasmic response regulator (RR) (648). The HK contains a periplasmic N-terminal domain that detects specific stimuli (sensing domain) and a C-terminal cytoplasmic transmitter domain that is comprised of a dimerization domain, a conserved histidine, and an ATP catalytic domain (649). HKs can have two or more transmembrane domains with little or no periplasmic domain while others are completely cytoplasmic. The cognate response regulator (RR) contains a conserved receiver domain and a variable output domain (650).

Figure 6.2: The *P. aeruginosa* virulence regulatory network

The pathogenic potential of *P. aeruginosa* is dictated by multiple virulence systems that are regulated transcriptionally, post-transcriptionally and post-translationally. The central mechanism for *P. aeruginosa* virulence regulation is QS, which controls expression of many virulence factors in a population density-dependent manner. Key activators of this system are LasR, RhlR, MvfR, VqsR, the cAMP receptor protein Vfr, and the stationary phase sigma factor RpoS. Las system repressors include RsaL, the H-NS protein MvaT, the sigma factor RpoN, and the small RNA binding protein RsmA, while others like QscR repress both the Las and Rhl systems. Other regulators such as AmpR affect QS genes by an unknown mechanism. QS plays a role in regulating critical pathogenic mechanisms including biofilm formation, secretion systems, production of numerous virulence factors, efflux pumps, antibiotic resistance and motility.

Acute *P. aeruginosa* infections can lead to chronic infections in response to largely unidentified signals. A key regulatory pathway that controls this lifestyle switch is the RetS-LadS-GacSA-RsmA pathway. RetS and LadS are hybrid sensor proteins that, in response to external signals, either activate or repress the GacSA TCS. The GacA regulator then activates transcription of two rgRNAs, rgRsmZ and rgRsmY that sequester and inhibit activity of the small RNA binding protein, RsmA. RsmA is a key activator/repressor that post-transcriptionally regulates numerous acute and chronic infection phenotypes including multiple QS-regulated virulence factors, biofilm formation, type 2, type 3 and type 6 secretion systems, and motility.

Another major phenotypic change associated with the switch from acute to chronic phases of infection is the formation of biofilm. This is associated with extensive changes in transcription. Three key TCS involved in activating biofilm formation are BfiSR, MifR and BfmSR. Cyclic-di-GMP is another major player influencing this process, whose levels are controlled by diguanylate cyclases and phosphodiesterases. QS and the *cup* genes enhance biofilms, while regulators like AmpR repress it. An important component of *P. aeruginosa* biofilms are extracellular polysaccharides such as alginate, Pel and Psl. Alginate production is under the control of the master regulator ECF AlgT/U, whose activity is regulated transcriptionally by AmpR, post-translationally by MucA and MucB, and by regulated intermembrane proteolysis involving MucP, AlgW, ClpXP and others. AlgT/U activates the alginate biosynthetic operon via AlgR, AlgB and AmrZ. In addition, biofilm formation is also affected by iron concentration, a process governed by the master repressor of iron uptake, Fur. Fur controls uptake of iron by regulating the sigma factor PvdS, thereby modulating siderophore levels. Fur also modulates transcription of two key regulatory RNAs, asPrrF1 and asPrrF2. These two sRNAs are involved not only in regulating iron-uptake related genes, but also enzymes of the TCA cycle, and genes involved in anthranilate synthesis. Anthralinate, a precursor for synthesis of PQS, is a key regulatory molecule of the PQS signaling system in *P. aeruginosa* that is involved in expression of QS-regulated virulence factors.

Details on the individual interactions and the appropriate references can be found in the text. Some of the interactions labeled as indirect are regulated by unknown mechanisms and warrant further investigation. In the figure, some regulators and phenotypes have been mentioned more than once.

Upon receiving a signal, two HK monomers dimerize and cross-phosphorylate at the conserved histidine residue, and the phosphate is subsequently transferred to an aspartate residue in the receiver domain of the cognate RR (651). The phosphotransfer is catalyzed by the receiver domain and results in a conformational change that activates the output domain, which often binds DNA and modulates gene expression or enzymatic activity (592,650,652). Variations to this model occur in phosphorelays where a sensor kinase first transfers the phosphoryl group to a RR that has no output domain. This P~RR then transfers the phosphoryl group to a histidine-containing phosphotransfer protein (HPT), and this in turn serves as a phosphate donor to a terminal RR which has an output domain mediating a cellular response (213). In other cases, the sensor kinase and the RR lacking an output domain are fused into one protein (hybrid sensor kinase) (653). Other variations include the TCS connectors, a group of proteins that modulate the phosphorylation state and activity of sensor HK and RR and establish regulatory links between otherwise independent signal transduction pathways (654).

P. aeruginosa, equipped with 55 HKs, 89 RRs and 14 HK-RR hybrids, possesses one of the largest pool of TCS proteins identified in any microorganism thus far analyzed (25). This provides the bacterium with a sophisticated capability to regulate diverse metabolic adaptations, virulence and antibiotic resistance processes that are hallmark of *P. aeruginosa* infections. One of the critical TCSs is GacSA (GacS-PA0928, GacA-PA2586) that is central to expression of virulence factors, secondary metabolites, biofilm formation and QS (190,655), and the switch between acute and chronic infections (656,657). GacS is a hybrid sensor HK that contains a HK domain, a RR domain and a histidine phosphotransfer (Hpt) domain (210,658). GacS phosphorylation is under the

control of two hybrid sensor kinases, RetS (PA4856) (210) and LadS (PA3974) (209) (Figure 6.2). RetS can directly interact with GacS and prevent GacS phosphorylation (209,659) while LadS phosphorylates GacS (209). Phosphorylated GacA positively regulates the transcription of two small regulatory RNAs, rgRsmZ (PA3621.1) and rgRsmY (PA0527.1), which block the negative regulator RNA-binding protein RsmA (PA0905). RsmA positively regulates genes of the Type 3 secretion system, type IV pili formation and iron homeostasis while repressing QS, Type 6 secretion, and potentially other transcription factors (29,391,458). The GacSA TCS is also involved in antibiotic resistance to three different families of antibiotics: tobramycin, ciprofloxacin and tetracycline (660) apparently through RsmA/rgRsmZ.

In *P. aeruginosa*, PhoPQ (PA1179-PA1180) together with PmrAB (PA4776-PA4777) are two TCSs that respond to limiting concentrations of cations, and regulate resistance to polymyxin B and cationic antimicrobial peptides through the regulation of the *arnBCADTEF-pmrE* (PA3552-PA3559) LPS modification operon (661,662). PhoQ is involved in swarming and twitching motility as well as in biofilm formation and is required for virulence without affecting the T3SS or QS systems (Figure 6.2) (663). The HK PhoQ activates the RR PmrA independently of PmrB suggesting an interaction between these TCSs (664). In addition, increased resistance to antibiotics including polymyxin B, aminoglycosides and quinolones in *phoQ* mutants suggests crosstalk between PhoPQ and other TCSs (665,666).

Formation of biofilms

Biofilms are surface-associated multicellular bacterial communities encapsulated in a self-produced extracellular matrix composed of polysaccharides, proteins and nucleic

acids that mediate cell-to-cell and cell-to-surface interactions (667). *P. aeruginosa* biofilms, typically associated with poor patient prognosis, signify the switch from an acute to a chronic infection. Biofilms can be formed on abiotic (environment), or biotic (wounds, surgical implants, cystic fibrosis lung) surfaces (668). Biofilm formation and maintenance is tightly regulated in response to environmental cues, conferring enhanced resistance against antimicrobial agents and immune defense mechanisms on the biofilm bacteria (451). Formation of biofilms is a multi-stage process that is initiated by the surface attachment of planktonic bacteria to form a monolayer, clonal growth/aggregation leading to the formation of microcolonies, maturation to form mushroom-shaped structures, and dispersal (171,669,670). As can be imagined, this complex transition in the bacterial lifestyle is accompanied by drastic changes in gene regulation.

Surface attachment by *P. aeruginosa* to form microcolonies has been attributed to Type IV pili, flagella, free DNA, alginate, and Pel and Psl polysaccharides, though pili, alginate and flagella mutants also form biofilms (670,671). Attachment is a reversible process and the commitment to form biofilms is partly under positive SadB (PA5346) regulation (672). SadB upregulates both Pel polysaccharide production, and the chemotaxis-like cluster CheIV (PA0408-PA0417) that is thought to regulate flagellar motion by an unknown mechanism (673).

The Cup fimbriae, encoded by three distinct gene clusters *cupA* (PA2128-PA2133), *cupB* (PA4081-PA4086), and *cupC* (PA0992, PA0993, PA0994) in *P. aeruginosa* PAO1, have been demonstrated to play a role in different stages of biofilm formation on biotic and abiotic surfaces (674). Regulation of the *cup* genes is complex involving a phase variation-dependent repression of *cupA* expression by a H-NS member

MvaT (675,676). MvaT also regulates the *cupB* and *cupC* loci to a lesser extent (675). The *cupB* and *cupC* clusters are under the primary regulation of the RocS1-RocR-RocA1 (PA3946-PA3948) three-component system (274). This system is similar to the *Bordetella pertussis* BvgASR system (677) and consists of the hybrid sensor kinase RocS1, the response regulator RocA1, and the RocA1-repressor RocR (274,288). RocR has been hypothesized to bind c-di-GMP via its EAL (diguanylate phosphodiesterase) domain and prevent phosphotransfer from RocS1 to RocA1, thus preventing RocA1 activation (274,288). *P. aeruginosa* PA14 has a fourth *cup* cluster (*cupD*) on the pathogenicity island PAPI-I that is controlled positively by the response regulator RcsB (PA4080), and negatively by the EAL-domain containing response regulator PvrR (678). In addition, diguanylate cyclases and phosphodiesterases of the *wsp* gene cluster (PA3702-PA3708) (679,680) MorA (PA4601) (681) and TpbA-TpbB (PA3885, PA1120) (682) modulate intracellular levels of c-di-GMP to exert a regulatory effect on the *cup* gene clusters (Figure 6.2).

The main components of the extracellular polymeric substance matrix of biofilms are Pel and Psl polysaccharides, alginate, and free DNA (451,670). Both *pel* and *psl* gene loci are post-transcriptionally regulated by the RetS-LadS (PA4856 and PA3974, respectively) system via *rsmY* and *rzmZ* (209,210,659) and by c-di-GMP levels, either directly (679) or by binding the transcriptional regulator FleQ (PA1097). (683) The *pel* operon is also repressed by the *las* QS system via the tyrosine phosphatase TpbA (PA3885) (682). A membrane-bound sensor, PpyR (PA2663) enhances biofilm formation via the *psl* operon, and virulence via modulating QS (684). Although alginate is a major component of biofilms and affects biofilm structure, it is not essential for biofilm

formation (670). Alginate regulation is discussed in a separate section (see below). QS regulates cell lysis in biofilms (685-687) thereby controlling the release of extracellular DNA, a major component of the biofilm matrix (688,689). The QS system also regulates rhamnolipid production (362) that promotes motility and hence formation of the cap in the mushroom structure of mature biofilms (690), and maintenance of biofilm channels (691). BfiRS (PA4196-PA4197), BfmRS (PA4101-PA4102) and MifR (PA5511) are TCSs shown to regulate biofilm development and maturation by sequential phosphorylation (275). They activate biofilm formation at different transition stages: reversible to irreversible attachment (BfiRS), irreversible attachment to maturation stage-1 (BfmRS), and maturation stage-1 to mushroom structure formation (MifR) (Figure 6.2) (275). The BfiRS system may function in conjunction with the GacSA TCS and feed into the Rsm loop of regulation to control biofilm formation (275). SagS (PA2824), the cognate sensor of HptB (PA3345), modulates biofilm development (by controlling BifS phosphorylation) and other virulence phenotypes (by modulating rgsRmZ levels) depending on whether the cells are in the planktonic or biofilm phase (692).

Analyses of clinical isolates reveal a positive correlation between expression of *lasR*, *rhlR* and acute virulence factors (693,694) suggesting that QS is required for virulence *in vivo*. QS is also important when *P. aeruginosa* grows as biofilms in the CF lung (695). *In vivo* studies show that *lasI* and *rhlI* mutants produce milder chronic lung infections compared to their wild-type counterparts (696), and form more susceptible biofilms (697). However, in some *in vitro* studies, there was no apparent difference in the biofilms formed by the QS mutants and the wild-type strains (698,699). This discrepancy in the requirement of QS for biofilm formation and establishment of a successful chronic

infection is probably not surprising since QS regulates many different functions. Further, it has been demonstrated that the CF environment selects for strains with *lasR* mutations, though the *rhl* system is intact (700). Although *lasR* is higher up in the QS hierarchy, studies have shown that secondary mutations can reestablish *rhl* expression in *las* mutants (701). This suggests that in CF biofilms, the *rhl* system is more important and *lasR* inactivation serves to downregulate the acute virulence factors (702).

A major cause of antibiotic resistance in biofilms has recently been attributed to the phenomenon of persistence. Persister cells are small subpopulations of antibiotic-sensitive cells that have acquired transient antibiotic tolerance (703). When the antibiotic levels drop, the persisters grow into a population of sensitive cells, again with a small sub-population of persisters (704,705). Many genes involved in the formation of persisters have been identified in *P. aeruginosa* PA14, including two transcriptional regulators AlgR (PA5261) and PilH (PA0409) (706). However, this topic is outside the scope of this dissertation but has been extensively reviewed elsewhere (83,707).

Alginate production

In the lungs, especially of patients with cystic fibrosis, *P. aeruginosa* can convert from a non-mucoid to an alginate-overproducing mucoid phenotype signaling chronic infection (708). Chronic *P. aeruginosa* infection seems to be localized to foci within the anaerobic mucus environment in the lung's respiratory zone (709-712). These foci lead to tissue damage decreasing lung function and the appearance of the mucoid phenotype correlates with poor patient prognosis (713,714). The exopolysaccharide alginate is a linear polymer of β -D-mannuronic acid and α -L-guluronic acid (715), which stimulates production of IgG and IgA antibodies (716). Although production of alginate is

metabolically taxing, it protects the bacteria from phagocytosis and antibodies thus conferring a survival advantage (717,718). Conversion to mucoidy occurs when biofilms are treated with activated polymorphonuclear leukocytes (569), hydrogen peroxide (569), antibiotics (719), and nutrient starvation (720,721).

A complex regulatory pathway controls alginate biosynthesis. The central player is the σ^E family extracytoplasmic function sigma factor AlgT/U (PA0762) (465,722) whose activity is inhibited post-transcriptionally by the anti-sigma factor MucA (PA0763), and by MucB (PA0764) (570,723,724). Loss of function mutations in *mucA* or *mucB* result in a mucoid phenotype (466,723,725) due to release of AlgT/U from MucA by a regulated intramembrane proteolytic pathway (reviewed in (582,726)). It was recently demonstrated that MucA proteolysis is regulated not only by AlgW (PA4446) but also by MucD (PA0766) by activating the MucP protease (PA3649) (727). In addition, AmpR links alginate production with antibiotic resistance and QS by negatively regulating *algT/U* expression (Figure 6.2) (226). AlgT/U regulates alginate production at least in part by autoregulation (465), controlling expression of the transcriptional regulators *algR* (481,482), *algB* (481,725), *amrZ* (728), and the *algD* (PA3540) alginate biosynthetic operon (481,729,730). AlgB (731), AlgR (732-734), and AmrZ (735) all directly bind to the *algD* operon to activate transcription. The alternative sigma factor RpoN (PA4462) is also required for high levels of *algT/U* and *algD* expression (736).

The *algB* (PA5483) and *algR* (PA5261) genes respectively encode NtrC and LytR subfamily of TCS RRs (737). Interestingly, aspartic acid phosphorylation in the regulatory domain is not essential for alginate production (738). Transcriptome analysis of a PAO*mucA22* mucoid strain (PDO300) (570) identified seven predicted

transcriptional regulators, PA1235, PA1261, PA1637 (KdpE), PA2881, PA3420, PA3771, and PA5431, and one sensor kinase, EraS (PA1979), whose expression was downregulated in an *algB* mutant but not in a strain containing a mutation in its cognate TCS sensor, KinB (PA5484) (731). In addition to regulating the *algD* operon, AlgR directly activates transcription of *algC* (PA5322), which encodes a phosphomannomutase/phosphoglucomutase essential for Psl, alginate and rhamnolipid synthesis (Figure 6.2) (739-742). AlgR also is important for mature biofilm formation, possibly by directly repressing *rhl*-QS (743), type IV pilus formation by binding to the *fimTU-pilVWXYIY2E* promoter (744,745), and hydrogen cyanide (HCN) production by binding to the *hcnA* (PA2193) promoter (480). Interestingly, in contrast to alginate production, the phosphorylation site is required for regulating cyanide production and twitching motility (744,746). AlgR has also been shown to indirectly regulate the cyclic AMP/Vfr-dependent pathway (747). The AlgR regulon has been characterized by several transcriptome studies (480,743,748). Two other regulators of alginate production in *P. aeruginosa* are Alg44 (PA3542) (749) and a diguanylate cyclase, MucR (PA1727) (750). MucR produces a pool of c-di-GMP in the vicinity of the PilZ domain of Alg44 (PA3542), which then positively regulates alginate production (Figure 6.2) (749-751).

Regulation of iron uptake

Iron is critical for growth of all organisms and *P. aeruginosa* is no exception. Transcriptome studies reveal that a large number of genes are regulated in response to iron (144,145). Biologically useful iron (Fe^{2+}) in the environment is scarce and is available mostly in the insoluble Fe^{3+} form. To help scavenge this free iron, bacteria produce siderophores that bind extracellular iron and transport them back into the cell via

TonB-dependent receptors on the cell surface (752). *P. aeruginosa* produces two siderophores, pyoverdine and pyochelin, and can also subvert siderophores produced by other organisms to take up heme (753,754). However, excess free iron in the cell leads to formation of toxic reactive oxygen species and so cells tightly regulate the uptake (755). The ferric uptake regulator (Fur, PA4769) is a conserved protein in *P. aeruginosa* and other Gram-negative bacteria, and is a major iron acquisition regulator (756). Fur dimerizes rapidly after synthesis and it takes a minimum of two dimers to bind promoters of genes under Fur regulation in *P. aeruginosa* (757). Fur control the iron regulon directly by binding the Fur box (758), and indirectly by modulating expression of other regulators including the pyochelin uptake regulator PchR (PA4227), ECF sigma factors like PvdS, TCS regulators and small regulatory RNAs (asPrrF1 (PA4704.1), asPrrF2 (PA4704.2; Figure 6.2) (758-760).

Iron concentrations in the cell also influence expression of virulence factors in *P. aeruginosa*. PvdS, for example, is critical in linking iron and virulence by controlling the production of pyoverdine, an outer membrane pyoverdine receptor (FpvA (PA2398)) and two important extracellular virulence factors (PrpL (PA4175) and exotoxin A (PA1148); Figure 6.2) (761-763). Also, *pvdS* mutants showed reduced virulence in a rabbit endocarditis model (764). Human lactoferrin inhibits *P. aeruginosa* biofilm formation indicating a role for iron in the process (765). Iron chelation by lactoferrin induces twitching motility in *P. aeruginosa* negating colonization and ultimately, biofilm formation (765,766). Intracellular iron concentrations are one of the signals for biofilm development in a process involving Fur but not the iron uptake regulatory RNAs asPrrF1

and asPrrF2 (767). Further, high levels of iron suppress the PQS system, release of extracellular DNA and biofilm formation (768).

The link between iron and QS systems in *P. aeruginosa* is complex. QS systems are enhanced under limiting iron concentrations (376,769,770) and major QS regulators are also involved in regulating iron responsive genes (168,224,573). MvfR, for example, has been demonstrated to control transcription of iron-related genes and itself has an iron-starvation (IS) box in its promoter, a site recognized by PvdS (PA2426) to turn on transcription under low iron conditions (144,771). It was recently demonstrated that iron levels affect activity of the MvfR signaling molecule HAQ, adding another layer of complexity to the role of iron in QS (772). Another major QS and virulence regulator, VqsR regulates phenazine production by modulating *phnAB* expression (282). Moreover, the small regulatory RNAs asPrrF1 and asPrrF2, which are negatively regulated by Fur, positively regulate PQS production (Figure 6.2) (773). PQS has been shown to accumulate in the outer membrane and in membrane vesicles (576). PQS chelates iron and this facilitates pyochelin and pyoverdine in scavenging iron (378,384).

Thus, iron uptake regulation in *P. aeruginosa* is a complex affair and involves multiple regulators that affect expression of numerous genes either by themselves or through other regulators. In addition, the interconnections between iron uptake mechanisms and other virulence systems such as QS and biofilm formation demonstrates the versatility of this bacterium in being able to pragmatically read environmental signals to accordingly modulate gene expression.

Toxins and exoproteins

Exoproteins are an important component of bacterial survival because not only do they allow the bacteria to interact with their immediate environment and other organisms in the vicinity, but also because they play a critical role in virulence. *P. aeruginosa* has a large complement of secreted proteins and five (type I, II, III, V and VI) of the seven secretion systems characterized in bacteria (774). A majority of the secreted proteins are toxins that aid in *P. aeruginosa* virulence, most of which, including LasA, LasB, PrpL, ToxA, and phospholipases (PlcH (PA0844), PlcN (PA3319), PlcB (PA0026)), are secreted via the Xcp type II secretion system (T2SS) (774). Effector molecules that are crucial for evading the host phagocytic response are secreted via a dedicated T3SS (206), whereas the type I system (T1SS) secretes the alkaline protease AprA (445,775) and the hemophore HasAp (PA3407) (776). Substrates of the recently identified T6SS are just being discovered (777). In addition, c-di-GMP levels, modulated by the diguanylate cyclase WspR (PA3702) is involved in the switch between T3SS and T6SS independent of RetS but dependent on rgRsmY and rgRsmZ (Figure 6.2) (778).

HasAp, a T1SS-secreted heme-uptake protein in *P. aeruginosa*, is under QS control (168). QS is also known to regulate PrpL that targets the human lactoferrin (see section above) (761). Thus, QS in *P. aeruginosa* not only regulates enzymes to degrade the human lactoferrin but also produces proteins to retrieve the iron from the degraded lactoferrin. The other known T1SS substrate, AprA, is regulated by a novel LTTR named BexR (PA2432) that controls bistability in *P. aeruginosa* (Figure 6.2) (232). Inactivation of QS has been demonstrated to reduce expression of T2SS-secreted proteases, chitinases and lipases (626,779) due to downregulation of the Xcp T2SS (780,781). The TCS

PhoBR (PA5360-PA5361) regulates other T2SS-secreted exoproteins such as PlcH, PlcC, PlcN and the Hxc T2SS secreted alkaline phosphatase LapA (782,783). Microarray analysis revealed that a novel cell-surface signaling system PUMA3 regulates Hxc T2SS genes (784). The three T6SS systems (HSI-I, HSI-II and HSI-III) in *P. aeruginosa* are differentially regulated by the QS systems (785). Whereas LasR and MvfR negatively regulate the HSI-I system, they positively regulate expression of the functionally redundant HSI-II and HSI-III (Figure 6.2) (785). In addition, a putative regulator Sfa3 (SfnR) in *P. aeruginosa* PA14 (an ortholog of PA2359 in PAO1) potentially regulates the HSI-III cluster (785). HSI-I expression is also regulated by RetS via RsmA (459).

P. aeruginosa T3SS is regulated in a complex and multi-tiered process, and is probably the most well understood (325). Expression of T3SS is regulated transcriptionally and post-transcriptionally in response to host cell contact and environmental Ca^{2+} levels (786,787). ExsA (PA1713), an AraC member regulates expression of the 43 genes that form the T3SS in *P. aeruginosa* by binding as a monomer to an A-rich 8-bp region upstream of the -35 in the promoter of genes under its regulation (786,788). ExsA autoregulates its own expression and is also activated by PsrA (PA3006), a member of the TetR family (307,308). Two anti-activators (ExsD (PA1714) and PtrA (PA2808)) also regulate ExsA-mediated activation (Figure 6.2). T3SS transcription is coupled to secretion and involves the anti-activator ExsD and the anti-anti-activator ExsC (PA1710) that regulate ExsA function. Under non-inducing conditions (high Ca^{2+}), ExsE (PA1711) binds the anti-anti-activator ExsC, allowing the anti-activator ExsD to bind ExsA and inhibit transcription. Under Ca^{2+} delimiting conditions, ExsE is secreted freeing ExsC to bind ExsD. Free ExsA then activates T3SS expression (Figure 6.2) (789).

Though this is the primary mode of control, T3SS can also be triggered by stress due to DNA damage (RecA-mediated activation of PtrB) (331), high salt (790,791), metabolic stress (29,791,792), alginate regulators AlgT/U, AlgR and MucA (793), the MexEF-OprN efflux pump regulator MexT (PA2492) via PtrC (794), and the RetS/LadS/Gac-Rsm TCSs (209,210,493). Under low oxygen conditions, the anaerobic regulator Anr activates the response regulator NarL (PA3879), which in turn represses rgRsmY and rgRsmZ expression, allowing RsmA to activate T3SS (546). Expression of T3SS, however, happens only in a subset of the population even under inducing conditions (791,795). Multiple levels of control allow fine-tuning of T3SS expression allowing *P. aeruginosa* to sense various environmental conditions and regulate expression in conjunction with other virulence factors.

Regulatory RNAs in *P. aeruginosa* virulence

RNAs other than messenger RNAs, transfer RNAs or ribosomal RNAs are termed small RNAs (sRNAs) and affect all steps in gene expression pathways in both prokaryotes and eukaryotes (796). In general, sRNA-mediated regulation occurs in one of two ways: base pairing with DNA or mRNA, or by affecting the activity of a protein or protein complex (796). Not surprisingly, virulence gene expression in *P. aeruginosa* also relies on small regulatory RNA (rgRNA)-mediated post-transcriptional regulation. The importance of rgRNAs in regulation of bacterial virulence is well established (619,797,798). Most of the *P. aeruginosa* rgRNAs that have been characterized play a role in virulence gene regulation (discussed below). A recent study identified about 150 novel sRNAs using sRNA-Seq in *P. aeruginosa* PAO1 and PA14, which includes both strain-specific and shared ones (799).

Perhaps the most well characterized system involves the rgRNAs, rgRsmY and rgRsmZ (whose roles in virulence regulation have been discussed in the TCS, and toxins and exoproteins sections). These are two functionally redundant rgRNAs in *P. aeruginosa* that play a critical role in the switch between acute and chronic infections (210,800). GacA of the GacSA TCS positively regulates expression of rgRsmY and rgRsmZ, which then bind to and sequester the small RNA binding protein RsmA via the GGA motif (548,549,801), leading to derepression of the genes that RsmA represses (190,458,545,802). The consequences of RsmA sequestration results in dysregulation of the expression of numerous virulence factors, as discussed in the TCS section. Given the importance of this regulatory process in *P. aeruginosa* pathogenesis, regulation of expression of rgRsmY and rgRsmZ is multi-tiered. The histidine phosphotransfer protein HptB is phosphorylated by a phosphorelay involving the three sensor kinases PA2824, PA1611 and PA1976 (803). Phosphorylated HptB then transfers the phosphate to an anti-sigma factor PA3374, to negatively regulate expression of *rsmY* (803,804). In another mode of regulation, the BfiSR TCS activates expression of the ribonuclease CafA (PA4477), which specifically targets rgRsmZ (547). Further regulation is achieved by the global regulators of the H-NS family of proteins MvaT and MvaU, which bind to AT-rich regions upstream of the *rsmZ* gene repressing their expression (390). In addition to all this, there is a negative autoregulatory feedback mechanism, the details of which have not been elucidated yet (805). Upon synthesis, rgRsmY is stabilized by Hfq binding, either alone or in conjunction with RsmA (647,806).

Another example of post-transcriptional regulation by sequestering a RNA-binding protein links virulence with metabolism. *P. aeruginosa* Crc (PA5332) is a RNA-

binding protein that recognizes CA-motifs around the ribosome binding sites of the mRNA of carbon compound catabolism genes. Crc thus represses genes whose products help utilize less preferred carbon sources (807-809). When less preferred substrates such as mannitol have to be utilized, expression of catabolic genes is achieved by sequestration of Crc by the rgRNA, rgCrcZ (810). Expression of rgCrcZ is under the control of the TCS CbrAB (PA4725-PA4726), which in conjunction with Crc plays a role in carbon compound catabolism, biofilm formation, antibiotic resistance, secretion systems and swarming (811-817).

P. aeruginosa antisense small RNAs (asRNAs) can also act by base-pairing with target mRNAs, thus inhibiting translation (818). One such example is asPhrS (*PA3305.1*) that plays a role in PQS and pyocyanin expression (819). Transcriptome studies indicate an extensive overlap between the genes that are positively regulated by the transcriptional regulator PqsR (also known as MvfR, PA1003) and asPhrS suggesting that asPhrS regulates *pqsR* mRNA (224,819). Interestingly, it was shown that asPhrS specifically targets a region in the RBS of a small ORF (*uof*) that is present upstream of PqsR (819). Since translation of *pqsR* and *uof* are coupled, asPhrS regulates *pqsR* translation by modulating translation of *uof* (819). Expression of asPhrS is under the control of the oxygen responsive regulator Anr (819). Hfq controls asPhrS expression indirectly by regulating Anr expression, whose mechanism of action is yet to be elucidated (819,820).

Small asRNAs also play a role in regulation of iron uptake and involves base pairing by the sRNAs asPrrF1 and asPrrF2, which are the *P. aeruginosa* orthologs of *E. coli* RyhB (758,821). Expression of asPrrF1 and asPrrF2 is repressed by Fur when iron concentrations are high (758). Under iron starvation conditions, asPrrF1 and asPrrF2 are

expressed and base pair with the mRNA of target genes, which include the superoxide dismutase *sodB* (PA4366), genes involved in the TCA cycle, and anthranilate and catechol degradation (107). Thus, asPrrF1 and asPrrF2 link carbon metabolism, iron uptake and QS-mediated virulence. Another asRNA gene, asPrrH is located in the same locus as asPrrF1 and asPrrF2. The asPrrH asRNA (at 325 nt) is longer than asPrrF1 (116 nt) and asPrrF2 (114 nt), and the coding region of asPrrH overlaps with the asPrrF1 terminator, the intergenic region between asPrrF1 and asPrrF2, and the 5' end of the asPrrF2 ORF (822). The expression of asPrrH is maximal in the stationary phase of growth, similar to asPrrF1 and asPrrF2, and under iron-deplete conditions (822). Heme represses asPrrH expression and this involves the outer membrane heme receptors PhuR (PA4710) and HasR (PA3408) (822). Interestingly, under conditions of heme starvation, asPrrH expression leads to the repression of *achAB* and *sdhCDAB*, which are also targets of the PrrF asRNAs (822). In addition to these targets, asPrrH also represses NirL, a protein involved in biosynthesis of heme, under heme and iron limitation (822).

Conclusions and perspectives

P. aeruginosa is a very versatile bacterium that can thrive in a wide range of habitats. This is achieved by an intricately interlinked regulatory system of transcriptional regulators, sigma factors, small RNAs, and their regulons. The exquisite control of gene expression is exemplified in the virulence regulatory network (Figure 6.2), which demonstrates that none of the virulence mechanisms are isolated. Expression of individual virulence networks is under transcriptional and post-transcriptional regulation of multiple regulatory systems, either directly or indirectly. Furthermore, some signaling cascades inversely regulate the acute and chronic virulence phenotypes depending on the

signals sensed. The next critical phase of research should focus on the signals that the bacteria recognizes in order to achieve gene regulation.

The extent of crossregulation between the transcriptional regulators highlights the global nature of the regulation where individual subnetworks (such as the QS network, alginate network, etc.) are interlinked to form a hyperconnected network (Figure 6.2). Given the complexity of the connections, one can expect the response of a cell to be elaborate even when faced with a simple stress condition. A fundamental point in a network setting is that one should evaluate the role of individual players (such as a regulator) not in isolation, but with the knowledge that the entire network will react to what it does. In other words, local changes can have global effects. This, in turn, results in very subtle cause-effect relationships. Studying the functions of a regulator by generating deletion or overexpression strains is often performed under the assumption that other regulators will remain static. In reality, however, such modifications can lead to aberrant changes across the network in ways that were initially unintended. Moreover, there is a possibility that such changes can occur because the network connections might not always be obvious. This can be attributed, in part, to as yet unelucidated implicit players in the network that dictate or otherwise influence cellular response. This is a likely explanation for the many ‘global’ regulators in *P. aeruginosa* and in similar bacteria that have complex regulatory networks. In such cases, many of the phenotypes observed with single regulator mutant strains can be part of a ripple effect that propagates through the network affecting disparate phenotypes.

A simplified model of gene regulatory network treats genes as being on or off i.e., taking binary values. It is therefore no surprise that Boolean networks (discrete

dynamical network models) have been used to model and study gene regulatory circuits (823-825). Probabilistic Boolean networks, which take into account molecular and genetic noise (826,827) and stochastic Boolean networks, which permit the modeling of gene perturbations (828), provide important insights into the dynamical behavior of the system. Though they are computationally complex, they are a valuable addition to the numerous other programs that are available to analyze gene regulatory networks (613,829,830).

Dynamical systems theory helps us to analyze the behavior of complex systems that can frequently be expressed by time-differential equations. When the behavior of a dynamical system depends very sensitively on small changes in initial conditions, then the system is said to be chaotic, i.e., capable of exhibiting chaotic behavior. Researchers have investigated whether regulatory networks can have subsystems that are capable of exhibiting chaotic behavior (831). It has been shown that competition between two or more subnetworks of comparable importance can lead to chaos (832-834). In fact, chaos has been shown to be possible in biochemical systems with only two feedback loops and positive feedback is known to be necessary for chaotic behavior (835). So, one would expect chaotic subsystems in a regulatory network as complex as the one that controls *P. aeruginosa* virulence (Figure 6.2). In spite of this predisposition, gene regulatory networks seldom exhibit chaotic behavior. This could be because the competitions between opposing nodes are not strong enough (836), or that chaotic behaviors are short-lived due to triggering of other pathways such as cell-cell communication (837). Another possibility is that the natural random variability of biochemical systems masks the chaotic

behavior (836). However, maintaining a low level of chaos in such a complex network is probably a combination of the above mentioned and, potentially, as yet unknown factors.

In gene regulatory networks, a particular dynamical system is characterized by time-evolving variables (chemical concentrations, gene expression, etc.) and by parameters (temperature, ambient chemical concentrations, etc). A network can exhibit chaotic or non-chaotic behavior depending on the parameters that influence it (838). Environmental factors such as the temperature or the nutritional status of the cells parameterize the relationship between transcription factors and the genes that they regulate. While it is understood that some choices of parameters can induce chaotic behaviors, the parameter may not even be achievable, such as very high temperatures (838). Mutations can also alter relationships in regulatory networks by causing changes in existing links or forming new ones. In a dynamically robust (non-chaotic) system, small, finite changes in the parameters lead to only qualitative changes in the dynamical behavior. However, there are boundaries in the parameter space where the behavior of the system changes qualitatively, and may include the possibility of chaotic dynamics. Predicting whether a network will be stable or chaos-prone under some conditions has proven to be difficult and remains poorly characterized. Recent work has identified the minimum number, types and interactions among three and four nodes/subnetworks that can lead to chaos in a gene regulatory network (836). Such minimal subnetworks have been termed ‘chaotic motifs’ and networks with these motifs can exhibit chaotic behavior under the right parameters (836). Analysis of the network in Figure 6.2 does not readily show such chaotic motifs. This could be because the network is incomplete (lack of data on the interactions among the *P. aeruginosa* regulators) or due to errors in the inferred

interactions. Though *P. aeruginosa* virulence regulation has been extensively studied, there is yet much to learn. Thus, absence of empirical evidence does not preclude a propensity to chaos and is worth further investigation.

Depending on an elaborate network to achieve gene regulation is likely an adaptive mechanism by *P. aeruginosa*. Possessing alternate pathways to regulate the same phenotype ensures a rapid response to stimuli even if one of the pathways is affected, thus enhancing survival. Such examples can be seen throughout the network. As discussed in the section on toxins and exoproteins, expression of T3SS genes can be regulated at multiple levels, in response to various different signals and stress conditions, and is not entirely dependent on any one signal. However, the extent of contributions of the individual regulators and consequently, the fine balance that exists in some regulatory cascades within the network, are sometimes not easily apparent. Network dependence is also a probable reason for regulator genes being non-essential, in the sense that deleting a transcriptional regulator gene typically does not affect cell viability due to the presence alternate regulatory mechanisms. Having key regulators modulate different related phenotypes has the added advantage in allowing the cells to adapt to external signals by modulating one or a few regulators instead of individually regulating different virulence systems. A case in point is AmpR that positively regulates acute virulence factors while downregulating chronic infection phenotypes (225). Also of importance is the co-regulation of metabolism and virulence. Studies have identified regulators like CbrB that, with its cognate sensor CbrA, not only regulate carbon metabolism but also virulence phenotypes via the rgRNA, rgCrcZ and the RNA-binding protein Crc (811,812).

Moreover, there is crosstalk between CbrA and regulators other than CbrB, highlighting the complexity of the system (811).

The plethora of transcriptome studies using microarrays, or deep sequencing will add to the database of genes that are differentially expressed in response to regulator mutations or specific growth conditions. Differentiating the direct effect of a change from a ripple effect can, at least partly, be achieved by metaanalysis studies that look at multiple transcriptomes, identifying effects unique to each condition and differentiating them from the so-called ripple (28,400). Network analyses can help us understand the relationship between different regulators, group them based on function and, more importantly, help identify critical nodes and prominent players. This can serve as a means of target identification in attempting to deal with *P. aeruginosa* infections. In Figure 6.2, we see that some parts of the network are more densely connected than others, with central cores containing most of the links. A case in point is LasR of the QS subnetwork. It is well known that QS is central to virulence regulation in *P. aeruginosa* and targeting key regulators will have a better chance of therapeutic success. Recently, inhibitors of a key QS regulator were shown to reduce pathogenicity in *Vibrio cholera* (586).

Future directions

Taking the AmpR project forward will involve working on two main aspects: mechanistic and application-oriented.

The mechanistic basis of the regulatory process, such as the effectors recognized by *P. aeruginosa* AmpR, is not known. This will be aided by resolving the AmpR structure. Further, the details of the AmpR regulatory cascade remains largely unsolved. For example, data from my dissertation identified the QS master regulator LasR to be a

direct target of AmpR. However, this does not fully explain the extensive AmpR regulon. The role of sRNAs in the AmpR regulon needs to be elucidated.

Data from my dissertation has identified AmpR to be a major regulator of pathogenesis and metabolism in *P. aeruginosa*. So, an application-oriented extension of my project will be to identify inhibitors of AmpR function that will help manage *P. aeruginosa* infections in the clinical setting. These have been discussed in more detail in the following section.

Identifying effectors that modulate AmpR activity

It has been well established that LTTRs regulate expression of target genes in response to effectors (103,104). Analysis of the *P. aeruginosa* AmpR protein sequence reveals a predicted N-terminal DNA-binding HTH domain (amino acids 1-63), and C-terminal effector-binding domain (amino acids 90-290). *In vitro* analyses have shown that the anhydro-MurNAc tripeptide activates *C. freundii* AmpR whereas in the presence of antibiotics, anhydro-MurNAc-pentapeptides positively activate *E. cloacae* AmpR (121,410,839). In *C. freundii*, UDP-MurNAc-pentapeptide represses AmpR activity (121,410). In *P. aeruginosa*, although these effectors have been suggested to play a role in AmpC β -lactamase induction (73,348), there is no supporting experimental evidence.

To date, AmpR is believed to be a soluble cytoplasmic protein (410). In the purification of AmpR from *C. freundii*, most of the protein was found to be in the insoluble fraction but this was attributed to protein aggregation (410). However, research in the Mathee lab shows that *P. aeruginosa* AmpR is an inner membrane protein, with a single predicted transmembrane domain between the HTH and effector-binding domains

(O. Caille, personal communication). Since the effector-binding domain is in the periplasm, the activating and repressing effectors molecules have to be found in periplasm. Based on what is known about *E. coli* cell-wall synthesis and recycling, one could predict the putative effectors to be one of the following molecules: GlcNAc-anhydroMurNAc tri- or tetra-peptides, GlcNAc-anhydroMurNAc, anhydroMurNAc-tri- or tetra-peptides, free peptides (tri- and tetrapeptides) GlcNAc-anhydroMurNAc-pentapeptide or anhydroMurNAc-pentapeptide (pentapeptides only in the presence of antibiotics) (Chapter 1, Figure 3). Identification of AmpR effector molecules can therefore be addressed with three specific aims:

- a. Analysis of specific cellular fractions by HPLC to identify the muramyl peptide effectors.
- b. Determine the role of effectors in activation and repression of AmpR using *in vitro* transcription assays.
- c. Crystallizing the AmpR protein in the presence and absence of the effector molecules.

Experimental Design to identify effectors of P. aeruginosa AmpR

Construction of *lysA* strain of PAO1 Previous work in the lab to label *P. aeruginosa* diaminopimelate (DAP) peptidoglycan showed that PAO1 was very refractory to labeling (840). This is due to the enzymatic activity of LysA (*PA5277* encoding a diaminopimelate decarboxylase) that converts DAP to L-lysine. In the presence of LysA, DAP cannot be labeled with $^3\text{[H]}$. Labeling was achieved in *E. coli* only in the absence of *lysA* (840). In order to label the *P. aeruginosa* peptidoglycan

precursors, the *lysA* gene will have to be deleted in PAO1. The permease AmpG has been demonstrated to transport the muramyl degradation products (GlcNAc-anhydroMurNAc-peptides) into the cytoplasm for recycling (121). We propose that an AmpG homolog, AmpP, is also involved in this process in *P. aeruginosa* and transports UDP-MurNAc-pentapeptide into the periplasm (Chapter 1, Figure 2). These genes should also be deleted in the *lysA* mutant strain background (PAO Δ *lysA* Δ *ampG* and PAO Δ *lysA* Δ *ampP*) to ensure isolation of a high concentration of peptidoglycan degradation products from the periplasm and cytoplasm.

Labeling, fractionation and HPLC analysis To isolate labeled muropeptides, PAO Δ *lysA*, PAO Δ *lysA* Δ *ampG* and PAO Δ *lysA* Δ *ampP* should be grown in LB broth containing $^3\text{[H]}$, thus labeling the DAP in the peptidoglycan layer. The periplasmic and cytoplasmic contents can then be isolated using commercially available kits (Epicentre Biotechnologies). Log/late-log phase cells, with and without exposure to sub-MIC β -lactams, should be used for the fractionation process. Spheroplasts can be generated by treating the cells with lysozyme and EDTA followed by osmotic shocking. This will release the periplasmic contents to form spheroplasts (841), which can be purified and lysed to obtain the cytoplasmic contents. The cytoplasmic, periplasmic and whole cell fractions from PAO1 and the mutant strains can then be analyzed by HPLC to determine the fractions that contain the putative effectors. As controls to ensure proper fractionation, the different fractions can be subjected to Western blot analysis using antibodies against β -lactamase (periplasmic fraction) and $\sigma 70$ (cytoplasmic fraction).

HPLC analysis of the different cellular fractions will identify the fractions containing the putative effectors. Fractions that contain radioactivity (due to DAP) and absorb UV (due to uridine) are the ones that contain the compounds of interest.

Purification of AmpR For the *in vitro* transcription assays, purified AmpR is required. For purification of AmpR, expression clones (pMMB67EH-Gm, P_{tac}) containing a C-terminal 6-His tag of the whole ORF (290 amino acids) should be used. This overexpression construct in PAO Δ *ampR* has already been generated (DBS39, DBS176). The overexpressed protein can be purified by affinity chromatography on Ni-NTA matrix. We already have data from radioactive mobility-shift assays of the *ampC* promoter with AmpR-containing membrane fractions of overexpression strains (O. Caille, unpublished data).

***In vitro* transcription assay** The *ampC* promoter from PAO1 can be cloned into pUC19-*spf*' and used as the template for the *in vitro* transcription assay. This plasmid is a derivative of pUC19 that contains a *spf* transcription terminator downstream of the multiple cloning site (842). Transcription termination will occur at *spf* to produce discrete-sized transcripts. Purified *E. coli* RNA polymerase (USB-Affymetrix) can be used for the assay. This approach should work because AmpR activation of *ampC* occurs in *E. coli* (105).

Testing effector molecules to influence β -lactamase production Purified AmpR with the template (target promoter cloned in pUC19-*spf*') can be incubated in a Tris-HCl buffer containing DTT, KCl, MgCl₂ and glycerol. Following this, RNA polymerase and NTPs (with α^{32} -labeled UTP, other bases unlabeled) should be added

and incubated to allow *in vitro* transcription. The samples must then be DNase-treated, spiked with unlabeled tRNA followed by the phenol-chloroform protocol to isolate nucleic acids. The samples should then be run on a denaturing polyacrylamide gel, and exposed to film to locate the bands.

Once this assay has been standardized for AmpR concentration and conditions, effector molecule fractions can be tested for activation or repression of *ampC* expression (incubated in the first step with AmpR). Activation of *ampC* expression by the effectors will lead to a higher concentration of mRNA, whereas repression will have the opposite effect. Pure effectors can be synthesized for this experiment.

Crystallization of AmpR with and without effectors This aspect of the project can be performed in collaboration with Emerald Biostructures. The non-effector bound AmpR can be crystallized to determine the pocket where the effector molecule would bind. Modeling and docking studies can then be done to correlate our *in vitro* data on effector molecules and compare with the *in vivo* and *in vitro* analysis. Subsequently, co-crystallization can be done. Emerald Biostructures also provides analysis of potential inhibitors that are likely to bind. The process to crystallize AmpR in collaboration with Emerald Biostructures has already been initiated.

Identification of small molecule inhibitors of AmpR

Once the AmpR effectors are determined, the next logical step would be to design/identify inhibitors of AmpR function. Small molecule inhibitors of AmpR can be potential therapeutic agents against *P. aeruginosa* virulence and reduce resistance to β -lactams. The screening can be performed at the National Screening Laboratory for the

Regional Centers for Biodefense and Emerging Infectious Diseases (NSRB; nsrb.med.harvard.edu), which has a library of over 100,000 structurally diverse compounds. Reporter strains that harbor promoter fusions of *ampC* (AmpR-positively regulated) or *mexE* (AmpR negatively-regulated) can be used to determine small molecule potentiators or inhibitors of AmpR function. Following the initial screen to identify compounds of interest, further assays such as qPCRs, MICs, *in vitro* production of various virulence factors, cytotoxicity, virulence in *C. elegans* can be performed to validate the efficacy of the inhibitor compounds. After the compounds have been identified and their inhibitory roles confirmed, one could model the compound with AmpR to identify potential interaction domains.

The studies that have identified AmpR effectors in Enterobacteriaceae members (121,410,839) consider AmpR to be only a positive regulator of *ampC* expression. However, data from my dissertation demonstrates that AmpR is a major transcriptional regulator that affects expression of diverse phenotypes. Whether AmpR-mediated regulation of the non-AmpC phenotypes is in response to the cell wall intermediates or other, as yet unidentified, effectors is a major question that needs to be answered. Answering this question will also help synthesize effector mimics that can potentially be used to inactivate AmpR function in the clinical setting, thus reducing virulence and rendering the cells sensitive to β -lactam antibiotics.

Known, putative and novel small regulatory RNAs in the AmpR regulon

Microarrays, RNA-Seq, qPCR and complementary studies have identified some known sRNAs (rgRsmZ, asPrrF1, rgRgsA) to be under AmpR regulation (Chapters 4, 5).

This clearly shows that the AmpR regulon includes sRNAs. However, recent studies have identified over 500 novel sRNAs in *P. aeruginosa* PAO1 (843,844) whose function and regulation have not been elucidated. Given the extensive nature of the AmpR regulon, it is very likely that many more sRNAs are AmpR-regulated. The next phase of research will involve determining the sRNAs that are regulated by AmpR. Further, detailing the mode of action of the AmpR-regulated sRNAs will make for exciting research.

Moreover, it is possible that there are novel sRNAs that have not been identified yet. Quantitative analysis of the RNA-Seq data will provide insights into novel ncRNAs. These can be regulatory RNAs or antisense RNAs and are explained below.

- a. Regulatory RNAs: RNA-Seq reads that map to intergenic regions, or even within ORFs, need to be analyzed further *in silico* (look for putative promoters and terminators, secondary structures of the reads, differential expression between the conditions) and *in vitro* (deletion and overexpression phenotypic analyses, Northern blot analysis) in PAO1 and the *ampR* mutant strains to confirm presence, and AmpR-mediated regulation.
- b. Antisense RNAs (asRNAs): These are RNA-Seq reads that map to within the pre-annotated ORFs in the *Pseudomonas* Genome Database (27), but in the opposite strand to the ORF in the RNA-Seq data. In addition to the *in silico* and *in vitro* analyses mentioned in the previous section, potential targets of asRNAs can be determined by BLAST analysis of the asRNA sequence against the PAO1 genome.

With the extensive use of high-throughput transcriptomics, gene regulation studies are now focusing on the role of non-coding RNAs in bacteria. rgRNAs have been

shown to be extensively involved in gene regulation in *P. aeruginosa* and other bacteria (390,458,758,810,819,845). Moreover, the interplay of rgRNAs and transcriptional regulators in controlling critical functions in bacteria is being increasingly appreciated. Given the important regulatory role of AmpR in *P. aeruginosa* virulence and metabolism, it is not surprising that rgRNAs such as rgRsmZ are AmpR-regulated (Chapter 5). Elucidating the mechanism of regulation of AmpR, the regulatory interaction of AmpR with rgRNAs and the signals that determine AmpR-mediated regulation would be the natural extension of my work. Other groups, by crystallizing the effector-binding domain, have initiated biochemical studies detailing AmpR (612). Moreover, techniques such as RNA-Seq allow for the entire transcriptome to be sequenced, giving us an unprecedented insight into non-coding RNAs, asRNAs and sRNAs involved in regulation. Preliminary studies using prediction software and complementary experiments have already advanced our understanding (390,458,519,647,819,846). Given the many different ways in which small RNAs can modulate gene expression (818) and potentially undiscovered ones, we can look forward to exciting new discoveries in bacterial gene regulation in the coming years.

APPENDICES

Supplementary information for Chapter 2

Supplementary Fig 1: Core set of gene differentially regulated in all the studies

Supplementary Table 1: Comparison of all the genes that are differentially regulated in the 18 transcriptomes. The list of ORFs are divided into core and accessory gene set, as defined in Mathee *et al* (PMID 18287045).

ORF	Product name	Function class
Transcriptional core set ORFs present in the <i>P. aeruginosa</i> core genome		
PA0044	exoenzyme T	Secreted Factors (toxins, enzymes, alginate)
PA0045	hypothetical protein	Hypothetical, unclassified, unknown
PA0059	osmotically inducible protein OsmC	Adaptation, Protection
PA0070	hypothetical protein	Membrane proteins
PA0078	hypothetical protein	Hypothetical, unclassified, unknown
PA0082	hypothetical protein	Hypothetical, unclassified, unknown
PA0084	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0085	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0140	alkyl hydroperoxide reductase subunit F	Adaptation, Protection
PA0141	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0162	histidine porin OpcC	Membrane proteins
PA0183	arylsulfatase	Central intermediary metabolism
PA0184	probable ATP-binding component of ABC transporter	Transport of small molecules
PA0185	probable permease of ABC transporter	Membrane proteins
PA0186	probable binding protein component of ABC transporter	Transport of small molecules
PA0229	dicarboxylic acid transporter PcaT	Membrane proteins
PA0284	hypothetical protein	Hypothetical, unclassified, unknown
PA0290	hypothetical protein	Hypothetical, unclassified, unknown
PA0291	Anaerobically-induced outer membrane porin OprE precursor	Membrane proteins
PA0424	multidrug resistance operon repressor MexR	Transcriptional regulators
PA0447	glutaryl-CoA dehydrogenase	Fatty acid and phospholipid metabolism
PA0460	hypothetical protein	Hypothetical, unclassified, unknown
PA0471	probable transmembrane sensor	Two-component regulatory systems
PA0472	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA0514	heme d1 biosynthesis protein NirL	Hypothetical, unclassified, unknown
PA0517	probable c-type cytochrome precursor	Biosynthesis of cofactors, prosthetic groups and carriers
PA0518	cytochrome c-551 precursor	Biosynthesis of cofactors, prosthetic groups and carriers
PA0519	nitrite reductase precursor	Energy metabolism
PA0524	nitric-oxide reductase subunit B	Energy metabolism
PA0572	hypothetical protein	Hypothetical, unclassified, unknown
PA0604	probable binding protein component of ABC transporter	Transport of small molecules
PA0605	probable permease of ABC transporter	Membrane proteins
PA0672	heme oxygenase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0688	probable binding protein component of ABC transporter	Transport of small molecules
PA0762	sigma factor AlgU	Transcriptional regulators
PA0763	anti-sigma factor MucA	Transcriptional regulators
PA0764	negative regulator for alginate biosynthesis MucB	Transcriptional regulators
PA0775	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0848	probable alkyl hydroperoxide reductase	Adaptation, Protection
PA0849	thioredoxin reductase 2	Nucleotide biosynthesis and metabolism
PA0852	chitin-binding protein ChpD precursor	Secreted Factors (toxins, enzymes, alginate)
PA0866	aromatic amino acid transport protein AroP2	Transport of small molecules
PA0874	hypothetical protein	Hypothetical, unclassified, unknown
PA0929	two-component response regulator	Transport of small molecules
PA0938	hypothetical protein	Hypothetical, unclassified, unknown
PA0958	Outer membrane porin OprD precursor	Transport of small molecules
PA0962	probable dna-binding stress protein	Adaptation, Protection
PA1071	branched-chain amino acid transport protein BraF	Transport of small molecules
PA1156	NrdA, catalytic component of class Ia ribonucleotide reductase	Nucleotide biosynthesis and metabolism
PA1179	two-component response regulator PhoP	Transcriptional regulators
PA1180	two-component sensor PhoQ	Two-component regulatory systems
PA1230	hypothetical protein	Membrane proteins
PA1245	hypothetical protein	Membrane proteins
PA1246	alkaline protease secretion protein AprD	Secreted Factors (toxins, enzymes, alginate)
PA1249	alkaline metalloproteinase precursor	Secreted Factors (toxins, enzymes, alginate)

ORF	Product name	Function class
PA1289	hypothetical protein	Hypothetical, unclassified, unknown
PA1300	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA1317	cytochrome o ubiquinol oxidase subunit II	Energy metabolism
PA1318	cytochrome o ubiquinol oxidase subunit I	Energy metabolism
PA1319	cytochrome o ubiquinol oxidase subunit III	Energy metabolism
PA1323	hypothetical protein	Hypothetical, unclassified, unknown
PA1324	hypothetical protein	Hypothetical, unclassified, unknown
PA1346	hypothetical protein	Hypothetical, unclassified, unknown
PA1408	hypothetical protein	Membrane proteins
PA1541	probable drug efflux transporter	Membrane proteins
PA1544	transcriptional regulator Anr	Transcriptional regulators
PA1546	oxygen-independent coproporphyrinogen III oxidase	Biosynthesis of cofactors, prosthetic groups and carriers
PA1555	probable cytochrome c	Energy metabolism
PA1556	probable cytochrome c oxidase subunit	Energy metabolism
PA1557	probable cytochrome oxidase subunit (cbb3-type)	Energy metabolism
PA1657	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1673	hypothetical protein	Hypothetical, unclassified, unknown
PA1691	translocation protein in type III secretion	Protein secretion/export apparatus
PA1696	translocation protein in type III secretion	Protein secretion/export apparatus
PA1698	Type III secretion outer membrane protein PopN precursor	Membrane proteins
PA1699	conserved hypothetical protein in type III secretion	Protein secretion/export apparatus
PA1700	conserved hypothetical protein in type III secretion	Protein secretion/export apparatus
PA1701	conserved hypothetical protein in type III secretion	Protein secretion/export apparatus
PA1705	regulator in type III secretion	Protein secretion/export apparatus
PA1706	type III secretion protein PcrV	Protein secretion/export apparatus
PA1707	regulatory protein PcrH	Secreted Factors (toxins, enzymes, alginate)
PA1708	translocator protein PopB	Protein secretion/export apparatus
PA1709	Translocator outer membrane protein PopD precursor	Protein secretion/export apparatus
PA1710	ExsC, exoenzyme S synthesis protein C precursor .	Translation, post-translational modification, degradation
PA1711	ExsE	Hypothetical, unclassified, unknown
PA1712	exoenzyme S synthesis protein B	Translation, post-translational modification, degradation
PA1713	transcriptional regulator ExsA	Protein secretion/export apparatus
PA1714	ExsD	Hypothetical, unclassified, unknown
PA1715	type III export apparatus protein	Protein secretion/export apparatus
PA1716	Type III secretion outer membrane protein PscC precursor	Protein secretion/export apparatus
PA1717	type III export protein PscD	Protein secretion/export apparatus
PA1719	type III export protein PscF	Protein secretion/export apparatus
PA1720	type III export protein PscG	Protein secretion/export apparatus
PA1721	type III export protein PscH	Protein secretion/export apparatus
PA1722	type III export protein PscI	Protein secretion/export apparatus
PA1723	type III export protein PscJ	Protein secretion/export apparatus
PA1761	hypothetical protein	Hypothetical, unclassified, unknown
PA1793	peptidyl-prolyl cis-trans isomerase B	Translation, post-translational modification, degradation
PA1869	probable acyl carrier protein	Fatty acid and phospholipid metabolism
PA1894	hypothetical protein	Hypothetical, unclassified, unknown
PA1902	phenazine biosynthesis protein PhzD	Secreted Factors (toxins, enzymes, alginate)
PA1904	probable phenazine biosynthesis protein	Secreted Factors (toxins, enzymes, alginate)
PA1982	quinoprotein alcohol dehydrogenase	Carbon compound catabolism
PA2080	hypothetical protein	Hypothetical, unclassified, unknown
PA2081	kynurenine formamidase, KynB	Hypothetical, unclassified, unknown
PA2087	hypothetical protein	Hypothetical, unclassified, unknown
PA2088	hypothetical protein	Hypothetical, unclassified, unknown
PA2111	hypothetical protein	Hypothetical, unclassified, unknown
PA2112	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2113	pyroglutamate porin OpdO	Transport of small molecules
PA2114	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2143	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA2146	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2158	probable alcohol dehydrogenase (Zn-dependent)	Putative enzymes
PA2159	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2166	hypothetical protein	Hypothetical, unclassified, unknown
PA2171	hypothetical protein	Hypothetical, unclassified, unknown
PA2173	hypothetical protein	Hypothetical, unclassified, unknown
PA2177	probable sensor/response regulator hybrid	Two-component regulatory systems
PA2191	adenylate cyclase ExoY	Secreted Factors (toxins, enzymes, alginate)
PA2193	hydrogen cyanide synthase HcnA	Central intermediary metabolism
PA2194	hydrogen cyanide synthase HcnB	Central intermediary metabolism
PA2195	hydrogen cyanide synthase HcnC	Central intermediary metabolism
PA2247	2-oxoisovalerate dehydrogenase (alpha subunit)	Amino acid biosynthesis and metabolism
PA2248	2-oxoisovalerate dehydrogenase (beta subunit)	Amino acid biosynthesis and metabolism
PA2260	hypothetical protein	Hypothetical, unclassified, unknown
PA2261	probable 2-ketogluconate kinase	Carbon compound catabolism
PA2262	probable 2-ketogluconate transporter	Membrane proteins
PA2263	probable 2-hydroxyacid dehydrogenase	Putative enzymes
PA2264	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2265	gluconate dehydrogenase	Carbon compound catabolism
PA2266	probable cytochrome c precursor	Carbon compound catabolism
PA2299	probable transcriptional regulator	Transcriptional regulators
PA2302	probable non-ribosomal peptide synthetase	Putative enzymes
PA2304	hypothetical protein	Hypothetical, unclassified, unknown
PA2305	probable non-ribosomal peptide synthetase	Putative enzymes
PA2307	probable permease of ABC transporter	Membrane proteins
PA2309	hypothetical protein	Hypothetical, unclassified, unknown
PA2310	hypothetical protein	Putative enzymes
PA2331	hypothetical protein	Membrane proteins
PA2385	PvdQ	Adaptation, Protection
PA2386	L-ornithine N5-oxygenase	Adaptation, Protection
PA2389	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2392	PvdP	Adaptation, Protection
PA2393	probable dipeptidase precursor	Central intermediary metabolism
PA2394	PvdN	Adaptation, Protection
PA2396	pyoverdine synthetase F	Secreted Factors (toxins, enzymes, alginate)
PA2397	pyoverdine biosynthesis protein PvdE	Membrane proteins
PA2403	hypothetical protein	Membrane proteins
PA2404	hypothetical protein	Membrane proteins
PA2405	hypothetical protein	Hypothetical, unclassified, unknown
PA2407	probable adhesion protein	Motility & Attachment
PA2408	probable ATP-binding component of ABC transporter	Transport of small molecules
PA2409	probable permease of ABC transporter	Membrane proteins
PA2410	hypothetical protein	Hypothetical, unclassified, unknown
PA2411	probable thioesterase	Adaptation, Protection
PA2412	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2413	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH	Adaptation, Protection
PA2414	L-sorbose dehydrogenase	Carbon compound catabolism
PA2415	hypothetical protein	Membrane proteins
PA2424	PvdL	Adaptation, Protection
PA2425	PvdG	Adaptation, Protection
PA2426	sigma factor PvdS	Transcriptional regulators
PA2433	hypothetical protein	Hypothetical, unclassified, unknown
PA2468	ECF sigma factor FoxI	Transcriptional regulators
PA2505	tyrosine porin OpdT	Transport of small molecules
PA2523	probable two-component response regulator	Transcriptional regulators
PA2548	hypothetical protein	Hypothetical, unclassified, unknown
PA2588	probable transcriptional regulator	Transcriptional regulators

ORF	Product name	Function class
PA2592	probable periplasmic spermidine/putrescine-binding protein	Transport of small molecules
PA2630	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2634	isocitrate lyase	Putative enzymes
PA2687	two-component sensor PfeS	Two-component regulatory systems
PA2747	hypothetical protein	Hypothetical, unclassified, unknown
PA2760	probable outer membrane protein precursor	Transport of small molecules
PA2808	two-component response repressor, PtrA	Transcriptional regulators
PA2850	organic hydroperoxide resistance protein	Adaptation, Protection
PA2896	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA3235	conserved hypothetical protein	Membrane proteins
PA3278	hypothetical protein	Membrane proteins
PA3281	hypothetical protein	Membrane proteins
PA3283	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3284	hypothetical protein	Hypothetical, unclassified, unknown
PA3287	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3328	probable FAD-dependent monooxygenase	Putative enzymes
PA3369	hypothetical protein	Membrane proteins
PA3371	hypothetical protein	Hypothetical, unclassified, unknown
PA3413	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3414	hypothetical protein	Hypothetical, unclassified, unknown
PA3416	probable pyruvate dehydrogenase E1 component, beta chain	Energy metabolism
PA3446	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3447	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3448	probable permease of ABC transporter	Membrane proteins
PA3450	probable antioxidant protein	Adaptation, Protection
PA3460	probable acetyltransferase	Putative enzymes
PA3461	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3515	hypothetical protein	Hypothetical, unclassified, unknown
PA3516	probable lyase	Putative enzymes
PA3517	probable lyase	Carbon compound catabolism
PA3518	hypothetical protein	Hypothetical, unclassified, unknown
PA3519	hypothetical protein	Hypothetical, unclassified, unknown
PA3520	hypothetical protein	Hypothetical, unclassified, unknown
PA3523	probable RND efflux membrane fusion protein precursor	Transport of small molecules
PA3531	bacterioferritin	Transport of small molecules
PA3573	probable major facilitator superfamily (MFS) transporter	Adaptation, Protection
PA3600	conserved hypothetical protein	Translation, post-translational modification, degradation
PA3690	probable metal-transporting P-type ATPase	Membrane proteins
PA3691	hypothetical protein	Hypothetical, unclassified, unknown
PA3709	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3790	Putative copper transport outer membrane porin OprC precursor	Transport of small molecules
PA3842	probable chaperone	Secreted Factors (toxins, enzymes, alginate)
PA3899	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA3900	probable transmembrane sensor	Membrane proteins
PA3904	hypothetical protein	Hypothetical, unclassified, unknown
PA3936	probable permease of ABC taurine transporter	Membrane proteins
PA3937	probable ATP-binding component of ABC taurine transporter	Transport of small molecules
PA3938	probable periplasmic taurine-binding protein precursor	Transport of small molecules
PA3954	hypothetical protein	Hypothetical, unclassified, unknown
PA4067	Outer membrane protein OprG precursor	Membrane proteins
PA4139	hypothetical protein	Hypothetical, unclassified, unknown
PA4156	probable TonB-dependent receptor	Transport of small molecules
PA4168	second ferric pyoverdine receptor FpvB	Transport of small molecules
PA4171	probable protease	Putative enzymes
PA4179	probable porin	Membrane proteins
PA4217	flavin-containing monooxygenase	Putative enzymes
PA4218	probable transporter	Membrane proteins

ORF	Product name	Function class
PA4219	hypothetical protein	Membrane proteins
PA4220	hypothetical protein	Hypothetical, unclassified, unknown
PA4221	Fe(III)-pyochelin outer membrane receptor precursor	Transport of small molecules
PA4222	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4224	pyochelin biosynthetic protein PchG	Transport of small molecules
PA4225	pyochelin synthetase	Secreted Factors (toxins, enzymes, alginate)
PA4226	dihydroaeruginic acid synthetase	Secreted Factors (toxins, enzymes, alginate)
PA4227	transcriptional regulator PchR	Transcriptional regulators
PA4228	pyochelin biosynthesis protein PchD	Secreted Factors (toxins, enzymes, alginate)
PA4229	pyochelin biosynthetic protein PchC	Secreted Factors (toxins, enzymes, alginate)
PA4230	salicylate biosynthesis protein PchB	Secreted Factors (toxins, enzymes, alginate)
PA4231	salicylate biosynthesis isochorismate synthase	Secreted Factors (toxins, enzymes, alginate)
PA4296	two-component response regulator, PprB	Transcriptional regulators
PA4359	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4429	probable cytochrome c1 precursor	Energy metabolism
PA4430	probable cytochrome b	Energy metabolism
PA4431	probable iron-sulfur protein	Putative enzymes
PA4467	hypothetical protein	Membrane proteins
PA4468	superoxide dismutase	Adaptation, Protection
PA4469	hypothetical protein	Hypothetical, unclassified, unknown
PA4470	fumarate hydratase	Energy metabolism
PA4471	hypothetical protein	Hypothetical, unclassified, unknown
PA4480	rod shape-determining protein MreC	Cell wall / LPS / capsule
PA4501	Glycine-glutamate dipeptide porin OpgP	Transport of small molecules
PA4563	30S ribosomal protein S20	Central intermediary metabolism
PA4570	hypothetical protein	Hypothetical, unclassified, unknown
PA4590	protein activator	Transport of small molecules
PA4613	catalase	Adaptation, Protection
PA4670	ribose-phosphate pyrophosphokinase	Carbon compound catabolism
PA4673	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4708	Heme-transport protein, PhuT	Transport of small molecules
PA4709	probable hemin degrading factor	Putative enzymes
PA4710	Haem/Haemoglobin uptake outer membrane receptor PhuR precursor	Transport of small molecules
PA4719	probable transporter	Membrane proteins
PA4738	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4739	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4876	osmotically inducible lipoprotein OsmE	Membrane proteins
PA4880	probable bacterioferritin	Central intermediary metabolism
PA4896	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA4909	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4981	probable amino acid permease	Membrane proteins
PA5261	alginate biosynthesis regulatory protein AlgR	Secreted Factors (toxins, enzymes, alginate)
PA5311	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA5315	50S ribosomal protein L33	Translation, post-translational modification, degradation
PA5407	hypothetical protein	Hypothetical, unclassified, unknown
PA5469	conserved hypothetical protein	Membrane proteins
PA5482	hypothetical protein	Membrane proteins
PA5530	probable MFS dicarboxylate transporter	Membrane proteins

Transcriptional core set ORFs present in the *P. aeruginosa* PAO1 accessory genome

PA0203	probable binding protein component of ABC transporter	Transport of small molecules
PA0204	probable permease of ABC transporter	Membrane proteins
PA0205	probable permease of ABC transporter	Membrane proteins
PA0206	probable ATP-binding component of ABC transporter	Transport of small molecules
PA0633	hypothetical protein	Related to phage, transposon, or plasmid
PA0691	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA1414	hypothetical protein	Hypothetical, unclassified, unknown
PA1592	hypothetical protein	Hypothetical, unclassified, unknown
PA1718	type III export protein PscE	Protein secretion/export apparatus
PA2033	hypothetical protein	Hypothetical, unclassified, unknown
PA2034	hypothetical protein	Hypothetical, unclassified, unknown
PA2184	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2190	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2308	probable ATP-binding component of ABC transporter	Transport of small molecules
PA2398	ferripyoverdine receptor	Transport of small molecules
PA2399	pyoverdine synthetase D	Secreted Factors (toxins, enzymes, alginate)
PA2400	PvdJ	Adaptation, Protection
PA2402	probable non-ribosomal peptide synthetase	Putative enzymes
PA2452	hypothetical protein	Hypothetical, unclassified, unknown
PA3370	hypothetical protein	Membrane proteins
PA3477	transcriptional regulator RhIR	Adaptation, Protection
PA3841	exoenzyme S	Secreted Factors (toxins, enzymes, alginate)
PA3866	pyocin protein	Adaptation, Protection
PA3923	hypothetical protein	Hypothetical, unclassified, unknown
PA4193	probable permease of ABC transporter	Membrane proteins
PA4223	probable ATP-binding component of ABC transporter	Membrane proteins
PA4306	Type IVb pilin, Flp	Motility & Attachment
PA4555	type 4 fimbrial biogenesis protein PilY2	Motility & Attachment
PA4748	triosephosphate isomerase	Central intermediary metabolism

Supplementary Table 2: Functional categorization of genes that are uniquely expressed under the different study conditions.

Code	Class	Percentage of differentially regulated unique genes (minus the core set)									Total %
		Iron	QS	Oxi. Stress	PCP	Copper	Sulfate	Osmotic	Biofilms	RsmA	
a	DNA replication, recombination, modification and repair	0	1	1	0	0	0	0	0	0	0.4
b	Fatty acid and phospholipid metabolism	0	2	0	1	0	0	0	2	1	1.2
c	Hypothetical, unclassified, unknown	27	32	26	31	14	14	3	40	1	29.7
d	Membrane proteins	22	12	8	11	33	14	0	12	8	13.3
e	Amino acid biosynthesis and metabolism	3	3	2	4	0	0	3	2	6	2.5
f	Translation, post-translational modification, degradation	1	1	3	5	2	0	3	4	1	2.4
g	Cell wall / LPS / capsule	1	1	1	3	0	0	0	5	6	2.2
h	Transport of small molecules	10	7	9	12	19	17	0	4	8	7.7
i	Energy metabolism	4	3	15	3	7	3	0	3	7	5.1
j	Biosynthesis of cofactors, prosthetic groups and carriers	4	2	2	3	1	0	0	0	3	1.7
k	Adaptation, Protection	3	2	4	0	5	0	3	3	14	3.1
l	Transcriptional regulators	7	6	3	7	10	3	37	5	3	5.8
m	Two-component regulatory systems	1	1	0	0	4	0	10	2	1	1.1
n	Secreted Factors (toxins, enzymes, alginate)	7	2	0	1	1	0	0	1	7	2.2
o	Putative enzymes	7	10	7	9	3	34	3	7	0	8.0
p	Chaperones & heat shock proteins	1	0	0	1	0	0	0	0	0	0.2
q	Central intermediary metabolism	1	3	3	4	0	0	0	1	0	2.1
r	Nucleotide biosynthesis and metabolism	0	1	0	0	0	14	0	1	1	0.7
s	Carbon compound catabolism	1	6	4	0	0	14	0	1	11	3.9
t	Motility & Attachment	1	0	1	0	0	0	0	2	13	1.2
u	Chemotaxis	1	0	1	1	0	0	0	1	0	0.4
v	Related to phage, transposon, or plasmid	0	5	10	1	0	0	0	0	1	3.2
w	Non-coding RNA gene	0	0	0	0	0	0	0	0	0	0.1
x	Protein secretion/export apparatus	1	1	0	0	0	0	33	1	1	1.1
y	Cell division	0	0	0	0	0	0	0	1	0	0.3
zA	Antibiotic resistance and susceptibility	0	0	0	1	0	0	0	0	1	0.2
zB	Transcription, RNA processing and degradation	0	0	0	0	0	0	3	1	0	0.4

Note: QS- quorum sensing, Oxi. Stress- oxidative stress, PCP- pentachlorophenol stress

Supplementary Table 3: List of genes that are uniquely expressed in the various transcriptome studies. The various conditions include iron response, quorum sensing, oxidative stress, pentachlorophenol stress, copper stress, sulfate starvation, osmotic shock, biofilm formation, alginate production and loss of the post-transcriptional regulator RsmA.

Iron		
ORF	Product name	Function class
PA0013	conserved hypothetical protein	Membrane proteins
PA0027	hypothetical protein	Hypothetical, unclassified, unknown
PA0128	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0203	probable binding protein component of ABC transporter	Transport of small molecules
PA0223	probable dihydronicotinate synthetase	Cell wall / LPS / capsule
PA0224	probable aldolase	Putative enzymes
PA0251	hypothetical protein	Hypothetical, unclassified, unknown
PA0252	hypothetical protein	Hypothetical, unclassified, unknown
PA0265	succinate-semialdehyde dehydrogenase	Central intermediary metabolism
PA0266	4-aminobutyrate aminotransferase	Central intermediary metabolism
PA0296	probable glutamine synthetase	Putative enzymes
PA0346	hypothetical protein	Hypothetical, unclassified, unknown
PA0417	probable chemotaxis protein	Chemotaxis
PA0433	hypothetical protein	Hypothetical, unclassified, unknown
PA0460	hypothetical protein	Hypothetical, unclassified, unknown
PA0470	Ferrichrome receptor FiuA	Transport of small molecules
PA0471	probable transmembrane sensor	Two-component regulatory systems
PA0472	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA0500	biotin synthase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0527	transcriptional regulator Dnr	Transcriptional regulators
PA0539	hypothetical protein	Membrane proteins
PA0605	probable permease of ABC transporter	Membrane proteins
PA0672	heme oxygenase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0707	transcriptional regulator ToxR	Transcriptional regulators
PA0725	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0774	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0801	hypothetical protein	Membrane proteins
PA0802	hypothetical protein	Membrane proteins
PA0818	hypothetical protein	Hypothetical, unclassified, unknown
PA0842	probable glycosyl transferase	Putative enzymes
PA0844	hemolytic phospholipase C precursor	Secreted Factors (toxins, enzymes, alginate)
PA0848	probable alkyl hydroperoxide reductase	Adaptation, Protection
PA0930	two-component sensor	Transport of small molecules
PA0931	ferric enterobactin receptor PirA	Transport of small molecules
PA0968	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1003	Transcriptional regulator MvrR	Biosynthesis of cofactors, prosthetic groups and carriers
PA1108	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1134	hypothetical protein	Hypothetical, unclassified, unknown
PA1148	exotoxin A precursor	Secreted Factors (toxins, enzymes, alginate)
PA1211	hypothetical protein	Hypothetical, unclassified, unknown
PA1247	alkaline protease secretion protein AprE	Secreted Factors (toxins, enzymes, alginate)
PA1248	Alkaline protease secretion outer membrane protein AprF precursor	Secreted Factors (toxins, enzymes, alginate)
PA1249	alkaline metalloproteinase precursor	Secreted Factors (toxins, enzymes, alginate)
PA1300	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA1301	probable transmembrane sensor	Membrane proteins
PA1302	probable heme utilization protein precursor	Membrane proteins
PA1316	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1317	cytochrome o ubiquinol oxidase subunit II	Energy metabolism
PA1318	cytochrome o ubiquinol oxidase subunit I	Energy metabolism
PA1319	cytochrome o ubiquinol oxidase subunit III	Energy metabolism
PA1320	cytochrome o ubiquinol oxidase subunit IV	Energy metabolism
PA1321	cytochrome o ubiquinol oxidase protein CyoE	Energy metabolism
PA1363	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA1365	probable siderophore receptor	Transport of small molecules
PA1431	regulatory protein RsaL	Secreted Factors (toxins, enzymes, alginate)
PA1556	probable cytochrome c oxidase subunit	Energy metabolism
PA1559	hypothetical protein	Hypothetical, unclassified, unknown
PA1654	probable aminotransferase	Putative enzymes
PA1655	probable glutathione S-transferase	Putative enzymes
PA1687	spermidine synthase	Amino acid biosynthesis and metabolism
PA1908	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1911	probable transmembrane sensor	Membrane proteins
PA1917	hypothetical protein	Hypothetical, unclassified, unknown
PA1972	conserved hypothetical protein	Membrane proteins
PA2002	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2021	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA2034	hypothetical protein	Hypothetical, unclassified, unknown
PA2038	hypothetical protein	Hypothetical, unclassified, unknown
PA2039	hypothetical protein	Membrane proteins
PA2041	probable amino acid permease	Transport of small molecules
PA2131	fimbrial subunit CupA4	Motility & Attachment
PA2181	hypothetical protein	Hypothetical, unclassified, unknown
PA2258	transcriptional regulator PtxR	Secreted Factors (toxins, enzymes, alginate)
PA2383	probable transcriptional regulator	Transcriptional regulators
PA2384	hypothetical protein	Hypothetical, unclassified, unknown
PA2387	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA2388	probable transmembrane sensor	Membrane proteins
PA2390	probable ATP-binding/permease fusion ABC transporter	Membrane proteins
PA2391	probable outer membrane protein precursor	Membrane proteins
PA2395	PvdO	Adaptation, Protection
PA2406	hypothetical protein	Hypothetical, unclassified, unknown
PA2420	probable porin	Membrane proteins
PA2427	hypothetical protein	Hypothetical, unclassified, unknown
PA2437	hypothetical protein	Membrane proteins
PA2441	hypothetical protein	Hypothetical, unclassified, unknown
PA2451	hypothetical protein	Hypothetical, unclassified, unknown
PA2467	Anti-sigma factor FoxR	Membrane proteins
PA2490	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2531	probable aminotransferase	Amino acid biosynthesis and metabolism
PA2637	NADH dehydrogenase I chain A	Energy metabolism
PA2638	NADH dehydrogenase I chain B	Energy metabolism
PA2686	two-component response regulator PfeR	Transcriptional regulators
PA2688	Ferric enterobactin receptor, outer membrane protein PfeA precursor	Membrane proteins
PA2691	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2753	hypothetical protein	Hypothetical, unclassified, unknown
PA2761	hypothetical protein	Membrane proteins
PA2767	probable enoyl-CoA hydratase/isomerase	Putative enzymes
PA2776	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2910	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2916	hypothetical protein	Membrane proteins
PA2917	probable transcriptional regulator	Transcriptional regulators
PA3049	ribosome modulation factor	Translation, post-translational modification, degradation
PA3165	histidinol-phosphate aminotransferase	Amino acid biosynthesis and metabolism
PA3221	CsaA protein	Protein secretion/export apparatus
PA3222	hypothetical protein	Membrane proteins
PA3233	hypothetical protein	Hypothetical, unclassified, unknown
PA3355	hypothetical protein	Membrane proteins
PA3397	ferredoxin--NADP+ reductase	Biosynthesis of cofactors, prosthetic groups and carriers
PA3407	heme acquisition protein HasAp	Transport of small molecules
PA3408	Haem uptake outer membrane receptor HasR precursor	Transport of small molecules
PA3409	probable transmembrane sensor	Membrane proteins
PA3410	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA3436	hypothetical protein	Hypothetical, unclassified, unknown
PA3530	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3553	AmC	Adaptation, Protection
PA3556	inner membrane L-Ara4N transferase AmT	Adaptation, Protection
PA3558	AmF	Membrane proteins
PA3608	polyamine transport protein PotB	Membrane proteins
PA3749	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3767	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3768	probable metallo-oxidoreductase	Putative enzymes
PA3775	hypothetical protein	Membrane proteins
PA3811	heat shock protein HscB	Chaperones & heat shock proteins
PA3812	probable iron-binding protein IscA	Biosynthesis of cofactors, prosthetic groups and carriers
PA3814	L-cysteine desulfurase (pyridoxal phosphate-dependent)	Amino acid biosynthesis and metabolism
PA3885	hypothetical protein	Hypothetical, unclassified, unknown
PA3901	Fe(III) dicitrate transport protein FecA	Membrane proteins
PA4090	hypothetical protein	Hypothetical, unclassified, unknown
PA4091	4-hydroxyphenylacetate 3-monooxygenase lar ge chain	Carbon compound catabolism
PA4158	ferric enterobactin transport protein FepC	Transport of small molecules
PA4175	protease IV	Secreted Factors (toxins, enzymes, alginate)
PA4223	probable ATP-binding component of ABC transporter	Membrane proteins
PA4224	pyochelin biosynthetic protein PchG	Transport of small molecules
PA4225	pyochelin synthetase	Secreted Factors (toxins, enzymes, alginate)

ORF	Product name	Function class
PA4226	dihydroaeruginic acid synthetase	Secreted Factors (toxins, enzymes, alginate)
PA4227	transcriptional regulator PchR	Transcriptional regulators
PA4228	pyochelin biosynthesis protein PchD	Secreted Factors (toxins, enzymes, alginate)
PA4229	pyochelin biosynthetic protein PchC	Secreted Factors (toxins, enzymes, alginate)
PA4230	salicylate biosynthesis protein PchB	Secreted Factors (toxins, enzymes, alginate)
PA4231	salicylate biosynthesis isochorismate synthase	Secreted Factors (toxins, enzymes, alginate)
PA4288	probable transcriptional regulator	Transcriptional regulators
PA4357	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4358	probable ferrous iron transport protein	Membrane proteins
PA4359	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4370	Insulin-cleaving metalloproteinase outer membrane protein precursor	Membrane proteins
PA4371	hypothetical protein	Hypothetical, unclassified, unknown
PA4372	hypothetical protein	Hypothetical, unclassified, unknown
PA4500	probable binding protein component of ABC transporter	Transport of small molecules
PA4503	probable permease of ABC transporter	Membrane proteins
PA4513	probable oxidoreductase	Putative enzymes
PA4514	probable outer membrane receptor for iron transport	Transport of small molecules
PA4515	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4516	hypothetical protein	Hypothetical, unclassified, unknown
PA4517	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4519	ornithine decarboxylase	Amino acid biosynthesis and metabolism
PA4629	hypothetical protein	Hypothetical, unclassified, unknown
PA4630	hypothetical protein	Hypothetical, unclassified, unknown
PA4633	probable chemotaxis transducer	Adaptation, Protection
PA4654	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA4675	probable TonB-dependent receptor	Transport of small molecules
PA4687	ferric iron-binding periplasmic protein HitA	Transport of small molecules
PA4688	iron (III)-transport system permease HitB	Membrane proteins
PA4706	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4773	hypothetical protein	Hypothetical, unclassified, unknown
PA4785	probable acyl-CoA thiolase	Putative enzymes
PA4826	hypothetical protein	Hypothetical, unclassified, unknown
PA4833	conserved hypothetical protein	Membrane proteins
PA4838	hypothetical protein	Hypothetical, unclassified, unknown
PA4858	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4860	probable permease of ABC transporter	Membrane proteins
PA4861	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4862	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4883	hypothetical protein	Hypothetical, unclassified, unknown
PA4892	urease accessory protein UreF	Biosynthesis of cofactors, prosthetic groups and carriers
PA4893	urease accessory protein UreG	Biosynthesis of cofactors, prosthetic groups and carriers
PA4894	hypothetical protein	Membrane proteins
PA4895	probable transmembrane sensor	Membrane proteins
PA4897	hypothetical protein	Hypothetical, unclassified, unknown
PA4973	thiamin biosynthesis protein ThiC	Biosynthesis of cofactors, prosthetic groups and carriers
PA5119	glutamine synthetase	Amino acid biosynthesis and metabolism
PA5124	two-component sensor NtrB	Two-component regulatory systems
PA5125	two-component response regulator NtrC	Transcriptional regulators
PA5150	probable short-chain dehydrogenase	Putative enzymes
PA5217	probable binding protein component of ABC iron transporter	Transport of small molecules
PA5287	ammonium transporter AmtB	Membrane proteins
PA5309	probable oxidoreductase	Putative enzymes
PA5312	probable aldehyde dehydrogenase	Putative enzymes
PA5313	probable pyridoxal-dependent aminotransferase	Putative enzymes
PA5314	hypothetical protein	Hypothetical, unclassified, unknown
PA5386	probable 3-hydroxyacyl-CoA dehydrogenase	Putative enzymes
PA5479	proton-glutamate symporter	Membrane proteins
PA5526	hypothetical protein	Hypothetical, unclassified, unknown
PA5531	TonB protein	Transport of small molecules

ORF	Product name	Function class
Quorum Sensing		
PA0006	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0048	probable transcriptional regulator	Transcriptional regulators
PA0049	hypothetical protein	Hypothetical, unclassified, unknown
PA0050	hypothetical protein	Hypothetical, unclassified, unknown
PA0051	potential phenazine-modifying enzyme	Putative enzymes
PA0052	hypothetical protein	Hypothetical, unclassified, unknown
PA0055	hypothetical protein	Hypothetical, unclassified, unknown
PA0079	hypothetical protein	Hypothetical, unclassified, unknown
PA0080	hypothetical protein	Hypothetical, unclassified, unknown
PA0097	hypothetical protein	Hypothetical, unclassified, unknown
PA0105	cytochrome c oxidase, subunit II	Energy metabolism
PA0106	cytochrome c oxidase, subunit I	Energy metabolism
PA0107	conserved hypothetical protein	Energy metabolism
PA0108	cytochrome c oxidase, subunit III	Energy metabolism
PA0112	hypothetical protein	Membrane proteins
PA0113	probable cytochrome c oxidase assembly factor	Energy metabolism
PA0119	probable dicarboxylate transporter	Transport of small molecules
PA0122	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0129	gamma-aminobutyrate permease	Transport of small molecules
PA0130	probable aldehyde dehydrogenase	Putative enzymes
PA0131	hypothetical protein	Hypothetical, unclassified, unknown
PA0132	beta-alanine-pyruvate transaminase	Amino acid biosynthesis and metabolism
PA0144	hypothetical protein	Hypothetical, unclassified, unknown
PA0145	hypothetical protein	Hypothetical, unclassified, unknown
PA0153	protocatechuate 3,4-dioxygenase, beta subunit	Carbon compound catabolism
PA0154	protocatechuate 3,4-dioxygenase, alpha subunit	Carbon compound catabolism
PA0160	hypothetical protein	Hypothetical, unclassified, unknown
PA0167	probable transcriptional regulator	Transcriptional regulators
PA0191	probable transcriptional regulator	Transcriptional regulators
PA0200	hypothetical protein	Hypothetical, unclassified, unknown
PA0209	conserved hypothetical protein	Putative enzymes
PA0210	malonate decarboxylase delta subunit	Carbon compound catabolism
PA0211	malonate decarboxylase beta subunit	Carbon compound catabolism
PA0212	malonate decarboxylase gamma subunit	Carbon compound catabolism
PA0213	hypothetical protein	Carbon compound catabolism
PA0214	probable acyl transferase	Carbon compound catabolism
PA0221	probable aminotransferase	Putative enzymes
PA0226	probable CoA transferase, subunit A	Carbon compound catabolism
PA0227	probable CoA transferase, subunit B	Carbon compound catabolism
PA0228	beta-ketoadipyl CoA thiolase PcaF	Fatty acid and phospholipid metabolism
PA0234	hypothetical protein	Hypothetical, unclassified, unknown
PA0235	4-hydroxybenzoate transporter PcaK	Membrane proteins
PA0239	hypothetical protein	Membrane proteins
PA0241	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA0251	hypothetical protein	Hypothetical, unclassified, unknown
PA0264	hypothetical protein	Hypothetical, unclassified, unknown
PA0265	succinate-semialdehyde dehydrogenase	Central intermediary metabolism
PA0266	4-aminobutyrate aminotransferase	Central intermediary metabolism
PA0269	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0270	hypothetical protein	Hypothetical, unclassified, unknown
PA0271	hypothetical protein	Hypothetical, unclassified, unknown
PA0274	hypothetical protein	Hypothetical, unclassified, unknown
PA0287	3-guanidinopropionate transport protein	Transport of small molecules
PA0297	probable glutamine amidotransferase	Amino acid biosynthesis and metabolism
PA0298	probable glutamine synthetase	Putative enzymes
PA0299	putrescine aminotransferase	Putative enzymes
PA0300	polyamine transport protein	Transport of small molecules
PA0301	polyamine transport protein	Transport of small molecules
PA0302	polyamine transport protein PotG	Transport of small molecules
PA0303	polyamine transport protein PotH	Membrane proteins
PA0304	polyamine transport protein PotI	Membrane proteins
PA0305	hypothetical protein	Hypothetical, unclassified, unknown
PA0321	probable acetyl/polyamine aminohydrolase	Putative enzymes
PA0363	phosphopantetheine adenyltransferase	Central intermediary metabolism
PA0364	probable oxidoreductase	Putative enzymes
PA0385	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA0399	cystathionine beta-synthase	Amino acid biosynthesis and metabolism
PA0436	probable transcriptional regulator	Transcriptional regulators
PA0439	probable oxidoreductase	Putative enzymes
PA0440	probable oxidoreductase	Putative enzymes
PA0441	dihydropyrimidinase	Nucleotide biosynthesis and metabolism
PA0443	probable transporter	Membrane proteins
PA0444	N-carbamoyl-beta-alanine amidohydrolase	Nucleotide biosynthesis and metabolism
PA0453	hypothetical protein	Hypothetical, unclassified, unknown
PA0456	probable cold-shock protein	Transcriptional regulators
PA0459	probable ClpA/B protease ATP binding subunit	Translation, post-translational modification, degradation
PA0467	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0468	hypothetical protein	Hypothetical, unclassified, unknown
PA0469	hypothetical protein	Hypothetical, unclassified, unknown
PA0473	probable glutathione S-transferase	Putative enzymes
PA0474	hypothetical protein	Hypothetical, unclassified, unknown
PA0480	probable hydrolase	Putative enzymes
PA0482	malate synthase G	Central intermediary metabolism
PA0489	probable phosphoribosyl transferase	Putative enzymes
PA0497	hypothetical protein	Hypothetical, unclassified, unknown
PA0498	hypothetical protein	Hypothetical, unclassified, unknown
PA0512	conserved hypothetical protein	Biosynthesis of cofactors, prosthetic groups and carriers
PA0513	probable transcriptional regulator	Biosynthesis of cofactors, prosthetic groups and carriers
PA0527	transcriptional regulator Dnr	Transcriptional regulators
PA0542	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0610	transcriptional regulator PrtN	Transcriptional regulators
PA0614	hypothetical protein	Hypothetical, unclassified, unknown
PA0615	hypothetical protein	Hypothetical, unclassified, unknown
PA0616	hypothetical protein	Related to phage, transposon, or plasmid
PA0617	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0618	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0619	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0620	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0621	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0622	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0623	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0624	hypothetical protein	Related to phage, transposon, or plasmid
PA0625	hypothetical protein	Related to phage, transposon, or plasmid
PA0626	hypothetical protein	Related to phage, transposon, or plasmid
PA0627	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0628	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0629	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0630	hypothetical protein	Related to phage, transposon, or plasmid
PA0631	hypothetical protein	Related to phage, transposon, or plasmid
PA0632	hypothetical protein	Related to phage, transposon, or plasmid
PA0635	hypothetical protein	Related to phage, transposon, or plasmid
PA0636	hypothetical protein	Related to phage, transposon, or plasmid
PA0637	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0638	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0639	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0640	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0641	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0642	hypothetical protein	Related to phage, transposon, or plasmid
PA0643	hypothetical protein	Related to phage, transposon, or plasmid
PA0644	hypothetical protein	Related to phage, transposon, or plasmid
PA0645	hypothetical protein	Related to phage, transposon, or plasmid
PA0646	hypothetical protein	Related to phage, transposon, or plasmid
PA0647	hypothetical protein	Related to phage, transposon, or plasmid
PA0648	hypothetical protein	Related to phage, transposon, or plasmid
PA0671	hypothetical protein	Hypothetical, unclassified, unknown
PA0683	probable type II secretion system protein	Protein secretion/export apparatus
PA0701	probable transcriptional regulator	Transcriptional regulators
PA0717	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0718	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0719	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0720	helix destabilizing protein of bacteriophage Pfl	DNA replication, recombination, modification and repair
PA0721	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0723	coat protein B of bacteriophage Pfl	Related to phage, transposon, or plasmid
PA0736	hypothetical protein	Membrane proteins

ORF	Product name	Function class
PA0740	SDS hydrolase SdsA1	Putative enzymes
PA0745	probable enoyl-CoA hydratase/isomerase	Putative enzymes
PA0746	probable acyl-CoA dehydrogenase	Putative enzymes
PA0747	probable aldehyde dehydrogenase	Putative enzymes
PA0776	hypothetical protein	Hypothetical, unclassified, unknown
PA0783	sodium/proline symporter PutP	Membrane proteins
PA0826	hypothetical protein	Hypothetical, unclassified, unknown
PA0841	hypothetical protein	Hypothetical, unclassified, unknown
PA0845	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0854	fumarate hydratase	Energy metabolism
PA0867	hypothetical protein	Hypothetical, unclassified, unknown
PA0870	aromatic amino acid aminotransferase	Amino acid biosynthesis and metabolism
PA0871	pterin-4-alpha-carbinolamine dehydratase	Amino acid biosynthesis and metabolism
PA0885	probable C4-dicarboxylate transporter	Transport of small molecules
PA0910	hypothetical protein	Hypothetical, unclassified, unknown
PA0938	hypothetical protein	Hypothetical, unclassified, unknown
PA0943	hypothetical protein	Hypothetical, unclassified, unknown
PA0997	Homologous to beta-keto-acyl-acyl-carrier protein synthase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0998	Homologous to beta-keto-acyl-acyl-carrier protein synthase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0999	3-oxoacyl-[acyl-carrier-protein] synthase III	Biosynthesis of cofactors, prosthetic groups and carriers
PA1018	hypothetical protein	Hypothetical, unclassified, unknown
PA1019	cis,cis-muconate transporter MucK	Membrane proteins
PA1025	probable porin	Membrane proteins
PA1049	pyridoxine 5'-phosphate oxidase	Biosynthesis of cofactors, prosthetic groups and carriers
PA1051	probable transporter	Membrane proteins
PA1070	branched-chain amino acid transport protein BraG	Transport of small molecules
PA1072	branched-chain amino acid transport protein BraE	Membrane proteins
PA1073	branched-chain amino acid transport protein BraD	Membrane proteins
PA1074	branched-chain amino acid transport protein BraC	Transport of small molecules
PA1106	hypothetical protein	Hypothetical, unclassified, unknown
PA1113	probable ATP-binding/permease fusion ABC transporter	Membrane proteins
PA1123	hypothetical protein	Hypothetical, unclassified, unknown
PA1127	probable oxidoreductase	Adaptation, Protection
PA1130	rhamnosyltransferase 2	Cell wall / LPS / capsule
PA1131	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1137	probable oxidoreductase	Putative enzymes
PA1143	hypothetical protein	Hypothetical, unclassified, unknown
PA1144	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1150	pyocin S2	Adaptation, Protection
PA1166	hypothetical protein	Hypothetical, unclassified, unknown
PA1183	C4-dicarboxylate transport protein	Transport of small molecules
PA1186	hypothetical protein	Hypothetical, unclassified, unknown
PA1190	conserved hypothetical protein	Membrane proteins
PA1197	hypothetical protein	Hypothetical, unclassified, unknown
PA1206	hypothetical protein	Hypothetical, unclassified, unknown
PA1207	glutathione-regulated potassium-ef flux system protein KefB	Transport of small molecules
PA1220	hypothetical protein	Hypothetical, unclassified, unknown
PA1223	probable transcriptional regulator	Transcriptional regulators
PA1228	hypothetical protein	Hypothetical, unclassified, unknown
PA1236	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1239	hypothetical protein	Hypothetical, unclassified, unknown
PA1242	hypothetical protein	Hypothetical, unclassified, unknown
PA1250	alkaline proteinase inhibitor AprI	Secreted Factors (toxins, enzymes, alginate)
PA1262	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1266	probable oxidoreductase	Putative enzymes
PA1338	gamma-glutamyltranspeptidase precursor	Amino acid biosynthesis and metabolism
PA1340	probable permease of ABC transporter	Transport of small molecules
PA1341	probable permease of ABC transporter	Membrane proteins
PA1342	probable binding protein component of ABC transporter	Transport of small molecules
PA1344	probable short-chain dehydrogenase	Putative enzymes
PA1367	hypothetical protein	Hypothetical, unclassified, unknown
PA1377	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1379	probable short-chain dehydrogenase	Putative enzymes
PA1386	probable ATP-binding component of ABC transporter	Transport of small molecules
PA1395	hypothetical protein	Hypothetical, unclassified, unknown
PA1399	probable transcriptional regulator	Transcriptional regulators
PA1400	probable pyruvate carboxylase	Energy metabolism
PA1416	conserved hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA1418	probable sodium:solute symport protein	Transport of small molecules
PA1419	probable transporter	Membrane proteins
PA1426	hypothetical protein	Hypothetical, unclassified, unknown
PA1431	regulatory protein RsaL	Secreted Factors (toxins, enzymes, alginate)
PA1440	hypothetical protein	Hypothetical, unclassified, unknown
PA1519	probable transporter	Membrane proteins
PA1537	probable short-chain dehydrogenase	Putative enzymes
PA1538	probable flavin-containing monooxygenase	Putative enzymes
PA1540	conserved hypothetical protein	Membrane proteins
PA1542	hypothetical protein	Hypothetical, unclassified, unknown
PA1568	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1569	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1575	hypothetical protein	Hypothetical, unclassified, unknown
PA1576	probable 3-hydroxyisobutyrate dehydrogenase	Putative enzymes
PA1608	probable chemotaxis transducer	Adaptation, Protection
PA1609	beta-ketoacyl-ACP synthase I	Fatty acid and phospholipid metabolism
PA1610	beta-hydroxydecanoyl-ACP dehydrase	Fatty acid and phospholipid metabolism
PA1617	probable AMP-binding enzyme	Putative enzymes
PA1650	probable transporter	Membrane proteins
PA1746	hypothetical protein	Hypothetical, unclassified, unknown
PA1759	probable transcriptional regulator	Transcriptional regulators
PA1763	hypothetical protein	Hypothetical, unclassified, unknown
PA1768	hypothetical protein	Hypothetical, unclassified, unknown
PA1772	probable methyltransferase	Biosynthesis of cofactors, prosthetic groups and carriers
PA1774	CfrX protein	Hypothetical, unclassified, unknown
PA1775	conserved cytoplasmic membrane protein, CmpX protein	Membrane proteins
PA1776	ECF sigma factor SigX	Transcriptional regulators
PA1781	assimilatory nitrite reductase large subunit	Central intermediary metabolism
PA1784	hypothetical protein	Hypothetical, unclassified, unknown
PA1789	hypothetical protein	Hypothetical, unclassified, unknown
PA1790	hypothetical protein	Hypothetical, unclassified, unknown
PA1812	membrane-bound lytic murein transglycosylase D precursor	Amino acid biosynthesis and metabolism
PA1824	conserved hypothetical protein	Membrane proteins
PA1852	hypothetical protein	Hypothetical, unclassified, unknown
PA1853	probable transcriptional regulator	Transcriptional regulators
PA1871	LasA protease precursor	Secreted Factors (toxins, enzymes, alginate)
PA1875	probable outer membrane protein precursor	Protein secretion/export apparatus
PA1891	hypothetical protein	Membrane proteins
PA1892	hypothetical protein	Hypothetical, unclassified, unknown
PA1893	hypothetical protein	Putative enzymes
PA1895	hypothetical protein	Membrane proteins
PA1896	hypothetical protein	Hypothetical, unclassified, unknown
PA1897	hypothetical protein	Hypothetical, unclassified, unknown
PA1898	quorum-sensing control repressor	Transcriptional regulators
PA1901	phenazine biosynthesis protein PhzC	Secreted Factors (toxins, enzymes, alginate)
PA1903	phenazine biosynthesis protein PhzE	Secreted Factors (toxins, enzymes, alginate)
PA1905	probable pyridoxamine 5'-phosphate oxidase	Secreted Factors (toxins, enzymes, alginate)
PA1922	probable TonB-dependent receptor	Transport of small molecules
PA1924	hypothetical protein	Hypothetical, unclassified, unknown
PA1927	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Amino acid biosynthesis and metabolism
PA1935	hypothetical protein	Hypothetical, unclassified, unknown
PA1942	hypothetical protein	Hypothetical, unclassified, unknown
PA1946	binding protein component precursor of ABC ribose transporter	Transport of small molecules
PA1947	ribose transport protein RbsA	Transport of small molecules
PA1955	hypothetical protein	Hypothetical, unclassified, unknown
PA1974	hypothetical protein	Hypothetical, unclassified, unknown
PA1976	probable two-component sensor	Two-component regulatory systems
PA1977	hypothetical protein	Membrane proteins
PA1978	probable transcriptional regulator	Transcriptional regulators
PA1979	probable two-component sensor	Two-component regulatory systems
PA1980	probable two-component response regulator	Transcriptional regulators
PA1981	hypothetical protein	Hypothetical, unclassified, unknown
PA1983	cytochrome c550	Energy metabolism
PA1984	probable aldehyde dehydrogenase	Putative enzymes
PA1991	probable iron-containing alcohol dehydrogenase	Putative enzymes
PA1992	probable two-component sensor	Two-component regulatory systems
PA2000	probable CoA transferase, subunit B	Amino acid biosynthesis and metabolism
PA2001	acetyl-CoA acetyltransferase	Central intermediary metabolism

ORF	Product name	Function class
PA2002	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2003	3-hydroxybutyrate dehydrogenase	Carbon compound catabolism
PA2004	conserved hypothetical protein	Membrane proteins
PA2006	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2012	methylcrotonyl-CoA carboxylase, alpha-subunit (biotin-containing)	Carbon compound catabolism
PA2013	putative 3-methylglutaconyl-CoA hydratase	Carbon compound catabolism
PA2014	methylcrotonyl-CoA carboxylase, beta-subunit	Carbon compound catabolism
PA2015	putative isovaleryl-CoA dehydrogenase	Carbon compound catabolism
PA2018	Resistance-Nodulation-Cell Division (RND) multidrug ef flux transporter	Transport of small molecules
PA2030	hypothetical protein	Hypothetical, unclassified, unknown
PA2031	hypothetical protein	Hypothetical, unclassified, unknown
PA2041	probable amino acid permease	Transport of small molecules
PA2060	probable permease of ABC transporter	Transport of small molecules
PA2066	hypothetical protein	Hypothetical, unclassified, unknown
PA2067	probable hydrolase	Putative enzymes
PA2068	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2069	probable carbamoyl transferase	Putative enzymes
PA2079	probable amino acid permease	Transport of small molecules
PA2091	hypothetical protein	Membrane proteins
PA2109	hypothetical protein	Hypothetical, unclassified, unknown
PA2110	hypothetical protein	Hypothetical, unclassified, unknown
PA2116	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2119	alcohol dehydrogenase (Zn-dependent)	Putative enzymes
PA2122	hypothetical protein	Hypothetical, unclassified, unknown
PA2129	chaperone CupA2	Motility & Attachment
PA2134	hypothetical protein	Hypothetical, unclassified, unknown
PA2160	probable glycosyl hydrolase	Putative enzymes
PA2161	hypothetical protein	Hypothetical, unclassified, unknown
PA2169	hypothetical protein	Hypothetical, unclassified, unknown
PA2172	hypothetical protein	Hypothetical, unclassified, unknown
PA2185	non-heme catalase KatN	Adaptation, Protection
PA2198	hypothetical protein	Hypothetical, unclassified, unknown
PA2201	hypothetical protein	Hypothetical, unclassified, unknown
PA2209	hypothetical protein	Hypothetical, unclassified, unknown
PA2210	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2211	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2212	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2213	probable porin	Membrane proteins
PA2214	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2217	probable aldehyde dehydrogenase	Putative enzymes
PA2225	hypothetical protein	Hypothetical, unclassified, unknown
PA2228	hypothetical protein	Hypothetical, unclassified, unknown
PA2249	branched-chain alpha-keto acid dehydrogenase (lipoamide component)	Amino acid biosynthesis and metabolism
PA2250	lipoamide dehydrogenase-V al	Amino acid biosynthesis and metabolism
PA2258	transcriptional regulator PtxR	Secreted Factors (toxins, enzymes, alginate)
PA2274	hypothetical protein	Hypothetical, unclassified, unknown
PA2300	chitinase	Carbon compound catabolism
PA2303	hypothetical protein	Hypothetical, unclassified, unknown
PA2313	hypothetical protein	Membrane proteins
PA2322	gluconate permease	Transport of small molecules
PA2328	hypothetical protein	Hypothetical, unclassified, unknown
PA2329	probable ATP-binding component of ABC transporter	Transport of small molecules
PA2330	hypothetical protein	Hypothetical, unclassified, unknown
PA2334	probable transcriptional regulator	Transcriptional regulators
PA2359	probable transcriptional regulator	Transcriptional regulators
PA2423	hypothetical protein	Hypothetical, unclassified, unknown
PA2439	hypothetical protein	Membrane proteins
PA2453	hypothetical protein	Hypothetical, unclassified, unknown
PA2474	hypothetical protein	Hypothetical, unclassified, unknown
PA2499	probable deaminase	Putative enzymes
PA2507	catechol 1,2-dioxygenase	Carbon compound catabolism
PA2508	muconolactone delta-isomerase	Carbon compound catabolism
PA2509	muconate cycloisomerase I	Carbon compound catabolism
PA2511	probable transcriptional regulator	Transcriptional regulators
PA2512	anthranilate dioxygenase large subunit	Carbon compound catabolism
PA2513	anthranilate dioxygenase small subunit	Carbon compound catabolism
PA2514	anthranilate dioxygenase reductase	Carbon compound catabolism
PA2515	cis-1,2-dihydroxycyclohexa-3,4-diene carboxylate dehydrogenase	Carbon compound catabolism

ORF	Product name	Function class
PA2516	toluate 1,2-dioxygenase electron transfer component	Energy metabolism
PA2517	toluate 1,2-dioxygenase beta subunit	Carbon compound catabolism
PA2518	toluate 1,2-dioxygenase alpha subunit	Carbon compound catabolism
PA2519	transcriptional regulator XylS	Transcriptional regulators
PA2529	hypothetical protein	Hypothetical, unclassified, unknown
PA2554	probable short-chain dehydrogenase	Putative enzymes
PA2555	probable AMP-binding enzyme	Putative enzymes
PA2557	probable AMP-binding enzyme	Fatty acid and phospholipid metabolism
PA2564	hypothetical protein	Hypothetical, unclassified, unknown
PA2566	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2567	hypothetical protein	Hypothetical, unclassified, unknown
PA2570	LecA	Adaptation, Protection
PA2587	probable FAD-dependent monooxygenase	Biosynthesis of cofactors, prosthetic groups and carriers
PA2591	probable transcriptional regulator	Transcriptional regulators
PA2595	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2625	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2629	adenylosuccinate lyase	Amino acid biosynthesis and metabolism
PA2654	probable chemotaxis transducer	Adaptation, Protection
PA2660	hypothetical protein	Hypothetical, unclassified, unknown
PA2667	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2682	conserved hypothetical protein	Putative enzymes
PA2711	probable periplasmic spermidine/putrescine-binding protein	Transport of small molecules
PA2717	chloroperoxidase precursor	Central intermediary metabolism
PA2719	hypothetical protein	Hypothetical, unclassified, unknown
PA2753	hypothetical protein	Hypothetical, unclassified, unknown
PA2754	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2769	hypothetical protein	Hypothetical, unclassified, unknown
PA2776	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2778	hypothetical protein	Hypothetical, unclassified, unknown
PA2779	hypothetical protein	Hypothetical, unclassified, unknown
PA2780	hypothetical protein	Hypothetical, unclassified, unknown
PA2781	hypothetical protein	Hypothetical, unclassified, unknown
PA2801	hypothetical protein	Hypothetical, unclassified, unknown
PA2805	hypothetical protein	Hypothetical, unclassified, unknown
PA2819	hypothetical protein	Hypothetical, unclassified, unknown
PA2838	probable transcriptional regulator	Transcriptional regulators
PA2847	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2851	translation elongation factor P	Translation, post-translational modification, degradation
PA2876	orotidine 5'-phosphate decarboxylase	Nucleotide biosynthesis and metabolism
PA2892	GCse, alpha-subunit (biotin-containing)	Putative enzymes
PA2905	precorrin isomerase CobH	Biosynthesis of cofactors, prosthetic groups and carriers
PA2915	hypothetical protein	Hypothetical, unclassified, unknown
PA2922	probable hydrolase	Putative enzymes
PA2924	histidine transport system permease HisQ	Membrane proteins
PA2927	hypothetical protein	Hypothetical, unclassified, unknown
PA2938	probable transporter	Membrane proteins
PA2939	probable aminopeptidase	Secreted Factors (toxins, enzymes, alginate)
PA2942	probable magnesium chelatase	Biosynthesis of cofactors, prosthetic groups and carriers
PA2950	hypothetical protein	Hypothetical, unclassified, unknown
PA2960	type 4 fimbrial biogenesis protein PilZ	Motility & Attachment
PA2966	acyl carrier protein	Fatty acid and phospholipid metabolism
PA2967	3-oxoacyl-[acyl-carrier-protein] reductase	Fatty acid and phospholipid metabolism
PA2968	malonyl-CoA-[acyl-carrier-protein] transacylase	Fatty acid and phospholipid metabolism
PA3032	cytochrome c SsrI	Energy metabolism
PA3035	probable glutathione S-transferase	Putative enzymes
PA3036	hypothetical protein	Hypothetical, unclassified, unknown
PA3062	PeIC	Cell wall / LPS / capsule
PA3066	hypothetical protein	Hypothetical, unclassified, unknown
PA3079	hypothetical protein	Membrane proteins
PA3131	probable aldolase	Central intermediary metabolism
PA3139	probable amino acid aminotransferase	Amino acid biosynthesis and metabolism
PA3186	Glucose/carbohydrate outer membrane porin OprB precursor	Transport of small molecules
PA3188	probable permease of ABC sugar transporter	Transport of small molecules
PA3189	probable permease of ABC sugar transporter	Transport of small molecules
PA3208	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3209	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3213	hypothetical protein	Hypothetical, unclassified, unknown
PA3221	CsaA protein	Protein secretion/export apparatus

ORF	Product name	Function class
PA3234	probable sodium:solute symporter	Membrane proteins
PA3236	probable glycine betaine-binding protein precursor	Adaptation, Protection
PA3237	hypothetical protein	Hypothetical, unclassified, unknown
PA3249	probable transcriptional regulator	Transcriptional regulators
PA3270	hypothetical protein	Hypothetical, unclassified, unknown
PA3283	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3295	probable HIT family protein	Putative enzymes
PA3301	hypothetical protein	Putative enzymes
PA3309	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3313	hypothetical protein	Transport of small molecules
PA3326	probable Clp-family ATP-dependent protease	Translation, post-translational modification, degradation
PA3329	hypothetical protein	Hypothetical, unclassified, unknown
PA3330	probable short chain dehydrogenase	Putative enzymes
PA3331	cytochrome P450	Adaptation, Protection
PA3332	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3333	3-oxoacyl-[acyl-carrier-protein] synthase III	Fatty acid and phospholipid metabolism
PA3334	probable acyl carrier protein	Fatty acid and phospholipid metabolism
PA3335	hypothetical protein	Hypothetical, unclassified, unknown
PA3336	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3337	ADP-L-glycero-D-mannoheptose 6-epimerase	Cell wall / LPS / capsule
PA3361	fucose-binding lectin PA-III	Motility & Attachment
PA3362	hypothetical protein	Membrane proteins
PA3363	aliphatic amidase regulator	Carbon compound catabolism
PA3364	aliphatic amidase expression-regulating protein	Transcriptional regulators
PA3365	probable chaperone	Chaperones & heat shock proteins
PA3366	aliphatic amidase	Carbon compound catabolism
PA3394	NosF protein	Energy metabolism
PA3422	hypothetical protein	Hypothetical, unclassified, unknown
PA3431	conserved hypothetical protein	Membrane proteins
PA3432	hypothetical protein	Membrane proteins
PA3458	probable transcriptional regulator	Transcriptional regulators
PA3466	probable ATP-dependent RNA helicase	Transcription, RNA processing and degradation
PA3467	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3478	rhamnosyltransferase chain B	Secreted Factors (toxins, enzymes, alginate)
PA3479	rhamnosyltransferase chain A	Secreted Factors (toxins, enzymes, alginate)
PA3480	probable deoxycytidine triphosphate deaminase	Nucleotide biosynthesis and metabolism
PA3507	probable short-chain dehydrogenase	Putative enzymes
PA3524	lactoylglutathione lyase	Central intermediary metabolism
PA3529	probable peroxidase	Adaptation, Protection
PA3535	probable serine protease	Putative enzymes
PA3542	alginate biosynthesis protein Alg44	Cell wall / LPS / capsule
PA3555	ArnD	Adaptation, Protection
PA3559	probable nucleotide sugar dehydrogenase	Putative enzymes
PA3560	phosphotransferase system, fructose-specific IIBC component	Carbon compound catabolism
PA3562	probable phosphotransferase system enzyme I	Central intermediary metabolism
PA3564	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3565	probable transcriptional regulator	Transcriptional regulators
PA3568	probable acetyl-coa synthetase	Putative enzymes
PA3569	3-hydroxyisobutyrate dehydrogenase	Carbon compound catabolism
PA3570	methylmalonate-semialdehyde dehydrogenase	Amino acid biosynthesis and metabolism
PA3572	hypothetical protein	Hypothetical, unclassified, unknown
PA3581	glycerol uptake facilitator protein	Transport of small molecules
PA3582	glycerol kinase	Central intermediary metabolism
PA3586	probable hydrolase	Putative enzymes
PA3595	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3608	polyamine transport protein PotB	Membrane proteins
PA3613	hypothetical protein	Hypothetical, unclassified, unknown
PA3614	hypothetical protein	Hypothetical, unclassified, unknown
PA3618	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3621	ferredoxin I	Energy metabolism
PA3661	hypothetical protein	Hypothetical, unclassified, unknown
PA3662	hypothetical protein	Hypothetical, unclassified, unknown
PA3674	hypothetical protein	Hypothetical, unclassified, unknown
PA3682	hypothetical protein	Hypothetical, unclassified, unknown
PA3686	adenylate kinase	Nucleotide biosynthesis and metabolism
PA3692	probable outer membrane protein precursor	Membrane proteins
PA3699	probable transcriptional regulator	Transcriptional regulators
PA3710	probable GMC-type oxidoreductase	Carbon compound catabolism

ORF	Product name	Function class
PA3718	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3724	elastase LasB	Secreted Factors (toxins, enzymes, alginate)
PA3737	thiol:disulfide interchange protein DsbC	Translation, post-translational modification, degradation
PA3779	hypothetical protein	Hypothetical, unclassified, unknown
PA3780	hypothetical protein	Membrane proteins
PA3781	probable transporter	Membrane proteins
PA3789	hypothetical protein	Membrane proteins
PA3795	probable oxidoreductase	Putative enzymes
PA3836	hypothetical protein	Hypothetical, unclassified, unknown
PA3837	probable permease of ABC transporter	Membrane proteins
PA3838	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3839	probable sodium:sulfate symporter	Membrane proteins
PA3840	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3865	probable amino acid binding protein	Transport of small molecules
PA3880	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3887	Na ⁺ /H ⁺ antiporter NhaP	Membrane proteins
PA3906	hypothetical protein	Hypothetical, unclassified, unknown
PA3908	hypothetical protein	Hypothetical, unclassified, unknown
PA3913	probable protease	Putative enzymes
PA3940	probable DNA binding protein	DNA replication, recombination, modification and repair
PA3959	hypothetical protein	Hypothetical, unclassified, unknown
PA3960	hypothetical protein	Hypothetical, unclassified, unknown
PA3966	hypothetical protein	Membrane proteins
PA3967	hypothetical protein	Hypothetical, unclassified, unknown
PA3984	apolipoprotein N-acyltransferase	Cell wall / LPS / capsule
PA4021	probable transcriptional regulator	Transcriptional regulators
PA4023	probable transport protein	Transport of small molecules
PA4024	ethanolamine ammonia-lyase lar ge subunit	Central intermediary metabolism
PA4025	probable ethanolamine ammonia-lyase light chain	Carbon compound catabolism
PA4031	inorganic pyrophosphatase	Central intermediary metabolism
PA4055	riboflavin synthase alpha chain	Biosynthesis of cofactors, prosthetic groups and carriers
PA4057	NrdR	Nucleotide biosynthesis and metabolism
PA4058	hypothetical protein	Hypothetical, unclassified, unknown
PA4070	probable transcriptional regulator	Transcriptional regulators
PA4072	probable amino acid permease	Transport of small molecules
PA4073	probable aldehyde dehydrogenase	Putative enzymes
PA4078	probable nonribosomal peptide synthetase	Adaptation, Protection
PA4079	probable dehydrogenase	Putative enzymes
PA4091	4-hydroxyphenylacetate 3-monooxygenase lar ge chain	Carbon compound catabolism
PA4092	4-hydroxyphenylacetate 3-monooxygenase small chain	Carbon compound catabolism
PA4093	hypothetical protein	Hypothetical, unclassified, unknown
PA4094	probable transcriptional regulator	Transcriptional regulators
PA4120	probable transcriptional regulator	Transcriptional regulators
PA4121	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4122	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4123	5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase	Carbon compound catabolism
PA4124	homoprotocatechuate 2,3-dioxygenase	Carbon compound catabolism
PA4125	5-carboxymethyl-2-hydroxymuconate isomerase	Carbon compound catabolism
PA4126	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA4127	2-oxo-hept-3-ene-1,7-dioate hydratase	Carbon compound catabolism
PA4128	conserved hypothetical protein	Putative enzymes
PA4129	hypothetical protein	Hypothetical, unclassified, unknown
PA4130	probable sulfite or nitrite reductase	Central intermediary metabolism
PA4131	probable iron-sulfur protein	Putative enzymes
PA4132	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4133	cytochrome c oxidase subunit (cbb3-type)	Energy metabolism
PA4134	hypothetical protein	Hypothetical, unclassified, unknown
PA4136	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA4137	probable porin	Transport of small molecules
PA4140	hypothetical protein	Hypothetical, unclassified, unknown
PA4141	hypothetical protein	Hypothetical, unclassified, unknown
PA4142	probable secretion protein	Protein secretion/export apparatus
PA4147	transcriptional regulator AcoR	Transcriptional regulators
PA4148	probable short-chain dehydrogenase	Putative enzymes
PA4150	probable dehydrogenase E1 component	Carbon compound catabolism
PA4151	acetoin catabolism protein AcoB	Carbon compound catabolism
PA4152	probable hydrolase	Carbon compound catabolism
PA4153	2,3-butanediol dehydrogenase	Carbon compound catabolism

ORF	Product name	Function class
PA4175	protease IV	Secreted Factors (toxins, enzymes, alginate)
PA4180	probable acetolactate synthase large subunit	Amino acid biosynthesis and metabolism
PA4205	hypothetical protein	Membrane proteins
PA4206	probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein	Transport of small molecules
PA4207	probable Resistance-Nodulation-Cell Division (RND) efflux transporter	Membrane proteins
PA4208	probable outer membrane protein precursor	Membrane proteins
PA4209	probable phenazine-specific methyltransferase	Putative enzymes
PA4210	probable phenazine biosynthesis protein	Secreted Factors (toxins, enzymes, alginate)
PA4211	probable phenazine biosynthesis protein	Secreted Factors (toxins, enzymes, alginate)
PA4288	probable transcriptional regulator	Transcriptional regulators
PA4293	two-component sensor PprA	Two-component regulatory systems
PA4294	hypothetical protein	Hypothetical, unclassified, unknown
PA4297	TadG	Membrane proteins
PA4298	hypothetical protein	Hypothetical, unclassified, unknown
PA4316	exodeoxyribonuclease I	DNA replication, recombination, modification and repair
PA4324	hypothetical protein	Hypothetical, unclassified, unknown
PA4328	hypothetical protein	Hypothetical, unclassified, unknown
PA4329	pyruvate kinase II	Energy metabolism
PA4348	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4351	probable acyltransferase	Fatty acid and phospholipid metabolism
PA4352	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4357	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4358	probable ferrous iron transport protein	Membrane proteins
PA4377	hypothetical protein	Hypothetical, unclassified, unknown
PA4394	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4433	50S ribosomal protein L13	Translation, post-translational modification, degradation
PA4436	probable transcriptional regulator	Transcriptional regulators
PA4496	probable binding protein component of ABC transporter	Transport of small molecules
PA4497	probable binding protein component of ABC transporter	Transport of small molecules
PA4498	probable metalloprotease	Translation, post-translational modification, degradation
PA4500	probable binding protein component of ABC transporter	Transport of small molecules
PA4502	probable binding protein component of ABC transporter	Transport of small molecules
PA4503	probable permease of ABC transporter	Membrane proteins
PA4504	probable permease of ABC transporter	Membrane proteins
PA4505	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4506	probable ATP-binding component of ABC dipeptide transporter	Transport of small molecules
PA4520	probable chemotaxis transducer	Adaptation, Protection
PA4569	octaprenyl-diphosphate synthase	Energy metabolism
PA4577	hypothetical protein	Hypothetical, unclassified, unknown
PA4588	glutamate dehydrogenase	Amino acid biosynthesis and metabolism
PA4596	probable transcriptional regulator	Transcriptional regulators
PA4606	conserved hypothetical protein	Adaptation, Protection
PA4611	hypothetical protein	Hypothetical, unclassified, unknown
PA4632	hypothetical protein	Hypothetical, unclassified, unknown
PA4633	probable chemotaxis transducer	Adaptation, Protection
PA4654	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA4676	probable carbonic anhydrase	Putative enzymes
PA4677	hypothetical protein	Hypothetical, unclassified, unknown
PA4681	hypothetical protein	Hypothetical, unclassified, unknown
PA4682	hypothetical protein	Hypothetical, unclassified, unknown
PA4683	hypothetical protein	Hypothetical, unclassified, unknown
PA4695	acetolactate synthase isozyme III small subunit	Biosynthesis of cofactors, prosthetic groups and carriers
PA4715	probable aminotransferase	Putative enzymes
PA4733	acetyl-coenzyme A synthetase	Carbon compound catabolism
PA4757	conserved hypothetical protein	Membrane proteins
PA4758	carbamoyl-phosphate synthase small chain	Amino acid biosynthesis and metabolism
PA4759	dihydrodipicolinate reductase	Amino acid biosynthesis and metabolism
PA4770	L-lactate permease	Transport of small molecules
PA4773	hypothetical protein	Hypothetical, unclassified, unknown
PA4779	hypothetical protein	Membrane proteins
PA4782	hypothetical protein	Hypothetical, unclassified, unknown
PA4808	L-seryl-tRNA(ser) selenium transferase	Translation, post-translational modification, degradation
PA4817	hypothetical protein	Hypothetical, unclassified, unknown
PA4851	hypothetical protein	Hypothetical, unclassified, unknown
PA4852	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4860	probable permease of ABC transporter	Membrane proteins
PA4862	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4867	urease beta subunit	Central intermediary metabolism

ORF	Product name	Function class
PA4884	hypothetical protein	Hypothetical, unclassified, unknown
PA4893	urease accessory protein UreG	Biosynthesis of cofactors, prosthetic groups and carriers
PA4894	hypothetical protein	Membrane proteins
PA4899	probable aldehyde dehydrogenase	Putative enzymes
PA4901	benzoylformate decarboxylase	Carbon compound catabolism
PA4908	hypothetical protein	Hypothetical, unclassified, unknown
PA4910	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4911	probable permease of ABC branched-chain amino acid transporter	Membrane proteins
PA4912	probable permease of ABC branched chain amino acid transporter	Membrane proteins
PA4913	probable binding protein component of ABC transporter	Transport of small molecules
PA4943	probable GTP-binding protein	Putative enzymes
PA4979	probable acyl-CoA dehydrogenase	Putative enzymes
PA4983	probable two-component response regulator	Transcriptional regulators
PA4984	probable transcriptional regulator	Transcriptional regulators
PA4985	hypothetical protein	Hypothetical, unclassified, unknown
PA4986	probable oxidoreductase	Putative enzymes
PA4997	transport protein MshA	Fatty acid and phospholipid metabolism
PA5020	probable acyl-CoA dehydrogenase	Putative enzymes
PA5025	homocysteine synthase	Amino acid biosynthesis and metabolism
PA5027	hypothetical protein	Hypothetical, unclassified, unknown
PA5028	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5030	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA5051	arginyl-tRNA synthetase	Translation, post-translational modification, degradation
PA5059	probable transcriptional regulator	Transcriptional regulators
PA5082	probable binding protein component of ABC transporter	Transport of small molecules
PA5096	probable binding protein component of ABC transporter	Transport of small molecules
PA5097	probable amino acid permease	Membrane proteins
PA5098	histidine ammonia-lyase	Amino acid biosynthesis and metabolism
PA5106	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5112	esterase EstA	Secreted Factors (toxins, enzymes, alginate)
PA5129	glutaredoxin	Energy metabolism
PA5130	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5137	hypothetical protein	Hypothetical, unclassified, unknown
PA5149	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5152	probable ATP-binding component of ABC transporter	Transport of small molecules
PA5153	probable periplasmic binding protein	Transport of small molecules
PA5154	probable permease of ABC transporter	Membrane proteins
PA5155	probable permease of ABC transporter	Membrane proteins
PA5161	dTDP-D-glucose 4,6-dehydratase	Carbon compound catabolism
PA5162	dTDP-4-dehydrothiamine reductase	Cell wall / LPS / capsule
PA5163	glucose-1-phosphate thymidyltransferase	Cell wall / LPS / capsule
PA5164	dTDP-4-dehydrothiamine 3,5-epimerase	Cell wall / LPS / capsule
PA5168	probable dicarboxylate transporter	Membrane proteins
PA5169	probable C4-dicarboxylate transporter	Membrane proteins
PA5174	probable beta-ketoacyl synthase	Fatty acid and phospholipid metabolism
PA5180	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5220	hypothetical protein	Hypothetical, unclassified, unknown
PA5237	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5240	thioredoxin	Translation, post-translational modification, degradation
PA5249	hypothetical protein	Membrane proteins
PA5275	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5285	hypothetical protein	Hypothetical, unclassified, unknown
PA5286	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5288	nitrogen regulatory protein P-II 2	Central intermediary metabolism
PA5289	hypothetical protein	Hypothetical, unclassified, unknown
PA5297	pyruvate dehydrogenase (cytochrome)	Central intermediary metabolism
PA5303	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5309	probable oxidoreductase	Putative enzymes
PA5324	probable transcriptional regulator	Transcriptional regulators
PA5325	hypothetical protein	Hypothetical, unclassified, unknown
PA5328	probable cytochrome c (mono-heme type)	Energy metabolism
PA5329	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5338	guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase	Nucleotide biosynthesis and metabolism
PA5344	probable transcriptional regulator	Transcriptional regulators
PA5348	probable DNA-binding protein	DNA replication, recombination, modification and repair
PA5351	Rubredoxin 1	Carbon compound catabolism
PA5353	glycolate oxidase subunit GlcF	Central intermediary metabolism
PA5355	glycolate oxidase subunit GlcD	Central intermediary metabolism

ORF	Product name	Function class
PA5380	GbdR	Transcriptional regulators
PA5381	hypothetical protein	Hypothetical, unclassified, unknown
PA5400	probable electron transfer flavoprotein alpha subunit	Energy metabolism
PA5409	hypothetical protein	Membrane proteins
PA5427	alcohol dehydrogenase	Energy metabolism
PA5434	tryptophan permease	Membrane proteins
PA5435	probable transcarboxylase subunit	Central intermediary metabolism
PA5436	probable biotin carboxylase subunit of a transcarboxylase	Central intermediary metabolism
PA5442	conserved hypothetical protein	Membrane proteins
PA5445	probable coenzyme A transferase	Putative enzymes
PA5451	membrane subunit of A-band LPS efflux transporter	Cell wall / LPS / capsule
PA5452	phosphomannose isomerase/GDP-mannose WbpW	Cell wall / LPS / capsule
PA5461	hypothetical protein	Hypothetical, unclassified, unknown
PA5475	hypothetical protein	Hypothetical, unclassified, unknown
PA5491	probable cytochrome	Energy metabolism
PA5506	hypothetical protein	Hypothetical, unclassified, unknown
PA5507	hypothetical protein	Hypothetical, unclassified, unknown
PA5508	probable glutamine synthetase	Putative enzymes
PA5509	hypothetical protein	Hypothetical, unclassified, unknown
PA5510	probable transporter	Membrane proteins
PA5522	probable glutamine synthetase	Putative enzymes
PA5523	probable aminotransferase	Putative enzymes
PA5533	hypothetical protein	Hypothetical, unclassified, unknown
PA5536	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5542	hypothetical protein	Putative enzymes
PA5543	hypothetical protein	Hypothetical, unclassified, unknown
PA5544	conserved hypothetical protein	Membrane proteins
PA5545	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5546	conserved hypothetical protein	Putative enzymes

ORF	Product name	Function class
Oxidative stress		
PA0007	hypothetical protein	Membrane proteins
PA0069	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0105	cytochrome c oxidase, subunit II	Energy metabolism
PA0106	cytochrome c oxidase, subunit I	Energy metabolism
PA0107	conserved hypothetical protein	Energy metabolism
PA0109	hypothetical protein	Membrane proteins
PA0122	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0128	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0158	RND; triclosan efflux transporter, TriC	Antibiotic resistance and susceptibility
PA0171	hypothetical protein	Motility & Attachment
PA0173	probable methylesterase	Adaptation, Protection
PA0174	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0176	aerotaxis transducer Aer2	Adaptation, Protection
PA0177	probable purine-binding chemotaxis protein	Adaptation, Protection
PA0178	probable two-component sensor	Chemotaxis
PA0179	probable two-component response regulator	Chemotaxis
PA0182	probable short-chain dehydrogenase	Putative enzymes
PA0250	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0280	sulfate transport protein CysA	Transport of small molecules
PA0281	sulfate transport protein CysW	Membrane proteins
PA0282	sulfate transport protein CysT	Membrane proteins
PA0283	sulfate-binding protein precursor	Transport of small molecules
PA0335	hypothetical protein	Hypothetical, unclassified, unknown
PA0337	phosphoenolpyruvate-protein phosphotransferase PtsP	Transport of small molecules
PA0352	probable transporter	Membrane proteins
PA0382	DNA mismatch repair protein MicA	DNA replication, recombination, modification and repair
PA0385	hypothetical protein	Hypothetical, unclassified, unknown
PA0459	probable ClpA/B protease ATP binding subunit	Translation, post-translational modification, degradation
PA0492	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0493	probable biotin-requiring enzyme	Putative enzymes
PA0494	probable acyl-CoA carboxylase subunit	Putative enzymes
PA0495	hypothetical protein	Hypothetical, unclassified, unknown
PA0555	fructose-1,6-bisphosphate aldolase	Central intermediary metabolism
PA0582	dihydroneopterin aldolase	Biosynthesis of cofactors, prosthetic groups and carriers
PA0586	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0587	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0612	repressor, PtrB	Transcriptional regulators
PA0613	hypothetical protein	Hypothetical, unclassified, unknown
PA0614	hypothetical protein	Hypothetical, unclassified, unknown
PA0615	hypothetical protein	Hypothetical, unclassified, unknown
PA0616	hypothetical protein	Related to phage, transposon, or plasmid
PA0617	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0618	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0619	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0620	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0621	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0622	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0623	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0624	hypothetical protein	Related to phage, transposon, or plasmid
PA0625	hypothetical protein	Related to phage, transposon, or plasmid
PA0626	hypothetical protein	Related to phage, transposon, or plasmid
PA0627	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0628	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0629	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0630	hypothetical protein	Related to phage, transposon, or plasmid
PA0631	hypothetical protein	Related to phage, transposon, or plasmid
PA0634	hypothetical protein	Related to phage, transposon, or plasmid
PA0635	hypothetical protein	Related to phage, transposon, or plasmid
PA0636	hypothetical protein	Related to phage, transposon, or plasmid
PA0637	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0638	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0639	conserved hypothetical protein	Related to phage, transposon, or plasmid
PA0640	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0641	probable bacteriophage protein	Related to phage, transposon, or plasmid
PA0642	hypothetical protein	Related to phage, transposon, or plasmid
PA0643	hypothetical protein	Related to phage, transposon, or plasmid

ORF	Product name	Function class
PA0645	hypothetical protein	Related to phage, transposon, or plasmid
PA0646	hypothetical protein	Related to phage, transposon, or plasmid
PA0669	probable DNA polymerase alpha chain	Putative enzymes
PA0670	hypothetical protein	Hypothetical, unclassified, unknown
PA0671	hypothetical protein	Hypothetical, unclassified, unknown
PA0743	probable 3-hydroxyisobutyrate dehydrogenase	Carbon compound catabolism
PA0745	probable enoyl-CoA hydratase/isomerase	Putative enzymes
PA0746	probable acyl-CoA dehydrogenase	Putative enzymes
PA0747	probable aldehyde dehydrogenase	Putative enzymes
PA0873	transcriptional regulator PhhR	Transcriptional regulators
PA0887	acetyl-coenzyme A synthetase	Carbon compound catabolism
PA0887.1	aaa	Non-coding RNA gene
PA0889	arginine/ornithine transport protein AotQ	Membrane proteins
PA0905	RsmA, regulator of secondary metabolites	Translation, post-translational modification, degradation
PA0915	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0916	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0922	hypothetical protein	Hypothetical, unclassified, unknown
PA0975	probable radical activating enzyme	Putative enzymes
PA0976	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0983	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0996	probable coenzyme A ligase	Biosynthesis of cofactors, prosthetic groups and carriers
PA1000	Quinolone signal response protein	Biosynthesis of cofactors, prosthetic groups and carriers
PA1001	anthranilate synthase component I	Adaptation, Protection
PA1002	anthranilate synthase component II	Amino acid biosynthesis and metabolism
PA1041	probable outer membrane protein precursor	Membrane proteins
PA1112	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1150	pyocin S2	Adaptation, Protection
PA1151	pyocin S2 immunity protein	Adaptation, Protection
PA1172	cytochrome c-type protein NapC	Energy metabolism
PA1173	cytochrome c-type protein NapB precursor	Energy metabolism
PA1174	periplasmic nitrate reductase protein NapA	Energy metabolism
PA1175	NapD protein of periplasmic nitrate reductase	Energy metabolism
PA1176	ferredoxin protein NapF	Energy metabolism
PA1177	periplasmic nitrate reductase protein NapE	Energy metabolism
PA1228	hypothetical protein	Hypothetical, unclassified, unknown
PA1320	cytochrome o ubiquinol oxidase subunit IV	Energy metabolism
PA1321	cytochrome o ubiquinol oxidase protein CyoE	Energy metabolism
PA1372	hypothetical protein	Hypothetical, unclassified, unknown
PA1432	autoinducer synthesis protein LasI	Adaptation, Protection
PA1466	hypothetical protein	Hypothetical, unclassified, unknown
PA1552	probable cytochrome c	Energy metabolism
PA1553	probable cytochrome c oxidase subunit	Energy metabolism
PA1554	probable cytochrome oxidase subunit (cbb3-type)	Energy metabolism
PA1574	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1581	succinate dehydrogenase (C subunit)	Energy metabolism
PA1582	succinate dehydrogenase (D subunit)	Energy metabolism
PA1583	succinate dehydrogenase (A subunit)	Energy metabolism
PA1584	succinate dehydrogenase (B subunit)	Energy metabolism
PA1634	potassium-transporting ATPase, B chain	Transport of small molecules
PA1687	spermidine synthase	Amino acid biosynthesis and metabolism
PA1759	probable transcriptional regulator	Transcriptional regulators
PA1760	probable transcriptional regulator	Transcriptional regulators
PA1776	ECF sigma factor SigX	Transcriptional regulators
PA1837	hypothetical protein	Hypothetical, unclassified, unknown
PA1845	hypothetical protein	Hypothetical, unclassified, unknown
PA1852	hypothetical protein	Hypothetical, unclassified, unknown
PA1865	hypothetical protein	Hypothetical, unclassified, unknown
PA1930	probable chemotaxis transducer	Adaptation, Protection
PA1946	binding protein component precursor of ABC ribose transporter	Transport of small molecules
PA1951	hypothetical protein	Hypothetical, unclassified, unknown
PA2006	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2007	maleylacetate isomerase	Carbon compound catabolism
PA2008	fumarylacetate isomerase	Carbon compound catabolism
PA2009	homogentisate 1,2-dioxygenase	Carbon compound catabolism
PA2012	methylenetetrahydropteroyl-CoA carboxylase, alpha-subunit (biotin-containing)	Carbon compound catabolism
PA2288	hypothetical protein	Hypothetical, unclassified, unknown
PA2290	glucose dehydrogenase	Carbon compound catabolism
PA2321	gluconokinase	Energy metabolism

ORF	Product name	Function class
PA2322	gluconate permease	Transport of small molecules
PA2338	probable binding protein component of ABC maltose/mannitol transporter	Transport of small molecules
PA2339	probable binding-protein-dependent maltose/mannitol transport protein	Transport of small molecules
PA2340	probable binding-protein-dependent maltose/mannitol transport protein	Transport of small molecules
PA2341	probable ATP-binding component of ABC maltose/mannitol transporter	Transport of small molecules
PA2342	mannitol dehydrogenase	Carbon compound catabolism
PA2344	fructokinase	Carbon compound catabolism
PA2453	hypothetical protein	Hypothetical, unclassified, unknown
PA2552	probable acyl-CoA dehydrogenase	Putative enzymes
PA2553	probable acyl-CoA thiolase	Putative enzymes
PA2555	probable AMP-binding enzyme	Putative enzymes
PA2557	probable AMP-binding enzyme	Fatty acid and phospholipid metabolism
PA2573	probable chemotaxis transducer	Adaptation, Protection
PA2579	hypothetical protein	Putative enzymes
PA2619	initiation factor	Translation, post-translational modification, degradation
PA2637	NADH dehydrogenase I chain A	Energy metabolism
PA2638	NADH dehydrogenase I chain B	Energy metabolism
PA2639	NADH dehydrogenase I chain C,D	Energy metabolism
PA2640	NADH dehydrogenase I chain E	Energy metabolism
PA2641	NADH dehydrogenase I chain F	Energy metabolism
PA2642	NADH dehydrogenase I chain G	Energy metabolism
PA2643	NADH dehydrogenase I chain H	Energy metabolism
PA2644	NADH dehydrogenase I chain I	Energy metabolism
PA2645	NADH dehydrogenase I chain J	Energy metabolism
PA2646	NADH dehydrogenase I chain K	Energy metabolism
PA2647	NADH dehydrogenase I chain L	Energy metabolism
PA2648	NADH dehydrogenase I chain M	Energy metabolism
PA2649	NADH dehydrogenase I chain N	Energy metabolism
PA2653	probable transporter	Membrane proteins
PA2667	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2730	hypothetical protein	Hypothetical, unclassified, unknown
PA2746	hypothetical protein	Membrane proteins
PA2911	probable TonB-dependent receptor	Membrane proteins
PA2937	hypothetical protein	Hypothetical, unclassified, unknown
PA3007	repressor protein LexA	Adaptation, Protection
PA3008	hypothetical protein	Hypothetical, unclassified, unknown
PA3009	hypothetical protein	Hypothetical, unclassified, unknown
PA3080	hypothetical protein	Hypothetical, unclassified, unknown
PA3147	probable glycosyl transferase WbpJ	Putative enzymes
PA3149	probable glycosyltransferase WbpH	Putative enzymes
PA3150	LPS biosynthesis protein WbpG	Cell wall / LPS / capsule
PA3181	2-keto-3-deoxy-6-phosphogluconate aldolase	Central intermediary metabolism
PA3183	glucose-6-phosphate 1-dehydrogenase	Energy metabolism
PA3186	Glucose/carbohydrate outer membrane porin OprB precursor	Transport of small molecules
PA3187	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3188	probable permease of ABC sugar transporter	Transport of small molecules
PA3189	probable permease of ABC sugar transporter	Transport of small molecules
PA3190	probable binding protein component of ABC sugar transporter	Transport of small molecules
PA3194	phosphogluconate dehydratase	Energy metabolism
PA3237	hypothetical protein	Hypothetical, unclassified, unknown
PA3282	hypothetical protein	Hypothetical, unclassified, unknown
PA3311	conserved hypothetical protein	Membrane proteins
PA3404	probable outer membrane protein precursor	Protein secretion/export apparatus
PA3415	probable dihydrolipoamide acetyltransferase	Energy metabolism
PA3417	probable pyruvate dehydrogenase E1 component, alpha subunit	Energy metabolism
PA3418	leucine dehydrogenase	Amino acid biosynthesis and metabolism
PA3441	probable molybdopterin-binding protein	Transport of small molecules
PA3442	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3443	probable permease of ABC transporter	Membrane proteins
PA3444	conserved hypothetical protein	Putative enzymes
PA3445	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3446	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3449	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3496	hypothetical protein	Hypothetical, unclassified, unknown
PA3561	1-phosphofructokinase	Central intermediary metabolism
PA3574	probable transcriptional regulator	Transcriptional regulators
PA3581	glycerol uptake facilitator protein	Transport of small molecules
PA3582	glycerol kinase	Central intermediary metabolism

ORF	Product name	Function class
PA3584	glycerol-3-phosphate dehydrogenase	Central intermediary metabolism
PA3600	conserved hypothetical protein	Translation, post-translational modification, degradation
PA3607	polyamine transport protein PotA	Transport of small molecules
PA3608	polyamine transport protein PotB	Membrane proteins
PA3609	polyamine transport protein PotC	Membrane proteins
PA3610	polyamine transport protein PotD	Transport of small molecules
PA3616	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3617	RecA protein	DNA replication, recombination, modification and repair
PA3621	ferredoxin I	Energy metabolism
PA3672	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3712	hypothetical protein	Hypothetical, unclassified, unknown
PA3713	spermidine dehydrogenase, SpdH	Hypothetical, unclassified, unknown
PA3723	probable FMN oxidoreductase	Putative enzymes
PA3753	conserved hypothetical protein	Transport of small molecules
PA3767	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3787	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3808	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3870	molybdopterin biosynthetic protein A1	Biosynthesis of cofactors, prosthetic groups and carriers
PA3871	probable peptidyl-prolyl cis-trans isomerase, PpiC-type	Translation, post-translational modification, degradation
PA3872	respiratory nitrate reductase gamma chain	Energy metabolism
PA3873	respiratory nitrate reductase delta chain	Energy metabolism
PA3874	respiratory nitrate reductase beta chain	Energy metabolism
PA3875	respiratory nitrate reductase alpha chain	Energy metabolism
PA3876	nitrite extrusion protein 2	Membrane proteins
PA3877	nitrite extrusion protein 1	Membrane proteins
PA3901	Fe(III) dicitrate transport protein FecA	Membrane proteins
PA3913	probable protease	Putative enzymes
PA3922	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3935	taurine dioxygenase	Carbon compound catabolism
PA3967	hypothetical protein	Hypothetical, unclassified, unknown
PA3986	hypothetical protein	Hypothetical, unclassified, unknown
PA4055	riboflavin synthase alpha chain	Biosynthesis of cofactors, prosthetic groups and carriers
PA4131	probable iron-sulfur protein	Putative enzymes
PA4132	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4133	cytochrome c oxidase subunit (cbb3-type)	Energy metabolism
PA4141	hypothetical protein	Hypothetical, unclassified, unknown
PA4199	probable acyl-CoA dehydrogenase	Putative enzymes
PA4236	catalase	Adaptation, Protection
PA4390	hypothetical protein	Hypothetical, unclassified, unknown
PA4427	stringent starvation protein B	Adaptation, Protection
PA4432	30S ribosomal protein S9	Translation, post-translational modification, degradation
PA4496	probable binding protein component of ABC transporter	Transport of small molecules
PA4553	type 4 fimbrial biogenesis protein PilX	Motility & Attachment
PA4563	30S ribosomal protein S20	Central intermediary metabolism
PA4573	hypothetical protein	Hypothetical, unclassified, unknown
PA4582	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4583	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4584	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4585	RNA 3'-terminal phosphate cyclase	Transcription, RNA processing and degradation
PA4607	hypothetical protein	Hypothetical, unclassified, unknown
PA4612	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4642	hypothetical protein	Hypothetical, unclassified, unknown
PA4645	probable purine/pyrimidine phosphoribosyl transferase	Nucleotide biosynthesis and metabolism
PA4648	hypothetical protein	Hypothetical, unclassified, unknown
PA4651	probable pili assembly chaperone	Motility & Attachment
PA4672	peptidyl-RNA hydrolase	Translation, post-translational modification, degradation
PA4687	ferric iron-binding periplasmic protein HitA	Transport of small molecules
PA4688	iron (III)-transport system permease HitB	Membrane proteins
PA4702	hypothetical protein	Hypothetical, unclassified, unknown
PA4703	hypothetical protein	Hypothetical, unclassified, unknown
PA4732	glucose-6-phosphate isomerase	Carbon compound catabolism
PA4746	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4747	secretion protein SecG	Membrane proteins
PA4748	triosephosphate isomerase	Central intermediary metabolism
PA4753	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4759	dihydrodipicolinate reductase	Amino acid biosynthesis and metabolism
PA4763	DNA repair protein RecN	DNA replication, recombination, modification and repair
PA4839	biosynthetic arginine decarboxylase	Amino acid biosynthesis and metabolism

ORF	Product name	Function class
PA4840	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4843	probable two-component response regulator	Transcriptional regulators
PA5024	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5049	50S ribosomal protein L31	Translation, post-translational modification, degradation
PA5074	probable ATP-binding component of ABC transporter	Transport of small molecules
PA5167	probable c4-dicarboxylate-binding protein	Membrane proteins
PA5235	glycerol-3-phosphate transporter	Membrane proteins
PA5351	Rubredoxin I	Carbon compound catabolism
PA5380	GbdR	Transcriptional regulators
PA5401	hypothetical protein	Hypothetical, unclassified, unknown
PA5436	probable biotin carboxylase subunit of a transcarboxylase	Central intermediary metabolism
PA5446	hypothetical protein	Hypothetical, unclassified, unknown
PA5452	phosphomannose isomerase/GDP-mannose WbpW	Cell wall / LPS / capsule
PA5453	GDP-mannose 4,6-dehydratase	Cell wall / LPS / capsule
PA5465	hypothetical protein	Hypothetical, unclassified, unknown
PA5470	probable peptide chain release factor	Translation, post-translational modification, degradation
PA5471	hypothetical protein	Hypothetical, unclassified, unknown
PA5479	proton-glutamate symporter	Membrane proteins

ORF	Product name	Function class
PCP		
PA0224	probable aldolase	Putative enzymes
PA0246	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA0263	secreted protein Hep	Secreted Factors (toxins, enzymes, alginate)
PA0355	protease PfpI	Translation, post-translational modification, degradation
PA0417	probable chemotaxis protein	Chemotaxis
PA0425	RND multidrug efflux membrane fusion protein MexA precursor	Transport of small molecules
PA0426	RND multidrug efflux transporter MexB	Transport of small molecules
PA0427	Major intrinsic multiple antibiotic resistance efflux outer membrane protein OprM precursor	Antibiotic resistance and susceptibility
PA0445	probable transposase	Related to phage, transposon, or plasmid
PA0526	hypothetical protein	Hypothetical, unclassified, unknown
PA0579	30S ribosomal protein S21	Translation, post-translational modification, degradation
PA0726	hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown
PA0868	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1178	PhoP/Q and low Mg ²⁺ inducible outer membrane protein H1 precursor	Membrane proteins
PA1198	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1281	cobalamin (5'-phosphate) synthase	Biosynthesis of cofactors, prosthetic groups and carriers
PA1393	adenosine 5'-phosphosulfate (APS) kinase	Amino acid biosynthesis and metabolism
PA1493	sulfate-binding protein of ABC transporter	Transport of small molecules
PA1588	succinyl-CoA synthetase beta chain	Energy metabolism
PA1656	hypothetical protein	Hypothetical, unclassified, unknown
PA1658	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1660	hypothetical protein	Hypothetical, unclassified, unknown
PA1664	hypothetical protein	Hypothetical, unclassified, unknown
PA1780	assimilatory nitrite reductase small subunit	Central intermediary metabolism
PA2062	probable pyridoxal-phosphate dependent enzyme	Putative enzymes
PA2562	hypothetical protein	Hypothetical, unclassified, unknown
PA2658	hypothetical protein	Hypothetical, unclassified, unknown
PA2669	hypothetical protein	Hypothetical, unclassified, unknown
PA2906	probable oxidoreductase	Putative enzymes
PA2941	hypothetical protein	Hypothetical, unclassified, unknown
PA2951	electron transfer flavoprotein alpha-subunit	Energy metabolism
PA3136	probable secretion protein	Transport of small molecules
PA3137	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3159	probable UDP-glucose/GDP-mannose dehydrogenase WbpA	Putative enzymes
PA3162	30S ribosomal protein S1	Translation, post-translational modification, degradation
PA3266	cold acclimation protein B	Transcriptional regulators
PA3547	poly(beta-D-mannuronate) lyase precursor AlgL	Cell wall / LPS / capsule
PA3552	ArnB	Cell wall / LPS / capsule
PA3676	probable Resistance-Nodulation-Cell Division (RND) efflux transporter	Membrane proteins
PA3677	probable RND efflux membrane fusion protein precursor	Transport of small molecules
PA3678	probable transcriptional regulator	Transcriptional regulators
PA3679	hypothetical protein	Hypothetical, unclassified, unknown
PA3719	hypothetical protein	Hypothetical, unclassified, unknown
PA3720	hypothetical protein	Hypothetical, unclassified, unknown
PA3721	probable transcriptional regulator	Transcriptional regulators
PA3814	L-cysteine desulfurase (pyridoxal phosphate-dependent)	Amino acid biosynthesis and metabolism
PA3815	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3819	conserved hypothetical protein	Membrane proteins
PA3905	hypothetical protein	Hypothetical, unclassified, unknown
PA4033	hypothetical protein	Hypothetical, unclassified, unknown
PA4037	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4053	6,7-dimethyl-8-ribityllumazine synthase	Biosynthesis of cofactors, prosthetic groups and carriers
PA4178	hypothetical protein	Hypothetical, unclassified, unknown
PA4344	probable hydrolase	Putative enzymes
PA4386	GroES protein	Chaperones & heat shock proteins
PA4443	ATP sulfurylase small subunit	Central intermediary metabolism
PA4568	50S ribosomal protein L21	Translation, post-translational modification, degradation
PA4816	hypothetical protein	Hypothetical, unclassified, unknown
PA4847	biotin carboxyl carrier protein (BCCP)	Fatty acid and phospholipid metabolism
PA5013	branched-chain amino acid transferase	Amino acid biosynthesis and metabolism
PA5157	probable transcriptional regulator	Transcriptional regulators
PA5158	probable outer membrane protein precursor	Transport of small molecules
PA5159	multidrug resistance protein	Transport of small molecules
PA5160	drug efflux transporter	Membrane proteins
PA5182	hypothetical protein	Membrane proteins

ORF	Product name	Function class
PA5212	hypothetical protein	Hypothetical, unclassified, unknown
PA5216	probable permease of ABC iron transporter	Membrane proteins
PA5253	alginate regulatory protein AlgP	Transcriptional regulators
PA5312	probable aldehyde dehydrogenase	Putative enzymes
PA5326	hypothetical protein	Hypothetical, unclassified, unknown
PA5369	phosphate ABC transporter, periplasmic phosphate-binding protein, PstS	Transport of small molecules
PA5390	probable peptidic bond hydrolase	Putative enzymes
PA5481	hypothetical protein	Hypothetical, unclassified, unknown
PA5570	50S ribosomal protein L34	Central intermediary metabolism

ORF	Product name	Function class
Copper		
PA0149	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA0150	probable transmembrane sensor	Membrane proteins
PA0397	probable cation ef flux system protein	Transport of small molecules
PA0423	PasP	Secreted Factors (toxins, enzymes, alginate)
PA0450	probable phosphate transporter	Membrane proteins
PA0802	hypothetical protein	Membrane proteins
PA0913	probable Mg transporter MgtE	Transport of small molecules
PA0918	cytochrome b561	Energy metabolism
PA0931	ferric enterobactin receptor PirA	Transport of small molecules
PA1134	hypothetical protein	Hypothetical, unclassified, unknown
PA1286	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA1297	probable metal transporter	Membrane proteins
PA1301	probable transmembrane sensor	Membrane proteins
PA1302	probable heme utilization protein precursor	Membrane proteins
PA1363	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA1364	probable transmembrane sensor	Membrane proteins
PA1365	probable siderophore receptor	Transport of small molecules
PA1390	probable glycosyl transferase	Putative enzymes
PA1435	RND efflux membrane fusion protein precursor	Transport of small molecules
PA1436	probable RND efflux transporter	Membrane proteins
PA1437	probable two-component response regulator	Transcriptional regulators
PA1438	probable two-component sensor	Two-component regulatory systems
PA1562	aconitate hydratase 1	Energy metabolism
PA1909	hypothetical protein	Hypothetical, unclassified, unknown
PA1910	probable tonB-dependent receptor protein	Transport of small molecules
PA1911	probable transmembrane sensor	Membrane proteins
PA1912	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA2064	copper resistance protein B precursor	Adaptation, Protection
PA2065	copper resistance protein A precursor	Adaptation, Protection
PA2277	ArsR protein	Transcriptional regulators
PA2278	ArsB protein	Membrane proteins
PA2279	ArsC protein	Adaptation, Protection
PA2384	hypothetical protein	Hypothetical, unclassified, unknown
PA2390	probable ATP-binding/permease fusion ABC transporter	Membrane proteins
PA2391	probable outer membrane protein precursor	Membrane proteins
PA2395	PvdO	Adaptation, Protection
PA2406	hypothetical protein	Hypothetical, unclassified, unknown
PA2451	hypothetical protein	Hypothetical, unclassified, unknown
PA2466	Ferrioxamine receptor FoxA	Transport of small molecules
PA2467	Anti-sigma factor FoxR	Membrane proteins
PA2476	thiol:disulfide interchange protein DsbG	Translation, post-translational modification, degradation
PA2477	probable thiol:disulfide interchange protein	Membrane proteins
PA2478	probable thiol:disulfide interchange protein	Membrane proteins
PA2479	probable two-component response regulator	Transcriptional regulators
PA2480	probable two-component sensor	Two-component regulatory systems
PA2520	RND divalent metal cation ef flux transporter CzcA	Membrane proteins
PA2521	RND divalent metal cation ef flux membrane fusion protein CzcB precursor	Membrane proteins
PA2522	outer membrane protein precursor CzcC	Membrane proteins
PA2524	probable two-component sensor	Two-component regulatory systems
PA2664	flavohemoprotein	Energy metabolism
PA2686	two-component response regulator PfeR	Transcriptional regulators
PA2688	Ferric enterobactin receptor , outer membrane protein PfeA precursor	Membrane proteins
PA2807	hypothetical protein	Hypothetical, unclassified, unknown
PA2809	two-component response regulator , CopR	Transcriptional regulators
PA2810	two-component sensor , CopS	Two-component regulatory systems
PA2826	probable glutathione peroxidase	Adaptation, Protection
PA2953	electron transfer flavoprotein-ubiquinone oxidoreductase	Energy metabolism
PA3392	nitrous-oxide reductase precursor	Energy metabolism
PA3407	heme acquisition protein HasAp	Transport of small molecules
PA3408	Haem uptake outer membrane receptor HasR precursor	Transport of small molecules
PA3409	probable transmembrane sensor	Membrane proteins
PA3410	probable sigma-70 factor , ECF subfamily	Transcriptional regulators
PA3411	hypothetical protein	Hypothetical, unclassified, unknown
PA3412	hypothetical protein	Hypothetical, unclassified, unknown
PA3521	probable outer membrane protein precursor	Membrane proteins

ORF	Product name	Function class
PA3522	probable Resistance-Nodulation-Cell Division (RND) ef flux transporter	Membrane proteins
PA3530	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3601	conserved hypothetical protein	Translation, post-translational modification, degradation
PA3602	conserved hypothetical protein	Membrane proteins
PA3749	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3920	probable metal transporting P-type ATPase	Membrane proteins
PA3921	probable transcriptional regulator	Transcriptional regulators
PA4158	ferric enterobactin transport protein FepC	Transport of small molecules
PA4159	ferrienterobactin-binding periplasmic protein precursor FepB	Transport of small molecules
PA4160	ferric enterobactin transport protein FepD	Membrane proteins
PA4192	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4194	probable permease of ABC transporter	Membrane proteins
PA4195	probable binding protein component of ABC transporter	Transport of small molecules
PA4513	probable oxidoreductase	Putative enzymes
PA4514	probable outer membrane receptor for iron transport	Transport of small molecules
PA4515	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4655	ferrochelatase	Biosynthesis of cofactors, prosthetic groups and carriers
PA4675	probable TonB-dependent receptor	Transport of small molecules
PA4705	hypothetical protein	Hypothetical, unclassified, unknown
PA4706	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4707	probable permease of ABC transporter	Membrane proteins
PA4711	hypothetical protein	Hypothetical, unclassified, unknown
PA4818	conserved hypothetical protein	Membrane proteins
PA4819	probable glycosyl transferase	Putative enzymes
PA4820	hypothetical protein	Hypothetical, unclassified, unknown
PA4825	Mg(2+) transport ATPase, P-type 2	Transport of small molecules
PA5230	probable permease of ABC transporter	Membrane proteins
PA5231	probable ATP-binding/permease fusion ABC transporter	Membrane proteins
PA5300	cytochrome c5	Energy metabolism
PA5304	D-amino acid dehydrogenase, small subunit	Energy metabolism
PA5531	TonB protein	Transport of small molecules

ORF	Product name	Function class
Sulfate		
PA0192	probable TonB-dependent receptor	Membrane proteins
PA0193	hypothetical protein	Putative enzymes
PA0194	hypothetical protein	Putative enzymes
PA0197	hypothetical protein	Transport of small molecules
PA0198	transport protein ExbB	Transport of small molecules
PA0199	transport protein ExbD	Transport of small molecules
PA2085	probable ring-hydroxylating dioxygenase small subunit	Carbon compound catabolism
PA2086	probable epoxide hydrolase	Carbon compound catabolism
PA2092	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA2292	hypothetical protein	Hypothetical, unclassified, unknown
PA2293	hypothetical protein	Hypothetical, unclassified, unknown
PA2294	probable ATP-binding component of ABC transporter	Transport of small molecules
PA2295	probable permease of ABC transporter	Membrane proteins
PA2296	hypothetical protein	Hypothetical, unclassified, unknown
PA2297	probable ferredoxin	Energy metabolism
PA2298	probable oxidoreductase	Putative enzymes
PA2324	hypothetical protein	Putative enzymes
PA2325	hypothetical protein	Putative enzymes
PA2326	hypothetical protein	Putative enzymes
PA2346	conserved hypothetical protein	Putative enzymes
PA2347	hypothetical protein	Putative enzymes
PA2348	conserved hypothetical protein	Putative enzymes
PA2349	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2350	probable ATP-binding component of ABC transporter	Transport of small molecules
PA2351	probable permease of ABC transporter	Membrane proteins
PA2354	probable transcriptional regulator	Transcriptional regulators
PA2355	probable FMN _{H2} -dependent monooxygenase	Putative enzymes
PA2356	methanesulfonate sulfonate MsuD	Carbon compound catabolism
PA2357	NADH-dependent FMN reductase MsuE	Carbon compound catabolism

ORF	Product name	Function class
Osmotic shock		
PA0236	probable transcriptional regulator	Transcriptional regulators
PA0448	probable transcriptional regulator	Transcriptional regulators
PA1159	probable cold-shock protein	Transcriptional regulators
PA1226	probable transcriptional regulator	Transcriptional regulators
PA1243	probable sensor/response regulator hybrid	Two-component regulatory systems
PA1690	translocation protein in type III secretion	Protein secretion/export apparatus
PA1692	probable translocation protein in type III secretion	Protein secretion/export apparatus
PA1693	translocation protein in type III secretion	Protein secretion/export apparatus
PA1694	translocation protein in type III secretion	Protein secretion/export apparatus
PA1695	translocation protein in type III secretion	Protein secretion/export apparatus
PA1697	ATP synthase in type III secretion system	Protein secretion/export apparatus
PA1702	conserved hypothetical protein in type III secretion	Protein secretion/export apparatus
PA1703	type III secretory apparatus protein PerD	Protein secretion/export apparatus
PA1704	transcriptional regulator protein PerR	Transcriptional regulators
PA1724	type III export protein PscK	Protein secretion/export apparatus
PA1725	type III export protein PscL	Protein secretion/export apparatus
PA2147	catalase HP11	Adaptation, Protection
PA2152	probable trehalose synthase	Putative enzymes
PA2417	probable transcriptional regulator	Transcriptional regulators
PA2665	probable transcriptional regulator	Transcriptional regulators
PA3049	ribosome modulation factor	Translation, post-translational modification, degradation
PA3459	probable glutamine amidotransferase	Amino acid biosynthesis and metabolism
PA3462	probable sensor/response regulator hybrid	Two-component regulatory systems
PA3689	probable transcriptional regulator	Transcriptional regulators
PA3831	leucine aminopeptidase	Transcription, RNA processing and degradation
PA3843	hypothetical protein	Hypothetical, unclassified, unknown
PA4135	probable transcriptional regulator	Transcriptional regulators
PA4581	transcriptional regulator RtcR	Transcriptional regulators
PA5483	two-component response regulator AlgB	Transcriptional regulators
PA5484	probable two-component sensor	Two-component regulatory systems

ORF	Product name	Function class
Biofilms		
PA0026	phospholipase C, PlcB	Hypothetical, unclassified, unknown
PA0027	hypothetical protein	Hypothetical, unclassified, unknown
PA0028	hypothetical protein	Hypothetical, unclassified, unknown
PA0038	hypothetical protein	Hypothetical, unclassified, unknown
PA0040	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0046	hypothetical protein	Hypothetical, unclassified, unknown
PA0047	hypothetical protein	Hypothetical, unclassified, unknown
PA0061	hypothetical protein	Hypothetical, unclassified, unknown
PA0062	hypothetical protein	Hypothetical, unclassified, unknown
PA0074	serine/threonine protein kinase PpkA	Adaptation, Protection
PA0075	probable phosphoprotein phosphatase	Putative enzymes
PA0076	hypothetical protein	Hypothetical, unclassified, unknown
PA0083	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0086	hypothetical protein	Hypothetical, unclassified, unknown
PA0087	hypothetical protein	Hypothetical, unclassified, unknown
PA0088	hypothetical protein	Hypothetical, unclassified, unknown
PA0089	hypothetical protein	Hypothetical, unclassified, unknown
PA0090	probable ClpA/B-type chaperone	Translation, post-translational modification, degradation
PA0091	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0092	hypothetical protein	Hypothetical, unclassified, unknown
PA0094	hypothetical protein	Hypothetical, unclassified, unknown
PA0102	probable carbonic anhydrase	Central intermediary metabolism
PA0126	hypothetical protein	Hypothetical, unclassified, unknown
PA0134	probable guanine deaminase	Nucleotide biosynthesis and metabolism
PA0170	hypothetical protein	Hypothetical, unclassified, unknown
PA0224	probable aldolase	Putative enzymes
PA0237	probable oxidoreductase	Putative enzymes
PA0238	hypothetical protein	Hypothetical, unclassified, unknown
PA0252	hypothetical protein	Hypothetical, unclassified, unknown
PA0276	hypothetical protein	Membrane proteins
PA0320	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0327	hypothetical protein	Hypothetical, unclassified, unknown
PA0346	hypothetical protein	Hypothetical, unclassified, unknown
PA0347	glycerophosphoryl diester phosphodiesterase, periplasmic	Fatty acid and phospholipid metabolism
PA0355	protease PipI	Translation, post-translational modification, degradation
PA0376	sigma factor RpoH	Transcriptional regulators
PA0409	twitching motility protein PilH	Two-component regulatory systems
PA0410	twitching motility protein PilI	Chemotaxis
PA0411	twitching motility protein PilJ	Chemotaxis
PA0413	still frameshift probable component of chemotactic signal transduction system	Two-component regulatory systems
PA0414	probable methyltransferase	Chemotaxis
PA0430	5,10-methylenetetrahydrofolate reductase	Amino acid biosynthesis and metabolism
PA0431	hypothetical protein	Hypothetical, unclassified, unknown
PA0432	S-adenosyl-L-homocysteine hydrolase	Amino acid biosynthesis and metabolism
PA0446	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0451	conserved hypothetical protein	Membrane proteins
PA0452	probable stomatin-like protein	Membrane proteins
PA0462	hypothetical protein	Hypothetical, unclassified, unknown
PA0464	two-component sensor CreC	Two-component regulatory systems
PA0465	inner membrane protein CreD	Membrane proteins
PA0466	hypothetical protein	Hypothetical, unclassified, unknown
PA0490	hypothetical protein	Hypothetical, unclassified, unknown
PA0515	probable transcriptional regulator	Biosynthesis of cofactors, prosthetic groups and carriers
PA0516	heme d1 biosynthesis protein NirF	Energy metabolism
PA0523	nitric-oxide reductase subunit C	Energy metabolism
PA0526	hypothetical protein	Hypothetical, unclassified, unknown
PA0529	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0530	probable class III pyridoxal phosphate-dependent aminotransferase	Putative enzymes
PA0531	probable glutamine amidotransferase	Putative enzymes
PA0543	hypothetical protein	Hypothetical, unclassified, unknown
PA0547	probable transcriptional regulator	Transcriptional regulators
PA0557	hypothetical protein	Hypothetical, unclassified, unknown
PA0585	hypothetical protein	Hypothetical, unclassified, unknown
PA0588	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA0601	probable two-component response regulator	Transcriptional regulators

ORF	Product name	Function class
PA0603	probable ATP-binding component of ABC transporter	Transport of small molecules
PA0674	hypothetical protein	Hypothetical, unclassified, unknown
PA0675	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA0676	probable transmembrane sensor	Membrane proteins
PA0693	transport protein ExbB2	Membrane proteins
PA0696	hypothetical protein	Hypothetical, unclassified, unknown
PA0697	hypothetical protein	Hypothetical, unclassified, unknown
PA0698	hypothetical protein	Hypothetical, unclassified, unknown
PA0699	probable peptidyl-prolyl cis-trans isomerase, PpiC-type	Translation, post-translational modification, degradation
PA0707	transcriptional regulator ToxR	Transcriptional regulators
PA0732	hypothetical protein	Hypothetical, unclassified, unknown
PA0737	hypothetical protein	Hypothetical, unclassified, unknown
PA0738	conserved hypothetical protein	Membrane proteins
PA0752	conserved hypothetical protein	Membrane proteins
PA0753	hypothetical protein	Membrane proteins
PA0755	cis-aconitate porin OpdH	Membrane proteins
PA0765	positive regulator for alginate biosynthesis MucC	Transcriptional regulators
PA0788	hypothetical protein	Hypothetical, unclassified, unknown
PA0842	probable glycosyl transferase	Putative enzymes
PA0853	probable oxidoreductase	Putative enzymes
PA0877	probable transcriptional regulator	Transcriptional regulators
PA0878	hypothetical protein	Hypothetical, unclassified, unknown
PA0879	probable acyl-CoA dehydrogenase	Putative enzymes
PA0880	probable ring-cleaving dioxygenase	Putative enzymes
PA0882	hypothetical protein	Putative enzymes
PA0884	probable C4-dicarboxylate-binding periplasmic protein	Transport of small molecules
PA0886	probable C4-dicarboxylate transporter	Transport of small molecules
PA0921	hypothetical protein	Membrane proteins
PA0952	hypothetical protein	Hypothetical, unclassified, unknown
PA0985	pyocin S5	Membrane proteins
PA1029	hypothetical protein	Hypothetical, unclassified, unknown
PA1065	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1066	probable short-chain dehydrogenase	Putative enzymes
PA1078	flagellar basal-body rod protein FlgC	Cell wall / LPS / capsule
PA1079	flagellar basal-body rod modification protein FlgD	Cell wall / LPS / capsule
PA1080	flagellar hook protein FlgE	Cell wall / LPS / capsule
PA1081	flagellar basal-body rod protein FlgF	Cell wall / LPS / capsule
PA1084	flagellar P-ring protein precursor FlgI	Cell wall / LPS / capsule
PA1111	hypothetical protein	Hypothetical, unclassified, unknown
PA1114	hypothetical protein	Hypothetical, unclassified, unknown
PA1115	hypothetical protein	Membrane proteins
PA1118	hypothetical protein	Membrane proteins
PA1155	NrdB, tyrosyl radical-harboring component of class Ia ribonucleotide reductase	Nucleotide biosynthesis and metabolism
PA1168	hypothetical protein	Hypothetical, unclassified, unknown
PA1178	PhoP/Q and low Mg ²⁺ inducible outer membrane protein H1 precursor	Membrane proteins
PA1195	hypothetical protein	Hypothetical, unclassified, unknown
PA1216	hypothetical protein	Hypothetical, unclassified, unknown
PA1217	probable 2-isopropylmalate synthase	Amino acid biosynthesis and metabolism
PA1247	alkaline protease secretion protein AprE	Secreted Factors (toxins, enzymes, alginate)
PA1285	probable transcriptional regulator	Transcriptional regulators
PA1333	hypothetical protein	Hypothetical, unclassified, unknown
PA1345	hypothetical protein	Hypothetical, unclassified, unknown
PA1404	hypothetical protein	Hypothetical, unclassified, unknown
PA1470	probable short-chain dehydrogenase	Putative enzymes
PA1471	hypothetical protein	Hypothetical, unclassified, unknown
PA1514	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1517	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1518	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1523	xanthine dehydrogenase	Nucleotide biosynthesis and metabolism
PA1524	xanthine dehydrogenase	Nucleotide biosynthesis and metabolism
PA1560	hypothetical protein	Hypothetical, unclassified, unknown
PA1600	probable cytochrome c	Energy metabolism
PA1601	probable aldehyde dehydrogenase	Putative enzymes
PA1602	probable oxidoreductase	Carbon compound catabolism
PA1603	probable transcriptional regulator	Transcriptional regulators
PA1606	hypothetical protein	Hypothetical, unclassified, unknown
PA1625	conserved hypothetical protein	Membrane proteins
PA1659	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA1730	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1780	assimilatory nitrite reductase small subunit	Central intermediary metabolism
PA1786	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1791	hypothetical protein	Hypothetical, unclassified, unknown
PA1847	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA1870	hypothetical protein	Hypothetical, unclassified, unknown
PA1889	hypothetical protein	Hypothetical, unclassified, unknown
PA1931	probable ferredoxin	Energy metabolism
PA1932	probable hydroxylase molybdopterin-containing subunit	Carbon compound catabolism
PA1933	probable hydroxylase large subunit	Carbon compound catabolism
PA2017	hypothetical protein	Hypothetical, unclassified, unknown
PA2021	hypothetical protein	Hypothetical, unclassified, unknown
PA2022	probable nucleotide sugar dehydrogenase	Putative enzymes
PA2024	probable ring-cleaving dioxygenase	Putative enzymes
PA2042	probable transporter (membrane subunit)	Transport of small molecules
PA2071	elongation factor G	Translation, post-translational modification, degradation
PA2076	probable transcriptional regulator	Transcriptional regulators
PA2107	hypothetical protein	Hypothetical, unclassified, unknown
PA2136	hypothetical protein	Hypothetical, unclassified, unknown
PA2140	probable metallothionein	Central intermediary metabolism
PA2149	hypothetical protein	Hypothetical, unclassified, unknown
PA2155	probable phospholipase	Putative enzymes
PA2167	hypothetical protein	Hypothetical, unclassified, unknown
PA2168	hypothetical protein	Hypothetical, unclassified, unknown
PA2170	hypothetical protein	Hypothetical, unclassified, unknown
PA2174	hypothetical protein	Hypothetical, unclassified, unknown
PA2175	hypothetical protein	Hypothetical, unclassified, unknown
PA2176	hypothetical protein	Hypothetical, unclassified, unknown
PA2178	hypothetical protein	Hypothetical, unclassified, unknown
PA2179	hypothetical protein	Hypothetical, unclassified, unknown
PA2181	hypothetical protein	Hypothetical, unclassified, unknown
PA2186	hypothetical protein	Hypothetical, unclassified, unknown
PA2188	probable alcohol dehydrogenase (Zn-dependent)	Putative enzymes
PA2243	hypothetical protein	Hypothetical, unclassified, unknown
PA2365	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2366	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2367	hypothetical protein	Hypothetical, unclassified, unknown
PA2369	hypothetical protein	Membrane proteins
PA2441	hypothetical protein	Hypothetical, unclassified, unknown
PA2444	serine hydroxymethyltransferase	Amino acid biosynthesis and metabolism
PA2445	glycine cleavage system protein P2	Central intermediary metabolism
PA2446	glycine cleavage system protein H2	Amino acid biosynthesis and metabolism
PA2459	hypothetical protein	Hypothetical, unclassified, unknown
PA2462	hypothetical protein	Hypothetical, unclassified, unknown
PA2485	hypothetical protein	Hypothetical, unclassified, unknown
PA2486	hypothetical protein	Hypothetical, unclassified, unknown
PA2491	probable oxidoreductase	Putative enzymes
PA2493	RND multidrug efflux membrane fusion protein MexE precursor	Transport of small molecules
PA2494	RND multidrug efflux transporter MexF	Transport of small molecules
PA2495	Multidrug efflux outer membrane protein OprN precursor	Membrane proteins
PA2533	probable sodium:alanine symporter	Membrane proteins
PA2536	probable phosphatidate cytidyltransferase	Fatty acid and phospholipid metabolism
PA2562	hypothetical protein	Hypothetical, unclassified, unknown
PA2569	hypothetical protein	Hypothetical, unclassified, unknown
PA2618	hypothetical protein	Translation, post-translational modification, degradation
PA2635	hypothetical protein	Hypothetical, unclassified, unknown
PA2658	hypothetical protein	Hypothetical, unclassified, unknown
PA2659	hypothetical protein	Hypothetical, unclassified, unknown
PA2708	hypothetical protein	Hypothetical, unclassified, unknown
PA2718	probable transcriptional regulator	Transcriptional regulators
PA2761	hypothetical protein	Membrane proteins
PA2777	conserved hypothetical protein	Membrane proteins
PA2782	hypothetical protein	Hypothetical, unclassified, unknown
PA2799	hypothetical protein	Hypothetical, unclassified, unknown
PA2803	hypothetical protein	Hypothetical, unclassified, unknown
PA2804	hypothetical protein	Hypothetical, unclassified, unknown
PA2815	probable acyl-CoA dehydrogenase	Putative enzymes
PA2816	hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA2827	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA2880	hypothetical protein	Hypothetical, unclassified, unknown
PA2881	probable two-component response regulator	Transcriptional regulators
PA2882	probable two-component sensor	Two-component regulatory systems
PA2883	hypothetical protein	Hypothetical, unclassified, unknown
PA2895	hypothetical protein	Hypothetical, unclassified, unknown
PA2931	probable transcriptional regulator	Transcriptional regulators
PA2939	probable aminopeptidase	Secreted Factors (toxins, enzymes, alginate)
PA2969	fatty acid biosynthesis protein PlsX	Fatty acid and phospholipid metabolism
PA2970	50S ribosomal protein L32	Translation, post-translational modification, degradation
PA3023	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3031	hypothetical protein	Hypothetical, unclassified, unknown
PA3079	hypothetical protein	Membrane proteins
PA3082	glycine betaine transmethylese	Amino acid biosynthesis and metabolism
PA3105	general secretion pathway protein D	Protein secretion/export apparatus
PA3121	3-isopropylmalate dehydratase lar ge subunit	Amino acid biosynthesis and metabolism
PA3205	hypothetical protein	Hypothetical, unclassified, unknown
PA3218	hypothetical protein	Hypothetical, unclassified, unknown
PA3219	hypothetical protein	Hypothetical, unclassified, unknown
PA3221	CsaA protein	Protein secretion/export apparatus
PA3229	hypothetical protein	Hypothetical, unclassified, unknown
PA3249	probable transcriptional regulator	Transcriptional regulators
PA3273	hypothetical protein	Hypothetical, unclassified, unknown
PA3274	hypothetical protein	Hypothetical, unclassified, unknown
PA3280	Pyrophosphate-specific outer membrane porin OprO precursor	Transport of small molecules
PA3296	alkaline phosphatase	Central intermediary metabolism
PA3309	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3319	non-hemolytic phospholipase C precursor	Secreted Factors (toxins, enzymes, alginate)
PA3326	probable Clp-family ATP-dependent protease	Translation, post-translational modification, degradation
PA3327	probable non-ribosomal peptide synthetase	Adaptation, Protection
PA3329	hypothetical protein	Hypothetical, unclassified, unknown
PA3330	probable short chain dehydrogenase	Putative enzymes
PA3331	cytochrome P450	Adaptation, Protection
PA3332	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3333	3-oxoacyl-[acyl-carrier-protein] synthase III	Fatty acid and phospholipid metabolism
PA3334	probable acyl carrier protein	Fatty acid and phospholipid metabolism
PA3335	hypothetical protein	Hypothetical, unclassified, unknown
PA3336	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA3337	ADP-L-glycero-D-mannoheptose 6-epimerase	Cell wall / LPS / capsule
PA3368	probable acetyltransferase	Putative enzymes
PA3376	probable ATP-binding component of ABC transporter	Transport of small molecules
PA3377	conserved hypothetical protein	Transport of small molecules
PA3380	conserved hypothetical protein	Transport of small molecules
PA3381	probable transcriptional regulator	Transcriptional regulators
PA3382	phosphonate transport protein PhnE	Membrane proteins
PA3383	binding protein component of ABC phosphonate transporter	Transport of small molecules
PA3384	ATP-binding component of ABC phosphonate transporter	Transport of small molecules
PA3389	probable ring-cleaving dioxygenase	Putative enzymes
PA3397	ferredoxin--NADP+ reductase	Biosynthesis of cofactors, prosthetic groups and carriers
PA3431	conserved hypothetical protein	Membrane proteins
PA3432	hypothetical protein	Membrane proteins
PA3436	hypothetical protein	Hypothetical, unclassified, unknown
PA3476	autoinducer synthesis protein RhlI	Adaptation, Protection
PA3478	rhamnosyltransferase chain B	Secreted Factors (toxins, enzymes, alginate)
PA3484	hypothetical protein	Hypothetical, unclassified, unknown
PA3540	GDP-mannose 6-dehydrogenase AlgD	Cell wall / LPS / capsule
PA3541	alginate biosynthesis protein Alg8	Cell wall / LPS / capsule
PA3542	alginate biosynthesis protein Alg44	Cell wall / LPS / capsule
PA3543	alginate biosynthetic protein AlgK precursor	Cell wall / LPS / capsule
PA3544	Alginate production outer membrane protein AlgE precursor	Membrane proteins
PA3545	alginate-c5-mannuronan-epimerase AlgG	Cell wall / LPS / capsule
PA3546	alginate biosynthesis protein AlgX	Cell wall / LPS / capsule
PA3547	poly(beta-d-mannuronate) lyase precursor AlgL	Cell wall / LPS / capsule
PA3548	alginate o-acetyltransferase AlgI	Cell wall / LPS / capsule
PA3549	alginate o-acetyltransferase AlgJ	Cell wall / LPS / capsule
PA3550	alginate o-acetyltransferase AlgF	Cell wall / LPS / capsule
PA3551	phosphomannose isomerase / guanosine 5'-diphospho-D-mannose pyrophosphorylase	Cell wall / LPS / capsule
PA3553	ArmC	Adaptation, Protection

ORF	Product name	Function class
PA3554	AmA	Putative enzymes
PA3555	AmD	Adaptation, Protection
PA3556	inner membrane L-Ara4N transferase AmT	Adaptation, Protection
PA3557	AmE	Adaptation, Protection
PA3559	probable nucleotide sugar dehydrogenase	Putative enzymes
PA3569	3-hydroxyisobutyrate dehydrogenase	Carbon compound catabolism
PA3577	hypothetical protein	Hypothetical, unclassified, unknown
PA3578	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3598	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3651	phosphatidate cytidyltransferase	Fatty acid and phospholipid metabolism
PA3655	elongation factor Ts	Translation, post-translational modification, degradation
PA3688	hypothetical protein	Hypothetical, unclassified, unknown
PA3692	probable outer membrane protein precursor	Membrane proteins
PA3724	elastase LasB	Secreted Factors (toxins, enzymes, alginate)
PA3743	tRNA (guanine-N1)-methyltransferase	Transcription, RNA processing and degradation
PA3744	16S rRNA processing protein	Transcription, RNA processing and degradation
PA3819	conserved hypothetical protein	Membrane proteins
PA3820	secretion protein SecF	Protein secretion/export apparatus
PA3821	secretion protein SecD	Membrane proteins
PA3840	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3890	probable permease of ABC transporter	Membrane proteins
PA3905	hypothetical protein	Hypothetical, unclassified, unknown
PA3906	hypothetical protein	Hypothetical, unclassified, unknown
PA3907	hypothetical protein	Hypothetical, unclassified, unknown
PA3908	hypothetical protein	Hypothetical, unclassified, unknown
PA3909	hypothetical protein	Hypothetical, unclassified, unknown
PA3910	hypothetical protein	Hypothetical, unclassified, unknown
PA3929	cyanide insensitive terminal oxidase	Energy metabolism
PA3944	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA3952	hypothetical protein	Hypothetical, unclassified, unknown
PA3957	probable short-chain dehydrogenase	Putative enzymes
PA3964	hypothetical protein	Hypothetical, unclassified, unknown
PA4017	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4040	hypothetical protein	Hypothetical, unclassified, unknown
PA4063	hypothetical protein	Hypothetical, unclassified, unknown
PA4110	beta-lactamase precursor	Adaptation, Protection
PA4111	hypothetical protein	Hypothetical, unclassified, unknown
PA4112	probable sensor/response regulator hybrid	Two-component regulatory systems
PA4154	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4155	hypothetical protein	Hypothetical, unclassified, unknown
PA4190	probable FAD-dependent monooxygenase	Putative enzymes
PA4238	DNA-directed RNA polymerase alpha chain	Transcription, RNA processing and degradation
PA4243	secretion protein SecY	Membrane proteins
PA4244	50S ribosomal protein L15	Translation, post-translational modification, degradation
PA4246	30S ribosomal protein S5	Translation, post-translational modification, degradation
PA4247	50S ribosomal protein L18	Translation, post-translational modification, degradation
PA4248	50S ribosomal protein L6	Translation, post-translational modification, degradation
PA4255	50S ribosomal protein L29	Translation, post-translational modification, degradation
PA4261	50S ribosomal protein L23	Translation, post-translational modification, degradation
PA4262	50S ribosomal protein L4	Transcription, RNA processing and degradation
PA4272	50S ribosomal protein L10	Translation, post-translational modification, degradation
PA4293	two-component sensor PprA	Two-component regulatory systems
PA4317	hypothetical protein	Membrane proteins
PA4344	probable hydrolase	Putative enzymes
PA4345	hypothetical protein	Hypothetical, unclassified, unknown
PA4348	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4350	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4351	probable acyltransferase	Fatty acid and phospholipid metabolism
PA4354	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4355	probable major facilitator superfamily (MFS) transporter	Membrane proteins
PA4356	xenobiotic reductase	Adaptation, Protection
PA4357	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4371	hypothetical protein	Hypothetical, unclassified, unknown
PA4407	cell division protein FtsZ	Cell division
PA4408	cell division protein FtsA	Cell division
PA4409	cell division protein FtsQ	Cell division
PA4410	D-alanine--D-alanine ligase	Cell wall / LPS / capsule
PA4411	UDP-N-acetylmuramate--alanine ligase	Cell wall / LPS / capsule

ORF	Product name	Function class
PA4412	UDP-N-acetylglucosamine--N-acetylmuramyl undecaprenol N-acetylglucosamine trans	Carbon compound catabolism
PA4413	cell division protein FtsW	Cell division
PA4414	UDP-N-acetylmuramoylalanine--D-glutamate ligase	Cell wall / LPS / capsule
PA4415	phospho-N-acetylmuramoyl-pentapeptide-transferase	Cell wall / LPS / capsule
PA4416	UDP-N-acetylmuramoylalanyl-D-glutamyl-2, 6-diaminopimelate--D-alanyl-D-alanyl liq	Cell wall / LPS / capsule
PA4419	cell division protein FtsL	Cell division
PA4421	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4498	probable metalloproteinase	Translation, post-translational modification, degradation
PA4500	probable binding protein component of ABC transporter	Transport of small molecules
PA4502	probable binding protein component of ABC transporter	Transport of small molecules
PA4503	probable permease of ABC transporter	Membrane proteins
PA4504	probable permease of ABC transporter	Membrane proteins
PA4505	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4506	probable ATP-binding component of ABC dipeptide transporter	Transport of small molecules
PA4507	hypothetical protein	Membrane proteins
PA4525	type 4 fimbrial precursor PilA	Motility & Attachment
PA4526	type 4 fimbrial biogenesis protein PilB	Motility & Attachment
PA4569	octaprenyl-diphosphate synthase	Energy metabolism
PA4575	hypothetical protein	Hypothetical, unclassified, unknown
PA4588	glutamate dehydrogenase	Amino acid biosynthesis and metabolism
PA4589	probable outer membrane protein precursor	Membrane proteins
PA4621	probable oxidoreductase	Putative enzymes
PA4623	hypothetical protein	Hypothetical, unclassified, unknown
PA4632	hypothetical protein	Hypothetical, unclassified, unknown
PA4657	hypothetical protein	Hypothetical, unclassified, unknown
PA4658	hypothetical protein	Hypothetical, unclassified, unknown
PA4671	probable ribosomal protein L25	Adaptation, Protection
PA4674	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4691	hypothetical protein	Hypothetical, unclassified, unknown
PA4697	hypothetical protein	Hypothetical, unclassified, unknown
PA4713	hypothetical protein	Hypothetical, unclassified, unknown
PA4742	tRNA pseudouridine 55 synthase	Translation, post-translational modification, degradation
PA4743	ribosome-binding factor A	Translation, post-translational modification, degradation
PA4744	translation initiation factor IF-2	Translation, post-translational modification, degradation
PA4773	hypothetical protein	Hypothetical, unclassified, unknown
PA4781	probable two-component response regulator	Transcriptional regulators
PA4782	hypothetical protein	Hypothetical, unclassified, unknown
PA4785	probable acyl-CoA thiolase	Putative enzymes
PA4786	probable short-chain dehydrogenase	Putative enzymes
PA4811	nitrate-inducible formate dehydrogenase, beta subunit	Energy metabolism
PA4813	lipase LipC	Fatty acid and phospholipid metabolism
PA4844	probable chemotaxis transducer	Adaptation, Protection
PA4858	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4862	probable ATP-binding component of ABC transporter	Transport of small molecules
PA4870	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4877	hypothetical protein	Hypothetical, unclassified, unknown
PA4879	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA4881	hypothetical protein	Hypothetical, unclassified, unknown
PA4896	probable sigma-70 factor, ECF subfamily	Transcriptional regulators
PA4933	hypothetical protein	Membrane proteins
PA5033	hypothetical protein	Hypothetical, unclassified, unknown
PA5040	Type 4 fimbrial biogenesis outer membrane protein PilQ precursor	Motility & Attachment
PA5041	type 4 fimbrial biogenesis protein PilP	Motility & Attachment
PA5042	type 4 fimbrial biogenesis protein PilO	Motility & Attachment
PA5043	type 4 fimbrial biogenesis protein PilN	Motility & Attachment
PA5044	type 4 fimbrial biogenesis protein PilM	Motility & Attachment
PA5075	probable permease of ABC transporter	Membrane proteins
PA5097	probable amino acid permease	Membrane proteins
PA5099	probable transporter	Membrane proteins
PA5116	probable transcriptional regulator	Transcriptional regulators
PA5124	two-component sensor NtrB	Two-component regulatory systems
PA5125	two-component response regulator NtrC	Transcriptional regulators
PA5128	secretion protein SecB	Protein secretion/export apparatus
PA5129	glutaredoxin	Energy metabolism
PA5139	hypothetical protein	Hypothetical, unclassified, unknown
PA5169	probable C4-dicarboxylate transporter	Membrane proteins
PA5170	arginine/ornithine antiporter	Membrane proteins
PA5180	conserved hypothetical protein	Hypothetical, unclassified, unknown

ORF	Product name	Function class
PA5182	hypothetical protein	Membrane proteins
PA5183	hypothetical protein	Membrane proteins
PA5192	phosphoenolpyruvate carboxykinase	Carbon compound catabolism
PA5202	hypothetical protein	Hypothetical, unclassified, unknown
PA5209	hypothetical protein	Hypothetical, unclassified, unknown
PA5212	hypothetical protein	Hypothetical, unclassified, unknown
PA5220	hypothetical protein	Hypothetical, unclassified, unknown
PA5232	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5262	alginate biosynthesis protein AlgZ/FimS	Secreted Factors (toxins, enzymes, alginate)
PA5275	conserved hypothetical protein	Hypothetical, unclassified, unknown
PA5287	ammonium transporter AmtB	Membrane proteins
PA5299	hypothetical protein	Hypothetical, unclassified, unknown
PA5322	phosphomannomutase AlgC	Cell wall / LPS / capsule
PA5360	two-component response regulator PhoB	Transcriptional regulators
PA5361	two-component sensor PhoR	Two-component regulatory systems
PA5366	ATP-binding component of ABC phosphate transporter	Membrane proteins
PA5367	membrane protein component of ABC phosphate transporter	Membrane proteins
PA5368	membrane protein component of ABC phosphate transporter	Membrane proteins
PA5415	serine hydroxymethyltransferase	Amino acid biosynthesis and metabolism
PA5422	hypothetical protein	Hypothetical, unclassified, unknown
PA5441	hypothetical protein	Hypothetical, unclassified, unknown
PA5460	hypothetical protein	Hypothetical, unclassified, unknown
PA5473	conserved hypothetical protein	Membrane proteins
PA5475	hypothetical protein	Hypothetical, unclassified, unknown
PA5476	citrate transporter	Membrane proteins
PA5498	probable adhesin	Motility & Attachment
PA5499	transcriptional regulator np20	Transcriptional regulators
PA5524	probable short-chain dehydrogenase	Putative enzymes
PA5525	probable transcriptional regulator	Transcriptional regulators
PA5526	hypothetical protein	Hypothetical, unclassified, unknown
PA5549	glucosamine--fructose-6-phosphate aminotransferase	Central intermediary metabolism
PA5555	ATP synthase gamma chain	Energy metabolism
PA5556	ATP synthase alpha chain	Energy metabolism
PA5557	ATP synthase delta chain	Energy metabolism
PA5558	ATP synthase B chain	Energy metabolism
PA5559	atp synthase C chain	Energy metabolism
PA5560	ATP synthase A chain	Energy metabolism
PA5568	conserved hypothetical protein	Membrane proteins

ORF	Product name	Function class
Alginate		
PA2867	probable chemotaxis transducer	Adaptation, Protection
PA3819	conserved hypothetical protein	Membrane proteins
PA5182	hypothetical protein	Membrane proteins
RsmA		
PA0105	cytochrome c oxidase, subunit II	Energy metabolism
PA0108	cytochrome c oxidase, subunit III	Energy metabolism
PA0129	gamma-aminobutyrate permease	Transport of small molecules
PA0173	probable methyltransferase	Adaptation, Protection
PA0210	malonate decarboxylase delta subunit	Carbon compound catabolism
PA0212	malonate decarboxylase gamma subunit	Carbon compound catabolism
PA0235	4-hydroxybenzoate transporter PcaK	Membrane proteins
PA0396	twitching motility protein PilU	Motility & Attachment
PA0652	transcriptional regulator Vfr	Transcriptional regulators
PA0673	hypothetical protein	Hypothetical, unclassified, unknown
PA0706	chloramphenicol acetyltransferase	Antibiotic resistance and susceptibility
PA0710	lactoylglutathione lyase	Central intermediary metabolism
PA0723	coat protein B of bacteriophage Pf1	Related to phage, transposon, or plasmid
PA0843	phospholipase accessory protein PlcR precursor	Secreted Factors (toxins, enzymes, alginate)
PA0844	hemolytic phospholipase C precursor	Secreted Factors (toxins, enzymes, alginate)
PA0905	RsmA, regulator of secondary metabolites	Translation, post-translational modification, degradation
PA0994	usher CupC3	Motility & Attachment
PA1001	anthranilate synthase component I	Adaptation, Protection
PA1092	flagellin type B	Motility & Attachment
PA1148	exotoxin A precursor	Secreted Factors (toxins, enzymes, alginate)
PA1151	pyocin S2 immunity protein	Adaptation, Protection
PA1173	cytochrome c-type protein NapB precursor	Energy metabolism
PA1247	alkaline protease secretion protein AprE	Secreted Factors (toxins, enzymes, alginate)
PA1251	probable chemotaxis transducer	Adaptation, Protection
PA1338	gamma-glutamyltranspeptidase precursor	Amino acid biosynthesis and metabolism
PA1393	adenosine 5'-phosphosulfate (APS) kinase	Amino acid biosynthesis and metabolism
PA1409	acetylpolymine aminohydrolase	Carbon compound catabolism
PA1635	potassium-transporting ATPase, C chain	Transport of small molecules
PA1695	translocation protein in type III secretion	Protein secretion/export apparatus
PA1758	para-aminobenzoate synthase component I	Biosynthesis of cofactors, prosthetic groups and carriers
PA1770	phosphoenolpyruvate synthase	Energy metabolism
PA1781	assimilatory nitrite reductase large subunit	Central intermediary metabolism
PA1947	ribose transport protein RbsA	Transport of small molecules
PA1949	ribose operon repressor RbsR	Carbon compound catabolism
PA1983	cytochrome c550	Energy metabolism
PA2007	maleylacetoacetate isomerase	Carbon compound catabolism
PA2009	homogentisate 1,2-dioxygenase	Carbon compound catabolism
PA2064	copper resistance protein B precursor	Adaptation, Protection
PA2129	chaperone CupA2	Motility & Attachment
PA2337	transcriptional regulator MtlR	Transcriptional regulators
PA2493	RND multidrug efflux membrane fusion protein MexE precursor	Transport of small molecules
PA2494	RND multidrug efflux transporter MexF	Transport of small molecules
PA2495	Multidrug efflux outer membrane protein OprN precursor	Membrane proteins
PA2509	muconate cycloisomerase I	Carbon compound catabolism
PA2926	histidine transport protein HisP	Amino acid biosynthesis and metabolism
PA3540	GDP-mannose 6-dehydrogenase AlgD	Cell wall / LPS / capsule
PA3543	alginate biosynthetic protein AlgK precursor	Cell wall / LPS / capsule
PA3544	Alginate production outer membrane protein AlgE precursor	Membrane proteins
PA3549	alginate O-acetyltransferase AlgJ	Cell wall / LPS / capsule
PA3603	diacylglycerol kinase	Fatty acid and phospholipid metabolism
PA4110	beta-lactamase precursor	Adaptation, Protection
PA4127	2-oxo-hept-3-ene-1,7-diolate hydratase	Carbon compound catabolism
PA4138	tyrosyl-tRNA synthetase	Amino acid biosynthesis and metabolism
PA4158	ferric enterobactin transport protein FepC	Transport of small molecules
PA4205	hypothetical protein	Membrane proteins
PA4210	probable phenazine biosynthesis protein	Secreted Factors (toxins, enzymes, alginate)
PA4307	chemotactic transducer PctC	Adaptation, Protection
PA4310	chemotactic transducer PctB	Adaptation, Protection
PA4356	xenobiotic reductase	Adaptation, Protection

ORF	Product name	Function class
PA4522	beta-lactamase expression regulator AmpD	Cell wall / LPS / capsule
PA4527	still frameshift type 4 fimbrial biogenesis protein PilC	Motility & Attachment
PA4855	phosphoribosylamine-glycine ligase	Nucleotide biosynthesis and metabolism
PA4953	chemotaxis protein MotB	Membrane proteins
PA4954	chemotaxis protein MotA	Adaptation, Protection
PA5041	type 4 fimbrial biogenesis protein PilP	Motility & Attachment
PA5042	type 4 fimbrial biogenesis protein PilO	Motility & Attachment
PA5043	type 4 fimbrial biogenesis protein PilN	Motility & Attachment
PA5044	type 4 fimbrial biogenesis protein PilM	Motility & Attachment
PA5243	delta-aminolevulinic acid dehydratase	Biosynthesis of cofactors, prosthetic groups and carriers
PA5355	glycolate oxidase subunit GlcD	Central intermediary metabolism
PA5361	two-component sensor PhoR	Two-component regulatory systems
PA5501	permease of ABC zinc transporter ZnuB	Membrane proteins

Supplementary information for Chapter 3

Supplementary Table 1: Family-wise list of transcription factors found in *P. aeruginosa* PAO1. The table shows the genomic location of the protein (core/RGP) (Mathee *et al.* 2008) and the different PFAM domains (Winzor *et al.* 2011, <http://pfam.sanger.ac.uk/> and <http://expasy.org/prosite/>). ORF (PA) numbers, protein name, homolog and amino acid information are from the *Pseudomonas* database (Winzor *et al.* 2011).

PA #	Protein name	# of amino acids	Subfamily	Nearest Homolog	Core/RGP	Domain 1	Domain 1 Details	Domain 2	Domain 2 Details	Other Domains	Domain Details
AraC											
PA0306					Core						
PA0163		265		52% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC				
PA0248	PobR	288		87% similar to putative regulator PobR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA0416	ChpD	264		40% similar to regulatory protein mmsR which is a member of the AraC/XylS family of transcriptional regulators [<i>Pseudomonas aeruginosa</i>]	Core	PF02311	AraC_binding	PF00165	HTH_AraC		
PA0564		303		57% similarity to hypothetical transcriptional regulator HI1052 from [<i>Haemophilus influenzae</i>]	Core	PF00165	HTH_AraC				
PA0748		306		55% similar to regulatory protein mmsR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA0780	PruR	250		54% similar to lumQ gene product [<i>Photobacterium leiognathi</i>]	Core	PF00165	HTH_AraC				
PA0791		262		50% similarity to hypothetical yeaM gene product of [<i>Escherichia coli</i>], a putative regulatory protein.	Core	PF00165	HTH_AraC				
PA0831	OruR	339		100% identical to OruR, transcriptional regulator required for growth on ornithine as the sole carbon source [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA0864		262		50% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC				
PA0893	ArgR	329			0 Core	PF00165	HTH_AraC				
PA1109		259		52% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC				

PA1182		333	47% similar to transcriptional regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC
PA1229	YeaM	268	67% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC
PA1235		260	51% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC
PA1261		224	62% similar to a region of probable transcriptional regulator LumQ [<i>Photobacterium leiognathi</i>]	Core	PF00165	HTH_AraC
PA1380		321	60% similar to putative regulatory protein YqhC [<i>Escherichia coli</i>]	RGP 15	PF00165	HTH_AraC
PA1599		260	56% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC
PA1619		274	45% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF00165	HTH_AraC
PA1713	ExsA	278	71% similar to thermoregulatory transcription factor LcrF [<i>Yersinia pestis</i>] ; 72% similar to transcriptional activator VirF [<i>Yersinia enterocolitica</i>] ; 99% similar to exoenzyme S synthesis regulatory protein ExsA [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC
PA1850		334	57% similar to AraC-like protein [<i>Azorhizobium caulinodans</i>]	Core	PF00165	HTH_AraC
PA2047		329	51% similar to putative regulatory protein YqhC [<i>Escherichia coli</i>]	RGP 20	PF00165	HTH_AraC
PA2096		344	51% similar to hypothetical transcriptional regulator CY21B4.12 [<i>Mycobacterium tuberculosis</i>]	Core	PF00165	HTH_AraC
PA2227	VqsM	325	47% similar to regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC
PA2276		296	62% similar (over C-terminal 3/4ths of ORF) to yqhC gene product of [<i>Escherichia coli</i>], a putative transcriptional regulator.	Core	PF00165	HTH_AraC
PA2281		284	64% similar to the C-terminal end of regulatory protein PchR <i>Synechocystis</i> sp.	Core	PF00165	HTH_AraC
PA2332		301	46% similar to putative regulator HI1052 [<i>Haemophilus influenzae</i> Rd]	Core	PF00165	HTH_AraC
PA2337	MtlR	301	84% similar to putative activator protein MtlR [<i>Pseudomonas fluorescens</i>]	Core	PF00165	HTH_AraC
PA2488		254	47% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC

PA2489		271	46% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF00165	HTH_AraC				
PA2511		333	50% similar to putative regulatory protein OxoS [<i>Pseudomonas putida</i>]	Core	PF00165	HTH_AraC				
PA2519	XylS	318	70% similar to positive regulatory protein XylS [TOL plasmid]	Core	PF00165	HTH_AraC				
PA2556		361	42% similar to regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA2588		342	48% similar to regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA2696		297	52% similar to regulatory protein GdhBR [<i>Pantoea citrea</i>]	Core	PF00165	HTH_AraC				
PA2704		339	54% similar to transcriptional regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC				
PA2917		278	47% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF00165	HTH_AraC				
PA3027		343	49% similar to transcriptional regulator OruR [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC	PF08281	Sigma70_r4_2	PF09312	SurA_N
PA3094		345	80% similar to putative virulence regulator [<i>Pseudomonas alcaligenes</i>]	Core	PF00165	HTH_AraC				
PA3215		337	52% similar to transcriptional regulator PhbR [<i>Pseudomonas</i> sp. 61-3]	Core	PF00165	HTH_AraC				
PA3220		254	48% similar to probable transcriptional regulator LumQ [<i>Photobacterium leiognathi</i>]	Core	PF00165	HTH_AraC	PF07883	Cupin_2		
PA3269		284	49% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF00165	HTH_AraC	PF02311	AraC_binding		
PA3423		247	52% similar to a region of putative araC-type regulatory protein [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC	PF07366	SnoaL		
PA3571	MmsR	307		Core	PF00165	HTH_AraC	PF00165	HTH_AraC	PF07883; PF03079	Cupin_2; ARD
PA3782		317	49% similar to a region of ArgR regulatory protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00165	HTH_AraC	PF01965	DJ-1_PfpI		
PA3830		270	51% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC	PF07883	Cupin_2	PF02311	AraC_binding
PA3898		293	54% similar to regulatory protein GdhBR [<i>Pantoea citrea</i>]	Core	PF00165	HTH_AraC				
PA3927		262	53% similar to putative regulatory protein YeaM [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC	PF02311	AraC_binding	PF07883	Cupin_2
PA4070		316	45% similar to the regulatory protein for 2-phenylethylamine catabolism FeaR [<i>Escherichia coli</i>]	Core	PF00165	HTH_AraC				
PA4094		319	42% similar to nitrilase regulator	Core	PF00165	HTH_AraC				

[[<i>Rhodococcus rhodochrous</i>]]										
PA4120		303	63% similar to the HpaA gene product of <i>[Escherichia coli]</i>	Core	PF00165	HTH_AraC	PF02311	AraC_bindi ng	PF07883	Cupin_2
PA4184		340	48% similar to ArgR regulatory protein <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC	PF01965	DJ-1_PfpI		
PA4227	PchR	296	100% similar to regulatory protein PchR <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC				
PA4288		267	51% similar to putative regulatory protein YeaM <i>[Escherichia coli]</i>	Core	PF00165	HTH_AraC	PF07883	Cupin_2	PF02311	AraC_bin ding
PA4436		299	45% similar to ArgR regulatory protein <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC				
PA4787		333	42% similar to regulator OruR <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC				
PA5032		357	53% similar to regulator OruR <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC				
PA5324		356	46% similar to hypothetical transcriptional regulator <i>[Mycobacterium tuberculosis]</i>	Core	PF00165	HTH_AraC	PF09312	SurA_N		
PA5342		266	46% similar to putative transcriptional regulator <i>[Streptomyces coelicolor]</i>	Core	PF00165	HTH_AraC	PF02311	AraC_bindi ng	PF07883	Cupin_2
PA5380	GbdR	367	59% similar to ArgR regulatory protein <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC	PF01965	DJ-1_PfpI		
PA5389	CdhR	336	58% similar to ArgR regulatory protein <i>[Pseudomonas aeruginosa]</i>	Core	PF00165	HTH_AraC	PF01965	DJ-1_PfpI		
ArsR										
PA0279	YdfF	232	59% similar to hypothetical protein YdfF <i>[Bacillus subtilis]</i>	Core	PS00846	Bacterial regulatory proteins, arsR family signature				
PA0547		333	49% similar to a region of unknown protein <i>[Streptomyces fradiae]</i> Transposon Tn4556	Core	PS00846	Bacterial regulatory proteins, arsR family signature	PF01022	HTH_5, Bacterial regulatory protein, arsR family.		
PA2277	ArsR	116	82% identical to arsR gene product of <i>[P. aeruginosa]</i> ; 73% similar (over 3/4ths of ORF length) to arsR gene product of <i>[Escherichia coli]</i>	Core						
PA4354		100	76% similar to a region of hypothetical protein <i>[Agrobacterium radiobacter]</i>	Core	PS00846	Bacterial regulatory proteins, ArsR family				

AsnC						
PA0513	NirG	147	77% similar to NirG protein [<i>Pseudomonas stutzeri</i>]	Core	PF01037	AsnC family
PA2028		157	62% similar to AzlB protein [<i>Bacillus subtilis</i>]	RGP20	PF01037	AsnC family
PA2082		158	57% similar to leucine-responsive regulatory protein Lrp [<i>Escherichia coli</i>]	Core	PF01037	AsnC family
PA2246	BkdR	153	64% similar to BkdR protein [<i>P. putida</i>]	Core	PF01037	AsnC family
PA2577		145	55% similar to hypothetical protein Rv3291c [<i>Mycobacterium tuberculosis</i>]	Core	PF01037	AsnC family
PA3965		169	63% similar to Leucine-responsive regulatory protein Lrp [<i>Escherichia coli</i>]	Core	PF01037	AsnC family
PA4508		157	70% similar to transcription regulatory protein PdhR [<i>Ralstonia eutropha</i>]	Core	PF01037	AsnC family
PA4784		155	57% similar to putative transcriptional regulator [<i>Bacillus subtilis</i>]	Core	PF01037	AsnC family
PA5308	Lrp, DadR	162	79% similar to global response regulator Lrp [<i>Escherichia coli</i>]	Core	PF01037	AsnC family
Cro/ cI						
PA0048		127	55% similar to a region of TrbA [plasmid RK2, [<i>Escherichia coli</i>]]	Core		
PA0225		179	51% similar to putative epoxidase [<i>Methanobacterium thermoautotrophicum</i>]	Core		
PA0535		184	48% similar to putative aldehyde dehydrogenase [<i>Azotobacter vinelandii</i>]	Core		
PA0611	PrtR	256		0	Core	
PA0906		68	70% similar to hypothetical repressor protein AF1793 [<i>Archaeoglobus fulgidus</i>]	Core		
PA1359		103		0	Core	
PA1879		238	60% similar to regulator PhnR [<i>Salmonella typhimurium</i>]	Core		
PA1884		72	73% similar to hypothetical protein YozG [<i>Bacillus subtilis</i>]	Core		
PA2312		114		Core		
PA2780		193	54% similar to hypothetical protein [<i>Bacillus</i> sp.]	Core		
PA2785		183	51% similar to putative transcriptional regulator protein [<i>Bacillus</i> sp.]	Core		

PA3260		218	Small portion 64% similar to <i>PvuII</i> restriction endonuclease regulator <i>pvuII</i> C of [<i>Proteus vulgaris</i>] ; 49% similar to hypothetical <i>hipB</i> protein of [<i>Escherichia coli</i>]	Core			
PA4077		237	66% similar to pyocin synthesis negative regulatory protein PrtR [<i>Pseudomonas aeruginosa</i>]	Core			
PA4499		187	44% similar to hypothetical protein YcjC [<i>Escherichia coli</i>]	Core			
PA4987		199	53% similar to aldehyde dehydrogenase [<i>Azotobacter vinelandii</i>] ; 48% similar to hypothetical <i>ycjC</i> gene product of [<i>Escherichia coli</i>]	Core			
PA5301	YcjC	182	62% similar to hypothetical protein YcjC [<i>Escherichia coli</i>]	Core			
PA5403		68	77% similar to putative transcriptional regulator YozG [<i>Bacillus subtilis</i>]	Core			
DksA/TraR							
PA0612	PtrB	66	59% similar to hypothetical protein Ybil (over N-terminal 75% of ORF) [<i>Escherichia coli</i>]	RGP 3	PF01258	zf-dskA_traR, Prokaryotic dksA/traR C4-type zinc finger	
PA4723	DksA	148	86% similar to dosage-dependent dnaK suppressor protein DksA [<i>Escherichia coli</i>]	Core	PF01258	zf-dskA_traR, Prokaryotic dksA/traR C4-type zinc finger	
PA4870	Ybil	88	84% similar to hypothetical protein Ybil [<i>Escherichia coli</i>]	Core	PF01258	zf-dskA_traR, Prokaryotic dksA/traR C4-type zinc finger	
PA5536		134	59% similar to dnaK suppressor protein DksA [<i>Escherichia coli</i>]	Core	PF01258	zf-dskA_traR, Prokaryotic dksA/traR C4-type zinc finger	
RpoN-binding							
PA0873	PhhR	519		Core	PF00158	Sigma-54 interaction domain	PAS, PAS fold

PA1097	FleQ	490	66% similar to sigma 54 transcriptional activator [<i>Vibrio cholerae</i>]	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF06490	FleQ, Flagellar regulatory protein FleQ	PF00158	Sigma-54 interaction domain
PA1099	FleR	473	68% similar to two-component response regulator [<i>Vibrio cholerae</i>]	Core	PF00158	Sigma-54 interaction domain	PF00072	Response regulator receiver domain	PF02954	HTH_8, Bacterial regulatory protein, Fis family
PA1196		466	53% similar to arginine catabolism positive regulator RocR [<i>Bacillus subtilis</i>]	Core	PF07728	AAA_5, ATPase family associated with various cellular activities (AAA)	PF08448	PAS_4, PAS fold	PF02954; PF00158	HTH_8, Bacterial regulatory protein, Fis family; Sigma-54 interaction domain
PA1335		425	67% similar to C4-dicarboxylate transport transcription regulator DctD [<i>Rhizobium meliloti</i>]	Core	PF02954	HTH_8, Fis family	PF00158	Sigma54_activat,	PF00072	Response regulator
PA1663		503	55% similar to response regulator of hydrogenase 3 activity HydG [<i>Escherichia coli</i>]	Core	PF00158	Sigma-54 interaction domain	PF02954	HTH_8, Bacterial regulatory protein, Fis family		
PA1945		442	57% similar to hydG gene product of [<i>Escherichia coli</i>]	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF00158	Sigma-54 interaction domain		
PA2005		481	58% similar to Acetoacetate metabolism regulatory protein AtoC [<i>Escherichia coli</i>] ; 53% similar to nifA gene product of [<i>Azotobacter vinelandii</i>]	Core	PF00158	Sigma-54 interaction domain	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF08448	PAS_4, PAS fold
PA2354		376	58% similar to transcriptional protein FtrC [<i>Caulobacter crescentus</i>]	Core	PF00158	Sigma-54 interaction domain	PF07728	AAA_5, ATPase family associated with various cellular		

PA2359		361	60% similar to a region of transcriptional protein FtrC [<i>Caulobacter crescentus</i>]	Core	PF07728	AAA_5, ATPase family associated with various cellular activities (AAA)	PF00158	Sigma-5 4 interaction domain	
PA2449		511	58% similar phenylalanine hydroxylase gene cluster transcription activator PhhR [<i>Pseudomonas aeruginosa</i>]	Core	PF00158	Sigma-54 interaction domain			
PA2665		517	63% similar to putative 2-component transcriptional regulator YgaA [<i>Escherichia coli</i>]	Core	PF00158	Sigma-54 interaction domain	PF01590	GAF, GAF domain	
PA3932		324	55% similar to regulator for prp operon PrpR [<i>Escherichia coli</i>]	Core	PF00158	Sigma-54 interaction domain			
PA4021		643	57% similar to transcription factor AcoR [<i>Alcaligenes eutrophus</i>]	Core	PF00158	Sigma-54 interaction domain	PF07728	AAA_5, ATPase family associated with various cellular activities (AAA)	PF02954 HTH_8, Bacterial regulatory protein, Fis family
PA4147	AcoR	625	59% similar to the acoR gene product of [<i>Alcaligenes eutrophus</i>]	Core	PF00158	Sigma-54 interaction domain	PF02954	HTH_8, Bacterial regulatory protein, Fis family	
PA4493	RoxR	186	67% similar to putative photosynthetic response regulator PrrA [<i>Rhodobacter sphaeroides</i>]; 66% similar to RegA [<i>Rhodovulum sulfidophilum</i>]	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF00072	Response regulator receiver domain	
PA4547	PilR	445		Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF00072	Response regulator receiver domain	PF00158 Sigma-54 interaction domain

PA4581	RtcR	531		79% similar to regulator RtcR [<i>Escherichia coli</i>]	Core	PF00158	Sigma-54 interaction domain	PF06956	RtcR, Regulator of RNA terminal phosphate cyclase		
PA4726	CbrB	478		99% similar to regulator CbrB [<i>Azotobacter vinelandii</i> DJ; 90% similar to NifA [<i>Alcaligenes faecalis</i>]	Core	PF07728	AAA_5, ATPase family associated with various cellular activities (AAA)	PF00158	Sigma-54 interaction domain	PF00072	Response regulator receiver domain
PA4853	Fis	107		80% similar to Fis protein [<i>Escherichia coli</i>]	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family				
PA5125	NtrC	476		79% similar to <i>S.typhimurium</i> NtrC	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF07728	AAA_5, ATPase family associated with various cellular activities (AAA)	PF00158; PF00072	Sigma-54 interaction domain; Response regulator receiver domain
PA5166		462		67% similar to C4-dicarboxylate transport system regulatory protein DctD [<i>Rhizobium meliloti</i>]	Core	PF00072	Response regulator receiver domain	PF00158	Sigma-54 interaction domain		
PA5483	AlgB	449		100% similar to AlgB [<i>Azotobacter vinelandii</i>]; 97% response regulator <i>Desulfomicrobium baculatum</i> DSM 4028]	Core	PF02954	HTH_8, Bacterial regulatory protein, Fis family	PF00158	Sigma-54 interaction domain	PF00072	Response regulator receiver domain
PA5511	MifR	447		69% similar to DctD gene product [<i>R. meliloti</i>]	Core	PF00158	Sigma-54 interaction domain	PF00072	Response regulator receiver domain		
GntR											
PA0120		228	FadR	45% similar to putative transcriptional regulator LldR [<i>Escherichia coli</i>]; Part of two gene operon	Core	PF07729	FCD, FCD domain.				

PA0121		244	FadR	44% similar to hypothetical transcriptional regulator [<i>Streptomyces ambofaciens</i>]	Core	PF07729	FCD, FCD domain.						
PA0253		158	MarR	55% similar to MarR regulator [<i>Salmonella typhimurium</i>]	Core	PF01047	MarR family						
PA0275		228	GntR	48% similar to hypothetical protein Rv3676 [<i>Mycobacterium tuberculosis</i>]	Core	PF00027	cNMP_bindin g, Cyclic nucleotide- binding domain.						
PA0424	MexR	147	MarR	48% similar to HosA protein [<i>Escherichia coli</i>]	Core	PF01047	MarR family						
PA0487		252		54% similar to molybdate uptake regulatory protein ModE [<i>Escherichia coli</i>]	Core	PF03459	TOBE domain to bind ligands	PF00126	HTH_1	PF03459	TOBE		
PA0797		240	FadR	48% similar YdhC protein [[<i>Bacillus subtilis</i>]]; 3 gene operon, 1st gene, the other two genes are involved in citrate synthase activity	Core	PF07729	FCD, FCD domain.						
PA0942		186	MarR	51% similar to a region of regulatory protein PecS [<i>Erwinia chrysanthemi</i>]	Core	PF01047	MarR family						
PA1142		238	HutC	65% similar to PhnR, probable repressor protein in phnXWRSTUV locus for uptake and metabolism of 2-aminoethylphosphonate [<i>Salmonella typhimurium</i>]	Core	PF07702	UTRA, UTRA domain.						
PA1269		222	FadR	44% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF07729	FCD, FCD domain.						
PA1285		149	MarR	56% similar to hypothetical protein Rv1049 [<i>Mycobacterium tuberculosis</i>]	Core	PF01047	MarR family						
PA1374		168	GntR superfamily	51% similar to hypothetical protein Rv3095 [<i>Mycobacterium tuberculosis</i>]	Core	PF01638	HxlR-like HTH			Weak MarR			
PA1467		323		52% similar to putative repressor [<i>Streptomyces peucetius</i>]	Core								
PA1490		258		59% similar to repressor GlpR [<i>Pseudomonas aeruginosa</i>]	Core								
PA1520		259	FadR	48% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF07729	FCD, FCD domain.						
PA1526		219	GntR superfamily	46% similar to hypothetical protein [<i>Escherichia coli</i>]; 1st gene in a 2-gene operon (2nd gene - Hypo)	Core		No discernible second domain						
PA1603		140	MarR	50% similar to HosA protein [<i>Escherichia coli</i>]	Core	PF01047	MarR family						

PA1627		215	FadR	70% similar to unknown ORF [<i>Pseudomonas marginalis</i> pv. <i>alfalfae</i>]; 1st gene in a 2-gene operon; the other gene is probable major facilitator superfamily (MFS) transporter	Core	PF07729	FCD, FCD domain.
PA1653		158	MarR	61% similar to PetP protein <i>Rhodobacter capsulatus</i>	Core	PF01047	MarR family
PA2032	YjiR	474	MocR	72% similar to putative regulator YjiR [<i>Escherichia coli</i>]	RGP 20	PF00155	Aminotransferase class I and II.
PA2100		477	MocR	49% similar to hypothetical transcriptional regulator [<i>Rhodobacter sphaeroides</i>]; Human homolog	RGP 21	PF00155	Aminotransferase class I and II.
PA2299		249	HutC	53% similar to putative transcriptional regulator YvoA [<i>Bacillus subtilis</i>]; 3rd gene in a 3-gene operon ; The other two code for probable ferridoxin and oxidoreductase	Core	PF07702	UTRA, UTRA domain.
PA2320	GntR, GnuR	343		56% similarity to GntR [<i>Escherichia coli</i>]	Core	PF00532	Peripla_BP_1, Periplasmic binding proteins and sugar binding domain of the LacI family.
PA2692		174	Rrf2 subfamily	55% similar to hypothetical protein [<i>Bacillus halodurans</i>]; Homologous to NsrR [<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> PC1]	Core	PF02082	Rrf2, Transcriptional I regulator.
PA2802		239	HutC	56% similar to probable repressor protein PhnR [<i>Salmonella typhimurium</i>]	Core	PF07702	UTRA, UTRA domain.
PA2825	OspR	163	MarR	69% similar to hypothetical protein [<i>Acinetobacter</i> sp. ADP1]	Core	PF01047	MarR family
PA2849	OhrR	151	MarR	74% similar to hypothetical protein [<i>Acinetobacter</i> sp. ADP1]	Core	PF01047	MarR family
PA2897		479	MocR	59% similar to putative regulator YjiR [<i>Escherichia coli</i>]; Human Homolog;	Core	PF00155	Aminotransferase class I and II.
PA3067		147	MarR	50% similar to hypothetical protein Rv0880 [<i>Mycobacterium tuberculosis</i>]		PF01047	MarR family
PA3249		298	HutC	60% similar to regulator PhnR [<i>Salmonella typhimurium</i>]	Core	PF07702	UTRA, UTRA domain.
PA3341		144	MarR	62% similar to transcriptional regulator SlyA [<i>Salmonella typhimurium</i>]	Core	PF01047	MarR family
PA3381	PhnF	240	HutC	58% similar to PhnF protein [<i>Escherichia coli</i>]; 10th gene in a 13-gene operon	Core	PF07702	UTRA, UTRA domain.

PA3458		157	MarR	48% similar to transcriptional regulator YkoM [<i>Bacillus subtilis</i>]	Core	PF01047	MarR family
PA3583	GlpR	251			Core		
PA3757		247	HutC	55% similar to putative transcriptional regulator YvoA [<i>Bacillus subtilis</i>]; First gene in a 5-gene operon involved in N-acetylglucosamine catabolism	Core	PF07702	UTRA, UTRA domain.
PA3815	IscR	163	Rrf2 subfamily	84% similar to unknown hypothetical protein [<i>A. vinelandii</i>]	Core	PF02082	Rrf2, Transcriptional regulator
PA4132		471	MocR	64% similar to putative transcriptional regulator [<i>Escherichia coli</i>]	Core	PF00155	Aminotransferase class I and II.
PA4135		140	MarR	73% similar to homoprotocatechuate degradation operon regulator HpcR [<i>Escherichia coli</i>]	Core	PF01047	MarR family
PA4165		496	MocR	53% similar to putative regulatory protein MocR [<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>]; Human Homolog	Core	PF00155	Aminotransferase class I and II.
PA4169		142	Rrf2 subfamily	57% similar to hypothetical protein YwnA [<i>Bacillus subtilis</i>]	Core	PF02082	Rrf2, Transcriptional regulator
PA4185		242	FadR	47% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF07729	FCD, FCD domain.
PA4764	Fur	134	GntR superfamily	77% similar to Regulatory protein Fur [<i>Escherichia coli</i>]	Core	PF01475	FUR, Ferric uptake regulator family
PA4769	LldR	257	FadR	66% similar to transcriptional regulator for pyruvate dehydrogenase complex PdhR [<i>Escherichia coli</i>]	Core	PF07729	FCD, FCD domain.
PA4906	VanR	237	FadR	66% similar to hypothetical protein AF009672 [<i>Acinetobacter</i> sp. ADP1]; Part of a 2-gene operon, upstream gene is a probable dehydrogenase	Core	PF07729	FCD, FCD domain.
PA5105	HutC	250	HutC	94% similar to hutC gene product of <i>Pseudomonas putida</i> ; 1st gene in a 2-gene operon (2nd gene - Hypo)	Core	PF07702	UTRA, UTRA domain.
PA5157	MarR	156	MarR	60% similar to MarR protein [<i>Escherichia coli</i>]	Core	PF01047	MarR family
PA5283		458	MocR	48% similar to putative transcriptional regulator [<i>Bacillus subtilis</i>] (Human homolog)	Core	PF00155	Aminotransferase class I and II.

PA5356	GlcC	251	FadR	78% similar to GlcC protein [<i>Escherichia coli</i>]; 1st gene in a 3-gene operon; 2nd-Hyp and 3rd- 4-hydroxybenzoate-octaprenyl transferase	Core	PF07729	FCD, FCD domain.		
PA5431		491	MocR	52% similar to hypothetical regulator MocR [<i>Rhizobium meliloti</i>]; 1st gene in a 2-gene operon (2nd gene - Hypo)	Core	PF00155	Aminotransferase class I and II.		
PA5499	Np20	167		54% similar to zinc-uptake regulator Zur [<i>Escherichia coli</i>]; PMID: 8971720; 1st gene in a 3-gene operon; 2nd & 3rd involved in Zn transport	Core	PF01475	FUR, Ferric uptake regulator family.		
PA5525		246	FadR	49% similar to <i>nta</i> operon transcriptional regulator [<i>Escherichia coli</i>]; 2nd gene in a part of 2-gene operon; downstream gene is a probable short-chain dehydrogenase	Core	PF07729	FCD, FCD domain.		
PA5550	GlmR	257		60% similar to regulator for glucitol operon, SrlR [<i>Escherichia coli</i>]	Core				
PA0268		473	MocR	55% similar to putative regulatory protein MocR [<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>]; Has a human homolog	Core	PF00155	Aminotransferase class I and II.		
H-NS (silencing)									
PA2667	MvaU	117		64% similar to P16 subunit of heteromeric transcriptional activator MvaT [<i>Pseudomonas mevalonii</i>]	Core				
PA4315	MvaT	124		82% similar to heteromeric transcriptional activator MvaT P16 subunit [<i>Pseudomonas mevalonii</i>]	Core	PB003306	Pfam-B_3306	PF00816.14	Histone_HNS
IclR									
PA0155	PcaR	279		88% similar to regulatory protein PcaR [<i>Pseudomonas putida</i>]	other				
PA0236		259		49% similar to repressor protein IclR [<i>Escherichia coli</i>]	Core				
PA1015		266		42% similar to glycerol operon regulatory protein GylR [<i>Streptomyces coelicolor</i>]	Core				
PA1630		288		58% similar to hypothetical protein [<i>Bordetella pertussis</i>]	Core				
PA2010		267		49% similar to putative transcriptional regulatory protein OhbR [<i>Pseudomonas aeruginosa</i>]	Core				
PA3174		242			0 Core				
PA3508		277		57% similar to hypothetical protein [<i>Acinetobacter</i> sp. ADP1]	RGP34				

PA4157		262	51% similar to putative transcriptional regulatory protein OhbR [<i>Pseudomonas aeruginosa</i>]	Core						
PA4341		256	45% similar to transcriptional regulator for mhp operon [<i>Escherichia coli</i>]	Core						
LacI/GalR										
PA1949	RbsR	337	62% similar to regulator for rbs operon RbsR [<i>Escherichia coli</i>]	Core						
PA2259	PtxS	340		0	Core					
PA3184	HexR	285	76% similar to HexR protein [<i>Escherichia coli</i>]	Core	PF01418	RpiR family	PF01380	SIS (Sugar ISomerase) domain		
PA3563	FruR	329	62% similar to transcriptional repressor of fru operon FruR [<i>Escherichia coli</i>]	Core						
PA4596		172	74% similar to nfxB gene product of [<i>Pseudomonas aeruginosa</i>]	Core						
PA4600	NfxB	187	95% similar to nfxB (excluding frameshift) [<i>P. aeruginosa</i>]	Core						
PA5438		293	63% similar to putative regulator HexR [<i>Pseudomonas aeruginosa</i>]	Core	PF01418	RpiR family	PF01380	SIS (Sugar ISomerase) domain		
LuxR										
PA0034		207	75% similar to BvgA positive transcription regulator, putative [<i>Bordetella pertussis</i>];	Core	PF00072	Response regulator	PF00196	GerE		
PA0533		496	44% similar to a region of putative sensory transduction histidine kinase [<i>Methanobacterium thermoautotrophicum</i>]	Core	PF00196	GerE	PF00989	PAS	PF08448; PB00136 0; PB00254 6	PAS_4; Pfam-B_1360; Pfam-B_2546
PA0601		210	66% similar to VsrD protein [<i>Burkholderia solanacearum</i>]	Core	PF00072	Response regulator	PF08266	GerE	PB00104 1	Pfam-B_1041
PA1136		243	42% similar to transcriptional activator LasR [<i>Pseudomonas aeruginosa</i>]	Core	PF03472	Autoinducer binding	PF00196	GerE		
PA1347		230	65% similar to a region of putative regulator FimZ [<i>Salmonella typhimurium</i>]	Core	PF00196	GerE	PF08448	PAS_4		
PA1397		210	56% similar to nitrate/nitrite response regulator NarP (sensor NarQ) [<i>Escherichia coli</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA1430	LasR	239	100% identical to transcription activator LasR [<i>Pseudomonas aeruginosa</i>]	Core	PF03472	Autoinducer binding	PF00196	GerE		

PA1484		268	50% similar to DMSO reductase regulatory protein DorX [<i>Rhodobacter sphaeroides</i>]	Core	PF00196	GerE	PB000146	Pfam-B_146		
PA1759		901	44% similar to regulator AcoK [<i>Klebsiella pneumoniae</i>]	Core	PF00196	GerE	PB008287	Pfam-B_8287		
PA1760		907	44% similar to regulator AcoK [<i>Klebsiella pneumoniae</i>]	Core	PF00196	GerE	PB001429	Pfam-B_1429	PB008287	Pfam-B_8287
PA1898	QscR, PhzR	237	29% identity, 46% similarity to LasR [<i>Pseudomonas aeruginosa</i>]; 27% identity, 45% similarity to LuxR [<i>Vibrio fischeri</i>]; 33% identity, 49% similarity to SolR [<i>Ralstonia solanacearum</i>]; 49% similar to positive regulatory protein PhzR [<i>Pseudomonas aureofaciens</i>]; 53% similar, 32% identity to regulatory protein RhIR [<i>Pseudomonas aeruginosa</i>]	Core	PF03472	Autoinducer binding	PF00196	GerE		
PA1978	ErbR, AgmR	221	100% identical to <i>agmR</i> gene product of [<i>P. aeruginosa</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA1980	EraR, ExaE	225	57% similar to uhpA gene product of [<i>Salmonella typhimurium</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA2376		213	54% similar to nitrate/nitrite regulatory protein NarL [<i>Pseudomonas aeruginosa</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA2586	GacA	214	99% similar to response regulator GacA [<i>Pseudomonas aeruginosa</i>]	Core	PF00072	Response regulator	PF00196	GerE	PB000645	Pfam-B_645
PA2591	VqsR	268	46% similar to DMSO reductase regulatory protein DorX [<i>Rhodobacter sphaeroides</i>]	Core	PF00196	GerE	PB000146	Pfam-B_146		
PA2899		212	53% similar to biosynthesis positive regulator RcsB [<i>Escherichia coli</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA3045		207	77% similar to putative transcription regulator BvgA [<i>Bordetella pertussis</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA3420		827		Core	PF00196	GerE	PB010363	Pfam-B_10363		
PA3477	RhlR	241		Core	PF03472	Autoinducer binding	PF00196	GerE		
PA3599		261	47% similar to dorX gene product of [<i>Rhodobacter sphaeroides</i>]	Core	PF00196	GerE	PB000146	Pfam-B_146	PB000645	Pfam-B_645
PA3604	ErdR	217	61% similar to glycerol metabolism activator (AGMR protein) [<i>P. aeruginosa</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA3714		213	62% similar to RcsB protein [<i>Erwinia amylovora</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA3771		325	54% similar to a region of putative regulatory protein [<i>Streptomyces coelicolor</i>]	Core	PF00196	GerE	PB005070	Pfam-B_5070		
PA3879	NarL	219	74% similar to E. coli NarL protein.	Core	PF00072	Response	PF00196	GerE		

regulator										
PA3921		906	42% similar to regulator AcoK [<i>Klebsiella pneumoniae</i>]	Core	PF00196	GerE	PB001429	Pfam-B_1429	PB001429; PB004550; PB009525; PB010363	Pfam-B_1429; Pfam-B_4550; Pfam-B_9525; Pfam-B_10363
PA3948	RocA1	209	74% similar to <i>bygA</i> gene product of [<i>Bordetella pertussis</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA4074		222	54% similar to regulator protein MoaR [<i>Klebsiella aerogenes</i>]	Core	PF03472	Autoinducer binding	PF00196	GerE	PB000146	Pfam-B_146
PA4080	RcsB	214	63% similar to rcsB gene product of [<i>Escherichia coli</i>]	Core	PF00072	Response regulator	PF00196	GerE		
PA4196	bfiR	214	62% similar to transcriptional regulatory protein FixJ [<i>Azorhizobium caulinodans</i>]	Core	PF00196	GerE, Bacterial regulatory proteins, luxR family	PF08281	Sigma70_r4_2, Sigma-70, region 4	PF00072	Response regulator receiver domain
PA4296	PprB	275	54% similar to NtrC-type response regulator [<i>Eubacterium acidaminophilum</i>] ; 43% similar to regulatory components of sensory transduction system <i>Synechocystis</i> sp.	Core	PF00072	Response regulator	PF00196	GerE, Bacterial regulatory proteins, LuxR family		
PA4806		227	46% similar to DMSO reductase regulatory protein DorX [<i>Rhodobacter sphaeroides</i>]	Core	PF00196	GerE				
LysR										
PA0032		304	49% similar to glycine cleavage system transcriptional activator protein GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	
PA0037	TrpI	295	97% similarity to TrpI protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	
PA0056		306	55% similar to regulator GstR [<i>Rhizobium leguminosarum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	
PA0123		302	51% similar to transcriptional activator PtxR [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	
PA0133		305	47% similar to transcriptional regulator OxyR [<i>Mycobacterium xenopi</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	
PA0152	PcaQ	275	56% similar to regulatory protein PcaQ [<i>Agrobacterium tumefaciens</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr	ate	

PA0159		312	52% similar to regulator GstR [<i>Bradyrhizobium japonicum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0181		310	48% similar to putative transcriptional regulator YeaT [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0191		305	59% similar to transcriptional control factor SdsB <i>Pseudomonas</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0207		297	51% similar to glycine cleavage activator protein GcvA [<i>Escherichia coli</i>]	RGP 1	PF00126	HTH_1	PF03466	LysR_substr ate
PA0217	MdcR	309	65% similar to malonate decarboxylase operon regulator MdcR [<i>Klebsiella pneumoniae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0218		306	49% similar to putative transcriptional regulator YvbU [<i>Bacillus subtilis</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0233		313	61% similar to putative transcriptional regulator [<i>Rhizobium leguminosarum</i> bv. <i>trifolii</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0272		310	44% similar to MexT protein [<i>Pseudomonas</i> <i>aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0289	GpuR	320	39% similar to LysR-type regulatory protein [<i>Comamonas</i> sp. JS765]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0448		302	55% similar to GcvA gene product of <i>E. coli</i> .	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0477		308	47% similar to DNA-binding protein NahR <i>Pseudomonas putida</i> ; 48% similar to MexT protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0479		316	46% similar to putative transcriptional regulator YhaJ [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0491		308	46% similar to oxidative stress transcriptional regulator OxyR [<i>Xanthomonas campestris</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0528		292	49% similar to positive regulator CynR [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0701		328	43% similar to iron-regulated virulence regulator [<i>Vibrio cholerae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0708		284	53% similar to putative transcriptional regulator YneJ [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0739		304	75% similar to SdsB (regulator of sdsA) <i>Pseudomonas</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0784		309	50% similar to glycine cleavage system transcriptional activator GcvA [<i>Haemophilus</i> <i>influenzae</i> Rd]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0815		317	46% similar to putative transcriptional regulator YjiE [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate

PA0816		294	60% similar to Brg1 [<i>Bordetella pertussis</i>], a transcriptional regulator that is controlled by the oxygen-responsive transcriptional regulator Btr under anaerobic conditions.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0876		314	54% similar to putative transcriptional regulator <i>Synechocystis</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA0877		298	60% similar to hypothetical protein [<i>Acinetobacter lwoffii</i> K24]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1003	MvfR, PqsR	332	99% identical to previously identified hypothetical protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1067		304	54% similar to regulator GstR [<i>Bradyrhizobium japonicum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1128		303	60% similar to YcaN, hypothetical transcriptional regulator [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1138		291	49% similar to transcription activator [<i>Azospirillum brasilense</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1141		300	51% similar to probable transcriptional regulator LrhA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1145		300	40% similar to positive transcriptional control factor SdsB <i>Pseudomonas</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1184		296	59% similar to glycine cleavage activator protein GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1201		304	60% similar to putative transcriptional regulator <i>Synechocystis</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1223		297	53% similar to regulator SyrM1 [<i>Rhizobium</i> sp. NGR234]	RGP 11	PF00126	HTH_1	PF03466	LysR_substr ate
PA1264		288	47% similar to regulator HexA [<i>Erwinia carotovora</i> subsp. <i>carotovora</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1309		286	52% similar to putative transcriptional regulator YwfK [<i>Bacillus subtilis</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1312		303	57% similar to PtxR [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1328		302	55% similar to a region of putative transcriptional regulator YcaN [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1399		306	49% similar to putative regulatory protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1413	YneJ	290	69% similar to putative transcriptional regulator YneJ [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1422	GbuR	297	49% similar to regulatory protein cfxR [<i>Alcaligenes eutrophus</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate

PA1570		293	54% similar to transcriptional regulator protein RbcR [<i>Chromatium vinosum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1738		304	62% similar to putative transcriptional regulator YcaN [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1754	CysB	324		Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1826		301	50% similar to regulator of catechol degradation (CatM) [<i>Acinetobacter calcoaceticus</i>]		PF00126	HTH_1	PF03466	LysR_substr ate
PA1853		287	80% similar to ORF286, hypothetical protein in the 30-kb denitrification gene cluster [<i>Pseudomonas stutzeri</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1859		295	69% similar to putative transcriptional regulator YafC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1915		515		Core	PF07905	PucR		
PA1961		311	50% similar to putative transcriptional regulator YafC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA1998	DhcR	306	52% similar to ybhD, yfeR, and yfhT hypothetical transcriptional regulatory proteins [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2054	CynR	295	66% similar to cynR gene product of [<i>Escherichia coli</i>]	RGP 20	PF00126	HTH_1	PF03466	LysR_substr ate
PA2056		300	54% similar to regulator GsrR [<i>Bradyrhizobium japonicum</i>]	RGP 20	PF00126	HTH_1	PF03466	LysR_substr ate
PA2076		300	44% similar to putative LysR-type transcriptional regulator <i>Pseudomonas putida</i>	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2115		317	47% similar to putative transcriptional regulator YrdL [<i>Bacillus subtilis</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2121		301	50% similar to transcriptional activator nahR of [<i>Pseudomonas putida</i> -plasmid]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2123		310	55% similar to putative transcriptional regulator YhcS [<i>Haemophilus influenzae</i> Rd]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2206		315	54% similar to galactose binding protein regulator [<i>Azospirillum brasilense</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2220	OprR	306	Identical to putative transcriptional regulator OprR [<i>Pseudomonas aeruginosa</i>]	RGP 23	PF00126	HTH_1	PF03466	LysR_substr ate
PA2258	PtxR	312		Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2267		298	45% similar to putative transcriptional activator MauR [<i>Paracoccus denitrificans</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2316		297	55% similar to hypothetical yafC gene product of [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate

PA2334		303	56% similar to positive transcriptional control factor SdsB <i>Pseudomonas</i> sp.	RGP 53	PF00126	HTH_1	PF03466	LysR_substr ate
PA2383		306	50% similar to positive regulator of gev operon GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2417		312	53% similar to transcriptional regulator PtxR [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2432	BexR	304	55% similar to putative transcriptional regulator <i>Synechocystis</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2447		307	51% similar to hypothetical protein [<i>Haemophilus influenzae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2469		304	52% similar to galactose binding protein regulator [<i>Azospirillum brasilense</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2492	MexT	347	100% similar to a region of MexT protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2497		292	52% similar to transcriptional regulator HexA [<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2510	CatR	290	81% similar to catR regulatory protein <i>Pseudomonas putida</i>	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2534		303	58% similar to putative transcriptional regulator YhjC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2547		305	56% similar to regulator GstR [<i>Bradyrhizobium japonicum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2551		310	54% similar to transcriptional activator NahR [<i>Pseudomonas putida</i> plasmid NAH7]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2601		298	46% similar to activator, hydrogen peroxide-inducible genes, OxyR [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2681		309	53% similar to regulator PtxR [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2758		295	46% similar to DNA binding protein HpkR [<i>Pseudomonas syringae</i> pv. <i>syringae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2834		319	47% similar to putative regulatory protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2838		323	53% similar to regulator GstR [<i>Bradyrhizobium japonicum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2846		284	56% similar to 2,2-dialkylglycine decarboxylase repressor protein [<i>Burkholderia cepacia</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2848		339	49% similar to regulator GstR [<i>Bradyrhizobium japonicum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2877		297	62% similar to putative transcriptional regulator YhjC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate

PA2879	HpkR	296	74% similar to putative DNA binding protein HpkR [<i>Pseudomonas syringae</i> pv. <i>syringae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2921		329	50% similar to regulator TsaR [<i>Comamonas testosteroni</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA2930		308	52% similar to putative regulatory protein TrpI <i>Pseudomonas syringae</i>	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3122	YibL	297	93% similar to hypothetical gene product YibL [<i>Azotobacter vinelandii</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3124		303	59% similar to hypothetical gene product YafC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3135		306	55% similar to putative transcriptional regulator <i>Synechocystis</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3225		309	48% similar to putative transcriptional regulator YeeY [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3321		304	52% similar to regulatory protein MexT [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3398		308	59% similar to putative transcriptional regulator YeiE [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3433	Ywbl	297	59% similar to putative transcriptional regulator Ywbl [[<i>Bacillus subtilis</i>]]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3565		306	50% similar to GsR [<i>Rhizobium leguminosarum</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3587	MetR	306	63% similar to regulator MetR [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3594		295	55% similar to putative transcriptional regulator YnfL [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3630		302	53-56% similar to numerous transcriptional regulators	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3711		301	43% similar to putative transcriptional regulator YgiP [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3776		302	52% similar to regulator BudR [<i>Klebsiella terrigena</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3778		311	46% similar to regulator CbbRI [<i>Rhodobacter capsulatus</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3845		298	52% similar to positive regulator GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3895		317	55% similar to putative transcriptional regulator <i>Synechocystis</i> sp.	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA3995		297	51% similar to the HexA gene product of [<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4109	AmpR	296		Core	PF00126	HTH_1	PF03466	LysR_substr ate

PA4145		296	48% similar to transcriptional activator PtxR [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4174		307	55% similar to putative transcriptional regulator YhjC [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4203		284	61% similar to putative transcriptional regulator YneJ [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4363	IciA	300	56% similar to inhibitor of chromosome initiation IciA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4902		298	51% similar to putative regulatory protein [<i>Pseudomonas stutzeri</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4914		312	53% similar to positive regulator GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA4989		294	46% similar to DNA binding protein HpkR [<i>Pseudomonas syringae</i> pv. <i>syringae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5029	YnfL	301	72% similar to putative transcriptional regulator YnfL [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5085		318	47% similar to regulatory protein LysR [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5179		293	47% similar to putative LysR-type transcriptional regulator [<i>Mycobacterium leprae</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5189		302	51% similar to putative regulatory protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5218		304	57% similar to hypothetical ynfL gene product of [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5293		305	53% similar to positive regulator GcvA [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5344	OxyR	310	60% similar to oxyR gene product of [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5382	YeiE	297	64% similarity to putative transcriptional regulator YeiE [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5428		302	56% similar to putative transcriptional regulator YjiE [<i>Escherichia coli</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5437		311	62% similar to regulator CbbRI [<i>Rhodobacter capsulatus</i>]	Core	PF00126	HTH_1	PF03466	LysR_substr ate
PA5261	AlgR	248	88% similar to encystment and alginate biosynthesis regulatory protein [<i>Azotobacter vinelandii</i>]	Core	PF00072	Response regulator receiver domain	PF04397	LytTR, LytT DNA- binding domain
MerR								

PA2016	LiuR, GnyR	134	85% identical to transcriptional regulator, putative [P. putida KT2440] ; 59% similar to putative transcriptional regulator YbbI [Escherichia coli]	Core	PF00376	MerR	PF09278	MerR-DNA-bind		
PA2273	SoxR	156	Identical to unpublished SoxR of P. aeruginosa (gi 2495412 sp Q51506) ; 77% similar to SoxR gene product of [Escherichia coli]	Core	PB000200	Pfam-B_200	PF00376	MerR	PB016009; PF09278	Pfam-B_16009; MerR-DNA-binding
PA2718		162	52% similar to putative transcriptional regulator YbbI [Escherichia coli]	Core	PF00376	MerR	PF09278	MerR-DNA-bind		
PA3689	YhdM	156	66% similar to putative regulatory protein YhdM [Haemophilus influenzae]	Core	PF00376	MerR	PF09278	MerR-DNA-bind		
PA4659		299	47% similar to putative transcriptional regulator YcgE [Escherichia coli]	Core	PB000200	Pfam-B_200	PF00376	MerR		
PA4778	CueR, YbbI	132	65% similar to putative transcriptional regulator YbbI [Escherichia coli]	Core	PF00376	MerR	PF05103	DivIVA	PF09278	MerR-DNA-binding
PA4878		270	47% similar to a region of multi-drug efflux transporter regulator TipA [Streptomyces lividans]	Core	PF00376	MerR	PF06445	AraC-binding domain		
PA5116		141	49% similar to activator SoxR [Escherichia coli]	Core	PF00376	MerR	PF09278	MerR-DNA-bind		
OmpC family										
PA5200	AmgR	247	OmpR similar E.coli	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain		
OmpR-PhoP class										
PA4032		238	52% similar to hypothetical protein Rv1033c (response regulator homolog TrcR) [Mycobacterium tuberculosis]; 51% similar to phosphate regulatory protein PhoB [Bradyrhizobium japonicum]		PF0072	Response regulator receiver domain	PF00486	Transcriptional regulatory protein, C terminal		
PA4381		227	91% similar to ColR [Pseudomonas fluorescens]	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptional regulatory protein, C terminal		
RpiR										

PA5506		285	44% similar to RpiR protein [<i>Escherichia coli</i>]	Core	PF01418	RpiR family	PF01380	SIS (Sugar Isomerase) domain
TetR								
PA0167		221	60% similar to putative regulator YcdC [<i>Escherichia coli</i>]	Core	PF08362	TetR_C_3, YcdC-like protein, C-terminal region		
PA0243		222	44% similar to putative transcriptional regulator [<i>Methanobacterium thermoautotrophicum</i>]	Core	PF00440	TetR_N, Bacterial regulatory proteins, tetR family		
PA0294	AguR	221	49% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core	PF00440	TetR_N, Bacterial regulatory proteins, tetR family		
PA0367		215	53% similar to hypothetical protein <i>Pseudomonas putida</i>	Core				
PA0436		206	54% similar to putative tet operon regulator YcdC [<i>Escherichia coli</i>]	Core	PF08362	TetR_C_3, YcdC-like protein, C-terminal region		
PA0475		189	47% similar to hypothetical protein Rv0196 [<i>Mycobacterium tuberculosis</i>]	Core				
PA0828		227	50% similar to a region of putative transcriptional regulator [<i>Aquifex aeolicus</i>]	Core				
PA0839		196	48% similar to hypothetical protein [<i>Escherichia coli</i>]	Core				
PA1226		202	45% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core				
PA1241		186	46% similar to hypothetical protein YxaF [<i>Bacillus subtilis</i>]	Core				
PA1283		186	48% similar to hypothetical protein <i>Synechocystis</i> sp.	Core				
PA1290		197	54% similar to unidentified reading frame L (ORFL TetC protein) in transposon Tn10 [<i>Escherichia coli</i>]	Core				
PA1315		204	49% similar to MtrR protein [<i>Neisseria gonorrhoeae</i>]	Core				

PA1403		210	43% similar to a region of hypothetical protein Rv1963c [<i>Mycobacterium tuberculosis</i>]	Core		
PA1504		216	57% similar to putative tet operon regulator YcdC [<i>Escherichia coli</i>]	Core	PF08362	TetR_C_3, YcdC-like protein, C-terminal region
PA1539		282	53% similar to a region of hypothetical protein YijC [<i>Escherichia coli</i>]		PF00440	
PA1836		193	45% similar to putative repressor [<i>Bacillus megaterium</i>]	Core		
PA1864		216	54% similar to putative tet operon regulator YcdC [<i>Escherichia coli</i>]	Core	PF08362	TetR_C_3, YcdC-like protein, C-terminal region
PA2020		210	72% similar to transcriptional regulator AmrR [<i>Burkholderia pseudomallei</i>]	Core	PF08361	TetR_C_2, MAATS-type transcriptional repressor, C-terminal region
PA2196		194	63% similar to hypothetical protein [<i>Escherichia coli</i>]	Core		
PA2270		196	52% similar to hypothetical protein <i>Synechocystis</i> sp.	Core		
PA2484		204	65% similar to SocA3 protein <i>Myxococcus xanthus</i>	Core		
PA2766		193		Core		
PA2885	AtuR	198	50% similar to hypothetical protein Rv3557c [<i>Mycobacterium tuberculosis</i>]	Core		
PA2931	CifR	196	63% similar to socA3 protein <i>Myxococcus xanthus</i>	Core		
PA2957		212	44% similar to putative transcriptional regulator [<i>Streptomyces coelicolor</i>]	Core		
PA3006	PsrA	233	90% similar to PsrA <i>Pseudomonas putida</i>	Core		
PA3034		185	54% similar to putative transcriptional regulator <i>Streptomyces lividans</i>	Core		
PA3133		180		Core		

PA3574	NalD	212	46% similar to acrR gene product, a probable transcriptional regulator from <i>[Escherichia coli]</i>	Core	PF08361	TetR_C_2, MAATS-type transcriptional repressor, C-terminal region		
PA3678		212	47% similar to putative transcriptional regulator <i>[Streptomyces coelicolor]</i>	Core				
PA3699		237	65% similar to a region of hypothetical protein Rv3066 <i>[Mycobacterium tuberculosis]</i>	Core				
PA3721	NalC	213	43% similar to putative transcriptional regulator <i>[Streptomyces coelicolor]</i>	Core				
PA3973		215	45% similar to hypothetical protein Rv3557c <i>[Mycobacterium tuberculosis]</i>	Core				
PA4831		186	54% similar to a region of acrAB operon repressor AcrR <i>[Escherichia coli]</i>	Core				
PA4890	DesT	209	52% similar to hypothetical protein YijC <i>[Escherichia coli]</i>	Core				
PA4984		215	46% similar to regulatory protein BetI <i>[Escherichia coli]</i>	Core				
PA5059		205	86% similar to PhaD <i>[Pseudomonas sp.]</i> ; 99% similar to hypothetical protein 3 (phaC2 3' region)	Core				
PA5374	BetI	197	71% similar to <i>E. coli betI</i> gene product	Core				
CRP								
PA0652	Vfr	214		Core	PF00325	Crp	PF00027.22	cNMP_binding
PA1544	Anr	244	100% identical to transcriptional activator Anr <i>[Pseudomonas aeruginosa]</i>	Core	PF00325	Crp	PF00027.22	cNMP_binding
Unclassified								
PA0116		157	65% similar to hypothetical protein Rv3095 <i>[Mycobacterium tuberculosis]</i>	Core	PF01638.10	HxlR		
PA0463	CreB	229	68% similar to <i>E. coli</i> catabolic regulation response regulator <i>creB</i> gene product ; 71% similar to <i>Aeromonas jandaei</i> response regulator protein	Core	PF00486	Transcriptional regulatory protein, C-terminal	PF00072	Response regulator receiver domain
PA0527	Dnr	227	100% identical to Dnr protein <i>[Pseudomonas aeruginosa]</i>	Core	PF00027	cNMP_binding		
PA0610	PrtN	104		Core	PF11112.1	PyocinActivator		

PA0707	ToxR, RegA	259	99% identity to ToxR (exotoxin A regulatory protein) [<i>P. aeruginosa</i>]	Core	PF07720	TPR_3, tetratricopeptide repeat	
PA0756		223	72% similarity to trans-activating transcriptional regulatory protein tctD [<i>Salmonella typhimurium</i>]	Core	PF00486		Response regulator receiver domain
PA0929		239	53% similar to Response-regulator BaeR protein [<i>Escherichia coli</i>]; 64% similar to PfeR protein [<i>Pseudomonas aeruginosa</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	Response regulator receiver domain
PA1157		236	63% similar to transcriptional regulatory protein RstA (RstB sensor) [<i>Escherichia coli</i>] ; 64% similar to response regulator OmpR (sensor, EnvZ)	Core	PF00072	Response regulator receiver domain	Transcriptional regulatory protein, C terminal
PA1179	PhoP	225	71% similar to regulatory protein PhoP [<i>Salmonella typhimurium</i>] ; 62% similar to trans-activating protein tctD [<i>Salmonella typhimurium</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	Response regulator receiver domain
PA1431	RsaL	80		??			
PA1437		229	81% similar to transcriptional activator protein CzcR [<i>Ralstonia eutropha</i>] ; 74% similar to transcriptional activator protein CopR [<i>Pseudomonas syringae</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	Response regulator receiver domain
PA1607		146	60% similar to hypothetical protein Rv3095 [<i>Mycobacterium tuberculosis</i>]	Core	PF01638.10	HxlR	
PA1637	KdpE	230	69% similar to <i>kdpE</i> gene product of [<i>E. coli</i>]		PF00486	Transcriptional regulatory protein, C terminal	Response regulator receiver domain
PA1704	PcrR	144	64% similar to LcrR [<i>Yersinia enterocolitica</i>] ; 99% similar to PcrR [<i>Pseudomonas aeruginosa</i>] ; 64% similar to low calcium response locus protein LcrR [<i>Yersinia pestis</i>]	Core	PF09621.3	LcrR	
PA1799	ParR	235	64% similar to RstA [<i>Escherichia coli</i>]	Core	PF00072	Response regulator receiver domain	Transcriptional regulatory protein, C terminal
PA2479		226	64% similar to putative 2-component transcriptional regulator YgiX [<i>Escherichia coli</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	Response regulator receiver domain

PA2523		224	75% similar to regulator protein CzcR [<i>Ralstonia eutropha</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA2572		447	57% similar to a region of putative DNA-binding protein HupR1 <i>Rhodobacter capsulatus</i>	Core	PF00072	Response regulator receiver domain		
PA2657		223	62% similar to putative 2-component transcriptional regulator YgiX [<i>Escherichia coli</i>]	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptional regulatory protein, C terminal
PA2686	PfeR	305	PfeR activator of the ferric enterobactin receptor (PfeA)	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA2713		159	61% similar to hypothetical protein Rv3095 [<i>Mycobacterium tuberculosis</i>]	Core	PF01638.10	HxlR		
PA2736		117		RGP 28	PF01047.15	MarR		
PA2809	CopR	226	82% similar to transcriptional activator CopR <i>Pseudomonas syringae</i>	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptional regulatory protein, C terminal
PA2881								
PA3007	LexA	204	77% similar to LexA protein [<i>Escherichia coli</i>]	Core	PF01726	LexA_DNA_bind	PF00717.16	Peptidase_S24
PA3077		223	68% similar to ColR [<i>Pseudomonas fluorescens</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA3192	GltR	242	99% similar to GltR [<i>Pseudomonas aeruginosa</i>]	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA3204		225	65% similar to <i>E. coli</i> cpxR gene product.	Core	PF00486	Transcriptional regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA3385	AmrZ	108		Core	PF03869	Arc		

PA3864	DauR	210	44% similar to hypothetical protein [<i>Streptomyces coelicolor</i>] A3(2)]	Core	PF08348 .4	PAS_6		
PA4057	NrdR, YbaD	154	82% similar to hypothetical ybaD gene product of [<i>Escherichia coli</i>]	Core	PF03477 .9	ATP-cone		
PA4101	BfmR	246	63% similar to glucose uptake regulatory gene glrR [<i>Pseudomonas aeruginosa</i>]	RGP 44	PF00072	Response regulator receiver domain	PF00486	Transcriptio nal regulatory protein, C terminal
PA4182		212	51% similar to hypothetical protein YKL070w [<i>Saccharomyces cerevisiae</i>]	Core	PF04299 .5	FMN_bind_2		
PA4776	PmrA	221	70% similar to putative 2-component transcriptional regulator YgiX [<i>Escherichia coli</i>]	Core	PF00486	Transcriptiona l regulatory protein, C terminal	PF00072	Response regulator receiver domain
PA4885	IrlR	229	79% similar to IrlR gene product of <i>Burkholderia pseudomallei</i> ; 81% similar to <i>czcR</i> [<i>Ralstonia eutropha</i>]; 78% similar to transcriptional activatorprotein CopR [<i>Pseudomonas syringae</i>]	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptio nal regulatory protein, C terminal
PA4983		244	63% similar to DmsR [<i>Rhodobacter sphaeroides</i>]	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptio nal regulatory protein, C terminal
PA5360	PhoB	229	96% similar to <i>phoB</i> gene product of [<i>P. aeruginosa</i>]	Core	PF00072	Response regulator receiver domain	PF00486	Transcriptio nal regulatory protein, C terminal

Supplementary Table 2: Transcription factor interacting partners. Genes that are differentially regulated by each transcriptional regulator were identified using the *Pseudomonas* database (Winsor *et al.*, 2009), published data (Madan Babu *et al.*, 2006) and data mining using STRING 8.3 (<http://string.embl.de/>). The data presented in this table was used as the input data for Cytoscape (www.cytoscape.org) to generate Fig 3.2.

ORF #	Gene Name	No. of regulated genes	Genes regulated
PA0652	<i>vfr</i>	96	PA0066, PA0165, PA0224, PA0265, PA0347, PA0376, PA0551, PA0552, PA0589, PA0653, PA0689, PA0783, PA0836, PA1092, PA1097, PA1183, PA1288, PA1337, PA1384, PA1430, PA1562, PA1580, PA1581, PA1582, PA1583, PA1584, PA1585, PA1586, PA1587, PA1588, PA1589, PA1629,
PA1544	<i>anr</i>	77	PA0836, PA1172, PA1173, PA1174, PA1175, PA1176, PA1317, PA1318, PA1319, PA1320, PA1321, PA1337, PA1475, PA1476, PA1477, PA1479, PA1480, PA1481, PA1482, PA1483, PA1546, PA1561, PA1562, PA1581, PA1582, PA1583, PA1584, PA1585, PA1586, PA1588, PA1589, PA1780,
PA2738	<i>himA</i>	57	PA0066, PA0353, PA0482, PA0836, PA0888, PA0962, PA0993, PA0994, PA1326, PA1339, PA1376, PA1580, PA1585, PA1586, PA1588, PA1589, PA1629, PA1631, PA1818, PA2634, PA2637, PA2638, PA2639, PA2640, PA2641, PA2642, PA2643, PA2644, PA2645, PA2646, PA2647, PA2648,
PA4983		52	PA0265, PA0482, PA0599, PA0854, PA1183, PA1317, PA1318, PA1319, PA1320, PA1321, PA1376, PA1562, PA1580, PA1581, PA1582, PA1583, PA1584, PA1585, PA1586, PA1587, PA1588, PA1589, PA1787, PA2623, PA2634, PA2637, PA2638, PA2639, PA2640, PA2641, PA2642, PA2643,
PA3879	<i>narL</i>	37	PA1172, PA1173, PA1174, PA1175, PA1176, PA1475, PA1476, PA1477, PA1479, PA1480, PA1481, PA1482, PA1483, PA1780, PA1781, PA2611, PA2637, PA2638, PA2639, PA2640, PA2641, PA2642, PA2643, PA2644, PA2645, PA2646, PA2647, PA2648, PA2649, PA3724, PA3872, PA3873,
PA5308	<i>lrp</i>	36	PA0059, PA0316, PA0353, PA0993, PA0994, PA1070, PA1071, PA1072, PA1073, PA1074, PA1217, PA1326, PA1760, PA2443, PA3118, PA3120, PA3121, PA3167, PA3700, PA3972, PA4097, PA4503, PA4504, PA4505, PA4506, PA4695, PA4696, PA5013, PA5035, PA5036, PA5213, PA5214,
PA4853	<i>fis</i>	26	PA1155, PA1156, PA1581, PA1582, PA1583, PA1584, PA1585, PA1586, PA1587, PA1588, PA1589, PA1787, PA1804, PA3769, PA3770, PA4022, PA4343, PA4538, PA4740, PA4741, PA4742, PA4743, PA4744, PA4745, PA4746, PA4852
PA5360	<i>phoB</i>	22	PA0688, PA3127, PA3296, PA3372, PA3373, PA3374, PA3375, PA3376, PA3377, PA3378, PA3379, PA3380, PA3381, PA3382, PA3383, PA3384, PA3981, PA4874, PA5361, PA5365, PA5366, PA5367
PA1754	<i>cysB</i>	21	PA0280, PA0281, PA0282, PA0932, PA1393, PA1493, PA1756, PA1838, PA2356, PA2357, PA2709, PA3444, PA3540, PA3935, PA3936, PA3937, PA3938, PA4130, PA4442, PA4443, PA4513
PA4764	<i>fur</i>	17	PA1165, PA1618, PA1902, PA1922, PA2466, PA2688, PA3899, PA3900, PA4158, PA4159, PA4160, PA4161, PA4228, PA4231, PA4468, PA5521, PA5531
PA3563	<i>fruR</i>	16	PA0551, PA0552, PA1376, PA1498, PA1770, PA1780, PA1781, PA2611, PA2623, PA2634, PA3181, PA3194, PA3560, PA3561, PA3562, PA5192
PA0893	<i>argR</i>	14	PA0895, PA0896, PA0897, PA0898, PA0899, PA0901, PA2042, PA3068, PA3537, PA3934, PA4976, PA4977, PA5152, PA5170
PA3007	<i>lexA</i>	14	PA0576, PA0577, PA0579, PA1886, PA2585, PA3008, PA3138, PA3617, PA4232, PA4234, PA4660, PA4763, PA4821, PA5443
PA0253		12	PA0254, PA0272, PA1928, PA1929, PA2002, PA2077, PA2113, PA2965, PA3703, PA3805, PA5237, PA5489
PA2510	<i>catR</i>	12	PA2513, PA2518, PA2740, PA3279, PA3280, PA3656, PA4408, PA4417, PA4418, PA4759, PA5045, PA5259
PA3898		12	PA0305, PA0854, PA1384, PA1619, PA2028, PA2854, PA3183, PA3764, PA4378, PA4468, PA4997, PA5157

PA0424	<i>mexR</i>	11	PA0425, PA0426, PA2020, PA2492, PA2495, PA3168, PA3574, PA3720, PA3721, PA4600, PA4964
PA1097	<i>fleQ</i>	11	PA1098, PA1099, PA1100, PA1443, PA1452, PA1453, PA1454, PA1455, PA2020, PA3385, PA4462
PA1179	<i>phoP</i>	11	PA1180, PA1343, PA1559, PA1560, PA1979, PA3552, PA3559, PA4773, PA4777, PA5199, PA5361
PA0272		10	PA1928, PA1929, PA2002, PA2077, PA2113, PA2965, PA3703, PA3805, PA5237, PA5489
PA0762	<i>algU</i>	10	PA0376, PA0576, PA0766, PA3337, PA4572, PA5011, PA5012, PA5077, PA5078, PA5261
PA1430	<i>lasR</i>	9	PA1432, PA1871, PA2586, PA2587, PA3476, PA3477, PA3622, PA3724, PA3861
PA3204		9	PA0766, PA3644, PA3645, PA3646, PA3725, PA3737, PA4953, PA4954, PA5489
PA0527	<i>dnr</i>	8	PA1544, PA1546, PA1781, PA1920, PA2020, PA3391, PA3392, PA3879
PA0547		8	PA0960, PA0962, PA0963, PA2326, PA4943, PA4946, PA4967, PA5471
PA0873	<i>phhR</i>	8	PA0292, PA1750, PA3000, PA3139, PA3166, PA4462, PA5039, PA5434
PA3477	<i>rhlR</i>	8	PA3478, PA3479, PA3622, PA3724, PA3861, PA4407, PA4408, PA4409
PA5125	<i>ntrC</i>	8	PA0888, PA1339, PA5075, PA5119, PA5124, PA5287, PA5288, PA5484
PA0001	<i>dnaA</i>	7	PA0002, PA0003, PA0376, PA1155, PA1156, PA3769, PA3770
PA1003	<i>myfR</i>	7	PA1430, PA1690, PA1725, PA2227, PA2587, PA3477, PA3861
PA1099	<i>fleR</i>	7	PA1100, PA1101, PA1430, PA1454, PA1713, PA1714, PA4462
PA2273	<i>soxR</i>	7	PA2740, PA3280, PA3656, PA3718, PA4417, PA4759, PA5259
PA1619		6	PA0305, PA0854, PA3183, PA4468, PA4615, PA5157
PA1998	<i>dheR</i>	5	PA1999, PA2000, PA5384, PA5388, PA5389
PA2016	<i>liuR</i>	5	PA2320, PA2886, PA2888, PA2891, PA2893
PA2020		5	PA2642, PA3391, PA3721, PA4671, PA5471
PA2259	<i>ptxS</i>	5	PA2260, PA2261, PA2262, PA2263, PA4315
PA3583	<i>glpR</i>	5	PA0347, PA3581, PA3582, PA3584, PA5235
PA4280	<i>birA</i>	5	PA0420, PA0500, PA0501, PA0503, PA0504
PA0487		4	PA1861, PA1863, PA4210, PA4811
PA0610	<i>priN</i>	4	PA0611, PA0612, PA0651, PA4590
PA0780	<i>pruR</i>	4	PA0781, PA0782, PA0783, PA0982
PA1432	<i>lasI</i>	4	PA1898, PA2227, PA3477, PA4296
PA1760		4	PA2163, PA2339, PA2340, PA2341
PA1949	<i>rbsR</i>	4	PA1946, PA1947, PA1948, PA1950
PA1979	<i>eraS</i>	4	PA1980, PA2586, PA3948, PA5483
PA2551		4	PA1217, PA3118, PA3120, PA3121
PA2586	<i>gacA</i>	4	PA3476, PA3477, PA3622, PA4856
PA2665		4	PA1779, PA3697, PA4811, PA4893
PA3540	<i>algD</i>	4	PA5125, PA5200, PA5261, PA5483

PA5200	<i>amgR</i>	4	PA0045, PA1288, PA5261, PA5361
PA5261	<i>algR</i>	4	PA5262, PA5322, PA5483, PA5484
PA5356	<i>glcC</i>	4	PA0482, PA5352, PA5353, PA5355
PA5380	<i>gbdR</i>	4	PA5398, PA5399, PA5410, PA5411
PA0037	<i>trpI</i>	3	PA0609, PA3166, PA4590
PA0152	<i>pcaQ</i>	3	PA0153, PA0154, PA0247
PA0928	<i>gacS</i>	3	PA1898, PA2586, PA3006
PA0996	<i>pqsA</i>	3	PA1003, PA1430, PA3477
PA1184		3	PA5213, PA5214, PA5215
PA1637	<i>kdpE</i>	3	PA1633, PA1634, PA1635
PA1713	<i>exsA</i>	3	PA1714, PA1716, PA3841
PA1898	<i>qscR</i>	3	PA2586, PA3006, PA3476
PA2054	<i>cynR</i>	3	PA2052, PA2053, PA2500
PA2118	<i>ada</i>	3	PA1686, PA3306, PA3972
PA2258	<i>ptxR</i>	3	PA2259, PA2426, PA3479
PA2320	<i>gntR</i>	3	PA2321, PA3181, PA3194
PA2809	<i>copR</i>	3	PA2810, PA5199, PA5361
PA2885	<i>atuR</i>	3	PA2886, PA2888, PA2891
PA3006	<i>psrA</i>	3	PA3007, PA3571, PA3622
PA3184		3	PA3193, PA3194, PA5036
PA3476	<i>rhII</i>	3	PA3477, PA4723, PA5261
PA3587	<i>metR</i>	3	PA1843, PA4602, PA5124
PA3899		3	PA2914, PA3900, PA3901
PA4080		3	PA2978, PA3816, PA5563
PA4109	<i>ampR</i>	3	PA4110, PA4393, PA4522
PA4341		3	PA0247, PA4127, PA4152
PA4363	<i>iciA</i>	3	PA0001, PA0002, PA0003
PA4462	<i>rpoN</i>	3	PA4547, PA5125, PA5483
PA4547	<i>pilR</i>	3	PA4549, PA4556, PA5124
PA4600	<i>nfxB</i>	3	PA4964, PA5263, PA5348
PA4769		3	PA1587, PA5015, PA5016
PA5085		3	PA0836, PA2683, PA5277
PA5344	<i>oxyR</i>	3	PA0139, PA0140, PA0962
PA5374	<i>betI</i>	3	PA3933, PA5372, PA5373

PA5550	<i>glmR</i>	3	PA2274, PA4457, PA5552
PA0036	<i>trpB</i>	2	PA0037, PA3587
PA0155	<i>pcaR</i>	2	PA0159, PA4974
PA0159		2	PA1294, PA4974
PA0287	<i>gpuP</i>	2	PA0289, PA1422
PA0288	<i>gpuA</i>	2	PA0289, PA1422
PA0289	<i>gpuR</i>	2	PA1418, PA1421
PA0370		2	PA2273, PA2510
PA0409	<i>pilH</i>	2	PA0416, PA3587
PA0416	<i>chpD</i>	2	PA0417, PA1456
PA0425	<i>mexA</i>	2	PA2020, PA3721
PA0482	<i>glcB</i>	2	PA2273, PA2809
PA0506		2	PA3006, PA3571
PA0519	<i>nirS</i>	2	PA0527, PA1544
PA0520	<i>nirQ</i>	2	PA0527, PA1544
PA0576	<i>rpoD</i>	2	PA0652, PA1544
PA0611	<i>priR</i>	2	PA0612, PA4590
PA0831	<i>oruR</i>	2	PA2020, PA4402
PA0888	<i>aotJ</i>	2	PA0893, PA4315
PA1085	<i>flgJ</i>	2	PA1097, PA1099
PA1588	<i>sucC</i>	2	PA2273, PA2510
PA1589	<i>sucD</i>	2	PA2273, PA2510
PA1710	<i>exsC</i>	2	PA1713, PA3006
PA1957		2	PA0761, PA4919
PA1978	<i>erbR</i>	2	PA1982, PA3583
PA2012	<i>liuD</i>	2	PA2016, PA2885
PA2014	<i>liuB</i>	2	PA2016, PA2885
PA2219	<i>opdE</i>	2	PA2273, PA2510
PA2227	<i>vqsM</i>	2	PA3476, PA4296
PA2272	<i>pbpC</i>	2	PA2273, PA2510
PA2492	<i>mexT</i>	2	PA2493, PA2495
PA2523		2	PA2809, PA2810
PA2570	<i>lecA</i>	2	PA3477, PA4315
PA2571		2	PA3587, PA5261

PA2686	<i>pfeR</i>	2	PA2687, PA2688
PA2810	<i>copS</i>	2	PA5200, PA5360
PA2951	<i>etfA</i>	2	PA3006, PA3571
PA2952	<i>etfB</i>	2	PA3006, PA3571
PA2953		2	PA3006, PA3571
PA3574	<i>nalD</i>	2	PA0425, PA0426
PA4227	<i>pchR</i>	2	PA4228, PA4229
PA4581	<i>rtcR</i>	2	PA4583, PA4585
PA4726	<i>cbrB</i>	2	PA5105, PA5124
PA5199	<i>amgS</i>	2	PA5200, PA5360
PA0017		1	PA2586
PA0024	<i>hemF</i>	1	PA0527
PA0041		1	PA0652
PA0044	<i>exoT</i>	1	PA1713
PA0176	<i>aer2</i>	1	PA1544
PA0186		1	PA1754
PA0240		1	PA3782
PA0254		1	PA0272
PA0286	<i>desA</i>	1	PA4890
PA0292	<i>aguA</i>	1	PA0294
PA0293	<i>aguB</i>	1	PA0294
PA0296	<i>spuI</i>	1	PA4726
PA0297	<i>spuA</i>	1	PA4726
PA0298	<i>spuB</i>	1	PA4726
PA0299	<i>spuC</i>	1	PA4726
PA0328		1	PA0893
PA0408	<i>pilG</i>	1	PA0416
PA0410	<i>pilI</i>	1	PA0416
PA0411	<i>pilJ</i>	1	PA0416
PA0412	<i>pilK</i>	1	PA0416
PA0413	<i>chpA</i>	1	PA0416
PA0414	<i>chpB</i>	1	PA0416
PA0415	<i>chpC</i>	1	PA0416
PA0523	<i>norC</i>	1	PA0527

PA0572		1	PA0652
PA0705	<i>migA</i>	1	PA5499
PA0755	<i>opdH</i>	1	PA0756
PA0756		1	PA0757
PA0763	<i>mucA</i>	1	PA5483
PA0779		1	PA2665
PA0844	<i>plcH</i>	1	PA5380
PA0870	<i>phhC</i>	1	PA0873
PA0871	<i>phhB</i>	1	PA0873
PA0872	<i>phhA</i>	1	PA0873
PA0887	<i>acsA</i>	1	PA1980
PA0889	<i>aotQ</i>	1	PA0893
PA0890	<i>aotM</i>	1	PA0893
PA0891		1	PA0893
PA0892	<i>aotP</i>	1	PA0893
PA0905	<i>rsmA</i>	1	PA2586
PA0932	<i>cysM</i>	1	PA2354
PA0969	<i>tolQ</i>	1	PA2686
PA1000	<i>pqsE</i>	1	PA1003
PA1002	<i>phnB</i>	1	PA5499
PA1077	<i>flgB</i>	1	PA1097
PA1078	<i>flgC</i>	1	PA1097
PA1082	<i>flgG</i>	1	PA1097
PA1084	<i>flgI</i>	1	PA1097
PA1093		1	PA1097
PA1094	<i>fliD</i>	1	PA1097
PA1098	<i>fleS</i>	1	PA1099
PA1174	<i>napA</i>	1	PA3879
PA1178	<i>oprH</i>	1	PA1179
PA1196		1	PA3861
PA1418		1	PA1422
PA1421	<i>gbuA</i>	1	PA1422
PA1458		1	PA5261
PA1461	<i>motD</i>	1	PA2259

PA1543	<i>apt</i>	1	PA1544
PA1546	<i>hemN</i>	1	PA3879
PA1557	<i>ccoN2</i>	1	PA5261
PA1609	<i>fabB</i>	1	PA4890
PA1610	<i>fabA</i>	1	PA4890
PA1690	<i>pscU</i>	1	PA4109
PA1712	<i>exsB</i>	1	PA1713
PA1714	<i>exsD</i>	1	PA2519
PA1725	<i>pseL</i>	1	PA4109
PA1796	<i>folD</i>	1	PA4600
PA1850		1	PA2063
PA1920	<i>nrdD</i>	1	PA3879
PA1977		1	PA1978
PA1980	<i>eraR</i>	1	PA1982
PA1999	<i>dhcA</i>	1	PA5389
PA2000	<i>dhcB</i>	1	PA5389
PA2011	<i>liuE</i>	1	PA2016
PA2013	<i>liuC</i>	1	PA2016
PA2015	<i>liuA</i>	1	PA2016
PA2018		1	PA2020
PA2193	<i>hcnA</i>	1	PA5261
PA2354		1	PA3444
PA2491		1	PA2492
PA2495	<i>oprN</i>	1	PA4600
PA2507	<i>catA</i>	1	PA2510
PA2508	<i>catC</i>	1	PA2510
PA2511		1	PA2512
PA2515	<i>xylL</i>	1	PA2519
PA2519	<i>xylS</i>	1	PA3571
PA2522	<i>czcC</i>	1	PA2809
PA2561		1	PA5360
PA2585	<i>uvrC</i>	1	PA2586
PA2587	<i>pqsH</i>	1	PA5499
PA2611	<i>cysG</i>	1	PA4723

PA2637	<i>nuoA</i>	1	PA2686
PA2664	<i>fhp</i>	1	PA2665
PA2696		1	PA1850
PA3082	<i>gbt</i>	1	PA5380
PA3094		1	PA5322
PA3168	<i>gyrA</i>	1	PA4600
PA3175		1	PA5105
PA3176	<i>gltS</i>	1	PA3184
PA3182	<i>pgl</i>	1	PA3184
PA3183	<i>zwf</i>	1	PA3184
PA3257	<i>prc</i>	1	PA5483
PA3266	<i>capB</i>	1	PA3168
PA3385	<i>amrZ</i>	1	PA5262
PA3391	<i>nosR</i>	1	PA3587
PA3479	<i>rhlA</i>	1	PA5261
PA3545	<i>algG</i>	1	PA5483
PA3547	<i>algL</i>	1	PA5483
PA3569	<i>mmsB</i>	1	PA3571
PA3570	<i>mmsA</i>	1	PA3571
PA3581	<i>glpF</i>	1	PA3583
PA3594		1	PA3004
PA3689		1	PA3690
PA3710		1	PA3782
PA3720		1	PA3721
PA3721	<i>nalC</i>	1	PA4598
PA3724	<i>lasB</i>	1	PA4723
PA3782		1	PA5065
PA3815	<i>iscR</i>	1	PA4236
PA3875	<i>narG</i>	1	PA3879
PA3878	<i>narX</i>	1	PA3879
PA3946	<i>two-compo</i>	1	PA3948
PA3947	<i>rocR</i>	1	PA3948
PA4101	<i>bfinR</i>	1	PA4208

PA4222		1	PA4227
PA4223		1	PA4227
PA4224	<i>pchG</i>	1	PA4227
PA4225	<i>pchF</i>	1	PA4227
PA4226	<i>pchE</i>	1	PA4227
PA4293	<i>pprA</i>	1	PA4296
PA4295	<i>fppA</i>	1	PA4296
PA4296	<i>pprB</i>	1	PA4305
PA4446	<i>algW</i>	1	PA5483
PA4498		1	PA4499
PA4525	<i>pilA</i>	1	PA4547
PA4550	<i>fimU</i>	1	PA5261
PA4597	<i>oprJ</i>	1	PA4600
PA4598	<i>mexD</i>	1	PA4600
PA4599	<i>mexC</i>	1	PA4600
PA4723	<i>dkcA</i>	1	PA5338
PA4725	<i>cbrA</i>	1	PA4726
PA4844		1	PA5360
PA4888	<i>desB</i>	1	PA4890
PA4889		1	PA4890
PA5092	<i>hutI</i>	1	PA5105
PA5098	<i>hutH</i>	1	PA5105
PA5100	<i>hutU</i>	1	PA5105
PA5124	<i>ntrB</i>	1	PA5125
PA5157		1	PA1619
PA5255	<i>algQ</i>	1	PA5261
PA5259	<i>hemD</i>	1	PA5261
PA5260	<i>hemC</i>	1	PA5261
PA5292	<i>pchP</i>	1	PA5380
PA5373	<i>betB</i>	1	PA5374
PA5384		1	PA5389
PA5388		1	PA5389
PA5483	<i>algB</i>	1	PA5484
PA5549	<i>glmS</i>	1	PA5550

Supplementary information for Chapter 4

Table S1: Key to identifying the AmpR-dependent, and AmpR- and β -lactam dependent genes from the venn diagram (Fig 4.4). Condition A: PAO1 uninduced vs PAO1 induced; Condition B: PAO Δ *ampR* uninduced vs PAO Δ *ampR* induced; Condition C: PAO1 uninduced vs PAO Δ *ampR* uninduced; Condition D: PAO1 induced vs PAO Δ *ampR* induced. NA- not applicable.

Regulation	Condition	Condition	Condition	AmpR Regulation
AmpR dependent, β-lactam independent	C- up	D- up	--	Negative
	C- down	D- down	--	Positive
	C- up	B- up	--	Negative
	C- down	B- down	--	Positive
	A- up	B- down	C- down	Positive
	A- down	C- up	D- up	Negative
AmpR dependent, β-lactam dependent	A- down	B- up	--	Negative
	A- up	B- down	--	Positive
	A- up	D- down	--	Positive
	A- down	D- up	B- up	Negative
AmpR independent, β-lactam dependent	C- down	D- up	--	NA
	C- up	D- down	--	NA
	A- down	B- down	--	NA
	A- up	B- up	--	NA
	A- up	D- up	--	NA
	A- up	C- down	D- up	NA
	B- up	D- down	C- up	NA

Table S2: β -lactam-dependent genes. The 206 β -lactam stress-dependent genes are separated into upregulated and downregulated genes with the corresponding corrected *p*-values for the fold change (FC) observed. The genes are arranged based on either functional categorization. Locus tag annotations are from the *Pseudomonas* Genome database (31).

A

Downregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA4078		probable nonribosomal peptide synthetase	-3.6	4.07E-03
Amino acid biosynthesis and metabolism				
PA5098	<i>hutH</i>	histidine ammonia-lyase	-21.4	2.15E-03
PA5100	<i>hutU</i>	urocanase	-10.5	2.61E-03
PA3700	<i>lysS</i>	lysyl-tRNA synthetase	-2.1	5.78E-03
Carbon compound catabolism				
PA0792	<i>prpD</i>	propionate catabolic protein PrpD	-3.8	8.44E-03
PA2300	<i>chiC</i>	chitinase	-20.2	4.17E-04
Cell wall / LPS / capsule ; Adaptation, Protection				
PA1130	<i>rhlC</i>	rhamnosyltransferase 2	-5.4	5.32E-03
Central intermediary metabolism				
PA5549	<i>glmS</i>	glucosamine--fructose-6-phosphate aminotransferase	-2.5	2.28E-03
Energy metabolism				
PA3930	<i>cioA</i>	cyanide insensitive terminal oxidase	-3.8	6.69E-03
Hypothetical, unclassified, unknown				
PA0088		hypothetical protein	-3.2	4.39E-03
PA0122		conserved hypothetical protein	-9.2	6.61E-03
PA0271		hypothetical protein	-3.0	3.10E-03
PA1494		conserved hypothetical protein	-2.5	4.94E-03
PA1896		hypothetical protein	-2.7	1.76E-03
PA2066		hypothetical protein	-4.8	5.07E-03
PA2111		hypothetical protein	-2.3	2.78E-03
PA2223		hypothetical protein	-3.4	9.59E-03
PA2274		hypothetical protein	-8.7	3.92E-03
PA2441		hypothetical protein	-3.4	5.33E-03
PA2448		hypothetical protein	-7.9	5.07E-03
PA2684		conserved hypothetical protein	-2.2	2.78E-03
PA3229		hypothetical protein	-2.1	4.66E-03
PA3734		hypothetical protein	-4.1	8.70E-03
PA3928		hypothetical protein	-3.5	7.28E-03
PA4035		hypothetical protein	-2.6	2.61E-03
PA4141		hypothetical protein	-8.7	3.29E-03
PA5106		conserved hypothetical protein	-10.4	4.45E-03
PA5201		conserved hypothetical protein	-2.5	2.15E-03
PA5220		hypothetical protein	-9.4	4.71E-03

Membrane proteins				
PA0070		hypothetical protein	-2.0	7.24E-03
PA1895		hypothetical protein	-3.3	3.71E-03
PA5183		hypothetical protein	-4.3	4.45E-03
PA1212		probable major facilitator superfamily (MFS) transporter	-2.9	7.31E-03
PA2068		probable major facilitator superfamily (MFS) transporter	-11.7	3.42E-03
PA2327		probable permease of ABC transporter	-5.8	6.57E-03
PA4034	<i>aqpZ</i>	aquaporin Z	-26.1	2.15E-03
PA5095		probable permease of ABC transporter	-12.0	2.15E-03
PA5097		probable amino acid permease	-20.7	2.78E-03
PA5099		probable transporter	-12.4	4.21E-03
Motility & Attachment				
PA0171		hypothetical protein	-3.0	3.68E-03
PA3361	<i>lecB</i>	fucose-binding lectin PA-IIL	-2.1	9.12E-03
Protein secretion/export apparatus				
PA1718	<i>pseE</i>	type III export protein PseE	-3.2	3.71E-03
PA3820	<i>secF</i>	secretion protein SecF	-2.4	9.63E-03
Putative enzymes				
PA0130		probable aldehyde dehydrogenase	-2.1	9.12E-03
PA0372		probable zinc protease	-2.3	2.91E-03
PA1914		conserved hypothetical protein	-14.8	7.12E-03
PA2067		probable hydrolase	-12.0	1.76E-03
PA2069		probable carbamoyl transferase	-35.4	2.66E-03
PA4715		probable aminotransferase	-4.7	6.10E-03
PA5093		probable histidine/phenylalanine ammonia-lyase	-12.5	3.73E-03
PA0075	<i>pppA</i>	PppA	-2.9	5.96E-03
Secreted Factors (toxins, enzymes, alginate)				
PA0852	<i>cbpD</i>	chitin-binding protein CbpD precursor	-6.7	3.41E-03
PA1901	<i>phzC2</i>	phenazine biosynthesis protein PhzC	-9.2	5.62E-03
PA1902	<i>phzD2</i>	phenazine biosynthesis protein PhzD	-10.7	4.07E-03
PA1903	<i>phzE2</i>	phenazine biosynthesis protein PhzE	-17.1	5.81E-03
PA1904	<i>phzF2</i>	probable phenazine biosynthesis protein	-15.8	3.98E-03
PA1905	<i>phzG2</i>	probable pyridoxamine 5'-phosphate oxidase	-19.8	3.44E-03
PA1871	<i>lasA</i>	LasA protease precursor	-7.9	6.80E-03
Transcription, RNA processing and degradation				
PA4238	<i>rpoA</i>	DNA-directed RNA polymerase alpha chain	-2.0	3.91E-03
Translation, post-translational modification, degradation				
PA2619	<i>infA</i>	initiation factor	-3.0	4.45E-03
PA4241	<i>rpsM</i>	30S ribosomal protein S13	-2.6	9.03E-03
PA0090	<i>clpV1</i>	ClpV1	-3.6	4.02E-03
Transport of small molecules				
PA0603		probable ATP-binding component of ABC transporter	-2.0	7.74E-03

PA5094		probable ATP-binding component of ABC transporter	-12.7	2.78E-03
PA5096		probable binding protein component of ABC transporter	-11.5	4.94E-03

Upregulated genes

Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA0176	<i>aer2</i>	aerotaxis transducer Aer2	4.9	7.74E-03
PA0177		probable purine-binding chemotaxis protein	3.1	9.87E-03
PA1646		probable chemotaxis transducer	2.6	7.41E-03
PA2570	<i>lecA</i>	LecA	41.7	1.60E-03
PA2654		probable chemotaxis transducer	3.2	2.78E-03
PA2788		probable chemotaxis transducer	2.1	4.77E-03
PA3236		probable glycine betaine-binding protein precursor	11.3	2.78E-03
PA3349		probable chemotaxis protein	2.4	5.78E-03
PA4310	<i>pctB</i>	chemotactic transducer PctB	2.6	3.40E-03
PA4566	<i>obg</i>	GTP-binding protein Obg	2.6	4.12E-03
PA4633		probable chemotaxis transducer	3.0	3.54E-03
PA5072		probable chemotaxis transducer	2.2	4.36E-03
Amino acid biosynthesis and metabolism				
PA4759	<i>dapB</i>	dihydrodipicolinate reductase	2.6	9.12E-03
PA5379	<i>sdaB</i>	L-serine dehydratase	4.1	5.30E-03
PA5398	<i>dgcA</i>	DgcA, Dimethylglycine catabolism	8.4	2.15E-03
PA5399	<i>dgcB</i>	DgcB, Dimethylglycine catabolism	7.3	2.78E-03
PA5410	<i>gbcA</i>	GbcA	13.8	2.96E-03
PA5411	<i>gbcB</i>	GbcB	7.1	2.78E-03
PA5416	<i>soxB</i>	sarcosine oxidase beta subunit	11.6	2.95E-03
PA5417	<i>soxD</i>	sarcosine oxidase delta subunit	15.8	2.78E-03
PA5419	<i>soxG</i>	sarcosine oxidase gamma subunit	6.5	7.74E-03
PA5429	<i>aspA</i>	aspartate ammonia-lyase	2.4	9.12E-03
Carbon compound catabolism				
PA4124	<i>hpcB</i>	homoprotocatechuate 2,3-dioxygenase	4.0	8.81E-03
PA5418	<i>soxA</i>	sarcosine oxidase alpha subunit	7.4	3.46E-03
Cell wall / LPS / capsule				
PA1078	<i>flgC</i>	flagellar basal-body rod protein FlgC	3.4	4.45E-03
PA1079	<i>flgD</i>	flagellar basal-body rod modification protein FlgD	3.0	5.78E-03
PA1080	<i>flgE</i>	flagellar hook protein FlgE	3.3	7.19E-03
PA1087	<i>flgL</i>	flagellar hook-associated protein type 3 FlgL	2.8	7.41E-03
PA1094	<i>fliD</i>	flagellar capping protein FliD	2.2	9.23E-03
PA1959	<i>bacA</i>	bacitracin resistance protein	4.2	7.71E-03
PA4700	<i>mrcB</i>	penicillin-binding protein 1B	3.5	3.71E-03
Central intermediary metabolism				

PA4640	<i>mgoB</i>	malate:quinone oxidoreductase	2.5	4.32E-03
PA4880		probable bacterioferritin	4.2	9.12E-03
PA5421	<i>fdhA</i>	glutathione-independent formaldehyde dehydrogenase	5.2	9.59E-03
Chaperones & heat shock proteins				
PA3126	<i>ibpA</i>	heat-shock protein IbpA	2.7	9.90E-03
Chemotaxis ; Adaptation, Protection ; Motility & Attachment				
PA1452	<i>flhA</i>	flagellar biosynthesis protein FlhA	2.7	8.47E-03
DNA replication, recombination, modification and repair				
PA3745	<i>rpsP</i>	30S ribosomal protein S16	4.3	9.29E-03
PA4763	<i>recN</i>	DNA repair protein RecN	2.5	6.57E-03
Energy metabolism				
PA2953		electron transfer flavoprotein-ubiquinone oxidoreductase	2.7	8.27E-03
PA5400		probable electron transfer flavoprotein alpha subunit	4.2	5.76E-03
PA5491		probable cytochrome	2.3	4.70E-03
Hypothetical, unclassified, unknown				
PA0026	<i>plcB</i>	phospholipase C, PlcB	2.9	7.51E-03
PA0277		conserved hypothetical protein	6.6	4.66E-03
PA0572		hypothetical protein	2.8	9.87E-03
PA0578		conserved hypothetical protein	4.7	9.29E-03
PA0613		hypothetical protein	6.2	5.45E-03
PA0718		hypothetical protein of bacteriophage Pfl	3.2	9.59E-03
PA0922		hypothetical protein	2.6	7.55E-03
PA1029		hypothetical protein	2.7	4.45E-03
PA1030		hypothetical protein	3.2	8.39E-03
PA1093		hypothetical protein	2.3	7.43E-03
PA1095		hypothetical protein	2.5	3.64E-03
PA1096		hypothetical protein	2.3	2.15E-03
PA1441		hypothetical protein	3.0	2.69E-03
PA1967		hypothetical protein	3.7	8.26E-03
PA1970		hypothetical protein	15.6	1.38E-03
PA2171		hypothetical protein	2.6	9.12E-03
PA2288		hypothetical protein	2.9	2.28E-03
PA2366		uricase PuuD	10.2	4.33E-03
PA2367		hypothetical protein	8.4	4.45E-03
PA2368		hypothetical protein	8.6	4.45E-03
PA2370		hypothetical protein	4.6	5.83E-03
PA2485		hypothetical protein	2.7	9.12E-03
PA2759		hypothetical protein	2.5	8.33E-03
PA2762		hypothetical protein	5.1	2.95E-03
PA2971		conserved hypothetical protein	3.3	1.92E-03
PA3179		conserved hypothetical protein	2.4	2.15E-03
PA3274		hypothetical protein	2.9	9.95E-03
PA3414		hypothetical protein	3.2	8.52E-03
PA3500		conserved hypothetical protein	4.2	3.92E-03
PA3501		hypothetical protein	5.6	6.67E-03

PA3502		hypothetical protein	5.4	7.01E-03
PA3510		hypothetical protein	7.6	3.57E-03
PA3513		hypothetical protein	5.4	6.65E-03
PA3520		hypothetical protein	7.3	4.74E-03
PA3691		hypothetical protein	2.6	9.12E-03
PA3952		hypothetical protein	2.1	9.12E-03
PA4354		conserved hypothetical protein	21.5	3.18E-03
PA4359		conserved hypothetical protein	2.7	9.95E-03
PA4623		hypothetical protein	20.0	1.39E-03
PA4635		conserved hypothetical protein	5.2	3.73E-03
PA4746		conserved hypothetical protein	5.5	9.20E-03
PA4826		hypothetical protein	3.8	7.33E-03
PA4852		conserved hypothetical protein	4.1	3.95E-03
PA4881		hypothetical protein	106.7	7.22E-04
PA4908		hypothetical protein	3.7	5.81E-03
PA4917		hypothetical protein	2.8	7.43E-03
PA4985		hypothetical protein	2.9	3.54E-03
PA5378		hypothetical protein	2.8	7.69E-03
PA5395		conserved hypothetical protein	2.2	9.63E-03
PA5396		hypothetical protein	11.4	3.54E-03
PA5397		hypothetical protein	13.1	2.78E-03
PA5401		hypothetical protein	8.3	4.57E-03
PA5446		hypothetical protein	2.7	9.12E-03
PA5465		hypothetical protein	3.0	6.65E-03
PA5471		hypothetical protein	5.1	6.26E-03
Membrane proteins				
PA0109		hypothetical protein	2.1	7.33E-03
PA0241		probable major facilitator superfamily (MFS) transporter	2.6	9.51E-03
PA1073	<i>braD</i>	branched-chain amino acid transport protein BraD	2.6	2.78E-03
PA2369		hypothetical protein	3.1	7.12E-03
PA3512		probable permease of ABC transporter	5.3	4.21E-03
PA4933		hypothetical protein	5.8	8.81E-03
PA4940		conserved hypothetical protein	4.6	8.27E-03
PA5409		hypothetical protein	2.3	2.15E-03
Nucleotide biosynthesis and metabolism				
PA3654	<i>pyrH</i>	uridylate kinase	3.5	6.24E-03
PA5420	<i>purU2</i>	formyltetrahydrofolate deformylase	5.4	9.79E-03
Protein secretion/export apparatus				
PA4276	<i>secE</i>	secretion protein SecE	4.2	5.13E-03
Putative enzymes				
PA2152		probable trehalose synthase	2.5	9.12E-03
PA2491		probable oxidoreductase	2.1	9.59E-03
PA3503		hypothetical protein	3.9	5.71E-03
PA3504		probable aldehyde dehydrogenase	5.2	4.86E-03
PA3507		probable short-chain dehydrogenase	5.1	4.12E-03
PA3509		probable hydrolase	6.0	2.78E-03

PA3511		probable short-chain dehydrogenase	6.8	4.02E-03
PA4986		probable oxidoreductase	2.7	5.71E-03
Secreted Factors (toxins, enzymes, alginate)				
PA3478	<i>rhlB</i>	rhamnosyltransferase chain B	6.0	3.71E-03
PA3479	<i>rhlA</i>	rhamnosyltransferase chain A	20.5	1.60E-03
PA5112	<i>estA</i>	esterase EstA	2.1	3.73E-03
Transcription, RNA processing and degradation				
PA3743	<i>trmD</i>	tRNA (guanine-N1)-methyltransferase	17.9	3.35E-03
PA4264	<i>rpsJ</i>	30S ribosomal protein S10	3.3	5.38E-03
Transcriptional regulators				
PA1196		probable transcriptional regulator	2.6	9.89E-03
PA3508		probable transcriptional regulator	5.8	5.71E-03
PA4296	<i>pprB</i>	two-component response regulator, PprB	3.3	8.60E-03
Translation, post-translational modification, degradation				
PA0580	<i>gcp</i>	O-sialoglycoprotein endopeptidase	3.3	8.70E-03
PA0905	<i>rsmA</i>	RsmA, regulator of secondary metabolites	2.3	9.63E-03
PA2741	<i>rplT</i>	50S ribosomal protein L20	2.2	7.71E-03
PA4542	<i>clpB</i>	ClpB protein	2.1	9.90E-03
PA4567	<i>rpmA</i>	50S ribosomal protein L27	4.5	5.62E-03
PA4568	<i>rplU</i>	50S ribosomal protein L21	7.0	4.81E-03
PA4742	<i>truB</i>	tRNA pseudouridine 55 synthase	3.5	9.10E-03
PA4932	<i>rplI</i>	50S ribosomal protein L9	4.6	8.70E-03
PA4934	<i>rpsR</i>	30S ribosomal protein S18	4.2	9.29E-03
PA5316	<i>rpmB</i>	50S ribosomal protein L28	2.3	5.97E-03
PA5470		probable peptide chain release factor	5.0	6.86E-03
Transport of small molecules				
PA1074	<i>braC</i>	branched-chain amino acid transport protein BraC	2.9	4.45E-03
PA1260		amino acid ABC transporter periplasmic binding protein	3.3	3.73E-03
PA1435		probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein precursor	5.2	4.50E-03
PA2019		Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein precursor	2.0	9.89E-03
PA2812		probable ATP-binding component of ABC transporter	3.7	2.40E-03
PA3514		probable ATP-binding component of ABC transporter	8.1	4.20E-03
PA5468		probable citrate transporter	2.7	4.37E-03

Table S3: AmpR-dependent genes. The 313 AmpR-dependent genes are separated into upregulated and downregulated genes with the corresponding corrected *p*-values for the fold change (FC) observed. The genes are arranged based on either (A) functional categorization, or (B) fold change. Locus tag annotations are from the *Pseudomonas* Genome database (31).

Downregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA0074	<i>ppkA</i>	serine/threonine protein kinase PpkA	-2.3	8.41E-03
PA1127		probable oxidoreductase	-2.0	4.74E-03
PA2826		probable glutathione peroxidase	-2.2	5.96E-03
PA3109		hypothetical protein	-2.6	9.59E-03
PA5117	<i>typA</i>	regulatory protein TypA	-3.6	8.06E-03
Amino acid biosynthesis and metabolism				
PA0132		beta-alanine--pyruvate transaminase	-3.9	9.29E-03
PA1337	<i>ansB</i>	glutaminase-asparaginase	-2.4	3.35E-03
PA1812	<i>mltD</i>	membrane-bound lytic murein transglycosylase D precursor	-2.1	5.32E-03
PA2443	<i>sdaA</i>	L-serine dehydratase	-10.6	4.02E-03
PA2444	<i>glyA2</i>	serine hydroxymethyltransferase	-7.1	1.76E-03
PA2629	<i>purB</i>	adenylosuccinate lyase	-2.3	9.89E-03
PA5323	<i>argB</i>	acetylglutamate kinase	-2.6	4.45E-03
PA5415	<i>glyA1</i>	serine hydroxymethyltransferase	-2.1	5.91E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA4664	<i>hemK</i>	probable methyl transferase	-2.0	9.51E-03
Carbon compound catabolism				
PA0208	<i>mdcA</i>	malonate decarboxylase alpha subunit	-2.4	3.91E-03
PA2414		L-sorbose dehydrogenase	-2.2	8.90E-03
Cell division				
PA0857	<i>bolA</i>	morphogene protein BolA	-2.3	2.78E-03
Cell wall / LPS / capsule				
PA2232	<i>pslB</i>	probable phosphomannose isomerase/GDP-mannose pyrophosphorylase	-3.2	8.70E-03
PA2234	<i>pslD</i>	PslD	-3.0	8.27E-03
PA3646	<i>lpxD</i>	UDP-3-O-[3-hydroxy-lauroyl] glucosamine N-acyltransferase	-2.1	4.78E-03
PA5077	<i>mdoH</i>	periplasmic glucans biosynthesis protein MdoH	-2.2	4.86E-03
PA5163	<i>rmlA</i>	glucose-1-phosphate thymidyltransferase	-2.1	4.66E-03
PA5322	<i>algC</i>	phosphomannomutase AlgC	-2.3	4.45E-03
Central intermediary metabolism				
PA2023	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	-2.5	4.75E-03
PA2193	<i>hcnA</i>	hydrogen cyanide synthase HcnA	-7.3	4.68E-03
PA2194	<i>hcnB</i>	hydrogen cyanide synthase HcnB	-5.3	7.08E-03
PA2195	<i>hcnC</i>	hydrogen cyanide synthase HcnC	-4.6	4.02E-03
PA2442	<i>gcvT2</i>	glycine cleavage system protein T2	-4.8	3.36E-03

PA2445	<i>gcvP2</i>	glycine cleavage system protein P2	-14.3	5.03E-03
PA4100		probable dehydrogenase	-2.7	6.81E-03
Energy metabolism				
PA0854	<i>fumC2</i>	fumarate hydratase	-2.4	9.59E-03
PA4470	<i>fumC1</i>	fumarate hydratase	-17.3	5.97E-03
PA5553	<i>atpC</i>	ATP synthase epsilon chain	-2.2	5.71E-03
Fatty acid and phospholipid metabolism				
PA0798	<i>pmtA</i>	phospholipid methyltransferase	-2.3	5.91E-03
PA1869		probable acyl carrier protein	-6.0	4.66E-03
Hypothetical, unclassified, unknown				
PA0062		hypothetical protein	-4.9	2.91E-03
PA0076		hypothetical protein	-2.5	3.34E-03
PA0078		hypothetical protein	-2.9	8.66E-03
PA0079		hypothetical protein	-2.9	5.97E-03
PA0080		hypothetical protein	-2.5	4.94E-03
PA0169		hypothetical protein	-3.3	2.95E-03
PA0170		hypothetical protein	-3.5	4.20E-03
PA0269		conserved hypothetical protein	-3.4	3.18E-03
PA0270		hypothetical protein	-3.0	8.85E-03
PA0370		conserved hypothetical protein	-2.5	3.73E-03
PA0371		hypothetical protein	-2.5	3.68E-03
PA0468		hypothetical protein	-2.2	6.12E-03
PA0567		conserved hypothetical protein	-3.2	9.87E-03
PA0713		hypothetical protein	-3.1	7.40E-03
PA0732		hypothetical protein	-2.9	4.45E-03
PA0737		hypothetical protein	-2.0	9.86E-03
PA0943		hypothetical protein	-2.0	3.65E-03
PA1471		hypothetical protein	-6.5	2.78E-03
PA1605		hypothetical protein	-2.3	7.41E-03
PA1657		conserved hypothetical protein	-8.4	3.19E-03
PA1658		conserved hypothetical protein	-9.7	1.60E-03
PA1659		hypothetical protein	-7.9	1.91E-03
PA1660		hypothetical protein	-4.7	8.44E-03
PA1661		hypothetical protein	-3.6	3.95E-03
PA1664		hypothetical protein	-6.3	4.73E-03
PA1665		hypothetical protein	-7.6	2.00E-03
PA1666		hypothetical protein	-6.1	3.68E-03
PA1667		hypothetical protein	-6.0	2.90E-03
PA1668		hypothetical protein	-4.1	3.98E-03
PA1669		hypothetical protein	-4.0	5.46E-03
PA1732		conserved hypothetical protein	-2.6	6.70E-03
PA1892		hypothetical protein	-2.6	9.29E-03
PA1894		hypothetical protein	-3.8	2.55E-03
PA1897		hypothetical protein	-2.8	6.00E-03
PA1951		hypothetical protein	-2.1	9.36E-03
PA1968		hypothetical protein	-2.5	6.60E-03
PA1969		hypothetical protein	-2.5	7.41E-03
PA2030		hypothetical protein	-4.1	8.89E-03

PA2031		hypothetical protein	-3.2	8.70E-03
PA2034		hypothetical protein	-4.8	5.91E-03
PA2080	<i>kynU</i>	kynureninase KynU	-7.2	2.29E-03
PA2081	<i>kynB</i>	kynurenine formamidase, KynB	-5.9	1.73E-03
PA2222		hypothetical protein	-3.1	4.94E-03
PA2235	<i>pslE</i>	hypothetical protein	-3.0	3.18E-03
PA2236	<i>pslF</i>	hypothetical protein	-2.8	6.78E-03
PA2238	<i>pslH</i>	hypothetical protein	-2.5	7.89E-03
PA2242	<i>pslL</i>	hypothetical protein	-2.7	3.41E-03
PA2289		conserved hypothetical protein	-2.3	7.15E-03
PA2384		hypothetical protein	-5.7	9.36E-03
PA2412		conserved hypothetical protein	-2.2	3.95E-03
PA2452		hypothetical protein	-5.1	3.95E-03
PA2630		conserved hypothetical protein	-2.8	9.59E-03
PA2702		hypothetical protein	-2.0	4.45E-03
PA2703		hypothetical protein	-2.2	9.03E-03
PA3042		hypothetical protein	-2.9	8.70E-03
PA3413		conserved hypothetical protein	-3.6	7.60E-03
PA3484		hypothetical protein	-2.1	7.63E-03
PA3572		hypothetical protein	-4.7	1.86E-03
PA3791		hypothetical protein	-2.1	9.75E-03
PA4033		hypothetical protein	-26.9	4.02E-03
PA4154		conserved hypothetical protein	-3.8	5.96E-03
PA4182		hypothetical protein	-2.6	5.46E-03
PA4204	<i>ppgL</i>	periplasmic gluconolactonase, PpgL	-3.3	2.15E-03
PA4384		hypothetical protein	-3.0	4.66E-03
PA4458		conserved hypothetical protein	-2.0	2.15E-03
PA4459		conserved hypothetical protein	-2.7	6.00E-03
PA4469		hypothetical protein	-9.7	9.29E-03
PA4625		hypothetical protein	-2.9	8.25E-03
PA5023		conserved hypothetical protein	-2.2	4.66E-03
PA5052		hypothetical protein	-2.0	8.60E-03
PA5104		conserved hypothetical protein	-2.5	4.45E-03
PA5180		conserved hypothetical protein	-6.4	3.95E-03
PA5202		hypothetical protein	-2.3	6.86E-03
PA5463		hypothetical protein	-2.0	8.59E-03
Membrane proteins				
PA0605		probable permease of ABC transporter	-3.0	4.74E-03
PA0606		probable permease of ABC transporter	-2.4	4.43E-03
PA2403		hypothetical protein	-3.3	7.28E-03
PA2415		hypothetical protein	-2.3	2.78E-03
PA2763		hypothetical protein	-3.6	8.47E-03
PA2811		probable permease of ABC-2 transporter	-4.6	6.12E-03
PA3692		probable outer membrane protein precursor	-7.8	3.46E-03
PA3821	<i>secD</i>	secretion protein SecD	-2.0	6.09E-03
PA4467		hypothetical protein	-9.9	6.69E-03
PA4503		probable permease of ABC transporter	-4.0	5.32E-03
PA4504		probable permease of ABC transporter	-4.2	4.76E-03

PA5182		hypothetical protein	-3.2	3.34E-03
Nucleotide biosynthesis and metabolism				
PA4855	<i>purD</i>	phosphoribosylamine--glycine ligase	-2.0	8.66E-03
PA5496	<i>nrdJb</i>	class II ribonucleotide-diphosphate reductase subunit, NrdJb	-3.0	5.62E-03
PA5497	<i>nrdJa</i>	class II ribonucleotide-diphosphate reductase subunit, NrdJa	-3.3	8.44E-03
Protein secretion/export apparatus				
PA0077	<i>icmF1</i>	IcmF1	-3.0	8.66E-03
		Translocator outer membrane protein PopD		
PA1709	<i>popD</i>	precursor	-6.2	1.38E-03
PA1711	<i>exsE</i>	ExsE	-3.6	3.35E-03
PA5210		probable secretion pathway ATPase	-2.3	3.31E-03
Putative enzymes				
PA0507		probable acyl-CoA dehydrogenase	-3.8	6.26E-03
PA0534		conserved hypothetical protein	-3.1	7.91E-03
PA0853		probable oxidoreductase	-2.6	4.45E-03
PA1662		probable ClpA/B-type protease	-5.0	3.41E-03
PA1893		hypothetical protein	-2.9	4.77E-03
PA2237	<i>pslG</i>	probable glycosyl hydrolase	-2.4	6.82E-03
PA2239	<i>pslI</i>	probable transferase	-3.0	4.02E-03
PA2263		probable 2-hydroxyacid dehydrogenase	-2.2	3.95E-03
PA2317		probable oxidoreductase	2.9	2.78E-03
PA2682		conserved hypothetical protein	-3.0	7.58E-03
PA3427		probable short-chain dehydrogenases	-3.8	8.59E-03
PA3454		probable acyl-CoA thiolase	-3.9	4.33E-03
PA4171		probable protease	-2.4	4.86E-03
PA5150		probable short-chain dehydrogenase	-2.0	5.46E-03
PA5181		probable oxidoreductase	-8.6	5.96E-03
Secreted Factors (toxins, enzymes, alginate)				
PA0085	<i>hcpI</i>	Hcp1	-3.7	3.09E-03
PA0263	<i>hcpC</i>	secreted protein Hcp	-7.7	4.66E-03
PA1707	<i>pcrH</i>	regulatory protein PcrH	-3.8	3.98E-03
PA3841	<i>exoS</i>	exoenzyme S	-3.5	1.16E-03
PA3842		probable chaperone	-2.9	5.73E-03
Transcription, RNA processing and degradation				
PA4740	<i>pnp</i>	polyribonucleotide nucleotidyltransferase	-2.1	5.49E-03
Transcriptional regulators				
PA0479		probable transcriptional regulator	-2.1	9.90E-03
PA1663		probable transcriptional regulator	-3.9	5.13E-03
PA2281		probable transcriptional regulator	-2.1	9.89E-03
PA2588		probable transcriptional regulator	-27.9	3.77E-03
PA5105	<i>hutC</i>	histidine utilization repressor HutC	-3.1	8.27E-03
PA5483	<i>algB</i>	two-component response regulator AlgB	-2.4	7.83E-03
Translation, post-translational modification, degradation				
PA0594	<i>surA</i>	peptidyl-prolyl cis-trans isomerase SurA	-2.4	6.26E-03
		serine/threonine phosphoprotein phosphatase		
PA1670	<i>stpI</i>	StpI	-2.3	3.81E-03

Transport of small molecules				
PA2987		probable ATP-binding component of ABC transporter	-2.1	3.68E-03
PA4206	<i>mexH</i>	probable RND efflux membrane fusion protein precursor	-21.4	2.54E-03
PA4500		probable binding protein component of ABC transporter	-5.2	3.73E-03
PA4502		probable binding protein component of ABC transporter	-4.9	3.41E-03
PA4505		probable ATP-binding component of ABC transporter	-3.3	3.71E-03
PA4506		probable ATP-binding component of ABC dipeptide transporter	-3.8	2.99E-03
Two-component regulatory systems				
PA5484		probable two-component sensor	-3.0	7.01E-03
Upregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA0180		probable chemotaxis transducer	2.1	5.78E-03
PA0923	<i>dinB</i>	DNA Polymerase IV, DinB	4.5	6.05E-03
PA2652		probable chemotaxis transducer	2.6	8.67E-03
PA3007	<i>lexA</i>	repressor protein LexA	2.7	7.28E-03
PA3348		probable chemotaxis protein methyltransferase	2.2	3.64E-03
PA4309	<i>pctA</i>	chemotactic transducer PctA	3.2	7.83E-03
PA4356	<i>xenB</i>	xenobiotic reductase	3.2	5.96E-03
PA4378	<i>inaA</i>	InaA protein	3.0	6.68E-03
PA4520		probable chemotaxis transducer	2.1	3.69E-03
Amino acid biosynthesis and metabolism				
PA0245	<i>aroQ2</i>	3-dehydroquinate dehydratase	2.9	3.71E-03
PA4565	<i>proB</i>	glutamate 5-kinase	2.9	5.03E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA4919	<i>pncB1</i>	nicotinate phosphoribosyltransferase	6.3	3.95E-03
Carbon compound catabolism				
PA0153	<i>pcaH</i>	protocatechuate 3,4-dioxygenase, beta subunit	3.3	3.42E-03
PA0154	<i>pcaG</i>	protocatechuate 3,4-dioxygenase, alpha subunit	2.2	6.25E-03
PA0226		probable CoA transferase, subunit A	4.4	9.97E-03
PA0230	<i>pcaB</i>	3-carboxy-cis,cis-muconate cycloisomerase	3.3	7.08E-03
PA0231	<i>pcaD</i>	beta-ketoadipate enol-lactone hydrolase	3.7	5.16E-03
PA0232	<i>pcaC</i>	gamma-carboxymuconolactone decarboxylase	4.1	8.44E-03
PA2862	<i>lipA</i>	lactonizing lipase precursor	3.6	5.97E-03
PA4123	<i>hpcC</i>	5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase	6.7	3.18E-03
PA4125	<i>hpcD</i>	5-carboxymethyl-2-hydroxymuconate isomerase	5.8	6.12E-03

Cell wall / LPS / capsule				
PA1082	<i>flgG</i>	flagellar basal-body rod protein FlgG	2.9	8.52E-03
PA3160	<i>wzz</i>	O-antigen chain length regulator	3.7	8.81E-03
PA4480	<i>mreC</i>	rod shape-determining protein MreC	4.8	6.34E-03
Central intermediary metabolism				
PA2813		probable glutathione S-transferase	2.6	9.10E-03
PA3629	<i>adhC</i>	alcohol dehydrogenase class III	3.2	4.98E-03
Chemotaxis				
PA0179		probable two-component response regulator	3.2	9.57E-03
DNA replication, recombination, modification and repair				
PA0577	<i>dnaG</i>	DNA primase	4.7	8.39E-03
PA3617	<i>recA</i>	RecA protein	3.3	5.46E-03
Energy metabolism				
PA0105	<i>coxB</i>	cytochrome c oxidase, subunit II	2.5	8.21E-03
PA3621	<i>fdxA</i>	ferredoxin I	3.1	5.32E-03
PA4430		probable cytochrome b	2.2	2.00E-03
Hypothetical, unclassified, unknown				
PA0124		hypothetical protein	2.8	3.81E-03
PA0128		conserved hypothetical protein	3.3	7.28E-03
PA0466		hypothetical protein	3.1	9.12E-03
PA0529		conserved hypothetical protein	3.0	8.58E-03
PA0614		hypothetical protein	6.7	8.26E-03
PA0615		hypothetical protein	6.5	5.78E-03
PA0717		hypothetical protein of bacteriophage Pfl	3.5	3.44E-03
PA0729		hypothetical protein	9.4	3.41E-03
PA0910		hypothetical protein	8.5	6.26E-03
PA0911		hypothetical protein	6.8	7.83E-03
PA1016		hypothetical protein	2.6	7.58E-03
PA1164		conserved hypothetical protein	2.1	3.66E-03
PA1299		conserved hypothetical protein	3.0	3.41E-03
PA1333		hypothetical protein	2.6	5.97E-03
PA1439		conserved hypothetical protein	2.2	7.28E-03
PA1545		hypothetical protein	2.7	6.65E-03
PA1744		hypothetical protein	3.1	5.62E-03
PA2362		hypothetical protein	2.4	9.87E-03
PA2365		conserved hypothetical protein	9.4	4.50E-03
PA2487		hypothetical protein	11.6	2.26E-03
PA2765		hypothetical protein	14.6	1.60E-03
PA2780		hypothetical protein	2.6	4.66E-03
PA2901		hypothetical protein	16.5	4.43E-03
PA3008		hypothetical protein	4.1	7.71E-03
PA3306		hypothetical protein	14.9	2.90E-03
PA3353		hypothetical protein	2.3	6.40E-03
PA3371		hypothetical protein	2.5	9.87E-03
PA3499		hypothetical protein	2.4	6.97E-03
PA3505		hypothetical protein	5.7	3.92E-03

PA3649		conserved hypothetical protein	2.1	6.31E-03
PA3741		hypothetical protein	7.1	6.52E-03
PA3880		conserved hypothetical protein	2.4	5.62E-03
PA3922		conserved hypothetical protein	2.4	8.59E-03
PA3923		hypothetical protein	3.6	6.26E-03
PA3951		conserved hypothetical protein	2.1	8.33E-03
PA4121		conserved hypothetical protein	4.9	6.75E-03
PA4326		hypothetical protein	3.1	7.63E-03
PA4379		conserved hypothetical protein	5.0	3.41E-03
PA4582		conserved hypothetical protein	7.6	3.19E-03
PA4584		conserved hypothetical protein	3.7	7.29E-03
PA4918		hypothetical protein	16.9	2.59E-03
PA4921		hypothetical protein	4.2	3.77E-03
PA5292	<i>pchP</i>	phosphorylcholine phosphatase	2.3	9.89E-03
PA5543		hypothetical protein	2.0	5.81E-03
Membrane proteins				
PA0229	<i>pcaT</i>	dicarboxylic acid transporter PcaT	4.6	6.78E-03
PA0240		probable porin	2.3	8.70E-03
PA0465	<i>creD</i>	inner membrane protein CreD	4.7	9.95E-03
		branched-chain amino acid transport protein		
PA1072	<i>braE</i>	BraE	2.8	4.45E-03
PA1436		probable RND efflux transporter	3.9	6.31E-03
		Multidrug efflux outer membrane protein		
PA2495	<i>oprN</i>	OprN precursor	102.6	1.05E-04
PA2988		conserved hypothetical protein	2.2	5.73E-03
PA3041		hypothetical protein	4.1	5.13E-03
		probable ATP-binding/permease fusion ABC transporter		
PA3228			3.5	1.38E-03
PA3234		probable sodium:solute symporter	3.3	8.62E-03
PA3235		conserved hypothetical protein	3.0	5.47E-03
		probable major facilitator superfamily (MFS) transporter		
PA3336			2.3	3.19E-03
PA3526		probable outer membrane protein precursor	2.3	3.64E-03
PA3575		hypothetical protein	2.2	6.11E-03
PA3602		conserved hypothetical protein	2.3	6.70E-03
		probable major facilitator superfamily (MFS) transporter		
PA4126			2.4	9.20E-03
		probable permease-ABC branched-chain amino acid transporter		
PA4911			5.1	2.78E-03
		branched chain amino acid ABC transporter		
PA4912		membrane protein	6.6	2.15E-03
PA5561	<i>atpI</i>	ATP synthase protein I	2.6	5.05E-03
Motility & Attachment				
PA1092	<i>fliC</i>	flagellin type B	2.5	9.63E-03
PA1099	<i>fleR</i>	two-component response regulator	3.0	7.63E-03
Non-coding RNA gene				
PA3621.1	<i>rsmZ</i>	regulatory RNA RsmZ	4.8	9.25E-03

Protein secretion/export apparatus				
PA2863	<i>lipH</i>	lipase modulator protein	2.2	4.85E-03
Putative enzymes				
PA0242		hypothetical protein	2.6	7.19E-03
PA0244		hypothetical protein	2.9	4.52E-03
PA1296		probable 2-hydroxyacid dehydrogenase	3.2	3.44E-03
PA3498		probable oxidoreductase	2.4	7.51E-03
PA3506		probable decarboxylase	4.8	5.76E-03
PA3628		probable esterase	2.9	5.71E-03
PA3817		probable methyltransferase	4.2	5.32E-03
PA5048		probable nuclease	3.2	9.01E-03
Related to phage, transposon, or plasmid				
PA0616		hypothetical protein	6.9	7.25E-03
PA0617		probable bacteriophage protein	7.8	8.66E-03
PA0618		probable bacteriophage protein	7.2	7.74E-03
PA0619		probable bacteriophage protein	5.9	9.87E-03
PA0620		probable bacteriophage protein	7.5	5.76E-03
PA0621		conserved hypothetical protein	7.7	6.53E-03
PA0622		probable bacteriophage protein	6.0	4.45E-03
PA0623		probable bacteriophage protein	6.3	7.01E-03
PA0633		hypothetical protein	7.5	5.76E-03
PA0634		hypothetical protein	6.6	7.15E-03
PA0637		conserved hypothetical protein	7.0	8.11E-03
PA0638		probable bacteriophage protein	6.3	9.02E-03
PA0641		probable bacteriophage protein	5.6	9.59E-03
PA0643		hypothetical protein	6.0	8.59E-03
Secreted Factors (toxins, enzymes, alginate)				
PA0843	<i>plcR</i>	phospholipase accessory protein PlcR precursor	2.2	2.16E-03
Transcription, RNA processing and degradation				
PA2840		probable ATP-dependent RNA helicase ribosomal large subunit pseudouridine	3.5	4.17E-04
PA2975	<i>rluC</i>	synthase C	6.5	6.17E-03
PA3744	<i>rimM</i>	16S rRNA processing protein	6.2	4.66E-03
PA4275	<i>nusG</i>	transcription antitermination protein NusG	2.7	9.69E-03
Transcriptional regulators				
PA0762	<i>algU</i>	sigma factor AlgU	5.4	3.35E-03
PA1300		probable sigma-70 factor, ECF subfamily	2.5	9.59E-03
PA5380	<i>gbdR</i>	GbdR	2.6	5.75E-03
		negative regulator for alginate biosynthesis		
PA0764	<i>mucB</i>	MucB	2.5	8.79E-03
PA0610	<i>prtN</i>	transcriptional regulator PrtN	6.1	5.58E-03
PA0611	<i>prtR</i>	transcriptional regulator PrtR	2.9	8.52E-03
PA0612	<i>ptrB</i>	repressor, PtrB	6.0	2.78E-03
Transport of small molecules				
PA0913	<i>mgtE</i>	probable Mg transporter MgtE	3.1	6.25E-03
		branched-chain amino acid transport protein		
PA1070	<i>braG</i>	BraG	2.5	4.02E-03

PA1071	<i>braF</i>	branched-chain amino acid transport protein BraF	2.2	7.55E-03
PA1256		amino acid ABC transporter ATP binding protein	2.3	8.26E-03
PA3038		probable porin	2.3	4.85E-03
PA3889		probable binding protein component of ABC transporter	2.2	9.95E-03
PA4909		probable ATP-binding component of ABC transporter	3.7	3.92E-03
PA4910		branched chain amino acid ABC transporter ATP binding protein	5.7	4.16E-03
PA4913		probable binding protein component of ABC transporter	4.7	5.71E-03
PA2493	<i>mexE</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexE precursor	6.9	2.72E-03
PA2494	<i>mexF</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexF	88.7	7.22E-04
PA0958	<i>oprD</i>	Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor	2.2	3.49E-03
PA5376		probable ATP-binding component of ABC transporter	2.2	6.12E-03
Two-component regulatory systems				
PA0757		probable two-component sensor	2.3	3.77E-03
PA1098	<i>fleS</i>	two-component sensor	2.5	6.65E-03

Table S4: AmpR, β -lactam-dependent genes. The 207 genes that are specifically dependent on AmpR, and β -lactam stress are separated into upregulated and downregulated genes with the corresponding corrected *p*-values for the fold change (FC) observed. The genes are arranged based on either (A) functional categorization, or (B) fold change. Locus tag annotations are from the *Pseudomonas* Genome database (31).

Downregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA4671		probable ribosomal protein L25	-2.6	9.07E-03
Amino acid biosynthesis and metabolism				
PA0390	<i>metX</i>	homoserine O-acetyltransferase	-2.5	6.29E-03
PA0932	<i>cysM</i>	cysteine synthase B	-2.0	2.90E-03
PA1750		phospho-2-dehydro-3-deoxyheptonate aldolase	-2.4	8.53E-03
PA4846	<i>aroQ1</i>	3-dehydroquinate dehydratase	-2.3	6.79E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA0024	<i>hemF</i>	coproporphyrinogen III oxidase, aerobic	-3.2	6.65E-03
PA4280	<i>birA</i>	BirA bifunctional protein	-3.0	9.14E-03
PA5118	<i>thiI</i>	thiazole biosynthesis protein ThiI	-2.9	7.95E-03
PA3652	<i>uppS</i>	undecaprenyl pyrophosphate synthetase	-2.3	6.65E-03
PA0342	<i>thyA</i>	thymidylate synthase	-2.1	8.99E-03
Carbon compound catabolism				
PA0227		probable CoA transferase, subunit B	-3.1	6.65E-03
PA5192	<i>pckA</i>	phosphoenolpyruvate carboxykinase	-3.0	6.04E-04
PA4670	<i>prs</i>	ribose-phosphate pyrophosphokinase	-4.0	9.10E-03
Cell division				
PA5563	<i>soj</i>	chromosome partitioning protein Soj	-2.1	9.71E-03
Cell wall / LPS / capsule				
PA4450	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-2.1	9.73E-03
PA4545	<i>comL</i>	competence protein ComL	-2.1	7.95E-03
PA4481	<i>mreB</i>	rod shape-determining protein MreB	-4.4	6.51E-03
Central intermediary metabolism				
PA4563	<i>rpsT</i>	30S ribosomal protein S20	-5.9	9.92E-03
Chaperones & heat shock proteins				
PA5193	<i>yrfI</i>	heat shock protein HSP33	-2.5	6.65E-03
DNA replication, recombination, modification and repair				
PA3620	<i>mutS</i>	DNA mismatch repair protein MutS	-2.6	9.22E-03
Hypothetical, unclassified, unknown				
PA0045		hypothetical protein	-4.2	7.48E-03
PA0046		hypothetical protein	-3.4	9.10E-03
PA1768		hypothetical protein	-3.4	7.86E-03
PA2318		hypothetical protein	-3.1	8.53E-03
PA2427		hypothetical protein	-2.2	9.12E-03
PA2451		hypothetical protein	-2.2	9.58E-03
PA2486		hypothetical protein	-4.0	7.00E-03

PA2660		hypothetical protein	-2.6	9.73E-03
PA2823		conserved hypothetical protein	-2.0	9.10E-03
PA3453		conserved hypothetical protein	-2.8	9.34E-03
PA3472		hypothetical protein	-5.4	8.53E-03
PA3483		hypothetical protein	-2.0	7.09E-03
PA3664		conserved hypothetical protein	-2.1	7.95E-03
PA3906		hypothetical protein	-2.4	9.39E-03
PA3908		hypothetical protein	-2.2	7.86E-03
PA4004		conserved hypothetical protein	-2.7	6.65E-03
PA4122		conserved hypothetical protein	-3.0	7.53E-03
PA4438		conserved hypothetical protein	-3.4	6.65E-03
PA4451		conserved hypothetical protein	-3.7	7.09E-03
PA4460		conserved hypothetical protein	-2.1	8.44E-03
PA4492		conserved hypothetical protein	-2.2	8.57E-03
PA4574		conserved hypothetical protein	-2.2	6.33E-03
PA4673		conserved hypothetical protein	-2.8	7.09E-03
PA4753		conserved hypothetical protein	-2.3	8.09E-03
PA4817		hypothetical protein	-2.0	7.48E-03
PA5441		hypothetical protein	-2.0	7.09E-03
Membrane proteins				
PA3794		hypothetical protein	-2.3	4.37E-03
PA3828		conserved hypothetical protein	-2.7	9.24E-03
PA5568		conserved hypothetical protein	-3.2	6.35E-03
PA4205	<i>mexG</i>	hypothetical protein	-	1.18E-03
PA4207	<i>mexI</i>	probable Resistance-Nodulation-Cell Division (RND) efflux transporter	10.8	2.29E-03
PA4208	<i>opmD</i>	probable outer membrane protein precursor	10.8	1.60E-03
PA4292		probable phosphate transporter	-2.4	9.10E-03
Nucleotide biosynthesis and metabolism				
PA0944	<i>purN</i>	phosphoribosylaminoimidazole synthetase	-2.2	9.73E-03
PA3527	<i>pyrC</i>	dihydroorotase	-2.5	8.09E-03
PA5298		xanthine phosphoribosyltransferase	-3.2	9.75E-03
PA5336	<i>gmk</i>	guanylate kinase	-3.0	8.86E-03
Protein secretion/export apparatus				
PA1708	<i>popB</i>	translocator protein PopB	-2.0	9.63E-03
PA5128	<i>secB</i>	secretion protein SecB	-2.5	7.43E-03
Putative enzymes				
PA0386		probable oxidase	-2.4	7.33E-03
PA4073		probable aldehyde dehydrogenase	-3.3	8.33E-03
PA4209	<i>phzM</i>	probable phenazine-specific methyltransferase	-3.8	3.95E-03
PA4217	<i>phzS</i>	flavin-containing monooxygenase	-	2.90E-03
PA4389		probable short-chain dehydrogenase	-2.1	7.86E-03
Secreted Factors (toxins, enzymes, alginate)				
PA4210	<i>phzA1</i>	probable phenazine biosynthesis protein	-3.5	3.81E-03
PA4211	<i>phzB1</i>	probable phenazine biosynthesis protein	20.7	5.11E-03
Transcription, RNA processing and degradation				
PA0592	<i>ksgA</i>	rRNA (adenine-N6,N6)-dimethyltransferase	-2.6	8.56E-03

PA4745	<i>nusA</i>	N utilization substance protein A	-2.3	8.39E-03
PA4262	<i>rplD</i>	50S ribosomal protein L4	-3.0	6.33E-03
Transcriptional regulators				
PA4853	<i>fis</i>	DNA-binding protein Fis	-3.3	6.65E-03
PA0463	<i>creB</i>	two-component response regulator CreB	-2.1	8.39E-03
PA3604		probable two-component response regulator	-2.2	6.96E-03
Translation, post-translational modification, degradation				
PA0579	<i>rpsU</i>	30S ribosomal protein S21	-3.5	9.32E-03
PA2851	<i>efp</i>	translation elongation factor P	-4.3	6.96E-03
PA2970	<i>rpmF</i>	50S ribosomal protein L32	-3.8	7.34E-03
PA3656	<i>rpsB</i>	30S ribosomal protein S2	-4.0	9.34E-03
PA3742	<i>rplS</i>	50S ribosomal protein L19	-2.7	6.83E-03
PA4239	<i>rpsD</i>	30S ribosomal protein S4	-2.4	6.51E-03
PA4244	<i>rplO</i>	50S ribosomal protein L15	-2.6	6.01E-03
PA4246	<i>rpsE</i>	30S ribosomal protein S5	-2.4	4.37E-03
PA4247	<i>rplR</i>	50S ribosomal protein L18	-2.9	7.53E-03
PA4248	<i>rplF</i>	50S ribosomal protein L6	-2.7	8.90E-03
PA4249	<i>rpsH</i>	30S ribosomal protein S8	-2.8	6.58E-03
PA4251	<i>rplE</i>	50S ribosomal protein L5	-2.4	6.33E-03
PA4253	<i>rplN</i>	50S ribosomal protein L14	-3.0	9.10E-03
PA4254	<i>rpsQ</i>	30S ribosomal protein S17	-2.4	4.37E-03
PA4257	<i>rpsC</i>	30S ribosomal protein S3	-2.7	8.90E-03
PA4261	<i>rplW</i>	50S ribosomal protein L23	-2.9	3.81E-03
PA4263	<i>rplC</i>	50S ribosomal protein L3	-2.8	6.94E-03
PA4266	<i>fusA1</i>	elongation factor G	-2.1	9.73E-03
PA4272	<i>rplJ</i>	50S ribosomal protein L10	-3.7	6.33E-03
PA4274	<i>rplK</i>	50S ribosomal protein L11	-2.0	5.03E-03
PA4665	<i>prfA</i>	peptide chain release factor 1	-2.2	6.65E-03
PA4672		peptidyl-tRNA hydrolase	-3.3	9.10E-03
PA4935	<i>rpsF</i>	30S ribosomal protein S6	-3.7	6.74E-03
PA5049	<i>rpmE</i>	50S ribosomal protein L31	-4.0	8.29E-03
PA5569	<i>rnpA</i>	ribonuclease P protein component	-3.5	5.03E-03
PA3818		extragenic suppressor protein SuhB	-2.7	9.10E-03
PA4743	<i>rbfA</i>	ribosome-binding factor A	-2.8	9.34E-03
Transport of small molecules				
PA2983		probable tolQ-type transport protein	-2.7	6.74E-03
PA3407	<i>hasAp</i>	heme acquisition protein HasAp	15.8	1.39E-03

Upregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected p-value
Adaptation, Protection				
PA1001	<i>phnA</i>	anthranilate synthase component I	5.7	7.31E-03
PA2573		probable chemotaxis transducer	4.3	5.19E-03
PA3866		pyocin protein	2.1	9.34E-03
PA4110	<i>ampC</i>	beta-lactamase precursor	2.0	3.84E-02

Amino acid biosynthesis and metabolism				
PA1217		probable 2-isopropylmalate synthase	13.8	6.51E-03
PA3418	<i>ldh</i>	leucine dehydrogenase	5.1	8.29E-03
PA1002	<i>phnB</i>	anthranilate synthase component II	4.7	5.30E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA0502		probable biotin biosynthesis protein bioH	2.3	7.53E-03
PA1000	<i>pqsE</i>	Quinolone signal response protein	3.6	6.94E-03
Carbon compound catabolism				
PA0743		probable 3-hydroxyisobutyrate dehydrogenase	6.1	8.10E-03
PA2290	<i>gcd</i>	glucose dehydrogenase	3.9	4.89E-03
Central intermediary metabolism				
PA4130		probable sulfite or nitrite reductase	3.4	9.10E-03
Energy metabolism				
PA1177	<i>napE</i>	periplasmic nitrate reductase protein NapE	5.5	8.53E-03
PA1317	<i>cyoA</i>	cytochrome o ubiquinol oxidase subunit II	3.9	8.51E-03
PA3032	<i>snrI</i>	cytochrome c SnrI	5.2	3.81E-03
PA3415		probable dihydrolipoamide acetyltransferase	8.1	6.51E-03
PA3416		probable pyruvate dehydrogenase E1 component, beta chain	8.2	6.96E-03
Hypothetical, unclassified, unknown				
PA0028		hypothetical protein	2.5	8.55E-03
PA0484		conserved hypothetical protein	3.9	6.79E-03
PA0586		conserved hypothetical protein	4.7	6.33E-03
PA0587		conserved hypothetical protein	4.5	9.10E-03
PA0588		conserved hypothetical protein	3.6	8.61E-03
PA0959		hypothetical protein	2.3	8.93E-03
PA1043		hypothetical protein	2.3	6.65E-03
PA1088		hypothetical protein	3.1	9.73E-03
PA1090		hypothetical protein	2.6	5.03E-03
PA1091	<i>fgtA</i>	flagellar glycosyl transferase, FgtA	3.1	3.60E-03
PA1135		conserved hypothetical protein	3.8	5.57E-03
PA1213		hypothetical protein	5.5	5.30E-03
PA1214		hypothetical protein	4.9	8.55E-03
PA1216		hypothetical protein	15.9	6.99E-03
PA1218		hypothetical protein	10.8	7.00E-03
PA1219		hypothetical protein	5.0	4.37E-03
PA1221		hypothetical protein	4.9	6.15E-03
PA1356		hypothetical protein	2.6	7.87E-03
PA1728		hypothetical protein	5.0	8.53E-03
PA1784		hypothetical protein	6.3	6.83E-03
PA1887		hypothetical protein	9.8	7.53E-03
PA1888		hypothetical protein	12.0	7.86E-03
PA2166		hypothetical protein	8.9	5.57E-03
PA2174		hypothetical protein	5.3	9.10E-03
PA2226		hypothetical protein	2.5	8.16E-03
PA2328		hypothetical protein	8.7	1.04E-03
PA2330		hypothetical protein	17.5	8.29E-03
PA2374		hypothetical protein	4.1	6.96E-03

PA2381		hypothetical protein	7.5	8.44E-03
PA2566		conserved hypothetical protein	4.0	9.34E-03
PA2699		hypothetical protein	3.9	9.10E-03
PA2747		hypothetical protein	12.1	7.31E-03
PA2937		hypothetical protein	4.3	8.53E-03
PA3089		hypothetical protein	2.9	9.50E-03
PA3688		hypothetical protein	4.9	9.10E-03
PA4132		conserved hypothetical protein	2.4	7.81E-03
PA4298		hypothetical protein	14.1	8.29E-03
PA4362		hypothetical protein	3.1	8.56E-03
PA4421		conserved hypothetical protein	2.1	7.37E-03
PA4573		hypothetical protein	4.2	9.51E-03
PA4583		conserved hypothetical protein	2.1	9.12E-03
PA4608		hypothetical protein	2.8	9.73E-03
PA4648		hypothetical protein	13.5	4.37E-03
PA4650		hypothetical protein	2.1	6.65E-03
PA4653		hypothetical protein	3.8	3.60E-03
PA4685		hypothetical protein	2.6	5.03E-03
PA4702		hypothetical protein	6.3	4.37E-03
PA4738		conserved hypothetical protein	15.6	7.30E-04
PA4739		conserved hypothetical protein	16.8	6.65E-03
PA5061		conserved hypothetical protein	4.2	6.51E-03
PA5101		hypothetical protein	3.2	8.55E-03
PA5191		hypothetical protein	2.2	8.23E-03
PA5481		hypothetical protein	5.9	7.42E-03
PA1874		hypothetical protein	22.1	5.09E-03
Membrane proteins				
PA0007		hypothetical protein	2.6	9.73E-03
PA1042		conserved hypothetical protein	2.7	9.05E-03
PA1119		probable outer membrane protein precursor	2.2	9.22E-03
PA2072		conserved hypothetical protein	2.2	9.80E-03
PA3370		hypothetical protein	3.7	7.86E-03
PA3789		hypothetical protein	2.9	7.09E-03
PA5482		hypothetical protein	11.1	6.65E-03
PA4614	<i>mscL</i>	conductance mechanosensitive channel	2.3	5.57E-03
PA4297	<i>tadG</i>	TadG	3.5	7.64E-03
PA4301	<i>tadB</i>	TadB	4.0	6.33E-03
Motility & Attachment				
PA4299	<i>tadD</i>	TadD	16.9	5.03E-03
PA4305	<i>rcpC</i>	RcpC	4.6	9.73E-03
PA4306	<i>flp</i>	Type IVb pilin, Flp	35.7	3.60E-03
PA4651		probable pili assembly chaperone	11.6	1.42E-03
Protein secretion/export apparatus				
PA1877		probable secretion protein	8.6	9.73E-03
PA1875		probable outer membrane protein precursor	11.5	8.53E-03
PA4302	<i>tadA</i>	TadA ATPase	7.1	6.51E-03
PA4304	<i>rcpA</i>	RcpA	8.7	7.33E-03
Putative enzymes				

PA0704		probable amidase	3.6	8.56E-03
PA0804		probable oxidoreductase	2.5	9.10E-03
PA0838		probable glutathione peroxidase	2.4	9.34E-03
PA1880		probable oxidoreductase	4.1	9.50E-03
PA2024		probable ring-cleaving dioxygenase	6.1	2.46E-03
PA5546		conserved hypothetical protein	4.6	7.09E-03
Related to phage, transposon, or plasmid				
PA0644		hypothetical protein	5.0	9.73E-03
Secreted Factors (toxins, enzymes, alginate)				
PA4175	<i>piv</i>	protease IV	3.8	9.50E-03
PA1876		probable ATP-binding/permease fusion ABC transporter	9.0	5.57E-03
Transcription, RNA processing and degradation				
PA4585	<i>rtcA</i>	RNA 3'-terminal phosphate cyclase	2.1	9.12E-03
Transcriptional regulators				
PA3622	<i>rpoS</i>	sigma factor RpoS	2.2	6.65E-03
PA5059		probable transcriptional regulator	4.0	6.33E-03
PA0175		probable chemotaxis protein methyltransferase	5.9	5.03E-03
PA4781		probable two-component response regulator	2.8	9.10E-03
PA2329		probable ATP-binding component of ABC transporter	14.7	7.53E-03
PA3858		probable amino acid-binding protein	2.3	5.19E-03
PA4497		probable binding protein component of ABC transporter	2.3	9.78E-03
Two-component regulatory systems				
PA4293	<i>pprA</i>	two-component sensor PprA	4.1	8.90E-03

Table S5: Regulation of QS-related genes by AmpR. List of genes that are significantly differentially regulated in PAO1 and PAO Δ *ampR* in the presence and absence of β -lactam stress. For sake of clarity, only significant fold changes (FC) and their corresponding corrected *p*-values in the various conditions are shown. Annotations for the locus tags are from the *Pseudomonas* Genome database (31).

Locus Tag	Gene Name	Product Name	AmpR dependent		AmpR, β -lactam dependent	
			FC	<i>p</i> -value	FC	<i>p</i> -value
Adaptation, protection						
PA4520		probable chemotaxis transducer	2.1	3.7E-03		
PA1127		probable oxidoreductase	-	4.7E-03		
Amino acid biosynthesis and metabolism						
PA0132		beta-alanine--pyruvate transaminase	-	9.3E-03		
PA1812	<i>mltD</i>	membrane-bound lytic murein transglycosylase D precursor	3.9	5.3E-03		
PA2629	<i>purB</i>	adenylosuccinate lyase	-	9.9E-03		
Carbon compound catabolism						
PA0153	<i>pcaH</i>	protocatechuate 3,4-dioxygenase, beta subunit	2.1	3.4E-03		
PA0154	<i>pcaG</i>	protocatechuate 3,4-dioxygenase, alpha subunit	-	6.2E-03		
PA0226		probable CoA transferase, subunit A	2.2	1.0E-02		
PA4123	<i>hpcC</i>	5-carboxy-2-hydroxymuconate semialdehyde dehydrogenase	4.4	3.2E-03		
PA4125	<i>hpcD</i>	5-carboxymethyl-2-hydroxymuconate isomerase	6.7	6.1E-03		
PA0227		probable CoA transferase, subunit B	5.8		-3.1	6.6E-03
Cell wall / LPS / capsule						
PA5163	<i>rmlA</i>	glucose-1-phosphate thymidyltransferase	-	4.7E-03		
Central intermediary metabolism						
PA4130		probable sulfite or nitrite reductase			3.4	9.1E-03
Energy metabolism						
PA0105	<i>coxB</i>	cytochrome c oxidase, subunit II	-	8.2E-03		
PA3621	<i>fdxA</i>	ferredoxin I	2.5	5.3E-03		
PA3032	<i>snr1</i>	cytochrome c SnrI	3.1		5.2	3.8E-03
PA0854	<i>fumC2</i>	fumarate hydratase	-	9.6E-03		
Hypothetical, unclassified, unknown						
PA0079		hypothetical protein	-	6.0E-03		
PA0080		hypothetical protein	2.9	4.9E-03		
PA0269		conserved hypothetical protein	-	3.2E-03		
PA0270		hypothetical protein	2.5	8.9E-03		
PA0468		hypothetical protein	-	6.1E-03		

PA0614		hypothetical protein	6.7	8.3E-03	
PA0615		hypothetical protein	6.5	5.8E-03	
PA0910		hypothetical protein	8.5	6.3E-03	
PA0943		hypothetical protein	-	3.7E-03	
PA1892		hypothetical protein	2.0	9.3E-03	
PA2030		hypothetical protein	-	8.9E-03	
PA2031		hypothetical protein	4.1	8.7E-03	
PA2780		hypothetical protein	3.2	4.7E-03	
PA3572		hypothetical protein	2.6	1.9E-03	
PA3880		conserved hypothetical protein	4.7	5.6E-03	
PA4121		conserved hypothetical protein	2.4	6.7E-03	
PA5543		hypothetical protein	4.9	5.8E-03	
PA1768		hypothetical protein	2.0	7.9E-03	
PA1784		hypothetical protein		6.8E-03	
PA2328		hypothetical protein		6.3	
PA2330		hypothetical protein		8.7	
PA2566		conserved hypothetical protein		17.5	
PA2660		hypothetical protein		4.0	
PA4122		conserved hypothetical protein		-2.6	
PA4132		conserved hypothetical protein		-3.0	
PA4298		hypothetical protein		2.4	
PA4817		hypothetical protein		14.1	
PA1897		hypothetical protein		-2.0	
PA0717		hypothetical protein of bacteriophage Pfl	-	6.0E-03	
Membrane proteins					
PA3789		hypothetical protein		2.9	7.1E-03
PA4126		probable major facilitator superfamily (MFS) transporter	2.4	9.2E-03	
PA4297	<i>tadG</i>	TadG		3.5	7.6E-03
PA1072	<i>braE</i>	branched-chain amino acid transport protein BraE	2.8	4.4E-03	
PA3234		probable sodium:solute symporter	3.3	8.6E-03	
PA4911		probable permease of ABC branched-chain amino acid transporter	5.1	2.8E-03	
PA4912		branched chain amino acid ABC transporter membrane protein	6.6	2.1E-03	
Non-coding RNA gene					
PA3621.1	<i>rsmZ</i>	regulatory RNA RsmZ	4.8	9.2E-03	

Protein secretion/export apparatus						
PA1875		probable outer membrane protein precursor			11.5	8.5E-03
Putative enzymes						
PA1893		hypothetical protein	-	4.8E-03		
PA2682		conserved hypothetical protein	-	7.6E-03		
PA4073		probable aldehyde dehydrogenase			-3.3	8.3E-03
PA5546		conserved hypothetical protein			4.6	7.1E-03
Related to phage, transposon, or plasmid						
PA0616		hypothetical protein	6.9	7.2E-03		
PA0617		probable bacteriophage protein	7.8	8.7E-03		
PA0618		probable bacteriophage protein	7.2	7.7E-03		
PA0619		probable bacteriophage protein	5.9	9.9E-03		
PA0620		probable bacteriophage protein	7.5	5.8E-03		
PA0621		conserved hypothetical protein	7.7	6.5E-03		
PA0622		probable bacteriophage protein	6.0	4.4E-03		
PA0623		probable bacteriophage protein	6.3	7.0E-03		
PA0637		conserved hypothetical protein	7.0	8.1E-03		
PA0638		probable bacteriophage protein	6.3	9.0E-03		
PA0641		probable bacteriophage protein	5.6	9.6E-03		
PA0643		hypothetical protein	6.0	8.6E-03		
PA0644		hypothetical protein			5.0	9.7E-03
Secreted Factors (toxins, enzymes, alginate)						
PA4175	<i>piv</i>	protease IV			3.8	9.5E-03
PA5380	<i>gbdR</i>	GbdR	2.6	5.8E-03		
PA5059		probable transcriptional regulator			4.0	6.3E-03
Translation, post-translational modification, degradation						
PA2851	<i>efp</i>	translation elongation factor P			-4.3	7.0E-03
Transport of small molecules						
PA1070	<i>braG</i>	branched-chain amino acid transport protein BraG	2.5	4.0E-03		
PA4910		branched chain amino acid ABC transporter ATP binding protein	5.7	4.2E-03		
PA4913		probable binding protein component of ABC transporter	4.7	5.7E-03		
PA2329		probable ATP-binding component of ABC transporter			14.7	7.5E-03
PA4497		probable binding protein component of ABC transporter			2.3	9.8E-03

Table S6: Regulation of biofilm-specific genes by AmpR. Significantly differentially regulated genes in PAO1 and PAOΔ*ampR* in the presence and absence of β-lactam antibiotics. For sake of clarity, only significant fold changes (FC) and their corresponding corrected *p*-values are shown. Locus tag annotations are from the *Pseudomonas* Genome database (31).

Locus Tag	Gene Name	Product Name	AmpR dependent		AmpR, β-lactam dependent	
			FC	<i>p</i> -value	FC	<i>p</i> -value
Adaptation, Protection						
PA4356	<i>xenB</i>	xenobiotic reductase	3.2	6.0E-03		
PA4671		probable ribosomal protein L25 serine/threonine protein kinase			-2.6	9.1E-03
PA0074	<i>ppkA</i>	PpkA	-2.3	8.4E-03		
Amino acid biosynthesis and metabolism						
PA2444	<i>glyA2</i>	serine hydroxymethyltransferase	-7.1	1.8E-03		
PA5415	<i>glyA1</i>	serine hydroxymethyltransferase	-2.1	5.9E-03		
		probable 2-isopropylmalate synthase				
PA1217					13.8	6.5E-03
Carbon compound catabolism						
		phosphoenolpyruvate carboxykinase				
PA5192	<i>pckA</i>				-3.0	6.0E-04
Cell wall / LPS / capsule						
PA5322	<i>algC</i>	phosphomannomutase AlgC	-2.3	4.4E-03		
Central intermediary metabolism						
			-			
PA2445	<i>gcvP2</i>	glycine cleavage system protein P2	14.3	5.0E-03		
Hypothetical, unclassified, unknown						
PA0062		hypothetical protein	-4.9	2.9E-03		
PA0076		hypothetical protein	-2.5	3.3E-03		
PA0170		hypothetical protein	-3.5	4.2E-03		
PA0529		conserved hypothetical protein	3.0	8.6E-03		
PA0737		hypothetical protein	-2.0	9.9E-03		
PA1471		hypothetical protein	-6.5	2.8E-03		
PA2365		conserved hypothetical protein	9.4	4.5E-03		
PA3484		hypothetical protein	-2.1	7.6E-03		
PA4154		conserved hypothetical protein	-3.8	6.0E-03		
PA4154		conserved hypothetical protein	2.5	8.7E-03		
PA5202		hypothetical protein	-2.3	6.9E-03		
PA0028		hypothetical protein			2.5	8.5E-03
PA0046		hypothetical protein			-3.4	9.1E-03
PA0588		conserved hypothetical protein			3.6	8.6E-03
PA1216		hypothetical protein			15.9	7.0E-03
PA2174		hypothetical protein			5.3	9.1E-03
PA3688		hypothetical protein			4.9	9.1E-03
PA4421		conserved hypothetical protein			2.1	7.4E-03
PA4421		conserved hypothetical protein			2.9	3.4E-03
PA5441		hypothetical protein			-2.0	7.1E-03
Membrane proteins						
PA5182		hypothetical protein	-3.2	3.3E-03		

PA5568		conserved hypothetical protein			-3.2	6.3E-03
PA3821	<i>secD</i>	secretion protein SecD	-2.0	6.1E-03		
Protein secretion/export apparatus						
PA5128	<i>secB</i>	secretion protein SecB			-2.5	7.4E-03
Putative enzymes						
PA0853		probable oxidoreductase	-2.6	4.4E-03		
PA2024		probable ring-cleaving dioxygenase			6.1	2.5E-03
Transcription, RNA processing and degradation						
PA3744	<i>rimM</i>	16S rRNA processing protein	6.2	4.7E-03		
PA4262	<i>rplD</i>	50S ribosomal protein L4			-3.0	6.3E-03
Transcriptional regulators						
PA4781		probable two-component response regulator			2.8	9.1E-03
Translation, post-translational modification, degradation						
PA2970	<i>rpmF</i>	50S ribosomal protein L32			-3.8	7.3E-03
PA4244	<i>rplO</i>	50S ribosomal protein L15			-2.6	6.0E-03
PA4246	<i>rpsE</i>	30S ribosomal protein S5			-2.4	4.4E-03
PA4247	<i>rplR</i>	50S ribosomal protein L18			-2.9	7.5E-03
PA4248	<i>rplF</i>	50S ribosomal protein L6			-2.7	8.9E-03
PA4261	<i>rplW</i>	50S ribosomal protein L23			-2.9	3.8E-03
PA4272	<i>rplJ</i>	50S ribosomal protein L10			-3.7	6.3E-03
PA4743	<i>rbfA</i>	ribosome-binding factor A			-2.8	9.3E-03

Table S7: Genes in RGPs that are AmpR-regulated. AmpR regulates genes that are part of the different RGPs both in the presence and absence of a β -lactam antibiotic, listed here with the corresponding fold changes (FC) and *p*-value. Locus tag annotations are from the *Pseudomonas* Genome database (31).

AmpR-Dependent					
Locus Tag	Gene Name	Product Name	Function Class	FC	Corrected <i>p</i>-Value
RGP01					
PA0208	<i>mdcA</i>	malonate decarboxylase alpha subunit	Carbon compound catabolism	-2.4	3.91E-03
RGP02					
PA0263	<i>hcpC</i>	secreted protein Hcp	Secreted Factors (toxins, enzymes, alginate)	-7.7	4.66E-03
RGP03					
PA0614		hypothetical protein	Hypothetical, unclassified, unknown	6.7	8.26E-03
PA0615		hypothetical protein	Hypothetical, unclassified, unknown	6.5	5.78E-03
PA0616		hypothetical protein	Related to phage, transposon, or plasmid	6.9	7.25E-03
PA0617		probable bacteriophage protein	Related to phage, transposon, or plasmid	7.8	8.66E-03
PA0618		probable bacteriophage protein	Related to phage, transposon, or plasmid	7.2	7.74E-03
PA0619		probable bacteriophage protein	Related to phage, transposon, or plasmid	5.9	9.87E-03
PA0620		probable bacteriophage protein	Related to phage, transposon, or plasmid	7.5	5.76E-03
PA0621		conserved hypothetical protein	Related to phage, transposon, or plasmid	7.7	6.53E-03
PA0622		probable bacteriophage protein	Related to phage, transposon, or plasmid	6.0	4.45E-03
PA0623		probable bacteriophage protein	Related to phage, transposon, or plasmid	6.3	7.01E-03
RGP04					
PA0641		probable bacteriophage protein	Related to phage, transposon, or plasmid	5.6	9.59E-03
PA0643		hypothetical protein	Related to phage, transposon, or plasmid	6.0	8.59E-03
RGP05					
PA0729		hypothetical protein	Hypothetical, unclassified, unknown	9.4	3.41E-03
PA0717		hypothetical protein of bacteriophage Pfl	Hypothetical, unclassified, unknown	3.5	3.44E-03
RGP09					
PA1092	<i>fliC</i>	flagellin type B	Motility & Attachment	2.5	9.63E-03

RGP20					
PA2030		hypothetical protein	Hypothetical, unclassified, unknown	-4.1	8.89E-03
PA2031		hypothetical protein	Hypothetical, unclassified, unknown	-3.2	8.70E-03
PA2034		hypothetical protein	Hypothetical, unclassified, unknown	-4.8	5.91E-03
RGP23					
PA2232	<i>pslB</i>	probable phosphomannose isomerase/GDP-mannose pyrophosphorylase	Cell wall / LPS / capsule	-3.2	8.70E-03
PA2234	<i>pslD</i>	PslD	Cell wall / LPS / capsule ; Transport of small molecules	-3.0	8.27E-03
PA2222		hypothetical protein	Hypothetical, unclassified, unknown	-3.1	4.94E-03
PA2235	<i>pslE</i>	hypothetical protein	Hypothetical, unclassified, unknown	-3.0	3.18E-03
RGP31					
PA3160	<i>wzz</i>	O-antigen chain length regulator	Cell wall / LPS / capsule	3.7	8.81E-03
RGP34					
PA3499		hypothetical protein	Hypothetical, unclassified, unknown	2.4	6.97E-03
PA3505		hypothetical protein	Hypothetical, unclassified, unknown	5.7	3.92E-03
PA3498		probable oxidoreductase	Putative enzymes	-24.6	7.22E-04
PA3506		probable decarboxylase	Putative enzymes	4.8	5.76E-03
RGP44					
PA4100		probable dehydrogenase	Central intermediary metabolism	-2.7	6.81E-03
RGP62					
PA5150		probable short-chain dehydrogenase	Putative enzymes	-2.0	5.46E-03

AmpR-β-lactam Dependent

Locus Tag	Gene Name	Product Name	Function Class	FC	Corrected <i>p</i> -Value
RGP04					
PA0644		hypothetical protein	Related to phage, transposon, or plasmid	5.0	9.73E-03
RGP09					

PA1088		hypothetical protein	Hypothetical, unclassified, unknown	3.1	9.73E-03
PA1090		hypothetical protein	Hypothetical, unclassified, unknown	2.6	5.03E-03
PA1091	<i>fgtA</i>	flagellar glycosyl transferase, FgtA	Hypothetical, unclassified, unknown	3.1	3.60E-03
RGP20					
PA2024		probable ring-cleaving dioxygenase	Putative enzymes	6.1	2.46E-03
RGP23					
PA2226		hypothetical protein	Hypothetical, unclassified, unknown	2.5	8.16E-03
RGP37					
PA3866		pyocin protein	Adaptation, Protection	2.1	9.34E-03
RGP42					
PA4673		conserved hypothetical protein	Hypothetical, unclassified, unknown	-2.8	7.09E-03

Table S8: Exclusively AmpR-, and AmpR- β -lactam-dependent genes. The list of 219 genes, derived from the lists in Tables S3A and 4A, that are specifically dependent on AmpR, both without and with β -lactam stress, and are not differentially regulated in any of the 20 other published transcriptome studies (see text for details). Genes are listed as either up- or down-regulated with the corresponding corrected *p*-values for the fold change (FC) observed. Locus tag annotations are from the *Pseudomonas* Genome database (31).

AmpR-dependent; Downregulated				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA2703		hypothetical protein	-2.2	9.03E-03
PA3109		hypothetical protein	-2.6	9.59E-03
PA5117	<i>typA</i>	regulatory protein TypA	-3.6	8.06E-03
Amino acid biosynthesis and metabolism				
PA1337	<i>ansB</i>	glutaminase-asparaginase	-2.4	3.35E-03
PA2443	<i>sdaA</i>	L-serine dehydratase	-10.6	4.02E-03
PA5323	<i>argB</i>	acetylglutamate kinase	-2.6	4.45E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA4664	<i>hemK</i>	probable methyl transferase	-2.0	9.51E-03
Carbon compound catabolism				
PA0208	<i>mdcA</i>	malonate decarboxylase alpha subunit	-2.4	3.91E-03
Cell division				
PA0857	<i>bolA</i>	morphogene protein BolA	-2.3	2.78E-03
Cell wall / LPS / capsule				
PA2232	<i>pslB</i>	probable phosphomannose isomerase/GDP-mannose pyrophosphorylase	-3.2	8.70E-03
PA2235	<i>pslE</i>	hypothetical protein	-3.0	3.18E-03
PA2236	<i>pslF</i>	hypothetical protein	-2.8	6.78E-03
PA2238	<i>pslH</i>	hypothetical protein	-2.5	7.89E-03
PA3646	<i>lpxD</i>	UDP-3-O-[3-hydroxylauroyl] glucosamine N-acyltransferase	-2.1	4.78E-03
PA5077	<i>mdoH</i>	periplasmic glucans biosynthesis protein MdoH	-2.2	4.86E-03
PA4625		hypothetical protein	-2.9	8.25E-03
PA2234	<i>pslD</i>	PslD	-3.0	8.27E-03
Central intermediary metabolism				
PA2023	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	-2.5	4.75E-03
PA4100		probable dehydrogenase	-2.7	6.81E-03
PA2442	<i>gcvT2</i>	glycine cleavage system protein T2	-4.8	3.36E-03
Energy metabolism				
PA5553	<i>atpC</i>	ATP synthase epsilon chain	-2.2	5.71E-03
Fatty acid and phospholipid metabolism				
PA0798	<i>pmtA</i>	phospholipid methyltransferase	-2.3	5.91E-03
Hypothetical, unclassified, unknown				
PA0169		hypothetical protein	-3.3	2.95E-03
PA0370		conserved hypothetical protein	-2.5	3.73E-03
PA0371		hypothetical protein	-2.5	3.68E-03
PA0713		hypothetical protein	-3.1	7.40E-03
PA1605		hypothetical protein	-2.3	7.41E-03

PA1732		conserved hypothetical protein	-2.6	6.70E-03
PA1968		hypothetical protein	-2.5	6.60E-03
PA1969		hypothetical protein	-2.5	7.41E-03
PA2222		hypothetical protein	-3.1	4.94E-03
PA2242	<i>pslL</i>	hypothetical protein	-2.7	3.41E-03
PA2289		conserved hypothetical protein	-2.3	7.15E-03
PA3042		hypothetical protein	-2.9	8.70E-03
PA4182		hypothetical protein	-2.6	5.46E-03
PA4204	<i>ppgL</i>	periplasmic gluconolactonase, PpgL	-3.3	2.15E-03
PA4384		hypothetical protein	-3.0	4.66E-03
PA4458		conserved hypothetical protein	-2.0	2.15E-03
PA4459		conserved hypothetical protein	-2.7	6.00E-03
PA5023		conserved hypothetical protein	-2.2	4.66E-03
PA5052		hypothetical protein	-2.0	8.60E-03
PA5104		conserved hypothetical protein	-2.5	4.45E-03
PA5463		hypothetical protein	-2.0	8.59E-03
PA0567		conserved hypothetical protein	-3.2	9.87E-03
PA3791		hypothetical protein	-2.1	9.75E-03
Membrane proteins				
PA2763		hypothetical protein	-3.6	8.47E-03
PA0606		probable permease of ABC transporter	-2.4	4.43E-03
PA2811		probable permease of ABC-2 transporter	-4.6	6.12E-03
Nucleotide biosynthesis and metabolism				
PA5496	<i>nrdJb</i>	class II ribonucleotide-diphosphate reductase subunit, NrdJb	-3.0	5.62E-03
PA5497	<i>nrdJa</i>	class II ribonucleotide-diphosphate reductase subunit, NrdJa	-3.3	8.44E-03
Protein secretion/export apparatus				
PA0077	<i>icmF1</i>	IcmF1	-3.0	8.66E-03
PA5210		probable secretion pathway ATPase	-2.3	3.31E-03
Putative enzymes				
PA0507		probable acyl-CoA dehydrogenase	-3.8	6.26E-03
PA0534		conserved hypothetical protein	-3.1	7.91E-03
PA2317		probable oxidoreductase	2.9	2.78E-03
PA3427		probable short-chain dehydrogenases	-3.8	8.59E-03
PA3454		probable acyl-CoA thiolase	-3.9	4.33E-03
PA5181		probable oxidoreductase	-8.6	5.96E-03
PA2237	<i>pslG</i>	probable glycosyl hydrolase	-2.4	6.82E-03
PA2239	<i>pslI</i>	probable transferase	-3.0	4.02E-03
PA2702		hypothetical protein	-2.0	4.45E-03
Transcriptional regulators				
PA0479		probable transcriptional regulator	-2.1	9.90E-03
PA2281		probable transcriptional regulator	-2.1	9.89E-03
PA5105	<i>hutC</i>	histidine utilization repressor HutC	-3.1	8.27E-03
Translation, post-translational modification, degradation				
PA0594	<i>surA</i>	peptidyl-prolyl cis-trans isomerase SurA	-2.4	6.26E-03
Transport of small molecules				
PA2987		probable ATP-binding component of ABC transporter	-2.1	3.68E-03

AmpR-dependent; Upregulated				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Adaptation, Protection				
PA4378	<i>inaA</i>	InaA protein	3.0	6.68E-03
PA2652		probable chemotaxis transducer	2.6	8.67E-03
PA3348		probable chemotaxis protein methyltransferase	2.2	3.64E-03
PA4309	<i>pctA</i>	chemotactic transducer PctA	3.2	7.83E-03
PA0923	<i>dinB</i>	DNA Polymerase IV, DinB	4.5	6.05E-03
Amino acid biosynthesis and metabolism				
PA0245	<i>aroQ2</i>	3-dehydroquinate dehydratase	2.9	3.71E-03
PA4565	<i>proB</i>	glutamate 5-kinase	2.9	5.03E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA4919	<i>pncB1</i>	nicotinate phosphoribosyltransferase	6.3	3.95E-03
Carbon compound catabolism				
PA0230	<i>pcaB</i>	3-carboxy-cis,cis-muconate cycloisomerase	3.3	7.08E-03
PA0231	<i>pcaD</i>	beta-ketoadipate enol-lactone hydrolase	3.7	5.16E-03
PA0232	<i>pcaC</i>	gamma-carboxymuconolactone decarboxylase	4.1	8.44E-03
PA2862	<i>lipA</i>	lactonizing lipase precursor	3.6	5.97E-03
Cell wall / LPS / capsule				
PA3160	<i>wzz</i>	O-antigen chain length regulator	3.7	8.81E-03
PA1082	<i>flgG</i>	flagellar basal-body rod protein FlgG	2.9	8.52E-03
Central intermediary metabolism				
PA2813		probable glutathione S-transferase	2.6	9.10E-03
PA3629	<i>adhC</i>	alcohol dehydrogenase class III	3.2	4.98E-03
DNA replication, recombination, modification and repair				
PA0577	<i>dnaG</i>	DNA primase	4.7	8.39E-03
Hypothetical, unclassified, unknown				
PA0124		hypothetical protein	2.8	3.81E-03
PA0729		hypothetical protein	9.4	3.41E-03
PA0911		hypothetical protein	6.8	7.83E-03
PA1016		hypothetical protein	2.6	7.58E-03
PA1164		conserved hypothetical protein	2.1	3.66E-03
PA1299		conserved hypothetical protein	3.0	3.41E-03
PA1439		conserved hypothetical protein	2.2	7.28E-03
PA1545		hypothetical protein	2.7	6.65E-03
PA1744		hypothetical protein	3.1	5.62E-03
PA2362		hypothetical protein	2.4	9.87E-03
PA2487		hypothetical protein	11.6	2.26E-03
PA2765		hypothetical protein	14.6	1.60E-03
PA2901		hypothetical protein	16.5	4.43E-03
PA3306		hypothetical protein	14.9	2.90E-03
PA3353		hypothetical protein	2.3	6.40E-03
PA3499		hypothetical protein	2.4	6.97E-03
PA3505		hypothetical protein	5.7	3.92E-03
PA3649		conserved hypothetical protein	2.1	6.31E-03
PA3741		hypothetical protein	7.1	6.52E-03
PA3951		conserved hypothetical protein	2.1	8.33E-03

PA4326		hypothetical protein	3.1	7.63E-03
PA4379		conserved hypothetical protein	5.0	3.41E-03
PA4918		hypothetical protein	16.9	2.59E-03
PA4921		hypothetical protein	4.2	3.77E-03
PA5292	<i>pchP</i>	phosphorylcholine phosphatase	2.3	9.89E-03
Membrane proteins				
PA2988		conserved hypothetical protein	2.2	5.73E-03
PA3526		probable outer membrane protein precursor	2.3	3.64E-03
PA3575		hypothetical protein	2.2	6.11E-03
PA5561	<i>atpI</i>	ATP synthase protein I	2.6	5.05E-03
PA0240		probable porin	2.3	8.70E-03
PA3228		probable ATP-binding/permease fusion ABC transporter	3.5	1.38E-03
Motility & Attachment				
PA1099	<i>fleR</i>	two-component response regulator	3.0	7.63E-03
Non-coding RNA gene				
PA3621.1	<i>rsmZ</i>	regulatory RNA <i>rsmZ</i>	4.8	9.25E-03
Protein secretion/export apparatus				
PA2863	<i>lipH</i>	lipase modulator protein	2.2	4.85E-03
Putative enzymes				
PA0242		hypothetical protein	2.6	7.19E-03
PA0244		hypothetical protein	2.9	4.52E-03
PA1296		probable 2-hydroxyacid dehydrogenase	3.2	3.44E-03
PA3498		probable oxidoreductase	2.4	7.51E-03
PA3506		probable decarboxylase	4.8	5.76E-03
PA3628		probable esterase	2.9	5.71E-03
PA3817		probable methyltransferase	4.2	5.32E-03
PA5048		probable nuclease	3.2	9.01E-03
Secreted Factors (toxins, enzymes, alginate)				
PA0843	<i>plcR</i>	phospholipase accessory protein PlcR precursor	2.2	2.16E-03
Transcription, RNA processing and degradation				
PA2840		probable ATP-dependent RNA helicase	3.5	4.17E-04
PA2975	<i>rluC</i>	ribosomal large subunit pseudouridine synthase C	6.5	6.17E-03
PA4275	<i>nusG</i>	transcription antitermination protein NusG	2.7	9.69E-03
Transport of small molecules				
PA1256		amino acid ABC transporter ATP binding protein	2.3	8.26E-03
PA5376		probable ATP-binding component of ABC transporter	2.2	6.12E-03
Two-component regulatory systems				
PA0757		probable two-component sensor	2.3	3.77E-03
PA1098	<i>fleS</i>	two-component sensor	2.5	6.65E-03

AmpR-β-lactam dependent; Downregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Amino acid biosynthesis and metabolism				
PA0390	<i>metX</i>	homoserine O-acetyltransferase	-2.5	6.29E-03

PA1750		phospho-2-dehydro-3-deoxyheptonate aldolase	-2.4	8.53E-03
PA4846	<i>aroQ1</i>	3-dehydroquinate dehydratase	-2.3	6.79E-03
PA0932	<i>cysM</i>	cysteine synthase B	-2.0	2.90E-03
Biosynthesis of cofactors, prosthetic groups and carriers				
PA0024	<i>hemF</i>	coproporphyrinogen III oxidase, aerobic	-3.2	6.65E-03
PA4280	<i>birA</i>	BirA bifunctional protein	-3.0	9.14E-03
PA5118	<i>thiI</i>	thiazole biosynthesis protein ThiI	-2.9	7.95E-03
PA3652	<i>uppS</i>	undecaprenyl pyrophosphate synthetase	-2.3	6.65E-03
PA0342	<i>thyA</i>	thymidylate synthase	-2.1	8.99E-03
Cell division				
PA5563	<i>soj</i>	chromosome partitioning protein Soj	-2.1	9.71E-03
Cell wall / LPS / capsule				
PA4450	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-2.1	9.73E-03
PA4545	<i>comL</i>	competence protein ComL	-2.1	7.95E-03
PA4481	<i>mreB</i>	rod shape-determining protein MreB	-4.4	6.51E-03
Chaperones & heat shock proteins				
PA5193	<i>yrfI</i>	heat shock protein HSP33	-2.5	6.65E-03
DNA replication, recombination, modification and repair				
PA3620	<i>mutS</i>	DNA mismatch repair protein MutS	-2.6	9.22E-03
Hypothetical, unclassified, unknown				
PA3472		hypothetical protein	-5.4	8.53E-03
PA4451		conserved hypothetical protein	-3.7	7.09E-03
PA4438		conserved hypothetical protein	-3.4	6.65E-03
PA2318		hypothetical protein	-3.1	8.53E-03
PA3453		conserved hypothetical protein	-2.8	9.34E-03
PA4004		conserved hypothetical protein	-2.7	6.65E-03
PA4574		conserved hypothetical protein	-2.2	6.33E-03
PA4492		conserved hypothetical protein	-2.2	8.57E-03
PA3664		conserved hypothetical protein	-2.1	7.95E-03
PA4460		conserved hypothetical protein	-2.1	8.44E-03
PA2823		conserved hypothetical protein	-2.0	9.10E-03
PA3483		hypothetical protein	-2.0	7.09E-03
Membrane proteins				
PA3828		conserved hypothetical protein	-2.7	9.24E-03
PA3794		hypothetical protein	-2.3	4.37E-03
PA4292		probable phosphate transporter	-2.4	9.10E-03
Nucleotide biosynthesis and metabolism				
PA5298		xanthine phosphoribosyltransferase	-3.2	9.75E-03
PA5336	<i>gmk</i>	guanylate kinase	-3.0	8.86E-03
PA3527	<i>pyrC</i>	dihydroorotase	-2.5	8.09E-03
PA0944	<i>purN</i>	phosphoribosylaminoimidazole synthetase	-2.2	9.73E-03
Putative enzymes				
PA0386		probable oxidase	-2.4	7.33E-03
PA4389		probable short-chain dehydrogenase	-2.1	7.86E-03
Transcription, RNA processing and degradation				
PA0592	<i>ksgA</i>	rRNA (adenine-N6,N6)-dimethyltransferase	-2.6	8.56E-03
PA4745	<i>nusA</i>	N utilization substance protein A	-2.3	8.39E-03

PA4853	<i>fis</i>	DNA-binding protein Fis	-3.3	6.65E-03
PA3604		probable two-component response regulator	-2.2	6.96E-03
PA0463	<i>creB</i>	two-component response regulator CreB	-2.1	8.39E-03
Translation, post-translational modification, degradation				
PA3656	<i>rpsB</i>	30S ribosomal protein S2	-4.0	9.34E-03
PA4935	<i>rpsF</i>	30S ribosomal protein S6	-3.7	6.74E-03
PA5569	<i>rnpA</i>	ribonuclease P protein component	-3.5	5.03E-03
PA4253	<i>rplN</i>	50S ribosomal protein L14	-3.0	9.10E-03
PA4263	<i>rplC</i>	50S ribosomal protein L3	-2.8	6.94E-03
PA4257	<i>rpsC</i>	30S ribosomal protein S3	-2.7	8.90E-03
PA4239	<i>rpsD</i>	30S ribosomal protein S4	-2.4	6.51E-03
PA4254	<i>rpsQ</i>	30S ribosomal protein S17	-2.4	4.37E-03
PA4251	<i>rplE</i>	50S ribosomal protein L5	-2.4	6.33E-03
PA4665	<i>prfA</i>	peptide chain release factor 1	-2.2	6.65E-03
PA4274	<i>rplK</i>	50S ribosomal protein L11	-2.0	5.03E-03
PA3818		extragenic suppressor protein SuhB	-2.7	9.10E-03
Transport of small molecules				
PA2983		probable tolQ-type transport protein	-2.7	6.74E-03
AmpR-β-lactam dependent; Upregulated genes				
Locus Tag	Gene Name	Product Name	FC	Corrected <i>p</i> -value
Biosynthesis of cofactors, prosthetic groups and carriers				
PA0502		probable biotin biosynthesis protein bioH	2.3	7.53E-03
Hypothetical, unclassified, unknown				
PA5191		hypothetical protein	2.2	8.23E-03
PA0959		hypothetical protein	2.3	8.93E-03
PA1043		hypothetical protein	2.3	6.65E-03
PA2226		hypothetical protein	2.5	8.16E-03
PA1090		hypothetical protein	2.6	5.03E-03
PA4685		hypothetical protein	2.6	5.03E-03
PA1356		hypothetical protein	2.6	7.87E-03
PA4608		hypothetical protein	2.8	9.73E-03
PA3089		hypothetical protein	2.9	9.50E-03
PA1091	<i>fgtA</i>	flagellar glycosyl transferase, FgtA	3.1	3.60E-03
PA1088		hypothetical protein	3.1	9.73E-03
PA4362		hypothetical protein	3.1	8.56E-03
PA5101		hypothetical protein	3.2	8.55E-03
PA1135		conserved hypothetical protein	3.8	5.57E-03
PA2699		hypothetical protein	3.9	9.10E-03
PA0484		conserved hypothetical protein	3.9	6.79E-03
PA2374		hypothetical protein	4.1	6.96E-03
PA1728		hypothetical protein	5.0	8.53E-03
PA2381		hypothetical protein	7.5	8.44E-03
PA1874		hypothetical protein	22.1	5.09E-03
Membrane proteins				
PA2072		conserved hypothetical protein	2.2	9.80E-03
PA1042		conserved hypothetical protein	2.7	9.05E-03

PA4614	<i>mscL</i>	conductance mechanosensitive channel	2.3	5.57E-03
PA1119		probable outer membrane protein precursor	2.2	9.22E-03
Protein secretion/export apparatus				
PA1877		probable secretion protein	8.6	9.73E-03
Putative enzymes				
PA0838		probable glutathione peroxidase	2.4	9.34E-03
PA0804		probable oxidoreductase	2.5	9.10E-03
PA0704		probable amidase	3.6	8.56E-03
Secreted Factors (toxins, enzymes, alginate) ; Protein secretion/export apparatus ; Transport of small molecules				
PA1876		probable ATP-binding/permease fusion ABC transporter	9.0	5.57E-03
Transport of small molecules				
PA3858		probable amino acid-binding protein	2.3	5.19E-03

Table S9: Phenotypic microarray analysis of PAO1 and PAOΔ*ampR*. Phenotypic microarray analysis was performed using Biolog plates, as explained in the text. Phenotypes gained indicate negative regulation by AmpR of these phenotypes, whereas phenotypes lost are those that are positively regulated by AmpR.

Phenotypes Gained		
Plate no.	Substrate	Nature of substrate
PM05	L-Histidine	Nutritional supplement
PM05	L-Leucine	Nutritional supplement
PM05	L-Serine	Nutritional supplement
PM05	L-Citrulline	Nutritional supplement
PM05	(-)-Shikimic acid	Nutritional supplement
PM05	Spermidine	Nutritional supplement
PM05	Pyridoxal	Nutritional supplement

Phenotypes Lost		
Plate no.	Substrate	Nature of substrate
PM02A	L-Pyroglutamic acid	C-Source, amino acid
PM03B	Uric acid	N-Source, other
PM11C	Amoxicillin	wall, lactam
PM11C	Potassium tellurite	toxic anion
PM11C	Cephalothin	wall, cephalosporin
PM12B	Penicillin G	wall, lactam
PM12B	Oxacillin	wall, lactam
PM12B	Paromomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM12B	Vancomycin	wall
PM12B	Sisomicin	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM12B	Novobiocin	DNA topoisomerase
PM12B	2,4-Diamino-6,7-diisopropylpteridine	folate antagonist, vibriostatic agent
PM12B	Tobramycin	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM12B	Spectinomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM12B	Spiramycin	protein synthesis, 50S ribosomal subunit, macrolide
PM12B	Rifampicin	RNA polymerase
PM14A	Sodium metavanadate	toxic anion, PO ₄ analog
PM14A	Sodium orthovanadate	toxic anion, PO ₄ analog
PM15B	Guanidine hydrochloride	membrane, chaotropic agent
PM15B	Cefmetazole	wall, cephalosporin
PM15B	D- Cycloserine	wall
PM15B	Alexidine	membrane, electron transport, biguanide
PM15B	3,4- Dimethoxybenzyl alcohol	oxidizing agent, free radical-peroxidase substrate

PM15B	2- Nitroimidazole	nitro compound, oxidizing agent, ribonucleotide DP reductase inhibitor
PM16A	Cefotaxime	wall, cephalosporin
PM16A	Streptomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside
PM16A	Chromium (III) chloride	toxic cation
PM17A	4-Aminopyridine	ion channel inhibitor, K ⁺
PM17A	Lithium chloride	toxic cation
PM18C	Azathioprine	nucleic acid analog, purine
PM18C	Poly-L-lysine	membrane, detergent, cationic
PM18C	Pentachlorophenol	respiration, ionophore, H ⁺
PM18C	Sodium Bromate	toxic anion
PM18C	Sodium periodate	toxic anion, oxidizing agent
PM18C	Semicarbazide hydrochloride	amine oxidase inhibitor, carcinogen
PM18C	5-Fluoro-5'-deoxyuridine	nucleic acid analog, pyrimidine, DNA synthesis
PM19	Coumarin	DNA intercalator
PM19	Harmane	imidazoline binding sites, agonist
PM19	7-Hydroxycoumarin	DNA intercalator
PM19	Phenethicillin	wall, lactam
PM19	Lauryl sulfobetaine (N-Dodecyl-N,N-dimethyl-3- ammonio-1-propanesulfonate)	membrane, detergent, zwitterionic
PM20B	Amitriptyline	membrane, transport
PM20B	Orphenadrine	anti-cholinergic
PM20B	Ciprofloxacin	DNA topoisomerase
PM20B	Oxytetracycline	protein synthesis, 30S ribosomal subunit, tetracycline
PM20B	Captan	fungicide, carbamate
PM20B	Tolylfluanid	fungicide, phenylsulphamide

Table S10: Primers used in this work. All primers were designed as part of this study. qRT in the primer name indicates that the primer was designed for qPCR.

Primer Name	Sequence
KMampRUF1	5' - GGAATTCAGCCTGGAACCTCCGCAATCTCGACGAGA -3'
KMampRUR1	5' - GAGCATTCTAGCTAGCTAGGTTCCGCCACCGAAGTGAGTACC -3'
KMampRDF2	5' - CTAGCTAGCTAGAATGCTCAATGGGGTCGAACCAATCTCTGCTCC -3'
KMampRDR2	5' - CGGGATCCGATGCTCGGGTTGGAATAGA -3'
DBS_ampRF2	5' - GGGGTACCGAAGGAGATATACCTTGGTTTCGACCCCATTTGCCGCTGAAC -3'
DBS_ampRR2	5' - CGAGCTCTCTCCCCCGCGCCTCAAC -3'
DBS_qRT_clpXF	5' - TGCGATTACGATGTGGAGA -3'
DBS_qRT_clpXR	5' - CCCTCGATGAGCTTCAGCA -3'
DBS_qRT_ampCF	5' - CGCCGTACAACCGGTGAT -3'
DBS_qRT_ampCR	5' - CGGCCGTCTCTTTTCGA -3'
DBS_qRT_ampGF	5' - GGCTTGACCTATGCGTTGTT -3'
DBS_qRT_ampGR	5' - CGACGGGTTGACGTGGAT -3'
DBS_qRT_ampPF	5' - ACCGCCGCCTGGTCAT -3'
DBS_qRT_ampPR	5' - CAGGCCGATGGGAATGC -3'
DBS_qRT_ampDF	5' - TCAGGAGCCCGATGCATTTTCGATT -3'
DBS_qRT_ampDR	5' - ACGCAGGTTGAAATTCGGTGAAGG -3'
DBS_qRT_ampDh2F2	5' - CGCAGCGTGTTCACATC -3'
DBS_qRT_ampDh2R2	5' - GGGTGAAGGCCAGTAGAACGAA -3'
DBS_qRT_ampDh3F2	5' - CGACGGCGTGTTCACCTT -3'
DBS_qRT_ampDh3R2	5' - CAGGATGTTCTTCGCCAGTTG -3'
DBS_qRT_nagZF	5' - ATGCAAGGCTCTCTGATGCTCGAC -3'
DBS_qRT_nagZR	5' - ATCATCTTCGCTCGCAACATCGAG -3'
DBS_qRT_mexTF	5' - ACCTCATGGGTTGTGACTGTATCC -3'
DBS_qRT_mexTR	5' - TAGGATCACTGACAGGCATAGCCA -3'
DBS_qRT_mexRF	5' - GTTCGTTGCATAGCGTTGTCCTCA -3'
DBS_qRT_mexRR	5' - TTGATAACCGCCATCGAGCTAAA -3'
DBS_qRT_oprDF2	5' - TTTCCGCAGGTAGCACTCAGT -3'
DBS_qRT_oprDR2	5' - CTTCGCTTCGGCCTGATC -3'
DBS_qRT_lasAF	5' - TTCTGTGATCGATTTCGGCTCGGTT -3'
DBS_qRT_lasAR	5' - ACCCGGGAAGACAACTATCAGCTT -3'
DBS_qRT_lecAF	5' - CACCATTGTGTTTCCTGGCGTTCA -3'
DBS_qRT_lecAR	5' - AGAAGGCAACGTCGACTCGTTGAT -3'
DBS_qRT_lecBF	5' - AGACAGCGTAACAATCGAACGAGC -3'
DBS_qRT_lecBR	5' - AGGACGCATCGTTCAGCCAATCTA -3'
DBS_qRT_rpoSF	5' - CGGCGAGTTGGTCATCATCAAACA -3'
DBS_qRT_rpoSR	5' - ATCGATTGCCCTACCTTGACCTGT -3'
DBS_qRT_fleRSF	5' - TAGAGAAGATGCGCAAGTACGGCA -3'
DBS_qRT_fleRSR	5' - TGTCGGTCGACGGAGATATAGCAA -3'
DBS_qRT_pcrHF	5' - CGCTCAACCGCTTCATCCAGAAAT -3'
DBS_qRT_pcrHR	5' - TAGAGGTATCCATGAACCAGCCGA -3'
DBS_qRT_exsEF	5' - CCGTTTCGATCTGCATTTTCGGCTT -3'

DBS_qRT_exsER	5' - CAACTGACCCTCGAATGCTTCGA -3'
DBS_qRT_rsmZF2	5' - GACGGTACTGGGTGTCAAAGG -3'
DBS_qRT_rsmZR2	5' - TGGTAAATTTTCCTCCCGGTGTA -3'
DBS_qRT_prtRF	5' - TCCCTGCACCCATGTGAAATCTCT -3'
DBS_qRT_prtRR	5' - ATCGGCAATCTACAGACCGATGGA -3'
DBS_qRT_creBF	5' - GCATATCCTGATCGTCGAAGATG -3'
DBS_qRT_creBR	5' - GGCCCTGCAGGGCGTAGA -3'
DBS_qRT_creDF	5' - CGCCATCGCCCTACTCAT -3'
DBS_qRT_creDR	5' - GGCGATCGCGGATCAG -3'
DBS_qRT_prtNF	5' - CTGGTTTCGCAACCTGAACAAGGA -3'
DBS_qRT_prtNR	5' - TAGTCACAAGGCCATGCAGTTCGT -3'
DBS_qRT_algTF	5' - AACCAGGAACAGGATCAGCAACT -3'
DBS_qRT_algTR	5' - AGATACTGGGATTGATCGTGCGGT -3'
DBS_qRT_PA3602F	5' - ACGAATTCATCAGCCACTCGATGC -3'
DBS_qRT_PA3602R	5' - TGTTC AACGTCTCGGCGATGAGTT -3'
DBS_qRT_mexEF	5' - TCATCGAACAACCGCTGAACGAGT -3'
DBS_qRT_mexER	5' - ATGAAGGCGCACTGGTGAAGAAAG -3'
DBS_qRT_PA4121F	5' - ATCAAGACACCGAATACCCGCAAC -3'
DBS_qRT_PA4121R	5' - TACGTGATCGTCAACGAGTTCAGC -3'
DBS_qRT_popBF	5' - AACTCAAGCTGACCGAAATCGAGC -3'
DBS_qRT_popBR	5' - ACAACCAGCAGAAGATCAGGGAGT -3'
DBS_qRT_hcnAF	5' - TTGAACGTCAACACGATATCCAGCCC -3'
DBS_qRT_hcnAR	5' - AGACCGTGCTCAACGTGCTCAAT -3'
DBS_qRT_flgCF	5' - GTCGAGGAAATGGCCGACATGATT -3'
DBS_qRT_flgCR	5' - ACCGCCAAACAGATGATGCAGAAG -3'
DBS_qRT_PA2069F	5' - AAACCAGGTGCTGGGCATGTTCAA -3'
DBS_qRT_PA2069R	5' - AACGCACCGAGTTCATGCCATT -3'
DBS_qRT_PA2331F	5' - TTATATCCCGAATCTGCTCGGCGT -3'
DBS_qRT_PA2331R	5' - ATGGCTGCGACTTCTGCGTT -3'

Supplementary information for Chapter 5

Supplementary Table 1: Key to identifying AmpR-regulated genes. Using the data from Figure 5.2, AmpR-regulated genes, both in the presence and absence of β -lactam stress, were identified using this table. Condition I: PAO1 uninduced vs. PAO1 induced; Condition II: PAO Δ ampR uninduced vs. PAO Δ ampR induced; Condition III: PAO1 uninduced vs. PAO Δ ampR uninduced; Condition IV: PAO1 induced vs. PAO Δ ampR induced. Out of the 1703 genes in Figure 5.2, 1623 genes were categorized here. Eighty genes in the following categories were omitted from further analysis for the following reasons: regulated in all four conditions [category O (24 genes)]; could not be assigned unambiguously [category H (44 genes), category K (1 gene), category M (4 genes), category N (7 genes)].

Regulation	Venn Category	# Genes Regulated (Total in Category)	Condition (Expression)	Regulation
AmpR-dependent, β-lactam independent (654)	B	135 (281)	II (up)	Negative
	B	146 (281)	II (down)	Positive
	C	132 (171)	III (up)	Negative
	C	39 (171)	III (down)	Positive
	J	70 (185)	III (up), IV (up)	Negative
	J	115 (185)	III (down), IV (down)	Positive
	M	2 (12)	I (down), III (up), IV (up)	Negative
	M	6 (12)	I (up), III (down), IV (down)	Positive
	N	2 (27)	II (up), III (up), IV (up)	Negative
	N	7 (27)	II (down), III (down), IV (down)	Positive
AmpR-β-lactam-dependent (483)	D	239 (392)	IV (up)	Negative
	D	153 (392)	IV (down)	Positive
	H	72 (116)	I (up), IV (down)	Positive
	L	11 (26)	I (up), II (down), IV (down)	Positive
	L	8 (26)	I (down), II (up), IV (up)	Negative
AmpR-independent, β-lactam-dependent (486)	A	26 (108)	I (down)	Negative
	A	82 (108)	I (up)	Positive
	G	49 (65)	II (down), III (up)	Negative
	G	16 (65)	II (up), III (down)	Positive
	F	16 (47)	I (down), III (down)	Negative
	F	31 (47)	I (up), III (up)	Positive
	I	84 (198)	II (down), IV (down)	Negative
	I	114 (198)	II (up), IV (up)	Positive
	E	19 (50)	I (down), II (down)	Negative
	E	31 (50)	I (up), II (up)	Positive
	L	6 (26)	I (down), II (down), IV (down)	Negative
	L	1 (26)	I (up), II (up), IV (up)	Positive
	N	8 (27)	II (down), III (up), IV (down)	Negative
	N	3 (27)	II (up), III (down), IV (up)	Positive

Supplementary Table 2: AmpR-dependent genes. The positively and negatively AmpR-regulated genes are classified based on information in Supplementary Table 1. Only genes that are ≥ 2.0 , Bonferroni correction (<0.05) are listed. Functional categorization, gene ID and gene/product names are from the *Pseudomonas* Genome database (Winsor et al 2011). Lack of expression of a gene under any of the four conditions is denoted as NA.

Gene ID	Gene name	Product Name	Condition 1		Condition 2		Condition 3		Condition 4	
			FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value
Genes positively regulated by AmpR (313)										
Adaptation, Protection										
PA1001	<i>phnA</i>	anthranilate synthase component I	NA	NA	NA	NA	-20.0	8.86E-11	-81.2	0.00E+00
PA2385	<i>pvdQ</i>	3-oxo-C12-homoserine lactone acylase PvdQ	NA	NA	NA	NA	-11.2	1.10E-07	NA	NA
PA2386	<i>pvdA</i>	L-ornithine N5-oxygenase	NA	NA	NA	NA	-5.3	0.00E+00	-7.2	6.05E-14
PA2392	<i>pvdP</i>	PvdP	NA	NA	NA	NA	- 102.6	0.00E+00	-18.5	0.00E+00
PA2394	<i>pvdN</i>	PvdN	NA	NA	NA	NA	-7.8	1.74E-08	-67.1	5.35E-14
PA2395	<i>pvdO</i>	PvdO	NA	NA	NA	NA	-82.9	0.00E+00	-2.9	7.65E-06
PA2411		probable thioesterase	NA	NA	NA	NA	-5.2	9.31E-14	-23.4	0.00E+00
PA2413	<i>pvdH</i>	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH	NA	NA	NA	NA	-3.5	5.94E-10	-4.1	1.05E-10
PA2424	<i>pvdL</i>	PvdL	NA	NA	NA	NA	-5.2	1.80E-05	-7.1	1.19E-05
PA2425	<i>pvdG</i>	PvdG	NA	NA	NA	NA	-7.6	5.66E-07	-37.6	2.58E-08
PA2788		probable chemotaxis transducer	NA	NA	NA	NA	-12.4	2.08E-06	NA	NA
PA2850	<i>ohr</i>	organic hydroperoxide resistance protein	NA	NA	- 30.6	4.59E-08	NA	NA	NA	NA
PA2867		probable chemotaxis transducer	NA	NA	-2.6	6.69E-08	NA	NA	NA	NA
PA3327		probable non-ribosomal peptide synthetase	NA	NA	NA	NA	-22.3	0.00E+00	- 113.0	1.03E-14
PA3331		cytochrome P450	NA	NA	NA	NA	-15.7	7.89E-14	- 160.0	0.00E+00
PA3450		probable antioxidant protein	NA	NA	NA	NA	-4.3	8.61E-07	NA	NA

PA3475	<i>pheC</i>	cyclohexadienyl dehydratase precursor	NA	NA	NA	NA	-3.8	1.58E-06	-3.6	7.51E-09
PA4236	<i>kata</i>	catalase	NA	NA	NA	NA	-2.1	1.58E-09	NA	NA
PA4468	<i>sodM</i>	superoxide dismutase	NA	NA	NA	NA	-34.8	3.26E-07	NA	NA
PA4566	<i>obg</i>	GTP-binding protein Obg	NA	NA	-2.2	1.38E-11	NA	NA	NA	NA
Amino acid biosynthesis and metabolism										
PA0292	<i>aguA</i>	agmatine deiminase	NA	NA	-6.9	5.28E-12	NA	NA	NA	NA
PA0297	<i>spuA</i>	probable glutamine amidotransferase	NA	NA	-2.2	2.29E-06	NA	NA	NA	NA
PA1002	<i>phnB</i>	anthranilate synthase component II	NA	NA	NA	NA	-31.4	1.36E-06	NA	NA
PA1217		probable 2-isopropylmalate synthase	NA	NA	NA	NA	-39.7	3.85E-08	NA	NA
PA1757	<i>thrH</i>	homoserine kinase	NA	NA	-3.6	1.65E-07	NA	NA	NA	NA
PA3121	<i>leuC</i>	3-isopropylmalate dehydratase large subunit	NA	NA	-	5.82E-09	NA	NA	NA	NA
PA3418	<i>ldh</i>	leucine dehydrogenase	NA	NA	NA	NA	-5.9	3.35E-07	NA	NA
PA3736	<i>hom</i>	homoserine dehydrogenase	NA	NA	-2.2	2.53E-06	NA	NA	NA	NA
PA5092	<i>hutI</i>	imidazolone-5-propionate hydrolase HutI	NA	NA	-	5.35E-06	NA	NA	NA	NA
Antibiotic Resistance and susceptibility										
PA5471		hypothetical protein	NA	NA	-3.1	2.96E-12	NA	NA	NA	NA
Biosynthesis of cofactors, prosthetic groups and carriers										
PA0350	<i>folA</i>	dihydrofolate reductase	NA	NA	-5.1	2.38E-08	NA	NA	NA	NA
PA0582	<i>folB</i>	dihydroneopterin aldolase	NA	NA	-3.6	1.11E-06	NA	NA	NA	NA
PA0996	<i>pqsA</i>	probable coenzyme A ligase	NA	NA	NA	NA	-17.3	3.99E-15	-	0.00E+00
PA0997	<i>pqsB</i>	PqsB	NA	NA	NA	NA	-25.2	4.83E-14	-8.4	0.00E+00
PA0998	<i>pqsC</i>	PqsC	NA	NA	NA	NA	-81.3	7.75E-14	-89.2	0.00E+00
PA0999	<i>pqsD</i>	3-oxoacyl-[acyl-carrier-protein] synthase III	NA	NA	NA	NA	-5.9	1.24E-13	-18.5	0.00E+00
PA1000	<i>pqsE</i>	Quinolone signal response protein	NA	NA	NA	NA	-8.2	0.00E+00	-	0.00E+00

PA3627	<i>ygbB</i>	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	NA	NA	-2.4	3.63E-07	NA	NA	NA	NA
PA4055	<i>ribC</i>	riboflavin synthase alpha chain	NA	NA	-2.7	1.46E-05	NA	NA	NA	NA
Carbon compound catabolism										
PA0211	<i>mdcD</i>	malonate decarboxylase beta subunit	NA	NA	-20.3	1.18E-05	NA	NA	NA	NA
PA0810		probable haloacid dehalogenase	NA	NA	-25.0	9.50E-07	NA	NA	NA	NA
PA2300	<i>chiC</i>	chitinase	NA	NA	NA	NA	-232.8	0.00E+00	-14.9	0.00E+00
PA2507	<i>cata</i>	catechol 1,2-dioxygenase	NA	NA	-8.0	5.38E-09	NA	NA	NA	NA
PA2514	<i>antC</i>	anthranilate dioxygenase reductase	NA	NA	-8.7	3.03E-09	-2.1	1.90E-05	-31.2	0.00E+00
PA5418	<i>soxA</i>	sarcosine oxidase alpha subunit	NA	NA	-15.9	5.01E-07	NA	NA	NA	NA
Cell wall / LPS / capsule ; Motility & Attachment										
PA1078	<i>flgC</i>	flagellar basal-body rod protein FlgC	NA	NA	-2.9	1.24E-06	NA	NA	NA	NA
PA1094	<i>fliD</i>	flagellar capping protein FliD	NA	NA	-2.0	2.20E-08	NA	NA	NA	NA
Central intermediary metabolism										
PA0654	<i>speD</i>	S-adenosylmethionine decarboxylase proenzyme	NA	NA	-2.9	4.36E-12	NA	NA	NA	NA
PA2193	<i>hcnA</i>	hydrogen cyanide synthase HcnA	NA	NA	NA	NA	-150.1	6.88E-14	-9.7	0.00E+00
PA2194	<i>hcnB</i>	hydrogen cyanide synthase HcnB	NA	NA	NA	NA	-4.5	7.21E-10	-5.0	5.09E-14
PA2195	<i>hcnC</i>	hydrogen cyanide synthase HcnC	NA	NA	NA	NA	-3.9	5.31E-10	-12.2	0.00E+00
PA2393		probable dipeptidase precursor	NA	NA	NA	NA	-17.1	1.15E-10	-64.0	2.08E-13
PA3629	<i>adhC</i>	alcohol dehydrogenase class III	NA	NA	-2.3	2.79E-07	NA	NA	NA	NA
PA4130		probable sulfite or nitrite reductase	NA	NA	NA	NA	-12.0	0.00E+00	-32.1	0.00E+00
PA5058	<i>phaC</i> ₂	poly(3-hydroxyalkanoic acid) synthase 2	NA	NA	NA	NA	-6.9	1.10E-05	-25.6	5.40E-06
PA5421	<i>fdhA</i>	glutathione-independent formaldehyde dehydrogenase	NA	NA	-4.7	1.87E-06	NA	NA	NA	NA

Chemotaxis										
PA0415	<i>chpC</i>	probable chemotaxis protein	NA	NA	-2.4	1.26E-05	NA	NA	NA	NA
DNA replication, recombination, modification and repair										
PA3867		probable DNA invertase	NA	NA	-22.8	3.04E-06	NA	NA	NA	NA
Energy metabolism										
PA1175	<i>napD</i>	NapD protein of periplasmic nitrate reductase	NA	NA	NA	NA	-3.8	1.75E-05	-43.5	1.74E-09
PA1320	<i>cyoD</i>	cytochrome o ubiquinol oxidase subunit IV	NA	NA	NA	NA	-35.2	2.73E-07	NA	NA
PA3930	<i>cioA</i>	cyanide insensitive terminal oxidase	NA	NA	NA	NA	-4.0	5.69E-10	-9.7	0.00E+00
PA4133		cytochrome c oxidase subunit (cbb3-type)	NA	NA	NA	NA	-24.3	5.31E-14	-38.0	0.00E+00
PA5490	<i>cc4</i>	cytochrome c4 precursor	NA	NA	-2.1	0.00E+00	NA	NA	NA	NA
Fatty acid and phospholipid metabolism										
PA3333	<i>fabH2</i>	3-oxoacyl-[acyl-carrier-protein] synthase III	NA	NA	NA	NA	-147.6	3.15E-14	-37.0	0.00E+00
PA3334		probable acyl carrier protein	2.8	0.00E+00	NA	NA	-118.2	0.00E+00	-359.1	1.64E-13
PA4050	<i>pgpA</i>	phosphatidylglycerophosphatase A	NA	NA	-3.6	7.94E-09	NA	NA	NA	NA
PA4351		probable acyltransferase	NA	NA	NA	NA	-30.4	2.03E-06	-27.7	2.09E-06
Hypothetical, unclassified, unknown										
PA0050		hypothetical protein	NA	NA	NA	NA	-4.5	0.00E+00	-4.4	8.02E-15
PA0057		hypothetical protein	NA	NA	-24.7	1.11E-06	NA	NA	NA	NA
PA0066		conserved hypothetical protein	NA	NA	-5.1	7.49E-08	NA	NA	NA	NA
PA0122		conserved hypothetical protein	NA	NA	NA	NA	-14.7	1.07E-13	-42.9	1.48E-13
PA0128		conserved hypothetical protein	NA	NA	-5.1	5.15E-12	NA	NA	NA	NA
PA0160		hypothetical protein	NA	NA	-47.1	5.36E-12	-2.4	1.52E-06	-77.2	5.35E-14
PA0200		hypothetical protein	NA	NA	NA	NA	-4.0	0.00E+00	-9.7	0.00E+00

PA0264	hypothetical protein	NA	NA	-	7.91E-06	NA	NA	NA	NA
PA0284	hypothetical protein	NA	NA	21.0	NA	-64.6	8.56E-13	-39.2	1.19E-08
PA0320	conserved hypothetical protein	NA	NA	-	1.11E-09	NA	NA	NA	NA
PA0387	conserved hypothetical protein	NA	NA	37.4	4.49E-06	NA	NA	NA	NA
PA0433	hypothetical protein	NA	NA	-2.7	1.26E-05	NA	NA	NA	NA
PA0569	hypothetical protein	NA	NA	-	NA	-25.1	1.87E-05	NA	NA
PA0570	hypothetical protein	NA	NA	20.1	8.47E-07	NA	NA	NA	NA
PA0587	conserved hypothetical protein	NA	NA	25.2	NA	-4.9	1.59E-06	NA	NA
PA0591	conserved hypothetical protein	NA	NA	NA	2.87E-06	NA	NA	NA	NA
PA0614	hypothetical protein	NA	NA	-3.6	1.53E-13	NA	NA	NA	NA
PA0671	hypothetical protein	NA	NA	-3.1	7.38E-07	NA	NA	NA	NA
PA0719	hypothetical protein of bacteriophage Pfl	NA	NA	-3.3	4.83E-09	NA	NA	NA	NA
PA0774	conserved hypothetical protein	NA	NA	-	5.47E-06	NA	NA	NA	NA
PA0805	hypothetical protein	NA	NA	34.8	3.70E-13	NA	NA	NA	NA
PA0812	hypothetical protein	NA	NA	-4.0	1.81E-07	NA	NA	NA	NA
PA0814	conserved hypothetical protein	NA	NA	-2.7	5.78E-08	NA	NA	NA	NA
PA0826	hypothetical protein	NA	NA	-8.7	2.61E-07	NA	NA	NA	NA
PA0910	hypothetical protein	NA	NA	30.2	1.50E-14	NA	NA	NA	NA
PA0911	hypothetical protein	NA	NA	-	6.56E-14	NA	NA	NA	NA
PA0983	conserved hypothetical protein	NA	NA	27.4	NA	-73.9	2.82E-14	-49.9	9.97E-11
PA1134	hypothetical protein	NA	NA	-2.4	8.47E-07	NA	NA	NA	NA
PA1216	hypothetical protein	NA	NA	-3.1	NA	-38.0	8.26E-08	-76.9	1.03E-14
PA1299	conserved hypothetical protein	NA	NA	NA	2.58E-07	NA	NA	NA	NA
PA1371	hypothetical protein	NA	NA	-4.3	NA	-2.6	1.79E-05	NA	NA

PA1377	conserved hypothetical protein	NA	NA	-2.9	1.22E-05	NA	NA	NA	NA
PA1378	hypothetical protein	NA	NA	NA	NA	-26.0	1.28E-05	NA	NA
PA1533	conserved hypothetical protein	NA	NA	NA	NA	-2.0	2.85E-07	NA	NA
PA1539	hypothetical protein	NA	NA	- 25.8	6.10E-07	NA	NA	NA	NA
PA1656	hypothetical protein	NA	NA	NA	NA	-5.9	2.94E-09	NA	NA
PA1657	conserved hypothetical protein	2.7	2.58E-13	NA	NA	-79.2	1.86E-14	- 226.7	0.00E+00
PA1664	hypothetical protein	NA	NA	NA	NA	-33.5	5.43E-07	-50.9	6.21E-11
PA1888	hypothetical protein	NA	NA	NA	NA	-28.5	4.59E-06	NA	NA
PA2031	hypothetical protein	NA	NA	NA	NA	-27.8	6.11E-06	-28.2	1.73E-06
PA2033	hypothetical protein	NA	NA	NA	NA	-26.0	1.30E-05	NA	NA
PA2066	hypothetical protein	NA	NA	NA	NA	-40.7	2.57E-08	-33.7	1.43E-07
PA2126	conserved hypothetical protein	NA	NA	- 27.5	2.44E-07	NA	NA	NA	NA
PA2274	hypothetical protein	NA	NA	NA	NA	-37.5	1.00E-07	NA	NA
PA2330	hypothetical protein	NA	NA	NA	NA	-31.0	1.59E-06	NA	NA
PA2362	hypothetical protein	NA	NA	NA	NA	-34.3	3.99E-07	-3.4	4.54E-06
PA2363	hypothetical protein	NA	NA	NA	NA	-9.5	1.30E-05	-6.8	1.01E-05
PA2366	uricase PuuD	NA	NA	NA	NA	-55.7	3.99E-11	-8.8	5.10E-07
PA2367	hypothetical protein	NA	NA	NA	NA	-8.1	3.77E-11	-57.7	2.93E-12
PA2380	hypothetical protein	NA	NA	- 37.0	1.43E-09	NA	NA	NA	NA
PA2412	conserved hypothetical protein	NA	NA	NA	NA	-13.3	1.86E-14	-10.7	0.00E+00
PA2427	hypothetical protein	NA	NA	NA	NA	-55.5	4.23E-11	NA	NA
PA2605	conserved hypothetical protein	NA	NA	-3.6	4.52E-06	NA	NA	NA	NA
PA2627	conserved hypothetical protein	NA	NA	- 28.2	1.70E-07	NA	NA	NA	NA
PA2661	hypothetical protein	NA	NA	- 39.2	4.15E-10	NA	NA	NA	NA
PA2669	hypothetical protein	NA	NA	-	3.67E-06	NA	NA	NA	NA

				22.5						
PA2679	hypothetical protein	NA	NA	-	2.38E-06	NA	NA	NA	NA	
PA2747	hypothetical protein	NA	NA	23.3	NA	-4.8	8.82E-09	NA	NA	
PA2781	hypothetical protein	NA	NA	-	6.75E-07	NA	NA	NA	NA	
PA2799	hypothetical protein	NA	NA	25.6	NA	-47.3	1.51E-09	-23.9	1.12E-05	
PA2941	hypothetical protein	NA	NA	-	1.13E-05	NA	NA	NA	NA	
PA2955	hypothetical protein	NA	NA	20.4	4.24E-06	NA	NA	NA	NA	
PA2980	conserved hypothetical protein	NA	NA	-3.3	1.11E-07	NA	NA	NA	NA	
PA3008	hypothetical protein	NA	NA	-2.9	1.13E-10	NA	NA	NA	NA	
PA3069	hypothetical protein	NA	NA	-2.3	NA	-42.1	1.40E-08	-58.1	2.42E-12	
PA3088	conserved hypothetical protein	NA	NA	NA	1.62E-05	NA	NA	NA	NA	
PA3089	hypothetical protein	NA	NA	-3.6	NA	-31.8	1.12E-06	NA	NA	
PA3127	hypothetical protein	NA	NA	NA	5.20E-06	NA	NA	NA	NA	
PA3199	conserved hypothetical protein	NA	NA	21.8	1.68E-05	NA	NA	NA	NA	
PA3209	conserved hypothetical protein	NA	NA	-4.3	2.04E-06	NA	NA	NA	NA	
PA3273	hypothetical protein	NA	NA	-	4.91E-06	NA	NA	NA	NA	
PA3289	hypothetical protein	NA	NA	21.9	NA	-28.5	4.50E-06	NA	NA	
PA3329	hypothetical protein	NA	NA	NA	NA	-14.7	0.00E+00	-	0.00E+00	
PA3332	conserved hypothetical protein	NA	NA	151.3	4.11E-08	-6.8	0.00E+00	-	1.40E-13	
PA3386	conserved hypothetical protein	NA	NA	30.8	1.66E-06	NA	NA	NA	NA	
PA3612	conserved hypothetical protein	NA	NA	-	NA	-63.9	1.13E-12	-32.4	2.64E-07	
PA3616	conserved hypothetical protein	NA	NA	23.9	1.13E-13	NA	NA	NA	NA	
PA3688	hypothetical protein	NA	NA	-3.1	NA	-4.8	1.89E-07	-	6.60E-14	

									104.1	
PA3707	<i>wspB</i>	hypothetical protein	NA	NA	-4.3	1.66E-06	NA	NA	NA	NA
PA3784		hypothetical protein	NA	NA	NA	NA	-4.4	1.62E-11	-8.8	5.04E-14
PA3785		conserved hypothetical protein	NA	NA	NA	NA	-3.0	7.44E-13	-3.6	3.86E-14
PA3786		hypothetical protein	NA	NA	NA	NA	-2.1	1.56E-05	-2.9	4.52E-08
PA3931		conserved hypothetical protein	NA	NA	NA	NA	-6.2	9.11E-10	-3.9	2.09E-07
PA4018		hypothetical protein	NA	NA	-	2.51E-08	NA	NA	NA	NA
					31.7					
PA4129		hypothetical protein	NA	NA	-	1.92E-11	-7.2	0.00E+00	-	1.03E-14
					44.8				440.6	
PA4132		conserved hypothetical protein	NA	NA	NA	NA	-4.8	0.00E+00	-10.5	0.00E+00
PA4134		hypothetical protein	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
							320.9		236.5	
PA4220		hypothetical protein	NA	NA	NA	NA	-41.9	1.51E-08	-76.4	0.00E+00
PA4294		hypothetical protein	NA	NA	NA	NA	-37.3	1.10E-07	-28.3	1.61E-06
PA4336		conserved hypothetical protein	NA	NA	-	6.69E-08	NA	NA	NA	NA
					29.9					
PA4471		hypothetical protein	NA	NA	-	4.37E-06	NA	NA	NA	NA
					22.1					
PA4536		hypothetical protein	2.8	1.34E-05	NA	NA	-26.3	1.16E-05	-4.9	2.99E-09
PA4607		hypothetical protein	NA	NA	NA	NA	-2.7	0.00E+00	-2.3	0.00E+00
PA4634		hypothetical protein	NA	NA	-	1.47E-07	NA	NA	NA	NA
					28.5					
PA4702		hypothetical protein	NA	NA	NA	NA	-5.7	1.10E-09	NA	NA
PA4800		hypothetical protein	NA	NA	-	4.98E-06	NA	NA	NA	NA
					21.9					
PA5139		hypothetical protein	NA	NA	-	1.49E-10	NA	NA	NA	NA
					41.0					
PA5220		hypothetical protein	NA	NA	NA	NA	-10.5	7.39E-14	-34.1	0.00E+00
PA5275		conserved hypothetical protein	NA	NA	-3.6	5.51E-07	NA	NA	NA	NA
PA5294		hypothetical protein	NA	NA	-	7.04E-08	NA	NA	NA	NA
					29.9					

PA5343		hypothetical protein	NA	NA	-3.1	3.10E-06	NA	NA	NA	NA
PA5359		hypothetical protein	NA	NA	NA	NA	-2.5	2.51E-10	-3.6	4.81E-14
PA5381		hypothetical protein	NA	NA	-4.3	1.51E-08	NA	NA	NA	NA
PA5414		hypothetical protein	NA	NA	-2.5	1.46E-06	NA	NA	NA	NA
PA5543		hypothetical protein	NA	NA	-	4.78E-06	NA	NA	NA	NA
Membrane proteins										
PA1540		conserved hypothetical protein	NA	NA	-	4.12E-07	NA	NA	NA	NA
PA2068		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	-87.3	4.55E-14	-	0.00E+00
PA2331		hypothetical protein	NA	NA	NA	NA	-5.2	1.33E-05	-51.2	5.50E-11
PA2568		hypothetical protein	NA	NA	-	5.28E-11	NA	NA	NA	NA
PA2653		probable transporter	NA	NA	-	1.49E-05	NA	NA	NA	NA
PA3234		probable sodium:solute symporter	NA	NA	-2.7	6.89E-07	NA	NA	NA	NA
PA3275		conserved hypothetical protein	NA	NA	NA	NA	-43.0	9.54E-09	NA	NA
PA3281		hypothetical protein	NA	NA	-	4.12E-06	NA	NA	NA	NA
PA3336		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	-7.0	1.47E-11	-16.6	5.35E-14
PA3608	<i>potB</i>	polyamine transport protein PotB	NA	NA	-	1.68E-05	NA	NA	NA	NA
PA3788		hypothetical protein	NA	NA	NA	NA	-59.7	7.14E-12	-5.8	1.56E-13
PA3794		hypothetical protein	NA	NA	-	6.39E-10	NA	NA	NA	NA
PA4205		hypothetical protein	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA4207	<i>mexI</i>	probable Resistance-Nodulation-Cell Division (RND) efflux transporter	NA	NA	NA	NA	-11.5	0.00E+00	-10.2	5.04E-14
PA4208	<i>opmD</i>	probable outer membrane protein precursor	NA	NA	NA	NA	-10.1	6.22E-14	-33.1	0.00E+00

PA4218	ampP	AmpP	NA	NA	NA	NA	-81.6	0.00E+00	-12.7	1.46E-12
PA4219		hypothetical protein	NA	NA	NA	NA	-49.9	5.03E-10	-42.4	2.86E-09
PA4223		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	-29.5	0.00E+00	-20.5	5.04E-14
PA4317		hypothetical protein	NA	NA	-2.0	4.43E-08	NA	NA	NA	NA
PA5097		probable amino acid permease	NA	NA	-8.0	2.80E-06	NA	NA	NA	NA
PA5099		probable transporter	NA	NA	-21.2	6.98E-06	NA	NA	NA	NA
PA5211		conserved hypothetical protein	NA	NA	NA	NA	-31.3	1.39E-06	NA	NA
PA5219		hypothetical protein	NA	NA	NA	NA	-9.5	3.72E-06	-36.1	4.92E-08
PA5469		conserved hypothetical protein	NA	NA	-22.9	2.90E-06	NA	NA	NA	NA
Motility & Attachment										
PA4306	flp	Type IVb pilin, Flp	NA	NA	-60.0	0.00E+00	-5.2	0.00E+00	-524.7	1.92E-13
Non-coding RNA gene										
PA1030.1	P8	P8	2.8	2.46E-06	NA	NA	-30.3	2.14E-06	-92.1	1.03E-14
PA2958.1	rgsA	RgsA	NA	NA	-2.9	9.71E-14	-2.0	5.61E-13	-3.4	0.00E+00
PA4277.3		tRNA-Tyr	NA	NA	-2.0	0.00E+00	-5.5	0.00E+00	-8.4	0.00E+00
PA4581.1	prfF1	tRNA-Arg	NA	NA	-3.1	3.62E-13	NA	NA	NA	NA
PA4704.1		regulatory RNA PrrF1	NA	NA	NA	NA	-3.9	0.00E+00	NA	NA
Protein secretion/export apparatus										
PA1715	pscB	type III export apparatus protein	NA	NA	-20.7	9.31E-06	NA	NA	NA	NA
PA1719	pscF	type III export protein PscF	NA	NA	NA	NA	-27.5	7.00E-06	-27.8	1.99E-06
PA2674		probable type II secretion system protein	NA	NA	-32.0	2.20E-08	NA	NA	NA	NA

PA3104	<i>xcpP</i>	secretion protein XcpP	NA	NA	NA	NA	-2.0	1.73E-07	NA	NA
PA4142		probable secretion protein	NA	NA	NA	NA	-45.1	3.80E-09	NA	NA
PA4304	<i>rcpA</i>	RcpA	NA	NA	NA	NA	-11.9	1.30E-07	-8.8	3.80E-08
Putative Enzymes										
PA0483		probable acetyltransferase	NA	NA	NA	NA	-26.6	1.01E-05	NA	NA
PA0829		probable hydrolase	NA	NA	-	2.40E-06	NA	NA	NA	NA
PA0953		probable thioredoxin	NA	NA	-3.1	1.02E-10	NA	NA	NA	NA
PA1662		probable ClpA/B-type protease	NA	NA	NA	NA	-9.3	1.64E-05	NA	NA
PA2067		probable hydrolase	NA	NA	NA	NA	-14.3	0.00E+00	-	6.29E-14
PA2069		probable carbamoyl transferase	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA2402		probable non-ribosomal peptide synthetase	NA	NA	NA	NA	167.1	4.50E-06	204.0	7.27E-08
PA2546		probable ring-cleaving dioxygenase	NA	NA	-	1.18E-05	NA	NA	NA	NA
PA3328		probable FAD-dependent monooxygenase	NA	NA	NA	NA	-85.1	1.18E-14	-25.3	0.00E+00
PA3330		probable short chain dehydrogenase	2.0	2.91E-08	NA	NA	-21.4	0.00E+00	-30.2	0.00E+00
PA3883		probable short-chain dehydrogenase	NA	NA	-	7.63E-06	NA	NA	NA	NA
PA4131		probable iron-sulfur protein	NA	NA	NA	NA	-8.5	3.50E-13	-21.0	0.00E+00
PA4209	<i>phzM</i>	probable phenazine-specific methyltransferase	NA	NA	NA	NA	-18.5	3.99E-15	-	3.15E-14
PA4217	<i>phzS</i>	flavin-containing monooxygenase	NA	NA	NA	NA	-7.7	0.00E+00	-	2.74E-13
PA5150		probable short-chain dehydrogenase	NA	NA	-	1.84E-06	NA	NA	NA	NA
Related to phage, transposon, or plasmid										
PA0616		hypothetical protein	NA	NA	-3.7	7.09E-13	NA	NA	NA	NA
PA0617		probable bacteriophage protein	NA	NA	-3.0	0.00E+00	NA	NA	NA	NA

PA0618		probable bacteriophage protein	NA	NA	-4.1	0.00E+00	NA	NA	NA	NA
PA0619		probable bacteriophage protein	NA	NA	-4.0	0.00E+00	NA	NA	NA	NA
PA0620		probable bacteriophage protein	NA	NA	-3.6	0.00E+00	NA	NA	NA	NA
PA0622		probable bacteriophage protein	NA	NA	-3.5	0.00E+00	NA	NA	NA	NA
PA0623		probable bacteriophage protein	NA	NA	-3.2	0.00E+00	NA	NA	NA	NA
PA0624		hypothetical protein	NA	NA	-3.4	0.00E+00	NA	NA	NA	NA
PA0625		hypothetical protein	NA	NA	-3.1	0.00E+00	NA	NA	NA	NA
PA0626		hypothetical protein	NA	NA	-3.2	0.00E+00	NA	NA	NA	NA
PA0627		conserved hypothetical protein	NA	NA	-3.3	6.47E-13	NA	NA	NA	NA
PA0628		conserved hypothetical protein	NA	NA	-2.7	5.82E-13	NA	NA	NA	NA
PA0629		conserved hypothetical protein	NA	NA	-3.5	0.00E+00	NA	NA	NA	NA
PA0630		hypothetical protein	NA	NA	-3.0	3.78E-13	NA	NA	NA	NA
PA0631		hypothetical protein	NA	NA	-3.0	2.27E-13	NA	NA	NA	NA
PA0633		hypothetical protein	NA	NA	-3.9	0.00E+00	NA	NA	NA	NA
PA0634		hypothetical protein	NA	NA	-3.8	0.00E+00	NA	NA	NA	NA
PA0636		hypothetical protein	NA	NA	-3.0	6.56E-13	NA	NA	NA	NA
PA0637		conserved hypothetical protein	NA	NA	-3.4	2.79E-13	NA	NA	NA	NA
PA0639		conserved hypothetical protein	NA	NA	-2.5	4.52E-13	NA	NA	NA	NA
PA0640		probable bacteriophage protein	NA	NA	-3.7	6.13E-13	NA	NA	NA	NA
PA0641		probable bacteriophage protein	NA	NA	-3.2	0.00E+00	NA	NA	NA	NA
PA0644		hypothetical protein	NA	NA	-2.7	1.04E-13	NA	NA	NA	NA
PA0645		hypothetical protein	NA	NA	-4.4	0.00E+00	NA	NA	NA	NA
PA0648		hypothetical protein	NA	NA	-2.8	0.00E+00	NA	NA	NA	NA
Secreted Factors (toxins, enzymes, alginate)										
PA0852	<i>cbpD</i>	chitin-binding protein CbpD precursor	NA	NA	NA	NA	-5.7	0.00E+00	-4.4	0.00E+00
PA1871	<i>lasA</i>	LasA protease precursor	NA	NA	NA	NA	-5.8	5.62E-11	-7.3	4.66E-12
PA1899	<i>phzA2</i>	probable phenazine biosynthesis protein	NA	NA	NA	NA	-	0.00E+00	-	1.50E-14
							203.0		352.5	

PA1900	<i>phzB2</i>	probable phenazine biosynthesis protein	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA1901	<i>phzC2</i>	phenazine biosynthesis protein PhzC	NA	NA	NA	NA	-8.4	9.74E-14	-	0.00E+00
PA1902	<i>phzD2</i>	phenazine biosynthesis protein PhzD	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA1903	<i>phzE2</i>	phenazine biosynthesis protein PhzE	NA	NA	NA	NA	-91.6	0.00E+00	-	4.11E-14
PA1904	<i>phzF2</i>	probable phenazine biosynthesis protein	2.1	5.56E-08	NA	NA	-13.8	4.10E-13	-21.4	0.00E+00
PA1905	<i>phzG2</i>	probable pyridoxamine 5'-phosphate oxidase	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA2396	<i>pvdF</i>	pyoverdine synthetase F	NA	NA	NA	NA	-4.4	5.82E-14	-5.8	2.73E-15
PA2399	<i>pvdD</i>	pyoverdine synthetase D	NA	NA	NA	NA	-6.0	2.42E-09	-4.0	1.33E-06
PA2939		probable aminopeptidase	NA	NA	NA	NA	-6.7	0.00E+00	-8.1	0.00E+00
PA3478	<i>rhlB</i>	rhamnosyltransferase chain B	NA	NA	NA	NA	-22.6	0.00E+00	-28.2	0.00E+00
PA3479	<i>rhlA</i>	rhamnosyltransferase chain A	NA	NA	NA	NA	-18.1	0.00E+00	-	1.73E-13
PA3724	<i>lasB</i>	elastase LasB	NA	NA	NA	NA	-16.5	8.67E-14	-18.8	4.06E-13
PA4175	<i>piv</i>	protease IV	NA	NA	NA	NA	-3.0	5.56E-07	NA	NA
PA4210	<i>phzA1</i>	probable phenazine biosynthesis protein	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA4211	<i>phzB1</i>	probable phenazine biosynthesis protein	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA4212	<i>phzC1</i>	phenazine biosynthesis protein PhzC	NA	NA	NA	NA	-41.3	0.00E+00	-	0.00E+00
PA4213	<i>phzD1</i>	phenazine biosynthesis protein PhzD	NA	NA	NA	NA	-	0.00E+00	-	0.00E+00
PA4214	<i>phzE1</i>	phenazine biosynthesis protein PhzE	NA	NA	NA	NA	-77.8	4.83E-14	-87.7	2.36E-14
PA4215	<i>phzF1</i>	probable phenazine biosynthesis protein	NA	NA	NA	NA	-21.9	4.29E-14	-15.6	0.00E+00
PA4216	<i>phzG1</i>	probable pyridoxamine 5'-phosphate oxidase	NA	NA	NA	NA	-10.9	9.50E-13	-12.7	0.00E+00

PA4225	<i>pchF</i>	pyochelin synthetase	NA	NA	NA	NA	-13.4	3.99E-15	-23.6	1.28E-14
PA4226	<i>pchE</i>	dihydroaeruginoic acid synthetase	NA	NA	NA	NA	-62.7	4.55E-14	-26.6	0.00E+00
PA4228	<i>pchD</i>	pyochelin biosynthesis protein PchD	NA	NA	NA	NA	-43.7	4.29E-14	-25.3	0.00E+00
PA4229	<i>pchC</i>	pyochelin biosynthetic protein PchC	NA	NA	NA	NA	- 125.0	0.00E+00	-85.5	0.00E+00
PA4230	<i>pchB</i>	salicylate biosynthesis protein PchB	NA	NA	NA	NA	- 193.1	0.00E+00	- 187.8	0.00E+00
PA4231	<i>pchA</i>	salicylate biosynthesis isochorismate synthase	NA	NA	NA	NA	- 125.5	0.00E+00	-95.4	4.64E-14
Transcription, RNA processing and degradation										
PA3743	<i>trmD</i>	tRNA (guanine-N1)-methyltransferase	NA	NA	-2.2	0.00E+00	NA	NA	NA	NA
Transcriptional Regulators										
PA1099	<i>fleR</i>	two-component response regulator	NA	NA	- 13.0	3.96E-11	NA	NA	NA	NA
PA1285		probable transcriptional regulator	NA	NA	NA	NA	-4.1	8.56E-13	NA	NA
PA1397		probable two-component response regulator	NA	NA	- 20.8	9.08E-06	NA	NA	NA	NA
PA1456	<i>cheY</i>	two-component response regulator CheY	NA	NA	-2.2	3.63E-07	NA	NA	NA	NA
PA1799	<i>parR</i>	two-component response regulator, ParR	NA	NA	- 30.9	3.92E-08	NA	NA	NA	NA
PA1859		probable transcriptional regulator	NA	NA	- 19.7	1.56E-05	NA	NA	NA	NA
PA2312		probable transcriptional regulator	NA	NA	- 22.6	3.45E-06	NA	NA	NA	NA
PA2426	<i>pvdS</i>	sigma factor PvdS	NA	NA	NA	NA	-7.6	3.49E-09	-38.2	1.93E-08
PA2588		probable transcriptional regulator	NA	NA	NA	NA	-9.7	8.45E-14	-2.9	2.32E-06
PA2622	<i>cspD</i>	cold-shock protein CspD	NA	NA	NA	NA	-2.1	1.99E-12	NA	NA
PA2686	<i>pfeR</i>	two-component response regulator PfeR	NA	NA	NA	NA	-38.6	6.29E-08	NA	NA
PA2834		probable transcriptional regulator	NA	NA	- 22.8	3.04E-06	NA	NA	NA	NA

PA3027		probable transcriptional regulator	NA	NA	-34.0	7.49E-09	NA	NA	NA	NA
PA3423		probable transcriptional regulator	NA	NA	-23.5	2.04E-06	NA	NA	NA	NA
PA3477	<i>rhlR</i>	transcriptional regulator RhlR	NA	NA	NA	NA	-4.5	0.00E+00	-3.6	0.00E+00
PA3622	<i>rpoS</i>	sigma factor RpoS	NA	NA	NA	NA	-2.3	7.89E-14	NA	NA
PA3948	<i>rocA1</i>	two-component response regulator RocA1	NA	NA	-4.3	1.68E-05	NA	NA	NA	NA
PA4145		probable transcriptional regulator	NA	NA	-29.5	8.43E-08	NA	NA	NA	NA
PA4296	<i>pprB</i>	two-component response regulator, PprB	NA	NA	NA	NA	-3.5	1.60E-05	NA	NA
PA4984		probable transcriptional regulator	NA	NA	-20.3	1.18E-05	NA	NA	NA	NA
PA5059		probable transcriptional regulator	NA	NA	NA	NA	-49.7	5.31E-10	NA	NA
PA5255	<i>algQ</i>	Alginate regulatory protein AlgQ	NA	NA	-2.1	7.66E-14	NA	NA	NA	NA
Translation, post-translational modification, degradation										
PA1712	<i>exsB</i>	exoenzyme S synthesis protein B	NA	NA	NA	NA	-45.7	3.00E-09	NA	NA
PA3326	<i>clpP2</i>	ClpP2	NA	NA	NA	NA	-5.5	1.50E-14	-5.1	9.71E-14
PA5018	<i>msrA</i>	peptide methionine sulfoxide reductase	NA	NA	-2.4	1.68E-05	NA	NA	NA	NA
PA5240	<i>trxA</i>	thioredoxin	NA	NA	-2.0	0.00E+00	NA	NA	NA	NA
PA5316	<i>rpmB</i>	50S ribosomal protein L28	NA	NA	-2.2	0.00E+00	NA	NA	NA	NA
Transport of small molecules										
PA2592		probable periplasmic spermidine/putrescine-binding protein	NA	NA	NA	NA	-3.4	4.14E-11	-2.8	8.45E-14
PA2983		probable tolQ-type transport protein	NA	NA	-2.2	1.56E-05	NA	NA	NA	NA
PA3373		conserved hypothetical protein	NA	NA	-5.1	1.15E-07	NA	NA	NA	NA
PA4206	<i>mexH</i>	probable Resistance-Nodulation-Cell Division (RND) efflux membrane fusion protein precursor	NA	NA	NA	NA	-11.3	0.00E+00	-7.8	3.15E-14
PA4221	<i>fptA</i>	Fe(III)-pyochelin outer membrane receptor precursor	NA	NA	NA	NA	-25.4	3.99E-15	-29.2	0.00E+00

PA4222		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	-11.6	1.85E-10	-66.6	4.11E-14
PA4224	<i>pchG</i>	pyochelin biosynthetic protein PchG	NA	NA	NA	NA	-56.3	3.15E-11	-13.6	3.69E-14
Two-component regulatory systems										
PA0409	<i>pilH</i>	twitching motility protein PilH	NA	NA	-3.5	4.93E-14	NA	NA	NA	NA

Genes negatively regulated by AmpR (341)										
Adaptation, Protection										
PA0140	<i>ahpF</i>	alkyl hydroperoxide reductase subunit F	NA	NA	3.0	4.76E-07	NA	NA	NA	NA
PA1008	<i>bcp</i>	bacterioferritin comigratory protein	NA	NA	NA	NA	2.4	2.35E-09	NA	NA
PA1457	<i>cheZ</i>	chemotaxis protein CheZ	NA	NA	2.2	3.03E-07	NA	NA	NA	NA
PA4356	<i>xenB</i>	xenobiotic reductase	NA	NA	NA	NA	10.5	0.00E+00	4.4	2.93E-10
PA4378	<i>inaA</i>	InaA protein	NA	NA	2.2	5.78E-06	NA	NA	NA	NA
PA5117	<i>typA</i>	regulatory protein TypA	NA	NA	NA	NA	3.2	6.38E-09	2.3	3.74E-07
Amino acid biosynthesis and metabolism										
PA0393	<i>proC</i>	pyrroline-5-carboxylate reductase	NA	NA	NA	NA	2.2	2.67E-06	2.9	3.65E-09
PA0904	<i>lysC</i>	aspartate kinase alpha and beta chain	NA	NA	NA	NA	2.0	8.49E-07	NA	NA
PA1004	<i>nadA</i>	quinolinate synthetase A	NA	NA	NA	NA	2.1	9.75E-06	NA	NA
PA1010	<i>dapA</i>	dihydrodipicolinate synthase	NA	NA	NA	NA	2.0	2.49E-10	NA	NA
PA1421	<i>gbuA</i>	guanidinobutyrase	NA	NA	28.5	3.92E-06	NA	NA	NA	NA
PA1685	<i>masA</i>	enolase-phosphatase E-1	NA	NA	3.1	2.66E-06	NA	NA	NA	NA
PA1818	<i>ldcA</i>	lysine-specific pyridoxal 5'-phosphate-dependent carboxylase, LdcA	NA	NA	NA	NA	2.2	2.51E-07	NA	NA
PA1999	<i>dhcA</i>	DhcA, dehydrocarnitine CoA transferase, subunit A	NA	NA	2.0	0.00E+00	NA	NA	NA	NA
PA2629	<i>purB</i>	adenylosuccinate lyase	NA	NA	NA	NA	2.2	1.80E-05	NA	NA

PA3167	<i>serC</i>	3-phosphoserine aminotransferase	NA	NA	NA	NA	2.2	6.38E-08	NA	NA
PA5140	<i>hisF1</i>	imidazoleglycerol-phosphate synthase, cyclase subunit	NA	NA	3.7	1.76E-06	NA	NA	NA	NA
PA5203	<i>gshA</i>	glutamate--cysteine ligase	NA	NA	2.4	1.37E-05	NA	NA	NA	NA
PA5373	<i>betB</i>	betaine aldehyde dehydrogenase	NA	NA	NA	NA	3.4	4.44E-07	5.1	1.87E-09
Biosynthesis of cofactors, prosthetic groups and carriers										
PA0510		probable uroporphyrin-III c-methyltransferase	NA	NA	48.9	6.85E-10	NA	NA	NA	NA
PA0518	<i>nirM</i>	cytochrome c-551 precursor	NA	NA	43.5	7.17E-09	NA	NA	NA	NA
PA1986	<i>pqqB</i>	pyrroloquinoline quinone biosynthesis protein B	NA	NA	NA	NA	7.4	5.76E-06	29.9	9.02E-08
PA3030	<i>mobA</i>	molybdopterin-guanine dinucleotide biosynthesis protein MobA	NA	NA	34.4	3.34E-07	NA	NA	NA	NA
PA3812	<i>iscA</i>	probable iron-binding protein IscA	NA	NA	2.5	3.47E-14	NA	NA	NA	NA
PA3917	<i>moaD</i>	molybdopterin converting factor, small subunit	NA	NA	2.8	4.77E-07	NA	NA	NA	NA
PA3976	<i>thiE</i>	thiamin-phosphate pyrophosphorylase	NA	NA	NA	NA	3.2	1.29E-07	NA	NA
PA4044	<i>dxs</i>	1-deoxyxylulose-5-phosphate synthase	NA	NA	NA	NA	2.8	1.69E-07	NA	NA
PA4053	<i>ribE</i>	6,7-dimethyl-8-ribityllumazine synthase	NA	NA	NA	NA	3.0	0.00E+00	NA	NA
PA4054	<i>ribB</i>	GTP cyclohydrolase II / 3,4-dihydroxy-2-butanone 4-phosphate synthase	NA	NA	NA	NA	2.6	2.53E-07	NA	NA
PA5259	<i>hemD</i>	uroporphyrinogen-III synthetase	NA	NA	4.1	0.00E+00	NA	NA	NA	NA
Carbon compound catabolism										
PA0210	<i>mdcC</i>	malonate decarboxylase delta subunit	NA	NA	NA	NA	4.2	3.66E-07	45.6	2.49E-11
PA0231	<i>pcaD</i>	beta-ketoadipate enol-lactone hydrolase	NA	NA	25.9	1.17E-05	NA	NA	NA	NA
PA2008	<i>fahA</i>	fumarylacetoacetase	NA	NA	NA	NA	2.3	2.26E-13	NA	NA
PA2011	<i>liuE</i>	3-hydroxy-3-methylglutaryl-CoA lyase	NA	NA	2.9	1.98E-11	NA	NA	NA	NA
PA2414		L-sorbose dehydrogenase	NA	NA	25.9	1.17E-05	NA	NA	NA	NA
PA3569	<i>mmsB</i>	3-hydroxyisobutyrate dehydrogenase	NA	NA	NA	NA	2.0	7.71E-08	2.4	6.55E-13

PA3636	<i>kdsA</i>	2-dehydro-3-deoxyphosphooctonate aldolase	NA	NA	NA	NA	2.0	1.74E-08	NA	NA
Cell division										
PA0857	<i>bolA</i>	morphogene protein BolA	NA	NA	2.1	7.84E-08	NA	NA	NA	NA
PA3245	<i>minE</i>	cell division topological specificity factor MinE	NA	NA	2.4	4.03E-14	NA	NA	NA	NA
PA4413	<i>ftsW</i>	cell division protein FtsW	NA	NA	2.5	5.64E-11	NA	NA	NA	NA
Cell wall / LPS / capsule										
PA1222		probable membrane-bound lytic murein transglycolase A	NA	NA	NA	NA	3.1	1.42E-05	NA	NA
PA3150	<i>wbpG</i>	LPS biosynthesis protein WbpG	NA	NA	2.0	9.14E-13	NA	NA	NA	NA
PA3552	<i>arnB</i>	ArnB	NA	NA	NA	NA	7.9	6.76E-10	NA	NA
PA3666	<i>dapD</i>	tetrahydrodipicolinate succinylase	NA	NA	NA	NA	2.3	7.51E-07	NA	NA
PA4001	<i>sltBI</i>	soluble lytic transglycosylase B	NA	NA	2.0	4.15E-07	NA	NA	NA	NA
PA4545	<i>comL</i>	competence protein ComL	NA	NA	NA	NA	2.5	5.05E-10	NA	NA
PA4624	<i>cdrB</i>	cyclic diguanylate-regulated TPS partner B, CdrB	NA	NA	2.6	1.51E-07	NA	NA	NA	NA
PA5077	<i>mdoH</i>	periplasmic glucans biosynthesis protein MdoH	NA	NA	2.1	7.64E-06	NA	NA	NA	NA
PA5164	<i>rmIC</i>	dTDP-4-dehydrorhamnose 3,5-epimerase	NA	NA	2.4	1.32E-07	NA	NA	NA	NA
PA5452	<i>wbpW</i>	phosphomannose isomerase/GDP-mannose WbpW	NA	NA	3.9	2.29E-06	NA	NA	NA	NA
Central intermediary metabolism										
PA0265	<i>gabD</i>	succinate-semialdehyde dehydrogenase	NA	NA	NA	NA	2.6	5.69E-10	3.7	1.95E-14
PA0266	<i>gabT</i>	4-aminobutyrate aminotransferase	NA	NA	NA	NA	2.6	3.31E-11	3.2	4.64E-14
PA2001	<i>atoB</i>	acetyl-CoA acetyltransferase	NA	NA	NA	NA	2.3	1.50E-14	2.4	4.11E-14
PA2442	<i>gcvT2</i>	glycine cleavage system protein T2	NA	NA	2.1	1.05E-11	NA	NA	NA	NA
PA2726		probable radical activating enzyme	NA	NA	NA	NA	18.9	3.08E-13	NA	NA
PA2813		probable glutathione S-transferase	NA	NA	NA	NA	6.7	0.00E+00	176.3	0.00E+00

PA3181		2-keto-3-deoxy-6-phosphogluconate aldolase	NA	NA	NA	NA	4.7	6.71E-08	103.2	0.00E+00
PA3182	<i>pgl</i>	6-phosphogluconolactonase	NA	NA	NA	NA	4.2	3.98E-06	2.8	3.58E-08
PA4031	<i>ppa</i>	inorganic pyrophosphatase	NA	NA	NA	NA	2.0	0.00E+00	NA	NA
PA5215	<i>gcvT1</i>	glycine-cleavage system protein T1	NA	NA	NA	NA	2.1	2.26E-11	NA	NA
PA5435		probable transcarboxylase subunit	NA	NA	NA	NA	2.2	2.89E-09	2.7	1.03E-14
PA5436		probable biotin carboxylase subunit of a transcarboxylase	NA	NA	NA	NA	2.1	1.13E-07	NA	NA
Chaperones & heat shock proteins										
PA2614	<i>lolA</i>	periplasmic chaperone LolA	NA	NA	NA	NA	2.3	1.29E-09	NA	NA
PA3810	<i>hscA</i>	heat shock protein HscA	NA	NA	2.1	1.26E-06	NA	NA	NA	NA
Chemotaxis ; Adaptation, Protection ; Motility & Attachment										
PA1445	<i>fliO</i>	flagellar protein FliO	NA	NA	6.9	6.54E-10	NA	NA	NA	NA
DNA replication, recombination, modification and repair										
PA3642	<i>rnhB</i>	ribonuclease HII	NA	NA	NA	NA	2.8	6.84E-07	NA	NA
PA3832	<i>holC</i>	DNA polymerase III, chi subunit	NA	NA	6.9	1.99E-10	NA	NA	NA	NA
Energy metabolism										
PA0362	<i>fdx1</i>	ferredoxin [4Fe-4S]	NA	NA	NA	NA	2.1	5.52E-06	2.6	4.56E-09
PA1555.1	<i>ccoQ2</i>	Cytochrome c oxidase, cbb3-type, CcoQ subunit	NA	NA	NA	NA	12.6	2.82E-14	3.1	1.91E-09
PA1581	<i>sdhC</i>	succinate dehydrogenase (C subunit)	NA	NA	NA	NA	2.5	0.00E+00	NA	NA
PA1586	<i>sucB</i>	dihydrolipoamide succinyltransferase (E2 subunit)	NA	NA	NA	NA	2.1	0.00E+00	NA	NA
PA1588	<i>sucC</i>	succinyl-CoA synthetase beta chain	NA	NA	NA	NA	2.4	3.00E-13	NA	NA
PA1589	<i>sucD</i>	succinyl-CoA synthetase alpha chain	NA	NA	NA	NA	2.7	1.47E-13	2.7	0.00E+00
PA1787	<i>acnB</i>	aconitate hydratase 2	NA	NA	NA	NA	2.0	4.01E-14	NA	NA
PA2638	<i>nuoB</i>	NADH dehydrogenase I chain B	NA	NA	NA	NA	2.0	2.02E-10	NA	NA
PA2639	<i>nuoD</i>	NADH dehydrogenase I chain C,D	NA	NA	NA	NA	2.1	3.62E-07	2.8	1.54E-10
PA2640	<i>nuoE</i>	NADH dehydrogenase I chain E	NA	NA	NA	NA	2.0	1.37E-08	2.2	2.07E-12
PA2642	<i>nuoG</i>	NADH dehydrogenase I chain G	NA	NA	NA	NA	2.1	4.26E-06	2.6	4.66E-12

PA2643	<i>nuoH</i>	NADH dehydrogenase I chain H	NA	NA	NA	NA	2.4	3.61E-07	NA	NA
PA2647	<i>nuoL</i>	NADH dehydrogenase I chain L	NA	NA	NA	NA	2.6	2.81E-08	NA	NA
PA2953		electron transfer flavoprotein-ubiquinone oxidoreductase	NA	NA	NA	NA	2.2	2.67E-07	2.1	2.01E-08
PA2994	<i>nqrF</i>	Na ⁺ -translocating NADH:quinone oxidoreductase, subunit Nqr6	NA	NA	NA	NA	2.3	6.89E-07	2.0	5.45E-06
PA2996	<i>nqrD</i>	Na ⁺ -translocating NADH:ubiquinone oxidoreductase subunit Nqr4	NA	NA	NA	NA	3.0	8.48E-06	NA	NA
PA3621	<i>fdxA</i>	ferredoxin I	NA	NA	NA	NA	2.0	7.87E-09	NA	NA
PA4333		probable fumarase	NA	NA	NA	NA	2.5	7.32E-09	NA	NA
PA4810	<i>fdnI</i>	nitrate-inducible formate dehydrogenase, gamma subunit	NA	NA	NA	NA	15.8	7.75E-14	3.1	5.18E-06
PA5129	<i>grx</i>	glutaredoxin	NA	NA	NA	NA	2.1	4.09E-08	3.6	5.09E-14
Fatty acid and phospholipid metabolism										
PA2537		probable acyltransferase	NA	NA	3.2	4.08E-06	NA	NA	NA	NA
PA4339		probable phospholipase	NA	NA	31.7	1.02E-06	NA	NA	NA	NA
PA4848	<i>accC</i>	biotin carboxylase	NA	NA	NA	NA	2.0	2.71E-11	NA	NA
Hypothetical, unclassified, unknown										
PA0141		conserved hypothetical protein	NA	NA	3.5	1.57E-06	NA	NA	NA	NA
PA0309		hypothetical protein	NA	NA	NA	NA	2.7	3.82E-06	NA	NA
PA0315		hypothetical protein	NA	NA	2.6	0.00E+00	NA	NA	NA	NA
PA0370		conserved hypothetical protein	NA	NA	NA	NA	3.2	3.40E-06	NA	NA
PA0392		conserved hypothetical protein	NA	NA	NA	NA	2.2	1.55E-06	NA	NA
PA0442		hypothetical protein	NA	NA	NA	NA	2.1	1.36E-05	4.1	3.52E-14
PA0490		hypothetical protein	NA	NA	46.6	1.87E-09	NA	NA	NA	NA
PA0754		hypothetical protein	NA	NA	4.8	1.17E-05	NA	NA	NA	NA
PA0760		conserved hypothetical protein	NA	NA	2.8	5.59E-07	NA	NA	NA	NA
PA0821		hypothetical protein	NA	NA	37.7	8.26E-08	NA	NA	NA	NA
PA0957		hypothetical protein	NA	NA	33.5	4.77E-07	NA	NA	NA	NA
PA1006		conserved hypothetical protein	-	4.96E-09	NA	NA	2.6	2.36E-06	54.3	2.58E-13

			37.5							
PA1063		hypothetical protein	NA	NA	54.3	6.55E-11	NA	NA	NA	NA
PA1069		hypothetical protein	NA	NA	2.0	3.96E-06	NA	NA	NA	NA
PA1106		hypothetical protein	NA	NA	4.1	6.47E-06	NA	NA	NA	NA
PA1191		hypothetical protein	NA	NA	34.6	3.12E-07	NA	NA	NA	NA
PA1333		hypothetical protein	- 68.5	0.00E+00	NA	NA	2.1	3.33E-06	132.2	0.00E+00
PA1469		hypothetical protein	NA	NA	NA	NA	10.5	3.94E-06	NA	NA
PA1638		conserved hypothetical protein	NA	NA	45.2	3.41E-09	NA	NA	NA	NA
PA1668		hypothetical protein	NA	NA	31.5	1.12E-06	NA	NA	NA	NA
PA1753		conserved hypothetical protein	NA	NA	3.5	5.64E-06	NA	NA	NA	NA
PA1755		hypothetical protein	NA	NA	NA	NA	37.8	8.26E-10	59.0	2.75E-14
PA1766		hypothetical protein	NA	NA	NA	NA	3.0	6.60E-09	NA	NA
PA1768		hypothetical protein	NA	NA	NA	NA	3.2	9.20E-09	NA	NA
PA1789		hypothetical protein	NA	NA	2.1	7.51E-06	NA	NA	NA	NA
PA1814		hypothetical protein	NA	NA	2.1	1.48E-05	NA	NA	NA	NA
PA1830		hypothetical protein	NA	NA	2.0	0.00E+00	NA	NA	NA	NA
PA1831		hypothetical protein	NA	NA	NA	NA	2.3	8.09E-14	NA	NA
PA1841		hypothetical protein	NA	NA	NA	NA	3.7	9.54E-07	NA	NA
PA1889		hypothetical protein	NA	NA	3.0	8.33E-06	NA	NA	NA	NA
PA1942		hypothetical protein	NA	NA	NA	NA	63.1	5.73E-13	355.5	8.45E-14
PA1957		hypothetical protein	NA	NA	38.9	5.04E-08	NA	NA	NA	NA
PA1969		hypothetical protein	NA	NA	2.2	2.21E-06	NA	NA	NA	NA
PA1970		hypothetical protein	NA	NA	NA	NA	54.8	5.95E-14	114.1	5.09E-14
PA2111		hypothetical protein	NA	NA	NA	NA	2.0	0.00E+00	2.2	0.00E+00
PA2145		hypothetical protein	NA	NA	NA	NA	32.9	1.19E-08	51.5	1.15E-12
PA2242	<i>pslL</i>	hypothetical protein	NA	NA	3.5	1.23E-05	NA	NA	NA	NA
PA2251		hypothetical protein	NA	NA	6.9	7.05E-06	NA	NA	NA	NA
PA2311		hypothetical protein	NA	NA	NA	NA	4.2	8.30E-07	43.0	9.49E-11

PA2345	conserved hypothetical protein	NA	NA	9.7	3.88E-06	NA	NA	NA	NA
PA2485	hypothetical protein	NA	NA	NA	NA	3.8	0.00E+00	7.7	2.07E-13
PA2486	hypothetical protein	NA	NA	3.5	8.78E-14	4.2	1.06E-05	175.5	0.00E+00
PA2651	conserved hypothetical protein	NA	NA	3.6	2.48E-07	NA	NA	NA	NA
PA2655	hypothetical protein	NA	NA	NA	NA	42.5	5.82E-11	3.1	4.39E-06
PA2713	conserved hypothetical protein	NA	NA	28.5	3.92E-06	NA	NA	NA	NA
PA2753	hypothetical protein	NA	NA	2.8	4.26E-07	NA	NA	NA	NA
PA2779	hypothetical protein	NA	NA	NA	NA	4.2	2.14E-10	5.1	0.00E+00
PA2819	hypothetical protein	NA	NA	5.5	5.95E-07	NA	NA	NA	NA
PA2827	conserved hypothetical protein	NA	NA	3.2	3.90E-09	NA	NA	NA	NA
PA2869	hypothetical protein	NA	NA	NA	NA	10.5	1.09E-08	NA	NA
PA2901	hypothetical protein	NA	NA	NA	NA	2.6	4.59E-09	NA	NA
PA3012	hypothetical protein	NA	NA	54.7	5.42E-11	NA	NA	NA	NA
PA3015	hypothetical protein	NA	NA	6.9	7.05E-06	NA	NA	NA	NA
PA3080	hypothetical protein	NA	NA	NA	NA	5.3	6.27E-06	37.4	1.82E-09
PA3110	hypothetical protein	NA	NA	2.8	3.97E-09	NA	NA	NA	NA
PA3229	hypothetical protein	NA	NA	NA	NA	76.8	0.00E+00	794.6	3.08E-13
PA3440	conserved hypothetical protein	NA	NA	NA	NA	2.5	6.26E-11	NA	NA
PA3572	hypothetical protein	NA	NA	NA	NA	2.1	3.56E-08	NA	NA
PA3580	conserved hypothetical protein	NA	NA	43.6	6.75E-09	NA	NA	NA	NA
PA3674	hypothetical protein	NA	NA	3.5	3.21E-07	NA	NA	NA	NA
PA3712	hypothetical protein	NA	NA	3.6	9.50E-11	NA	NA	NA	NA
PA3741	hypothetical protein	NA	NA	NA	NA	3.5	1.54E-05	64.3	6.69E-14
PA3850	hypothetical protein	NA	NA	NA	NA	2.9	6.39E-11	NA	NA
PA3869	hypothetical protein	NA	NA	NA	NA	34.2	5.91E-09	NA	NA
PA3906	hypothetical protein	NA	NA	NA	NA	2.4	2.61E-06	NA	NA
PA3962	hypothetical protein	NA	NA	NA	NA	3.4	3.42E-08	4.1	9.76E-08
PA3967	hypothetical protein	NA	NA	NA	NA	3.5	6.29E-06	34.8	6.97E-09
PA3988	hypothetical protein	NA	NA	NA	NA	2.0	3.75E-06	NA	NA

PA3998		conserved hypothetical protein	NA	NA	2.0	0.00E+00	NA	NA	NA	NA
PA4004		conserved hypothetical protein	NA	NA	NA	NA	4.2	6.38E-07	NA	NA
PA4045		conserved hypothetical protein	NA	NA	NA	NA	3.4	3.54E-07	4.1	4.86E-08
PA4049		hypothetical protein	NA	NA	4.1	1.08E-05	NA	NA	NA	NA
PA4111		hypothetical protein	NA	NA	5.5	3.62E-08	NA	NA	NA	NA
PA4204	<i>ppgL</i>	periplasmic gluconolactonase, PpgL	NA	NA	3.2	1.57E-06	NA	NA	NA	NA
PA4325		hypothetical protein	NA	NA	NA	NA	2.6	2.08E-07	NA	NA
PA4379		conserved hypothetical protein	NA	NA	NA	NA	2.0	1.67E-09	NA	NA
PA4405		hypothetical protein	NA	NA	NA	NA	5.3	6.71E-08	NA	NA
PA4438		conserved hypothetical protein	NA	NA	NA	NA	8.7	0.00E+00	2.7	3.97E-06
PA4458		conserved hypothetical protein	NA	NA	2.2	1.45E-07	NA	NA	NA	NA
PA4460		conserved hypothetical protein	NA	NA	NA	NA	2.2	1.74E-13	2.1	4.34E-08
PA4487		conserved hypothetical protein	NA	NA	2.9	6.16E-11	NA	NA	NA	NA
PA4488		conserved hypothetical protein	NA	NA	3.2	2.53E-07	NA	NA	NA	NA
PA4531		hypothetical protein	NA	NA	65.2	6.49E-13	NA	NA	NA	NA
PA4603		hypothetical protein	NA	NA	5.5	1.60E-08	NA	NA	NA	NA
PA4608		hypothetical protein	NA	NA	2.8	4.77E-07	NA	NA	NA	NA
PA4637		hypothetical protein	NA	NA	2.3	4.11E-06	NA	NA	NA	NA
PA4643		hypothetical protein	NA	NA	NA	NA	4.2	1.08E-06	NA	NA
PA4661	<i>pagL</i>	Lipid A 3-O-deacylase	NA	NA	NA	NA	2.1	0.00E+00	2.2	0.00E+00
PA4673		conserved hypothetical protein	NA	NA	NA	NA	2.4	1.17E-07	NA	NA
PA4684		hypothetical protein	NA	NA	NA	NA	3.5	1.82E-05	NA	NA
PA4691		hypothetical protein	NA	NA	33.7	4.45E-07	NA	NA	NA	NA
PA4692		conserved hypothetical protein	NA	NA	NA	NA	3.6	1.04E-05	NA	NA
PA4718		hypothetical protein	NA	NA	NA	NA	2.9	1.26E-05	NA	NA
PA4866		conserved hypothetical protein	NA	NA	26.4	9.58E-06	NA	NA	NA	NA
PA4881		hypothetical protein	NA	NA	NA	NA	192.1	2.82E-14	200.1	0.00E+00
PA5061		conserved hypothetical protein	NA	NA	2.0	0.00E+00	NA	NA	NA	NA
PA5137		hypothetical protein	NA	NA	NA	NA	7.4	4.76E-07	NA	NA

PA5184		hypothetical protein	NA	NA	NA	NA	4.2	6.16E-06	NA	NA
PA5209		hypothetical protein	NA	NA	NA	NA	4.0	1.85E-06	NA	NA
PA5222		hypothetical protein	NA	NA	2.2	8.02E-07	NA	NA	NA	NA
PA5257		hypothetical protein	NA	NA	2.1	9.47E-08	NA	NA	NA	NA
PA5340		hypothetical protein	NA	NA	NA	NA	3.4	9.35E-08	NA	NA
PA5406		hypothetical protein	NA	NA	NA	NA	2.9	1.06E-07	3.1	1.01E-05
PA5457		hypothetical protein	NA	NA	8.3	5.46E-07	NA	NA	NA	NA
PA5462		hypothetical protein	NA	NA	5.5	7.92E-11	NA	NA	NA	NA
PA5467		hypothetical protein	NA	NA	37.7	8.26E-08	NA	NA	NA	NA
PA5517		conserved hypothetical protein	NA	NA	6.9	7.51E-08	NA	NA	NA	NA
PA5532		hypothetical protein	NA	NA	27.0	7.20E-06	NA	NA	NA	NA
PA5537		hypothetical protein	NA	NA	32.8	6.40E-07	NA	NA	NA	NA
Membrane proteins										
PA0291	<i>oprE</i>	Anaerobically-induced outer membrane porin OprE precursor	NA	NA	NA	NA	2.0	4.13E-07	NA	NA
PA0303	<i>spuG</i>	polyamine transport protein PotH	NA	NA	NA	NA	3.2	6.85E-07	3.1	6.63E-10
PA0334		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	14.7	1.45E-05	NA	NA
PA0752		conserved hypothetical protein	NA	NA	11.1	5.64E-06	NA	NA	NA	NA
PA0789		probable amino acid permease	NA	NA	NA	NA	2.3	9.45E-08	NA	NA
PA1048		probable outer membrane protein precursor	NA	NA	2.6	0.00E+00	NA	NA	NA	NA
PA1073	<i>braD</i>	branched-chain amino acid transport protein BraD	NA	NA	NA	NA	2.7	8.22E-08	4.1	4.46E-07
PA1126		hypothetical protein	NA	NA	6.9	8.15E-08	NA	NA	NA	NA
PA1170		conserved hypothetical protein	NA	NA	NA	NA	7.4	3.21E-07	NA	NA
PA1577		hypothetical protein	NA	NA	NA	NA	25.6	6.69E-07	NA	NA
PA1767		hypothetical protein	NA	NA	2.3	1.49E-08	NA	NA	NA	NA
PA2114		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	2.3	7.75E-14	NA	NA

PA2241	<i>pslK</i>	PsIL	NA	NA	NA	NA	5.8	1.80E-05	NA	NA
PA2415		hypothetical protein	NA	NA	32.1	8.49E-07	NA	NA	NA	NA
PA2495	<i>oprN</i>	Multidrug efflux outer membrane protein OprN precursor	NA	NA	NA	NA	345.7	0.00E+00	496.8	3.36E-13
PA2710		hypothetical protein	NA	NA	5.5	1.24E-05	NA	NA	NA	NA
PA2811		probable permease of ABC-2 transporter	NA	NA	NA	NA	3.2	1.11E-07	2.5	7.47E-07
PA2868		hypothetical protein	NA	NA	NA	NA	8.4	9.75E-09	NA	NA
PA3041		hypothetical protein	NA	NA	8.3	0.00E+00	NA	NA	NA	NA
PA3145	<i>wbpL</i>	glycosyltransferase WbpL	NA	NA	2.4	1.66E-12	NA	NA	NA	NA
PA3602		conserved hypothetical protein	NA	NA	NA	NA	3.8	1.11E-08	NA	NA
PA3827		conserved hypothetical protein	NA	NA	2.3	4.77E-07	NA	NA	NA	NA
PA4338		hypothetical protein	NA	NA	5.1	8.11E-07	NA	NA	NA	NA
PA4355		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	8.4	1.07E-05	10.3	8.55E-11
PA4455		probable permease of ABC transporter	NA	NA	NA	NA	2.3	6.35E-10	NA	NA
PA4754		hypothetical protein	NA	NA	NA	NA	2.3	2.50E-07	2.8	1.21E-13
PA5155		amino acid (lysine/arginine/ornithine/histidine/octopine) ABC transporter membrane protein	NA	NA	NA	NA	3.5	1.57E-09	4.1	4.15E-09
PA5170	<i>arcD</i>	arginine/ornithine antiporter	NA	NA	2.1	8.49E-14	NA	NA	NA	NA
PA5230		probable permease of ABC transporter	NA	NA	2.4	8.24E-07	NA	NA	NA	NA
PA5231		probable ATP-binding/permease fusion ABC transporter	NA	NA	3.8	2.30E-11	NA	NA	NA	NA
PA5430		hypothetical protein	NA	NA	NA	NA	2.7	3.90E-06	NA	NA
PA5479	<i>glpP</i>	proton-glutamate symporter	NA	NA	NA	NA	2.3	8.05E-08	NA	NA
Motility & Attachment										
PA0171		hypothetical protein	NA	NA	NA	NA	4.2	1.32E-09	NA	NA

Non-coding RNA gene										
PA0887.1	<i>P7</i>	P7	NA	NA	NA	NA	6.3	0.00E+00	3.1	5.35E-14
PA1112.1			NA	NA	NA	NA	2.3	0.00E+00	NA	NA
PA3366.1	<i>amiL</i>	AmiL	NA	NA	NA	NA	2.5	1.82E-13	NA	NA
PA4406.1			NA	NA	2.5	0.00E+00	NA	NA	NA	NA
PA4541.1		tRNA-Lys	NA	NA	NA	NA	2.7	0.00E+00	NA	NA
Nucleotide biosynthesis and metabolism										
PA0401		noncatalytic dihydroorotase-like protein	NA	NA	2.6	6.88E-07	NA	NA	NA	NA
PA0945	<i>purM</i>	phosphoribosylaminoimidazole synthetase	NA	NA	NA	NA	2.0	7.03E-06	2.1	1.54E-06
PA1013	<i>purC</i>	phosphoribosylaminoimidazole-succinocarboxamide synthase	NA	NA	NA	NA	2.0	2.77E-06	NA	NA
PA2876	<i>pyrF</i>	orotidine 5'-phosphate decarboxylase	NA	NA	6.9	9.97E-07	NA	NA	NA	NA
PA2962	<i>tmk</i>	thymidylate kinase	NA	NA	NA	NA	3.4	1.11E-05	3.8	2.65E-12
PA4314	<i>purU1</i>	formyltetrahydrofolate deformylase	NA	NA	NA	NA	2.0	1.09E-06	NA	NA
PA4855	<i>purD</i>	phosphoribosylamine--glycine ligase	NA	NA	NA	NA	2.5	1.48E-05	NA	NA
PA5336	<i>gmk</i>	guanylate kinase	NA	NA	NA	NA	4.9	6.05E-07	NA	NA
Protein secretion/export apparatus										
PA0071	<i>tagR1</i>	TagR1	NA	NA	2.1	8.53E-09	NA	NA	NA	NA
PA0091	<i>vgrG1</i>	VgrG1	NA	NA	NA	NA	3.2	9.81E-06	NA	NA
PA3099	<i>xcpV</i>	general secretion pathway protein I	NA	NA	4.1	1.69E-05	NA	NA	NA	NA
PA3100	<i>xcpU</i>	General secretion pathway outer membrane protein H precursor	NA	NA	2.8	7.09E-07	NA	NA	NA	NA
PA4559	<i>lspA</i>	prolipoprotein signal peptidase	NA	NA	NA	NA	2.1	1.45E-05	NA	NA

PA5069	<i>tatB</i>	translocation protein TatB	NA	NA	NA	NA	2.6	2.32E-07	NA	NA
Putative enzymes										
PA0075	<i>pppA</i>	PppA	NA	NA	4.8	2.65E-07	NA	NA	NA	NA
PA0478		probable N-acetyltransferase	NA	NA	NA	NA	10.5	6.86E-09	28.7	1.71E-07
PA0597		probable nucleotidyl transferase	NA	NA	NA	NA	3.2	1.39E-05	3.1	2.62E-10
PA0766	<i>mucD</i>	serine protease MucD precursor	NA	NA	2.3	0.00E+00	NA	NA	NA	NA
PA0838		probable glutathione peroxidase	NA	NA	3.7	7.88E-10	NA	NA	NA	NA
PA0954		probable acylphosphatase	NA	NA	4.1	2.30E-07	NA	NA	NA	NA
PA1137		probable oxidoreductase	NA	NA	34.7	3.00E-07	NA	NA	NA	NA
PA1287		probable glutathione peroxidase	NA	NA	4.1	2.48E-07	NA	NA	NA	NA
PA1296		probable 2-hydroxyacid dehydrogenase	NA	NA	2.4	1.18E-05	NA	NA	NA	NA
PA1306		probable HIT family protein	NA	NA	NA	NA	2.9	8.81E-06	4.1	4.46E-07
PA1344		probable short-chain dehydrogenase	NA	NA	2.8	9.38E-07	NA	NA	NA	NA
PA1621		probable hydrolase	NA	NA	6.9	6.47E-06	NA	NA	NA	NA
PA1655		probable glutathione S-transferase	NA	NA	3.8	4.87E-11	NA	NA	NA	NA
PA1742		probable amidotransferase	NA	NA	NA	NA	2.5	9.88E-12	NA	NA
PA1821		probable enoyl-CoA hydratase/isomerase	NA	NA	NA	NA	2.7	2.61E-10	NA	NA
PA1984	<i>exaC</i>	NAD ⁺ dependent aldehyde dehydrogenase ExaC	NA	NA	NA	NA	7.7	8.72E-06	5.1	3.45E-09
PA2491		probable oxidoreductase	NA	NA	NA	NA	8.7	2.51E-13	7.4	0.00E+00
PA2553		probable acyl-CoA thiolase	NA	NA	NA	NA	2.1	2.38E-07	NA	NA
PA2554		probable short-chain dehydrogenase	NA	NA	NA	NA	2.8	4.57E-12	3.9	6.14E-14
PA2579	<i>kynA</i>	L-Tryptophan:oxygen 2,3-oxidoreductase (decyclizing) KynA	NA	NA	NA	NA	2.9	5.95E-14	NA	NA
PA3053		probable hydrolytic enzyme	NA	NA	33.9	4.04E-07	NA	NA	NA	NA
PA3106		probable short-chain dehydrogenase	NA	NA	4.8	5.49E-07	NA	NA	NA	NA
PA3460		probable acetyltransferase	NA	NA	6.5	3.58E-07	NA	NA	NA	NA
PA4022		probable aldehyde dehydrogenase	NA	NA	NA	NA	2.7	0.00E+00	NA	NA

PA4576		probable ATP-dependent protease	NA	NA	NA	NA	2.3	2.40E-06	NA	NA
PA5432		probable acetyltransferase	NA	NA	NA	NA	19.5	1.87E-05	NA	NA
Secreted Factors (toxins, enzymes, alginate)										
PA1844	<i>tseI</i>	TseI	NA	NA	29.4	2.66E-06	NA	NA	NA	NA
Transcription, RNA processing and degradation										
PA2626	<i>trmU</i>	tRNA methyltransferase	NA	NA	NA	NA	3.1	1.79E-10	NA	NA
PA3861	<i>rhl</i>	ATP-dependent RNA helicase RhlB	NA	NA	NA	NA	2.0	1.77E-05	NA	NA
PA4238	<i>rpoA</i>	DNA-directed RNA polymerase alpha chain	NA	NA	NA	NA	2.0	2.82E-14	NA	NA
PA4262	<i>rplD</i>	50S ribosomal protein L4	NA	NA	NA	NA	2.2	0.00E+00	NA	NA
Transcriptional regulators										
PA0233		probable transcriptional regulator	NA	NA	29.1	3.11E-06	NA	NA	NA	NA
PA0448		probable transcriptional regulator	NA	NA	3.3	2.81E-07	NA	NA	NA	NA
PA0601		probable two-component response regulator	NA	NA	5.5	1.68E-05	NA	NA	NA	NA
PA0764	<i>mucB</i>	negative regulator for alginate biosynthesis MucB	NA	NA	2.3	0.00E+00	NA	NA	NA	NA
PA1015		probable transcriptional regulator	NA	NA	NA	NA	21.9	5.20E-06	NA	NA
PA1850		probable transcriptional regulator	NA	NA	NA	NA	21.8	5.43E-06	NA	NA
PA2320	<i>gntR</i>	transcriptional regulator GntR	NA	NA	26.5	8.99E-06	NA	NA	NA	NA
PA3133		probable transcriptional regulator	NA	NA	50.4	3.53E-10	NA	NA	NA	NA
PA4174		probable transcriptional regulator	NA	NA	29.6	2.46E-06	NA	NA	NA	NA
PA5483	<i>algB</i>	two-component response regulator AlgB	NA	NA	3.1	6.72E-07	NA	NA	NA	NA
Translation, post-translational modification, degradation										
PA0579	<i>rpsU</i>	30S ribosomal protein S21	NA	NA	NA	NA	2.1	0.00E+00	NA	NA
PA1796	<i>folD</i>	5,10-methylene-tetrahydrofolate dehydrogenase / cyclohydrolase	NA	NA	NA	NA	2.4	9.36E-06	NA	NA
PA2740	<i>pheS</i>	phenylalanyl-tRNA synthetase, alpha-subunit	NA	NA	NA	NA	2.2	5.58E-06	NA	NA

PA2742	<i>rpmI</i>	50S ribosomal protein L35	NA	NA	NA	NA	2.0	2.93E-13	NA	NA
PA3655	<i>tsf</i>	elongation factor Ts	NA	NA	NA	NA	2.0	0.00E+00	NA	NA
PA3656	<i>rpsB</i>	30S ribosomal protein S2	NA	NA	NA	NA	2.2	0.00E+00	NA	NA
PA3818		extragenic suppressor protein SuhB	NA	NA	NA	NA	2.3	1.16E-06	NA	NA
PA4237	<i>rplQ</i>	50S ribosomal protein L17	NA	NA	NA	NA	3.3	6.55E-13	NA	NA
PA4244	<i>rplO</i>	50S ribosomal protein L15	NA	NA	NA	NA	2.3	5.02E-14	NA	NA
PA4245	<i>rpmD</i>	50S ribosomal protein L30	NA	NA	NA	NA	2.0	1.99E-13	NA	NA
PA4246	<i>rpsE</i>	30S ribosomal protein S5	NA	NA	NA	NA	2.0	1.86E-13	NA	NA
PA4248	<i>rplF</i>	50S ribosomal protein L6	NA	NA	NA	NA	2.5	2.48E-13	NA	NA
PA4250	<i>rpsN</i>	30S ribosomal protein S14	NA	NA	NA	NA	2.3	4.15E-13	NA	NA
PA4256	<i>rplP</i>	50S ribosomal protein L16	NA	NA	NA	NA	2.0	3.49E-13	NA	NA
PA4257	<i>rpsC</i>	30S ribosomal protein S3	NA	NA	NA	NA	2.2	4.21E-13	NA	NA
PA4258	<i>rplV</i>	50S ribosomal protein L22	NA	NA	NA	NA	2.2	8.86E-14	NA	NA
PA4260	<i>rplB</i>	50S ribosomal protein L2	NA	NA	NA	NA	2.0	2.77E-13	NA	NA
PA4267	<i>rpsG</i>	30S ribosomal protein S7	NA	NA	NA	NA	2.1	0.00E+00	NA	NA
PA4268	<i>rpsL</i>	30S ribosomal protein S12	NA	NA	NA	NA	2.0	3.30E-13	NA	NA
PA4272	<i>rplJ</i>	50S ribosomal protein L10	NA	NA	NA	NA	2.1	4.21E-13	NA	NA
PA4273	<i>rplA</i>	50S ribosomal protein L1	NA	NA	NA	NA	2.1	3.57E-13	NA	NA
PA4558		probable peptidyl-prolyl cis-trans isomerase, FkbP-type	NA	NA	NA	NA	2.1	1.80E-05	NA	NA
PA4567	<i>rpmA</i>	50S ribosomal protein L27	NA	NA	NA	NA	2.0	4.01E-14	NA	NA
PA4665	<i>prfA</i>	peptide chain release factor 1	NA	NA	NA	NA	2.7	6.05E-07	NA	NA
PA4743	<i>rbfA</i>	ribosome-binding factor A	NA	NA	NA	NA	3.0	2.94E-12	NA	NA
PA4932	<i>rplI</i>	50S ribosomal protein L9	NA	NA	NA	NA	2.3	4.08E-13	NA	NA
PA5254		probable peptidyl-prolyl cis-trans isomerase, FkbP-type	NA	NA	NA	NA	4.2	7.23E-07	NA	NA
Transport of small molecules										
PA0866	<i>aroP2</i>	aromatic amino acid transport protein AroP2	NA	NA	NA	NA	2.6	1.47E-11	2.7	3.52E-14

PA1071	<i>braF</i>	branched-chain amino acid transport protein BraF	NA	NA	NA	NA	3.0	1.38E-06	NA	NA
PA1074	<i>braC</i>	branched-chain amino acid transport protein BraC	NA	NA	NA	NA	2.2	8.45E-14	NA	NA
PA1862	<i>modB</i>	molybdenum transport protein ModB	NA	NA	NA	NA	6.3	2.88E-06	39.8	5.06E-10
PA2041		probable amino acid permease	NA	NA	NA	NA	7.0	1.27E-05	5.1	4.72E-07
PA2113	<i>opdO</i>	pyroglutamate porin OpdO	NA	NA	NA	NA	2.4	1.50E-14	2.2	0.00E+00
PA2408		probable ATP-binding component of ABC transporter	NA	NA	8.3	7.48E-08	NA	NA	NA	NA
PA2493	<i>mexE</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexE precursor	NA	NA	NA	NA	298.8	0.00E+00	113.0	2.70E-13
PA2494	<i>mexF</i>	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexF	NA	NA	NA	NA	578.6	1.82E-13	547.2	0.00E+00
PA2760	<i>oprQ</i>	OprQ	NA	NA	NA	NA	2.7	3.26E-13	NA	NA
PA2812		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	5.4	2.82E-14	4.4	1.95E-14
PA3187		probable ATP-binding component of ABC transporter	NA	NA	6.9	1.79E-11	NA	NA	NA	NA
PA3531	<i>bfrB</i>	bacterioferritin	NA	NA	NA	NA	2.1	5.09E-09	NA	NA
PA3838		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	5.9	1.89E-11	77.5	4.11E-14
PA4064		probable ATP-binding component of ABC transporter	NA	NA	29.1	3.04E-06	NA	NA	NA	NA
PA4466		probable phosphoryl carrier protein	NA	NA	2.1	4.31E-06	4.2	8.63E-08	3.1	1.60E-12
PA4500		probable binding protein component of ABC transporter	NA	NA	NA	NA	2.0	8.61E-06	NA	NA
PA4514		probable outer membrane receptor for iron transport	NA	NA	5.5	4.82E-06	NA	NA	NA	NA
PA4590	<i>pra</i>	protein activator	NA	NA	2.8	1.17E-05	NA	NA	NA	NA

PA5076		probable binding protein component of ABC transporter	NA	NA	NA	NA	2.5	2.42E-09	NA	NA
PA5152		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	3.1	3.78E-12	2.5	8.06E-13
PA5217		probable binding protein component of ABC iron transporter	NA	NA	2.7	2.60E-09	NA	NA	NA	NA

Supplementary Table 3: AmpR- β -lactam-dependent genes. The genes that are either positively or negatively regulated by AmpR when exposed to β -lactam stress are classified based on information in Supplementary Table 1. Only genes that are ≥ 2.0 , Bonferroni correction (<0.05) is listed. Functional categorization, gene ID and gene/product names are from the *Pseudomonas* Genome database (27). Lack of expression of a gene under any of the four conditions is denoted as NA.

Gene ID	Gene name	Product Name	Condition 1		Condition 2		Condition 3		Condition 4	
			FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value
Genes positively regulated by AmpR upon b-lactam exposure (236)										
Adaptation, Protection										
PA0176	<i>aer2</i>	aerotaxis transducer Aer2	NA	NA	NA	NA	NA	NA	-5.2	4.53E-07
PA0177		probable purine-binding chemotaxis protein	NA	NA	NA	NA	NA	NA	-73.9	4.27E-14
PA0848		probable alkyl hydroperoxide reductase	NA	NA	NA	NA	NA	NA	-4.9	3.48E-07
PA1561	<i>aer</i>	aerotaxis receptor Aer	NA	NA	NA	NA	NA	NA	-2.8	2.21E-07
PA2400	<i>pvdJ</i>	PvdJ	NA	NA	NA	NA	NA	NA	-4.4	8.65E-06
PA2703	<i>tsi2</i>	Tsi2	NA	NA	NA	NA	NA	NA	-30.7	5.57E-07
PA2826		probable glutathione peroxidase	4.3	6.54E-09	NA	NA	NA	NA	-2.9	1.13E-06
PA4110	<i>ampC</i>	beta-lactamase precursor	14.2	1.11E-13	NA	NA	NA	NA	-24.3	1.50E-14
PA4915		probable chemotaxis transducer	NA	NA	NA	NA	NA	NA	-12.7	7.54E-10
Amino acid biosynthesis and metabolism										
PA0407	<i>gshB</i>	glutathione synthetase	NA	NA	NA	NA	NA	NA	-4.4	1.72E-07
PA0546	<i>metK</i>	methionine adenosyltransferase	NA	NA	NA	NA	NA	NA	-2.2	1.95E-13
PA3537	<i>argF</i>	ornithine carbamoyltransferase, anabolic	3.8	5.55E-06	NA	NA	NA	NA	-3.9	1.92E-06
PA4519	<i>speC</i>	ornithine decarboxylase	NA	NA	NA	NA	NA	NA	-2.9	4.17E-08
Biosynthesis of cofactors, prosthetic groups and carriers										
PA0672	<i>hemO</i>	heme oxygenase	NA	NA	NA	NA	NA	NA	-3.4	1.93E-07
PA1049	<i>pdxH</i>	pyridoxine 5'-phosphate oxidase	NA	NA	NA	NA	NA	NA	-2.6	8.07E-06

PA3397	<i>fpr</i>	ferredoxin--NADP+ reductase	NA	NA	NA	NA	NA	NA	-2.8	0.00E+00
Carbon compound catabolism										
PA2509	<i>catB</i>	muconate cycloisomerase I	NA	NA	NA	NA	NA	NA	-44.8	9.76E-10
PA5350	<i>rubA2</i>	Rubredoxin 2	2.1	1.44E-05	NA	NA	NA	NA	-2.9	3.28E-09
PA5351	<i>rubA1</i>	Rubredoxin 1	2.5	0.00E+00	NA	NA	NA	NA	-2.3	6.60E-14
Cell division										
PA1462		probable plasmid partitioning protein	2.1	2.17E-07	NA	NA	NA	NA	-3.4	0.00E+00
Cell wall / LPS / capsule										
PA1077	<i>flgB</i>	flagellar basal-body rod protein FlgB	NA	NA	NA	NA	NA	NA	-88.0	2.36E-14
PA1079	<i>flgD</i>	flagellar basal-body rod modification protein FlgD	NA	NA	NA	NA	NA	NA	-2.9	8.59E-07
PA1101	<i>fliF</i>	Flagella M-ring outer membrane protein precursor	NA	NA	NA	NA	NA	NA	-2.7	2.92E-09
PA1130	<i>rhlC</i>	rhamnosyltransferase 2	NA	NA	NA	NA	NA	NA	-36.7	3.72E-08
Central intermediary metabolism										
PA0710	<i>gloA2</i>	lactoylglutathione lyase	NA	NA	NA	NA	NA	NA	-36.3	4.53E-08
PA4443	<i>cysD</i>	ATP sulfurylase small subunit	NA	NA	NA	NA	NA	NA	-2.6	2.74E-09
PA4865	<i>ureA</i>	urease gamma subunit	5.7	4.49E-06	NA	NA	NA	NA	-47.4	3.02E-10
PA4868	<i>ureC</i>	urease alpha subunit	NA	NA	NA	NA	NA	NA	-33.8	1.40E-07
Chemotaxis, Adaptation, Protection										
PA0178		probable two-component sensor	NA	NA	NA	NA	NA	NA	-5.2	1.88E-07
DNA replication, recombination, modification and repair										
PA4042	<i>xseB</i>	exodeoxyribonuclease VII small subunit	2.8	3.91E-06	NA	NA	NA	NA	-2.9	1.13E-06
Energy metabolism										
PA1317	<i>cyoA</i>	cytochrome o ubiquinol oxidase subunit II	NA	NA	NA	NA	NA	NA	-43.3	1.96E-09
PA1318	<i>cyoB</i>	cytochrome o ubiquinol oxidase subunit I	NA	NA	NA	NA	NA	NA	-5.8	4.86E-06

PA1319	<i>cyoC</i>	cytochrome o ubiquinol oxidase subunit III	NA	NA	NA	NA	NA	NA	-34.2	1.15E-07
PA1883		probable NADH-ubiquinone/plastoquinone oxidoreductase	NA	NA	NA	NA	NA	NA	-36.0	5.12E-08
PA1983	<i>exaB</i>	cytochrome c550	32.8	4.68E-07	NA	NA	NA	NA	-32.8	2.16E-07
PA2646	<i>nuoK</i>	NADH dehydrogenase I chain K	2.4	4.37E-06	-3.6	1.65E-07	NA	NA	-4.9	4.71E-13
PA2998	<i>nqrB</i>	Na ⁺ -translocating NADH:ubiquinone oxidoreductase subunit Nrq2	NA	NA	NA	NA	NA	NA	-2.4	1.05E-05
Fatty acid and phospholipid metabolism										
PA2856	<i>tesA</i>	acyl-CoA thioesterase I precursor	NA	NA	NA	NA	NA	NA	-2.4	8.32E-07
PA3223	<i>azoR3</i>	AzoR3, azoreductase 3	NA	NA	NA	NA	NA	NA	-5.8	9.35E-09
Hypothetical, unclassified, unknown										
PA0015		hypothetical protein	6.6	1.37E-13	NA	NA	NA	NA	-3.4	3.93E-13
PA0028		hypothetical protein	NA	NA	NA	NA	NA	NA	-47.6	2.73E-10
PA0052		hypothetical protein	NA	NA	NA	NA	NA	NA	-33.5	1.59E-07
PA0055		hypothetical protein	NA	NA	NA	NA	NA	NA	-4.9	2.57E-08
PA0065		hypothetical protein	5.7	2.08E-10	NA	NA	NA	NA	-2.6	1.08E-05
PA0115		conserved hypothetical protein	7.1	1.10E-10	NA	NA	NA	NA	-4.9	3.38E-09
PA0144		hypothetical protein	2.8	1.28E-05	NA	NA	NA	NA	-6.8	5.10E-11
PA0187		hypothetical protein	35.2	1.64E-07	NA	NA	NA	NA	-35.2	7.32E-08
PA0255		conserved hypothetical protein	5.1	1.24E-10	NA	NA	NA	NA	-2.9	5.10E-07
PA0271		hypothetical protein	NA	NA	NA	NA	NA	NA	-25.2	6.47E-06
PA0346		hypothetical protein	NA	NA	NA	NA	NA	NA	-39.6	1.04E-08
PA0384		hypothetical protein	27.8	4.08E-06	NA	NA	NA	NA	-27.8	1.99E-06
PA0419		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-5.8	6.94E-08
PA0431		hypothetical protein	NA	NA	NA	NA	NA	NA	-8.8	0.00E+00
PA0481		hypothetical protein	7.1	7.01E-11	NA	NA	NA	NA	-80.9	5.39E-15

PA0484	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-69.6	2.18E-14
PA0486	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-36.8	3.55E-08
PA0526	hypothetical protein	36.8	8.06E-08	NA	NA	NA	NA	-36.8	3.55E-08
PA0563	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.2	2.73E-15
PA0581	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-5.8	9.72E-10
PA0588	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.4	3.27E-12
PA0664	hypothetical protein	2.1	2.02E-07	NA	NA	NA	NA	-2.1	3.21E-08
PA0734	hypothetical protein	4.3	5.13E-08	NA	NA	NA	NA	-2.9	4.72E-06
PA0823	hypothetical protein	30.7	1.18E-06	NA	NA	NA	NA	-30.7	5.57E-07
PA0874	hypothetical protein	NA	NA	NA	NA	NA	NA	-26.9	3.04E-06
PA0938	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.2	0.00E+00
PA0980	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.9	7.65E-06
PA1036	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.6	1.47E-06
PA1040	hypothetical protein	NA	NA	NA	NA	NA	NA	-28.8	1.28E-06
PA1043	hypothetical protein	NA	NA	NA	NA	NA	NA	-3.4	9.38E-06
PA1117	hypothetical protein	14.2	1.62E-13	NA	NA	NA	NA	-4.9	6.65E-11
PA1133	hypothetical protein	40.9	1.30E-08	NA	NA	NA	NA	-40.9	5.61E-09
PA1154	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-26.9	3.04E-06
PA1198	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.4	0.00E+00
PA1215	hypothetical protein	NA	NA	NA	NA	NA	NA	-27.9	1.95E-06
PA1244	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.8	0.00E+00
PA1358	hypothetical protein	72.0	0.00E+00	NA	NA	NA	NA	-3.9	2.93E-07
PA1369	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.3	8.58E-07
PA1394	hypothetical protein	4.3	1.57E-08	- 34.4	6.00E-09	NA	NA	-84.5	4.11E-14
PA1440	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.1	1.05E-07
PA1531	hypothetical protein	NA	NA	NA	NA	NA	NA	-41.4	4.50E-09
PA1545	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.3	2.06E-09
PA1550	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.9	1.16E-08

PA1575	hypothetical protein	NA	NA	NA	NA	NA	NA	-26.0	4.49E-06
PA1728	hypothetical protein	3.2	9.16E-13	NA	NA	NA	NA	-2.6	3.96E-10
PA1745	hypothetical protein	NA	NA	NA	NA	NA	NA	-29.4	1.00E-06
PA1874	hypothetical protein	NA	NA	NA	NA	NA	NA	-12.7	7.98E-07
PA1967	hypothetical protein	NA	NA	NA	NA	NA	NA	-57.3	3.39E-12
PA2036	hypothetical protein	8.5	2.50E-06	NA	NA	NA	NA	-41.3	4.80E-09
PA2045	conserved hypothetical protein	55.0	2.39E-11	NA	NA	NA	NA	-55.0	9.82E-12
PA2198	hypothetical protein	8.5	3.67E-09	NA	NA	NA	NA	-62.5	3.43E-13
PA2288	hypothetical protein	2.2	1.42E-13	-3.4	6.77E-14	NA	NA	-5.5	0.00E+00
PA2365	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-4.9	8.50E-08
PA2372	hypothetical protein	NA	NA	NA	NA	NA	NA	-3.4	1.03E-07
PA2406	hypothetical protein	NA	NA	NA	NA	NA	NA	-25.6	5.40E-06
PA2410	hypothetical protein	2.8	1.67E-05	NA	NA	NA	NA	-3.2	1.12E-06
PA2423	hypothetical protein	NA	NA	NA	NA	NA	NA	-3.2	1.48E-07
PA2434	hypothetical protein	NA	NA	NA	NA	NA	NA	-26.3	3.95E-06
PA2487	hypothetical protein	26.3	8.04E-06	NA	NA	NA	NA	-26.3	3.95E-06
PA2496	hypothetical protein	NA	NA	NA	NA	NA	NA	-27.8	1.99E-06
PA2504	hypothetical protein	NA	NA	NA	NA	NA	NA	-81.4	0.00E+00
PA2564	hypothetical protein	NA	NA	NA	NA	NA	NA	-60.7	7.49E-13
PA2565	hypothetical protein	NA	NA	NA	NA	NA	NA	-33.7	1.43E-07
PA2566	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-42.3	2.99E-09
PA2706	hypothetical protein	4.3	8.85E-06	-	4.37E-06	NA	NA	-54.4	1.30E-11
PA2721	hypothetical protein	NA	NA	NA	NA	NA	NA	-44.9	9.43E-10
PA3198	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-3.4	4.14E-06
PA3288	hypothetical protein	NA	NA	NA	NA	NA	NA	-4.9	2.32E-08
PA3325	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-32.4	2.64E-07
PA3335	hypothetical protein	NA	NA	NA	NA	NA	NA	-76.3	2.18E-14
PA3390	hypothetical protein	5.7	4.49E-06	NA	NA	NA	NA	-47.4	3.02E-10

PA3446	conserved hypothetical protein	2.6	2.03E-06	NA	NA	NA	NA	-4.4	1.99E-11
PA3520	hypothetical protein	73.7	0.00E+00	NA	NA	NA	NA	-73.7	0.00E+00
PA3623	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.3	0.00E+00
PA3684	hypothetical protein	2.6	2.08E-10	NA	NA	NA	NA	-4.4	0.00E+00
PA3698	hypothetical protein	5.1	4.23E-13	NA	NA	NA	NA	-2.9	1.73E-08
PA3752	hypothetical protein	NA	NA	NA	NA	NA	NA	-36.8	3.55E-08
PA3755	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.1	1.67E-06
PA3793	hypothetical protein	2.0	1.28E-05	-6.5	9.93E-14	NA	NA	-6.8	2.58E-14
PA3797	conserved hypothetical protein	2.6	3.75E-07	NA	NA	NA	NA	-2.5	3.94E-07
PA3808	conserved hypothetical protein	2.5	2.72E-13	NA	NA	NA	NA	-2.3	7.31E-12
PA3905	hypothetical protein	2.5	3.24E-10	NA	NA	NA	NA	-13.6	0.00E+00
PA3911	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-27.8	1.99E-06
PA3928	hypothetical protein	84.0	5.05E-14	NA	NA	NA	NA	-84.0	5.24E-14
PA4009	hypothetical protein	NA	NA	NA	NA	NA	NA	-33.5	1.59E-07
PA4027	hypothetical protein	NA	NA	NA	NA	NA	NA	-24.1	1.05E-05
PA4059	hypothetical protein	NA	NA	NA	NA	NA	NA	-38.0	2.11E-08
PA4093	hypothetical protein	NA	NA	NA	NA	NA	NA	-34.4	1.03E-07
PA4183	hypothetical protein	NA	NA	NA	NA	NA	NA	-30.7	5.57E-07
PA4200	hypothetical protein	NA	NA	NA	NA	NA	NA	-24.9	7.55E-06
PA4327	hypothetical protein	NA	NA	NA	NA	NA	NA	-35.6	6.13E-08
PA4337	hypothetical protein	8.5	1.63E-05	NA	NA	NA	NA	-35.2	7.32E-08
PA4349	hypothetical protein	NA	NA	NA	NA	NA	NA	-40.0	8.38E-09
PA4352	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.3	3.69E-14
PA4357	conserved hypothetical protein	29.6	1.94E-06	NA	NA	NA	NA	-29.6	9.24E-07
PA4362	hypothetical protein	NA	NA	NA	NA	NA	NA	-3.9	1.00E-05
PA4364	hypothetical protein	35.7	1.30E-07	NA	NA	NA	NA	-35.7	5.77E-08
PA4530	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-71.5	0.00E+00
PA4533	hypothetical protein	NA	NA	NA	NA	NA	NA	-4.9	6.98E-09
PA4535	hypothetical protein	2.5	9.73E-11	NA	NA	NA	NA	-4.1	0.00E+00

PA4582	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.3	2.62E-11
PA4583	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-3.8	3.69E-14
PA4610	hypothetical protein	17.0	0.00E+00	NA	NA	NA	NA	-5.8	1.82E-12
PA4635	conserved hypothetical protein	2.8	6.39E-07	- 37.3	1.21E-09	NA	NA	-101.9	2.36E-14
PA4648	hypothetical protein	NA	NA	NA	NA	NA	NA	-26.2	4.21E-06
PA4650	hypothetical protein	NA	NA	NA	NA	NA	NA	-26.5	3.71E-06
PA4677	hypothetical protein	NA	NA	NA	NA	NA	NA	-5.4	5.23E-08
PA4679	hypothetical protein	2.4	1.88E-05	NA	NA	NA	NA	-2.4	5.13E-06
PA4714	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-5.8	4.40E-12
PA4736	hypothetical protein	2.5	1.02E-10	NA	NA	NA	NA	-3.9	0.00E+00
PA4790	conserved hypothetical protein	67.0	2.68E-14	NA	NA	NA	NA	-6.8	2.25E-09
PA4830	hypothetical protein	NA	NA	NA	NA	NA	NA	-53.2	2.26E-11
PA4840	conserved hypothetical protein	2.2	1.83E-09	-2.2	1.19E-05	NA	NA	-3.6	0.00E+00
PA4916	hypothetical protein	NA	NA	NA	NA	NA	NA	-3.9	8.92E-12
PA5026	hypothetical protein	NA	NA	NA	NA	NA	NA	-31.7	3.54E-07
PA5101	hypothetical protein	NA	NA	NA	NA	NA	NA	-27.0	2.91E-06
PA5123	hypothetical protein	NA	NA	NA	NA	NA	NA	-37.6	2.53E-08
PA5185	conserved hypothetical protein	3.5	4.36E-07	NA	NA	NA	NA	-4.9	2.31E-09
PA5286	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.1	2.92E-10
PA5333	conserved hypothetical protein	2.8	6.42E-09	NA	NA	NA	NA	-2.3	7.31E-07
PA5339	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.1	3.47E-10
PA5405	hypothetical protein	30.7	1.18E-06	NA	NA	NA	NA	-30.7	5.57E-07
PA5423	hypothetical protein	NA	NA	NA	NA	NA	NA	-2.2	9.04E-06
Membrane proteins									
PA0007	hypothetical protein	NA	NA	NA	NA	NA	NA	-25.2	6.36E-06
PA1118	hypothetical protein	3.1	1.26E-08	NA	NA	NA	NA	-5.4	1.09E-13
PA1131	probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	NA	NA	-62.3	3.57E-13

PA1297		probable metal transporter	8.5	1.12E-06	NA	NA	NA	NA	-43.9	1.46E-09
PA1541		probable drug efflux transporter	NA	NA	NA	NA	NA	NA	-58.4	2.11E-12
PA1547		hypothetical protein	4.3	1.46E-06	NA	NA	NA	NA	-5.8	2.81E-08
PA1650		probable transporter	NA	NA	NA	NA	NA	NA	-24.1	1.05E-05
PA1825		hypothetical protein	NA	NA	NA	NA	NA	NA	-5.8	1.02E-08
PA2306	<i>ambA</i>	AmbA	NA	NA	NA	NA	NA	NA	-4.4	5.03E-11
PA2327		probable permease of ABC transporter	NA	NA	NA	NA	NA	NA	-28.2	1.73E-06
PA2409		probable permease of ABC transporter	NA	NA	NA	NA	NA	NA	-8.8	7.14E-11
PA2501		hypothetical protein	3.5	1.11E-13	NA	NA	NA	NA	-4.9	5.59E-14
PA2663	<i>ppyR</i>	psl and pyoverdine operon regulator, PpyR	NA	NA	NA	NA	NA	NA	-27.8	1.99E-06
PA3235		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.9	7.64E-10
PA3370		hypothetical protein	48.9	3.87E-10	NA	NA	NA	NA	-48.9	1.59E-10
PA3432		hypothetical protein	NA	NA	NA	NA	NA	NA	-36.8	3.55E-08
PA3789		hypothetical protein	NA	NA	NA	NA	NA	NA	-30.4	6.24E-07
PA3897		hypothetical protein	23.9	2.37E-05	NA	NA	NA	NA	-23.9	1.17E-05
PA3920		probable metal transporting P-type ATPase	4.8	1.92E-07	NA	NA	NA	NA	-10.7	8.43E-11
PA3966		hypothetical protein	NA	NA	NA	NA	NA	NA	-2.4	2.22E-09
PA4046		hypothetical protein	NA	NA	NA	NA	NA	NA	-3.9	6.00E-07
PA4507		hypothetical protein	NA	NA	NA	NA	NA	NA	-34.0	1.24E-07
PA5132		hypothetical protein	11.3	6.08E-06	-21.4	6.46E-06	NA	NA	-35.1	7.75E-08
Motility & Attachment										
PA1461	<i>motD</i>	MotD	2.4	2.67E-06	NA	NA	NA	NA	-2.4	6.34E-07
PA3361	<i>lecB</i>	fucose-binding lectin PA-IIL	NA	NA	NA	NA	NA	NA	-4.9	1.13E-11
PA4086	<i>cupB1</i>	probable fimbrial subunit CupB1	25.2	1.31E-05	NA	NA	NA	NA	-25.2	6.47E-06
PA4305	<i>rcpC</i>	RcpC	NA	NA	NA	NA	NA	NA	-8.8	8.24E-11

PA4551	<i>pilV</i>	type 4 fimbrial biogenesis protein PilV	NA	NA	NA	NA	NA	NA	-25.7	5.09E-06
PA4555	<i>pilY2</i>	type 4 fimbrial biogenesis protein PilY2	NA	NA	NA	NA	NA	NA	-61.9	4.31E-13
Non-coding RNA gene										
PA2819.3		tRNA-Glu	2.8	1.77E-06	-57.6	0.00E+00	NA	NA	-94.5	0.00E+00
Nucleotide biosynthesis and metabolism										
PA0143	<i>nuh</i>	purine nucleosidase Nuh	NA	NA	NA	NA	NA	NA	-2.2	2.19E-06
PA0849	<i>trxB2</i>	thioredoxin reductase 2	NA	NA	NA	NA	NA	NA	-4.2	4.39E-14
Protein secretion/export apparatus										
PA1692		probable translocation protein in type III secretion	NA	NA	NA	NA	NA	NA	-26.9	3.04E-06
PA1705	<i>pcrG</i>	regulator in type III secretion	24.2	2.05E-05	NA	NA	NA	NA	-24.2	1.01E-05
PA1713	<i>exsA</i>	transcriptional regulator ExsA	NA	NA	NA	NA	NA	NA	-5.8	6.00E-07
PA1875		probable outer membrane protein precursor	NA	NA	NA	NA	NA	NA	-28.1	1.78E-06
PA3095	<i>xcpZ</i>	general secretion pathway protein M	NA	NA	NA	NA	NA	NA	-6.8	5.40E-13
PA3098	<i>xcpW</i>	general secretion pathway protein J	NA	NA	NA	NA	NA	NA	-7.8	1.18E-11
Putative enzymes										
PA0534		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-2.9	2.05E-07
PA0817		probable ring-cleaving dioxygenase	3.3	2.41E-09	NA	NA	NA	NA	-2.3	4.01E-06
PA1914		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-29.3	1.05E-06
PA2024		probable ring-cleaving dioxygenase	11.3	8.90E-11	-20.7	9.31E-06	NA	NA	-67.9	5.04E-14
PA2271		probable acetyltransferase	8.5	2.17E-06	NA	NA	NA	NA	-41.8	3.86E-09
PA2379		probable oxidoreductase	NA	NA	NA	NA	NA	NA	-5.8	1.41E-11
PA3295		probable HIT family protein	2.8	1.04E-06	NA	NA	NA	NA	-2.9	2.70E-07
PA4026		probable acetyltransferase	NA	NA	NA	NA	NA	NA	-5.8	8.72E-12

Transcription, RNA processing and degradation										
PA5197	<i>rimK</i>	ribosomal protein S6 modification protein	NA	NA	NA	NA	NA	NA	-3.9	1.63E-06
Transcriptional regulators										
PA0175		probable chemotaxis protein methyltransferase	NA	NA	NA	NA	NA	NA	-25.6	5.51E-06
PA0547		probable transcriptional regulator	2.0	4.43E-12	NA	NA	NA	NA	-2.7	1.75E-14
PA1109		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-27.6	2.19E-06
PA1359		probable transcriptional regulator	8.5	1.40E-09	NA	NA	NA	NA	-5.8	1.41E-08
PA1455	<i>fliA</i>	sigma factor FlhA	NA	NA	NA	NA	NA	NA	-2.9	2.85E-10
PA2809	<i>copR</i>	two-component response regulator, CopR	NA	NA	NA	NA	NA	NA	-31.6	3.64E-07
PA2877		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-24.1	1.05E-05
PA3034		probable transcriptional regulator	6.4	0.00E+00	NA	NA	NA	NA	-4.4	4.09E-12
PA3077		probable two-component response regulator	8.5	2.20E-09	NA	NA	NA	NA	-5.8	2.10E-08
PA3351	<i>flgM</i>	FlgM	2.0	0.00E+00	NA	NA	NA	NA	-2.4	4.81E-14
PA3630		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-31.6	3.70E-07
PA4070		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-30.2	6.91E-07
PA4077		probable transcriptional regulator	34.7	2.02E-07	NA	NA	NA	NA	-34.7	9.20E-08
PA4135		probable transcriptional regulator	2.2	1.47E-05	NA	NA	NA	NA	-3.4	4.26E-10
PA4203		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-33.6	1.50E-07
PA4987		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	-4.9	3.48E-07

Translation, post-translational modification, degradation										
PA1165	<i>pcpS</i>	PcpS	NA	NA	NA	NA	NA	NA	-39.4	1.11E-08
PA1670	<i>stpI</i>	serine/threonine phosphoprotein phosphatase StpI	NA	NA	NA	NA	NA	NA	-4.9	4.29E-06
PA2371	<i>ppiA</i>	probable ClpA/B-type protease	NA	NA	NA	NA	NA	NA	-28.2	1.73E-06
PA3227		peptidyl-prolyl cis-trans isomerase A	NA	NA	NA	NA	NA	NA	-2.9	6.82E-06
PA3601		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	-27.2	2.65E-06
PA3717	<i>gatC</i>	probable peptidyl-prolyl cis- trans isomerase, FkbP-type	11.3	2.72E-13	NA	NA	NA	NA	-3.9	2.65E-08
PA4482		Glu-tRNA(Gln) amidotransferase subunit C	NA	NA	NA	NA	NA	NA	-4.9	8.36E-14
Transport of small molecules										
PA0487	<i>oprD</i>	probable molybdenum transport regulator	8.5	2.16E-08	NA	NA	NA	NA	-5.8	1.46E-07
PA0958		Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor	NA	NA	NA	NA	NA	NA	-3.0	2.52E-13
PA3407	<i>hasAp</i>	heme acquisition protein HasAp	NA	NA	NA	NA	NA	NA	-34.9	8.55E-08
PA3753		conserved hypothetical protein	2.2	6.17E-07	- 75.1	0.00E+00	NA	NA	-150.5	0.00E+00

Genes negatively regulated by AmpR upon β -lactam exposure (247)										
Gene ID	Gene name	Product Name	Condition 1		Condition 2		Condition 3		Condition 4	
			Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value
Adaptation, Protection										
PA3553	<i>arnC</i>	ArnC	NA	NA	NA	NA	NA	NA	53.7	3.65E-13
PA3624	<i>pcm</i>	L-isoaspartate protein carboxylmethyltransferase type II	NA	NA	NA	NA	NA	NA	2.0	4.42E-08
PA4671		probable ribosomal protein L25	NA	NA	NA	NA	NA	NA	2.2	0.00E+00
Amino acid biosynthesis and metabolism										
PA5278	<i>dapF</i>	diaminopimelate epimerase	NA	NA	NA	NA	NA	NA	2.0	2.02E-06
PA2446	<i>gcvH2</i>	glycine cleavage system protein H2	NA	NA	NA	NA	NA	NA	2.1	4.91E-14
PA5413	<i>ltaA</i>	low specificity l-threonine aldolase	NA	NA	NA	NA	NA	NA	2.3	2.24E-06
PA2247	<i>bkdA1</i>	2-oxoisovalerate dehydrogenase (alpha subunit)	NA	NA	NA	NA	NA	NA	2.4	5.09E-14
PA3117	<i>asd</i>	aspartate semialdehyde dehydrogenase	NA	NA	NA	NA	NA	NA	2.4	1.73E-11
PA5067	<i>hisE</i>	phosphoribosyl-ATP pyrophosphohydrolase	NA	NA	NA	NA	NA	NA	3.6	4.64E-14
PA5417	<i>soxD</i>	sarcosine oxidase delta subunit	NA	NA	NA	NA	NA	NA	21.3	7.88E-06
PA3570	<i>mmsA</i>	methylmalonate-semialdehyde dehydrogenase	NA	NA	NA	NA	NA	NA	2.3	6.69E-14
PA2000	<i>dhcB</i>	DhcB, dehydrocarnitine CoA transferase, subunit B	NA	NA	NA	NA	NA	NA	2.7	0.00E+00
PA2250	<i>lpdV</i>	lipoamide dehydrogenase-Val	NA	NA	NA	NA	NA	NA	2.0	0.00E+00
PA4758	<i>carA</i>	carbamoyl-phosphate synthase small chain	NA	NA	NA	NA	NA	NA	2.2	1.07E-08
PA0402	<i>pyrB</i>	aspartate carbamoyltransferase	NA	NA	NA	NA	NA	NA	2.6	4.58E-07

Antibiotic resistance and susceptibility ; Membrane proteins										
PA0157	<i>triB</i>	Resistance-Nodulation-Cell Division (RND) triclosan efflux membrane fusion protein, TriB	NA	NA	NA	NA	NA	NA	4.1	3.03E-06
PA0551	<i>epd</i>	D-erythrose 4-phosphate dehydrogenase	NA	NA	NA	NA	NA	NA	2.5	3.97E-09
PA4529	<i>coaE</i>	dephosphocoenzyme A kinase	NA	NA	NA	NA	NA	NA	2.6	1.21E-07
PA1275	<i>cobD</i>	cobalamin biosynthetic protein CobD	NA	NA	NA	NA	NA	NA	6.2	6.55E-07
PA2905	<i>cobH</i>	precorrin isomerase CobH	NA	NA	NA	NA	NA	NA	65.5	5.09E-14
PA0024	<i>hemF</i>	coproporphyrinogen III oxidase, aerobic	-2.6	1.80E-05	2.8	5.65E-06	NA	NA	3.1	7.66E-08
PA5320	<i>coaC</i>	Phosphopantothenoylcysteine synthase/(R)-4'-phospho-N-pantothenoylcysteine decarboxylase	NA	NA	NA	NA	NA	NA	3.9	3.13E-09
Carbon compound catabolism										
PA2013	<i>liuC</i>	putative 3-methylglutaconyl-CoA hydratase	NA	NA	NA	NA	NA	NA	2.2	3.74E-06
PA2007	<i>maiA</i>	maleylacetoacetate isomerase	NA	NA	NA	NA	NA	NA	2.6	0.00E+00
PA1311	<i>phnX</i>	2-phosphonoacetaldehyde hydrolase	NA	NA	NA	NA	NA	NA	24.8	1.30E-06
PA2862	<i>lipA</i>	lactonizing lipase precursor	NA	NA	NA	NA	NA	NA	21.9	5.72E-06
Cell division										
PA4408	<i>ftsA</i>	cell division protein FtsA	NA	NA	NA	NA	NA	NA	2.2	1.49E-12
PA5563	<i>soj</i>	chromosome partitioning protein Soj	NA	NA	NA	NA	NA	NA	3.9	0.00E+00
Cell wall / LPS / capsule										
PA3643	<i>lpxB</i>	lipid A-disaccharide synthase	NA	NA	NA	NA	NA	NA	2.0	7.76E-07
PA5162	<i>rmlD</i>	dTDP-4-dehydrorhamnose reductase	NA	NA	NA	NA	NA	NA	2.5	4.76E-11

PA2236	<i>pslF</i>	PslF	NA	NA	NA	NA	NA	NA	3.6	2.60E-08
PA2238	<i>pslH</i>	PslH	NA	NA	NA	NA	NA	NA	5.6	5.14E-09
Central intermediary metabolism										
PA5046		malic enzyme	NA	NA	NA	NA	NA	NA	2.2	0.00E+00
PA2023	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	NA	NA	NA	NA	NA	NA	2.2	2.02E-11
PA0102		probable carbonic anhydrase	NA	NA	NA	NA	NA	NA	2.2	1.22E-06
PA4956	<i>rhdA</i>	thiosulfate:cyanide sulfurtransferase	NA	NA	NA	NA	NA	NA	3.4	3.14E-08
PA1780	<i>nirD</i>	assimilatory nitrite reductase small subunit	NA	NA	NA	NA	NA	NA	20.9	9.60E-06
PA0555	<i>fda</i>	fructose-1,6-bisphosphate aldolase	NA	NA	NA	NA	NA	NA	2.3	4.39E-14
PA1614	<i>gpsA</i>	glycerol-3-phosphate dehydrogenase, biosynthetic	NA	NA	NA	NA	NA	NA	5.1	3.36E-13
Chemotaxis										
PA1459		probable methyltransferase	NA	NA	NA	NA	NA	NA	2.8	2.68E-10
PA1448	<i>fliR</i>	flagellar biosynthetic protein FliR	NA	NA	NA	NA	NA	NA	26.4	5.58E-07
PA0410	<i>pilI</i>	twitching motility protein PilI	NA	NA	NA	NA	NA	NA	3.1	7.57E-07
DNA replication, recombination, modification and repair										
PA5147	<i>mutY</i>	A / G specific adenine glycosylase	NA	NA	NA	NA	NA	NA	2.4	9.25E-06
PA0357	<i>mutM</i>	formamidopyrimidine-DNA glycosylase	NA	NA	NA	NA	NA	NA	2.6	5.28E-06
PA5345	<i>recG</i>	ATP-dependent DNA helicase RecG	NA	NA	NA	NA	NA	NA	2.9	9.51E-06
PA5280	<i>sss</i>	site-specific recombinase Sss	NA	NA	NA	NA	NA	NA	6.2	4.40E-07
PA3738	<i>xerD</i>	integrase/recombinase XerD	NA	NA	NA	NA	NA	NA	22.9	3.50E-06
Energy metabolism										
PA2637	<i>nuoA</i>	NADH dehydrogenase I chain A	NA	NA	NA	NA	NA	NA	2.1	2.36E-14

PA5491		probable cytochrome	NA	NA	NA	NA	NA	NA	2.2	0.00E+00
PA4571		probable cytochrome c	NA	NA	NA	NA	NA	NA	2.3	2.66E-06
PA0794		probable aconitate hydratase	NA	NA	NA	NA	NA	NA	2.3	1.06E-05
PA3809	<i>fdx2</i>	ferredoxin [2Fe-2S]	NA	NA	NA	NA	NA	NA	2.3	5.04E-10
PA2641	<i>nuoF</i>	NADH dehydrogenase I chain F	NA	NA	NA	NA	NA	NA	2.7	1.73E-10
PA2649	<i>nuoN</i>	NADH dehydrogenase I chain N	NA	NA	NA	NA	NA	NA	4.0	1.27E-13
PA3195	<i>gapA</i>	glyceraldehyde 3-phosphate dehydrogenase	NA	NA	NA	NA	NA	NA	2.8	4.03E-06
PA3194	<i>edd</i>	phosphogluconate dehydratase	NA	NA	NA	NA	NA	NA	2.9	2.17E-06
PA1557	<i>ccoN2</i>	Cytochrome c oxidase, cbb3-type, CcoN subunit	NA	NA	NA	NA	NA	NA	2.1	7.13E-06
PA1555	<i>ccoP2</i>	Cytochrome c oxidase, cbb3-type, CcoP subunit	NA	NA	NA	NA	NA	NA	2.2	3.75E-07
Fatty acid and phospholipid metabolism										
PA2969	<i>plsX</i>	fatty acid biosynthesis protein PlsX	NA	NA	NA	NA	NA	NA	2.7	2.65E-08
PA0447	<i>gcdH</i>	glutaryl-CoA dehydrogenase	NA	NA	NA	NA	NA	NA	3.1	0.00E+00
PA0796	<i>prpB</i>	carboxyphosphoenolpyruvate phosphonmutase	NA	NA	NA	NA	NA	NA	2.2	9.11E-07
Hypothetical, unclassified, unknown										
PA0100		hypothetical protein	NA	NA	NA	NA	NA	NA	2.0	6.80E-06
PA0394		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.0	2.88E-08
PA4000		hypothetical protein	NA	NA	NA	NA	NA	NA	2.0	2.30E-12
PA0317		hypothetical protein	NA	NA	NA	NA	NA	NA	2.0	3.84E-06
PA4454		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.1	2.63E-12
PA3801		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.1	3.67E-13
PA3980		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.1	4.74E-06
PA5526		hypothetical protein	NA	NA	NA	NA	NA	NA	2.2	0.00E+00
PA0446		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.2	1.19E-10

PA3214		hypothetical protein	NA	NA	NA	NA	NA	NA	2.3	9.52E-06
PA5446		hypothetical protein	NA	NA	NA	NA	NA	NA	2.3	4.39E-14
PA2112		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.4	0.00E+00
PA5492		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.4	7.77E-09
PA3263		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.4	1.64E-06
PA3731		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.4	2.71E-08
PA2110		hypothetical protein	NA	NA	NA	NA	NA	NA	2.5	0.00E+00
PA2894		hypothetical protein	NA	NA	NA	NA	NA	NA	2.6	8.23E-06
PA2733		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.6	1.07E-08
PA3345		hypothetical protein	NA	NA	NA	NA	NA	NA	2.6	8.61E-07
PA2959		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.6	7.67E-11
PA2080	<i>kynU</i>	kynureninase KynU	NA	NA	NA	NA	NA	NA	2.7	2.41E-08
PA0388		hypothetical protein	NA	NA	NA	NA	NA	NA	2.7	1.56E-09
PA0542		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.7	1.56E-09
PA4697		hypothetical protein	NA	NA	NA	NA	NA	NA	2.7	6.53E-09
PA0598		hypothetical protein	NA	NA	NA	NA	NA	NA	2.7	9.96E-06
PA5303		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.7	5.35E-14
PA0462		hypothetical protein	NA	NA	NA	NA	NA	NA	2.8	2.70E-08
PA5422		hypothetical protein	NA	NA	NA	NA	NA	NA	2.9	5.19E-08
PA1114		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	1.10E-05
PA4322		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	1.10E-05
PA4335		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	4.39E-06
PA4841		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	7.57E-07
PA0385		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	7.51E-06
PA1639		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	8.98E-09
PA3284		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	5.46E-06
PA4605		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	1.07E-08
PA0553		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	8.20E-09
PA1034		hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	8.20E-09

PA4746	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.3	0.00E+00
PA3200	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.6	6.76E-06
PA5459	hypothetical protein	NA	NA	NA	NA	NA	NA	3.6	3.42E-06
PA4321	hypothetical protein	NA	NA	NA	NA	NA	NA	3.8	1.29E-06
PA3084	hypothetical protein	NA	NA	NA	NA	NA	NA	3.8	6.37E-10
PA2986	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.9	1.22E-08
PA0568	hypothetical protein	NA	NA	NA	NA	NA	NA	4.1	4.06E-07
PA1514	ureidoglycolate hydrolaseYbbT	NA	NA	NA	NA	NA	NA	4.1	1.66E-06
PA1837	hypothetical protein	NA	NA	NA	NA	NA	NA	4.1	1.22E-06
PA4062	hypothetical protein	NA	NA	NA	NA	NA	NA	4.1	7.19E-09
PA0389	hypothetical protein	NA	NA	NA	NA	NA	NA	4.8	4.91E-14
PA4579	hypothetical protein	NA	NA	NA	NA	NA	NA	4.8	5.72E-07
PA2230	hypothetical protein	NA	NA	NA	NA	NA	NA	5.1	3.56E-07
PA2441	hypothetical protein	NA	NA	NA	NA	NA	NA	5.1	1.76E-10
PA2632	hypothetical protein	NA	NA	NA	NA	NA	NA	5.1	1.10E-07
PA0022	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	6.2	5.95E-11
PA3670	hypothetical protein	NA	NA	NA	NA	NA	NA	6.7	8.82E-08
PA5145	hypothetical protein	NA	NA	NA	NA	NA	NA	7.2	2.60E-07
PA1193	hypothetical protein	NA	NA	NA	NA	NA	NA	7.2	2.12E-11
PA3680	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	7.2	6.79E-10
PA4617	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	11.3	1.67E-12
PA2670	hypothetical protein	NA	NA	NA	NA	NA	NA	20.8	1.02E-05
PA0977	hypothetical protein	NA	NA	NA	NA	NA	NA	21.1	8.70E-06
PA1123	hypothetical protein	NA	NA	NA	NA	NA	NA	21.3	7.88E-06
PA4182	hypothetical protein	NA	NA	NA	NA	NA	NA	21.4	7.49E-06
PA2874	hypothetical protein	NA	NA	NA	NA	NA	NA	21.5	7.10E-06
PA2422	hypothetical protein	NA	NA	NA	NA	NA	NA	21.7	6.39E-06
PA1517	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	22.1	5.14E-06
PA0104	hypothetical protein	NA	NA	NA	NA	NA	NA	22.9	3.43E-06

PA1489	hypothetical protein	NA	NA	NA	NA	NA	NA	23.0	3.33E-06
PA0709	hypothetical protein	NA	NA	NA	NA	NA	NA	23.0	3.23E-06
PA5407	hypothetical protein	NA	NA	NA	NA	NA	NA	23.5	2.52E-06
PA3066	hypothetical protein	NA	NA	NA	NA	NA	NA	23.9	2.09E-06
PA3283	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	24.0	1.96E-06
PA1139	hypothetical protein	NA	NA	NA	NA	NA	NA	24.2	1.79E-06
PA2292	hypothetical protein	NA	NA	NA	NA	NA	NA	24.3	1.72E-06
PA4985	hypothetical protein	NA	NA	NA	NA	NA	NA	25.1	1.13E-06
PA3991	hypothetical protein	NA	NA	NA	NA	NA	NA	25.9	7.26E-07
PA0711	hypothetical protein	NA	NA	NA	NA	NA	NA	27.0	4.15E-07
PA1234	hypothetical protein	NA	NA	NA	NA	NA	NA	27.2	3.81E-07
PA2625	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	29.1	1.42E-07
PA1761	hypothetical protein	NA	NA	NA	NA	NA	NA	31.2	4.51E-08
PA4169	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	31.9	3.22E-08
PA5408	hypothetical protein	NA	NA	NA	NA	NA	NA	38.7	9.39E-10
PA1135	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	39.1	7.60E-10
PA1593	hypothetical protein	NA	NA	NA	NA	NA	NA	43.3	8.24E-11
PA2264	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	47.7	8.25E-12
PA1641	hypothetical protein	NA	NA	NA	NA	NA	NA	49.1	4.05E-12
PA5533	hypothetical protein	NA	NA	NA	NA	NA	NA	55.6	1.27E-13
PA4753	conserved hypothetical protein	-30.0	2.19E-07	6.9	5.20E-14	NA	NA	108.6	0.00E+00
PA1518	conserved hypothetical protein	-24.8	2.96E-06	53.9	7.87E-11	NA	NA	53.9	3.13E-13
PA0469	hypothetical protein	-24.8	2.99E-06	4.8	2.38E-06	NA	NA	55.8	1.40E-13
PA0541	hypothetical protein	-4.9	4.55E-09	2.2	1.32E-05	NA	NA	8.2	4.39E-14
PA3767	conserved hypothetical protein	-4.2	4.40E-06	3.2	7.00E-07	NA	NA	7.2	1.03E-13
PA4612	conserved hypothetical protein	NA	NA	NA	NA	NA	NA	24.8	1.30E-06
PA0718	hypothetical protein of bacteriophage Pf1	NA	NA	NA	NA	NA	NA	23.5	2.52E-06

Membrane proteins										
PA3819		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.1	0.00E+00
PA1053		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.2	5.59E-14
PA5568		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	2.3	2.98E-09
PA5250		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.8	2.05E-10
PA2004		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	4.8	5.77E-09
PA2929		hypothetical protein	NA	NA	NA	NA	NA	NA	5.1	9.48E-08
PA3362		hypothetical protein	NA	NA	NA	NA	NA	NA	5.1	5.35E-09
PA3748		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	7.2	2.09E-06
PA2751		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	22.8	3.64E-06
PA2936		hypothetical protein	NA	NA	NA	NA	NA	NA	23.5	2.52E-06
PA2784		hypothetical protein	NA	NA	NA	NA	NA	NA	24.5	1.50E-06
PA2712		hypothetical protein	NA	NA	NA	NA	NA	NA	31.7	3.60E-08
PA1055		conserved hypothetical protein	-27.9	6.43E-07	5.5	1.75E-09	NA	NA	80.8	2.36E-14
PA0070	<i>tagQ1</i>	TagQ1	NA	NA	NA	NA	NA	NA	2.2	4.11E-14
PA0984		colicin immunity protein	NA	NA	NA	NA	NA	NA	3.1	8.30E-06
PA1911	<i>femR</i>	sigma factor regulator, FemR	NA	NA	NA	NA	NA	NA	21.6	6.86E-06
PA4233		probable major facilitator superfamily (MFS) transporter	NA	NA	NA	NA	NA	NA	2.1	8.92E-07
PA1341		amino acid ABC transporter membrane protein	NA	NA	NA	NA	NA	NA	2.3	8.39E-06
PA4503		probable permease of ABC transporter	NA	NA	NA	NA	NA	NA	2.6	4.97E-07
PA4765	<i>omlA</i>	Outer membrane lipoprotein OmlA precursor	NA	NA	NA	NA	NA	NA	3.1	7.54E-13
PA5504		D-methionine ABC transporter membrane protein	NA	NA	NA	NA	NA	NA	3.1	2.86E-10
PA2533		probable sodium:alanine symporter	NA	NA	NA	NA	NA	NA	3.6	1.95E-07
PA0890	<i>aotM</i>	arginine/ornithine transport protein AotM	NA	NA	NA	NA	NA	NA	3.6	1.85E-13

PA4707		probable permease of ABC transporter	NA	NA	NA	NA	NA	NA	10.3	1.26E-12
PA5216		probable permease of ABC iron transporter	NA	NA	NA	NA	NA	NA	12.3	6.54E-10
PA3585	<i>glpM</i>	membrane protein GlpM	NA	NA	NA	NA	NA	NA	20.7	1.05E-05
PA0786		probable transporter	NA	NA	NA	NA	NA	NA	22.4	4.59E-06
PA3888		probable permease of ABC transporter	NA	NA	NA	NA	NA	NA	37.9	1.43E-09
PA4990		SMR multidrug efflux transporter	NA	NA	NA	NA	NA	NA	20.6	1.16E-05
Motility & Attachment										
PA0406	<i>tonB3</i>	TonB3	NA	NA	NA	NA	NA	NA	6.2	8.79E-07
Non-coding RNA gene										
PA3368.1		tRNA-Arg	NA	NA	NA	NA	NA	NA	2.1	0.00E+00
PA3133.2		tRNA-Ala	NA	NA	NA	NA	NA	NA	2.4	0.00E+00
Nucleotide biosynthesis and metabolism										
PA0944	<i>purN</i>	phosphoribosylaminoimidazole synthetase	NA	NA	NA	NA	NA	NA	2.6	4.02E-10
PA3527	<i>pyrC</i>	dihydroorotase	NA	NA	NA	NA	NA	NA	3.6	2.67E-09
Protein secretion/export apparatus										
PA0086	<i>tagJ1</i>	TagJ1	NA	NA	NA	NA	NA	NA	2.2	7.28E-06
PA0089	<i>tssG1</i>	TssG1	NA	NA	NA	NA	NA	NA	2.6	9.13E-09
PA0077	<i>tssM1</i>	TssM1	NA	NA	NA	NA	NA	NA	2.6	1.82E-07
PA0080	<i>tssJ1</i>	TssJ1	-25.4	2.22E-06	5.5	1.88E-13	NA	NA	117.7	2.75E-14
PA1718	<i>pseE</i>	type III export protein PseE	NA	NA	NA	NA	NA	NA	33.5	1.37E-08
PA1699	<i>pcr1</i>	Pcr1	NA	NA	NA	NA	NA	NA	24.5	1.50E-06
Putative enzymes										
PA2631		probable acetyl transferase	NA	NA	NA	NA	NA	NA	2.1	5.03E-06
PA5312		probable aldehyde	NA	NA	NA	NA	NA	NA	2.1	4.94E-08

PA0506		dehydrogenase								
PA0506		probable acyl-CoA dehydrogenase	NA	NA	NA	NA	NA	NA	2.4	2.13E-11
PA0318		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	3.1	1.01E-05
PA5521		probable short-chain dehydrogenase	NA	NA	NA	NA	NA	NA	3.1	2.40E-11
PA0130		probable aldehyde dehydrogenase	NA	NA	NA	NA	NA	NA	3.3	4.83E-07
PA1047		probable esterase	NA	NA	NA	NA	NA	NA	3.4	5.13E-11
PA5313		probable pyridoxal-dependent aminotransferase	NA	NA	NA	NA	NA	NA	3.7	1.32E-09
PA0840		probable oxidoreductase	NA	NA	NA	NA	NA	NA	4.6	3.10E-07
PA2040		probable glutamine synthetase	NA	NA	NA	NA	NA	NA	5.5	4.76E-11
PA3427		probable short-chain dehydrogenases	NA	NA	NA	NA	NA	NA	7.2	1.16E-08
PA1537		probable short-chain dehydrogenase	NA	NA	NA	NA	NA	NA	23.1	3.10E-06
PA2550		probable acyl-CoA dehydrogenase	NA	NA	NA	NA	NA	NA	33.4	1.48E-08
PA5020		probable acyl-CoA dehydrogenase	NA	NA	NA	NA	NA	NA	38.0	1.36E-09
PA0656		probable HIT family protein	NA	NA	NA	NA	NA	NA	40.4	3.82E-10
PA3554	<i>arnA</i>	ArnA	NA	NA	NA	NA	NA	NA	3.5	3.74E-06
PA0705	<i>migA</i>	alpha-1,6-rhamnosyltransferase MigA	NA	NA	NA	NA	NA	NA	3.1	3.10E-06
PA2237	<i>pslG</i>	PslG	NA	NA	NA	NA	NA	NA	3.1	6.60E-07
Secreted Factors (toxins, enzymes, alginate)										
PA0085	<i>hcp1</i>	Hcp1	NA	NA	NA	NA	NA	NA	2.1	0.00E+00
PA2702	<i>tse2</i>	Tse2	NA	NA	NA	NA	NA	NA	4.1	6.90E-07
Transcription, RNA processing and degradation										
PA3823	<i>tgt</i>	queuine tRNA-ribosyltransferase	NA	NA	NA	NA	NA	NA	2.1	2.94E-06

Transcriptional regulators										
PA3285		probable sigma-70 factor, ECF subfamily	NA	NA	NA	NA	NA	NA	3.1	3.92E-06
PA0961		probable cold-shock protein	NA	NA	NA	NA	NA	NA	3.6	1.50E-14
PA0479		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	21.6	6.86E-06
PA1961		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	21.9	5.72E-06
PA3571	<i>mmsR</i>	transcriptional regulator MmsR	NA	NA	NA	NA	NA	NA	22.2	4.95E-06
PA1399		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	22.3	4.77E-06
PA2432	<i>bexR</i>	bistable expression regulator, BexR	NA	NA	NA	NA	NA	NA	22.4	4.42E-06
PA4436		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	22.8	3.64E-06
PA2718		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	28.0	2.47E-07
PA1142		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	28.6	1.77E-07
PA5116		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	32.1	2.86E-08
PA5342		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	42.7	1.13E-10
PA4983		probable two-component response regulator	NA	NA	NA	NA	NA	NA	5.1	1.20E-06
PA3363	<i>amiR</i>	aliphatic amidase regulator	NA	NA	NA	NA	NA	NA	4.1	9.26E-06
PA3184		probable transcriptional regulator	NA	NA	NA	NA	NA	NA	7.2	3.81E-09
PA2510	<i>catR</i>	transcriptional regulator CatR	NA	NA	NA	NA	NA	NA	23.5	2.52E-06
PA0155	<i>pcaR</i>	transcriptional regulator PcaR	NA	NA	NA	NA	NA	NA	24.4	1.57E-06
PA3192	<i>gltR</i>	two-component response regulator GltR	NA	NA	NA	NA	NA	NA	3.6	5.85E-07

Translation, post-translational modification, degradation										
PA3701	<i>prfB</i>	peptide chain release factor 2	NA	NA	NA	NA	NA	NA	2.0	4.71E-07
PA4498		probable metallopeptidase	NA	NA	NA	NA	NA	NA	2.2	1.08E-05
PA3262		probable peptidyl-prolyl cis-trans isomerase, FkbP-type	NA	NA	NA	NA	NA	NA	2.5	3.86E-14
PA0538	<i>dsbB</i>	disulfide bond formation protein	NA	NA	NA	NA	NA	NA	4.1	1.66E-06
PA4572	<i>fklB</i>	peptidyl-prolyl cis-trans isomerase FklB	NA	NA	NA	NA	NA	NA	4.1	2.75E-14
Transport of small molecules										
PA5074	<i>dctA</i>	probable ATP-binding component of ABC transporter	NA	NA	NA	NA	NA	NA	2.7	7.03E-06
PA1964		probable ATP-binding component of ABC transporter	NA	NA	NA	NA	NA	NA	5.1	2.66E-06
PA3188		probable permease of ABC sugar transporter	NA	NA	NA	NA	NA	NA	8.2	5.42E-11
PA1183		C4-dicarboxylate transport protein	NA	NA	NA	NA	NA	NA	20.9	9.85E-06
PA0295		probable periplasmic polyamine binding protein	NA	NA	NA	NA	NA	NA	25.8	7.82E-07
PA3372		conserved hypothetical protein	NA	NA	NA	NA	NA	NA	35.5	4.90E-09
PA0950	<i>wzx</i>	probable arsenate reductase	NA	NA	NA	NA	NA	NA	2.1	4.84E-07
PA3153		O-antigen translocase	NA	NA	NA	NA	NA	NA	2.4	5.39E-15

Table 4: β -lactam-dependent genes. The genes that are either positively or negatively regulated when exposed to β -lactam stress in the absence of AmpR are classified based on information in Supplementary Table 1. Only genes that are expressed ≥ 2.0 , FDR-corrected (Bonferroni, <0.05) are listed. Functional categorization, gene ID and gene/product names are from the *Pseudomonas* Genome database (27). Lack of expression of a gene under any of the four conditions is denoted as NA.

Gene ID	Gene name	Product Name	Condition 1		Condition 2		Condition 3		Condition 4	
			FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value	FC	Corrected <i>p</i> -value
Genes positively regulated in an AmpR-independent, β-lactam-dependent manner (278)										
Adaptation, Protection										
PA0059	<i>osmC</i>	osmotically inducible protein OsmC	NA	NA	9.7	0.00E+00	NA	NA	3.6	1.57E-10
Amino acid biosynthesis and metabolism										
PA0025	<i>aroE</i>	shikimate dehydrogenase	NA	NA	4.8	1.43E-06	NA	NA	3.6	2.88E-06
PA0896	<i>aruF</i>	arginine/ornithine succinyltransferase AI subunit	2.0	2.91E-10	NA	NA	NA	NA	NA	NA
PA1338	<i>ggt</i>	gamma-glutamyltranspeptidase precursor	NA	NA	2.6	9.04E-07	NA	NA	3.6	2.19E-11
PA2443	<i>sdaA</i>	L-serine dehydratase	NA	NA	2.3	1.95E-10	NA	NA	2.2	4.10E-11
PA2612	<i>serS</i>	seryl-tRNA synthetase	NA	NA	2.1	5.43E-07	NA	NA	3.0	1.27E-13
PA3107	<i>metZ</i>	o-succinylhomoserine sulfhydrylase	NA	NA	2.2	2.03E-07	NA	NA	2.3	5.30E-09
PA3997	<i>lipB</i>	lipoate-protein ligase B	2.0	2.60E-06	NA	NA	NA	NA	NA	NA
PA4449	<i>hisG</i>	ATP-phosphoribosyltransferase	NA	NA	2.2	8.79E-09	NA	NA	6.5	0.00E+00
PA4602	<i>glyA3</i>	serine hydroxymethyltransferase	2.0	1.64E-07	NA	NA	2.4	9.05E-13	NA	NA
PA4731	<i>panD</i>	aspartate 1-decarboxylase precursor	2.8	2.48E-05	71.8	1.50E-14	NA	NA	NA	NA
PA4846	<i>aroQ1</i>	3-dehydroquinate dehydratase	NA	NA	3.0	0.00E+00	NA	NA	4.2	0.00E+00
PA5015	<i>aceE</i>	pyruvate dehydrogenase	NA	NA	2.0	4.46E-08	NA	NA	2.6	0.00E+00
PA5171	<i>arcA</i>	arginine deiminase	2.1	2.39E-11	2.2	6.77E-14	NA	NA	NA	NA
PA5172	<i>arcB</i>	ornithine carbamoyltransferase,	NA	NA	2.4	0.00E+00	NA	NA	2.0	3.52E-14

PA5173	<i>arcC</i>	catabolic carbamate kinase	2.3	1.20E-08	NA	NA	NA	NA	NA	NA
PA5323	<i>argB</i>	acetylglutamate kinase	NA	NA	2.1	2.58E-10	NA	NA	2.1	1.24E-11
Biosynthesis of cofactors, prosthetic groups and carriers										
PA0583	<i>moaB2</i>	hypothetical protein	5.7	1.13E-06	NA	NA	NA	NA	NA	NA
PA3029		molybdopterin biosynthetic protein B2	3.1	6.26E-10	NA	NA	2.8	1.25E-07	NA	NA
PA4047		GTP cyclohydrolase II	3.1	7.10E-11	NA	NA	3.0	5.42E-10	NA	NA
PA4728	<i>folK</i>	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase	NA	NA	8.3	1.01E-11	NA	NA	84.0	3.86E-14
Carbon compound catabolism										
PA0232	<i>pcaC</i>	gamma-carboxymuconolactone decarboxylase	NA	NA	6.9	4.48E-11	NA	NA	5.1	2.50E-11
PA4127	<i>hpcG</i>	2-oxo-hept-3-ene-1,7-dioate hydratase	26.8	6.45E-06	NA	NA	NA	NA	NA	NA
Cell division										
PA4419	<i>ftsL</i>	cell division protein FtsL	NA	NA	2.4	1.98E-08	NA	NA	2.4	1.23E-09
PA5564	<i>gidB</i>	glucose inhibited division protein B	NA	NA	5.5	2.19E-14	NA	NA	2.5	4.45E-08
Cell wall / LPS / capsule										
PA2232	<i>pslB</i>	PslB	NA	NA	2.5	4.91E-09	NA	NA	2.6	2.02E-11
PA2235	<i>pslE</i>	PslE	NA	NA	2.4	3.42E-07	NA	NA	2.3	1.12E-07
PA3999	<i>dacC</i>	D-ala-D-ala-carboxypeptidase	NA	NA	2.2	8.78E-14	NA	NA	2.0	1.51E-13
PA4415	<i>mraY</i>	phospho-N-acetylmuramoyl-pentapeptide-transferase	2.0	4.46E-07	NA	NA	NA	NA	NA	NA
PA4668		probable lipoprotein localization protein LolB	3.6	3.65E-09	NA	NA	NA	NA	NA	NA
PA5163	<i>rmlA</i>	glucose-1-phosphate thymidyltransferase	NA	NA	2.5	5.49E-12	NA	NA	2.1	1.71E-10
PA5276	<i>lppL</i>	Lipopeptide LppL precursor	5.7	0.00E+00	NA	NA	4.2	4.55E-14	NA	NA

PA5322	<i>algC</i>	phosphomannomutase AlgC	NA	NA	2.6	1.06E-13	NA	NA	3.3	0.00E+00
Central intermediary metabolism										
PA3524	<i>gloA1</i>	lactoylglutathione lyase	2.0	5.32E-07	NA	NA	2.0	4.42E-06	NA	NA
PA4880		probable bacterioferritin	26.9	6.17E-06	NA	NA	NA	NA	NA	NA
Chemotaxis ; Adaptation, Protection ; Motility & Attachment										
PA1446	<i>fliP</i>	flagellar biosynthetic protein FliP	2.3	4.37E-06	NA	NA	NA	NA	NA	NA
DNA replication, recombination, modification and repair										
PA0002	<i>dnaN</i>	DNA polymerase III, beta chain	2.1	9.60E-12	NA	NA	NA	NA	NA	NA
PA0720		helix destabilizing protein of bacteriophage Pfl	8.5	1.98E-07	NA	NA	NA	NA	NA	NA
PA0965	<i>ruvC</i>	Holliday junction resolvase RuvC	2.8	3.41E-08	NA	NA	NA	NA	NA	NA
PA1534	<i>recR</i>	recombination protein RecR	4.7	9.40E-07	NA	NA	NA	NA	NA	NA
PA1815	<i>rnhA</i>	ribonuclease H	2.8	1.80E-11	NA	NA	NA	NA	NA	NA
PA2961	<i>holB</i>	DNA polymerase III, delta prime subunit	NA	NA	10.4	0.00E+00	NA	NA	2.6	3.54E-07
Energy metabolism										
PA1562	<i>acnA</i>	aconitate hydratase 1	NA	NA	4.0	1.28E-12	NA	NA	3.0	4.48E-11
PA2995	<i>nqrE</i>	Na+-translocating NADH:quinone oxidoreductase subunit Nqr5	NA	NA	2.2	8.00E-07	NA	NA	3.3	2.68E-13
PA2997		Na+-translocating NADH:ubiquinone oxidoreductase subunit Nqr3	2.5	4.15E-07	NA	NA	NA	NA	NA	NA
PA3183	<i>zwf</i>	glucose-6-phosphate 1-dehydrogenase	2.7	1.40E-06	2.8	3.20E-07	NA	NA	NA	NA
PA4975		NAD(P)H quinone oxidoreductase	5.7	2.50E-05	NA	NA	NA	NA	NA	NA
Fatty acid and phospholipid metabolism										
PA1806	<i>fabI</i>	NADH-dependent enoyl-ACP reductase	3.6	2.85E-07	NA	NA	NA	NA	NA	NA

PA4888	<i>desB</i>	acyl-CoA delta-9-desaturase, DesB	8.5	5.51E-06	NA	NA	NA	NA	NA	NA
Hypothetical, unclassified, unknown										
PA0047	<i>icp</i>	hypothetical protein	3.3	6.23E-08	3.7	2.40E-09	NA	NA	NA	NA
PA0061		hypothetical protein	NA	NA	62.5	1.98E-12	NA	NA	4.1	2.05E-07
PA0124		hypothetical protein	NA	NA	4.1	3.12E-07	NA	NA	3.1	1.45E-06
PA0127		hypothetical protein	5.7	3.64E-07	NA	NA	NA	NA	NA	NA
PA0168		conserved hypothetical protein	NA	NA	3.2	1.85E-06	NA	NA	3.6	2.39E-08
PA0169		hypothetical protein	NA	NA	2.5	3.34E-06	NA	NA	3.8	4.30E-11
PA0170		hypothetical protein	4.7	3.80E-10	NA	NA	4.2	2.56E-08	NA	NA
PA0261		hypothetical protein	NA	NA	27.5	6.00E-06	NA	NA	27.5	3.22E-07
PA0274		hypothetical protein	11.3	2.88E-06	NA	NA	NA	NA	NA	NA
PA0329		conserved hypothetical protein	NA	NA	3.7	0.00E+00	-2.5	2.41E-11	2.7	4.11E-14
PA0332		hypothetical protein	3.2	2.03E-10	NA	NA	NA	NA	NA	NA
PA0359		hypothetical protein	5.7	1.99E-05	NA	NA	14.7	0.00E+00	NA	NA
PA0404		conserved hypothetical protein	NA	NA	47.2	1.43E-09	NA	NA	47.2	1.09E-11
PA0460		hypothetical protein	2.0	2.16E-08	3.1	0.00E+00	NA	NA	NA	NA
PA0466		hypothetical protein	NA	NA	158.1	0.00E+00	NA	NA	158.1	0.00E+00
PA0505		hypothetical protein	NA	NA	59.3	7.93E-12	- 112.5	0.00E+00	NA	NA
PA0537		conserved hypothetical protein	2.2	2.33E-09	2.8	0.00E+00	NA	NA	NA	NA
PA0567		conserved hypothetical protein	NA	NA	43.0	8.53E-09	NA	NA	43.0	9.49E-11
PA0769		hypothetical protein	NA	NA	3.7	2.34E-12	NA	NA	4.1	1.03E-14
PA0778		inhibitor of cysteine peptidase	NA	NA	5.5	4.59E-08	NA	NA	67.6	4.91E-14
PA0803		hypothetical protein	NA	NA	31.0	1.35E-06	NA	NA	31.0	5.03E-08
PA0856		hypothetical protein	2.0	2.68E-10	2.0	7.18E-14	NA	NA	NA	NA
PA0925		hypothetical protein	8.5	7.30E-08	NA	NA	NA	NA	NA	NA
PA0926		hypothetical protein	4.3	1.98E-05	NA	NA	NA	NA	NA	NA
PA0948		hypothetical protein	NA	NA	32.6	7.05E-07	-	5.32E-06	NA	NA

						28.1				
PA0952		hypothetical protein	NA	NA	35.6	1.99E-07	NA	NA	35.6	4.55E-09
PA1324		hypothetical protein	NA	NA	3.5	8.70E-11	NA	NA	5.1	4.64E-14
PA1343		hypothetical protein	8.5	4.44E-07	NA	NA	10.5	3.29E-09	NA	NA
PA1395		hypothetical protein	8.5	1.04E-07	NA	NA	NA	NA	NA	NA
PA1439		conserved hypothetical protein	5.7	1.26E-08	NA	NA	NA	NA	NA	NA
PA1471		hypothetical protein	NA	NA	5.5	1.00E-10	NA	NA	4.1	1.84E-10
PA1560		hypothetical protein	NA	NA	2.8	2.53E-06	NA	NA	6.2	6.14E-14
PA1592		hypothetical protein	NA	NA	4.2	0.00E+00	NA	NA	3.5	0.00E+00
PA1744		hypothetical protein	32.8	4.68E-07	NA	NA	60.0	0.00E+00	NA	NA
PA1747		hypothetical protein	76.0	1.55E-13	36.2	1.57E-07	NA	NA	NA	NA
PA1774	<i>crfX</i>	CrfX protein	3.3	0.00E+00	NA	NA	NA	NA	NA	NA
PA1817		hypothetical protein	36.8	8.06E-08	NA	NA	22.5	3.81E-06	NA	NA
PA1855		hypothetical protein	NA	NA	30.8	1.47E-06	NA	NA	30.8	5.61E-08
PA1879		hypothetical protein	25.9	9.68E-06	NA	NA	NA	NA	NA	NA
PA1892		hypothetical protein	38.9	3.23E-08	37.1	1.08E-07	NA	NA	NA	NA
PA1963		hypothetical protein	53.8	4.28E-11	51.3	2.44E-10	NA	NA	NA	NA
PA2021		hypothetical protein	NA	NA	30.4	1.76E-06	NA	NA	30.4	6.97E-08
PA2029		hypothetical protein	NA	NA	4.1	7.35E-07	NA	NA	68.4	5.59E-14
PA2106		hypothetical protein	4.7	1.23E-05	3.7	9.34E-07	NA	NA	NA	NA
PA2127		conserved hypothetical protein	4.5	6.61E-07	NA	NA	NA	NA	NA	NA
PA2174		hypothetical protein	NA	NA	44.3	5.00E-09	NA	NA	44.3	5.00E-11
PA2226		hypothetical protein	NA	NA	27.3	6.44E-06	- 28.3	4.97E-06	NA	NA
PA2293		hypothetical protein	NA	NA	28.3	4.21E-06	NA	NA	28.3	2.05E-07
PA2368		hypothetical protein	50.6	1.81E-10	NA	NA	NA	NA	NA	NA
PA2484		conserved hypothetical protein	NA	NA	3.2	3.05E-06	NA	NA	3.6	4.57E-08
PA2560		hypothetical protein	2.4	3.23E-12	3.2	2.25E-12	NA	NA	NA	NA
PA2598		hypothetical protein	26.5	7.31E-06	NA	NA	NA	NA	NA	NA

PA2607	conserved hypothetical protein	4.3	3.08E-07	NA	NA	NA	NA	NA	NA
PA2658	hypothetical protein	NA	NA	4.1	1.41E-06	-3.8	1.07E-05	NA	NA
PA2730	hypothetical protein	NA	NA	5.8	0.00E+00	-3.3	5.40E-07	NA	NA
PA2736	hypothetical protein	2.0	2.48E-05	2.8	1.12E-09	NA	NA	NA	NA
PA2737	conserved hypothetical protein	NA	NA	2.4	1.68E-09	-2.7	1.02E-12	NA	NA
PA2792	hypothetical protein	NA	NA	4.1	6.88E-07	NA	NA	6.2	2.66E-10
PA2797	hypothetical protein	3.1	1.55E-13	2.1	9.12E-07	NA	NA	NA	NA
PA2864	conserved hypothetical protein	NA	NA	5.5	1.45E-07	NA	NA	4.1	1.86E-07
PA2872	hypothetical protein	7.1	1.37E-06	NA	NA	NA	NA	NA	NA
PA2883	hypothetical protein	2.8	6.09E-06	2.8	1.58E-05	NA	NA	NA	NA
PA2935	hypothetical protein	NA	NA	28.5	3.92E-06	NA	NA	28.5	1.88E-07
PA2956	conserved hypothetical protein	7.1	8.86E-11	NA	NA	NA	NA	NA	NA
PA2972	conserved hypothetical protein	NA	NA	5.5	6.36E-06	NA	NA	47.3	1.05E-11
PA3010	hypothetical protein	3.6	8.86E-11	NA	NA	NA	NA	NA	NA
PA3018	hypothetical protein	14.2	7.00E-09	49.8	4.60E-10	NA	NA	NA	NA
PA3022	hypothetical protein	NA	NA	2.8	1.46E-09	-2.9	1.20E-10	NA	NA
PA3031	hypothetical protein	NA	NA	3.2	1.79E-13	NA	NA	3.1	6.14E-14
PA3040	conserved hypothetical protein	NA	NA	4.8	0.00E+00	-3.3	5.85E-08	2.4	1.08E-08
PA3042	hypothetical protein	NA	NA	2.8	4.56E-08	NA	NA	3.1	1.06E-10
PA3051	hypothetical protein	5.7	1.85E-06	NA	NA	NA	NA	NA	NA
PA3056	hypothetical protein	2.8	3.07E-06	5.5	5.48E-07	NA	NA	NA	NA
PA3057	hypothetical protein	NA	NA	29.6	2.46E-06	NA	NA	29.6	1.05E-07
PA3072	hypothetical protein	58.0	6.07E-12	NA	NA	35.4	3.06E-09	NA	NA
PA3123	conserved hypothetical protein	NA	NA	3.2	1.34E-09	-2.7	1.19E-06	2.4	1.02E-07
PA3203	hypothetical protein	2.4	1.84E-06	NA	NA	NA	NA	NA	NA
PA3274	hypothetical protein	24.9	1.47E-05	NA	NA	NA	NA	NA	NA
PA3291	hypothetical protein	NA	NA	37.0	1.12E-07	NA	NA	37.0	2.25E-09
PA3307	hypothetical protein	NA	NA	2.3	1.30E-05	NA	NA	2.6	1.41E-07
PA3338	hypothetical protein	5.7	2.74E-06	NA	NA	NA	NA	NA	NA

PA3412		hypothetical protein	NA	NA	25.3	1.47E-05	NA	NA	25.3	9.73E-07
PA3425		hypothetical protein	NA	NA	39.7	3.64E-08	NA	NA	39.7	5.53E-10
PA3470		hypothetical protein	4.3	2.17E-09	NA	NA	3.2	1.71E-05	NA	NA
PA3501		hypothetical protein	32.8	4.68E-07	62.5	1.98E-12	NA	NA	NA	NA
PA3626		conserved hypothetical protein	2.5	7.56E-06	NA	NA	NA	NA	NA	NA
PA3668		conserved hypothetical protein	4.3	1.84E-05	NA	NA	NA	NA	NA	NA
PA3691		hypothetical protein	NA	NA	8.8	0.00E+00	NA	NA	5.9	2.01E-13
PA3716		hypothetical protein	2.1	1.01E-05	NA	NA	NA	NA	NA	NA
PA3762		hypothetical protein	2.4	6.16E-06	NA	NA	NA	NA	NA	NA
PA3952		hypothetical protein	NA	NA	2.8	5.60E-09	NA	NA	2.5	6.26E-09
PA3982		conserved hypothetical protein	NA	NA	3.7	7.18E-14	NA	NA	2.7	4.11E-14
PA3986		hypothetical protein	NA	NA	63.4	1.35E-12	- 27.4	7.38E-06	NA	NA
PA4028		hypothetical protein	NA	NA	61.7	2.79E-12	NA	NA	61.7	0.00E+00
PA4033	<i>mucE</i>	MucE	NA	NA	50.7	3.12E-10	NA	NA	50.7	1.72E-12
PA4090		hypothetical protein	NA	NA	47.0	1.53E-09	- 65.0	7.36E-13	NA	NA
PA4116	<i>bphO</i>	heme oxygenase, BphO	2.1	2.95E-06	NA	NA	NA	NA	NA	NA
PA4154		conserved hypothetical protein	14.2	4.24E-09	3.5	1.92E-08	NA	NA	NA	NA
PA4346		hypothetical protein	NA	NA	4.1	1.34E-11	NA	NA	2.1	8.80E-06
PA4388		hypothetical protein	NA	NA	37.2	1.01E-07	- 28.9	3.82E-06	NA	NA
PA4392		conserved hypothetical protein	NA	NA	5.5	1.84E-08	NA	NA	71.3	4.91E-14
PA4422		conserved hypothetical protein	NA	NA	3.5	7.56E-07	NA	NA	2.6	8.55E-06
PA4537		hypothetical protein	42.0	8.09E-09	NA	NA	25.6	6.69E-07	NA	NA
PA4570		hypothetical protein	31.9	6.88E-07	NA	NA	19.5	1.87E-05	NA	NA
PA4699		hypothetical protein	2.2	1.77E-07	2.6	1.11E-08	NA	NA	NA	NA
PA4870		conserved hypothetical protein	5.7	8.94E-07	NA	NA	NA	NA	NA	NA
PA4965		hypothetical protein	NA	NA	2.2	2.39E-06	NA	NA	2.8	8.32E-11
PA5062		conserved hypothetical protein	26.0	9.13E-06	NA	NA	31.7	2.33E-08	NA	NA

PA5078		conserved hypothetical protein	NA	NA	2.2	1.37E-08	NA	NA	2.0	1.37E-08
PA5108		hypothetical protein	2.1	3.93E-10	4.6	8.07E-14	NA	NA	NA	NA
PA5120		hypothetical protein	NA	NA	50.7	3.12E-10	NA	NA	50.7	1.72E-12
PA5148		conserved hypothetical protein	NA	NA	3.8	6.31E-14	NA	NA	2.8	2.75E-14
PA5178		conserved hypothetical protein	NA	NA	4.6	0.00E+00	NA	NA	2.9	0.00E+00
PA5208		conserved hypothetical protein	2.8	1.57E-06	NA	NA	NA	NA	NA	NA
PA5212		hypothetical protein	3.8	1.39E-14	2.8	1.06E-12	NA	NA	NA	NA
PA5229		conserved hypothetical protein	NA	NA	3.2	4.80E-08	NA	NA	2.4	1.57E-06
PA5258		hypothetical protein	2.0	9.23E-06	NA	NA	NA	NA	NA	NA
PA5271		hypothetical protein	NA	NA	4.1	8.78E-14	NA	NA	2.1	7.40E-08
PA5279		conserved hypothetical protein	2.4	1.88E-05	NA	NA	NA	NA	NA	NA
PA5330		hypothetical protein	2.2	5.08E-06	NA	NA	NA	NA	NA	NA
PA5456		hypothetical protein	3.8	2.79E-06	NA	NA	NA	NA	NA	NA
PA5461		hypothetical protein	2.0	1.62E-13	NA	NA	NA	NA	NA	NA
PA5494		hypothetical protein	2.6	0.00E+00	NA	NA	NA	NA	NA	NA
PA5519		conserved hypothetical protein	NA	NA	60.3	4.92E-12	NA	NA	5.1	2.58E-08
PA5545		conserved hypothetical protein	NA	NA	3.7	6.90E-10	NA	NA	2.7	1.84E-08
PA5551		hypothetical protein	NA	NA	26.8	7.87E-06	- 41.7	1.65E-08	NA	NA

Membrane proteins										
PA0013		conserved hypothetical protein	5.7	4.07E-08	NA	NA	NA	NA	NA	NA
PA0340		conserved hypothetical protein	NA	NA	4.8	1.01E-06	NA	NA	3.6	2.09E-06
PA0465	<i>creD</i>	inner membrane protein CreD	9.0	0.00E+00	27.7	0.00E+00	NA	NA	2.2	8.64E-10
PA0833		hypothetical protein	2.5	0.00E+00	2.9	2.19E-14	NA	NA	NA	NA
PA1119	<i>yfiB</i>	YfiB	NA	NA	2.3	1.23E-06	NA	NA	2.1	2.83E-06
PA1199		probable lipoprotein	3.2	1.11E-13	NA	NA	2.1	1.07E-05	NA	NA
PA1288		probable outer membrane protein precursor	2.0	5.81E-10	NA	NA	2.3	0.00E+00	NA	NA

PA1496		probable potassium channel	7.1	4.79E-06	NA	NA	NA	NA	NA	NA
PA1676		hypothetical protein	NA	NA	3.5	9.17E-09	NA	NA	5.1	7.91E-14
PA1773	<i>cmaX</i>	CmaX protein	2.1	1.30E-07	NA	NA	NA	NA	NA	NA
PA2757		hypothetical protein	NA	NA	30.8	1.47E-06	NA	NA	30.8	5.61E-08
PA2763		hypothetical protein	NA	NA	65.2	6.49E-13	NA	NA	65.2	4.64E-14
PA2775		hypothetical protein	3.1	4.06E-12	NA	NA	2.6	1.39E-07	NA	NA
PA2777		conserved hypothetical protein	NA	NA	3.3	5.77E-07	NA	NA	3.1	1.46E-07
PA2900		probable outer membrane protein precursor	2.5	2.11E-06	NA	NA	NA	NA	NA	NA
PA3278		hypothetical protein	4.5	0.00E+00	NA	NA	NA	NA	NA	NA
PA3369		hypothetical protein	NA	NA	49.1	6.39E-10	-76.2	0.00E+00	NA	NA
PA3558	<i>arnF</i>	ArnF	NA	NA	33.1	5.79E-07	NA	NA	33.1	1.77E-08
PA3692	<i>lptF</i>	Lipotoxon F, LptF	NA	NA	6.8	0.00E+00	NA	NA	10.2	1.74E-13
PA3890		probable permease of ABC transporter	NA	NA	51.6	2.10E-10	NA	NA	51.6	1.06E-12
PA4014		hypothetical protein	7.1	9.95E-12	NA	NA	6.3	5.86E-10	NA	NA
PA4034	<i>aqpZ</i>	aquaporin Z	NA	NA	11.1	4.03E-14	NA	NA	4.1	0.00E+00
PA4940		conserved hypothetical protein	2.8	1.92E-05	NA	NA	3.2	1.42E-06	NA	NA
PA5107	<i>blc</i>	outer membrane lipoprotein Blc	2.6	6.54E-09	2.9	1.70E-13	NA	NA	NA	NA
PA5154		probable permease of ABC transporter	3.5	7.84E-09	NA	NA	3.2	5.67E-07	NA	NA
PA5167		probable c4-dicarboxylate-binding protein	NA	NA	3.7	2.80E-14	NA	NA	12.3	0.00E+00
PA5182		hypothetical protein	NA	NA	2.6	8.07E-14	NA	NA	3.6	0.00E+00
PA5183		hypothetical protein	NA	NA	2.8	1.95E-10	NA	NA	3.4	0.00E+00
PA5424		conserved hypothetical protein	2.1	1.37E-10	2.8	5.20E-14	NA	NA	NA	NA
Non-coding RNA gene										
PA4272	<i>P27</i>	P27	2.8	0.00E+00	NA	NA	3.2	1.50E-14	NA	NA
PA4276		tRNA-Trp	NA	NA	2.1	4.62E-14	-2.0	3.12E-13	NA	NA

.1 PA4704	<i>prfF2</i>	regulatory RNA PrrF2	NA	NA	2.6	1.15E-13	-4.8	0.00E+00	NA	NA
.2 PA4758	<i>P32</i>	P32	2.8	1.45E-12	NA	NA	2.1	7.59E-06	NA	NA
.1 PA5181	<i>P34</i>	P34	NA	NA	2.8	9.88E-07	NA	NA	3.1	7.15E-09
Nucleotide biosynthesis and metabolism										
PA5426	<i>purE</i>	phosphoribosylaminoimidazole carboxylase, catalytic subunit	2.8	8.16E-08	NA	NA	NA	NA	NA	NA
Protein secretion/export apparatus										
PA0076	<i>tagF1</i>	TagF1	4.3	1.75E-09	3.7	3.28E-07	NA	NA	NA	NA
PA0081	<i>fha1</i>	Fha1	NA	NA	3.4	7.04E-10	NA	NA	2.5	3.90E-08
PA0088	<i>tssF1</i>	TssF1	NA	NA	2.5	2.35E-07	NA	NA	5.1	1.95E-14
PA3221	<i>csaA</i>	CsaA protein	11.3	2.41E-13	NA	NA	6.3	2.16E-06	NA	NA
Putative enzymes										
PA0745		probable enoyl-CoA hydratase/isomerase	NA	NA	2.1	4.49E-13	NA	NA	2.3	1.95E-14
PA1023		probable short-chain dehydrogenase	11.3	2.19E-05	NA	NA	NA	NA	NA	NA
PA1615		probable lipase	2.5	2.64E-09	3.3	0.00E+00	NA	NA	NA	NA
PA1683		probable sugar aldolase	NA	NA	3.7	7.49E-08	NA	NA	2.7	8.51E-07
PA1828		probable short-chain dehydrogenase	NA	NA	2.4	1.13E-06	NA	NA	3.6	2.47E-12
PA1832		probable protease	2.4	8.27E-07	NA	NA	NA	NA	NA	NA
PA2119		alcohol dehydrogenase (Zn-dependent)	3.1	3.80E-10	NA	NA	NA	NA	NA	NA
PA3302		conserved hypothetical protein	2.5	5.67E-07	NA	NA	2.3	1.09E-05	NA	NA
PA3568		probable acetyl-coa synthetase	NA	NA	2.3	6.74E-06	NA	NA	3.4	3.25E-11
PA4128		conserved hypothetical protein	11.3	5.08E-06	NA	NA	NA	NA	NA	NA
PA4615		probable oxidoreductase	3.1	1.11E-07	NA	NA	NA	NA	NA	NA

PA5234		probable oxidoreductase	5.1	1.11E-07	NA	NA	NA	NA	NA	NA
PA5281		probable hydrolase	5.7	2.29E-05	NA	NA	NA	NA	NA	NA
PA5546		conserved hypothetical protein	NA	NA	2.9	1.45E-07	-3.2	6.55E-09	NA	NA
Related to phage, transposon, or plasmid										
PA0986		conserved hypothetical protein	NA	NA	25.9	1.17E-05	NA	NA	25.9	7.26E-07
Secreted Factors (toxins, enzymes, alginate)										
PA0423	<i>pasP</i>	PasP	NA	NA	3.1	0.00E+00	-2.4	0.00E+00	NA	NA
Transcriptional regulators										
PA0403	<i>pyrR</i>	transcriptional regulator PyrR	NA	NA	3.0	2.47E-10	NA	NA	3.8	0.00E+00
PA0463	<i>creB</i>	two-component response regulator CreB	5.0	3.00E-08	NA	NA	4.7	1.27E-07	NA	NA
PA0491		probable transcriptional regulator	38.7	3.48E-08	NA	NA	NA	NA	NA	NA
PA0527	<i>dnr</i>	transcriptional regulator Dnr	4.0	3.81E-07	NA	NA	NA	NA	NA	NA
PA0535		probable transcriptional regulator	5.7	2.00E-09	NA	NA	NA	NA	NA	NA
PA0839		probable transcriptional regulator	NA	NA	4.8	8.22E-09	NA	NA	81.1	5.59E-14
PA1179	<i>phoP</i>	two-component response regulator PhoP	NA	NA	2.5	0.00E+00	NA	NA	2.1	0.00E+00
PA1261		probable transcriptional regulator	NA	NA	30.4	1.76E-06	NA	NA	30.4	6.97E-08
PA1300		probable sigma-70 factor, ECF subfamily	8.5	2.88E-06	NA	NA	NA	NA	NA	NA
PA1403		probable transcriptional regulator	5.7	7.62E-06	NA	NA	NA	NA	NA	NA
PA1437		probable two-component response regulator	NA	NA	49.6	5.05E-10	NA	NA	49.6	3.04E-12
PA2016	<i>liuR</i>	regulator of liu genes	NA	NA	2.0	2.08E-11	NA	NA	2.0	3.89E-12
PA2093		probable sigma-70 factor, ECF subfamily	42.2	7.35E-09	NA	NA	NA	NA	NA	NA

PA2798		probable two-component response regulator	2.1	6.55E-09	NA	NA	NA	NA	NA	NA
PA2885	<i>atuR</i>	putative repressor of <i>atu</i> genes	4.0	4.51E-08	NA	NA	NA	NA	NA	NA
PA2957		probable transcriptional regulator	2.6	1.03E-08	NA	NA	NA	NA	NA	NA
PA3458		probable transcriptional regulator	4.7	2.74E-08	NA	NA	NA	NA	NA	NA
PA3899		probable sigma-70 factor, ECF subfamily	4.7	9.09E-08	26.8	7.87E-06	NA	NA	NA	NA
PA4853	<i>fis</i>	DNA-binding protein Fis	2.6	1.63E-12	NA	NA	2.5	4.18E-11	NA	NA
PA5261	<i>algR</i>	alginate biosynthesis regulatory protein AlgR	NA	NA	2.9	0.00E+00	NA	NA	2.1	1.35E-13
PA5403		probable transcriptional regulator	34.7	2.02E-07	NA	NA	21.2	7.63E-06	NA	NA
PA5550	<i>glmR</i>	GlmR transcriptional regulator	3.8	6.16E-07	NA	NA	NA	NA	NA	NA
Translation, post-translational modification, degradation										
PA0019	<i>def</i>	polypeptide deformylase	NA	NA	2.1	7.20E-08	NA	NA	2.6	4.60E-13
PA0090	<i>clpV1</i>	ClpV1	NA	NA	2.1	1.99E-06	NA	NA	2.8	7.68E-11
PA0355	<i>pfpI</i>	protease PfpI	NA	NA	25.3	1.47E-05	NA	NA	25.3	9.73E-07
PA0767	<i>lepA</i>	GTP-binding protein LepA	2.0	1.04E-05	NA	NA	NA	NA	NA	NA
PA0837	<i>slyD</i>	peptidyl-prolyl cis-trans isomerase SlyD	2.4	6.71E-11	NA	NA	2.1	1.05E-07	NA	NA
PA1996	<i>ppiC1</i>	peptidyl-prolyl cis-trans isomerase C1	5.7	1.60E-06	NA	NA	NA	NA	NA	NA
PA2755	<i>eco</i>	ecotin precursor	NA	NA	2.4	2.42E-10	NA	NA	2.1	5.98E-09
PA2970	<i>rpmF</i>	50S ribosomal protein L32	2.2	6.56E-13	NA	NA	NA	NA	NA	NA
PA3114	<i>truA</i>	tRNA-pseudouridine synthase I	NA	NA	4.1	3.03E-09	NA	NA	4.1	7.91E-11
PA3600		conserved hypothetical protein	NA	NA	44.7	4.14E-09	NA	NA	44.7	3.99E-11
PA4255	<i>rpmC</i>	50S ribosomal protein L29	2.0	0.00E+00	NA	NA	2.4	3.12E-13	NA	NA
PA5256	<i>dsbH</i>	disulfide bond formation protein	NA	NA	8.3	1.17E-11	NA	NA	3.1	2.20E-07
PA5569	<i>rnpA</i>	ribonuclease P protein comp.	2.4	1.42E-13	NA	NA	2.1	2.75E-12	NA	NA

Transport of small molecules										
PA0970	<i>tolR</i>	TolR protein	2.3	5.18E-10	2.3	1.76E-07	NA	NA	NA	NA
PA1339		amino acid ABC transporter	2.4	1.84E-07	2.3	7.82E-07	NA	NA	NA	NA
PA1493	<i>cysP</i>	ATP binding protein								
		sulfate-binding protein of ABC transporter	3.0	6.40E-11	NA	NA	NA	NA	NA	NA
PA2291		probable glucose-sensitive porin	NA	NA	6.2	4.93E-06	NA	NA	9.2	2.65E-08
PA3186	<i>oprB</i>	Glucose/carbohydrate outer membrane porin OprB precursor	NA	NA	2.8	2.47E-12	NA	NA	5.9	0.00E+00
PA3189		probable permease of ABC sugar transporter	NA	NA	9.0	1.28E-13	NA	NA	2.7	5.17E-07
PA3190		probable binding protein component of ABC sugar transporter	NA	NA	4.2	8.78E-14	NA	NA	2.8	0.00E+00
PA3441		probable molybdopterin-binding protein	33.3	3.87E-07	31.7	1.02E-06	NA	NA	NA	NA
PA4235	<i>bfrA</i>	bacterioferritin	2.4	8.15E-08	NA	NA	NA	NA	NA	NA
PA4502		probable binding protein component of ABC transporter	NA	NA	2.4	2.09E-06	NA	NA	2.5	5.39E-08
PA4506		probable ATP-binding component of ABC dipeptide transporter	3.8	1.08E-05	NA	NA	NA	NA	NA	NA
Two-component regulatory systems										
PA3879	<i>narL</i>	two-component response regulator NarL	7.1	1.47E-07	NA	NA	NA	NA	NA	NA
PA5484	<i>kinB</i>	KinB	NA	NA	2.8	1.13E-05	NA	NA	2.8	9.37E-07

Genes negatively regulated in an AmpR-independent, β-lactam-dependent manner (208)										
Gene ID	Gene name	Product Name	Condition 1		Condition 2		Condition 3		Condition 4	
			Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value	Fold Change	Corrected <i>p</i> -value
Adaptation, Protection										
PA1150	<i>pys2</i>	pyocin S2	NA	NA	-4.4	0.00E+00	NA	NA	-3.8	0.00E+00
PA1151	<i>imm2</i>	pyocin S2 immunity protein	NA	NA	-3.1	0.00E+00	NA	NA	-3.2	2.47E-13
PA3007	<i>lexA</i>	repressor protein LexA	NA	NA	-4.0	0.00E+00	NA	NA	-2.6	3.15E-14
PA3866		pyocin protein	NA	NA	-3.3	0.00E+00	NA	NA	-2.4	4.27E-14
Amino acid biosynthesis and metabolism										
PA0009	<i>glyQ</i>	glycyl-tRNA synthetase alpha chain	NA	NA	-2.0	3.89E-07	2.4	9.54E-10	NA	NA
PA4565	<i>proB</i>	glutamate 5-kinase	NA	NA	-2.1	2.16E-10	2.1	8.47E-10	NA	NA
PA5098	<i>hutH</i>	histidine ammonia-lyase	NA	NA	-14.5	9.80E-12	3.8	1.55E-06	NA	NA
PA5100	<i>hutU</i>	urocanase	NA	NA	-13.7	0.00E+00	NA	NA	-5.4	3.61E-06
PA5143	<i>hisB</i>	imidazoleglycerol-phosphate dehydratase	-123.3	0.00E+00	-10.1	1.50E-14	NA	NA	NA	NA
Antibiotic resistance and susceptibility										
PA0807	<i>ampDh₃</i>	AmpDh3	-2.1	0.00E+00	-4.5	0.00E+00	NA	NA	NA	NA
PA3719	<i>armR</i>	antirepressor for MexR, ArmR	-29.2	3.39E-07	-54.1	1.22E-13	NA	NA	NA	NA
Biosynthesis of cofactors, prosthetic groups and carriers										
PA0381	<i>thiG</i>	thiamine biosynthesis protein, thiazole moiety	NA	NA	-8.7	1.21E-11	NA	NA	-7.8	1.57E-10
PA0512		conserved hypothetical protein	NA	NA	-25.5	7.28E-07	25.5	7.21E-07	NA	NA
PA1272	<i>cobO</i>	cob(I)alamin adenosyltransferase	NA	NA	-35.8	2.73E-09	NA	NA	-46.9	3.73E-10
PA1985	<i>pqqA</i>	pyrroloquinoline quinone biosynthesis protein A	-32.8	5.34E-08	-60.8	0.00E+00	NA	NA	NA	NA

PA3633	<i>ygbP</i>	4-diphosphocytidyl-2-C-methylerythritol synthase	NA	NA	-3.3	1.42E-05	NA	NA	-3.4	1.87E-06
PA4891	<i>ureE</i>	urease accessory protein UreE	NA	NA	-26.1	5.32E-07	26.1	5.25E-07	NA	NA
Carbon compound catabolism										
PA0887	<i>acsA</i>	acetyl-coenzyme A synthetase	NA	NA	-2.6	0.00E+00	2.1	2.74E-13	NA	NA
PA2508	<i>catC</i>	muconolactone delta-isomerase	NA	NA	-45.2	1.57E-11	NA	NA	-24.7	8.15E-06
PA2512	<i>antA</i>	anthranilate dioxygenase large subunit	NA	NA	-5.6	1.42E-13	NA	NA	-12.7	0.00E+00
PA2513	<i>antB</i>	anthranilate dioxygenase small subunit	NA	NA	-89.0	0.00E+00	NA	NA	-233.6	0.00E+00
PA4125	<i>hpcD</i>	5-carboxymethyl-2-hydroxymuconate isomerase	NA	NA	-22.3	4.01E-06	22.3	4.16E-06	NA	NA
Central intermediary metabolism										
PA4864	<i>ureD</i>	urease accessory protein	NA	NA	-20.8	8.97E-06	20.8	9.37E-06	NA	NA
DNA replication, recombination, modification and repair										
PA3617	<i>recA</i>	RecA protein	NA	NA	-2.9	0.00E+00	NA	NA	-2.1	4.64E-14
PA4232	<i>ssb</i>	single-stranded DNA-binding protein	NA	NA	-2.2	6.98E-14	2.2	6.88E-14	NA	NA
PA4763	<i>recN</i>	DNA repair protein RecN	NA	NA	-5.5	0.00E+00	NA	NA	-4.4	0.00E+00
Energy metabolism										
PA0523	<i>norC</i>	nitric-oxide reductase subunit C	NA	NA	-19.9	1.46E-05	19.9	1.53E-05	NA	NA
PA1172	<i>napC</i>	cytochrome c-type protein NapC	NA	NA	-36.7	1.67E-09	NA	NA	-48.1	2.21E-10
PA3929	<i>cioB</i>	cyanide insensitive terminal oxidase	-2.2	1.53E-06	NA	NA	-6.7	2.82E-14	NA	NA
Hypothetical, unclassified, unknown										
PA0014		hypothetical protein	-26.0	1.69E-06	-32.1	2.07E-08	NA	NA	NA	NA
PA0020		hypothetical protein	NA	NA	-2.0	4.45E-06	2.4	3.67E-08	NA	NA
PA0045		hypothetical protein	-3.2	1.43E-05	NA	NA	NA	NA	NA	NA
PA0053		hypothetical protein	NA	NA	-	4.03E-14	NA	NA	-28.2	1.73E-06

PA0116	hypothetical protein	NA	NA	-55.4	6.31E-14	NA	NA	-45.5	7.30E-10
PA0161	hypothetical protein	NA	NA	-4.3	2.49E-14	NA	NA	-2.9	5.14E-10
PA0250	conserved hypothetical protein	-3.0	1.96E-07	NA	NA	-2.7	6.91E-06	NA	NA
PA0277	conserved hypothetical protein	NA	NA	-4.1	3.07E-11	3.6	5.10E-10	NA	NA
PA0449	hypothetical protein	-6.4	2.39E-11	-2.7	1.38E-06	NA	NA	NA	NA
PA0468	hypothetical protein	NA	NA	-5.8	7.61E-06	NA	NA	-5.8	3.09E-06
PA0540	hypothetical protein	-30.5	1.70E-07	NA	NA	NA	NA	NA	NA
PA0578	conserved hypothetical protein	NA	NA	-	0.00E+00	NA	NA	-111.7	4.06E-14
PA0613	hypothetical protein	NA	NA	107.1	0.00E+00	NA	NA	-3.0	2.85E-13
PA0615	hypothetical protein	NA	NA	-7.4	0.00E+00	NA	NA	-2.3	7.47E-14
PA0808	hypothetical protein	NA	NA	-3.2	5.03E-13	NA	NA	-2.3	7.47E-14
PA0808	hypothetical protein	-2.1	1.17E-05	-10.8	0.00E+00	NA	NA	-3.9	2.85E-06
PA0819	hypothetical protein	NA	NA	-29.8	7.27E-08	NA	NA	-24.4	9.09E-06
PA0907	hypothetical protein	NA	NA	-3.8	0.00E+00	NA	NA	-2.9	0.00E+00
PA0908	hypothetical protein	NA	NA	-3.0	0.00E+00	NA	NA	NA	NA
PA0922	hypothetical protein	-2.4	0.00E+00	-6.1	0.00E+00	NA	NA	-3.9	0.00E+00
PA0922	hypothetical protein	NA	NA	-34.4	6.00E-09	34.4	5.49E-09	NA	NA
PA0940	hypothetical protein	NA	NA	-3.6	1.43E-12	NA	NA	-3.7	1.59E-13
PA0947	conserved hypothetical protein	NA	NA	-2.7	8.59E-10	NA	NA	-3.5	3.15E-14
PA0959	hypothetical protein	NA	NA	-4.3	1.94E-12	NA	NA	-3.6	2.22E-09
PA1030	hypothetical protein	NA	NA	-3.9	8.85E-06	NA	NA	NA	NA
PA1035	hypothetical protein	NA	NA	-27.7	2.22E-07	NA	NA	-45.5	7.30E-10
PA1039	conserved hypothetical protein	NA	NA	-3.6	3.47E-14	NA	NA	-2.7	6.05E-14
PA1095	hypothetical protein	NA	NA	-8.0	2.80E-14	NA	NA	-9.7	0.00E+00
PA1152	hypothetical protein	-36.8	7.05E-09	-27.3	2.79E-07	NA	NA	NA	NA
PA1168	hypothetical protein	NA	NA	-22.6	3.34E-06	22.6	3.46E-06	NA	NA
PA1314	hypothetical protein	NA	NA	-47.6	4.11E-12	4.2	5.43E-06	NA	NA
PA1332	hypothetical protein	NA	NA	-27.0	3.18E-07	27.0	3.14E-07	NA	NA
PA1362	hypothetical protein	NA	NA	-19.7	1.56E-05	19.7	1.64E-05	NA	NA
PA1402	hypothetical protein	NA	NA						

PA1536	conserved hypothetical protein	-21.9	1.33E-05	NA	NA	NA	NA	NA	NA
PA1574	conserved hypothetical protein	NA	NA	-4.0	2.49E-14	NA	NA	-2.4	3.07E-07
PA1679	hypothetical protein	NA	NA	-22.5	3.67E-06	NA	NA	-64.5	1.32E-13
PA1743	hypothetical protein	NA	NA	-36.5	1.84E-09	36.5	1.65E-09	NA	NA
PA1751	hypothetical protein	NA	NA	-46.4	8.13E-12	NA	NA	-38.0	2.11E-08
PA1842	hypothetical protein	NA	NA	-24.1	1.48E-06	NA	NA	-39.6	1.04E-08
PA1870	hypothetical protein	-22.2	1.13E-05	NA	NA	NA	NA	NA	NA
PA1936	hypothetical protein	NA	NA	-21.8	5.20E-06	NA	NA	-35.7	5.77E-08
PA2146	conserved hypothetical protein	NA	NA	-26.1	5.32E-07	26.1	5.25E-07	NA	NA
PA2381	hypothetical protein	-5.3	9.84E-14	NA	NA	-7.1	2.21E-14	NA	NA
PA2405	hypothetical protein	NA	NA	-26.5	4.12E-07	NA	NA	-43.5	1.74E-09
PA2436	hypothetical protein	NA	NA	-20.1	1.26E-05	20.1	1.33E-05	NA	NA
PA2450	hypothetical protein	NA	NA	-33.4	1.01E-08	NA	NA	-46.9	3.73E-10
PA2506	hypothetical protein	-21.6	1.54E-05	NA	NA	NA	NA	NA	NA
PA2569	hypothetical protein	NA	NA	-3.6	6.60E-13	3.0	1.10E-10	-2.4	3.79E-06
PA2606	conserved hypothetical protein	NA	NA	-48.7	2.31E-12	NA	NA	-59.9	1.10E-12
PA2660	hypothetical protein	NA	NA	-28.3	1.62E-07	NA	NA	-46.4	4.73E-10
PA2780	hypothetical protein	NA	NA	-3.6	7.89E-07	5.3	5.83E-09	NA	NA
PA3033	hypothetical protein	NA	NA	-3.6	3.20E-08	2.6	8.07E-06	NA	NA
PA3085	hypothetical protein	NA	NA	-3.6	1.20E-08	5.3	1.71E-11	NA	NA
PA3140	hypothetical protein	NA	NA	-92.9	0.00E+00	14.7	0.00E+00	NA	NA
PA3178	hypothetical protein	NA	NA	-69.5	1.04E-13	3.2	1.75E-06	-57.0	4.01E-12
PA3237	hypothetical protein	-21.3	1.79E-05	-19.7	1.56E-05	NA	NA	NA	NA
PA3250	hypothetical protein	NA	NA	-20.7	9.46E-06	NA	NA	-40.7	6.23E-09
PA3352	hypothetical protein	NA	NA	-2.6	4.03E-14	NA	NA	-2.5	1.03E-14
PA3413	conserved hypothetical protein	NA	NA	-2.3	2.49E-14	2.7	6.88E-14	NA	NA
PA3414	hypothetical protein	NA	NA	-5.8	0.00E+00	NA	NA	-5.8	0.00E+00
PA3435	conserved hypothetical protein	-2.7	2.76E-08	NA	NA	NA	NA	NA	NA
PA3451	hypothetical protein	-45.0	1.05E-10	-20.9	8.60E-06	NA	NA	NA	NA

PA3463	<i>wspD</i>	conserved hypothetical protein	-30.7	1.57E-07	-56.9	0.00E+00	NA	NA	NA	NA
PA3472		hypothetical protein	NA	NA	-4.3	1.24E-10	3.2	5.44E-08	NA	NA
PA3492		conserved hypothetical protein	NA	NA	-29.6	7.90E-08	14.7	1.72E-06	NA	NA
PA3530		conserved hypothetical protein	-4.2	3.94E-14	NA	NA	-2.9	2.10E-08	NA	NA
PA3536		hypothetical protein	NA	NA	-74.9	0.00E+00	NA	NA	-61.4	5.51E-13
PA3577		hypothetical protein	-20.7	2.38E-05	NA	NA	NA	NA	NA	NA
PA3611		hypothetical protein	NA	NA	-2.4	1.01E-06	2.3	2.15E-06	NA	NA
PA3634		conserved hypothetical protein	NA	NA	-3.6	4.57E-08	5.3	1.07E-10	NA	NA
PA3662		hypothetical protein	NA	NA	-3.6	1.16E-14	3.5	5.95E-14	NA	NA
PA3705		hypothetical protein	-30.8	1.47E-07	NA	NA	NA	NA	NA	NA
PA3722		hypothetical protein	NA	NA	-4.7	0.00E+00	NA	NA	-5.8	0.00E+00
PA3787		conserved hypothetical protein	-3.1	3.93E-06	NA	NA	-4.1	4.10E-07	NA	NA
PA3822		conserved hypothetical protein	NA	NA	-2.7	0.00E+00	2.5	1.50E-14	NA	NA
PA3951		conserved hypothetical protein	-23.4	6.01E-06	NA	NA	NA	NA	NA	NA
PA3990		conserved hypothetical protein	NA	NA	-85.9	0.00E+00	NA	NA	-46.9	3.73E-10
PA4029		conserved hypothetical protein	-5.3	3.49E-07	NA	NA	NA	NA	NA	NA
PA4298		hypothetical protein	-24.9	2.88E-06	NA	NA	NA	NA	NA	NA
PA4573		hypothetical protein	NA	NA	-42.9	5.28E-11	NA	NA	-46.9	3.73E-10
PA4574		conserved hypothetical protein	NA	NA	-6.5	1.69E-12	3.8	8.31E-09	-4.9	1.37E-08
PA4575		hypothetical protein	-34.6	2.25E-08	NA	NA	-34.6	3.55E-07	NA	NA
PA4580		conserved hypothetical protein	NA	NA	-8.0	2.80E-14	2.9	3.73E-07	-5.8	5.62E-10
PA4674		conserved hypothetical protein	NA	NA	-3.6	3.79E-14	NA	NA	-3.4	1.36E-13
PA4682		hypothetical protein	-24.8	2.95E-06	NA	NA	NA	NA	NA	NA
PA4739		conserved hypothetical protein	-2.1	5.81E-10	-4.3	7.18E-14	NA	NA	-2.9	5.40E-09
PA4782		hypothetical protein	NA	NA	-2.5	2.81E-08	14.7	9.31E-14	NA	NA
PA4793		hypothetical protein	NA	NA	-2.4	3.60E-06	2.3	7.35E-06	-2.2	1.20E-05
PA4794		hypothetical protein	NA	NA	-36.3	2.08E-09	NA	NA	-29.7	8.54E-07
PA4826		hypothetical protein	-2.8	0.00E+00	-10.8	0.00E+00	NA	NA	-2.9	2.70E-07

PA4917		hypothetical protein	NA	NA	-3.8	0.00E+00	NA	NA	-5.8	0.00E+00
PA4918		hypothetical protein	NA	NA	-3.7	0.00E+00	2.4	0.00E+00	NA	NA
PA5126		hypothetical protein	NA	NA	-46.8	6.33E-12	NA	NA	-61.4	5.51E-13
PA5227		conserved hypothetical protein	-2.6	1.34E-07	NA	NA	-2.4	6.82E-06	NA	NA
PA5246		conserved hypothetical protein	NA	NA	-27.7	2.22E-07	27.7	2.15E-07	NA	NA
PA5247		conserved hypothetical protein	NA	NA	-81.6	8.97E-14	NA	NA	-89.2	5.59E-14
PA5269		hypothetical protein	NA	NA	-2.9	1.72E-05	NA	NA	-2.9	5.34E-06
PA5289		hypothetical protein	NA	NA	-67.1	8.97E-14	NA	NA	-55.0	9.82E-12
PA5397		hypothetical protein	-26.7	1.17E-06	-24.7	1.06E-06	NA	NA	NA	NA
PA5402		hypothetical protein	-37.3	5.49E-09	NA	NA	NA	NA	NA	NA
PA5465		hypothetical protein	NA	NA	-3.6	1.11E-10	3.5	2.07E-10	NA	NA
PA5527		hypothetical protein	NA	NA	-3.4	8.49E-14	2.5	1.54E-09	NA	NA
Membrane proteins										
PA0109		hypothetical protein	NA	NA	-41.7	1.03E-10	NA	NA	-34.2	1.15E-07
PA0215		malonate transporter MadL	NA	NA	-43.3	4.46E-11	NA	NA	-70.9	2.73E-15
PA0738		conserved hypothetical protein	NA	NA	-35.6	3.01E-09	35.6	2.73E-09	NA	NA
PA0783	<i>putP</i>	sodium/proline symporter PutP	NA	NA	-3.0	2.25E-06	2.8	9.14E-06	NA	NA
PA0811		probable major facilitator superfamily (MFS) transporter	NA	NA	-10.1	2.57E-09	NA	NA	-6.8	4.92E-06
PA0985	<i>pyoS5</i>	pyocin S5	NA	NA	-5.0	0.00E+00	NA	NA	-3.2	6.05E-14
PA1042		conserved hypothetical protein	NA	NA	-43.8	3.32E-11	NA	NA	-47.9	2.46E-10
PA1072	<i>braE</i>	branched-chain amino acid transport protein BraE	NA	NA	-2.4	1.19E-05	3.4	6.21E-09	NA	NA
PA1513		hypothetical protein	-24.0	4.42E-06	NA	NA	NA	NA	NA	NA
PA1958		probable transporter	NA	NA	-30.4	5.15E-08	8.4	9.24E-06	-24.9	7.27E-06
PA2558		probable transport protein	-27.3	8.74E-07	NA	NA	27.3	7.61E-06	NA	NA
PA2576		hypothetical protein	NA	NA	-43.8	3.32E-11	9.5	3.00E-08	NA	NA
PA3231		hypothetical protein	NA	NA	-27.5	2.44E-07	27.5	2.37E-07	NA	NA

PA3526		probable outer membrane protein precursor	NA	NA	-5.1	1.00E-08	NA	NA	-5.4	5.44E-10
PA3647		probable outer membrane protein precursor	-2.0	2.74E-09	NA	NA	NA	NA	NA	NA
PA3747		conserved hypothetical protein	-2.7	1.34E-06	NA	NA	NA	NA	NA	NA
PA3837		probable permease of ABC transporter	NA	NA	-4.0	1.99E-06	NA	NA	-3.9	1.31E-06
PA5095		probable permease of ABC transporter	-27.7	6.91E-07	NA	NA	NA	NA	NA	NA
PA5482		hypothetical protein	NA	NA	-54.1	1.22E-13	NA	NA	-133.0	1.95E-14
Motility & Attachment										
PA0992	<i>cupC1</i>	fimbrial subunit CupC1	NA	NA	-21.3	6.93E-06	NA	NA	-34.9	8.55E-08
PA1080	<i>flgE</i>	flagellar hook protein FlgE	NA	NA	-3.1	1.98E-07	NA	NA	-2.9	5.90E-07
PA1081	<i>flgF</i>	flagellar basal-body rod protein FlgF	NA	NA	-3.9	3.21E-10	4.2	5.07E-11	NA	NA
PA1083	<i>flgH</i>	flagellar L-ring protein precursor FlgH	NA	NA	-10.8	5.76E-14	2.4	4.26E-06	NA	NA
PA1087	<i>flgL</i>	flagellar hook-associated protein type 3 FlgL	NA	NA	-12.3	3.71E-11	NA	NA	-9.7	8.51E-09
PA1092	<i>fliC</i>	flagellin type B	NA	NA	-2.5	0.00E+00	NA	NA	-2.3	0.00E+00
PA1100	<i>fliE</i>	flagellar hook-basal body complex protein FliE	-3.2	9.42E-11	-2.4	3.91E-08	NA	NA	NA	NA
PA1107		conserved hypothetical protein	-21.7	1.44E-05	NA	NA	NA	NA	NA	NA
PA1447	<i>fliQ</i>	flagellar biosynthetic protein FliQ	-26.3	1.46E-06	-64.9	0.00E+00	NA	NA	NA	NA
PA4299	<i>tadD</i>	TadD	-25.6	2.01E-06	NA	NA	-25.6	1.52E-05	NA	NA
Non-coding RNA gene										
PA0668.5		5S ribosomal RNA	-2.3	0.00E+00	NA	NA	NA	NA	NA	NA
PA0836.1	<i>P5</i>	P5	-33.3	4.23E-08	NA	NA	-33.3	5.98E-07	NA	NA
PA4451	<i>P35</i>	P35	-38.1	3.64E-09	NA	NA	-	7.76E-08	NA	NA

.1						38.1				
PA5471		PA5471 leader peptide	-56.3	3.33E-13	NA	NA	-56.3	3.15E-11	NA	NA
.1										
Putative enzymes										
PA0249		probable acetyltransferase	NA	NA	-29.4	8.89E-08	NA	NA	-32.1	2.92E-07
PA2580		conserved hypothetical protein	NA	NA	-22.2	4.12E-06	NA	NA	-24.3	9.60E-06
PA3172		probable hydrolase	NA	NA	-4.0	4.04E-08	3.3	6.66E-07	-3.4	1.20E-06
PA3968		probable pseudouridine synthase	-4.6	1.16E-06	NA	NA	-6.2	6.36E-07	NA	NA
PA4114		lysine decarboxylase	-4.2	1.76E-06	NA	NA	NA	NA	NA	NA
PA4709		probable hemin degrading factor	-39.9	1.44E-09	NA	NA	NA	NA	NA	NA
PA5445		probable coenzyme A transferase	NA	NA	-4.2	5.04E-10	2.5	5.91E-06	NA	NA
Related to phage, transposon, or plasmid										
PA0621		conserved hypothetical protein	-2.0	9.38E-13	-4.3	0.00E+00	NA	NA	-2.1	2.44E-13
PA0632		hypothetical protein	-2.1	0.00E+00	-5.7	0.00E+00	NA	NA	-2.2	0.00E+00
PA0635		hypothetical protein	-2.1	0.00E+00	-3.4	0.00E+00	NA	NA	NA	NA
PA0638		probable bacteriophage protein	-2.3	5.88E-13	-3.4	1.90E-13	NA	NA	NA	NA
PA0642		hypothetical protein	NA	NA	-3.8	5.73E-13	NA	NA	-2.0	0.00E+00
PA0643		hypothetical protein	NA	NA	-3.7	6.06E-13	NA	NA	-2.2	0.00E+00
PA0646		hypothetical protein	NA	NA	-3.7	7.28E-13	NA	NA	-2.1	0.00E+00
PA0647		hypothetical protein	-2.4	5.93E-13	-5.4	1.24E-13	NA	NA	NA	NA
PA0909		hypothetical protein	NA	NA	-6.7	0.00E+00	NA	NA	-4.2	0.00E+00
Transcription, RNA processing and degradation										
PA0733		probable pseudouridylate synthase	NA	NA	-31.6	2.70E-08	10.5	2.31E-06	NA	NA
Transcriptional regulators										
PA0367		probable transcriptional regulator	-4.6	5.84E-06	-3.6	3.31E-07	NA	NA	NA	NA

PA0424	<i>mexR</i>	multidrug resistance operon repressor MexR	NA	NA	-59.2	0.00E+00	NA	NA	-97.1	4.11E-14
PA0610	<i>prtN</i>	transcriptional regulator PrtN	-2.2	1.50E-13	-9.9	0.00E+00	NA	NA	-3.4	0.00E+00
PA0611	<i>prtR</i>	transcriptional regulator PrtR	NA	NA	-4.2	0.00E+00	NA	NA	-2.3	6.21E-09
PA0612	<i>ptrB</i>	repressor, PtrB	NA	NA	-5.1	0.00E+00	NA	NA	-2.1	3.14E-13
PA0708		probable transcriptional regulator	NA	NA	-20.5	1.05E-05	NA	NA	-25.2	6.47E-06
PA0831	<i>oruR</i>	transcriptional regulator OruR	-6.7	7.91E-07	NA	NA	NA	NA	NA	NA
PA0906		probable transcriptional regulator	NA	NA	-3.9	1.50E-14	2.3	4.71E-09	NA	NA
PA1603		probable transcriptional regulator	NA	NA	-41.4	1.21E-10	NA	NA	-34.0	1.28E-07
PA2277	<i>arsR</i>	ArsR protein	-26.9	1.05E-06	NA	NA	NA	NA	NA	NA
PA2332		probable transcriptional regulator	-20.9	2.22E-05	NA	NA	NA	NA	NA	NA
PA2387	<i>fpvI</i>	FpvI	NA	NA	-27.4	2.66E-07	NA	NA	-29.9	7.84E-07
PA2511		probable transcriptional regulator	NA	NA	-26.2	4.88E-07	NA	NA	-64.5	1.37E-13
PA3067		probable transcriptional regulator	NA	NA	-39.5	3.57E-10	NA	NA	-32.4	2.64E-07
PA3220		probable transcriptional regulator	NA	NA	-45.8	1.10E-11	NA	NA	-37.6	2.58E-08
PA3341		probable transcriptional regulator	-2.6	8.89E-08	NA	NA	-2.6	4.28E-07	NA	NA
PA4109	<i>ampR</i>	transcriptional regulator AmpR	-6.7	0.00E+00	-6.4	0.00E+00	NA	NA	NA	NA
PA4227	<i>pchR</i>	transcriptional regulator PchR	-7.4	8.51E-09	NA	NA	-5.0	2.15E-06	NA	NA
PA4764	<i>fur</i>	ferric uptake regulation protein	NA	NA	-2.2	0.00E+00	NA	NA	-2.6	0.00E+00
PA5308	<i>lrp</i>	leucine-responsive regulatory protein	-4.6	1.25E-07	NA	NA	NA	NA	NA	NA
PA5374	<i>betI</i>	transcriptional regulator BetI	NA	NA	-5.8	3.89E-15	3.9	0.00E+00	NA	NA
Translation, post-translational modification, degradation										
PA2618		hypothetical protein	NA	NA	-30.9	3.92E-08	NA	NA	-30.4	6.24E-07

PA3049	<i>rmf</i>	ribosome modulation factor	-2.3	0.00E+00	NA	NA	-2.5	0.00E+00	NA	NA
PA5049	<i>rpmE</i>	50S ribosomal protein L31	NA	NA	-2.1	0.00E+00	2.1	0.00E+00	NA	NA
PA5315	<i>rpmG</i>	50S ribosomal protein L33	NA	NA	-3.3	0.00E+00	2.2	0.00E+00	-2.3	0.00E+00
PA5470		probable peptide chain release factor	NA	NA	-12.3	0.00E+00	NA	NA	-4.9	4.93E-07
Transport of small molecules										
PA0892	<i>aotP</i>	arginine/ornithine transport protein AotP	NA	NA	-2.1	1.40E-08	2.5	1.36E-11	NA	NA
PA3790	<i>oprC</i>	Putative copper transport outer membrane porin OprC precursor	NA	NA	-4.0	9.12E-07	NA	NA	-4.3	7.52E-08

Table 5: Functional enrichment of genes: AmpR-, AmpR- β -lactam- and β -lactam-dependent genes (Appendix; Supplementary information for Chapter 5; Tables 2, 3 and 4) were tested for enrichment of functional categories using GOEAST (518). There was no significant enrichment in the AmpR- β -lactam-dependent and β -lactam-dependent gene sets.

AmpR +ve

GOID	Ontology	Term	# in list	# in genome	Log-odd ratio	<i>p</i>-value
GO:0050896	biological_process	response to stimulus	25	208	0.989	4.51E-02
GO:0006518	biological_process	peptide metabolic process	8	15	3.138	2.27E-04
GO:0009237	biological_process	siderophore metabolic process	8	9	3.875	1.51E-06
GO:0019184	biological_process	nonribosomal peptide biosynthetic process	8	12	3.46	2.85E-05
GO:0019290	biological_process	siderophore biosynthetic process	8	9	3.875	1.51E-06
GO:0019748	biological_process	secondary metabolic process	8	10	3.723	3.58E-06
GO:0043043	biological_process	peptide biosynthetic process	8	13	3.345	5.87E-05
GO:0043603	biological_process	cellular amide metabolic process	8	21	2.653	4.12E-03
GO:0043604	biological_process	amide biosynthetic process	8	17	2.958	6.79E-04
GO:0044550	biological_process	secondary metabolite biosynthetic process	8	10	3.723	3.58E-06
GO:0016999	biological_process	antibiotic metabolic process	4	6	3.46	3.31E-02
GO:0017000	biological_process	antibiotic biosynthetic process	4	6	3.46	3.31E-02
GO:0017144	biological_process	drug metabolic process	4	6	3.46	3.31E-02

AmpR -ve

GOID	Ontology	Term	# in list	# in genome	Log-odd ratio	p-value
GO:0005737	cellular_component	cytoplasm	14	48	1.714	1.85E-02
GO:0005840	cellular_component	ribosome	13	31	2.238	1.36E-03
GO:0030529	cellular_component	ribonucleoprotein complex	13	32	2.193	1.42E-03
GO:0043226	cellular_component	organelle	13	48	1.608	5.60E-02
GO:0043228	cellular_component	non-membrane-bounded organelle	13	48	1.608	5.60E-02
GO:0043229	cellular_component	intracellular organelle	13	48	1.608	5.60E-02
GO:0043232	cellular_component	intracellular non-membrane-bounded organelle	13	48	1.608	5.60E-02
GO:0044391	cellular_component	ribosomal subunit	13	31	2.238	1.36E-03
GO:0044422	cellular_component	organelle part	13	42	1.8	1.85E-02
GO:0044444	cellular_component	cytoplasmic part	14	44	1.84	8.04E-03
GO:0044446	cellular_component	intracellular organelle part	13	33	2.148	1.62E-03
GO:0006119	biological_process	oxidative phosphorylation	6	12	2.492	8.07E-02
GO:0016310	biological_process	phosphorylation	6	12	2.492	8.07E-02
GO:0015934	cellular_component	large ribosomal subunit	9	18	2.492	5.63E-03

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VITA

DEEPAK BALASUBRAMANIAN

1995-1998	University of Madras B.S. Microbiology
1998-2000	Madurai Kamaraj University M.S. Microbiology
2000-2006	GangaGen Biotechnologies Pvt. Ltd. Associate Scientist
2007-2013	Florida International University Doctoral Candidate in Biology

PUBLICATIONS

D. Balasubramanian, L. Schneper, H. Kumari and K. Mathee. 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. *Nucleic Acids Research*. 41(1): 1-20; doi: 10.1093/nar/gks1039

D. Balasubramanian, S.K. Murugapiran, E. Silva-Herzog, L. Schneper, X. Yang, G. Tatke, G. Narasimhan and K. Mathee. 2013. Transcriptional regulatory network in *Pseudomonas aeruginosa*. In *Bacterial Gene Regulation*. 195-248. M. Babu (Ed.), Horizon Press.

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D. Balasubramanian, and K. Mathee. 2009 Comparative transcriptome analyses of *Pseudomonas aeruginosa*. *Human Genomics*. 3(4):349-61.

AWARDS AND HONORS

- 2013 Dissertation Year Fellowship, University Graduate School, Florida International University.
- 2011 Student Traineeship Award (BALASU11H0), Cystic Fibrosis Foundation.
- 2011 NIH MBRS RISE BRI Student Summer Research Award, Florida International University.
- 2009 Student Traineeship Award (BALASU08H0), Cystic Fibrosis Foundation.
- 2000 Gold medal for Academic Excellence during Master's degree