THE REGULATION AND CHARACTERIZATION OF SURFING MOTILITY IN *PSEUDOMONAS AERUGINOSA*

by

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen associated with a high incidence of infections in hospitalized and cystic fibrosis (CF) patients. *P. aeruginosa* is highly adaptable and exhibits diverse lifestyle adaptations depending on its surrounding environment. Here I studied a complex motility lifestyle termed surfing that occurs in the presence of mucin, a glycoprotein that is found in large abundance in the CF lung, and showed that surfing was associated with broad-spectrum antibiotic resistance, conserved in several bacterial species, and regulated by a complex network of regulators. RNA-Seq revealed ~1,024 genes dysregulated in *P. aeruginosa* under surfing conditions, while a screen of the PA14 transposon mutant library revealed 192 mutants that exhibited surfing deficiency, 40 of which were regulatory genes, including the putative chemotaxis regulator, PA1463, and two-component regulator, *pfeR*. Both PA1463 and *pfeR* were found to be master regulators of *P. aeruginosa* surfing and mutants in these genes demonstrated dysregulation of the majority of other regulators influencing surfing. Using disk diffusion assays, I investigated the adaptive antibiotic resistance associated with surfing motility. *P. aeruginosa* surfing cells were significantly more resistant to several antibiotics including all tested aminoglycosides, carbapenems, polymyxins, fluoroquinolones, and trimethoprim, tetracycline, and chloramphenicol. To identify the genes mediating surfing-dependent antibiotic resistance, transposon mutants in antibiotic susceptibility genes that were dysregulated under surfing conditions were screened for altered susceptibility under surfing conditions. This revealed 65 mutants, including mutants in *armR, recG, atpB, clpS, nuoB,* that exhibited changes in susceptibility to one or more antibiotics, consistent with a contribution to the observed adaptive resistance. It was further demonstrated that other motile bacterial species, including *Escherichia coli*, *Salmonella enterica*, *Vibrio harveyi*, *Enterobacter cloacae*, *Proteus mirabilis*, and *Bacillus subtilis,* exhibited similar characteristics of surfing as observed for *P. aeruginosa* in the presence of mucin, including rapid surface growth, dependence on flagella, and broad-spectrum adaptive resistance. Therefore, surfing is a conserved motile lifestyle regulated by complex networks of regulators and leads to broad spectrum adaptive antibiotic resistance.

Lay summary

Pseudomonas aeruginosa is highly adaptable and exhibits diverse motile lifestyles. Here I studied a novel form of bacterial motility termed surfing that occurs in the presence of mucin as a wetting agent, and showed that surfing was associated with antibiotic resistance, conserved in several bacterial species, and regulated by a complex network of regulators. Using disk diffusion assays, *P. aeruginosa* surfing cells were found to be significantly more resistant to several antibiotics. RNASeq revealed over 1,000 dysregulated genes under surfing. I performed a comprehensive mutant library screen to identify 44 surfing-essential regulators, of which *pfeS* and PA1463 were identified as master regulators involved in surfing. It was further demonstrated that other motile bacterial species also characteristics of surfing including rapid surface spread, dependence on flagella, and broad-spectrum antibiotic resistance. Therefore, surfing is a conserved motile lifestyle regulated by complex networks of regulators and leads to broad spectrum adaptive antibiotic resistance.

Preface

A portion of the research presented in this thesis was drawn from published literature. I was the lead investigator, responsible for all major areas of concept formation, data collection and analysis, as well as manuscript composition. Below is a description of the contributions made by fellow scientists and collaborators. Dr. Robert Hancock was involved in the research presented in all chapters with regards to original conception, research planning, and extensive editing of all written work. The use of all bacterial strains presented in this thesis was appoved by UBC Risk Management Services under the UBC Biosafety Permit Number B14-0207.

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Chapter 1:

Twitching motility assays and associated plate imaging (Figure 1-1) were performed by Morgan Alford.

Chapter 2:

Sections of Chapter 2 have been derived from the following published papers and copyright permissions were granted:

Sun, E., Gill, E. E., Falsafi, R., Yeung, A., Liu, S., & Hancock, R. E. W. (2018). Broad-spectrum adaptive antibiotic resistance associated with *Pseudomonas aeruginosa* mucin-dependent surfing motility. *Antimicrobial Agents and Chemotherapy* 62:pii:e00848-18 (Sun et al 2018a).

Sun, E., Liu, S., & Hancock, R. E. W. (2018). Surfing motility: a conserved yet diverse adaptation among motile bacteria. *Journal of Bacteriol*ogy 200(23):pii:e00394-18. (Sun et al 2018b)

RNA preparation and RNA-Seq was carried out by Reza Falsafi with bioinformatic generation of read-count Tables performed by Dr. Erin Gill who assisted me in writing the methods section. Dr. Amy Yeung was involved in the original conception of the surfing and swimming antibiotic screens as well as the surfing assay including providing guidance on how to perform the screens and analyze the data.

Chapter 3:

Swarming assays and RNA extraction were performed by Shannon Coleman and biofilm assays and RNA extraction by Dr. Daniel Pletzer from our lab. RNA-Seq library preparation and quality control (QC) were performed by Reza Falsafi. Generation of RNA-Seq read count tables and assistance with bioinformatics analysis of data for surfing and swimming cells was done by Dr. Erin Gill. Dr. Amy Lee was responsible for uploading the raw data into GEO. Dr. Daniel Pletzer generated the pyoverdine and pyochelin mutant in PA14. Mutants of *pfe* genes in PAO1 as well as PAO6609 were generously provided by Dr. Keith Poole's lab.

Chapter 4:

RNA-Seq library preparation and QC were performed by Reza Falsafi. Dr. Maren Smith generated RNA-Seq read count tables and assisted with bioinformatics analysis for the *pfeR* and PA1463 mutants and their complemented derivatives.

Chapter 5:

A version of Chapter 5 has been published in Sun et al 2018a, and copyright permission was granted. RNA-Seq library preparation and QC were performed by Reza Falsafi. Generation of RNA-Seq read count tables and assistance with bioinformatics analysis was done by Dr. Erin Gill. Dr. Amy Lee uploaded data onto GEO. A summer student Nicole Liu, directed by me, contributed to the generation of complemented strains and performed liquid MICs.

Chapter 6:

A version of Chapter 6 has been published in Sun et al 2018b, and copyright permission was granted: Nicole Liu assisted in conducting motility assays, antibiotic susceptibility screens, and motility zone growth assays. Mutants in other bacterial species besides *Pseudomonas aeruginosa* were generously provide by the labs of Dr. Bonnie Bassler, Dr. Avigdor Eldar, and Dr. John Gunn, Dr. Rasika Harshey, Dr. Paul Orndorff, Dr. Fitnat Yildiz, and Dr. Paula Watnick.

Chapter 7:

All stringent response mutants, constructs and complemented strains were made by Dr. Daniel Pletzer. He was also responsible for performing all swarming assays and RT-qPCR under swarming conditions, as well as designing the original concepts, and curating experimental results. Research presented is this chapter is currently being drafted into a manuscript with Dr. Pletzer and I as joint first authors.

Chapter 8:

Sections of Chapter 8 have been published in Sun et al 2018a and copyright permission was granted.

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List of abbreviations

$3OC_{12}$-HSL	*N*-3-oxododecanoyl-homoserine lactone
Acyl-HSL	*N*-acylated homoserine lactone
AIDS	Acquired immunodeficiency syndrome
C4-HSL	Butanoyl-homoserine lactone
CDC	Centre for Disease Control
c-di-GMP	Cyclic di-GMP
CMC	Carboxymethyl cellulose
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
DGC	Diguanylate cyclase
ECF	Extracytoplasmic function
eDNA	Extracellular DNA
EPS	Extracellular polymeric substance
HAA	3-(hydroxyalkanoyloxy)alkanoic acid
HHQ	4-hydroxy-2-heptylquinoline
LPS	Lipopolysaccharide
MDR	Multidrug resistance
PDE	Phosphodiesterase
ppGpp	Guanosine 3’5’-bispyrophosphoate
pppGpp	3’-diphosphate-5’-triphosphate
PQS	2-heptyl-3,4-dihydroxyquinoline
QS	Quorum sensing
RND	Resistance nodulation division
ROS	Reactive oxygen species
SCFM	Synthetic cystic fibrosis media
T1SS	Type I secretion system
T2SS	Type II secretion system
T3SS	Type III secretion system
T5SS	Type V secretion system
T6SS	Type VI secretion system
TCA	Tricarboxylic acid
Usp	Universal stress protein

Acknowledgments

Funding for work presented in this thesis was provided by the John Richard Turner Scholarship as well as the Canadian Institute of Health Research (CIHR) and Cystic Fibrosis Canada grants to Dr. REW (Bob) Hancock.

I wish to thank Bob for the opportunity to work under him as his graduate student and make meaningful contributions to research in this area. I am very grateful for his endless patience and support throughout the years. He is an amazing mentor and researcher who continues to be a pioneer in his field. I wish to also thank my committee members, Dr. Erin Gaynor, Dr. Lindsay Eltis, and Dr. Michael Murphy, for their amazing support and guidance throughout the course of my degree.

Much of the work presented in this thesis could not have been done without the continuous support and guidance from current and past members of the Hancock lab. I am especially grateful for their energy and familial ambience. I would especially like to thank the bioinformatics team for their help compiling and managing all the RNA-Seq data, Reza for helping prepare all the libraries for RNA-Seq, and the microbiology group for their input and collaborations. The Hancock lab is such an amazing bunch that have made my experience rich and memorable, and I wish to thank all of them for their positive attitudes.

In addition to all the amazing people who have directly impacted my research and experience as a graduate student, I would also like to thank my family for their patience in letting me pursue this degree and their endless support.

Chapter 1: Introduction

1.1 *Pseudomonas aeruginosa*: a serious threat

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium found ubiquitously in the environment (Azam and Khan, 2018; Opperman and Shachar-Hill, 2016). *P. aeruginosa* can be isolated from both biotic and abiotic environments, from soil and water to organic tissue (Azam and Khan, 2018). *P. aeruginosa* was first discovered in France in 1882 by Carle Gessard who first identified the species by its production of colored pigments which appeared blue-green (Gessard, 1984). *P. aeruginosa* is primarily an opportunistic pathogen, meaning that it is a commensal organism that normally does not cause infections unless the host is compromised. It is a leading cause of death from hospital-acquired or nosocomial infections, primarily involved in pneumonia, urinary tract infections, device-related infections and sepsis (Inweregbu et al., 2005). For example, *P. aeruginosa* is the primary agent and cause of death associated with ventilator-associated pneumonia (Sawa, 2014). *P. aeruginosa* is also known to be involved in several other diseases including: skin infections, bacteremia, chronic respiratory infections associated with cystic fibrosis (CF), joint infections, and infections in burn victims and immunosuppressed patients suffering from cancer or AIDS (Azam and Khan, 2018; Opperman and Shachar-Hill, 2016). *Pseudomonas* carries a large array of genes and broad genetic potential that contributes to its relatively high adaptability and prevalence (Silby et al., 2011). Therefore, *P. aeruginosa* can colonize diverse niches and is involved in several types of infections.

In 2017, the World Health Organization (WHO) released a list of 12 bacterial species classified as high threats to human health and this included *P. aeruginosa* (WHO, 2017). The Centre for Disease Control (CDC) also recognizes *P. aeruginosa* as a "serious threat" (CDC, 2018) accounting for approximately 10% of all hospital acquired infections and chronic infections in CF patients (Bennett, 1974; van Ewijk et al., 2006). In addition, approximately 13% of all *Pseudomonas* infections are associated with multidrug resistance, exhibiting resistance to at least three groups of antibiotics (Azam and Khan, 2018). Its high intrinsic resistance, high adaptability and quick acquisition of resistance has made *P. aeruginosa* one of the world's greatest threats to human health.

1.1.1 Pseudomonas aeruginosa pathogenicity

P. aeruginosa pathogenesis involves a combination of multiple factors that together

contribute to the threat that *P. aeruginosa* poses on human health. Moreover, *P. aeruginosa* is also involved in both acute and chronic infections. During acute infections, *P. aeruginosa* tends to retain a motile lifestyle, but after a series of binary signals, *P. aeruginosa* can undertake a sessile lifestyle growing as a biofilm which contributes to chronic infections (Valentini et al., 2018). Acute infections are characterized by tissue damage and dissemination (Turner et al., 2014). Chronic infections, on the other hand, involve more localized, persistent infections. During chronic infections, *P. aeruginosa* experiences loss of flagella which are immunogenic, formation of biofilms, loss of lipopolysaccharide O-antigenic side chains, and production of copious alginate exopolysaccharide, and causes persistent inflammation due in part to the production of virulence factors (Hancock et al., 1983; Kipnis et al., 2006). Quorum sensing plays an important role in maintaining ongoing inflammation and enhancing biofilm formation (De Kievit et al., 2001; Smith et al., 2002). However, one key binary signal that promotes the switch between lifestyles is cyclic di-GMP (c-di-GMP). C-di-GMP is a secondary messenger that triggers the switch between motile to sessile lifestyle by regulating key aspects of biofilm formation such as the production of the extracellular polymeric substance (EPS) (Kuchma et al., 2007). Many other factors and regulators also reciprocally regulate the planktonic and chronic lifestyles (Gooderham and Hancock, 2009; Yeung et al., 2009). Biofilm formation plays key a role in the transition between acute to chronic infections.

Biofilms are colonial aggregations of bacterial cells encased in an extracellular matrix comprising primarily polysaccharides, proteins, and extracellular DNA (Azam and Khan, 2018). The centres of biofilms exhibit low growth rates due the limited availability of oxygen and nutrients, and the up-regulation of sigma factor S, which contributes to antimicrobial resistance (Borriello et al., 2004; Brown et al., 1988; Yang et al., 2008). Biofilm formation is considered a stress adaptation and follows a defined development program involving complex regulation. It normally begins with reversible attachment to a surface, which in *Pseudomonas* involves pili and flagella (Rasamiravaka et al., 2015). Subsequently there is tighter, almost irreversible, attachment accompanying the production of the exopolysacharride matrix involving in *P. aeruginosa* the expression of exopolysaccharides Pel and Psl, and (more controversially) alginate. Microcolony development leads into the maturation of the biofilm structures and biofilms demonstrate a high level of adaptive multidrug resistance (Taylor et al., 2014). During chronic infections, *P. aeruginosa* can also switch into a mucoidal variant, producing large amounts of

alginate which is a polymer of mannuronic and glucuronic acid (Pedersen et al., 1992). Alginate protects *Pseudomonas* from immune responses including phagocytosis and antimicrobials (Leid et al., 2005). Although it is found to be overexpressed in biofilms, it is not actually necessary for biofilm formation (Schurr, 2013).

1.1.2 Pseudomonas aeruginosa virulence

P. aeruginosa produces a wide range of virulence factors, both cell-associated, such as flagella, pili, and lipopolysaccharides, and extracellular, including proteases, exotoxins, lipases, phospholipases, phenazines, pyocyanin, and hydrogen cyanide (Gonçalves-de-Albuquerque et al., 2016; Kipnis et al., 2006; Son et al., 2007; Strateva and Mitov, 2011). Cell-associated virulence factors, adhesins, aid in binding to host cells to initiate colonization and promote growth on surfaces (Kipnis et al., 2006). Flagella and pili can adhere and tether to epithelial cells (Conrad et al., 2011). Once bound, bacteria can produce enzymes that damage the local tissue to promote spreading. Proteases including elastases (LasA, LasB), staphylolysins, phospholipase C, alkaline proteases promote host tissue damage (Gonçalves-de-Albuquerque et al., 2016; Sawa, 2014). Exotoxins can be injected into host cells using the type III secretion system (T3SS) or secreted into the medium by type II secretion system (T2SS), and these in turn promote necrosis and apoptosis in host cells (Kipnis et al., 2006). T3SS injects cytotoxins (ExoSTUY) into host cells, while the T2SS secretes elastases, alkaline proteases, exotoxin A, and phospholipase C, which can be cytotoxic to, or damage, epithelial cells (Kipnis et al., 2006).

P. aeruginosa also expresses pigmented signaling molecules that contribute to virulence (Jimenez et al., 2012). Pyoverdine is a siderophore that in conjunction with limited iron autoregulates the *pvd* biosynthesis and uptake genes. Once bound to Fe^{3+}, it is taken up across the outer membrane by the ferripyoverdine receptor FpvA in conjunction with TonB1 and other *pvd* genes are involved in reduction to release Fe^{2+} and active transport across the cytoplasmic membrane. In the inner membrane ferripyoverdine interacts with FpvR, an anti-σ factor (Adams et al., 2006) which then promotes the cytoplasmic expression of PvdS and FpvI (Rédly and Poole, 2005). PvdS up-regulates *toxA*, *prpL*, and pyoverdine production. FpvI up-regulates *fpvA* in a positive feedback loop resulting in increased pyoverdine production (Rédly and Poole, 2003).

Other pigmented signaling molecules include the phenazines. Phenazines are redox-active compounds such as pyocyanin produced in both acute and chronic infections. Pyocyanin can suppress lymphocyte proliferation, damage the epithelium, inactivate protease inhibitors, and

target specific host cell functions (Jimenez et al., 2012). Pyocyanin biosynthesis genes, *pyo*, are regulated by the Pqs quorum sensing (QS) system (Jimenez et al., 2012).

1.1.2.1 Secretion systems

P. aeruginosa has six known secretion systems (Filloux, 2011). There are two type I secretion systems (T1SS) (Strateva and Mitov, 2011). The Apr system involves the ABC transporter, AprD, outer membrane protein, AprF, and adaptor, AprE, that work together to secrete proteases such as AprA (Guzzo et al., 1991). A second T1SS is involved in iron acquisition involving HasD, HasE, and HasF. This system is used to secrete the hemophore, HasAp (Létoffé et al., 1998). The major type II secretion system (T2SS) is comprised of 11 genes in two operons (*xcpPQ*, *xcpRSTUVWXYZ*). It makes use of a pseudopilin to excrete several virulence factors such elastases (LasB), staphylolysins (LasA), protease IV, phospholipases (PlcH, PlcN) and exotoxin A (Braun et al., 1998; Lu et al., 1993; Voulhoux et al., 2001). Two other T2SSs identified in *P. aeruginosa* include the Hxc system which is homologous to the Xcp system and secretes the alkaline phosphatase, LapA, independent of the Xcp system, and the Txc system which secretes the chitin-biding protein, CpbE (Ball et al., 2002; Cadoret et al., 2014).The T3SS is the most complex system and is responsible for the secretion of four known effectors (not all are present in all *P. aeruginosa* strains), ExoS, ExoU, ExoT, and ExoY (Yahr et al., 1997). These effectors contribute to the initial stages of infection including tissue damage used to promote dissemination and induction of inflammation (Strateva and Mitov, 2011). ExoS is a cytotoxin with two active domains: C-terminus ADP-ribosyltransferase and N-terminus Rho GTPase-activating domain. Both domains act to disrupt host cytoskeletal organization and activate Toll-like receptors (TLR2, TLR4) to promote inflammation (Epelman et al., 2004). ExoT is similar to ExoS as it also has two active domains that can disrupt the host cytoskeleton; however, it is also involved in preventing phagocytosis and wound repair (Garrity-Ryan et al., 2000). ExoY is an adenylate cyclase that induces the production of the secondary messenger, cAMP (Yahr et al., 1998), resulting in increased cell gap junction permeability allowing bacterial virulence factors to spread and induce inflammation (Castellano and Eugenin, 2014; Hritonenko et al., 2011). ExoU is a phospholipase that disrupts host membrane integrity during acute infections (Kurahashi et al., 1999). The type V secretion system (T5SS) involves a two-step process: effectors leave the inner membrane through the Sec secretion system and are transported through the outer membrane through an integral autotransporter protein. The T5SS is involved in

secreting the protease, LepA, which induces inflammation, and the chaperone protein, CupB5 (Kida et al., 2008; Ruer et al., 2008). The type VI secretion system (T6SS) involves a phage-like injection mechanism into target cells and is primarily involved in interstrain and interspecies competition (2 of the *Pseudomonas* Type V1 systems), although one system attacks host cells (Hood et al., 2017; Lien and Lai, 2017; Logan et al., 2018).

1.2 The role of *P. aeruginosa* in cystic fibrosis

Cystic fibrosis is an autosomal recessive genetic disease resulting from mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which in turn results in irregular electrolyte secretion at mucosal surfaces (Folkesson et al., 2012; Oliver et al., 2000). There are more than 1,500 possible mutations that can result in CF, and 1 out of every 2,500 live births exhibit the disease phenotype (Folkesson et al., 2012). CF affects >70,000 people worldwide (Opperman and Shachar-Hill, 2016). It can result in pulmonary infections, bronchiectasis, pancreatic insufficiency, and diabetes mellitis (Opperman and Shachar-Hill, 2016). Not only are CF patients highly susceptible to infections by *P. aeruginosa* whereby >93% of CF patients between the ages of 18-24 acquire a *P. aeruginosa* infection (Son et al., 2007), but *P. aeruginosa* infections are also the primary cause of death in CF patients (Fothergill et al., 2012; Oliver et al., 2000).

The CFTR is a cAMP-regulated chloride ion channel which regulates electrolyte levels at epithelial surfaces such as the lung epithelium (Folkesson et al., 2012; Gellatly and Hancock, 2013). Irregular chloride levels result in the reduction of fluids within the lungs and increased dehydration leading to the impairment of mucociliary function and accumulation of thickened mucus (Folkesson et al., 2012; Gellatly and Hancock, 2013). This results in reduced microbial clearance and reduced lung immunity. The accumulation of rich mucus also promotes the growth of opportunistic pathogens such as *P. aeruginosa*. It also inhibits oxygen exchange and results in difficulties in breathing (Folkesson et al., 2012). Overall lung function decreases throughout the patient's life. Although the lung flora is a mixture of several organisms, *P. aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae* being the three major players in the CF lungs, CF patients more frequently suffer from infections by *P. aeruginosa* or *S. aureus* (Folkesson et al., 2012). More than half of CF patients develop a *P. aeruginosa* infection by the age of 20. During early life, CF patients tend to have a higher level of *S. aureus* colonization but as changes in physiology of the CF sputum occur, in addition to the use of antibiotics, there is

selective pressure that favours *P. aeruginosa* (Folkesson et al., 2012).

1.2.1 Cystic fibrosis lung environment

The CF sputum environment is relatively rich in amino acids, nicotinamide adenine dinucleotide (NAD), and glutathione which contribute to the growth of *P. aeruginosa*; however, it is relatively poor in cofactors including biotin, pantothenate, and riboflavin (Turner et al., 2015). Synthetic CF sputum medium (SCFM) was found to closely resemble the composition of *in vivo* CF sputum (Palmer et al., 2007; Turner et al., 2015). However, the original SCFM lacked major molecules such as mucin and extracellular DNA which were later introduced in a modified version of the SCFM (Yeung et al., 2012). Mucin is a major glycoprotein, produced by mucosal and submucosal glands, found in the CF lung in large abundance (Li et al., 1997). Mucin is a highly glycosylated polypeptide that acts as a surfactant in the lung sputum, regulating the viscosity of mucus (Gellatly and Hancock, 2013; Yeung et al., 2012). The number of carbohydrate chains and the amount of cross-linking between chains that occurs varies depending on the hydration level, iron concentration, and pH (Celli et al., 2007; Gellatly and Hancock, 2013). Mucin helps bind and trap bacteria, which are swept away by cilia out of the lungs. In the CF lungs, however, due to dehydration, the carbohydrate chains of mucin cannot fold properly, which disrupts its bacterial trapping properties. Instead, it binds tightly to the epithelium through MUC1 and MUC4, and impedes the cilia thus inhibiting their mucocilliary function (Gellatly & Hancock, 2013). *P. aeruginosa* lipopolysaccharides (LPS) also promote overproduction of mucin in the CF lungs by inducing the expression of the *MUC2* gene in epithelial cells (Li et al., 1997).

A study by Son et al (2007) revealed that a total of 437 genes are involved in *P. aeruginosa* pathogenesis *in vivo* in the CF lungs, and 323 are constitutively expressed. These genes appear to be mainly involved in the metabolism of fatty acids, choline, and glycerol, and virulence through phospholipase and lipase production (Son et al., 2007). Several strains of *P. aeruginosa* isolated from the lungs of CF patients were also found to be prone to developing auxotrophic phenotypes to certain amino acids, which in turn also had an effect on *Pseudomonas* metabolism (Oliver et al., 2000; Turner et al., 2015).

The CF lung is a complex environment that *P. aeruginosa* must adapt to with regards to nutritional changes, physiochemical changes, and challenges by the immune system and antimicrobial treatments (Folkesson et al., 2012). One key stress factor faced by *P. aeruginosa* in

the CF lung is the production of reactive oxygen species (ROS) released by host cells, resulting in oxidative stress (Folkesson et al., 2012). Oxidative stress can induce DNA, lipid, and protein damage, which can in turn induce mutations that select for stronger variants. As CF patients suffer from initial *S. aureus* infections, antibiotics are prescribed and enter the lung environment. This is a major factor that promotes the rise of and selection for drug-resistant *P. aeruginosa* (Folkesson et al., 2012).

1.2.2 Acute to chronic infections

During the early stages of CF infection, *P. aeruginosa* adopts a non-mucoidal, motile lifestyle and initiates infection by binding to the mucosal surface via adhesins such as flagella and type IV pili which also promotes inflammation in the surrounding tissues (Opperman and Shachar-Hill, 2016; Valentini et al., 2018). Therefore, during these early stages of CF infection, flagella and type IV pili play key roles in acute infectivity (Penesyan et al., 2015). Flagellar components such as the flagellar cap, FliD, bind to mucin (Arora et al., 1998) as well as airway epithelial cells (Bucior et al., 2012; Feldman et al., 1998). Adhesins at the end of pili also mediate attachment to epithelial cells. After attaching to the surface, bacteria can cause tissue damage leading to inflammation and produce virulence factors that are released into the extracellular space using the T1SS and T2SS, or directly into host cells using the T3SS (Kipnis et al., 2006). Multiple T2SS effectors, as well as T3SS effectors such as phospholipase ExoU and exotoxin ExoY, inhibit phagocytosis by host immune cells and promote significant tissue damage allowing the bacteria to mobilize further (Gonçalves-de-Albuquerque et al., 2016). In rare cases, bacteria can reach the bloodstream and result in septicemia (Turner et al., 2014). As the infection becomes chronic, *P. aeruginosa* tend to experience loss of flagella/motility, reduced expression of virulence factors and the T3SS, adopt an LPS-rough phenotype, and begin to form small colony variants (Valentini et al., 2018). Reducing the expression of motile appendages such as flagella reduces immunogenic recognition through TLR5 (Murray et al., 2007). Loss of LPS O-antigen prevents recognition by serotype-specific antibodies (Hancock et al., 1983). The production of an EPS layer also contributes to immune evasion by preventing the binding of antibodies (Tseng et al., 2013). *P. aeruginosa* can also develop into highly resistant, multicellular structures known as biofilms that especially resist phagocytosis. While acute infections are predominantly aggressive, chronic infections are adapted for long-term persistence and resistance to clearance (Valentini et al., 2018). Bacteria isolated from chronic cases have been

shown to be less virulent than those isolated from acute infections due to their loss of flagella and the down-regulation of virulence factors (Bragonzi et al., 2009).

As *P. aeruginosa* adapt to the CF lung and begin to transition into a more a persistent lifestyle, they produce the polysaccharide alginate (Folkesson et al., 2012). Alginate is produced in response to cell envelope stress as a means of increasing envelope integrity (Wood and Ohman, 2012). It also prevents complement activation and phagocytosis by host immune cells (Leid et al., 2005). A common mutation found in CF variants includes a mutation in *mucA,* an anti-σ-factor*,* which normally regulates alginate production through the *algD* operon (Pulcrano et al., 2012). The mutated *mucA* variant results in the overproduction of alginate, which in turn promotes increased *P. aeruginosa* survival in the CF lungs.

1.2.3 Pseudomonas diversity in CF isolates

P. aeruginosa isolated from CF patients exhibits extensive diversity. In a study done in Spain in 2013-2014, 79 isolates were recovered from 75 patients (López-Causapé et al., 2017). Of the 79 isolates, more than half exhibited multi-drug resistance to at least three different antibiotics and 16% exhibited extensive resistance (i.e. resistance to all tested antibiotics). Mutations in *mutS* and *mutL* were observed in about 15% of the isolates (López-Causapé et al., 2017). Mutations in these two genes promote a mutator phenotype (Oliver et al., 2002), increasing the rate at which mutations appear. Oliver et al (2000) identified the mutational rate of 128 *P. aeruginosa* isolates from 30 CF patients and determined that 36% of those patients were colonized with hypermutable or mutator strains. These hypermutable strains were not found in acutely infected patients, only in chronic cases (Oliver et al., 2000). Hypermutable strains arise due to loss of ability to repair mistakes accumulated during DNA synthesis (e.g. mutations in *mutL* and *mutS*), which result in an increased number of genetic alterations in the chromosome (Oliver et al., 2000). The high frequency of hypermutable strains in the CF lungs, which can be selected for by aggressive antibiotic therapy (Wiegand et al., 2008), is consistent with an advantage conferred by rapid adaptation, which is accomplished by such variants (Oliver et al., 2000). Common mutations were found in *mucA*, *lasR*, and *rpoN* which contribute to increased survivability in the CF lungs (Folkesson et al., 2012). As previously discussed, mutations in *mucA* promote the overproduction of alginate. *Pseudomonas* also tend to exhibit common mutations in *lasR* that promote modification of the lipopolysaccharide (LPS) specifically on Lipid A and loss of the O-antigen in order to avoid host and antibiotic recognition (Feltner et al.,

2016; Yang et al., 2000).

P. aeruginosa in the CF lungs can also exhibit diversity in metabolic states (Jørgensen et al., 2015; Opperman and Shachar-Hill, 2016). Opperman and Shachar-Hill (2016) identified two metabolic phenotypes of *P. aeruginosa* isolated from CF sputum. One phenotype includes increased flux through the tricaboxylic acid (TCA) cycle and Entner-Doudoroff Pathway (EDP) with low flux through the oxidative pentose phosphate pathway (OPPP) and another phenotype with high flux through OPPP and low flux through the TCA cycle (Opperman and Shachar-Hill, 2016). Metabolic diversity is likely a consequence of oxidative stress and nutrient-limiting conditions encountered in the CF lung environment (Opperman and Shachar-Hill, 2016). Therefore, several factors contribute to the rise of mutator variants and metabolically diverse cells in the CF lung that promote *Pseudomonas* survival and colonization.

1.3 Bacterial motility

Bacterial motility is used for finding a new environment through processes such as chemotaxis, contributes to virulence, and can promote social behaviours (Mitchell and Kogure, 2006). Bacteria can have several motile appendages including flagella, and pili or fimbriae. Flagella are long helical structures made of flagellin, locked in the right-hand helical conformation (Harshey, 2003; Mitchell and Kogure, 2006). Bacteria can have one polar flagellum or multiple flagella that promote movement through low viscosity media (Mitchell and Kogure, 2006). Pili, on the other hand, are helical structures that, in *Pseudomonas*, extend out from the poles, have tip-associated adhesins for attachment and promote movement on solid surfaces (Harshey, 2003). Bacteria also rely on motility to promote collective behaviours, including swarming (Harshey, 2003). Collective behaviours help optimize growth and survival by triggering specialized functions, promoting access to nutrients, and influencing defense against the host and desiccation.

1.3.1 Swimming

Figure 1-1 illustrates the different forms of motility as they appear *in vitro*. Swimming occurs under highly aqueous or low agar (0.2-0.35%) conditions and is dependent on flagella (Harshey, 2003; Yeung et al., 2012). *P. aeruginosa* swimming *in vitro* under low agar conditions occurs embedded within the agar, and can be observed to form a translucent halo. Increased viscosity or agar concentration inhibits swimming, which is dependent on high moisture content. The direction of swimming is influenced by chemotaxis, which is the ability of cells to sense

changes in the environment, including concentration gradients of specific nutrients, chemicals and/or oxygen. In the presence of a chemoattractant, flagella will be locked in a counter-clockwise rotation promoting a forward movement towards the attractant (Watari and Larson, 2010). Conversely, in the presence of repellant, flagella rotation will switch to a clockwise rotation allowing the bacteria to "tumble", or in the case of *Pseudomonas* twitch by Brownian motion, and change directions away from the repellant (Watari and Larson, 2010).

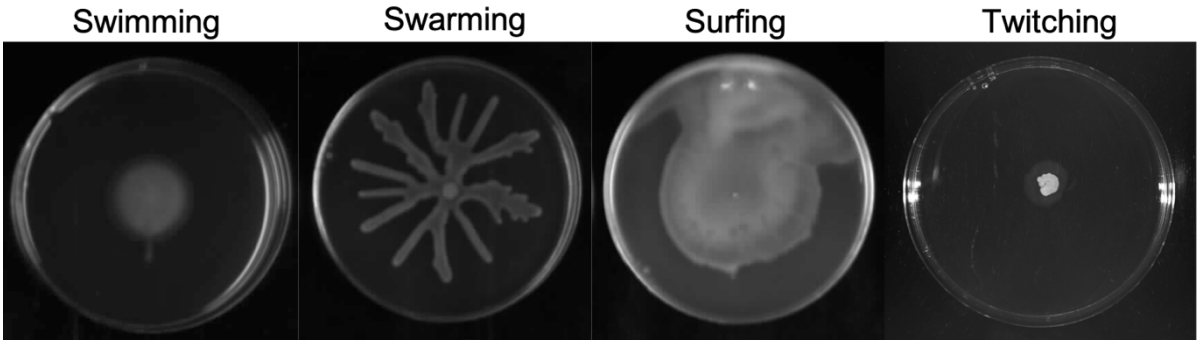


Figure 1-1. Types of *Pseudomonas aeruginosa* motility *in vitro*. Swimming motility was grown in 0.3% agar SCFM with ammonia. Swarming was grown on 0.5% agar SCFM without ammonia. Surfing was grown on 0.3% agar and 0.4% mucin SCFM. Twitching was grown on 1.5% agar BM2. All plates were inoculated with mid-log phase culture and incubated at 37°C for 15 hours.

1.3.2 Twitching

Twitching is pilus-dependent and involves movement on solid surfaces or the interstitial space between the plate and the agar (Yeung et al., 2012). Type IV pili uniquely function independently of flagella and work to help cells adhere and aggregate, as reviewed in (Burrows, 2012). Twitching relies on the retraction of pili at the cell poles (Harshey, 2003). Twitching begins after an assembled pilus attaches to the surface via adhesins at the tip. Movement occurs when the pilus retracts into the body of the cell, dragging the bacterial cell towards the point of attachment (Burrows, 2012). Twitching is also involved in *Pseudomonas* biofilm formation (O'Toole and Kolter, 1998) and plays a role in initial attachment and dispersal (Morgan et al., 2006; O'Toole and Kolter, 1998). Thus mutants deficient in pilus production and twitching exhibit poor biofilm formation (Conrad et al., 2011). Pilus deficient mutants also exhibit an inability to develop into mushroom-shaped structures, forming only the stalk but not the cap structure (Klausen et al., 2003). Therefore, twitching appears to be a mechanism by which cells stack on top of each other to form an aggregate (Klausen et al., 2003).

Twitching motility is affected by external factors such as nutrient levels, viscosity, and surface hydrophobicity as well as internal factors such as the rate of pilin and surfactant production (Burrows, 2012), and can be triggered by specific molecules such as phosphatidylethanolamine (Kearns et al., 2001). Twitching normally occurs in environments with intermediate viscosity or about 1% agar wt/vol (Burrows, 2012)

In addition to environmental cues and presence of specific molecules, twitching is also highly dependent on pilus biosynthesis. Assembly of pilus is regulated by PilT which has an ATPase at the base to provide rotary power (Harshey, 2003). Type IV pilus production is regulated by PilA, and controlled by sigma N and the two-component system PilRS (Burrows, 2012). Twitching is also regulated by c-di-GMP, a secondary messenger involved in regulating biofilm formation, through PilZ which is involved in regulating pilin assembly and FimX which senses environmental cues needed to trigger twitching motility (Amikam and Galperin, 2006; Navarro et al., 2009).

1.3.3 Swarming

Swarming is a complex, community-based motile adaptation that involves both flagella and pili (Köhler et al., 2000). Swarming normally occurs on semi-viscous surfaces with a poor nitrogen source (e.g. amino acids). It is dependent on rhamnolipids and 3-(hydroxyalkanoyloxy)alkanoic acid (HAA), a precursor to rhamnolipids (Caiazza et al., 2005). Swarming is associated with increased production of virulence factors and adaptive resistance (Overhage et al., 2008). Depending on the strain, *P. aeruginosa* swarming can appear dendritic (as shown in Figure 1-1 for strain PA14) or solar flare patterned (PAO1 strain). In *P. aeruginosa*, swarmer cells exhibit polar flagella but also express an alternative motor that facilitates their movement on surfaces (Toutain et al., 2005). When transitioning from swimming and swarming, *P. aeruginosa* can express more than one polar flagella (Kearns, 2010; Köhler et al., 2000). Cells at the swarming front (tips of the tendrils) appear to be significantly longer and more flagellated than cells in the swarm centre which exhibit a non-vegetative morphology (Harshey, 2003). Bacteria at the swarming front appear relatively inactive, but just behind the front cells are vigorously active (Harshey, 2003). Swarming cells group together as they align along their axes (Harshey, 2003). Swarming is dependent on QS in *P. aeruginosa*, in part because the Rhl and Las systems regulate the expression of rhamnolipids required for swarming motility (Köhler et al., 2000). QS regulation of swarming is highly nutrient dependent (Shrout et al., 2006;

Verstraeten et al., 2008). Shrout et al. (2006) demonstrated that depending on the carbon source, quorum sensing would differentially regulate the vigor of swarming motility which in turn affects the structure of a biofilm. Highly active swarmer cells tend to form less structured biofilms than poor swarmers. Therefore, swarming, which has also been shown to have an inverse regulation of certain genes in relation to biofilms (Caiazza et al., 2007), is also an influencer of other adaptations.

Through a comprehensive screen of a PA14 transposon mutant library, Yeung et al. (2009) identified approximately 233 mutants with altered swarming motility. Among the 233 mutants, 12% belong to regulatory genes. In addition to flagella and pili biosynthesis regulators, swarming was found to be dependent on QS (Rhl and Pqs) and other global regulatory systems such as CbrAB, NtrBC, and Arn (Yeung et al., 2009). Interestingly, the mutant in *gacA*, a global regulator involved in regulating the transition between motile to sessile lifestyle, exhibited hyperswarming phenotypes (Yeung et al., 2009). A mutant in *gacA* has previously been shown to exhibit a 10-fold decrease in its ability to form biofilms (Parkins et al., 2001). Conversely, several mutants including *ntrB*, *pilH*, and *arn* that exhibited swarming deficiencies also exhibited a biofilm overproduction phenotype (Yeung et al., 2009), which correlates to the previously reported inverse relationship observed between swarming and biofilm formation (Caiazza et al., 2007).

1.3.4 Sliding

Sliding motility, such as that exhibited by *P. aeruginosa*, was first reported in other bacteria such as *Bacillus subtilis* (Fall et al., 2006). Sliding is a passive form of motility, independent of either pili or flagella, and instead *Pseudomonas* relies on surfactants to propel itself across semi-solid surfaces (Murray and Kazmierczak, 2008). The production of surfactants acts as an expansion force needed to grow the motility zone (Harshey, 2003; Murray and Kazmierczak, 2008). Sliding motility can occur at agar concentrations of 0.3-0.7% wt/vol in both rich and minimal media (Harshey, 2003). Like swarming motility, sliding motility is regulated by the GacA/S system and the hybrid sensor-response regulator RetS as well as the secondary messenger, c-di-GMP (Murray and Kazmierczak, 2008).

1.3.5 Surfing

Swarming had been proposed to reflect the main form of bacterial motility exhibited by *P. aeruginosa* in the CF lungs, due to the conditional requirements for swarming (poor N source

and moderate viscosity) that were similar to those found in the CF lung; however, swarming models lacked a major component in the CF lung that is produced in large amounts, namely mucin (Yeung et al., 2012). The addition of mucin into swarm plates surprisingly induced a novel form of motility known as surfing. Surfing occurs on the surface and appears as a dense circular colony with a thick white outer edges and a blue-green centre (Yeung et al., 2012). The propagation of surfing, i.e how rapidly the surfing zone expands is dependent on the viscosity of the media and on the concentration of mucin (Yeung et al., 2012). It is not affected by the presence of ammonium (NH_4^+), which inhibits swarming motility (Yeung et al., 2012). It is also significantly faster than other forms of motility such as swimming. Mutant studies reveal that surfing is dependent on flagella but not type IV pili (Yeung et al., 2012). Electron microscopy showed that surfing cells in the centre of a surfing colony appeared to be motile and flagellated whereas edge cells were relatively immotile and atrichous (Yeung et al., 2012). Surfing was also shown to be dependent on the Las and Rhl QS systems (Yeung et al., 2012). In comparison to swarming, surfing was found to not be dependent on rhamnolipids, was less stringent with regards to growth conditions and viscosity parameters (Yeung et al., 2012). It was proposed that mucin acts as a wetting agent since replacing mucin with other wetting agents such as Tween-20 or carboxymethylcellulose (CMC) promoted surfing-like motility, albeit not as well as mucin. Yeung et al. (2012) found that the T3SS was down-regulated in surfing cells whereas the T2SS was up-regulated in both the centre and edge cells. There was an overall down-regulation of phenazines and up-regulation of pyoverdine and pyochelin at the edge and down-regulation in the centre relative to swimming. An up-regulation of genes involved in polymyxin resistance was also observed (Yeung et al., 2012). Therefore, surfing appears to be a novel motile lifestyle that involves complex regulation and adaptive phenotypes. Due to the very recent discovery of surfing, not much has been studied regarding this motility and, therefore, this thesis focussed on surfing motility.

1.4 *Pseudomonas* antimicrobial resistance

Antimicrobial agents, including antibiotics, are compounds/molecules that inhibit the growth of or kill microorganisms. Broad-spectrum antimicrobials work against several different species of microbes while a narrow-range antimicrobial targets specific species. Antimicrobials can work in several different ways, by inhibiting cell wall synthesis (e.g. penicillins), cell membrane function (e.g. polymyxins), protein synthesis (e.g. aminoglycosides), nucleic acid synthesis (e.g.

flouroquinolones), or as antimetabolites (e.g. nitrofurans) (Kapoor et al., 2017). Microorganisms can retaliate by developing mechanisms of resistance such as altering the target of the antimicrobial agents, altering membrane permeability or promoting efflux, or producing enzymes that degrade or modify an antibiotic. *P. aeruginosa* is notorious for its high intrinsic resistance and ability to adapt, mutate or acquire genetic elements that increase antimicrobial resistance. Resistance can be genetic (e.g. mutations, acquisition of genetic material) or adaptive (e.g. due to alterations in lifestyle that influence the expression of resisatnce genes). One of the greatest concerns concerning resistance is the evolution of superbugs, which are microorganisms that exhibit resistance to almost all drugs on the market (Breidenstein et al., 2011).

1.4.1 Intrinsic resistance

Bacteria have naturally occurring features or systems that allow them to evade antimicrobial action that collectively contribute to intrinsic resistance (Azam and Khan, 2018; Zhang and Feng, 2016). *P. aeruginosa* is equipped with features such as low outer membrane permeability, the production of multi-drug resistance (MDR) efflux pumps, and enzymes such as Class C β-lactamase that inactive certain antibiotics, and these collectively contribute to its high intrinsic resistance (Azam and Khan, 2018; Breidenstein et al., 2011). Low outer membrane permeability occurs due to the inefficiency of so-called porin proteins that form channels enabling antibiotic uptake, leading to low outer membrane permeability to antibiotics and consequent limited (slow) entry (Fernández et al., 2012).

There are several classes of drug efflux pumps known, including the multidrug and toxin compound extrusion (MATE), major facilitator superfamily (MFS), small multidrug resistance (SMR), and resistance nodulation division (RND) families (Azam and Khan, 2018). Major *P. aeruginosa* efflux pumps systems including the MexAB-OprM system belong to the RND family of efflux systems (Azam & Khan, 2018; Gellatly & Hancock, 2013). RND family efflux pumps span the outer membrane, periplasm, and inner membrane due to a tripartite structure involving a gated channel, adapter protein, and transporter respectively (Azam and Khan, 2018). *P. aeruginosa* constitutively expresses the MexAB-OprM system which contributes to resistance to a majority of β-lactams (Masuda et al., 2000) and conditionally, or due to multidrug resistance regulatory mutations, can express many other RND assemblies. Efflux pumps in general contribute to resistance against many classes of antibiotics including β-lactams, aminoglycosides, fluoroquinolones, tetracycline, chloramphenicol etc. (Azam and Khan, 2018).

Bacteria also encode enzymes that recognize antibiotics and degrade or modify/inactivate them. Mechanisms include chemical modifications, hydrolyzation, and reducing the affinity of an antibiotic to its target (Azam and Khan, 2018). *P. aeruginosa* expresses inducible AmpC β-lactamase that hydrolyzes most variants of β-lactams, and possibly PoxB which can break down certain carbapenems (Berrazeg et al., 2015; Zincke et al., 2016).

1.4.2 Acquired resistance

Acquired resistance normally occurs through the acquisition of extracellular genetic material through horizontal gene transfer as well as through mutations (Breidenstein et al., 2011). Horizontal gene transfer includes the exchange of plasmids, transposons, integrons, prophages, and resistance islands (Breidenstein et al., 2011). Mutations, on the other hand, occur due to replication errors which can be enhanced by certain chemicals or conditional inducers. For example, there is an increased frequency of mutations when bacteria are subjected to sub-inhibitory concentrations of certain antibiotics (Breidenstein et al., 2011). Mutations resulting in resistance can occur in the antimicrobial target to prevent recognition or reduce binding affinity or in the genes/mechanisms associated with intrinsic resistance. For example, certain mutations in the DNA gyrase genes (*gyrA*, *gyrB*) and topoisomerases IV genes (*parC*, *parE*) result in resistance to fluoroquinolones (Bagel et al., 1999). Mutations can also occur to upregulate the expression of efflux pumps which then promote efflux and reduce net uptake or can occur to increase the levels of enzymes that hydrolyze or modify antibiotics to promote resistance (Azam and Khan, 2018; Breidenstein et al., 2011). Overexpression mutants in *ampC*, β-lactamase, and/or derepression of the *mexABOprM* and *mexXY* efflux pumps, can arise during CF infections and promote increased resistance to β-lactams such piperacillin and ceftazidime or aminoglycosides and specific cephalosporins respectively (Berrazeg et al., 2015; Cabot et al., 2011). Those genes that mediate resistance when mutated are collectively known as the resistome for specific antibiotics (Breidenstein et al., 2011).

1.4.3 Adaptive resistance

Adaptive resistance is a form of reversible resistance that is induced as a consequence of certain adaptations to environmental changes or growth conditions (Azam and Khan, 2018; Breidenstein et al., 2011). Intrinsic and acquired resistance are often irreversible, in contrast to adaptive resistance which is transient and dependent on the triggering condition (Azam and Khan,

2018). Therefore, when conditions are reversed, susceptibility is restored (Breidenstein et al., 2011). Adaptive resistance was first discovered when exposure to tetracycline was found to induce plasmid-mediated tetracycline resistant gene expression (Bochner et al., 1980). Analogously, biofilms were found to have several mechanism of non-mutational resistance that were dependent on the biofilm growth state (de la Fuente-Núñez et al., 2013; Minami et al., 1980).

Adaptive resistance can involve the expression of efflux pumps, cell envelope proteins, and antibiotic-modifying enzymes, to name a few mechanisms (Breidenstein et al., 2011). It can occur as a consequence of stress responses and environmental cues. For example, polymyxins and aminoglycosides, which normally self-promote their uptake through binding to outer membrane LPS lipid A, induce the expression of *arn* genes which are involved in the modification (addition of aminoarabinose) of the lipid A, inhibiting self-promoted uptake of polycationic antibiotics (Fernández et al., 2010). The expression of *arn* genes is regulated by several two-component systems, which senses the presence of divalent cations and cationic compounds including antimicrobial peptides (Fernández et al., 2012). Exposure to chemical changes in the environment such as the buildup of ROS in the CF lung can also induce stress responses that promote the up-regulation of the MexXY-OprM efflux system, which in turn promotes resistance to aminoglycosides (Fraud and Poole, 2011).

As *Pseudomonas* exhibits high adaptability to diverse environments, it can deploy diverse responses that include changes in lifestyle (motile to sessile), changes in motility, and stress adaptations. These adaptations including swarming and biofilm formation can lead to adaptive resistance (de la Fuente-Núñez et al., 2013; Overhage et al., 2008).

1.4.4 Biofilm-associated resistance

Biofilms are highly resistant structures, which may be substantially related to their altered physiology and transcriptional patterns, including a dysregulation of regulatory and effector genes mediating resistance (Taylor et al., 2014). For example, some cells exhibit reduced metabolism and growth rate which contributes to increased persistence (Sultana et al., 2016). Thus, antibiotics such as aminoglycosides which target growing cells are ineffective against cells in the interior of a biofilm which may also exhibit persistence (Sultana et al., 2016). Persister cells are slow growing, non-dividing cells that can withstand high concentrations of antibiotics (Breidenstein et al., 2011). Additionally, accumulated mutations and mutator variants also

promote the up-regulation of resistance genes. Therefore, biofilms are often saturated with β-lactamases due to overexpression mutations as an extra layer of resistance (Breidenstein et al., 2011). Consequently, several characteristics of biofilm cells contribute to its high level of resistance against a broad range of antibiotics. Other likely contributors include adaptations to stressors that has been proposed to trigger biofilm development, and quorum sensing (Hall and Mah, 2017).

1.5 *Pseudomonas* pangenome organization and gene regulation

P. aeruginosa is known to have one of the largest bacterial genomes at 6.3 Mbp (5,567 genes) in comparison to other bacteria such as Gram-negative *E. coli* that has a genome size of 4.6 Mbp (4,279 genes) and the Gram-positive *S. aureus* with a genome size of 2.8 Mbp (2,594 genes) (Azam and Khan, 2018). Approximately 8.4-9.4% (468-521 genes) of the *P. aeruginosa* genome is predicted to encode regulatory genes, including two-component regulators and transcriptional regulators (Stover et al., 2000) which suggest additional complexity when compared to the estimated 5.8% regulators encoded in the *E. coli* genome (Azam and Khan, 2018). *P. aeruginosa* contains ~60 known two-component systems (Strateva and Mitov, 2011). The general pattern for these two-component systems involves a sensor kinase that in response to a signal autophosphorylates and then phosphorylates (usually activating) a response regulator. CbrAB is a global two-component system that regulates a large number of genes involved in virulence, antibiotic resistance, carbon metabolism and swarming (Yeung et al., 2011). CbrA is the sensor kinase which activates the regulator, CbrB. This system then regulates the expression of other major regulators such as *phoPQ*, *arn*, and *pmrAB* (Yeung et al., 2011).

Recent analysis has suggested that *P. aeruginosa* contains more than 690 predicted regulatory genes and >1,020 predicted regulatory interactions (Galán-Vásquez et al., 2011). Regulatory hierarchies are composed of origins at the top, where activating interactions/signals occur, while the lower end of the hierarchy is normally enriched in regulators of moderate subsets of effectors (Galán-Vásquez et al., 2011). Global regulators are those that regulate a large number of genes, often regulating other transcriptional factors and sigma factors, and target the promoters of genes with more than one sigma factor regulating them (Galán-Vásquez et al., 2011). Galan-Vasquez et al. (2011) predicted that the most influential transcriptional regulators in *P. aeruginosa* are LasR, RhlR, both involved in quorum sensing, Fur, MexT, and Anr, although this was likely biased by the interests of researchers who performed the analyzed

studies.

1.5.1 Gac/Rsm cascade

GacA/S is a two-component regulatory system involving a sensor kinase (GacS) and response regulator (GacA). GacA regulates the expression of two small regulatory RNAs, RsmY and RsmZ (Brencic et al., 2009). RsmY and RsmZ sequester RNA binding protein RsmA which is a translational repressor for several virulence factors including pyocyanin, hydrogen cyanide, and elastases, but an activator of Pel, Psl, and c-di-GMP synthesis (Heurlier et al., 2004). C-di-GMP levels are regulated by diguanylate cyclases (DGC) which produce c-di-GMP and phosphodiesterases (PDE) which hydrolyze c-di-GMP (Jimenez et al., 2012). One influential DGC is encoded by the gene *wspR*. Under specific conditions, WspF phosphorylates WspR to activate the production of c-di-GMP (Jimenez et al., 2012). FleQ and LasR can also regulate c-di-GMP levels. LasR regulates the tyrosine phosphatase, TpbA, which dephosphorylates and inactivates TpbB, a membrane-bound DGC, which in turn decreases c-di-GMP levels (Ueda and Wood, 2009). A decrease in c-di-GMP promotes an increase in EPS production and biofilm formation (Jimenez et al., 2012; Valentini and Filloux, 2016). The inactivation of RsmA, therefore, results in an increase in EPS synthesis and reduction of virulence factors (Gellatly & Hancock, 2013; Jimenez et al., 2012). RetS is a hybrid sensor/regulator that also regulates GacA by repressing it through the production of c-di-GMP produced by WspR (Goodman et al., 2009; Valentini and Filloux, 2016).

The Gac/Rsm regulatory system is involved in the transition between motile to sessile lifestyles (Valentini et al., 2018). RsmA is a key post-transcriptional regulator that positively regulates the expression of virulence factors, more highly expressed during acute infection, and negatively regulates genes involved in biofilm formation. RsmA-regulated virulence factors include flagella, type IV pili, rhamnolipids, and type II and III secretion systems and their effectors (Valentini et al., 2018). Conversely, RsmA down-regulates the expression of genes involved in EPS production such as *pel* and *psl* genes, quorum sensing, and the T6SS (Allsopp et al., 2017; Irie et al., 2010). Mutants in *rsmA* exhibit reduced virulence and ability to spread during acute infections in mice but exhibit increased persistence during chronic infections (Mulcahy et al., 2006). Increased RsmY/Z levels, as regulated by RsmA, promote attachment which triggers the initiation of biofilm formation (Valentini et al., 2018). RsmZ levels are subsequently reduced by the two-component system, BfiSR and MifSR, which further promote

growth of a biofilm by preventing reversion of the attachment (Petrova and Sauer, 2009). Mature biofilms exhibit high levels of RsmY/Z compared to planktonic cells. Decreased levels of RsmY/Z promote dispersal (Valentini et al., 2018).

RetS is a hybrid sensor kinase-response regulator that regulates GacAS in order to regulate the expression the two small RNAs, RsmY and RsmZ (Bordi et al., 2010). Consequently, it regulates biofilm formation and the expression of several virulence factors and the T2SS and T3SS. In turn, RetS is also regulated by HptB which can also regulate the expression of *rsmY* (Bordi et al., 2010). HptB up-regulates RetS which inhibits GacS and the production of RsmY and RsmZ, thus inhibiting biofilm production genes and activating the expression of virulence factors and the T3SS (Bordi et al., 2010). Therefore, the Gac/Rsm system acts as a global regulator that plays an important role in the transition between acute to chronic infection. It is worth mentioning however that there are many regulators that appear to independently regulate this switch between acute/motile (swarming) and chronic/sessile (biofilm) lifestyles (Yeung et al., 2009).

1.5.2 Quorum sensing system

Quorum sensing (QS) is a system that is conceptually conserved in Gram-negative bacteria (Kipnis et al., 2006). It involves the release of small signalling molecules in response to changes in cell density (Azam and Khan, 2018). Each signalling molecule, when it reaches a given concentration in the community, acts as a cofactor that binds to a specific transcriptional regulator (Kipnis et al., 2006). Quorum sensing is a form of intercellular communication that allows bacteria to develop community-based adaptations. In *P. aeruginosa*, more than 300 genes are regulated by quorum sensing (Azam and Khan, 2018). *P. aeruginosa* QS regulates the production of rhamnolipids, pyocyanin, elastases, alkaline proteases, and hydrogen cyanide. In addition, it also regulates genes involved in biofilm development and ROS defense (Winstanley et al., 2009). There are currently four known quorum sensing systems in *P. aeruginosa*: the N-acylated homoserine lactone (acyl-HSL) systems Rhl and Las, and the quinolone-based systems Pqs and Iqs (Azam and Khan, 2018; Gonçalves-de-Albuquerque et al., 2016). Table 1-1 defines the respective autoinducer molecules, synthases and regulators for each of the four systems. The systems have also been shown to overlap with one another as shown in Figure 1-2. Thus LasR bound to its autoinducer, $3OC_{12}$-HSL, for example, has been shown to activate the expression of *rhlR*, *rhlI*, *pqsR*, and the 2-heptyl-3,4-dihydroxyquinoline (PQS) autoinducer synthase operon

(McGrath et al., 2004; Pesci et al., 1997). Recently, it has been proposed that RhlR can also bind to an alternative ligand to induce Rhl-dependent gene expression to compensate for a lack of LasR-induced expression of its autoinducer synthase, *rhlI* (Mukherjee et al., 2017). The PQS autoinducer has also been found to regulate the Las and Rhl QS systems (McKnight et al., 2000). The Iqs system is regulated by the Las system, and is triggered by phosphate starvation (Lee et al., 2013).

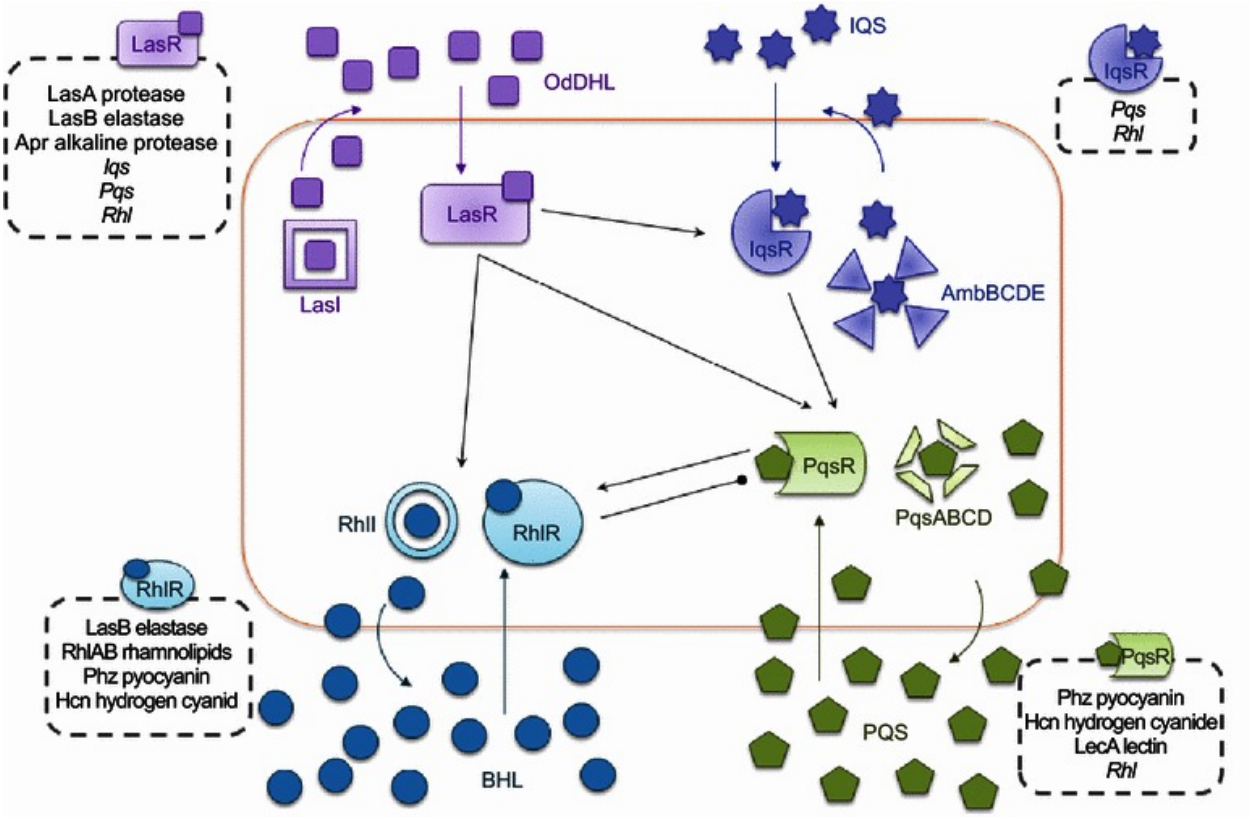


Figure 1-2. *Pseudomonas* quorum sensing systems. The Las (purple), Rhl (blue), Pqs (green) and Iqs (blue) quorum sensing systems including their respective autoinducer synthases, autoinducer molecules, and regulators. Arrows indicate the direction of binding. Dashed-line boxes list proteins of genes regulated by each regulator bound to its respective autoinducer. Modified and reprinted with permission from (Lee and Zhang, 2015).

The Pqs autoinducer, PQS, is made from anthranilate and α-keto-fatty acids that are converted into 4-hydroxy-2-heptylquinoline (HHQ) which in turn is catalyzed by PqsH to PQS which, when it reaches a certain threshold level, binds to PqsR (Jimenez et al., 2012; Kim et al., 2010). HHQ, however, can also act as an autoinducer by binding to PqsR and activating several PQS-regulated genes, all except phenazine and lectin genes (Xiao et al., 2006). PQS can also complex with iron to act a chelator. It sequesters iron closer to the cell to facilitate pyoverdine

and pyochelin function (Bredenbruch et al., 2006).

Table 1-1. *P. aeruginosa* quorum sensing systems including their respective autoinducers, synthases, and regulators (Kipnis et al., 2006; Kiratisin et al., 2002; Lee et al., 2013; Mukherjee et al., 2017; Strateva and Mitov, 2011).

Quorum sensing system	**Autoinducer molecule**	**Autoinducer synthase(s)**	**Regulator**
Las	*N*-3-oxo-dodecanoyl-homoserine lactone ($3OC_{12}$-HSL)	LasI	LasR
Rhl	*N*-butanoyl homoserine lactone (C_4-HSL)	RhlI	RhlR
Pqs	2-heptyl-3-hydroxy-4-quinolone (PQS)	PqsABCDE, PqsH	PqsR
Iqs	2-(2-hydroxy-phenyl)-thiazole-4-carbaldehyde (IQS)	AmbBCDE	undetermined

Quorum sensing links cell density to the regulation of the production of several virulence factors (Strateva and Mitov, 2011). QS transforms environmental signals into the expression of certain genes. The Las system is known to regulate genes such as *lasI, lasB, lasA, apr*, and *toxA* which are elastases, proteases and exotoxins (Kiratisin et al., 2002; Pearson et al., 1997). The Rhl system is known to regulate *rhlI* as well as *rhlAB*, which express rhamnolipids, and *rpoS*, the stationary phase sigma factor involved in regulating a large number of virulence factors and T3SS effectors (Mukherjee et al., 2017; Pearson et al., 1997). The *Pseudomonas* Pqs system is found to regulate a number of genes including some overlapping genes with the Rhl and Las systems including elastases, rhamnolipids, and pyocyanin (Strateva and Mitov, 2011). It also regulates genes involved in biofilm formation (Guo et al., 2014). The Pqs system has been shown to be up-regulated during persistent infections where *P. aeruginosa* variants frequently lose LasR regulation but retain active Rhl and Pqs regulation (Feltner et al., 2016). The Las system, however, has been shown to be crucial in enabling normal biofilm architecture, since mutants in the Las system develop abnormal (flat) biofilms (Davies et al., 1998). As infections progress from acute to chronic, there is selective pressure for non-functional mutations in *lasR* (Feltner et al., 2016).

Quorum sensing systems are also subjected to other levels of regulation outside of the inter-regulation among the four known systems. For example, QscR can form a complex with LasR and RhlR to delay expression of QS genes (Chugani et al., 2001). RsaL is a transcriptional repressor of *lasI* which inhibits expression of Las-dependent genes as well as directly repressing the expression of virulence genes involved in hydrogen cyanide and pyocyanin synthesis (Rampioni et al., 2006). QteE and QslA prevent activation of Rhl and Las QS during stationary

phase, whereby QteE controls the post-translation levels of LasR and RhlR, while QslA complexes with LasR to prevent DNA binding (Asfahl and Schuster, 2018).

1.5.3 Stringent response

The stringent stress response is a conserved mechanism in bacteria that occurs in response to environmental cues such as amino acid and nutrient starvation leading to transcriptional changes. It is mediated by the nucleotide secondary messengers, guanosine 3'5'-bispyrophosphoate (ppGpp) and 3'-diphosphate-5'-triphosphate (pppGpp), collectively known as (p)ppGpp (Boes et al., 2008; Khakimova et al., 2013; Vogt et al., 2011). In most Gram negative bacteria (p)ppGpp is an alarmone synthesized using ATP, primarily by the enzymes RelA and SpoT, whereby SpoT has both the ability to synthesize and hydrolyze (p)ppGpp (Khakimova et al., 2013). Amino acid starvation triggers a RelA-induced response, whereas SpoT responds to several forms of stimuli including membrane perturbations, carbon-limiting conditions, and inhibited fatty acid metabolism (Boes et al., 2008). When amino acid limitation occur, the lack of amino acids loading onto tRNAs results in an abundance of unloaded tRNAs entering the ribosome, which triggers the activation of RelA bound to the ribosome (Vogt et al., 2011). (P)ppGpp is synthesized, which in turn binds to RNA polymerase to redirect transcription, resulting in a global decrease in ribosomal protein synthesis, an increase in amino acid biosynthesis and proteolysis, a decrease in DNA replication, phospholipid, murein, and carbohydrate synthesis, and increased sigma S production (Vogt et al., 2011). Sigma S up-regulates the expression of glycolysis, oxidative stress response, stasis, and osmotic stress response proteins. Thus the stringent response copes with stress by reducing macromolecular synthesis and increasing stress coping mechanisms. The SpoT-induced stringent response, but not the RelA-induced response system, was also found to be involved in regulating the expression of universal stress response (*usp*) genes (Boes et al., 2008).

The *Pseudomonas* stringent response was found to play a role in the oxidative stress response, which is normally regulated by OxyR that detects hydrogen peroxide levels and activates transcription of the oxidative stress response genes, *katA*, *katB*, *ahpB*, and *ahlpCF*, encoding catalases and reductases (Khakimova et al., 2013). A stringent response mutant, Δ*relA*Δ*spoT*, was found to be more sensitive to ROS, and the stringent response was found to be involved in regulating QS, which in turn regulates the expression of *oxyR* (Khakimova et al., 2013). The stringent response also regulates the expression of several virulence factors since the

stringent response double mutant exhibits reduced production of certain secreted virulence factors (Vogt et al., 2011). The stringent response mutant was also shown to have attenuated virulence in mouse infection models; therefore, the stringent stress response appears to play a key role in *Pseudomonas* virulence (Pletzer et al., 2017).

The stringent response, (p)ppGpp specifically, was found to negatively regulate the production of HHQ and PQS involved in PQS quorum sensing (Schafhauser et al., 2014). More specifically, ppGpp appears to regulate the Las and Rhl systems which in turn regulate the PQS system. The Las system induces the expression of PqsH, involved in converting HHQ to PQS, and PqsR, the Pqs regulator. RhlR, however, inhibits PqsR and PqsABCD production, inhibiting the production of HHQ and Pqs-induced gene expression (Schafhauser et al., 2014). Therefore, an accumulation of HHQ in the stringent response mutants suggests that the stringent response regulates the PQS system primarily through ppGpp-induced transcription of *lasR* which in turn regulates the expression of *pqsH* involved in converting HHQ to PQS (Schafhauser et al., 2014).

1.5.4 Chemotaxis

Chemotaxis is the ability of bacteria to sense gradients of changes in the environment and results in bacterial movement away from increased concentrations, in the presence of a repellant, or towards increased concentrations of an attractant (Mitchell and Kogure, 2006). Chemotaxis involves two types of proteins, sensors and transducers, which work coordinatively to respond to specific changes in the environment (Porter et al., 2008). The chemotaxis sensor is a cluster of sensory proteins called methyl-accepting chemotaxis proteins (MCP) at the cell envelope that act to amplify and trigger response to chemotactic signals (Porter et al., 2008). The presence of a chemoattract will trigger the dephosphorylation of CheA, the sensor kinase, which dephosphorylates the response regulators, CheB and CheY, as shown in Figure 1-3 (Porter et al., 2008). CheY is bound to flagellar motor switch proteins and dephosphorylated CheY triggers a counter-clockwise rotation of the flagellar motor, allowing the bacterium to move towards the attractant or “run” (Porter et al., 2008). The presence or change in a chemical gradient can trigger a net change in the frequency of runs, or tumbles which reorient the bacterium (Mitchell and Kogure, 2006). The presence of a chemoattractant reduces the frequency of tumbles (NB. *Pseudomonas* does not tumble per se but instead reorients by Brownian motion), and promotes more runs, allowing the bacterium to move towards the chemoattractant (Mitchell and Kogure, 2006). CheB and CheR work together to regulate the methylation of MCP to trigger and reset the

system. The dephosphorylation of CheB results in the methylation of the MCP. CheR also methylates the MCP which phosphorylates CheA, triggering a clock-wise rotation or tumble (Porter et al., 2008). CheZ is another chemotaxis protein that regulates the phosphorylation status of CheY to reset it to a "stalled" state. Besides regulating swimming motility, *P. aeruginosa* also has a chemotaxis system that regulates pili retraction during twitching motility through a similar set of chemotaxis proteins encored by *chp* genes that work in a similar fashion as the Che proteins, illustrated in Figure 1-3 (Sampedro et al., 2015).

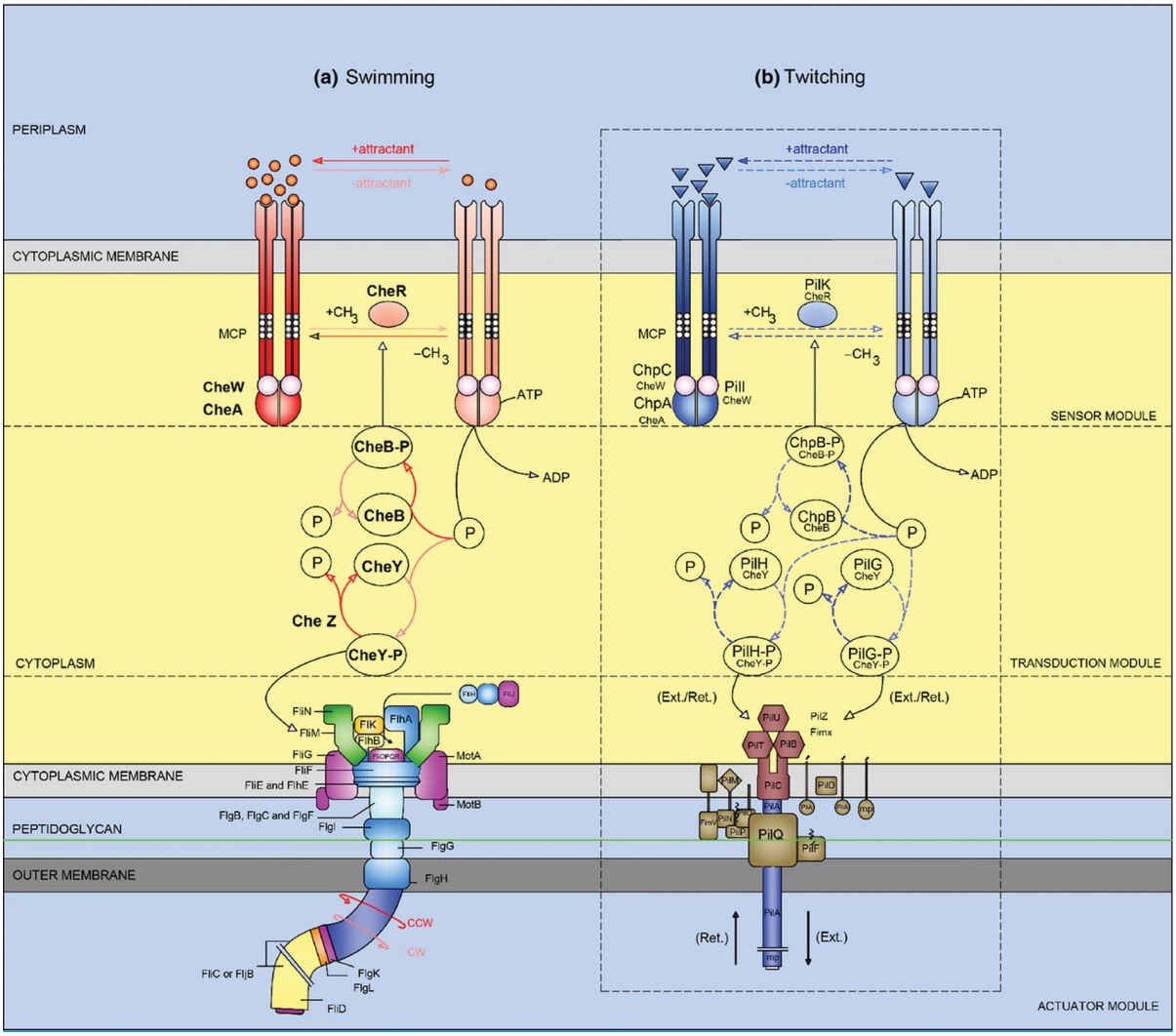


Figure 1-3. *Pseudomonas* chemotaxis regulation of swimming and twitching motility. Reprinted with permissions from (Sampredo et al., 2015).

1.6 Hypotheses and objectives

Due to the novelty of surfing motility, first discovered in *P. aeruginosa* under host-like conditions (Yeung et al., 2012), there is still much to be determined about how this motility is

regulated and how it may be linked to other aspects of the bacterium's physiology. I hypothesize that surfing motility is mediated by a complex network of regulators and is orchestrated by global regulatory systems such as quorum sensing and the stringent stress response. Surfing, being a social motility like swarming, is hypothesized to be similarly involved in broad-spectrum adaptive resistance as a result of the dysregulation of resistome genes. Surfing and its complex characteristics are also predicted to be conserved in other motile bacteria. Therefore, I pursued six specific aims in addressing my hypotheses. The first aim is to determine the transcriptomic profile of surfing cells collected from the edge and centre of a surfing colony in order to better characterize surfing cells within the motility zone (Chapter 3) as they have previously been shown to exhibit differential physical features (Yeung et al., 2012). This will allow me to determine if cells from the edge and centre of a surfing colony also exhibit differential transcriptomic profiles in addition to their physical differences. The second aim, also presented in Chapter 3, focuses on identifying master regulators involved in surfing motility and mapping a regulatory network. A comprehensive mutant library screen was used to determine surfing-essential genes, both effectors and regulators. Among the regulators, a network was determined by analyzing the expression profile of each surfing-essential regulator in each of the regulatory mutants. Two regulators, PfeRS and PA1463, were identified as potential master regulators involved in surfing. Therefore, the third aim of this study is to determine the roles of PfeRS and PA1463 in mediating surfing motility through the regulation of other surfing-essential regulatory genes (Chapter 4). Swarming, like surfing, is a social motility. Because swarming cells exhibit adaptive resistance (Overhage et al., 2008), Aim 4 sought to determine if surfing motility is associated with adaptive antibiotic resistance and to identify the resistome genes involved (Chapter 5). The fifth aim of this study is to determine the level of conservation of surfing motility in other motile bacterial species. As presented in Chapter 6, this included testing the conditional requirements for surfing, adaptive resistance, and dependence on key regulators in other Gram-negative and one Gram-positive bacterial species. Finally, Aim 6, presented in Chapter 7, is to determine the dependence of surfing on the stringent stress response and to identify surfing-essential regulators mediated by the stringent response that may be contributing to its regulation of surfing.

Chapter 2. Materials and methods

2.1 Bacterial strains

Bacterial strains used in the research presented in this thesis are listed in Table 2-1. *P. aeruginosa* PA14 mutants used in this study, unless otherwise stated, were derived from the PA14 Transposon Mutant Library (Liberati et al., 2006).

Table 2-1. List of bacterial strains used in this study.

Bacterial Species	Description	Reference
P. aeruginosa	Wild-type strain UCBPP-PA14	(Rahme et al., 1995)
P. aeruginosa	UCBPP-PA14 ΔPA1463-5	This study
P. aeruginosa	UCBPP-PA14 ΔPA1463-5/ PA1463-5::pUCp18	This study
P. aeruginosa	Wild-type strain PAO1	(Hancock and Carey, 1979)
P. aeruginosa	PA06609, spontaneous pyoverdine mutant	(Dean et al., 1996)
P. aeruginosa	PAO1/ *pfeRS*::pUCp18	This study
P. aeruginosa	Δ*pfeR;* parent strain PAO6609	(Dean et al., 1996)
P. aeruginosa	Δ*pfeA;* parent strain PAO6609	(Dean et al., 1996)
P. aeruginosa	Δ*pfeR* (PAO6609)/ *pfeRS*::pUCp18	This study
P. aeruginosa	PA14 Δ*pvd/pch*, pyoverdine and pyochelin mutant	(Pletzer et al., 2017)
P. aeruginosa	PAO1 Δ*relA*Δ*spoT*	(Pletzer et al., 2017)
P. aeruginosa	PAO1 Δ*relA*Δ*spoT* / *relA*+ chromosomal complement	(Pletzer et al., 2017)
P. aeruginosa	PAO1 Δ*relA*Δ*spoT* / *spoT*+ chromosomal complement	(Pletzer et al., 2017)
P. aeruginosa	Wild-type strain LESB58	(Cheng et al., 1996)
P. aeruginosa	LESB58 Δ*relA*Δ*spoT*	(Pletzer et al., 2017)
P. aeruginosa	LESB58 Δ*relA*Δ*spoT* / *relA*+ chromosomal complement	(Pletzer et al., 2017)
P. aeruginosa	LESB58 Δ*relA*Δ*spoT* / *spoT*+ chromosomal complement	(Pletzer et al., 2017)
P. aeruginosa	LESB58 Δ*relA*Δ*spoT* / *cueR*::pBBR5	This study
P. aeruginosa	LESB58 Δ*relA*Δ*spoT* / *pqsH*::pBBR5	This study
Enterobacter cloacae	Clinical strain FC1165	(Pollard et al., 2001)
Proteus mirabilis	Wild-type strain UNSW059300	(Gram et al., 1996)
P. mirabilis	Wild-type strain BA6163	(Mobley et al., 1996)
P. mirabilis	Strain BB2401 Δ*flaD*; parent strain BA6163	(Mobley et al., 1996)
P. mirabilis	Strain HI4320 Δ*mrpA*; parent strain BA6163	(Bahrani et al., 1994)
Salmonella enterica	Wild-type ATCC14028/ JSG210	(Prouty et al., 2002)
S. enterica	Strain KK105 *fliA::Tn10d*-Tet mutant derived from ATCC14028	(Prouty et al., 2001)
S. enterica	Strain JSG1240 *luxS::MudJ* mutant derived from ATCC14028	(Prouty et al., 2002)

Escherichia coli	Wild-type strain 0157:H7	(Nataro and Kaper, 1998)
E. coli	Wild- type strain MG1655	(Partridge et al., 2015)
E. coli	Strain RP3098 Δ*flhDC*; parent strain MG1655	(Partridge et al., 2015)
E. coli	Strain ORN172 Δ*fim*; deletion of entire *fim* region	(Woodall et al., 1993)
Vibrio harveyi	Wild-type strain BB120	(Nackerdien et al., 2008)
V. harveyi	Strain KM664 Δ*luxR::Tn5*; parent strain BB120	(Nackerdien et al., 2008)
Bacillus subtilis	Wild-type strain NCIB3610	(Pollak et al., 2016)
B. subtilis	Strain AES1403Δ*comA*::Cm; parent is NCIB 3610	(Pollak et al., 2016)
B. subtilis	Strain AES2135 Δ*comQXP*::tet; parent is 3610	(Pollak et al., 2016)
B. subtilis	∆*hag*::kan; parent is wild-type 3610	(Pollak et al., 2016)

2.2 Motility assays

Surfing, swimming and swarming assays were performed on either Luria Broth (LB; Difco), Basal Media 2 (BM2) (Yeung et al., 2012) or synthetic cystic fibrosis media (SCFM) (Palmer et al., 2007) with 0.5% glucose, containing 0.3-0.5% (wt/vol) agar with 0.4% (wt/vol) mucin for surfing, 0.5% agar without mucin for swarming, or 0.3% agar without mucin for swimming. Briefly, as described in more detail in Palmer et al., 2007, SCFM consists of each amino acid at an average concentration of 19mM, 1.3mM NaH_2PO_4, 1.25mM Na_2HPO_4, 0.35mM KNO_3, 10mM MOPS, 0.05M NaCl, 0.01M KCl, 1.75mM $CaCl_2$, 0.6mM $MgCl_2$, and 3.6μM $FeSO_4$. Other wetting agents tested besides mucin included carboxymethyl cellulose (CMC) added at 1.0%, and Tween-20 added at 0.01% wt/vol into LB with 0.3% agar. Bacterial species were sub-cultured 1 in 100 and grown to an OD_{600} of 0.4 - 0.5 in liquid LB medium, and 1 μL was inoculated onto the plates and incubated for 13-18 hours at 37ºC unless otherwise stated. Surfing and swarming plates were air-dried for approximately 1 hour before inoculation. Inoculation involved stabbing bacteria mid-way through the agar using the pipette tip. For the agar titration assay, bacterial species were grown on SCFM and LB with and without 0.4% mucin at varying agar concentrations (0.3%, 0.5%, 0.8%, and 1.0%). Bacterial cultures were grown and inoculated as described previously. Percent plate coverage was measured using ImageJ.

2.3 RNA-Seq

PA14 was grown in liquid LB medium overnight and sub-cultured to an OD_{600}=0.4-0.5. Mid-log phase cultures were used to inoculate SCFM (Palmer et al., 2007) for surfing and swimming plates, prepared as described above. Using sterile swabs, cells from the centre and edge of a surfing colony and centre of a swimming colony were collected into RNA protect bacteria reagent (Qiagen). Swimming liquid cultures were harvested at mid-log phase

(OD_{600}=0.5) for RNA extraction. RNA extraction was conducted using a RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Deoxyribonuclease treatment was performed using a TURBO DNA-free kit (Thermo Fisher) and rRNA depletion was performed using a RiboZero Bacteria Kit (Illumina). Single end cDNA libraries were constructed using a Kapa stranded Total RNA Kit (Kapa Biosystems) and libraries were sequenced on an Illumina HiSeq 2500 in rapid run mode with 100 bp reads that were base-called and de-multiplexed using built-in software on the sequencer. Fastq file quality control was performed using FastQC v0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and MulitQC v0.8.dev0 (Ewels et al., 2016). Fastq files were aligned to the UCBPP-PA14 genome (GenBank gene annotations) using bowtie-2 (Langmead and Salzberg, 2012). Bam-sam file conversion and sorting were performed with samtools (Li et al., 2009). Read count tables were generated with htseq-count v2.5 (Anders et al., 2015). Differential expression analysis was performed using DESeq2 (Love et al., 2014). Fold-changes in surfing were calculated relative to swimming. Gene annotations were taken from the *Pseudomonas* Genome Database (Winsor et al., 2016).

RNA-Seq was performed for the following samples using artificial sputum media (SCFM):

- PA14 WT surfing (SCFM + 0.3% agar + 0.4% mucin) compared to PA14 WT swimming in liquid culture (SCFM) – Results in Chapter 3
- PA14 WT surfing compared to PA14 WT swimming in low agar conditions (SCFM + 0.3% agar) – Results in Chapter 5
- PA14 ΔPA1463 operon (PA1463o) and ΔPA1463o::PA1463o/pUCp18 under surfing conditions compared to PA14 WT surfing – Results in Chapter 4
- PAO6609 WT surfing compared to PAO6609 WT swimming in low agar conditions – Results in Chapter 4
- PAO6609 Δ*pfeR* and ΔpfeR::*pfeRS*/pUCp18 under surfing conditions compared to PAO6609 WT surfing – Results in Chapter 4

2.4 Library Screen

A screen of the PA14 transposon mutant library (Liberati et al., 2006) was performed on large Corning square bioassay dishes (245 mm x 245 mm x 18 mm) using SCFM with 0.5% agar and 0.4% mucin. 96-pin stamps were used for high-throughput initial screens of approximately 5,500 mutants. Subsequent surfing screens as described in the motility assays (Section 2.2) were

performed on standard, circular petri dishes (100 mm x 15 mm) using SCFM with 0.3% agar and 0.4% mucin. Mutant phenotypes were divided into four different categories: hypersurfing, wild-type surfing, other forms of motility (e.g. swarming or swimming), or 1-directional motility which appeared as a single streak from the centre towards the edge of the plate. Surfing deficiency was considered: a complete lack of motility, exhibiting swimming or swarming, or 1-directional motility.

2.5 RT-qPCR

RNA was collected as described for RNA-Seq. Reaction samples were prepared using the qScript one-step SYBR green RT-qPCR Kit (QuantaBio) with 5 ng of RNA per 25 µL reaction amplified in a Roche LightCycler 96. Quantification analysis was done using the comparative Ct method (Schmittgen and Livak, 2008) using *rpoD* as the normalizing gene.

2.6 Growth curves

Bacterial strains were grown overnight (15-18 hours) in liquid LB, shaken at 750 rpm in 37°C. Overnight cultures were diluted to an OD_{600}=0.05 in a total volume of 100 µL and grown in round-bottom 96-well plates for 16 hours shaken at 567 cpm at 37°C in a TECAN Spectrofluor Plus. OD readings were taken every hour. Three replicates were done per sample.

2.7 Generating knock-out mutants and complements

Complemented mutants were generated as follows. PCR primers were used to amplify the desired genes from genomic DNA. The amplified products were cloned into a TOPO vector using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen). TOPO vectors containing amplified product were digested using two different enzymes, which differed depending on the gene of interest, and ligated into a desired vector containing the *lac* promoter. Vectors containing the desired genes were then transformed into their respective mutant using electroporation.

The knock-out mutant of the PA1463 operon was generated as previously described (Pletzer et al., 2014). Briefly, primers were used to amplify 500 bp regions up-stream and down-stream of the PA1463 operon and combined into a 1 kb fragment using an overlapping PCR reaction involving the forward primer that binds 500 bp up-stream and reverse primer that binds 500 bp down-stream of operon. The combined fragment was subsequently cloned into a Zero Blunt TOPO vector using the accompanying kit (Invitrogen) to be sequenced for verification that the correct fragment had been cloned. This 1 kb fragment was then cloned into a pEX18Gm suicide

vector using *BamH1/XbaI*. The vector was transformed into *E. coli* ST18 then transferred into PA14 via conjugation (mixing 100 µL of the transformed ST18 with 200 µL of PA14). Selection on 5% sucrose media was used to identify clean knock-out mutants. The knock-out region was then sequenced to verify that a complete deletion had been made.

2.8 Disk diffusion assay

Disk diffusion assays were performed on SCFM prepared as described by Palmer et al (2007) with 0.3% agar and 0.4% (wt/vol) mucin (surfing conditions), or with 0.3% agar without mucin (swimming conditions). Control disk diffusion assays were performed on SCFM 1.5% agar with and without 0.4% mucin. Bacterial strains were grown in Luria broth (LB; Difco) liquid medium overnight then sub-cultured to mid-log phase (OD_{600}=0.4-0.5). To assay motility, mid-log cultures were spotted on agar surfaces at four points around an antibiotic disk (Appendix Figure A-1) impregnated with 10uL of antibiotic at the concentrations indicated (Appendix Table A-1). Agar plates were air-dried at 37°C for 30 min before inoculation and application of antibiotic disks. Once inoculated, plates were incubated at 37°C for 15-18 hours. The zone of inhibition surrounding the antibiotic disk was measured in millimeters using a ruler. In the case of asymmetric zones of inhibition, the average of the four sides was taken. Disk diffusion controls or growth controls were spread as lawns on plates and antibiotic disks were applied to the centre. Two-way ANOVA was used to determine if any significant difference existed between surfing and swimming conditions. All statistical analysis was done using Graphpad Prism 7.

2.9 Antibiotic incorporation assay

Incorporation assays were done on SCFM (Palmer et al., 2007) using 0.3% agar with 0.4% mucin (surfing conditions) and 0.3% agar without mucin (swimming). Antibiotics were added into the agar before solidification. Once hardened, plates were air-dried for 30 minutes at 37°C before being inoculated with 1 µL of a sub-culture at an OD_{600}=0.4-0.5. Plates were incubated at 37°C for 15-18 hours. Spot inoculation involved stabbing bacteria midway into the agar. The percentage of area growth on the plates was measured using ImageJ. Two-way ANOVA was used to determine if significant differences occurred between the two conditions (surfing and swimming) and between concentrations for surfing.

2.10 Liquid minimal inhibitory concentration (MIC)

Liquid MICs were conducted as described by (Wiegand et al., 2008). This assay was

performed in liquid SCFM (Palmer et al., 2007) with and without 0.4% mucin. An inoculum of 2 to 7 x10^5 cells was used. Significant differences between MICs were taken as a 3-fold or greater change.

2.11 Motility zone growth assay

Motility growth assays were done on SCFM/0.3-0.5% agar (Palmer et al., 2007) without (swimming motility within the agar) or with 0.4% mucin (surfing motility on the agar surface). Motility assays were performed as previously described in Section 2.2. Measurements of the visible growth zone at 37ºC were taken every hour for 10 hours in the incubator to prevent interruption of incubation. Notches were drawn at the ends of the motility zones at each time point to ensure that measurements were consistently taken from the same sides of the motility colony. Measurements were taken using a ruler in mm.

2.12 Iron titration assay

2,2'-Dipyridyl (Sigma) was added into SCFM with 0.3% agar and 0.4% mucin at various concentrations. Bacterial strains were spot inoculated as described for the motility assays. Iron ($FeSO_4$) was added at various concentrations in addition to the dipyridyl at 50μM and 500μM. Plates were incubated at 37ºC for 15 hours before imaging.

Chapter 3. The transcriptomic profile and genes essential to surfing

3.1 Introduction

P. aeruginosa has a relatively large genome compared to other bacterial species and, therefore, high genetic potential. The *P. aeruginosa* transcriptional network is the third largest known bacterial regulatory network (Galán-Vásquez et al., 2011). *P. aeruginosa* has a genome size of 6.3 Mbp and about 5,567 predicted genes, 690 predicted regulators and more than a thousand predicted regulatory interactions (Azam & Khan, 2018; Galán-Vásquez et al., 2011). Galan-Vasquez et al. (2011) used computational predictions to estimate the most influential transcriptional factors in *P. aeruginosa* (based on published literature and thus biased towards well studied regulators) and identified *lasR,* Fur*, mexT,* Vfr*, algR,* Anr*,* Ihf*, ptxR, rhlR,* and *algW*. Gene regulation is well known to be crucial in mediating virulence and adaptability. *Pseudomonas* adaptions such as swarming motility and biofilm formation have been shown to exhibit unique transcriptomic profiles and rely on networks of regulators that work cooperatively to induce and maintain each adaptation. Biofilms and swarming have also been shown to have many inverse regulatory mechanisms (Caiazza et al., 2007; Yeung et al., 2009).

With regards to surfing motility, in relation to virulence and resistance, Yeung et al. (2012) previously found using qRT-PCR that genes involved in the T3SS are down-regulated in both the centre and edge while the T2SS is up-regulated (Yeung et al., 2012). Both pyoverdine and pyochelin biosynthesis genes appear to be up-regulated at the edge but down-regulated in the centre. Phenazine biosynthesis, however, is down-regulated in both population of cells (Yeung et al., 2012). A significantly high up-regulation in *oprH, phoP, arnB,* and *pmrB* was also found in both the centre and edge cells (Yeung et al., 2012). All of these genes have been shown to be associated with antimicrobial resistance (Bell et al., 1991; Macfarlane et al., 2000; Olaitan et al., 2014).

In regards to motility genes, transposon mutant screens revealed a surfing-dependence on flagella, particularly *fliC, fleR, fleS, fliQ, fliD, flgB,* and *flgC* (Yeung et al., 2012). FleRS is a two-component regulatory system found to involved in motility and adherence (Gellatly et al., 2018). Despite the dependence on flagellar biosynthesis genes, a dependence on correlated motor genes, *motABCD*, however, was not determined (Yeung et al., 2012). It is predicted that alternative motor proteins could be involved. Surfing was also shown to be independent of pili genes (Yeung et al., 2012). Surfing is, however, dependent on the Rhl and Las quorum sensing

systems. Disruption mutants in *rhlI* and *lasI* were shown to be surfing deficient whereas the addition of each respective autoinducer exogenously restored surfing motility in these mutants (Yeung et al., 2012).

In this chapter, I have extended these preliminary data to show that, like swarming and biofilm formation, surfing also exhibited a unique and extensive transcriptomic profile with distinct gene expression patterns in cells collected from the edge and centre. RNA-Seq revealed >1,000 genes dysregulated in the surf centre and at the surf edge compared to swimming cells. These differential expression patterns revealed that cells in the centre and edge were metabolically distinct. Here I have shown that surfing is dependent on a set of approximately 40 regulators, involved in various regulatory systems. Transposon mutant screens for cells with altered surfing, revealed that surfing was dependent on the GacAS master regulator, three of *Pseudomonas*' quorum sensing systems namely Las, Rhl, and Pqs, chemotaxis regulators, the two-component system CbrAB, and many others. Cross analysis of the expression levels of each surfing-essential regulator in each of their mutants revealed three regulatory systems that appeared to have relatively high influence on surfing regulation, specially PfeRS, PA1463, and CbrAB.

3.2 The *P. aeruginosa* surfing adaptation involved a transcriptionally diverse population of cells

Physically, a surfing colony exhibits differences in the centre and at the edge, with a thick white outer edge and a blue-green centre (Yeung et al., 2012). RNA-Seq data (NCBI GEO accession number GSE110044) on surfing cells collected from the centre and edge of surfing colonies revealed that surfing involves distinct cell populations with different transcriptomic profiles. There were 1,094 genes dysregulated at the edge and 1,617 genes in the centre of surf colonies (SCFM + 0.3% agar + 0.4% mucin) relative to swimming cells (SCFM + 0.3% agar) with 487 genes overlapping between the two surfing zones. Figure 3-1 shows a cohort of functionally grouped genes and their expression levels in the centre and at the edge. Interestingly, although surfing was shown to be independent of pili, certain pilus assembly genes were found to be expressed in the centre and at the edge in an inverse manner. Thus alternative pilus genes such as *cupE4*, *cpaB*, and *tadD* were up-regulated in the centre but down-regulated at the edge, while major Type IV pilus biosynthesis genes (*pilH, pilG*, and *pilJ*) were down-regulated in the centre but up-regulated at the edge. On the other hand, flagella biosynthesis genes were similarly

regulated in both the edge and centre. However, certain flagella genes such as *flgN* and *flgM*, which are negative regulators of flagellin synthesis, were down-regulated while structural genes (*flgK, flgE, flgJ*) as well as *flhA*, which is a positive regulator of flagella synthesis, were up-regulated in surfing cells. Therefore, regardless of the observed flagellation status previously reported (Yeung et al., 2012), surfing cells were actively expressing flagella assembly proteins throughout the surf colony.

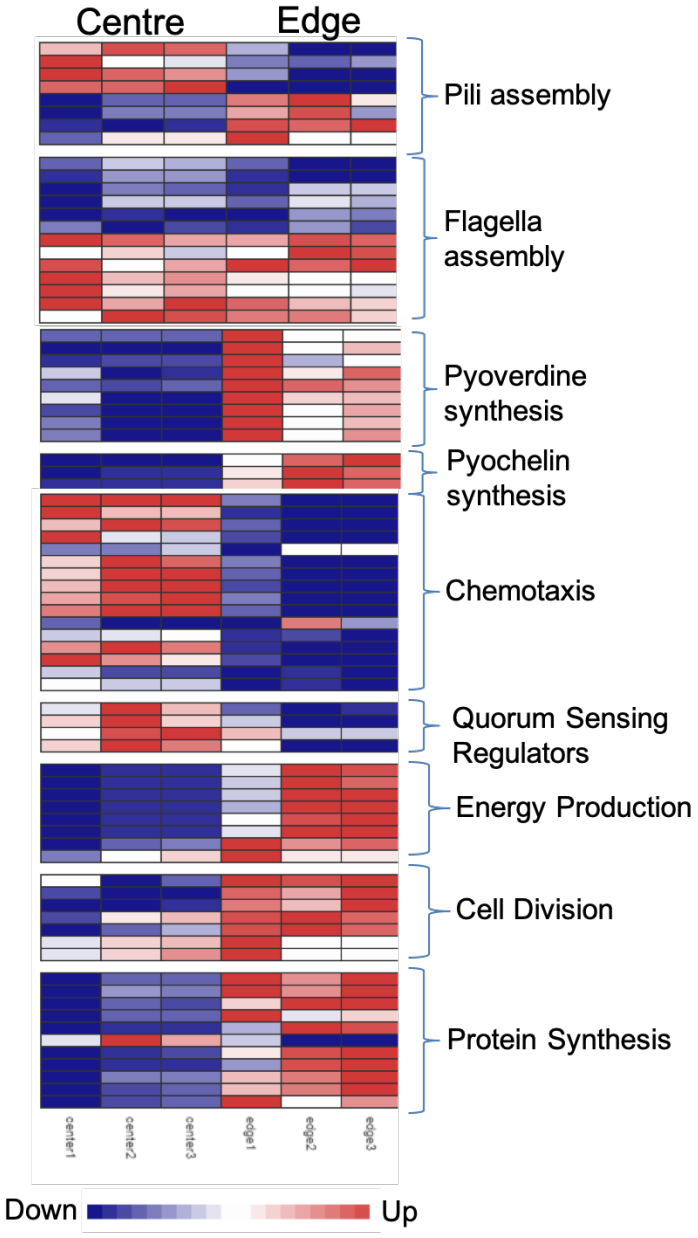


Figure 3-1. **Transcriptomic analysis of surfing cells revealed distinct expression patterns in cells collected from the edge and centre.** Heatmaps were generated using INVEX. Blue represents down-regulation and red up-regulation relative to a swimming cell control. Three

biological replicates were prepared for each sample group (e.g. edge and centre).

Coupled with flagella regulation was chemotaxis, which directly influences the rotation of the flagellar motor. According to the RNA-Seq data, chemotactic transducers, *pct* genes, were generally down-regulated at the centre and edge, while the *wbp* chemotaxis genes were generally up-regulated. Core chemotaxis genes (*che* genes) appeared to not be expressed at the edge but relatively more highly expressed in the centre relative to swimming cells. Therefore, centre cells appeared to be more chemotactic.

Centre and edge cells appeared to have an inverse relationship with regards to virulence gene expression. Quorum sensing regulators for Rhl, Las, and Pqs were more highly up-regulated in the centre, and there was a significant up-regulation of lipases and phospholipases in the centre as well. However, pyoverdine and pyochelin genes were relatively more expressed at the edge whereas they were significantly down-regulated in the centre. Therefore, the centre and edge had relatively distinct virulence characteristics.

Metabolic genes were mostly down-regulated in the centre and generally un-expressed at the edge. This same pattern was observed for F0F1 ATPase genes involved in energy production. The most dysregulation in terms of metabolic genes in the centre were genes involved in arginine and ornithine metabolism. Cells at the edge also revealed a modest down-regulation of these genes. Cells in the centre exhibited a relatively extensive down-regulation of nitrogen metabolism genes especially genes involved in nitrite reduction. In addition to metabolic activity, cell division and protein synthesis genes were also down-regulated in the centre and up-regulated at the edge, speaking to the differential metabolic states of the centre and edge cells.

3.3 Regulatory genes expressed in surfing compared to swarming and biofilms

A comparison between RNA-Seq data collected from surfing edge, swarming (courtesy of Shannon Coleman) and biofilm cells (courtesy of Dr. Daniel Pletzer) relative to swimming revealed 21 predicted and known regulators dysregulated in all three conditions (Table 3-1). In addition, surfing motility led to the dysregulation of 63 additional regulators that were also dysregulated under biofilm conditions but not during swarming motility, and 10 that were dysregulated under swarming motility conditions but not in biofilms. Thus, with regards regulation, surfing motility appeared to have more in common with biofilm formation when compared to swarming motility. Table 3-1 summarizes the regulators dysregulated under swarming and biofilm conditions that were also dysregulated in surfing.

Table 3-1. **Regulatory genes dysregulated in surfing as well as swarming and/or biofilm cells.** RNA-Seq was performed on surfing edge cells, swarming tip cells (by Shannon Coleman), and biofilm cells (by Dr. Daniel Pletzer) and analyzed relative to swimming as a control. The relative direction of dysregulation is shown as "-" for down-regulation, "+" for up-regulation, and "0" for no change. Fold-change cut-off of ± 1.5 was used. Gene annotations and descriptions come from www.pseudomonas.com (Winsor et al., 2016).

Gene ID	Gene name	Description	Direction of dysregulation (RNA-Seq)		
			Swarming	Surfing edge	Biofilm
PA0612	*ptrB*	Repressor, PtrB	-	+	+
PA3719	*armR*	Antirepressor for MexR, ArmR	+	-	+
PA3007	*lexA*	Repressor protein LexA	-	+	+
NA	*tpnC*	TpnA repressor protein	+	-	-
PA3410	*hasI*	RNA polymerase ECF-subfamily sigma-70 factor/HasI	-	+	-
PA2387	*fpvI*	RNA polymerase sigma factor/FpvI	+	-	-
PA1912	*femI*	ECF subfamily RNA polymerase sigma-70 factor/ECF sigma factor, FemI	-	-	-
PA1300		RNA polymerase ECF-subfamily sigma-70 factor/ECF sigma factor	+	-	-
PA4896		RNA polymerase sigma factor/probable sigma-70 factor, ECF subfamily	-	-	-
PA0520	*nirQ*	Regulatory protein NirQ	-	-	+
PA3932		Probable transcriptional regulator	-	-	+
PA3391	*nosR*	Regulatory protein NosR	-	-	+
PA2825	*ospR*	MarR family transcriptional regulator/OspR	-	+	+
PA2383		Probable transcriptional regulator	+	+	-
PA2320	*gntR*	Transcriptional regulator GntR	+	-	-
PA2303	*ambD*	Regulatory protein/AmbD	+	+	-
PA1243		Probable sensor/response regulator hybrid	+	+	+
PA1179	*phoP*	Two-component response regulator PhoP	-	-	+
NA	*rcsB*	Two-component response regulator	+	-	+
PA4659		MerR family transcriptional regulator	-	-	+
PA4843	*gcbA*	Two-component response regulator/GcbA	-	-	-
PA4878	*brlR*	Transcriptional regulator/BrlR	-	+	+
PA0424	*mexR*	Multidrug resistance operon repressor MexR	0	-	+
PA0149		Probable sigma-70 factor, ECF subfamily	0	-	-
PA0472	*fiuI*	RNA polymerase sigma factor/FiuI	0	-	-
PA3899	*fecI*	RNA polymerase sigma factor/FecI	0	-	-
PA2468	*foxI*	ECF subfamily RNA polymerase sigma-70 factor/ECF sigma factor FoxI	0	-	-
PA2426	*pvdS*	Extracytoplasmic-function sigma-70 factor/sigma	0	-	-

		factor PvdS			
PA0179	*cheY*	Chemotaxis protein, CheY	0	-	+
PA0448	*gcdR*	LysR family transcriptional regulator, GcdR	0	+	-
PA4165		Probable transcriptional regulator	0	+	-
PA4132		Conserved hypothetical protein	0	+	+
PA4112		Probable sensor/response regulator hybrid	0	-	-
PA3995		Probable transcriptional regulator	0	-	+
PA3973		Probable transcriptional regulator	0	-	+
PA3921		Probable transcriptional regulator	0	-	-
PA3782		Probable transcriptional regulator	0	-	-
PA3721	*nalC*	Transcriptional regulator/NalC	0	-	+
PA3385	*algZ*	Alginate and motility regulator Z	0	-	+
PA3341	*slyA*	MarR family transcriptional regulator/probable transcriptional regulator	0	-	+
PA3260		Probable transcriptional regulator	0	-	+
PA3160	*wzz*	O-antigen chain length regulator	0	+	+
PA3034		Probable transcriptional regulator	0	-	+
PA3006	*psrA*	Transcriptional regulator PsrA	0	-	+
PA2931	*cifR*	Transcriptional regulator/CifR	0	-	+
PA2846		LysR family transcriptional regulator	0	-	+
PA2718		MerR family transcriptional regulator	0	-	+
PA2696		Probable transcriptional regulator	0	-	+
PA2663	*ppyR*	Pyoverdine operon regulator, PpyR	0	-	+
PA2586	*gacA*	Response regulator GacA	0	-	+
PA2572		Probable two-component response regulator	0	-	+
PA2523	*czcR*	Two-component response regulator/CzcR	0	+	-
PA2519	*xylS*	Transcriptional regulator XylS	0	-	+
PA2511	*antR*	Transcriptional regulator/AntR	0	-	+
PA2376		Probable transcriptional regulator	0	-	+
PA2127	*cgrA*	CupA gene regulator A, CgrA	0	+	+
PA2005	*hbcR*	Transcriptional regulator/HbcR	0	+	-
PA1998	*dchR*	LTranscriptional regulator, DchR	0	-	+
PA1911	*femR*	Sigma factor regulator, FemR	0	-	-
PA1760		Probable transcriptional regulator	0	-	-
PA1430	*lasR*	Transcriptional regulator LasR	0	-	+
PA1399		Probable transcriptional regulator	0	-	+
PA1290		Probable transcriptional regulator	0	-	+
PA1269		Probable transcriptional regulator	0	-	+
PA1196	*ddaR*	Transcriptional regulator DdaR	0	-	+
PA0942		Probable transcriptional regulator	0	-	+
PA0929	*pirR*	Two-component response regulator	0	-	-
PA0877		Probable transcriptional regulator	0	-	+
PA0876		Probable transcriptional regulator	0	-	+
PA0873	*phhR/*	Transcriptional regulator PhhR	0	-	+
PA0839		Probable transcriptional regulator	0	-	+

PA4296	*pprB*	Two-component response regulator, PprB	0	-	+
NA	*pvrR*	Two-component response regulator	0	-	+
PA4596	*nfxB*	Transcriptional regulator NfxB	0	+	+
PA4726	*cbrB*	Two-component response regulator CbrB	0	-	+
PA4776	*pmrA*	Two-component regulator system response regulator PmrA	0	-	+
PA4777	*pmrB*	Two-component regulator system signal sensor kinase PmrB	0	-	+
PA4856	*retS*	RetS (Regulator of Exopolysaccharide and Type III Secretion)	0	+	-
PA5274	*rnk*	Nucleoside diphosphate kinase regulator	0	+	+
PA5324	*sphR*	Sphingosine-responsive Regulator, SphR	0	-	-
PA5342		Probable transcriptional regulator	0	+	-
PA5356	*glcC*	Transcriptional regulator GlcC	0	+	-
PA5382	*yeiE*	Probable transcriptional regulator	0	+	-
PA5438		Probable transcriptional regulator	0	-	+
PA5499	*np20*	Transcriptional regulator np20	0	-	+
PA1351		RNA polymerase ECF-subfamily sigma-70 factor	+	-	0
PA0367		Probable transcriptional regulator	+	-	0
PA3346	*hsbR*	HptB-dependent secretion and biofilm regulator HsbR	+	-	0
PA2917		Probable transcriptional regulator	-	-	0
PA2588		Probable transcriptional regulator	+	-	0
PA2032	*yjiR*	Transcriptional regulator	+	-	0
PA1836		Probable transcriptional regulator	+	-	0
PA1431	*rsaL*	Regulatory protein RsaL	+	-	0
PA5059	*phaD*	TetR family transcriptional regulator	+	+	0
PA5437		Probable transcriptional regulator	-	+	0

3.4 Mutant screens revealed more than 100 surfing-essential genes

From the screen of the PA14 transposon mutant library (Liberati et al., 2006), 5,307 mutants were screened under surfing conditions, i.e. on SCFM medium with 0.3-5% agar and 0.4% mucin. An initial large plate screen yielded 320 mutants that exhibited irregular surfing as shown in Figure 3-2. When retested using regular Petri dishes, none of the mutants initially identified as hypersurfers showed any significant difference in their motility zone when compared to the wild-type. Non-surfing behaviour was observed for 192 mutants, which exhibited either a complete lack of motility, alternative forms of motility such as swimming or swarming, or one-directional motility (Figure 3-2). Among these 192 mutants exhibiting surfing deficiency, 44 mutants belonged to regulatory genes as listed in Table 3-2. There were 13 regulators belonging to two-component regulatory systems, 10 regulators belonging to one of the four known quorum sensing

systems, and 4 regulators involved in chemotaxis.

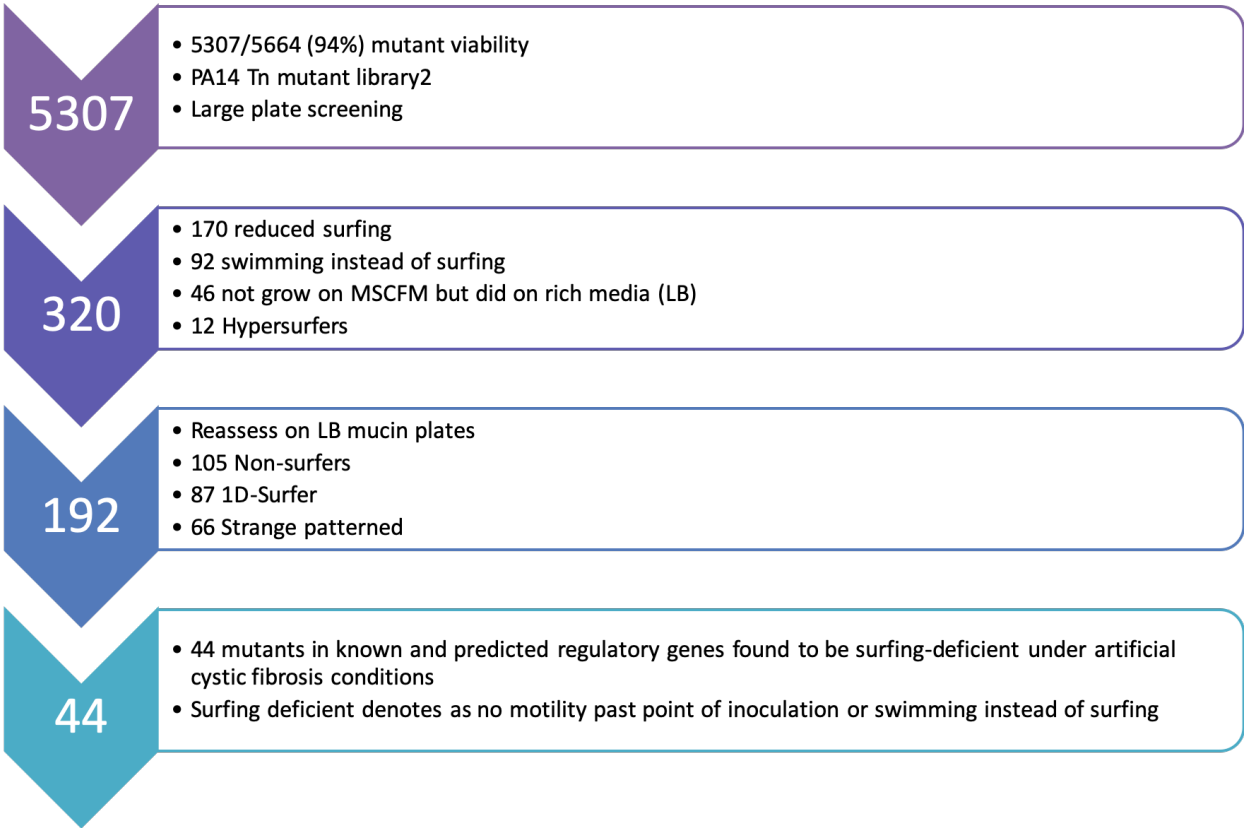


Figure 3-2. Sequential screening of the strain PA14 transposon mutant library revealed 44 regulators required for surfing motility. The library consisted of 5,664 mutants, 94% of which proved viable. Initial large plate screening yielded 320 mutants exhibiting irregular surfing (i.e. hypersurfing or surfing deficiency). Retesting on regular Petri dishes, led to the verification of 192 mutants as surfing deficient, exhibiting either not motility, alternative forms of motility (e.g. swarming), or one-directional (1D) motility phenomena. Among the 192, there were 44 mutants in regulatory genes.

Table 3-2. Regulators for which transposon mutant variants exhibited surfing deficiency. Surfing deficiency included no motility, a different form of surfing such as swimming or swarming, or One-directional (1D) motility. The mutant library screen was performed as described in Fig. 3.2. RNA-Seq data was collected from edge and centre cells of surfing colonies grown in SCFM with 0.5% agar/0.4% mucin using wild-type PA14. RNA-Seq fold-change had a cut-off of ± 1.5. Descriptions and gene annotations are from www.pseudomonas.com (Winsor et al., 2016).

Gene	Fold Change RNA-Seq		Gene Product Description	Surfing phenotype in Library Screen
	Centre	Edge		
cbrA	NC	NC	Two-component sensor	No motility
cbrB	NC	-1.95	Two-component response regulator	No motility
cheA	3.04	NC	Chemotaxis protein	No motility
cheW	3.14	NC	Chemotaxis protein	No motility

cheZ	NC	NC	Chemotaxis protein	No motility
cueR	NC	NC	Copper-responsive transcriptional regulator	Swimming
cysB	NC	NC	Transcriptional regulator	No motility
czcS	NC	NC	Putative heavy metal sensor histidine kinase	No motility
dipA	-1.98	-2.14	Putative sensory box protein	Swimming
fleQ	NC	NC	Transcriptional regulator	1D motility
fleR	NC	NC	Probably two-component response regulator	No motility
fleS	NC	NC	Two-component sensor	Swimming
flgM	-2.94	-3.21	Flagellin biosynthesis negative regulator FlgM	No motility
gacA	NC	NC	Response regulator	No motility
gacS	NC	NC	Sensor/response regulator hybrid	No motility
lasI	2.24	NC	Autoinducer synthesis protein	No motility
nirQ	-2.38	-2.27	Regulatory protein	1D motility
PA0034	NC	NC	Probably two-component response regulator	No motility
PA0475	NC	NC	Probable transcriptional regulator	No motility
PA1157	NC	NC	Probable two-component response regulator	No motility
PA1463	NC	NC	Hypothetical protein	No motility
PA2276	NC	NC	Probable transcriptional regulator	1D motility
PA2882	NC	NC	Probably two-component sensor	No motility
PA3197	NC	NC	Hypothetical protein	No motility
PA3348	NC	NC	Probable chemotaxis protein methyltransferase	1D motility
PA3599	NC	-1.81	Probably transcriptional regulator	No motility
PA3921	NC	NC	Probable transcriptional regulator	1D motility
PA4398	NC	NC	Two-component sensor	No motility
PA4831	NC	NC	Probably transcriptional regulator	1D motility
PA5392	NC	NC	Conserved hypothetical protein	No motility
pfeS	NC	NC	Two-component sensor	Swimming
pqsA	-3.03	3.29	Probable coenzyme A ligase	No motility
pqsB	-3.46	4.57	2-heptyl-4(1H)-quinolone synthase subunit B	No motility
pqsC	-2.72	5.56	2-heptyl-4(1H)-quinolone synthase subunit C	No motility
pqsD	-3.12	5.14	3-oxoacyl-ACP synthase	No motility
pqsE	-2.33	5.12	Quinolone signal response protein	No motility
pqsH	NC	NC	FAD-dependent monooxygenase	No motility
pqsR	NC	NC	Transcriptional regulator (Also called *mvfR*)	No motility
rcsB	NC	NC	Probable response regulator	No motility
rhlI	3.44	NC	Autoinducer synthesis protein	No motility
rhlR	NC	NC	Transcriptional regulator	No motility
rocA1	NC	NC	Two-component response regulator	Swimming
rpoN	NC	NC	RNA polymerase sigma-54	No motility
rsmA	NC	2.22	Regulator of secondary metabolite	No motility

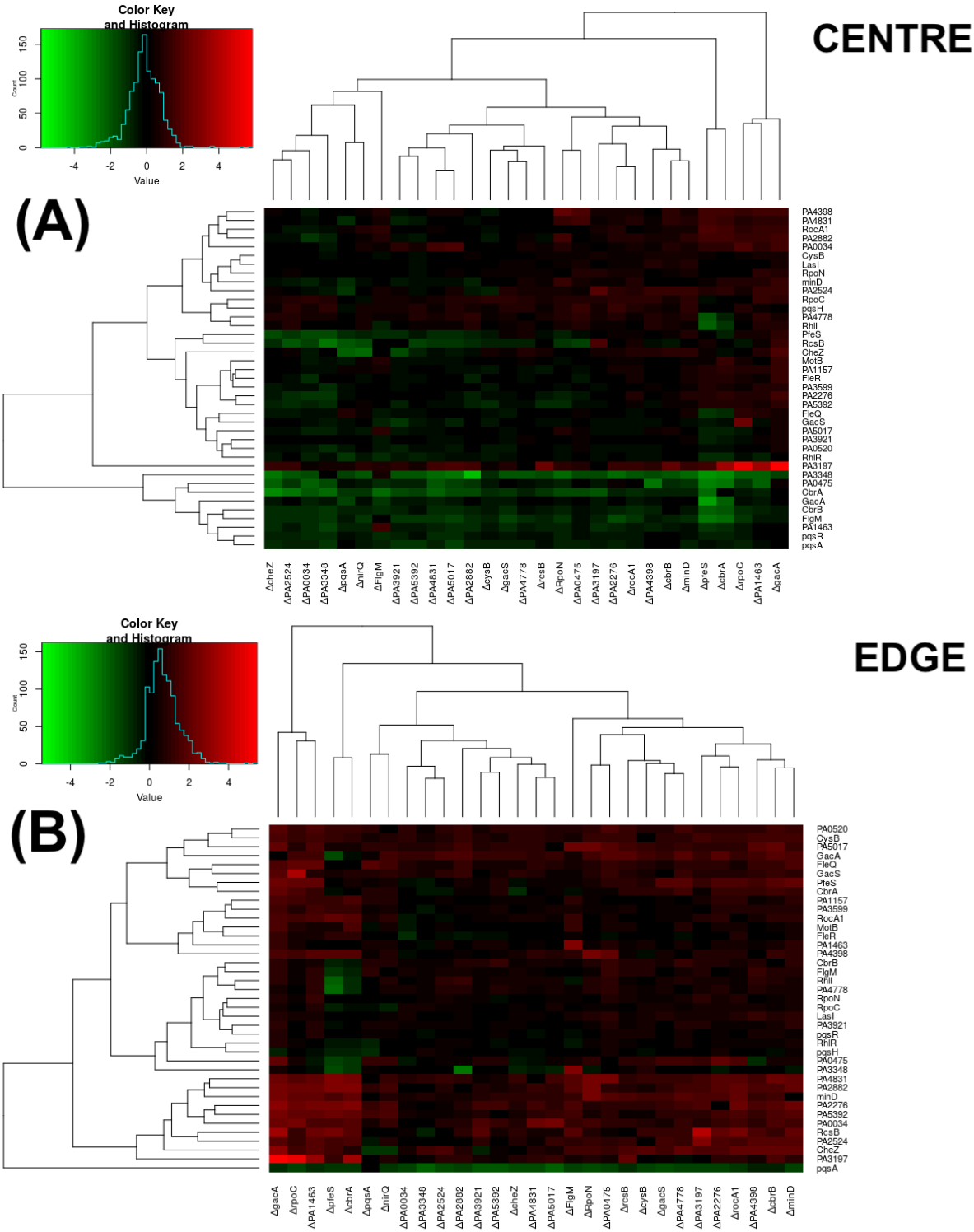
Non-regulatory or effectors genes are listed in Appendix Table A-2. The majority of genes required for surfing were hypothetical or metabolic genes, including several genes involved in biotin synthesis, *bioA, bioD, bioF*, PA0503. Among the effectors found to be important for

surfing, *algX*, an alginate biosynthesis gene involved in chronic infections during the transition from non-mucoidal to mucoidal variant, was identified. Virulence factors included a hydrogen cyanide synthase gene, *hcnC*, serine protease, *mucD*, and phospholipase C, *plcB*. Mutants in twitching motility genes, namely *pilT, pilU, pilW,* and *fimX* previously untested by Yeung et al. (2012) were also found to be surfing deficient raising the issue as to the potential for an ancilliary role for twitching in surfing. Previously identified flagellar genes were also confirmed. Additional flagellar genes found to be important for surfing included *fliK* and *fliL*. There were also mutants in two cell division proteins that exhibited surfing deficiency, *rrmJ* and *minD*. There were four surfing deficient mutants in genes involved in resistance to ROS including the oxidoreductases *rmd*, PA0545, PA3489, and PA1127. Other critical resistance genes included the following porins and efflux pumps: *oprO*, PA2454, and PA4455. There were also three *tonB*-dependent receptors found to be important for surfing: PA4168, PA2089, and PA1271.

3.5 Surfing was mediated by interactions between 44 regulators

To identify interdependent regulation (so-called regulatory hierarchy) among the essential regulators identified from the mutant library screen, RT-qPCR was performed for 39 regulator genes, in mutants affecting 29 regulators [NB. only the first gene from any particular operon or pathway was screened in order to avoid redundancy, e.g. both chemotaxis genes, *cheZ* and *cheA*, were screened as a representatives of their respective operons). Mutants from each pathway were selected based on the consistency of the surfing deficient phenotype. Figure 3-3 summarizes the results of this analysis as heatmaps clustered based on similar dysregulation patterns among mutants and among regulators, and a graph summarizing the total log fold-change among the other regulators that resulted in response to each disruption mutation. Among the tested regulators, major (also termed master) regulators found higher up in the regulatory hierarchy/network could be identified as those whose mutant resulted in the most dysregulation of the other regulators and regulators which themselves were relatively unaffected by disruption mutations in the other regulators. More specific regulators lower in the regulatory network were those that were most affected by mutants in other regulators while their own mutants demonstrated little to no dysregulation of the other tested regulators occurred. Patterns of dysregulation among the mutants when comparing results at the edge and centre varied greatly (Figure 3-3C,D). At both the centre and edge, mutants in *gacA, cbrA, PA1463, pfeS,* and *rpoC* were the five that demonstrated the greatest influence on expression of other regulators. In

contrast there was substantial variation in the influence of genes found lower in the regulatory network.



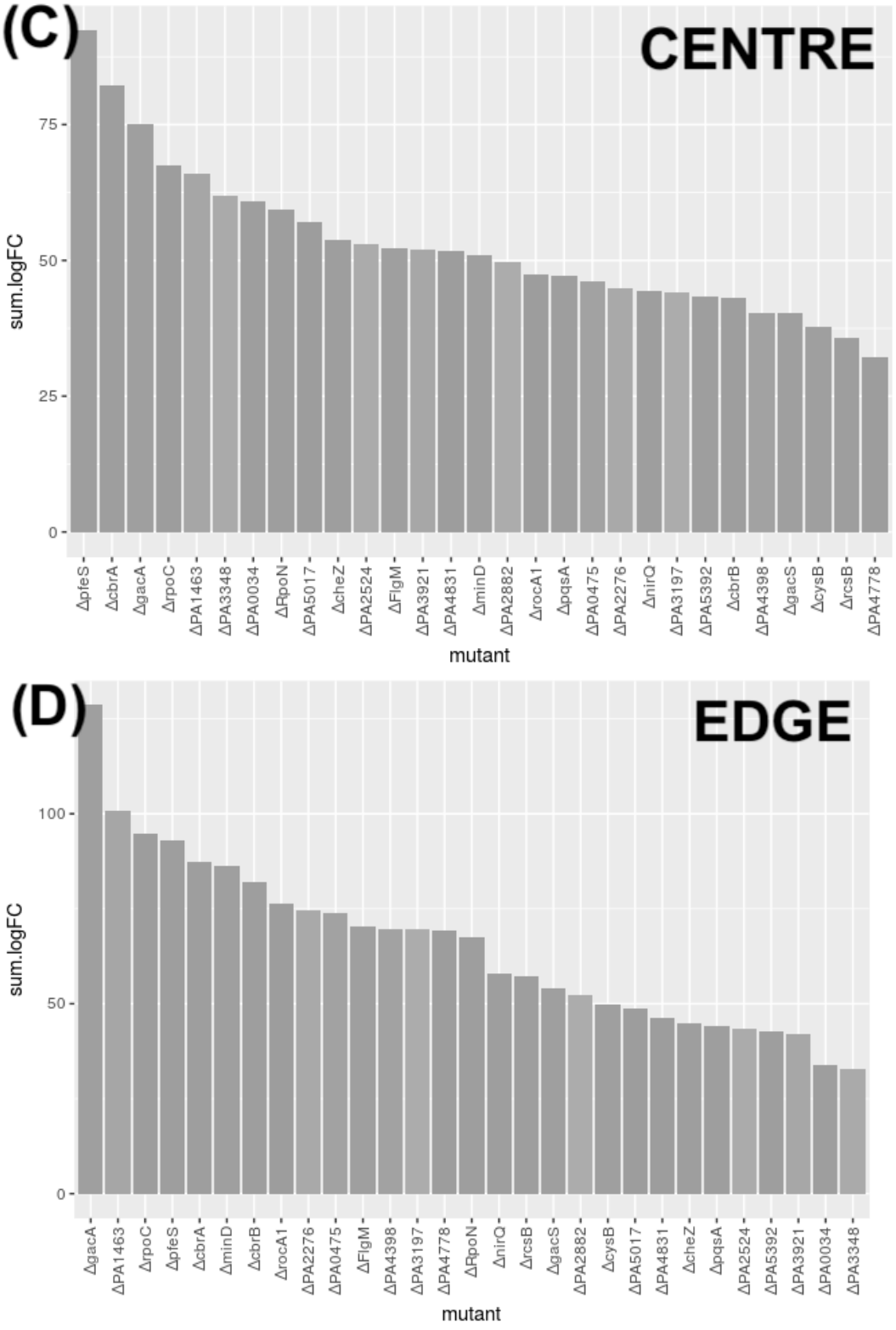


Figure 3-3. Gene expression profiles (RT-qPCR) of 39 regulators in mutants in 29 individual regulators required for surfing. Gene expression relative to that assessed in the centre of wild-type surfing colonies is shown in (A) and (C). Gene expression relative that

assessed at the edge of wild-type surfing colonies is shown in (B) and (D). (A) and (B) Heatmaps are hierarchically clustered based on similar gene expression profiles within each mutant and between each regulator. Green represents up-regulation and red represents down-regulation. (C) and (D) show the sum of the log fold change number of other regulators that change expression in each respective mutant, as identified in this checkerboard RT-qPCR assay. Regultors affecting the greatest number of other regulators influence these reglators and thus the highest bars are highest in the hierarchy.

3.6 Discussion

RNA-Seq revealed that surfing motility is a complex adaptation that involved large transcriptomic changes, with more than a thousand genes dysregulated. Surfing cells in the centre and at the edge exhibited distinct transcriptomic profiles. Analysis of these profiles were consistent with the possibility that cells in the centre appeared to be less metabolically active compared to those at the edge where cells appeared to be actively dividing and growing; this seems reasonable since nutrients would be more likely to have been consumed at the centre. Although motility genes specifically for flagella biosynthesis were generally expressed throughout a surfing colony, chemotaxis genes were more actively expressed in the centre. Quorum sensing genes were also more actively expressed in the centre which correlated with the higher density of cells in this region and the up-regulation of virulence factors such as elastases and lipases. However, edge cells were expressing more pyoverdine and pyochelin biosynthesis genes, indicating an active requirement for iron acquisition.

Among the approximate 106 regulators found to be dysregulated at the surfing edge, only 31 were found to also be dysregulated under swarming (as compared to data collected by Coleman) and 84 in biofilms (as compared to data collected by Pletzer) with 21 shared among the three conditions. Among the 21 shared regulators between the three conditions, 4 were dysregulated in the same direction (i.e. all down- or up-regulated). Two of the regulators were predicted sigma factors specifically from the sigma-70 family (FemI, PA4896). Sigma-70 or RpoD in *P. aeruginosa* is a major sigma factor involved in facilitating the recognition of diverse promoters by RNA polymerase. Sigma-70 sigma factors are divided into four different subgroups, homologous to RpoD (Potvin et al., 2008). The first group involves RpoD and sigma factors closely related to it, which all play similar functions and are essential for cell survival. The other groups, however, are non-essential and less homologous to RpoD. They play roles in stress responses, motility, and other adaptations (Potvin et al., 2008). FemI is an extracytoplasmic function (ECF) sigma factor that regulates the expression of a TonB-dependent transducer,

FemA (Llamas et al., 2008). FemA is involved in iron-uptake through the mycobactin siderophore produced by *Mycobacterium* species (Llamas et al., 2008). A lack of mycobactin in the system may have resulted in a shut-down of the FemI/FemA system. Both of these were down-regulated under surfing conditionsa ccording to the RNA-Seq data. RNA-Seq data also revealed that several other TonB-dependent receptors were down-regulated in surfing. The mutant library screen, however, revealed that surfing was dependent on 3 TonB-dependent receptors: PA4168, PA2089, and PA1271. Therefore, the three conditions, surfing, swarming, and biofilms, may have exhibited a similar shut down of the FemI/FemA system involved in iron acquisition and may rely on other mechanisms of high affinity iron acquisition.

The 2 other regulators similarly dysregulated among the three conditions were a sensor/response hybrid, PA1243, and a two-component response regulator, GcbA. PA1243 was up-regulated in all three conditions while GcbA was down-regulated. PA1243 was found to be a part of the sigma-22 or AlgT regulon (Wood and Ohman, 2012); however, no other studies on PA1243 have been performed. AlgT is an ECF sigma factor that is involved in the production of alginate and the transition of *P. aeruginosa* during chronic infection from non-mucoid to a mucoid variant (Wood and Ohman, 2012). GcbA, which was found to be down-regulated in all three conditions, is a diguanylate cyclase involved in the production of the secondary messenger, c-di-GMP (Petrova et al., 2014). Although it is found to be involved in the initial attachment stage of biofilms, it is also found to be non-essential for biofilm formation (Petrova et al., 2014). It is found to be involved in flagella-dependent motility and the switch between motile to sessile lifestyle (Petrova et al., 2014). Interestingly, these genes involved in alginate production and c-di-GMP synthesis were similarly dysregulated in all three distinct adaptations.

Among the 63 additional regulators dysregulated in both surfing and biofilm but not swarming, 14 shared the same direction of dysregulation and only two shared up-regulation, the O-antigen chain length regulator, *wzz*, and transcriptional regulator, *nfxB*. Wzz regulates the length of the O-antigen before it is attached to the LPS core (Daniels et al., 2002). NfxB is a repressor of the multidrug efflux system, *mexCD-oprJ* (Purssell and Poole, 2013). An up-regulation of NfxB results in reduced expression of *mexCD-oprJ*. The other down-regulated genes found in surfing and biofilms included several sigma factors such as FiuI, FecI, FoxI, and PvdS, as well as several predicted or putative transcriptional regulators, and the known regulators SphR and FemR. Surfing also shared an inverse dysregulation of 49 regulators

compared to biofilms. These regulators included NalC, GacA, LasR, and CbrA which are regulators found through the mutant library screen to be essential for mediating surfing motility.

Surfing shared 10 additional regulators with swarming but not biofilm cells. Among the ten, only two shared the same direction of dysregulation. PhaD was up-regulated in both swarming and surfing. PhaD (PA5059) in *Pseudomonas oleovorans* was found to be involved in synthesis of poly(3-hydroxyalkanoates) (PHAs), as a means of storing carbon under nutrient limiting conditions (Klinke et al., 2000). Nine of the regulators found to be dysregulated in both surfing and swarming exhibited an inverse relationship. A majority were putative transcriptional regulators and the known regulator RsaL. RsaL is a regulatory protein involved in regulating the expression of virulence genes through the regulation of quorum sensing (De Kievit et al., 2001). This gene was found to be up-regulated in swarming but down-regulated in surfing edge cells.

The mutant library screen revealed 192 mutants with an altered surfing phenotype. Among the 192 were approximately 40 mutants in regulator genes with several in overlapping operons. Surfing was found to be dependent on three quorum sensing systems, Rhl, Las, and Pqs as well as global regulatory systems such as the GacAS and CbrAB two-component systems. The screen also identified 13 uncharacterized or hypothetical regulators. Regulators found to be essential for surfing motility through the mutant library screen were subsequently tested for dysregulation in mutants of the other regulators using RT-qPCR. Results revealed a large distinction between the overall dysregulation when compared to wild-type edge and centre cells. Although relative to centre cells there was a mixture of dysregulated levels, relative to the edge, many of the regulators exhibited mostly up-regulation compared to the wild-type. In order to distinguish a potential hierarchy in regulation, a set of criteria was established. Regulators belonging higher up in the regulatory network had the following characteristics:

1. Mutants of these regulators resulted in a large fold-change relative to the wild-type
2. Regulator expression was relatively unaffected in a large number of mutants
3. Mutants of these regulators affected the expression levels of a large number of surfing-essential regulators

The following regulatory network was proposed based on these criteria as shown in Figure 3-4. Not all of the 40 regulators could be placed in the network due to contradicting characteristics at the edge and centre or contradicting results in relation to the proposed criteria shown above (i.e. mutants caused dysregulation in many genes but were also dysregulated in

many mutants). Therefore, Figure 3-4 highlights regulators that shared similar patterns of dysregulation relative to both centre and edge cells and that could be matched to the proposed criteria. Among the 13 regulators which shared similar effects in both the centre and edge, three regulators were identified as having the largest influence in the expression pattern of other surfing-essential regulators, therefore, higher up in the regulatory network. These regulators were PfeS, CbrA, and PA1463.

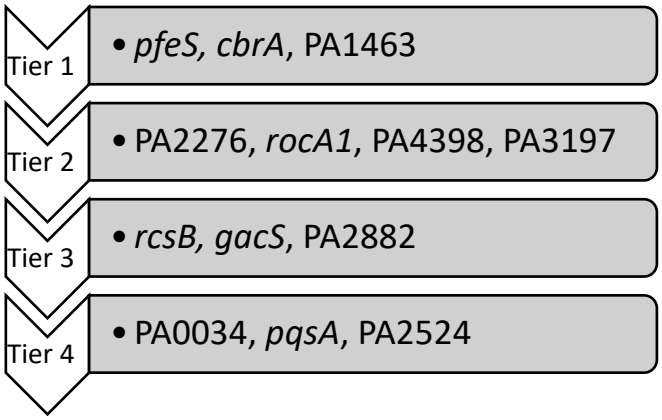


Figure 3-4. **Putative surfing regulatory network based on regulators that had the same expression patterns in centre and edge cells.** Each tier represents regulators whose mutant variant exhibited relatively the same number of dysregulated regulators and whose regulator's expression levels were affected in the same number of mutants. Tier 1 represents regulators who had the most effect on the expression of other regulators, exhibited the highest sum logFC, and which were the least affected in mutants of other regulators. Tier 4 represents the regulators whose mutants had the least effect on the expression of other regulators (0-3), were dysregulated in the greatest number of mutants, and which had a relatively low logFC relative to other mutants. Only 13 regulators exhibited a similar expression pattern between the centre and edge cells as shown here.

Chapter 4. PA1463 and PfeR as major regulators of surfing motility

4.1 Introduction

In Chapter 3, major regulators involved in surfing were identified through a mutant library screen. Despite expression profiles being different at the edge and centre, there was consensus on the importance of 3 major regulators: PfeS, CbrA, and PA1463. CbrA is a two-component sensor cognate to CbrB, the response regulator. The CbrAB two-component system regulates genes for carbon and nitrogen catabolism (Li and Lu, 2007). It was also found to be essential for mediating swarming motility, biofilm formation, and cytotoxicity (Yeung et al., 2014). However, CbrA was found to be involved in virulence during acute infections and antibiotic resistance independent of CbrB (Yeung et al., 2014, 2011). Consistent with its reduced virulence CbrA grown in amoeba was found to be involved in regulating the expression of virulence, iron acquisition, and redox response genes (Yeung et al., 2014). Here I found that transposon mutants in both *cbrA* and *cbrB* were surfing deficient and, therefore, essential for mediating surfing (Chapter 3 Table 3-2). The role of CbrAB has been extensively studied as a global regulator involved in swarming motility (Yeung et al., 2011). The role of PfeS, a sensor kinase, and PA1463, a putative chemotaxis regulator, however, had not been well established and, here I investigated PfeS and PA1463 as major regulators involved in surfing motility.

PfeRS is a two-component regulatory system known to regulate the expression of the ferric enterobactin receptor, *pfeA* (Dean & Poole, 1993). PfeA is normally involved in iron acquisition through the siderophore, enterobactin which is produced by *E. coli*. *P. aeruginosa* is known to produce two types of siderophores, pyoverdine (*pyo*) and pyochelin (*pch*) (Dean et al., 1996). However, *Pseudomonas* can also recognize and use siderophores produced by other bacterial species including enterobactin, ferrioxamine B, and aerobactins (Cornelis et al., 1987; Poole et al., 1990). Here it was shown that knocking out *pfeR* or *pfeA* resulted in surfing deficiency. However, given the lack of enterobactin in the experiments, it is proposed that PfeA may be responsive to other molecules, such as other types of iron chelators.

Here I found that PfeRS appeared to be involved in regulating a large subset of genes specifically under surfing motility. RT-qPCR data (Figure 3.3) revealed that the *pfeS* disruption mutant exhibited dysregulation of several other surfing-essential regulators. Here I performed RNA-Seq on a *pfeR* surfing-deficient knock-out mutant that revealed 1,856 genes dysregulated under surfing conditions indicating that this gene encoded a global regulator. The presence of a

homologous Fur binding site upstream of the *pfeRS* operon indicates that *pfeRS* expression is iron regulated (Dean et al., 1996). Consistent with this I showed here that surfing had a dependency on iron, whereby low iron conditions resulted in reduced surfing which could be restored to some extent by overexpressing the *pfeRS* operon.

PA1463 is an uncharacterized hypothetical chemotaxis protein based on its possession of a conserved CheW domain. It is in a predicted operon of 3 genes, PA1463-PA1464-PA1465 and the latter two genes have also been predicted to be involved in chemotaxis (Mao et al., 2009). It was recently proposed that PA1463 is homologous to the *Vibrio* sp. ParP protein, which plays a role in the localization of chemotactic proteins within the cell (Reinhardt and Bardy, 2018; Ringgaard et al., 2014). Specifically, Reinhardt and Bardy (2018) found that PA1463 regulates the localization of CheA and DipA, directly interacting with DipA. Mutants of both *dipA* and *parP* were found to be deficient in swimming and biofilm dispersal (Reinhardt and Bardy, 2018). DipA is a phosphodiesterase involved in the regulation of c-di-GMP levels (Roy et al., 2012). It reduces c-di-GMP levels and promotes a shift from sessile to motile lifestyle, thus, playing a key role in biofilm dispersion. Therefore, *dipA* mutants exhibit high levels of c-di-GMP, reduced swarming motility, and increased biofilm formation (Roy et al., 2012). Here I found that the PA1463 operon also played a key role in regulating several other regulators, and knocking-out the PA1463 operon resulted in the dysregulation of 827 genes under surfing conditions indicating its potential role as a master regulator.

4.2 Surfing is highly dependent on the *pfeRS* and PA1463 operons

Chromosomal deletions, in the PAO1 strain of *P. aeruginosa*, of *pfeR*, the cognitive response regulator of *pfeS*, and *pfeA*, an enterobactin receptor regulated by PfeRS, were obtained from Dr. Keith Poole, Queen's University (Dean et al., 1996); NB the *pfe* mutants were generated in a spontaneous pyoverdine deficient mutant (PAO6609). PA1463 is the first gene in a 3 gene operon PA1463-PA1464-PA1465 (Mao et al., 2009). A chromosomal deletion mutant of the whole PA1463 operon (PA1463o) was constructed in *P. aeruginosa* PA14. Both mutants in *pfeR* and PA1463o exhibited complete inhibition of surfing motility in SCFM/0.5% agar with mucin (Figure 4-1). Complementation using a high copy plasmid containing each respective operon restored the wild-type surfing phenotype. Conversely both a spontaneous pyoverdine deficient mutant (PAO6609) and a chromosomal deletion mutant of all *pyo/pch* (pyoverdine and pyochelin) genes continued to exhibit wild-type-like surfing, albeit lacking the normal blue/green

pigmentation in the centre of the motility zone. A mutant in *pfeA*, one of the genes known to be regulated by PfeRS, was also found to be surfing deficient when knocked-out. No substantial growth deficiencies were observed for any of the mutants tested, and although the *pfeA* knock-out exhibited slower growth during the exponential phase it reached the same OD_{600} as the other strains after 13 hours (Figure 4-1).

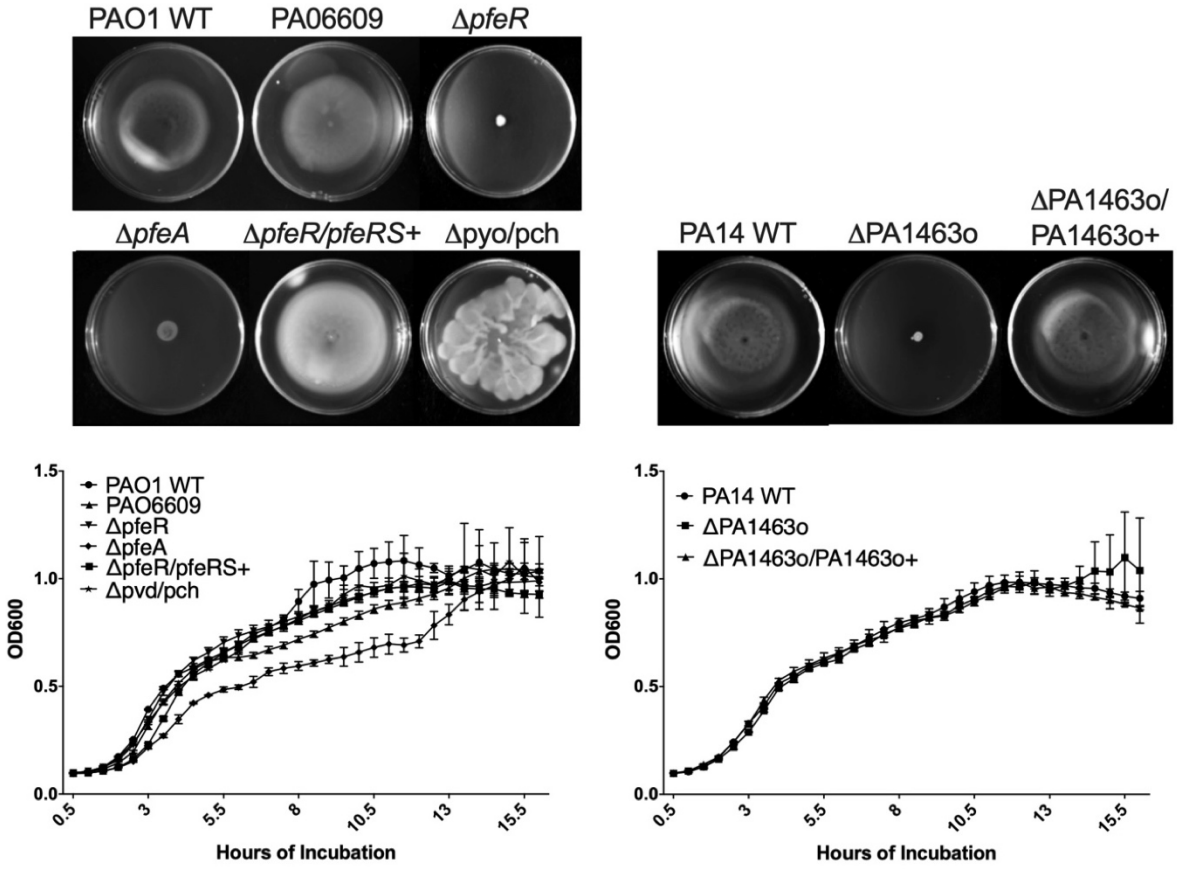


Figure 4-1. Surfing was inhibited in knock out mutations in *pfeR* or the PA1463 operon. All strains were grown on SCFM/0.5% agar with 0.4% mucin and incubated at 37°C for 18 hours. Knock-out mutants of *pfeR* and *pfeA* in the PAO6609 strain and PA1463o in the PA14 strain exhibited surfing deficiency. Mutants complemented with the cloned operons exhibited either wild-type surfing and in the case of Δ*pfeR/pfeRS*+ led to a modest increase in surfing motility (greater surface coverage in the same amount of time). Pyoverdine and pyochelin mutants exhibited similar surfing to the wild-type but lacked pigmentation. The complemented strain Δ*Pyo/pch* exhibited an irregular motility zone shape. Growth curves were generated in regular LB media over a period of 16 hours.

4.3 PfeR and PA1463 as master regulators in surfing

As previously shown in Chapter 3, both *pfeS* and PA1463 were found to be important for mediating surfing motility and their transposon mutants were responsible for the dysregulation of

a large subset of other surfing-essential regulators (Figure 3-3). Table 4-1 shows the RT-qPCR data collected on surfing-essential regulators in the context of the *pfeR* and PA1463o knock-out mutants and their complemented strains. In the *pfeR* mutant, 13 regulators were found to be down-regulated and two up-regulated among the 32 tested. Among these 15, 9 exhibited wild-type expression levels in the complemented strain. In the PA1463o knock-out, 14 regulators were down-regulated with only one significantly up-regulated gene. Thirteen of the dysregulated genes exhibited wild-type levels of expression in the complemented strain. These two mutants had different dysregulation patterns for the tested regulators. For example, *dipA* was significantly dysregulated in the PA1463o mutant but not in the *pfeR* mutant. It is also worthy of note that PA1463 was significantly down-regulated in the *pfeR* mutant but the complemented strain still exhibited significant down-regulation. PfeS, on the other hand, was not significantly dysregulated in the PA1463o mutant.

Table 4-1. Expression of surfing-essential regulators in the *pfeR* and PA1463 operon mutants and complements. RT-qPCR was performed on RNA collected from the *pfeR* and PA1463o knock-outs under surfing conditions (SCFM/0.5% agar with 0.4% mucin) relative to their respective wild-type/parent strains (PA14 for PA1463 and PAO6609 for *pfeR*) also grown under surfing conditions. A fold-change cut-off of ±2 was considered used as a cut-off as indicated with *. *RpoD* was used as the house-keeping gene.

	Δ*pfeR*		**ΔPA1463o**	
Gene	**FC Mutant**	**FC Complement**	**FC Mutant**	**FC Complement**
cbrA	-2.91*	1.42	-1.96	2.31*
cheZ	1.02	-1.80	-1.12	1.09
cueR	-2.70*	-5.02*	-1.98	1.14
cysB	-2.35*	-2.75*	-1.55	1.05
czcS	-4.39*	-1.38	-5.98*	-1.45
dipA	-1.05	1.06	-2.57*	1.57
fleQ	-1.50	1.03	-2.17*	-1.30
fleR	1.69	-1.26	1.32	-2.18*
flgM	-1.73	1.02	-1.43	1.10
gacA	-2.22*	1.29	-4.19*	1.50
lasI	-2.42*	-13.12*	1.24	1.08
motB	-1.03	-1.99	1.17	1.33
nirQ	-2.65*	-1.5	-5.86*	1.37
PA0034	-1.25	-1.15	-1.41	1.50
PA0475	-2.95*	-1.07	-5.84*	-1.31
PA1157	1.13	-1.07	-2.85*	-1.51
PA1463	-4.08*	-3.25*	-	-

PA2276	-3.67*	1.47	-3.40*	-1.05
PA2882	-2.82*	-1.97	-1.04	1.82
PA3197	1.09	-1.19	-2.63*	-1.52
PA3348	-1.30	-2.92*	-1.51	-1.02
PA3599	1.10	1.02	-4.59*	-1.55
PA3921	1.67	-1.07	-1.71	1.15
PA4398	2.75*	1.2	1.30	4.25*
PA4831	-1.32	-1.15	-3.18*	-2.08*
PA5392	-2.45*	-1.22	-2.75*	1.03
pfeS	-	-	-1.41	1.50
pqsA	1.67	-3.18*	4.31*	-2.25*
pqsR	1.51	-2.42*	-2.27*	-1.91
rcsB	-11.67*	-3.67*	-1.46	-1.02
rhlR	2.15*	-2.92*	1.25	-1.61
rocA1	1.9	1.27	1.40	1.79
rpoN	1.51	-1.33	-2.07*	-1.40

4.4 PfeR and PA1463 regulated a large subset of *Pseudomonas* genes

RNA-Seq performed on the PAO6609 (derived from the PAO1 WT) surfing motility edge relative to swimming cells revealed 499 significantly dysregulated genes as summarized in Appendix Table A-3. In comparison, as was shown in Chapter 3, the PA14 strain had 1,094 dysregulated genes in the surfing motility edge relative to swimming cells (NCBI GEO accession number GSE110044). The surfing-deficient Δ*pfeR* mutant relative to the PAO6609 WT under surfing conditions demonstrated 1,856 dysregulated genes (1,177 upregulated, 679 downregulated) where 6 genes exhibited inverse dysregulation compared to wild-type surfing as summarized in Table 4-2. Conversely, the complemented mutant demonstrated only 390 dysregulated genes relative to the WT.

Among the 1,856 genes found to be both dysregulated in the mutant relative to the wild-type under surfing conditions (Appendix Table A-4), there were several virulence genes including phosphatases, phospholipases, phenazines, and elastases. There were also several redox genes known to be involved in the oxidative stress response. There was a large subset of iron transport genes up-regulated including several pyochelin synthesis genes and *fptA*, the pyochelin-iron receptor. Several other iron transporters, including *fpvB*, the alternative pyoverdine transporter, were found to be down-regulated. TonB, involved in energization of siderophore-mediated iron acquisition in *Pseudomonas* (Poole et al., 1996), was found to be up-regulated. Also among the

genes dysregulated in the mutant were the *rhlI* and *rhlR* genes, that mediated the Rhl quorum sensing regulation, which had previously been shown to be essential for mediating surfing (Table 3-3). Table 4-2 shows 48 genes previously found to be essential for surfing initation through the mutant library screen (Chapter 3) that were dysregulated in the Δ*pfeR* mutant compared to the parent strain including genes involved in alginate biosynthesis (*algX*) and fimbriae assembly (*cupA, cupE*) which were ~4 fold down-regulated.

Table 4-2. Surfing-essential effectors dysregulated in the Δ*pfeR* and ΔPA1463o mutants. RNA-Seq was performed on the surfing deficient Δ*pfeR* and ΔPA1463 mutants compared to the parent strain PAO6609 and wild-type PA14 surfing respectively in SCFM/0.5% agar with 0.4% mucin. A log fold-change cut-off of ± 1.5 and p-value < 0.05 was used. Surfing-essential genes were found through the PA14 Tn mutant library screen as described in Chapter 3. Those genes were matched up to the dysregulated genes in both mutants to reveal 48 surfing-essential effector genes dysregulated in the Δ*pfeR* mutant and 17 in the ΔPA1463 mutant. Descriptions and gene annotations are from www.pseudomonas.com (Winsor et al., 2016).

Gene ID	Gene Name	Description	LogFC Mutant/ WT surf
***pfeR*/PAO6609**			
PA0104		Hypothetical protein	-2.52
PA0504	*bioD*	Dethiobiotin synthase	-1.57
PA0545		Hypothetical protein	-2.59
PA0551	*epd*	D-erythrose 4-phosphate dehydrogenase	1.56
PA0663		Hypothetical protein	1.55
PA0718		Hypothetical protein of bacteriophage Pf1	-2.44
PA0766	*mucD*	Serine protease	1.60
PA0817		Probable ring-cleaving dioxygenase	-2.30
PA1119	*yfiB*	YfiB	1.96
PA1187		Probable acyl-Coa dehydrogenase	-2.17
PA1271		Probable TonB-dependent receptor	1.75
PA1547		Hypothetical protein	-2.66
PA1875		Probable outer membrane protein precursor	4.30
PA1935		Hypothetical protein	-2.55
PA1982	*exaA*	Quinoprotein ethanol dehydrogenase	-1.91
PA2009	*hmgA*	Homogentisate 1,2-dioxygenase	2.68
PA2089		Hypothetical protein	-4.26
PA2120		Hypothetical protein	-2.43
PA2130	*cupA3*	Usher cupa3	-4.35
PA2195	*hcnC*	Hydrogen cyanide synthase	1.56
PA2576		Hypothetical protein	-2.15
PA2685	*vgrG4*	Vgrg4	1.59
PA2693		Conserved hypothetical protein	-2.18

PA2969	*plsX*	Fatty acid biosynthesis protein	2.14
PA3324		Probable short-chain dehydrogenase	-2.64
PA3325		Conserved hypothetical protein	-2.12
PA3387	*rhlG*	Beta-ketoacyl reductase	-2.02
PA3546	*algX*	Alginate biosynthesis protein	-4.73
PA3573		Probable major facilitator superfamily (MFS) transporter	-1.63
PA3735	*thrC*	Threonine synthase	1.52
PA3749		Probable major facilitator superfamily (MFS) transporter	-3.83
PA3818		Extragenic suppressor protein	2.54
PA3884		Hypothetical protein	-3.15
PA4001	*sltB1*	Soluble lytic transglycosylase B	1.53
PA4006	*nadD1*	Nicotinate mononucleotide adenylyltransferase	1.76
PA4144		Probable outer membrane protein precursor	-2.24
PA4168	*fpvB*	Second ferric pyoverdine receptor	1.76
PA4210	*phzA1*	Probable phenazine biosynthesis protein	5.38
PA4471		Hypothetical protein	5.22
PA4612		Conserved hypothetical protein	-2.27
PA4650	*cupE3*	Pilin subunit	2.04
PA4743	*rbfA*	Ribosome-binding factor A	2.91
PA4753		Conserved hypothetical protein	2.00
PA4981	*lysP*	Lysine-specific permease	-2.39
PA5015	*aceE*	Pyruvate dehydrogenase	1.71
PA5192	*pckA*	Phosphoenolpyruvate carboxykinase	1.78
PA5323	*argB*	Acetylglutamate kinase	2.48
PA5399	*dgcB*	Dimethylglycine catabolism	-2.93
PA5555	*atpG*	ATP synthase gamma chain	1.92
PA1463o/PA14 WT			
PA0062		Lipoprotein	1.95
PA0298	*spuB*	Glutamine synthetase	-2.07
PA0545		Hypothetical protein	-1.75
PA0766	*mucD*	Serine protease	1.73
PA1838	*cysI*	Sulfite reductase	1.53
PA2120		Hypothetical protein	-2.29
PA2693		Hypothetical protein	-1.77
PA2927		Hypothetical protein	1.82
PA3324		Short chain dehydrogenase	-1.51
PA3730		Hypothetical protein	-2.04
PA4144		Outer membrane protein	2.38
PA4552	*pilW*	Type 4 fimbrial biogenesis protein	2.11
PA4616		C4-dicarboxylate-binding protein	-1.66
PA4838		Hypothetical protein	4.61
PA5109		Hypothetical protein	1.99
PA5323	*argB*	Acetylglutamate kinase	1.64
PA5399		Ferredoxin	-3.49

RNA-Seq on the ΔPA1463o mutant relative to the PA14 WT under surfing conditions revealed 827 significantly dysregulated genes and only 81 that were dysregulated in the PA1463o complement strain relative to the wild-type. Of interest, four chemotaxis genes, namely *cheW*, *cheA*, PA0236, and PA0176, were up-regulated in the PA1463o mutant relative to WT surfing (Table 4-2, A-5). CheW and PA01776 exhibited a ~1.6-1.7 fold upregulation in the mutant whereas they were found to be down-regulated in wild-type surfing (Table 4-2). The mutant also exhibited significant downregulation in the PQS quorum sensing system (Appendix Table A-5), which had also been shown through the mutant library screen to be essential for mediating surfing motility (Table 3-2). More specifically, the mutant exhibited an up-regulation of the *pqsABCDE* operon and *pqsH*, which are involved in synthesizing the final autoinducer, PQS, under surfing conditions relative to the WT. Among the 827 dysregulated genes in the mutant, 17 were previously found to be essential for surfing initiation through the mutant library screen (Chapter 3) as summarized in Table 4-2 including genes involved in alginate biosynthesis (*mucD*) and pili assembly (*pilW*).

4.5 Surfing dependence on iron and PfeRS regulation

Due to the known involvement of the PfeRS system in iron acquisition (Dean et al., 1996; Dean & Poole, 1993), the dependency of surfing on iron was investigated. Dipyridyl is a chelator that binds iron to derecase its availability to bacteria. Titrating dipyridyl into SCFM/0.3% agar with mucin resulted in an initial shift from surfing occurring on the surface to swimming which occurred within the agar (Figure 4-2). Increasing the concentration of dipyridyl from 25μM to 50μM resulted in a shift from surfing to swimming. Increasing concentrations of dipyridyl or decreasing concentrations of iron resulted in reduced swimming, with a complete abolishment of motility and growth at 500μM dipyridyl. Adding iron ($FeSO_4$) into a system where surfing had been prevented (50μM dipyridyl) resulted in a gradual increase in surfing motility. Surfing was completely restored by adding 10μM $FeSO_4$. In 500μM of dipyridyl that completely inhibited motility and growth, addition of 200μM $FeSO_4$ restored wild-type surfing. Increasing iron concentrations led to an increase in the density of surface growth. Overexpression of *pfeRS* in the wild-type abolished the switch to swimming when dipyridyl was titrated into the system indicating a role for this transport system in efficiently assimilating iron in the context of surfing motility. In this case, surfing motility only became deficient at 250μM dipyridyl and growth was

absent at 500μM dipyridyl.

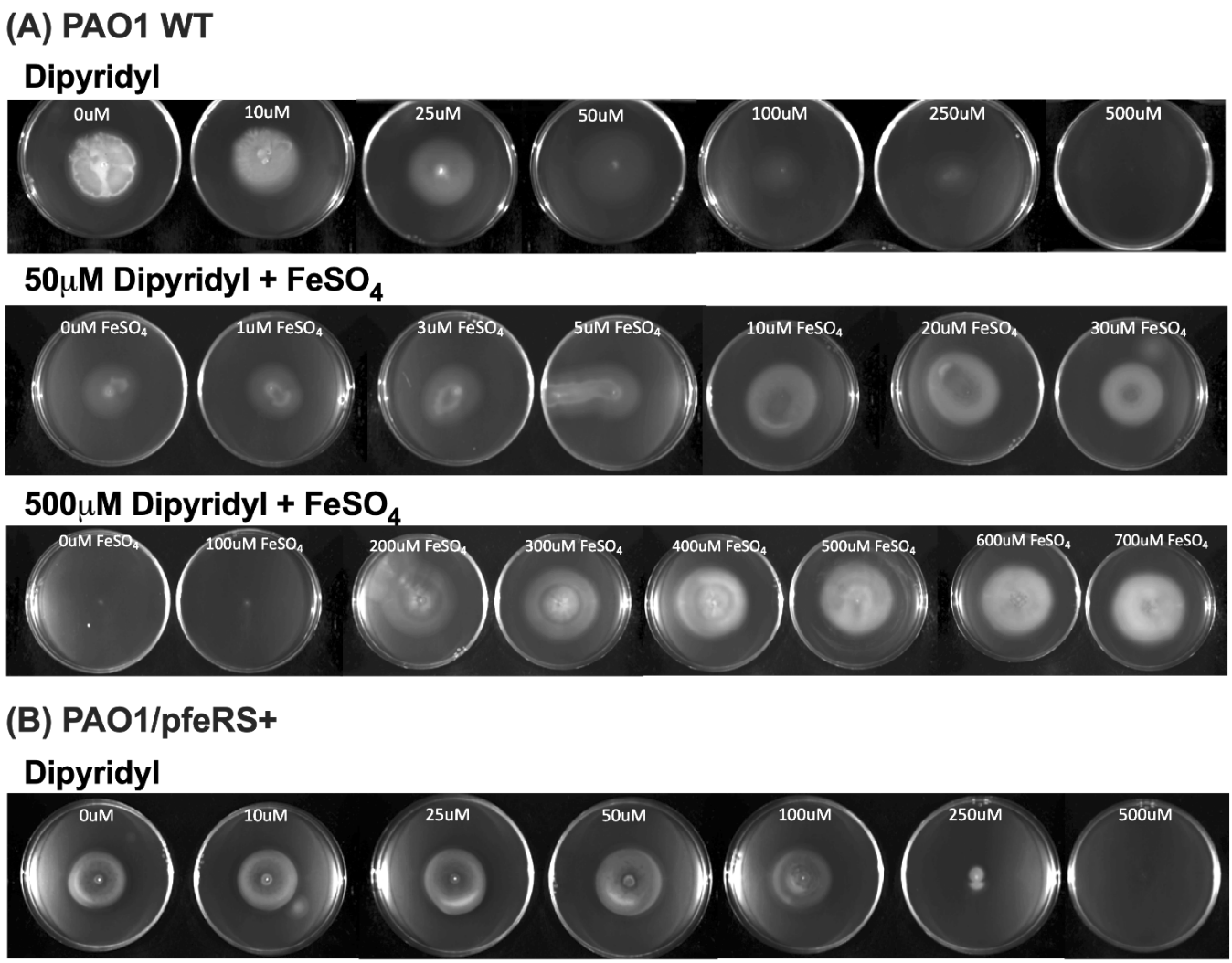


Figure 4-2. Surfing motility was dependent on iron and surfing persisted under more extreme iron-limiting conditions when *pfeRS* was overexpressed. Iron was removed from the medium using 2,2-dipyridyl. Cells were inoculated and grown at 37°C for 15 hours in SCFM with 0.3% agar and 0.4% mucin.

4.6 Discussion

The mutant library screen (Table 3-2) identified 44 regulators found to be essential for mediating surfing motility. Among these regulators, a checkerboard RT-qPCR assay measuring the expression levels of 39 of these regulators in transposon mutants in 29 of these regulators revealed 3 that appeared to act as master regulators affecting the expression levels of a large subset of other essential surfing regulators. Among the three, *pfeS* and PA1463 had not been previously investigated as master regulators. RNA-Seq revealed that knock out either the *pfeR*

regulator in PAO6609 or the PA1463 operon in PA14 resulted in the dysregulation of a large number of genes, 827 and 1,856 respectively. Both RT-qPCR (Table 4-1) and RNASeq (Table A-4, A-5) also revealed that these mutants in *pfeR* and PA1463o affected the expression levels, mainly down-regulating, of several essential surfing regulators (Table 3-2). Similarly, compared to wild type surfing cells, the Δ*pfeR* and ΔPA1463o mutants both exhibited down-regulation in the master regulator, *gacA*, the denitrification regulator, *nirQ* (Hayashi et al., 1998), the two-component sensor found to be involved in carbapenem resistance, *czcS* (Perron et al., 2004), and several hypothetical regulators, PA0475, PA2276, and PA5392. Interestingly, although the expression levels of *pfeS* and *pfeR* were unaffected in the PA1463o mutant, PA1463 was significantly down-regulated in the *pfeR* mutant. A *ΔpfeR* mutant affected the expression of more than twice the number of genes compared to ΔPA1463, including affecting the expression of PA1463 itself. Therefore, PfeRS appeared to be higher in the hierarchy of regulation than PA1463. Among the genes dysregulated in the mutants as found through RNA-Seq, 344 genes were similarly dysregulated between the mutants. This constitutes approximately half of the dysregulated genes in the PA1463 mutant.

PA1463, as previously mentioned, is potentially a homolog of the ParP partitioning protein and contains a homologous CheW domain, which functions in CheW in the localization of CheA and DipA (Ringgaard et al., 2014). Interestingly according to the RNA-Seq data, chemotaxis genes including *cheA* and *cheW* were found to dysregulated in the ΔPA1463o mutant, which indicates that PA1463o might also be involved in regulating the expression of *cheA* and *cheW*. Notably, *cheW* exhibited an inverse dysregulation in the mutant compared to the wild-type. DipA expression was also significantly down-regulated in the PA1463o mutant as also found through RT-qPCR (Table 4-1), which indicates that PA1463 also regulates *dipA* expression. DipA, in turn, regulates several adaptions by regulating the levels of c-di-GMP. Based on the RT-qPCR data, knock out of PA1463o also found affected the expression of several known master regulators including down-regulating the expression of *gacA* and *nirQ*. RNA-Seq revealed that several Pqs genes were also dysregulated in the ΔPA1463o mutant including the entire *pqsABCDE* operon and *pqsH*, all involved in the synthesis of the autoinducer PQS. As shown below, I found that the Pqs quorum sensing system played a key role in mediating surfing motility.

The knock-out of *pfeR* resulted in a massive dysregulation of >1,800 genes including the Rhl

quorum sensing regulators, *rhlI* and *rhlR*. In addition to the known regulation of *pfeA*, an iron acquisition receptor, it was found here to affect the expression of several other siderophore receptors as well as the genes mediating the synthesis of pyoverdine and pyochelin. Overexpressing the *pfeRS* operon in the PAO1 WT resulted in more resilient surfing in iron-limiting conditions, speaking to its role in mediating high-affinity iron acquisition by as yet unknown mechanisms. Although the role of PfeRS in iron-acquisition through PfeA has been extensively studied (Dean et al., 1996; Dean & Poole, 1993; Poole et al., 1990), it has not yet been shown to be involved in regulating other siderophores. Its role as a master regulator has also not been explored. Here I showed that knocking out *pfeR* resulted in massive transcriptional dysregulation including affecting the expression levels of several known global regulators like GacA, CbrA, RcsB, and PA1463 in the context of surfing. In addition to the many surfing-essential regulators found to be dysregulated in the two mutants, Δ*pfeR* and ΔPA1463o, several surfing-essential effectors were also found to be dysregulated, 48 in the *pfeR* mutant and 17 in the PA1463o knock-out. Both shared a dysregulation in genes involved in alginate and pili/fimbriae biosynthesis, which may suggest an important role for regulators *pfeRS* and PA1463 in the initiation of surfing motility.

Chapter 5. Broad-spectrum adaptive antibiotic resistance associated with *Pseudomonas aeruginosa* mucin-dependent surfing motility

5.1 Introduction

The rise of antibiotic resistance is a global concern. As the number of new antibiotics being discovered declines and the extensive and sometimes inappropriate use of antibiotics continues, more patients suffer and die from infections caused by antibiotic resistant bacteria (Bassetti et al., 2013; Ventola, 2015). As mentioned in Chapter 1, *P. aeruginosa* can deploy intrinsic, acquired and adaptive resistance mechanisms (Breidenstein et al., 2011; Taylor et al., 2014). Adaptive resistance refers to resistance that occurs due to environmental circumstances (e.g. exposure to stresses including antibiotics, complex adaptive growth states such as swarming or biofilm formation, etc.) and is thought to be largely due to transcriptional changes in genes that determine resistance/susceptibility and is reversible when environmental circumstances are reversed (Taylor et al., 2014).

Here I expanded on the original observation (Yeung et al., 2012) that surfing cells were polymyxin resistant to demonstrate that surfing cells exhibited multi-drug adaptive resistance, dependent on the complex adaptive changes that accompanied this motility phenotype. Compared to swimming, surfing adaptive cells were significantly more resistant to several classes of antibiotics including aminoglycosides, polymyxins, flouroquinolones, and carbapenems. Screening mutants in resistome genes that were found by me to be dysregulated under surfing conditions revealed changes in susceptibility under surfing conditions that may account for their contribution to the observed resistance.

5.2 Surfing cells exhibited broad-spectrum antibiotic resistance

Disk diffusion assay results (Figure 5-1), assessing how close surfing and swimming cells approached an antibiotic disk, revealed a significant decrease in the zone of inhibition (i.e. increased resistance) under surfing conditions (SCFM/0.3% agar, 0.4% mucin) when compared to swimming (SCFM/0.3% agar). This was observed for 12 of the 17 antibiotics tested with the exceptions of 3 of the β-lactams and 2 macrolides. Compared to swimming bacteria, and disk diffusion assays on solid 1.5% agar plates, surfing cells exhibited significant adaptive resistance to the tested aminoglycosides, carbapenems, polymyxins, fluoroquinolones, trimethoprim, tetracycline, and chloramphenicol, with complete resistance to 3 different aminoglycosides, imipenem, clarithromycin, and the polymyxins (Figure 5-1).

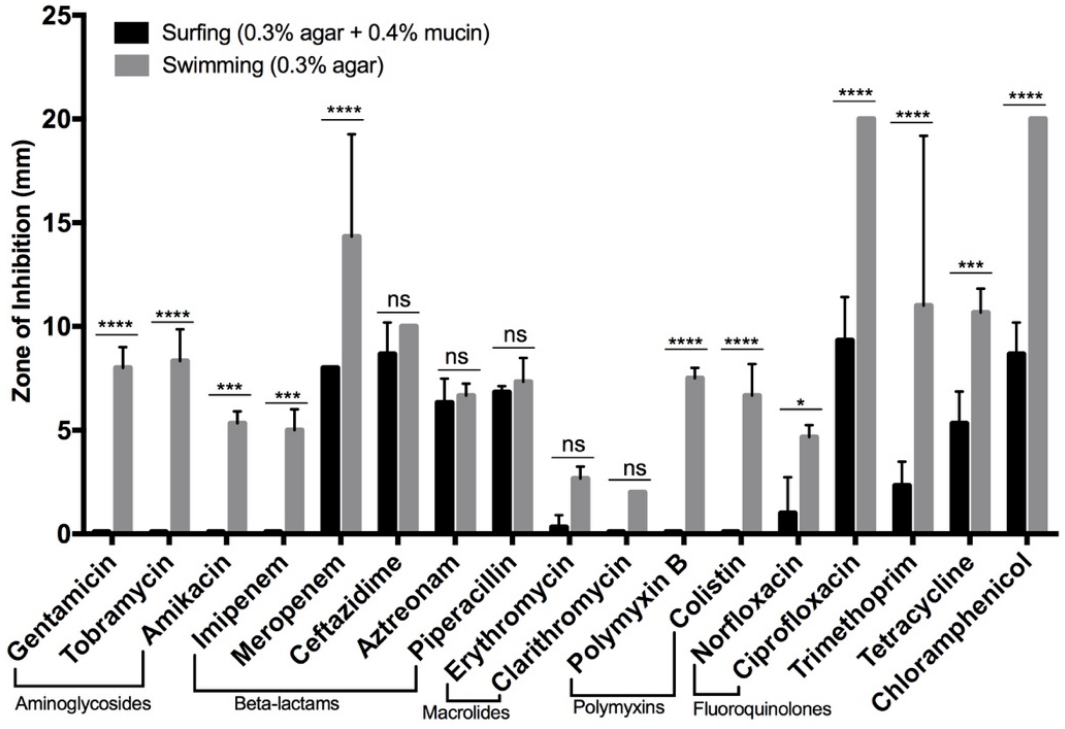


Figure 5-1. Multi-drug adaptive resistance of surfing colonies. The zones of inhibition (mm) under swimming (0.3% agar) and surfing (0.3% agar 0.4% mucin) conditions in SCFM were obtained using the motility disk diffusion method with 17 different antibiotics. Statistical significance between swimming and surfing was determined using two-way ANOVA based on 3 independent experiments: * $p<0.05$, ** $p<0.01$, *** $p< 10^{-3}$, **** $p<10^{-4}$.

5.3 Antibiotic incorporation assays to confirm adaptive resistance

To further investigate the adaptive resistance of surfing colonies, 5 selected antibiotics were incorporated into growth plates to determine how they affected the initiation and propagation of motility colonies. Four of the selected antibiotics, polymyxin B, imipenem, tobramycin and norfloxacin were chosen as representatives of their antibiotic classes that showed the greatest (or complete) resistance under surfing conditions compared to swimming, and no effect of mucin on MICs in liquid media (Table A-6). Tetracycline was chosen since disk diffusion results were more consistent when compared to trimethoprim and chloramphenicol. Antibiotic incorporation assays, revealed a concentration-dependent inhibition of surfing motility and showed that surfing motility proceeded at antibiotic concentrations that completely inhibited swimming (Figure 5-2).

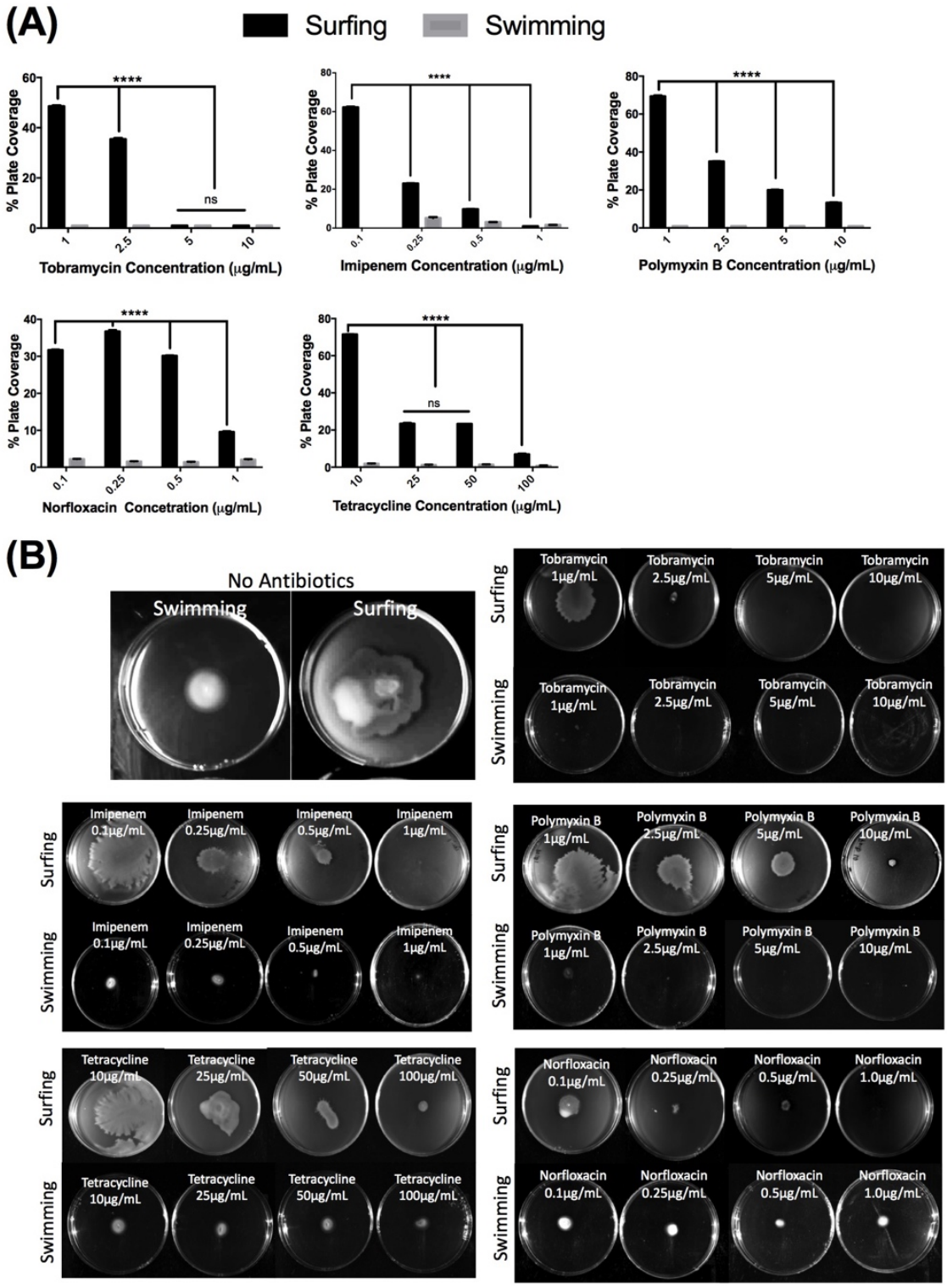


Figure 5-2. Antibiotic concentration dependent inhibition of surfing motility. Surfing motility colonies of wild-type PA14 were assessed with the antibiotic at varying concentrations incorporated into 25 mL of SCFM/0.3% agar containing 0.4% mucin (surfing) or no mucin (swimming). Incorporation assay results in part A are described as the % plate coverage, relative

to the control with no antibiotics, measured using Image J. Surfing colonies are represented by the black bars and swimming by the grey bars. Statistical significance between surfing and swimming was assessed using two-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p< 10^{-3}$, **** $p<10^{-4}$

For example, surfing on the agar surface occurred on 0.1 μM imipenem whereas swimming in-agar was completely abolished at this concentration. As the imipenem concentration increased, there was a clear reduction in the size of the surfing colony and at a concentration of 1 μM imipenem both surfing and swimming were completely inhibited. Indeed, for all five antibiotics tested, inhibition of surfing occurred with increasing concentrations but still occurred to some extent at concentrations much higher than those inhibiting swimming.

5.4 Adaptive antibiotic resistance was not due to the presence of mucin alone

To show that the observed resistances were attributable to the surfing adaptation rather than the presence of mucin, I examined the effect of mucin on antibiotic activity. Mucin itself could conceivably influence antibiotic diffusion or susceptibility. Therefore, as one control I assessed the effect of mucin on antibiotic susceptibility by testing its effects in a disk diffusion format using 1.5% agar, under which conditions surfing did not occur. I observed that for 9 out of the 12 antibiotics for which surfing cells demonstrated resistance (and 3 of 5 for which they did not demonstrate resistance), there were no significant differences in the diffusion zone between agar plates with and without mucin, indicating that per se mucin had a minimal influence on antibiotic susceptibility (Table 5-1). For those that show a mucin dependent alteration of susceptibility on 1.5% agar plates, tobramycin revealed increased susceptibility in the presence of mucin, the opposite of the effect of surfing conditions, while amikacin showed a partial but much lesser effect cf. surfing, and ciprofloxacin showed a significant reduction in susceptibility. From this, I concluded that, with the possible exception of ciprofloxacin, the surfing adaptation rather than altered antibiotic diffusion was responsible for the observed multidrug adaptive resistance phenotype.

I also assessed the broth dilution MIC of *P. aeruginosa* PA14 (Appendix Table A-6) in the presence and absence of mucin and observed increased MIC values accompanying mucin addition for gentamicin, amikacin and colistin, but no differences for other antibiotics in the same classes (tobramycin, polymyxin B). Conversely, for tetracycline mucin actually increased susceptibility by 4-fold. Overall these data suggested that the observed resistances for most antibiotics (Figure 5-1 and 5-2) was likely due to the adaptation accompanying surfing motility

rather than the presence per se of mucin. For this reason, I investigated these adaptive changes in greater detail.

Table 5-1. Mucin addition had little impact on antibiotic susceptibility at hiogher agar concentrations that prevent surfing motility. Disk diffusion assays were performed on SCFM with 1.5% agar in the absence or presence of 0.4% mucin. Bacterial cultures were spread as lawn and antibiotic disks applied on top. The zones of inhibition (mm) were measured after overnight incubation at 37°C. P-values were calculated using 2-way ANOVA.

	Zone of clearing on SCFM plates		
Antibiotic	**1.5% agar**	**1.5% agar + 0.4% mucin**	**P-value**
Gentamicin	8.7	8.0	1.0
Tobramycin	5.3	10.7	$<10^{-4}$
Amikacin	7.3	3.7	$<10^{-4}$
Imipenem	4.3	4.7	>1.0
Meropenem	10.7	9.0	0.6
Ceftazidime	10.7	9.3	0.7
Erythromycin	4.0	9.3	$<10^{-4}$
Clarithromycin	2.7	6.3	$<10^{-4}$
Aztreonam	6.3	6.7	>1.0
Piperacillin	8.0	6.7	0.7
Polymyxin B	3.0	3.0	>1.0
Colistin	4.0	2.3	0.6
Norfloxacin	5.0	3.7	0.7
Ciprofloxacin	9.7	6.3	0.0003
Trimethoprim	5.7	5.7	>1.0
Tetracycline	9.3	8.3	1.0
Chloramphenicol	9.7	10.3	1.0

5.5 Surfing-mediated antibiotic resistance is associated with multiple resistome genes

RNA-Seq data (NCBI GEO Accession: GSE110044), comparing the surfing colony edge and centre (SCFM 0.3% agar, 0.4% mucin) to swimming in agar (SCFM 0.3% agar), as previously discussed in Chapter 3, revealed that the surfing adaptation strongly affected gene expression. In total, when compared to swimming in liquid media, there were 1,467 genes dysregulated at the edge and 2,078 genes in the centre, with 816 genes commonly dysregulated between the two, while differences were consistent with the strong phenotypic differences in the blue-green centre and at the thick white edge of surfing colonies (Yeung et al., 2012). In particular these global gene expression data confirmed that the surfing adaptation was considerably different from swarming in that, out of the 1,467 genes dysregulated at the edge of

the surfing colony, only 215 (14.6%) matched those previously (Overhage et al., 2008) found to be dysregulated during swarming, while out of the 2,078 dysregulated in the centre, 217 (10.5%) overlapped with those from swarming cells.

To examine the possibility that adaptive resistance during surfing motility was due to the dysregulation of genes that influenced resistance, literature searches were conducted. This revealed 119 genes that when mutated led to increased susceptibility (intrinsic resistance genes) and 252 genes that when mutated mediated antibiotic resistance; collectively these form the resistomes for various antibiotics (Alvarez-Ortega et al., 2010; Breidenstein et al., 2008; Dötsch et al., 2009; Fernández et al., 2010; Gallagher et al., 2011; Schurek et al., 2008; Wiegand et al., 2008). Among the resistome genes, 65 were identified, through RNA-Seq gene expression data from surfing cells, that matched the direction of dysregulation of expression levels expected if they were to have a potential role in surfing mediated resistance. Thus to be potentially important for surfing-mediated resistance, one would expect genes for which mutants led to resistance to be transcriptionally downregulated, while intrinsic resistance genes for which mutants led to supersusceptibility would be expected to be upregulated. Available transposon mutants of these 65 resistome genes were tested for changes in susceptibility to certain antibiotics.

Table 5-2 shows the resistome genes dysregulated at the edge and/or centre for which transposon mutants showed a change in susceptibility, under surfing motility conditions, to at least one of the 5 tested antibiotics based on a disk diffusion assay. The mean zone of inhibition measurements are presented in Appendix Table A-7. Several of these genes showed a change in susceptibility to more than one antibiotic, possibly illustrating a contribution to broad-spectrum resistance under surfing conditions.

Table 5-2. Resistome genes and their corresponding changes, when mutated, in antibiotic susceptibility relative to the wild-type under surfing conditions. This group included 8 resistome genes similarly regulated in both the centre and at the edge. A further 10 resistome genes were dysregulated only at the edge of surfing colonies and affected in such a way as to influence resistance or susceptibility. Twenty resistome genes, dysregulated only in the centre of surfing colonies, were affected in such a way as to influence antibiotic resistance or susceptibility.

Gene	Gene function (Winsor et al., 2016)	RNA-Seq Fold Change		Antibiotic susceptibility, under surfing conditions, of mutant relative to WT[a]
		Centre	Edge	
armR	Anti-repressor for MexR	-3.2	-5.1	TOB^R, IMI^R, PXB^R, NFX^R, TET^R
atpB	ATP synthase A chain	-2.1	NC[b]	TOB^R, NFX^R

braB	Branched chain amino acid transporter	-4.2	NC	NFX^R
ccmF	Cytochrome C-type biogenesis protein	-2.2	NC	TOB^R, PXB^R, NFX^R
ccoO1	Cytochrome c oxidase, cbb3-type, CcoO subunit	-3.5	NC	TOB^R, NFX^R
clpS	ATP-dependent Clp protease adaptor protein	NC	-2.3	TOB^R, IMI^R, PXB^R, NFX^R
cycH	Cytochrome c-type biogenesis protein	NC	2.2	TOB^S, PXB^S
ddaH	Dimethylarginine dimethylaminohydrolase	4.9	3.4	IMI^S, TET^S
etfA	Electron transfer flavoprotein α-subunit	-6.2	NC	TOB^R, PXB^R, NFX^R
gidA	Glucose-inhibited division protein A	-2.2	NC	NFX^R
htpX	Heat shock protein	-2.0	NC	NFX^R
mutS	DNA mismatch repair protein	-2.5	NC	TOB^R, PXB^R
nalC	Transcriptional regulator	-5.3	-2.7	TOB^R, PXB^R, NFX^R
nuoB	NADH dehydrogenase I chain B	-2.8	NC	TOB^R, IMI^R, PXB^R, NFX^R
nuoF	NADH dehydrogenase I chain F	-2.4	NC	TOB^R
nuoG	NADH dehydrogenase I chain G	-2.1	NC	TOB^R, NFX^R
PA1348	Hypothetical protein	NC	-3.4	IMI^R, NFX^R
PA1428	Conserved hypothetical protein	-3.4	NC	TOB^R, NFX^R
PA1513	Hypothetical protein	NC	-3.0	TET^R
PA2047	Probable transcriptional regulator	NC	-2.0	TOB^R, NFX^R
PA2566	Conserved hypothetical protein	NC	-5.0	NFX^R
PA2571	Probable two-component sensor	NC	-2.7	TOB^R
PA3233	Hypothetical protein	2.5	NC	NFX^S
PA3576	Hypothetical protein	NC	-2.9	TOB^R, NFX^R, TET^R
PA3667	Probable pyridoxal-phosphate dependent enzyme	-1.7	-2.5	TET^R
PA4292	Probable phosphate transporter	-6.7	NC	TOB^R, IMI^R, PXB^R, NFX^R
PA4429	Probable cytochrome c1 precursor	-2.3	NC	TOB^R, PXB^R, NFX^R
PA4766	Conserved hypothetical protein	NC	-2.3	TOB^R
PA4781	Cyclic di-GMP phosphodiesterase	NC	-2.9	TOB^R, NFX^R
PA5130	Conserved hypothetical protein	NC	2.4	TOB^S, IMI^S, PXB^S, NFX^S, TET^S
pchF	Pyochelin synthetase	-2.2	NC	TOB^R
pckA	Phosphoenolpyruvate carboxykinase	-2.6	NC	TOB^R
recG	ATP-dependent DNA helicase	1.9	2.1	TOB^S, PXB^S, NFX^S, TET^S
rph	Ribonuclease PH	-2.3	NC	TOB^R
serA	D-3-phosphoglycerate dehydrogenase	-12.4	NC	TOB^R, PXB^R, NFX^R
thiG	Thiamine biosynthesis protein, thiazole moiety	-2.9	NC	IMI^R, NFX^R

[a] Antibiotic abbreviations are IMI - imipenem, TET - tetracycline, PXB - polymyxin B, TOB - tobramycin, NFX - norfloxacin. Superscript R indicates resistant; superscript S indicates supersusceptible.

[b]NC = no change in gene expression under the given condition

Five of the tested mutants, Δ*recG,* Δ*ddaH,* Δ*armR,* Δ*nalC*, and ΔPA3667, were similarly

dysregulated in the centre and edge of a surfing colony, with *recG* and *ddaH* both up-regulated and *armR*, *nalC*, and *PA3667* down-regulated. Complementation of selected resistome mutants with the respective cloned genes showed that this broad-spectrum effect could be significantly reversed either partially, completely or excessively (Table 5-3), while overexpression of some of these genes also revealed a change in susceptibility to other antibiotics as shown in Table 5-3. RT-qPCR data (Table A-9) verified the direction of dysregulation shown in the RNA-Seq data for selected resistome genes.

Table 5-3. **Complementation of selected resistome mutants that showed broad spectrum changes in surfing-dependent susceptibility led to restoration of antibiotic susceptibility.** Results show the average zones of inhibition of each mutant and its complemented equivalent against five antibiotics (n=3) cf. wild-type (n=6). Mutants of up-regulated resistome genes were tested against 10 µg/disk of antibiotic and down-regulated against 100 µg/disk. Standard deviations ranged between 0 and 2.5 mm. Statistical significance relative to wild-type was determined using two-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p< 10^{-3}$, **** $p<10^{-4}$

Strain	Zone of Inhibition (mm)				
	Imipenem	**Tetracycline**	**Polymyxin B**	**Tobramycin**	**Norfloxacin**
10 µg/disk antibiotic concentration					
Wild-type	5.7	5.0	5.6	3.3	1.0
Δ*recG*	7.3	8.7*	9.7**	12.5****	7.3****
Δ*recG*/*recG*$^+$	6.0	5.7	3.7	6.7*	3.0
Δ*ddaH*	9.0**	0***	5.3	3.0	2.3
Δ*ddaH*/*ddaH*$^+$	6.3	3.3	5.7	6.3	2.0
100 µg/disk antibiotic concentration					
Wild-type	12.3	6.7	10.3	12.0	14.7
Δ*PA1428*	12.7	7.7	8.0	7.0***	0.0****
Δ*PA1428*/*PA1428*$^+$	9.0*	7.3	9.0	11.6	13.3
Δ*PA2047*	12.3	7.0	5.7	7.3**	9.7***
Δ*PA2047*/*PA2047*$^+$	9.7	7.0	7.3	11.3	12.0
Δ*thiG*	6.3****	6.7	7.0	8.7	10.3**
Δ*thiG*/thiG$^+$	9.0*	8.7	8.3	11.0	15.0
Δ*atpB*	9.7	4.0	8.0	8.3*	9.7***
Δ*atpB*/*atpB*+	10.3	7.3	8.3	12.0	14.0
Δ*PA3667*	15.7	0.0****	7.7	10.0	12.0
Δ*PA3667*/*PA3667*$^+$	12.0	9.3	11.3*	11.0	9.7***
Δ*PA3576*	12.0	3.0*	6.0	8.3*	10.7*
Δ*PA3576*/*PA3576*$^+$	10.7	6.3	7.3	9.0*	12.3
Δ*PA3721*	10	2**	14.5****	0****	10**
Δ*PA3721*/*PA3721*$^+$	11.7	8.3	9.3	11.3	13.0
Δ*clpS*	8.3*	6.3	15****	6.7***	8.3****
Δ*clpS*/*clpS*$^+$	11.3	13.0****	11.5	11.7	12.3
Δ*armR*	0****	0****	1.0****	6.3****	0****

Δ*armR*/*armR*$^{+}$	12.3	12.3****	10.3	12.7	15.0

5.6 Discussion

P. aeruginosa is a highly adaptable organism that exhibits diverse lifestyles from coordinated forms of motility like swarming to community-based sessile structures like biofilms. Another lifestyle includes *P. aeruginosa* surfing motility that occurs on the surface of agar plates under artificial cystic fibrosis-like conditions where the mucin content is high (Yeung et al., 2012). In Chapter 3, I presented data that confirmed the role of this motility form as a complex adaptation influencing expression of hundreds of genes. Here I demonstrated that this novel form of motility is associated with multidrug adaptive resistance. Both disk diffusion and antibiotic incorporation assays revealed that cells undergoing surfing were significantly more resistant to multiple antibiotics compared to swimming, and the same concentrations of antibiotics that completely abolished swimming were found to be much less effective against surfing cells.

Antibiotic susceptibility was generally unaffected by mucin in the presence of high agar concentrations at which swimming and surfing do not occur, indicating that mucin had a minimal effect on susceptibility to most antibiotics (Table 5-1). MIC assays also confirmed that the observed adaptive resistance was dependent on surface growth associated with the surfing adaption, and not merely due to the presence of mucin. Indeed experiments measuring the resistance of surfing and swimming colonies to antibiotics incorporated into agar plates not only confirmed that surfing cells were considerably more resistant to the 5 tested antibiotics (Figure 5-2), but also that surfing cells could grow at concentrations above the liquid MICs in the presence of mucin (Table 5-S1), again consistent with the concept of adaptive resistance.

RNA-Seq data on cells collected from the centre of a surf colony revealed 10 genes, *ccoO1, atpB, nuoB,* PA4429*, eftA, serA, ccmF, thiG, nuoF,* and *pckA*, involved in metabolism and energy production, that were down-regulated and for which mutants exhibited increased resistance to certain antibiotics. Three of these genes, *ccoO1*, *atpB*, and PA4429, have also been shown to be dysregulated under swarming conditions as described previously . Mutants for these 10 metabolic genes that were down-regulated at the centre of surfing colonies showed an increased resistance to norfloxacin and/or tobramycin. Aminoglycosides are taken up by energy dependent mechanisms (Bryan & Kwan, 1983), and reduced metabolic activities have previously been shown in *P. aeruginosa* biofilms to contribute to resistance to tobramycin (Walters et al., 2010). Although norfloxacin has been shown to affect animal metabolism through interactions

with cytochrome P450 (McLellan et al., 1996), it has not been shown to affect metabolism in *P. aeruginosa* and the effects on susceptibility could reflect reduced DNA replication (involving the target topoisomerases) in these metabolically-challenged cells. Here I have demonstrated that reduced expression levels of certain metabolic resistome genes in the surf centre may contribute to adaptive resistance against tobramycin and/or norfloxacin.

To explain the mechanisms behind surfing-mediated resistance, I explored the contribution of resistome genes found to be dysregulated in surfing through RNA-Seq and transposon mutant screens. In total, 36 resistome genes were identified as being dysregulated under surfing conditions and exhibiting a change in susceptibility to certain antibiotics when mutated. Among the 36 resistome genes, there were 5 that showed the same direction of dysregulation (i.e. both down or up-regulated) in both the centre and edge of a surfing colony. For example, *recG* and *ddaH* were both up-regulated in the surfing centre and edge, and their mutants exhibited similar reduced resistance to tetracycline. The mutant in *recG* (encoding an ATP-dependent DNA helicase) also exhibited increased susceptibility to polymyxin B, tobramycin, and norfloxacin. Tetracycline and tobramycin target protein synthesis through the 30S ribosomal submit while polymyxin B targets the cell membrane, and norfloxacin targets DNA replication. The broad-spectrum activity observed by *recG* as a resistome gene against such diverse antibiotics may arise from its regulatory nature, since it is known that RecG transcriptionally regulates OxyR-controlled genes in *P. putida* (Yeom et al., 2012). Genes identified in the RecG regulon of *P. putida* included porins (*oprE, oprD,* PP0883) and thioredoxin reductase (*trxB*) involved in stress coping mechanisms (Yeom et al., 2012).

There were 3 genes, *armR*, *nalC*, and PA3667, that were down-regulated in both regions of the surf colony. NalC is known to negatively regulate the expression of *armR*, and ArmR inhibits the DNA binding activity of MexR (Starr et al., 2012; Wilke et al., 2008). MexR negatively regulates expression of the *mexAB-oprM* operon, which encodes for a major efflux pump in *P. aeruginosa*, involved in intrinsic and mutational broad-spectrum antibiotic resistance (Wilke et al., 2008). ArmR allosterically binds to MexR to alleviate its repression on the *mexAB-oprM* operon (Wilke et al., 2008). Interestingly, Starr et al. (2012) revealed that a knock-out mutant of *armR* still exhibited increased expression levels of the *mexAB-oprM* operon under certain conditions (Starr et al., 2012). Here I showed that mutants in *armR* and *nalC*, which are both down-regulated under surfing conditions, exhibited similar increases in resistance to tobramycin,

norfloxacin, and polymyxin B. The observed increases in resistance to these antibiotics might be attributed in part to increased expression levels of the *mexAB-oprM* operon.

There were 11 genes dysregulated at the edge and 20 genes at the centre of a surfing colony that exhibited a change in susceptibility to at least one of the tested antibiotics when mutated compared to the wild-type. PA5130 was a conserved hypothetical protein found to be up-regulated at the surfing edge and exhibited an increased susceptibility to all 5 of the tested antibiotics when mutated. The ATP-dependent protease adapter *clpS* which was downregulated at the edge, exhibited a significant increase in resistance to imipenem, polymyxin B, tobramycin, and norfloxacin. ClpS has been previously shown by our lab to contribute to antibiotic resistance, biofilm formation, and swarming motility (Fernández et al., 2012). More specifically, a transposon mutant variant of *clpS* was observed to have increased resistance to β-lactams through the increased expression of β-lactamase (Fernández et al., 2012). Here it was shown that *clpS* also had an effect on resistance to imipenem, polymyxin B, tobramycin, and norfloxacin under surfing conditions.

Swarming is another complex form of motility exhibited by *P. aeruginosa* found to be involved with major transcriptional changes (Overhage et al., 2008; Wang et al., 2013), substantially distinct from the transcriptional profile of surfing cells. Swarming cells have also previously been shown to be resistant to multiple antibiotics including polymyxin B, ciprofloxacin, and gentamicin, and *pvdQ* mutants influenced swarming-specific resistance (Overhage et al., 2008; Wang et al., 2013). Here surfing motility was also found to be associated with resistance to these same antibiotics and several others. Among the resistome genes identified in this study to be dysregulated under surfing conditions that showed contributions to adaptive antibiotic resistance (Table 5-2), *pchF* (Overhage et al., 2008; Tremblay & Déziel, 2010), *atpB*, *ccoO1*, and PA4429 (Tremblay & Déziel, 2010) were also shown to be dysregulated under swarming conditions (Overhage et al., 2008; Tremblay & Déziel, 2010). However our preliminary studies of the swarming resistome (Coleman and Hancock, 2018, manuscript in preparation) have indicated major differences compared to the surfing-associated resistome described here, and less than 15% of dysregulated genes were in common for the two motility adaptations. Thus the mechanistic overlap in swarming and surfing mediated adaptive resistance would appear to be minimal.

The biofilm growth state also leads to adaptive resistance (Domitrovic et al., 2016; Høiby et

al., 2010). Biofilms represent a very different adaptation being sessile rather than motile communities. Our preliminary analyses of gene expression in biofilm bacteria have suggested that there are considerable differences compared to surfing bacteria (D. Pletzer, E. Sun and R.E.W. Hancock, unpublished data, Table 3-1) with only 22-34% commonly dysregulated genes, and thus I would again anticipate different adaptations were involved in resistance. One overlapping gene is *nalC*, identified here as being dysregulated in surfing and mediating surfing-associated polymyxin B, tobramycin and norfloxacin resistance. Mutants in *nalC* were found in biofilms formed by clinical strains of *P. aeruginosa* isolated from prosthetic valves, and the such isolates were resistant to fluoroquinolones and carbapenems (Domitrovic et al., 2016). Other surfing resistome genes identified in our study included *nuoB*, *nuoF*, and *nuoG*. The *nuo* operon, *nuoA-N*, involved in nitrate sensing, has previously been shown to be activated during biofilm formation and important for regulating biofilm formation as well as motility (Southey-Pillig et al., 2005; Van Alst et al., 2007), but its role in biofilm mediated adaptive resistance was not studied. The other genes identified in this study as important for surfing-mediated resistance have not yet been shown to be involved in biofilm formation or related resistance.

In conclusion, surfing motility is a novel form of motility that results in a mucin-triggered lifestyle adaptation. Here I demonstrated how surfing cells exhibit increased resistance that can be attributed to a variety of transcriptomic changes resulting from that adaptation.

Chapter 6. Surfing motility: A conserved yet diverse adaptation among motile bacteria

6.1 Introduction

Bacteria are found in a broad array of dynamic abiotic and biotic environments. They can lead to both positive (biodegradation, normal flora, probiotics) and negative (infections, diseases) implications in humans. In order to thrive in so many different changing environments, bacteria must adapt. Motility is critical to their ability to colonize certain sites, to move towards more favorable environments and away from unfavorable conditions, and to form complex multicellular surface-associated structures such as biofilms (Harshey, 2003). Bacterial motility is also important to pathogenicity since it is involved in movement between body compartments, host cell adherence, colonization, formation of biofilms, and survival. It is often coupled with metabolism and the expression of virulence factors (Belas & Suvanasuthi, 2005; Haiko & Westerlund-Wikström, 2013; Rajagopala et al., 2007).

Here I examined whether surfing motility was conserved amongst other motile bacteria. Results revealed that the physical characteristics of surfing including rapid surface spreading and adaptation were observed in the investigated bacteria both under artificial cystic fibrosis host-like conditions and rich medium supplemented with mucin. However, other characteristics of surfing were found to be more variable.

6.2 Physical characteristics of surfing motility exhibited by multiple motile bacterial species

To determine if the physical characteristics of surfing were conserved in other Gram-negative motile bacteria, *Enterobacter cloacae, Proteus mirabilis, Salmonella enterica, E. coli,* and *Vibrio harveyi* were grown under the same conditions under which *P. aeruginosa* was originally reported to surf (i.e. artificial cystic fibrosis medium supplemented with mucin on semi-solid plates with 0.3% agar). The same basic physical characteristics of surfing were observed in all tested bacterial species (Figure 6-1). The addition of mucin to SCFM in 0.3% agar resulted in surface growth and a significantly larger area of spread in comparison to swimming without mucin that occurred within the agar. In contrast, on 1.5% agar plates without mucin, most bacteria grew as punctuate colonies with almost no spread. Unlike the other tested species, *P. mirabilis* as observed previously (Rauprich et al., 1996) exhibited swarming motility characterized by concentric rings on 1.5% agar without mucin (Rauprich et al., 1996). On mucin-supplemented media, *P. mirabilis* did not exhibit the same concentric phenotype, instead

demonstrating a larger, thicker spread similar to that observed for *P. aeruginosa* surfing.

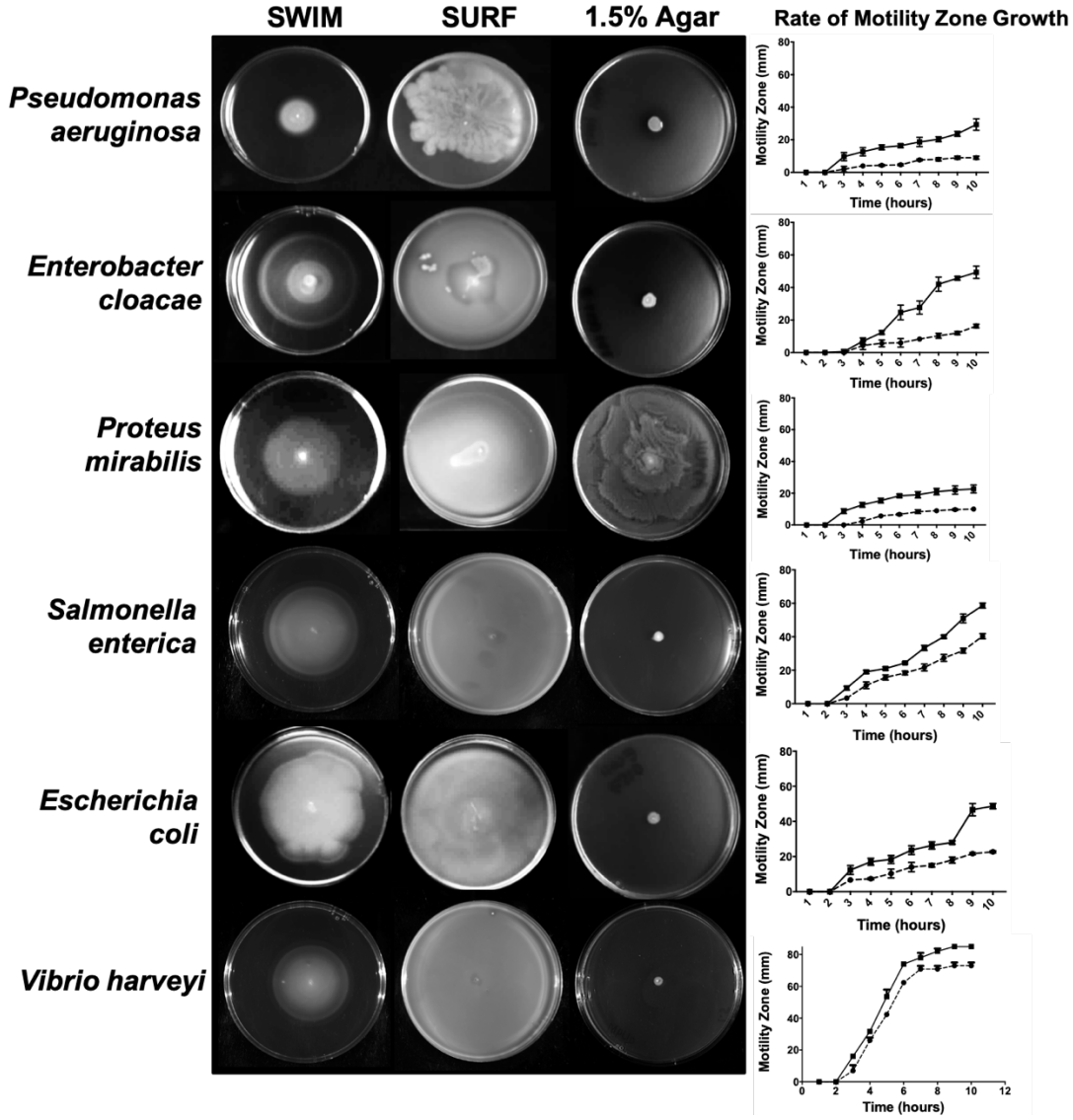


Figure 6-1. Mucin triggered rapid surface motility in a range of bacterial species. Bacterial strains were grown under swimming conditions (0.3% agar), surfing conditions (0.3% agar in the presence of 0.4% mucin), and solid medium conditions (1.5% agar) in SCFM medium. The rate of motility zone growth, depicted on the right graphs, was assessed as the diameter of the motility zone (mm) over 10 hours of incubation at 37°C and surfing is represented by the continuous lines and swimming by the dashed lines (N=3).

Overall, the physical characteristics of surfing first observed for *P. aeruginosa* were also observed for other motile Gram-negative bacterial species including *E. cloacae, P. mirabilis, S. enterica, E. coli,* and *V. harveyi*. The rate of motility zone growth was consistently faster in the presence of mucin and the motility zone eventually filled the plate (within ~10-15 hours). Although *S. enterica, E. coli* and *V. harveyi* exhibited more rapid swimming motility than *P.*

aeruginosa, their swimming zones (within agar) were marginally less than their surfing zones (surface-localized) at the same incubation time. Even though other species did not show the differential pigmentation observed during *P. aeruginosa* surfing (Yeung et al., 2012), surface growth on mucin supplemented media was quite thick throughout, as also observed for *P. aeruginosa* surfing.

6.3 Surfing motility demonstrated adaptability to various medium viscosities

P. aeruginosa surfing motility is not as stringent compared to other forms of motility such as swarming and swimming (Yeung et al., 2012). Swarming often occurs at a limited range of medium viscosities (e.g. 0.4-0.7% agar for *Pseudomonas*), and is dependent on specific medium conditions (not occurring in rich medium or with ammonium as an N source), while swimming is limited to very low viscosity media (≤ 0.3% agar) (Yeung et al., 2012). Agar titration assays in both minimal SCFM (Figure 6-2) and nutrient-rich LB (Appendix Figure A-2) media revealed that surfing was generally less dependent on growth conditions compared to swarming and swimming in all tested species, since for most it occurred at a broad variety of agar concentrations and in both nutrient-rich LB and defined SCFM media. In general, there was a decrease in the size of surfing colonies as agar concentration increased, however, surfing still occurred to a significant extent at high agar concentrations in all except *E. cloacae* in SCFM with mucin. *E. cloacae* did, however, exhibit significant surfing at up to 0.5% agar in LB (Appendix Figure A-3), in contrast to SCFM where surfing was only observed at 0.3% agar. Interestingly, although *E. cloacae* surfing was reduced at higher concentrations, at 1.0% agar it began to exhibit dendritic surface spread (a swarming like behavior) under conditions containing mucin. *P. mirabilis* had no significant change in the area of surfing from 0.3%-1.0% agar in SCFM and LB with mucin. Swimming, in general, was completely inhibited at concentrations higher than 0.3% in all except *P. mirabilis* that exhibited swimming in 0.3% and 0.5% agar, although swimming was completely inhibited at ≥ 0.8% agar. *P. mirabilis* also exhibited a difference in the conditions under which swarming (concentric rings) was observed. *P. mirabilis* began exhibiting a swarming phenotype at 0.8% agar in LB which was not observed in SCFM. However, at 1.5% agar in SCFM without mucin, swarming was indeed observed (Figure 6-1). In general, I observed that surfing manifested somewhat differently in each of the different bacterial species but tended to occur at higher agar concentrations than those that supported swimming and swarming.

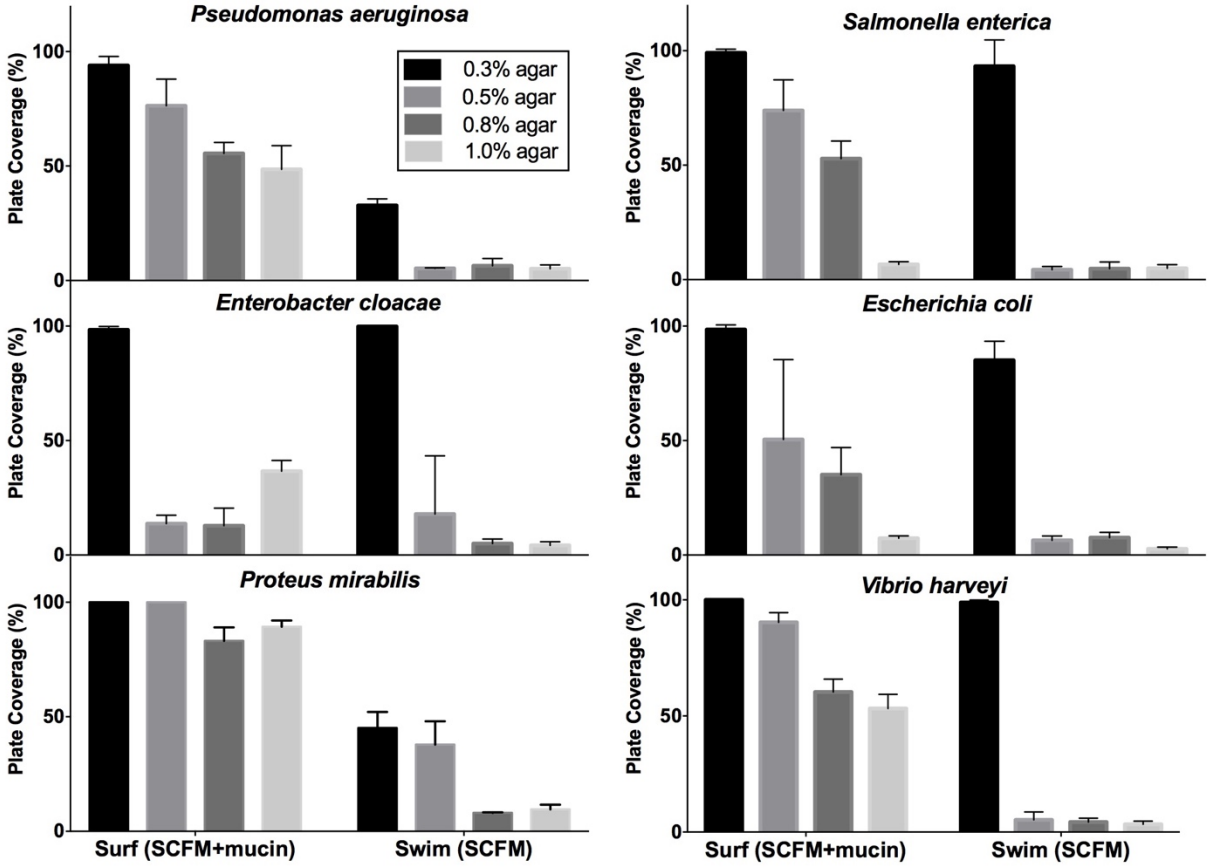


Figure 6-2. Effect of medium viscosity on surfing motility. Bacterial strains were point inoculated onto SCFM medium at varying agar concentrations, with and without mucin, and grown for 18 hours at 37°C to test the effects on surfing (Surf) and Swimming (Swim) motility. Percent plate coverage as a function of agar concentration was measured using ImageJ (N=3) and graphs appear on the left with representative images of motility zones on the right. Corresponding images are presented in Appendix Figure A-3.

6.4 Consistent surfing-like motility was not observed in alternative wetting agents

Yeung et al (2012) previously tested the role of mucin as a wetting agent by demonstrating that surfing-like phenotypes were observed in PA14 under artificial cystic fibrosis conditions containing Tween 20 detergent or carboxymethylcellulose. However, the observed surfing phenotypes were somewhat different from those observed under mucin conditions (Yeung et al., 2012). Here I demonstrated (Figure 6-3) that rich media containing either carboxymethylcellulose (CMC) or Tween-20 promoted distinct rapid surface motility in *P. aeruginosa* at the highest concentrations tested by Yeung et al (2012). CMC, despite being at a higher concentration (1.0% wt/vol) than Tween-20 (0.01%) or mucin (0.4%), was unable to promote any form of motility in *E. cloacae, S. enterica, E. coli,* or *V. harveyi.* For *P. mirabilis*,

CMC promoted a distinct spotty phenotype, quite different from surfing observed under mucin conditions (which displayed an even, thick circular motility zone). Tween-20, however, appeared to promote surfing-like motility to various extents in all of the tested bacteria except *E. coli*. Minimal surfing was observed in *E. coli* but there was an increased motility zone of growth with increased incubation time and increased Tween-20 concentration (data not shown). Tween-20 was able to promote surfing in some tested bacteria at the very low concentration of 0.01% wt/vol.

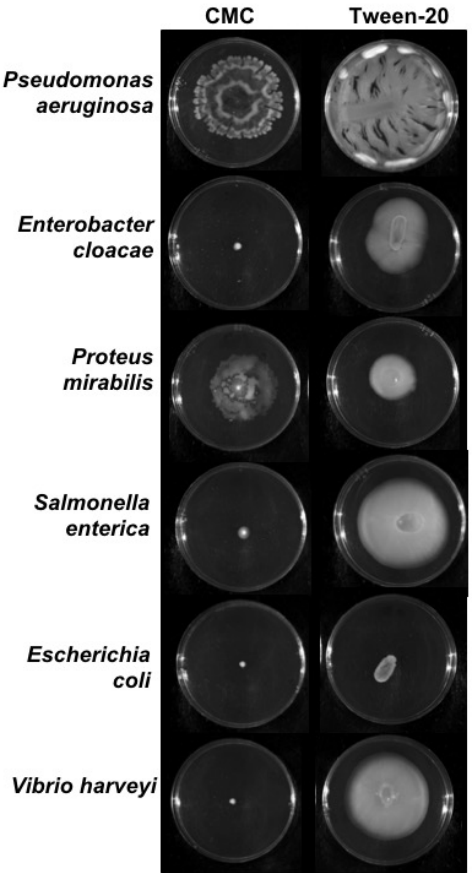


Figure 6-3. Effect of alternative wetting agents on surfing motility. Mucin was substituted with carboxymethyl cellulose (CMC) at 1% wt/vol or Tween-20 at 0.01% added into 0.3% agar LB.

6.5 Surfing cells exhibited distinct multiple antibiotic resistance

Surfing is a complex adaptive lifestyle in *Pseudomonas* causing large changes in gene expression and virulence properties (Yeung et al., 2012, Chapter 3). As with other complex lifestyle adaptations including swarming motility and biofilm formation (Drenkard, 2003; Overhage et al., 2008), *P. aeruginosa* exhibits increased resistance to a series of antibiotics when undergoing surfing motility (Yeung et al., 2012, Chapter 5). Here I studied surfing mediated

resistance based on the distance of closest approach of motility colonies to antibiotic-containing disks in the context of surfing conditions (Table 6-1).

Table 6-1. Surfing motility mediated diverse adaptive multi-drug resistance in different bacterial species. Antibiotic screens were done using the disk diffusion assays on plates containing LB ± 0.4% mucin with 0.3% agar. Statistical analysis to determine relative susceptibility was performed using two-way ANOVA to compare surfing and swimming circumstances, whereby increased resistance represented a lower mean zone of inhibition. R indicates an increased resistance and S indicates an increased susceptibility under surfing motility conditions relative to swimming.

		Relative Susceptibility					
Class	**Antibiotic**	***P. aeruginosa***	***E. cloacae***	***P. mirabilis***	***S. enterica***	***E. coli***	***V. harveyi***
Aminoglycosides	Gentamicin	R			R	R	
	Tobramycin	R		R	R		R
	Amikacin	R			R		
β-lactams	Imipenem	R	R				
	Meropenem	R	R			R	R
	Carbenicillin	R				R	
	Piperacillin			R		R	
	Aztreonam		R	R			
	Ceftazidime					R	
Macrolides	Erythromycin	R	S				
	Azithromycin	R				R	R
Quinolones	Ciprofloxacin	R	R		S	R	R
	Norfloxacin						R
Polymyxins	Polymyxin B	R	R		R	R	R
	Colistin	R				R	R
Others	Trimethoprim	R	R	R	R		R
	Tetracycline	R	R	R			R
	Chloramphenicol	R	R				R

P. aeruginosa strain PA14 exhibited increased resistance to aminoglycosides, carbapenems, polymyxins, macrolides, carbenicillin, ciprofloxacin, trimethoprim, tetracycline, and chloramphenicol, when compared to susceptibility under swimming conditions (Chapter 5; summarized in Table 6-1). The other tested bacterial species also showed increased resistance to multiple antibiotics under surfing conditions when compared to swimming motility. However, the antibiotics to which surfing colonies exhibited resistance varied substantially in different bacterial species, but was broad spectrum, affecting 5 to 14 of the 18 antibiotics tested from diverse classes. Furthermore, resistance rarely affected all members of a given class of antibiotics, indicating that there were likely multiple resistance mechanisms triggered, as found

in *Pseudomonas* (Chapter 5).

Thus, the patterns of susceptibility to particular classes of antibiotics, as observed for *P. aeruginosa* (e.g. resistance to all tested aminoglycosides, macrolides, carbapenem β-lactams, and polymyxins) were not generally observed in other species. For example, *S. enterica* showed increased resistance to all tested aminoglycosides as was seen in *P. aeruginosa*, but was only resistant to polymyxin but not colistin. Conversely, surfing *E. coli* and *V. harveyi* were resistant to both polymyxins but only one aminoglycoside each. *E. cloacae* was the only species among those tested to exhibit a similar surfing-mediated adaptive resistance to both carbapenems, imipenem and meropenem, as observed for *P. aeruginosa*, but did not demonstrate adaptive aminoglycoside resistance.

Some species also exhibited resistance to antibiotics for which *P. aeruginosa* demonstrated no surfing-mediated adaptive changes in susceptibility such as the β-lactams, piperacillin, aztreonam, and ceftazidime. *E. cloacae, E. coli,* and *P. mirabilis* exhibited increased resistance to at least one of these antibiotics under surfing conditions. Conversely, in the case of *E. cloacae* and *S. enterica* increased susceptibility was observed during surfing relative to swimming bacteria towards ciprofloxacin and erythromycin respectively (Table 6-1).

6.6 Surfing dependence on flagella was conserved

Yeung et al. (2012) previously demonstrated that mutants deficient in flagella biosynthesis genes in *P. aeruginosa* PA14 were surfing deficient. Here I also demonstrated (Figure 6-4) that this dependence on flagella was conserved in the following species: *S. enterica, E. coli,* and *P. mirabilis*. Mutants of flagella biosynthesis genes in each of these species exhibited complete inhibition of motility. On the other hand, pilus-deficient mutants of *E. coli* and *P. mirabilis* exhibited normal surfing, as was also observed in *P. aeruginosa* in this study (Figure 6-4) and previously (Yeung et al., 2012). An *E. coli fim* mutant did, however, exhibit slower growth of the surfing motility zone compared to the wild-type (data not shown) but still exhibited the physical characteristics of surfing motility. Therefore, surfing appeared to have a conserved dependence on flagella but not pili or fimbriae.

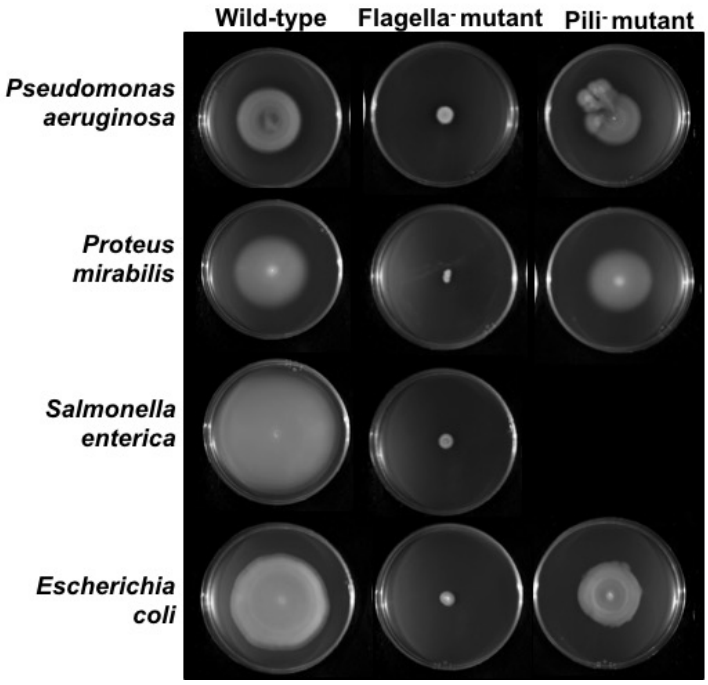


Figure 6-4. Surfing motility was dependent on flagella but not pili/fimbriae. Flagella deficient mutants in *P. aeruginosa* (Δ*fliC*), *S. enterica* (Δ*fliC*), *P. mirabilis* (Δ*flaD*), and *E. coli* (Δ*flhDC*) demonstrated complete inhibition of surfing motility in 0.3% agar SCFM supplemented with 0.4% agar after 13-15 hours of incubation. Pilus or fimbriae deficient mutants of *P. aeruginosa* (Δ*pilC*), *P. mirabilis* (Δ*mrpA*), and *E. coli* (Δ*fim*) still exhibited surfing motility under the same conditions.

6.7 Dependence on quorum sensing of *P. aeruginosa* surfing motility was not conserved

Surfing motility in *P. aeruginosa* PA14 is dependent on the Rhl and Las quorum sensing systems based the inhibitory effects of transposon mutants in the *rhlI* and *lasI* genes, which could be complemented by the addition of their respective homoserine lactones (Yeung et al., 2012). Additional screens of quorum sensing mutants (Figure 6-5) revealed that *P. aeruginosa* mutants in genes involved in the PQS quorum sensing system (*pqsABCDE, pqsR*) also exhibited surfing-deficiency. Indeed, certain mutants such as *pqsR* and *pqsB* exhibited swarming motility rather than surfing motility in the presence of mucin. Genetic complements were generated for *lasI* and *rhlI*, the autoinducer synthesis proteins. Addition of their respective autoinducers or genetic complementation of *lasI*, *rhlI,* and *pqsA* restored wild-type surfing (Figure 6-5; (Yeung et al., 2012)).

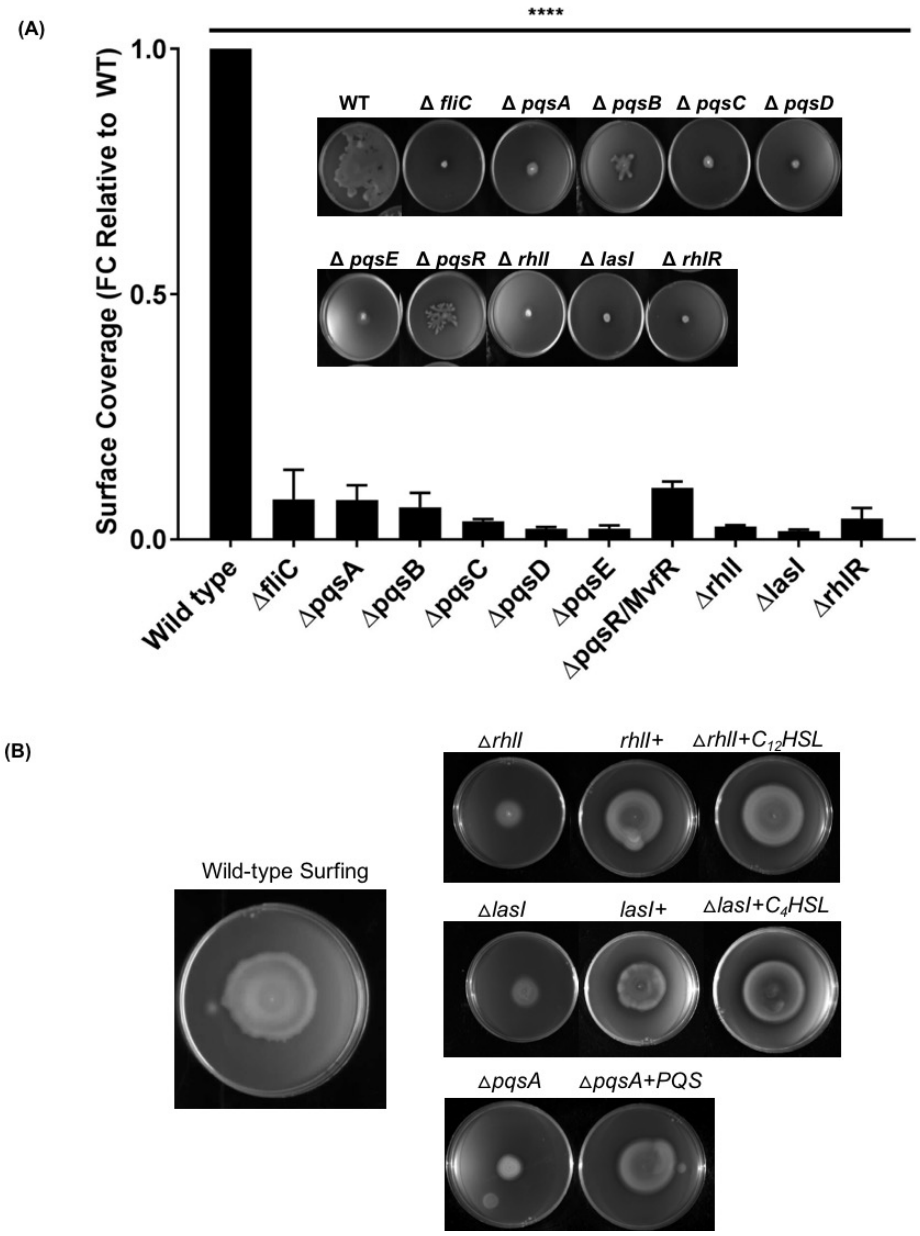


Figure 6-5. *P. aeruginosa* surfing was dependent on quorum sensing. (A) Quorum sensing PA14 mutants *(*Δ*pqsA,* Δ*pqsB,* Δ*pqsC,* Δ*pqsD,* Δ*pqsE,* Δ*pqsR,* Δ*lasI,* Δ*rhlI,* Δ*rhlR*) exhibited surfing deficiency as shown by the negative control *(*Δ*fliC*) or conversion to swarming. Surface coverage was determined by analyzing the % surface coverage using ImageJ relative to wild-type PA14. (B) Complements of quorum sensing mutants (*rhlI*+*, lasI*+) exhibited complete or partial surfing restoration. Addition of exogenous autoinducer molecules restored surfing with a slight increase in motility zone compared to wild-type. Differences in plate coverage area of the mutants cf. the wild-type were singificant by 2-way ANOVA at $p<0.0001$ (****).

To test if this dependence on quorum sensing was conserved in the other motile bacteria, quorum sensing mutants were obtained for *S. enterica* (Δ*luxS*), and *V. harveyi* (Δ*luxR*). Each of these quorum sensing mutants still exhibited normal surfing in SCFM with mucin (Figure 6-6).

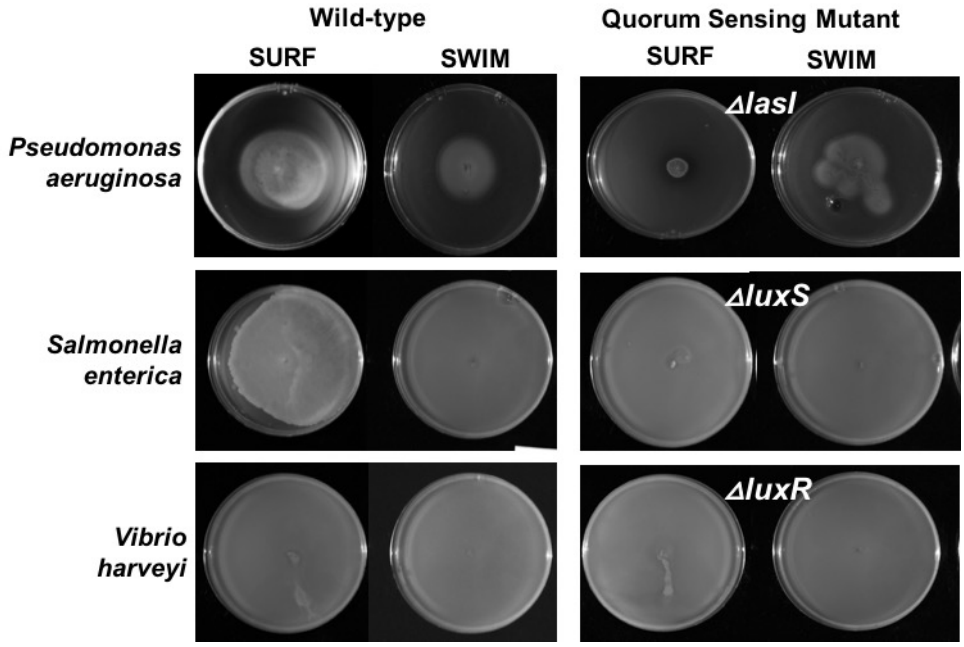


Figure 6-6. Surfing-dependence on quorum sensing did not extend to bacterial species other than *P. aeruginosa*. Motility assays were performed on SCFM containing 0.3% agar and 0.4% mucin (surfing), or 0.3% agar (swimming). Swimming for the three test species, *P. aeruginosa*, *S. enterica*, and *V. harveyi,* showed no dependence on quorum sensing since their respective quorum sensing mutants continued to exhibit wild-type swimming. Although the *P. aeruginosa lasI* mutant was surfing deficient, quorum sensing mutants from *S. enterica (*Δ*luxS)* and *V. harveyi* (Δ*luxR*) continued to show surfing.

6.8 Discussion

Surfing is a mucin-dependent adaptation that was first observed in *P. aeruginosa* (Yeung et al., 2012). Here I showed that *E. coli, S. enterica, P. mirabilis,* and *E. cloacae*, which are known to associate with the mucosa during infections, as well as the marine bacterium *V. harveyi*, exhibited similar physical characteristics to those reported for *P. aeruginosa* on artificial cystic fibrosis semi-solid medium containing mucin. The bacterial species selected for this study with the exception of *E. cloacae* had been previously reported to exhibit more than one form of motile adaptation, including swimming and swarming (Armbruster et al., 2013; Böttcher et al., 2016; Hejazi & Falkiner, 1997; Kearns & Losick, 2003; Kim & Surette, 2003). The surface adaptation observed in the presence of mucin was distinct from the characteristics of swimming which occurs within agar and swarming (as summarized in Chapter 8, Table 8-2), and unlike both motility processes surfing was dependent on the presence of mucin. For all tested organisms, surfing was faster than swimming motility. Interestingly, the conditions under which surfing

occurred were also observed to be less stringent than the conditions needed to display other motility forms such as swarming or swimming. In particular, swimming motility was only observed at low viscosities (0.3% agar), whereas surfing was observed at a range of viscosities (0.3-1.0%) in both minimal and rich media. Overall several characteristics of surfing that have been catalogued in *P. aeruginosa* (Yeung et al., 2012), including rapid surface spread, adaptability to various media viscosities, minimal growth substrate requirements, dependence on flagella, and multidrug adaptive resistance were observed for all the tested Gram-negative motile bacteria.

Previously (Yeung et al., 2012), it was shown that *P. aeruginosa* exhibited surfing-like motility in SCFM agar plates with carboxymethyl cellulose or Tween-20 instead of mucin; however, the appearance of these motility colonies were different from those observed under mucin conditions. Here I tested these two alternative wetting agents in rich medium (LB) at the concentrations previously tested (Yeung et al., 2012) and observed distinct surface motility phenotypes in *P. aeruginosa*. Carboxymethyl cellulose (CMC) was found to be ineffective at promoting surfing in any of the other tested species, however, Tween-20 was able to promote surfing in all except *E. coli* and *P. aeruginosa* where a swarming-like phenotype was observed instead. Neither wetting agent was able to induce surfing in *E. coli* under the conditions in which mucin induced surfing. Mucin was, therefore, the only agent able to consistently promote distinctive surfing motility in all the tested species.

Surfing was initially reported to be dependent on intact flagella but not pili in *P. aeruginosa* (Yeung et al., 2012). In this study, these findings were corroborated for other species as shown in Figure 6.4. This dependence of surfing motility on flagella was also found to be conserved in *P. mirabilis, E. coli,* and *S. enterica*. Pili or fimbriae mutants of *P. aeruginosa, E. coli,* and *P. mirabilis* were also screened, but did not exhibit surfing deficiency. Surfing was observed to be slower in an *E. coli* fimbriae mutant, but it still occurred to a diminished extent unlike the flagella mutants which exhibited complete inhibition of surfing. The type IV pili in *P. aeruginosa* (Köhler et al., 2000) and type 1 fimbriae in *E. coli* (Inoue et al., 2007) were previously found to be important in swarming motility, but as shown in this study did not play an obligate role in surfing motility.

Many of the tested bacterial species are known to cause a wide range of infections that are often difficult to treat. With regards to mucosal infections by these bacteria, adaptive resistance

accompanying a motile lifestyle in the presence of mucin could exacerbate this. Here I demonstrated that the surfing motility adaptation led to increased resistance (and in two cases enhanced susceptibility) to specific antibiotics when compared to bacteria undergoing swimming motility. All tested bacterial species exhibited a certain level of broad-spectrum resistance under surfing conditions, although the antibiotics for which adaptive resistance was observed differed greatly.

In this study, I also tested the importance of quorum sensing which had been previously reported to be involved in *P. aeruginosa* surfing (Yeung et al., 2012). Using transposon mutants, A dependence of surfing on the N-acyl homoserine lactone (AHL) Rhl and Las quorum sensing systems was demonstrated in *P. aeruginosa* (Yeung et al., 2012), as confirmed here. Mutants deficient in rhamnolipid production genes regulated by the Rhl system, namely *rhlA* and *rhlB* mutants, necessary for swarming motility in *P. aeruginosa*, were found to exhibit wild-type-like surfing and thus surfing was confirmed to be independent of rhamnolipids (Appendix Figure A-4, Yeung et al., 2012). Here it was demonstrated that surfing was also dependent on the PQS system in *P. aeruginosa*. Mutants displaying surfing deficiency included those in the PQS operon, *pqsABCDE*, involved in synthesizing the autoinducer, PQS, and *pqsR*, the transcriptional regulator that binds to and mediates responses to PQS. Interestingly, such mutants, e.g. the *pqsR* mutant, often exhibited a swarming phenotype rather than surfing in medium supplemented with mucin, possibly explaining the surface coverage observed previously (Yeung et al., 2012). Complementing the quorum sensing transposon mutants with the respective wild-type genes, as well as the addition of their respective autoinducers exogenously restored surfing to the wild-type-like level. Indeed high concentrations of the autoinducers actually further enhanced surfing to a level greater than that of the wild-type (i.e. demonstrating increased surface coverage in less time), as shown here and previously for the Rhl and Las autoinducers (Yeung et al., 2012). Therefore, it appears that each of the Rhl, Las, and PQS systems are required for surfing motility in *P. aeruginosa*. Although these data confirmed and extended information on the importance of quorum sensing in *P. aeruginosa* surfing, I did not observe this dependence on the AHL-based quorum sensing systems of *S. enterica* and *V. harveyi*. However, each of these AHL-based quorum sensing systems involved distinct autoinducers and have distinct regulons.

To further examine how conserved surfing motility is in other bacteria, I also tested the Gram-positive bacterium, *Bacillus subtilis* (Appendix Figure A-5). *B. subtilis* exhibited similar

surface spread as was observed in the other tested bacteria under conditions involving SCFM agar supplemented with mucin. In contrast, *B. subtilis* swimming occurred within the agar and at 1.5% agar exhibited no spread (Appendix Figure A-5a). *B. subtilis* mucin-dependent motility also exhibited similar characteristics as observed for the other bacteria including faster spreading than swimming, broad-spectrum antibiotic resistance, and adaptability to various agar concentrations. Indeed *B. subtilis* did exhibit surfing-like phenotypes at a range of viscosities (0.3-1.0% agar) in both LB and SCFM media supplemented with mucin, but it also exhibited significant surface spread at higher agar concentrations without mucin, especially on LB agar (Appendix Figure A-5d). This might reflect the type of swarming motility described by Kearns and Losick (2003), who previously described *B. subtilis* swarming at 0.5-0.7% agar. However, because *B. subtilis* swarming did not exhibit any visible features distinct from surfing, it was difficult to distinguish between the two forms of motility. There was, indeed a clear shift from embedded agar motility (swimming) at 0.3% agar to surface spread (potentially swarming) at higher agar concentrations in medium without mucin. In contrast in the presence of mucin, only surface motility was observed. The mucin-promoted motility was found to be partially dependent on flagella in that a flagellar mutant exhibited dendritic rather than circular surface spread, but no dependence on the Com quorum sensing system (mutants exhibited wild-type-like surfing) (Appendix Figure A-5b).

In conclusion, I observed that surfing motility demonstrated conserved features in other motile, mucosa-associated pathogens and was associated with broad-spectrum antibiotic resistance. However, the surfing adaptation could be differentially regulated in different bacterial species.

Chapter 7. Role of the stringent stress response in *Pseudomonas* surfing motility

7.1 Introduction

The stringent stress response plays a key adaptational role in *Pseudomonas* survival and virulence. The stringent response is regulated by the secondary messenger nucleotide, ppGpp, produced and hydrolyzed by the RelA and SpoT enzymes. RelA-induced ppGpp production is triggered by amino acid starvation, and a SpoT-induced response is triggered by environmental stress conditions such as limiting iron conditions, or carbon and fatty acid starvation (Vogt et al., 2011). Activation of the stringent stress response results in global transcriptomic changes that divert energetically costly processes such as growth and cell division to stress response and coping mechanisms (Boutte and Crosson, 2013). Vogt et al. (2011) previously showed that the stringent stress response plays an important role in *Pseudomonas* virulence in the rat lung model, while Pletzer et al (2017) showed its importance in a mouse abscess model, as well as expression of *spoT* and *relA* in abscess infections. A stringent response mutant (Δ*relA*Δ*spoT*) exhibited attenuated virulence and ability to survive under conditions of heat shock and oxidative stress (Pletzer et al., 2017; Vogt et al., 2011). The CF lung environment is a complex niche of stress factors that favour highly adaptable organisms like *P. aeruginosa*. It is often associated with high levels of inflammation, reactive oxygen species, and a diverse microbial community simultaneously producing synergistic and antagonistic compounds (Cifani et al., 2013; Harrison, 2007). Therefore, the stringent response is likely crucial for *Pseudomonas* survival and colonization of the CF lungs (Xu et al., 2016). The stringent stress response regulates oxidative stress tolerance (Khakimova et al., 2013), production of virulence factors during acute infections through the regulation of quorum sensing (van Delden et al., 2001; Schafhauser et al., 2014), as well as swarming and biofilm formation (Fuente-Núñez et al., 2014; Xu et al., 2016).

Since the stringent response has been proposed to play a key role in adaptability in the CF lung environment (Xu et al., 2016), I explored here its role in surfing motility. Mucin is a key component found in large abundances in the CF lung (Quinton, 2008) and a critical important inducer of surfing motility. Here I demonstrated the essential role of the stringent response in both strain PAO1 and the CF-adapted Liverpool epidemic strain LESB58.

LESB58 was first isolated in 1996 from chronically infected CF patients (Cheng et al., 1996). It promotes a stronger chronic infection than PAO1 or PA14 in mouse models (Fothergill et al.,

2012). Sequencing of its genome in 2009 revealed a number of genomic prophage islands which were found to be important in chronic infectivity, allowing LESB58 to out-compete other *P. aeruginosa* strains in chronic infection models in mice (Winstanley et al., 2009). Here, I explored the dependence of surfing motility on the stringent stress response in LESB58 and PAO1 and provided evidence it occurred through the regulation of the quorum sensing regulator, *pqsH*, and copper-resistance regulator, *cueR*. Both *pqsH* and *cueR* had been identified through the mutant library screen (Table 3-2) as essential regulators for surfing motility.

7.2 The stringent stress response regulated swarming and surfing, but not swimming

Three forms of motility, swarming, surfing and swimming, were investigated in the LESB58 and PAO1 wild-type and stringent response mutants (Δ*relA*Δ*spoT*) (Figure 7-1; Appendix Figure A-6).

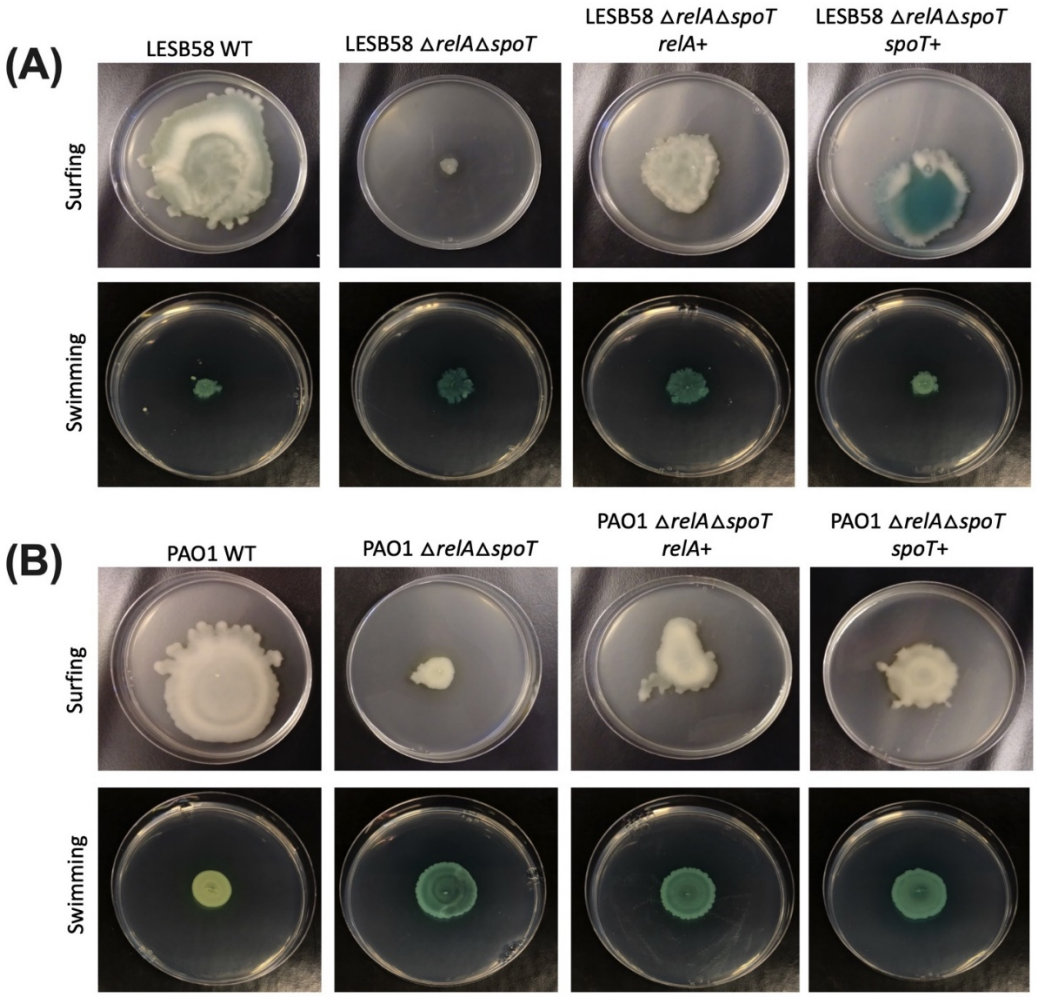


Figure 7-1. **Stringent response mutants exhibited surfing inhibition but wild-type swimming.** Stringent response mutants and complements in PAO1 (B) and LESB58 (A) under

swimming and surfing conditions. Surfing colonies were grown in KB media with 0.3% agar and 0.4% mucin at 37°C for 15 hours. Swimming colonies were grown in SCFM media with 0.3% agar at 37°C for 36 hours.

Both the LESB58 and PAO1 wild-type isolates exhibited all three forms of motility. However, the LESB58 strain exhibited extremely poor swimming motility, significantly less than either strains PAO1 and PA14, showing minimal swimming or in-agar motility zone growth even after 36 hours of incubation. Compared to the wild-type, the stringent response mutant exhibited a normal swimming motility phenotype in contrast to what was observed for surfing and swarming motility. The double-mutant exhibited complete inhibition of surfing in LESB58 and almost complete attenuation in strain PAO1 (Figure 7-1), and attenuation of swarming in both strains (Appendix Figure A-6; swarming performed by Daniel Pletzer). Complementing the mutant with either *relA* or *spoT* partly restored the swarming and surfing phenotypes, but to a lesser extent than the wild-type, whereby the two complemented isolates covered less surface area, while the *relA* complemented mutant formed a less structured swarming colony. Under surfing conditions, the *spoT*$^{+}$ complemented mutant also exhibited a stronger pigmentation level as it appeared bright blue-green. Therefore, the stringent response mutant exhibited inhibition of surfing and swarming but not swimming motility in both the PAO1 and LESB58 strains.

7.3 Differential gene expression in the stringent response mutant during motility

The expression of motility genes and surfing-essential regulators were explored by RT-qPCR in the context of swarming, swimming, and surfing in the LESB58 wild-type and Δ*relA*Δ*spoT* stringent response mutant as shown in Table 7-1. All three forms of motilities were dependent on flagellar biosynthesis; therefore, I determined the expression levels of key flagellar and chemotaxis regulators in the mutant vs. the wild-type. Under swarming conditions, the chemotaxis gene, *cheY*, and two-component regulator found to be involved in regulating flagellar biosynthesis genes, *fleR*, were found to be significantly down-regulated in the stringent response mutant relative to the wild-type. In addition, the rhamnolipid biosynthesis gene, *rhlB*, was also found to be down-regulated by 5.8-fold in the mutant. Swarming motility is highly dependent on the production of rhamnolipids as surfactants (Caiazza et al., 2005). Major quorum sensing regulators, including RhlR which regulates the expression of rhamnolipid biosynthesis genes, were found to be down-regulated in the mutant under swarming conditions. Under surfing conditions, *pqsH*, which is involved in the synthesis of the PQS autoinducer, as well as the

copper resistance regulator, *cueR*, were found to be significantly down-regulated in the stringent response mutant that exhibited surfing deficiency. Under swimming conditions, however, it did not appear to be affected in the stringent response mutant. In contrast under swimming conditions I still observed dysregulation between the mutant and wild-type in *rhlR, rhlB,* and *cueR*, which were all found to be down-regulated.

Table 7-1. **Differential gene expression levels of key surfing- and swarming-essential regulators and effectors in LESB58.** RT-qPCR was performed on RNA collected from the swarming (by Pletzer), swimming, and surfing edge cells in the stringent response mutant (Δ*relA*/Δ*spoT*) and wild-type. Dysregulation was observed in different regulators among the three forms of motility. A fold-change cutoff of ± 2.0 fold was considered to impute a meaningful change. Analysis was performed by comparison with the house-keeping gene, RpoD.

Gene	**Fold change in Δ*relA*/Δ*spoT* compared to WT**		
	LESB58		
	Swarming	Swimming	Surfing
fleQ	-1.0	1.1	1.7
fleR	-3.5	-1.4	1.1
cheY	-3.8	-1.5	1.7
rhlB	-5.8	-2.1	3.2
lasR	-4.3	1.8	2.1
rhlR	-3.5	-2.2	2.2
pqsH	-2.4	-1.1	-2.8
pqsR	1.3	-1.0	1.7
cueR	-1.9	-3.1	-4.4

7.4 Cross-complementation with the copper-transport regulator *cueR* or the quinolone synthase *pqsH* restored surfing motility in a stringent response mutant

As previously shown through the mutant library screen (Table 3-2), surfing is dependent on *cueR* and *pqsH*. These two surfing-essential regulators were also found to be down-regulated in the Δ*relA*Δ*spoT* mutant which exhibited surfing deficiency. To determine whether hierarchical regulation existed among these regulators, the *cueR* and *pqsH* genes were complemented into the Δ*relA*Δ*spoT* mutant. Surfing motility was restored to a wild-type-like level in the mutant complemented with wild type *cueR* and *pqsH* genes in LESB58 as shown in Figure 7-2.

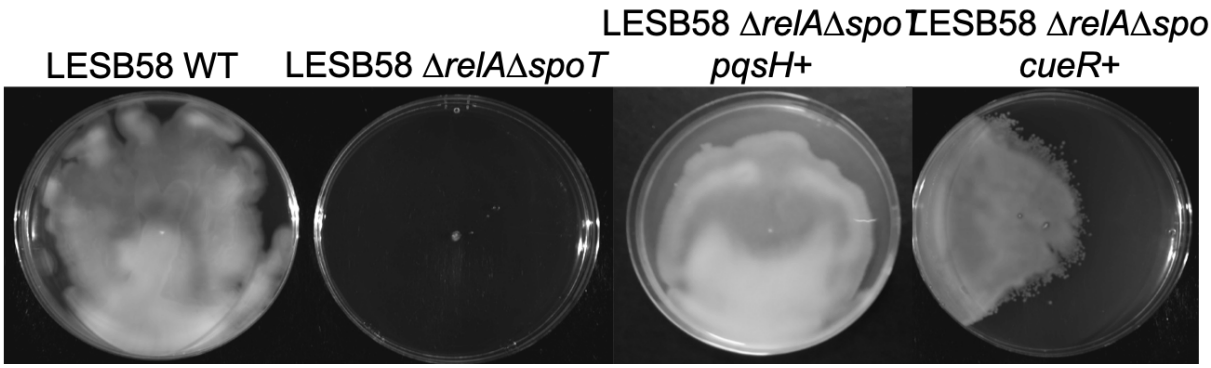


Figure 7-2. **Overexpression of *pqsH* and *cueR* in the stringent response mutant restored surfing in the LESB58 strain.** Surfing assays were done in SCFM with 0.3% agar and 0.4% mucin, grown at 37°C for 18 hours. *PqsH* and *cueR* were cloned into a high copy plasmid (pBBR5).

7.5 Discussion

The stringent response is a key adaptive mechanism used by bacteria like *P. aeruginosa* and plays an important role in adapting to diverse niches. Here I have shown that a mutant deficient in the production of ppGpp, the second messenger involved in regulating genes under the stringent stress response, is also deficient in certain forms of motility, particularly surfing (Figure 7-1), and swarming (Figure A-6). Complementing either *relA* or *spoT*, both of which can synthesize ppGpp, partially restored surfing and swarming motility. The *relA* complemented strain, for example, exhibited surfing but to a lesseer extent than the wild-type, while the *spoT* complement also exhibited reduced surfing and hyperpigmentation. These observations were made in both the LESB58 and PAO1 strains of *P. aeruginosa*. Swimming motility, although observed to be much slower in the LESB58 compared to PAO1, was not affected by the stringent response mutant, but the somewhat more substantative phenotype in the LESB58 strain might have been due to this general limitation in motility. Swarming, on the other hand, like surfing, was found to be attenuated in the stringent response mutant. Therefore, the stringent response appeared to play an important role in mediating surfing and swarming.

To determine the down-stream effects of the stringent response regulators on other key regulatory systems, the expression levels of surfing essential regulators were measured in the stringent response mutants compared to the wild-type. RT-qPCR analysis (Table 7-1) showed that the double mutant under swarming conditions exhibited down-regulation in several regulators for flagella synthesis, chemotaxis, and quorum sensing including the production of rhamnolipids which is required for swarming motility. Under surfing conditions, however, two

key regulators were found to be significantly down-regulated, the Pqs quorum sensing gene, *pqsH*, and the copper responsive regulator, *cueR*. Overexpressing these regulators in the double-mutant restored surfing motility, speaking to the potential hierarchical effect of the stringent response on these regulators. Therefore, the stringent response influenced the expression levels of *pqsH* and *cueR* which, as previously shown through the mutant library screen in Chapter 3, were essential regulators mediating surfing motility.

CueR is a dimeric transcriptional regulator with two domains: a sensor domain that responds to environmental stimuli such as the presence of copper and a DNA-binding domain (Bagchi, 2015). CueR was found to directly regulate the expression of several genes involved in drug resistance, copper resistance and virulence as well as several uncharacterized proteins (Bagchi, 2015; Thaden et al., 2010). It was previously found that *cueR* expression is regulated by the Las quorum sensing system (Thaden et al., 2010). Here I showed that it was likely also regulated by the stringent stress response. The *cueR* transposon mutant was shown in Chapter 3 to be surfing deficient. Complementation of *cueR* in the transposon mutant restored surfing to a wild-type-like state (Appendix Figure A-7). Therefore, *cueR* is an essential regulator for surfing motility found to be dysregulated in the stringent response mutant. Overexpressing the *cueR* regulator in the stringent response mutant restored surfing motility.

Similar data was obatined for *pqsH* whereby a *pqsH* disruption mutant in PA14 was found to be surfing deficient, but surfing was restored by complementing with the wild type *pqsH* gene (Figure A-7). PqsH plays a role in converting the precursor of the autoinducer HHQ to its final form, PQS. Previous studies showed that the stringent response negatively regulates the production of PQS (Schafhauser et al., 2014). However, here I found a down-regulation of *pqsH* under surfing conditions, whereas Schafhauser et al. (2014) reported an observed up-regulation in both *pqsA* and *pqsH* during stationary phase in liquid culture conditions, resulting in an accumulation of HHQ and PQS. Therefore, the stringent response appeared to be contextually regulating PQS production. Under surfing, which was reliant on the Pqs quorum sensing system (Figure 6-5), *pqsH* down-regulation was observed in the stringent response mutant which could limit the conversion of HHQ to PQS leading to low PQS levels. Surfing was highly dependent on PQS-regulated gene expression (Figure 6-5), but swimming was not dependent on the PQS system (Schafhauser et al. 2014). This again confirmed that stringent response regulation of the PQS system was context dependent. In conclusion, I observed that *pqsH* and *cueR* were both

down-regulated in the stringent response mutant and overexpressing either regulator in the mutant recovered surfing motility, indicating a role for the stringent stress response as a global regulatory system that potentially mediates surfing through *cueR* and *pqsH*.

Chapter 8. Conclusions

8.1 Surfing in comparison to other motile lifestyles

Surfing is a novel motile adaptation first discovered in the opportunistic pathogen, *P. aeruginosa* grown on 0.3-1% agar plates under artificial CF-like growth conditions in the presence of mucin (Yeung et al., 2012). *P. aeruginosa* is the leading cause of death in CF patients, accounting for more than half of all CF infections in adults. Motility is known to be important for *P. aeruginosa* virulence since strains deficient in flagella or pili biosynthesis exhibit significantly lower virulence in acute infection models as well as an attenuated ability to form biofilms (Drake and Montie, 1988; O'Toole and Kolter, 1998). It has been previously shown that under conditions of excess mucin *P. aeruginosa* exhibits a novel rapid form of surface motility, phenotypically distinct from other forms of motility (Yeung et al., 2012). Table 8-1 highlights key differences among the various motility adaptations.

Table 8-1. **Differences between motile adaptations in *P. aeruginosa*.** Observations were taken from cited papers or studies conducted in this thesis.

Property	Swimming	Swarming	Twitching	Surfing	Sliding
In vitro appearance	Circular, spread within agar	Dendritic (PA14, LESB58) or solar flared (PAO1), on surface	Semi-circular, spread in interstitial space between agar and plate	Semi-circular, on surface, blue-green centre and white outer edge[1]	Concentric and dendritic, on surface[2]
Viscosity requirements (% agar wt/vol)	≤ 0.3%	0.5-0.8%	1.5%	0.3-1.0%	0.3%-0.7%[2]
Appendage requirements	Flagella	Flagella and Type IV pili[3]	Type IV pili[4]	Flagella[1]	None
Involved in adaptive resistance	No	Yes[5]	Untested	Yes	Untested
Required for virulence *in vivo*	Untested	Untested	Yes[6]	Untested	Untested
Dependence on quorum sensing	No	Yes[3]	Yes[7]	Yes[1]	Untested

1. (Yeung et al., 2012)
2. (Murray and Kazmierczak, 2008)
3. (Köhler et al., 2000)

4. (Bradley, 1980)
5. (Overhage et al., 2008)
6. (Alarcon et al., 2009)
7. (Beatson et al., 2002)

Previously, Yeung et al. (2012) found that surfing motility, like swarming and swimming, is dependent on the presence of intact flagella. However, unlike swarming and twitching, it is not dependent on the type IV pili (Yeung et al., 2012). However, here I found, through a transposon mutant library screen, that surfing was dependent on three previously untested twitching genes, *pilT, pilU,* and *pilW*. PilT and PilU, normally involved in pili retraction, are also involved in adherence and cytotoxicity during acute infections (Comolli et al., 1999). Both *pilT* and *pilU* mutants exhibit a hyperfimbrial phenotype due to their inability to retract (Whitchurch and Mattick, 1994). Despite that, they are still deficient in twitching motility (Whitchurch and Mattick, 1994). PilW, on the other hand, is a membrane-bound protein found to be involved in protein secretion during type IV pili biosynthesis (Alm et al., 1996). Therefore, *pilW* mutants exhibit a type IV pilus deficient phenotype (Siryaporn et al., 2014). Like the *pilU* and *pilT* mutants, the *pilW* mutant also exhibits significantly reduced cell adherence and virulence (Siryaporn et al., 2014). Interestingly, it has been found that not all pilus deficient mutants, namely Δ*pilB* and Δ*pilC*, exhibit reduced virulence (Siryaporn et al., 2014). Therefore, among the many pilus biosynthesis and twitching genes, *pilU, pilT,* and *pilW* play key roles in colonization through surface adherence (Comolli et al., 1999; Siryaporn et al., 2014). Therefore, although surfing is not dependent on the structural pilus genes as previously described (Yeung et al., 2012), it was found to be dependent on key twitching genes involved in adherence and secretion.

Besides differential dependence of pili and flagellar genes, surfing motility also exhibited less stringent nutritional and environmental requirements compared to other forms of motility, being mainly dependent on mucin as a wetting agent. Swarming normally occurs on semi-aqueous conditions (0.5-0.8% wt/vol agar) with a poor nitrogen source such as casamino acids and is inhibited by ammonium. Swimming occurs in aqueous conditions (less than 0.3% agar) in the presence of ammonium as a nitrogen source. Twitching occurs on solid surfaces or the interstitial space between the agar and the plate in rich media. Sliding motility occurs on solid surfaces in strains that are deficient in both flagella and pili. Surfing, however, was found to be relatively flexible in medium viscosity. Surfing was observed in 0.3-1.0% agar in *P. aeruginosa*

whereas swimming was only observed at 0.3% agar (Figure 6-2). Surfing was also found to occur in both rich and minimal conditions in the absence and presence of ammonia (Yeung et al., 2012, Figure 6-2, A-2). The only key requirement for surfing was the presence of mucin since it was also found that mucin, as a wetting agent, uniquely induced surfing motility in *P. aeruginosa*. Tween-20, was also able to act as a substitute in inducing surfing-like behaviour in *E. cloacae, P. mirabilis, S. enterica,* and *V. harveyi*. However, in *P. aeruginosa*, substituting mucin with alternative wetting agents such as CMC or Tween-20 induced more swarming-like phenotypes. CMC was also ineffective at relatively high concentrations in being able to induce surfing among bacteria other than *Pseudomonas*.

Various conditions induce different adaptations that are regulated by distinct sets of regulators. Compared to other forms of motile adaptations like swarming and sessile lifestyles like biofilms, surfing was found to be dependent on and exhibited dysregulation of a unique cohort of regulators. RNA-Seq data collected from surfing, swarming, and biofilm cells revealed that the three adaptations only share dysregulation in 21 regulatory genes, not all in the same direction of dysregulation. Surfing and swarming cells exhibited dysregulation of 10 regulators not dysregulated in biofilms, while surfing cells and biofilms exhibited dysregulation in 63 other regulators not dysregulated under swarming conditions. Therefore, surfing had more dysregulated regulators in common with biofilm cells than swarming. Among the regulators found to be dysregulated in all three adaptations, two sigma-70 family regulators and a two-component sensor, *gcbA*, were found to be commonly down-regulated, while the putative two-component regulator, PA1243, was found to be up-regulated. Among the 10 regulators dysregulated in both swarming and surfing cells but not biofilms, only one regulator exhibited the same direction of dysregulation in the two motility forms. PhaD was up-regulated in both motile adaptations. PhaD is involved in polyhydroxyalkanoates synthesis in *P. oleovorans,* but its role in *P. aeruginosa* has not been well established (Klinke et al., 2000). Therefore, surfing and swarming might share a dependence on PHA production, which can be used for carbon storage during nutrient limiting conditions. Compared to biofilm cells, surfing exhibited a similar direction of dysregulation to 14 regulators in addition to the ones found to be similarly dysregulated in all three conditions. Among these 14 genes, surfing and biofilm cells shared similar expression level of regulators for various multidrug resistance genes and several sigma factors. However, surfing also shared an inverse dysregulation compared to biofilm cells for

major regulators like GacA, LasR, and CbrA. Therefore, these different adaptations appeared to have relatively unique regulatory networks and cascades that mediate them.

8.2 Regulation of surfing motility

Surfing was found to result in the dysregulation of 1,094 genes in cells collected from the edge of a surfing colony and 1,172 genes from the centre relative to swimming, and 1,617 genes were similarly dysregulated between the two. Cells in the centre and edge shared the same direction of dysregulation of flagellar biosynthesis genes, but they exhibited differential regulation on pilus genes. Primarily, core pilus genes were up-regulated at the edge but down-regulated in the centre. Alternative pilus genes, on the other hand, were inversely dysregulated. The centre and edge also exhibited inverse dysregulation in pyoverdine and pyochelin biosynthesis, chemotaxis, quorum sensing, energy production and cell division genes. Although centre genes exhibited an up-regulation of chemotaxis and quorum sensing regulators and effectors, which were relatively down-regulated in edge cells, centre cells may have been dividing and growing less compared to those at the edge, as suggested by an up-regulation of energy production, cell division and protein synthesis genes.

RNA-Seq revealed that surfing resulted in massive dysregulation of genes, which differed between centre and edge cells. The mutant library screen, on the other hand, revealed key genes involved in mediating the initiation of surfing motility. Transposon mutants in 192 genes were identified and verified as being surfing deficient, exhibiting either complete inhibition of motility, alternative motile phenotypes, or irregular growth patterns under surfing conditions. Among those genes, approximately 40 regulators were identified including known global regulators such as GacAS, CbrA, FleQ, LasI, PqsR, RhlR, and RpoN. Looking at the gene expression levels of each of the surfing-essential regulators in each of the transposon mutants for those same regulators revealed that three regulators consistently exhibited a large influence on the expression levels of the other regulators in both centre and edge cells, namely PfeS, PA1463 and CbrA. As CbrA had already been extensively studied for its role in motility and virulence as a global regulator (Yeung et al., 2011), here I focused on PfeS and PA1463 as master regulators in surfing motility.

Knocking out either the PfeR regulator or the PA1463 operon resulted in complete abolishment of surfing. Complementing either with its respective operon led to rescue of wild-type-like surfing phenotype. Both knock-out mutants were found to affect a large cohort of

surfing-essential regulators. Thus *pfeR* mutant exhibited dysregulation of 9 essential regulators while the PA1463o mutant led to changes in 13 regulators among 32 tested genes for which wild-type expression could be restored through complementation. Additionally, the *pfeR* mutant also revealed significant down-regulation (4.1-fold) of PA1463, whereas *pfeRS* was not significantly dysregulated in the PA1463o knock-out. Therefore, the *pfeRS* system may regulate the expression of the PA1463 operon. Five regulators were found to be dysregulated in both mutants, specifically *czcS*, *gacA*, *nirQ*, PA2276, and PA5392. RNA-Seq performed in these surfing deficient mutants revealed 827 genes dysregulated in the PA1463o mutant and 1,856 genes in the *pfeR* mutant relative to wild-type surfing cells. Therefore, both regulators have relatively large regulons (defined as all genes found to be dysregulated in the mutant, whether regulated either directly or indirectly), including regulating the expression of several key surfing-essential regulators.

8.3 Surfing as a conserved complex adaptation

Due to the known role of the PfeRS system in regulating iron acquisition, the dependence of surfing on iron was investigated. Titrating out iron from the system resulted in a reduction of surfing, and a switch from surfing to swimming. Exogenous addition of iron restored surfing motility. Overexpressing the *pfeRS* operon in the PAO1 wild-type resulted in increased persistence of surfing under iron limiting conditions, requiring a 5-fold higher concentration of the iron chelator dipyridyl to abolish surfing. The swich to swimming observed in the wild-type was also not observed in the *pfeRS* overexpression strain.

Surfing, as previously mentioned, is clearly a complex adaptation that involves several regulatory systems and a cascade of genes that mediate initiation and progression of the motility. Here I also explored surfing motility in the context of antibiotic resistance and how conserved it is among other motile bacterial species. Table 8-2 summarizes the major differences in motile adaptations among different bacterial strains.

Table 8-2. Comparison of motility in diverse species. Observations were taken from the cited papers or from the studies presented in Chapter 6.

Type of Motility	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Proteus mirabilis*	*Salmonella enterica*	*Escherichia coli*	*Vibrio harveyi*
Physical appearance on motility plates						
Swim	Within agar circular pattern[1,2]	Within agar circular pattern	Within agar circular pattern[3]	Within agar circular pattern	Within agar circular pattern	Within agar circular pattern

Swarm	Surface motility, dendritic or flared pattern[2]	Not described	Surface motility, concentric rings/terrace[4]	Surface motility, circular[5]	Surface motility, circular[5]	Surface motility, circular for related *Vibrio sp.*[6]
Surf	Surface motility, thick, circular pattern, blue-green centre and white outer edge[1]	Surface motility, circular pattern, thick throughout	Surface motility, circular pattern, thick throughout	Surface motility, circular pattern, thick throughout	Surface motility, circular pattern, thick throughout	Surface motility, circular pattern, thick throughout
Viscosity requirements (agar concentration %)						
Swim	≤ 0.3[1]	0.3	0.3-0.4[3]	0.3	0.3	0.3
Swarm	0.5-0.7[7,8]	0.5-0.8[9]	1.5-3[9]	0.5-0.8[5,9]	0.5-0.8[9]	1.5-3[9]
Surf	0.1-1.0[1]	0.3-0.5	0.3-1.0	0.3-1.0	0.3-1.0	0.3-1.0
Media/growth requirements						
Swim	Plates not dried	Plates not dried	Plates not dried	Plates not dried	Plates not dried	Plates not dried
Swarm	Poor N source not NH_4, minimal media, plates dried	Not described	High glutamine[10]	Glucose as C source[5], Plates dried[9]	30°C[5]	30°C for related *Vibrio sp.*[6]
Surf	Mucin[1]	Mucin	Mucin	Mucin	Mucin	Mucin
Dependence on Quorum Sensing						
Swim	No	Not described			Yes[11]	
Swarm	Yes[8]	Not described	No[12]			Yes for related *Vibrio sp.*[6]
Surf	Yes[1]	Untested	Untested	No	Untested	No
Rate of motility						
Swim	0.9mm/h	1.6mm/h	1mm/h	4mm/h	2.3mm/h	7.3mm/h
Swarm	Not described	Not described	1.2mm/h[4]	1.5mm/h[9]	Untested	Untested
Surf	2.9mm/h	4.9mm/h	2.3mm/h	5.9mm/h	4.9mm/h	9mm/h
Multidrug Resistance						
Swim	No	No	No	No	No	No
Swarm	Yes[13]	Not described	Not described	Yes[9,14]	Not described	Yes for related *Vibrio sp.*[6]
Surf	Yes	Yes	Yes	Yes	Yes	Yes
Dependence on flagella						
Swim	Yes[2]	Not described	No[15]	Yes[16]	Yes[11]	Yes for related *Vibrio sp.*[17]
Swarm	Yes[2]	Not described	Yes[15]	Yes[5,18]	Yes[5]	Yes[6,17]

Surf	Yes[1]	Untested	Untested	Yes	Untested	Untested
Dependence on Biosurfactant						
Swim						
Swarm	Rhamnolipid[19]	Untested	Capsular polysacch-aride[20]	None[21]	Capsular polysacch-aride[22]	Untested
Surf	None[1]	Untested	Untested	Untested	Untested	Untested

1. (Yeung et al., 2012)
2. (Rashid and Kornberg, 2000)
3. (Liaw et al., 2000)
4. (Rauprich et al., 1996)
5. (Harshey and Matsuyama, 1994)
6. (Jaques and McCarter, 2006)
7. (Overhage et al., 2008)
8. (Köhler et al., 2000)
9. (Butler et al., 2010)
10. (Armbruster et al., 2013)
11. (Sperandio et al., 2002)
12. (Schneider et al., 2002)
13. (Overhage et al., 2007)
14. (Kim and Surette, 2003)
15. (Gygi et al., 1997)
16. (Lockman and Curtis, 1990)
17. (Belas and Colwell, 1982)
18. (Stafford and Hughes, 2007)
19. (Caiazza et al., 2005)
20. (Gygi et al., 1995)
21. (Chen et al., 2007)
22. (Takeda et al., 2001)

Surfing *P. aeruginosa* cells were found to be more resistant to several classes of antibiotics including aminoglycosides, macrolides, polymyxins, and quinolones compared to swimming cells. Analgous but unique broad-spectrum resistance was also observed in several other bacterial species that exhibited surfing motility in the presence of mucin, including *E. coli, P. mirabilis, S. enterica, E. cloacae, V. harveyi,* and *B. subtilis*. Analysis of the *P. aeruginosa* resistome revealed 36 genes that when disrupted exhibited a change in susceptibility to certain test antibiotics. Among these resistome genes, 25 mutants exhibited a change in susceptibility to more than one antibiotic tested, speaking to their role in broad-spectrum resistance. Although various other bacterial species also exhibited surfing-mediated broad-spectrum resistance, the antibiotics to which resistance was observed varied greatly between species. Some species even showed increased susceptibility under surfing conditions to certain antibiotics that was not observed in *P. aeruginosa*. Besides antibiotic resistance, the different tested species also exhibited similar flexibility in medium conditions and dependence on flagella but not pili, as was found in *P. aeruginosa* surfing (Yeung et al., 2012). However, a dependence on quorum sensing

was not observed in the tested species, *S. enterica* and *V. harveyi*.

8.4 Concluding remarks

In conclusion, surfing motility, a novel form of bacterial motility dependent on mucin, first established in *P. aeruginosa*, was shown to be conserved in other motile bacteria, both Gram-negative and Gram-positive. Surfing mediated broad-spectrum adaptive resistance, also conserved in other bacteria. It is less stringent than other forms of motility in terms of viscosity and nutrient availability but was dependent on iron. Surfing is a complex motile lifestyle adaptation that involved a large array of regulators that coordinately work to mediate surfing motility. Surfing was found to be dependent on quorum sensing and the stringent stress response. PfeRS, a two-component system, and PA1463, a putative chemotaxis regulator, were found to be master regulators and essential for mediating surfing motility through the regulation of other surfing-essential genes.

8.5 Clinical implications

Surfing motility, as presented in this thesis, is a novel social adaptation of the pathogen, *P. aeruginosa*, that was first discovered using an artificial model of the cystic fibrosis lung sputum. This model mimics several key characteristics of the CF lung including abundant amino acid content and high levels of mucin. Mucin is an important component of the CF sputum that contributes to the disease phenotype by inhibiting mucociliary function and regulating the mucosal viscosity that uniquely promotes surfing. Other wetting agents tested showed poor consistently in promoting surfing motility in *P. aeruginosa*. Mucin, therefore, has unique properties that promote surfing as an adaptation that may optimally contribute to the survival of *P. aeruginosa* under CF conditions. Many characteristics of surfing also reflect *P. aeruginosa* acute infections including the up-regulation of several virulence factors, mainly those secreted by the type 2 secretion system as well as a dependence on key motility appendages. In additon, surfing shared more genetic and physical characterisitcs with biofilms rather than swarming. Biofilms are adaptations that occur in relatively high frequency under chronic CF lung infections. Therefore, key adaptive features that promote *Pseudomonas* survival in the CF lung conserved in biofilms may also be reflected in surfing motility as a consequence of increased survivability in the CF lung environment. Therefore, surfing is an adaptation that potentially contributes to acute CF infections and promotes *P. aeruginosa* survival and persistence in the CF lung.

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Appendix

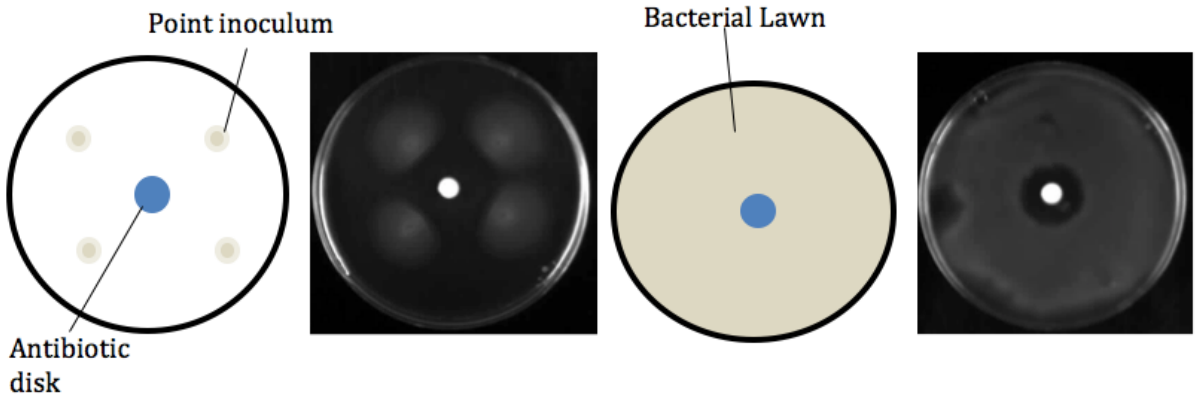


Figure A-1. Disk diffusion assay plate set-up. Mid-log phase (OD_{600}=0.4-0.5) cultures are inoculated at 1uL around the antibiotic disk at equal distances. Four-point inoculation was used for swim and surf antibiotic disk assays. Disk diffusion control assays were done using a bacterial lawn spread with 50uL of mid-log phase culture and dried antibiotic disks were applied to the centre of each plate.

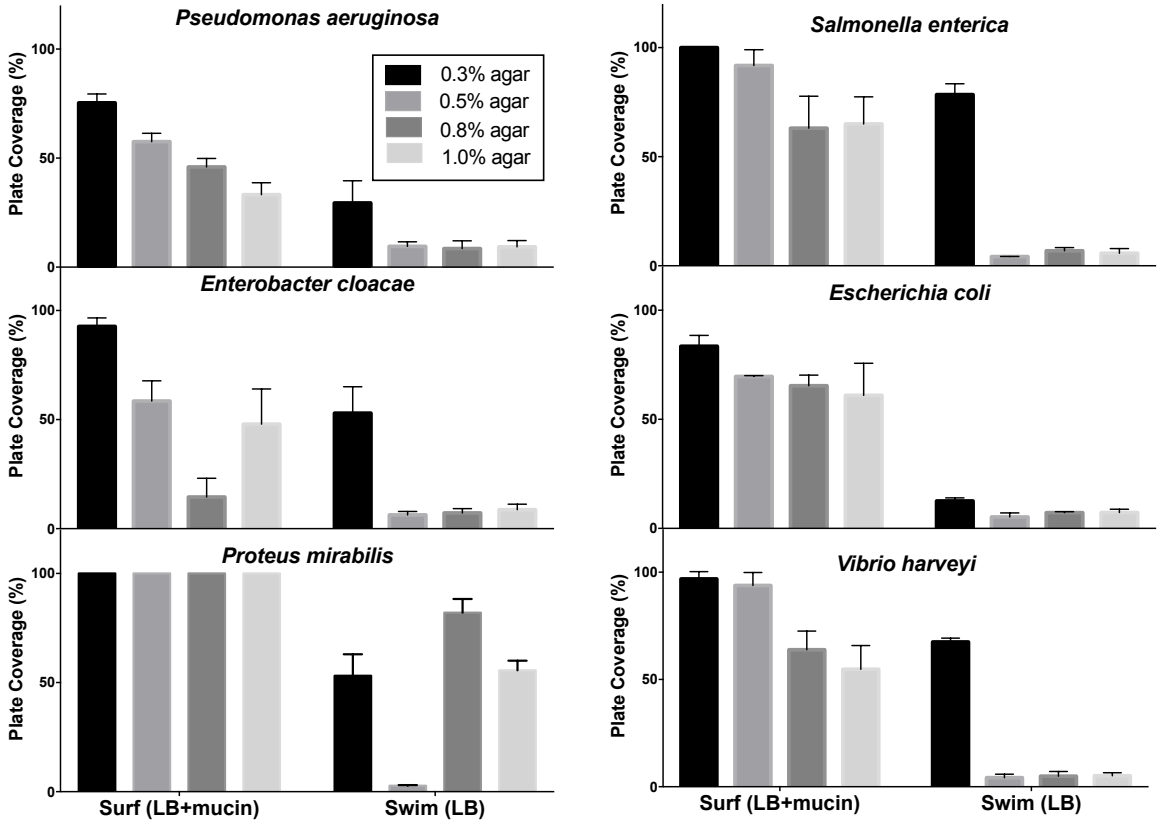


Figure A-2. Effect of viscosity in rich medium on surfing motility. Bacterial strains were point inoculated onto LB medium at varying agar concentrations with and without mucin and grown for 18 hours at 37°C to test the effects on surfing (Surf) and Swimming (Swim) motility. Percent plate coverage was measured using ImageJ (N=3).

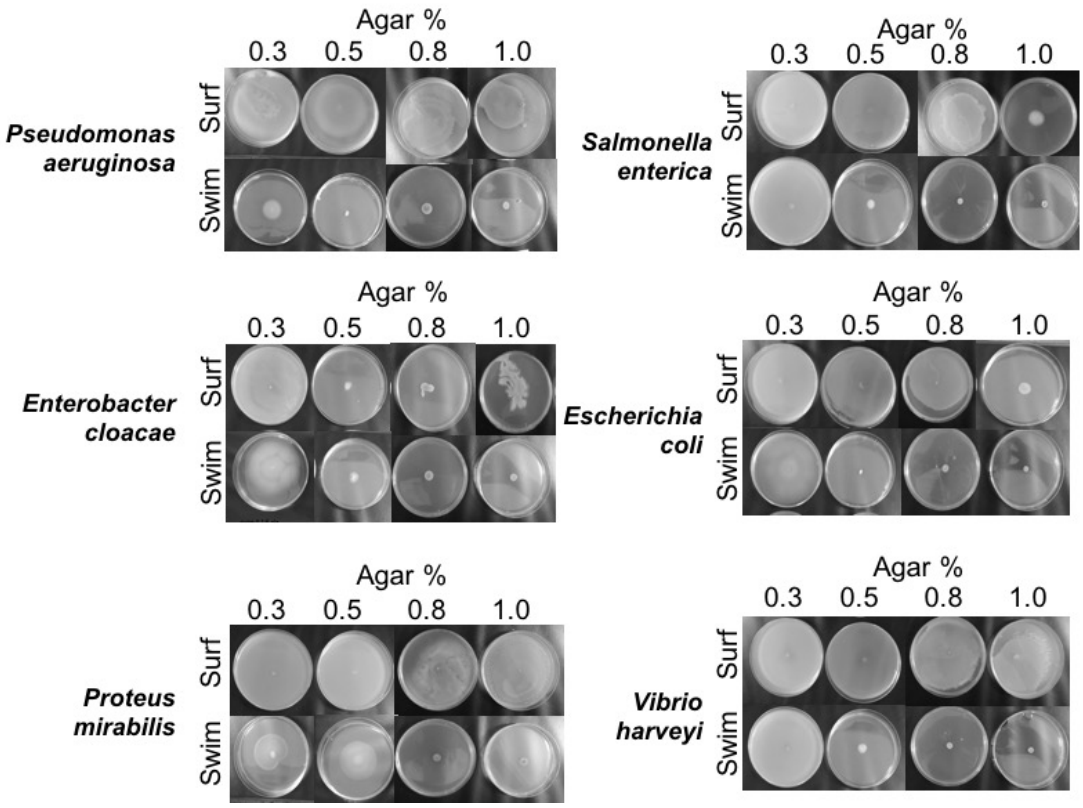


Figure A-3. Effect of medium viscosity on surfing motility. Bacterial strains were point inoculated onto SCFM medium at varying agar concentrations with and without mucin and grown for 18 hours at 37°C to test the effects on surfing (Surf) and Swimming (Swim) motility.

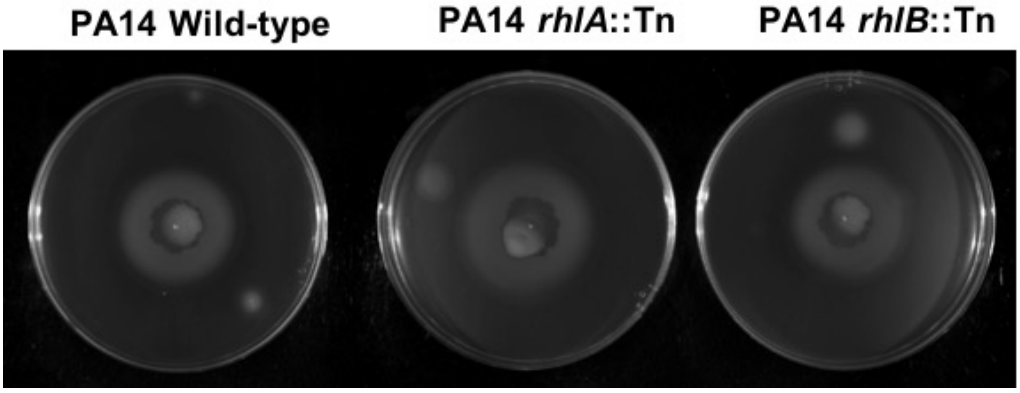
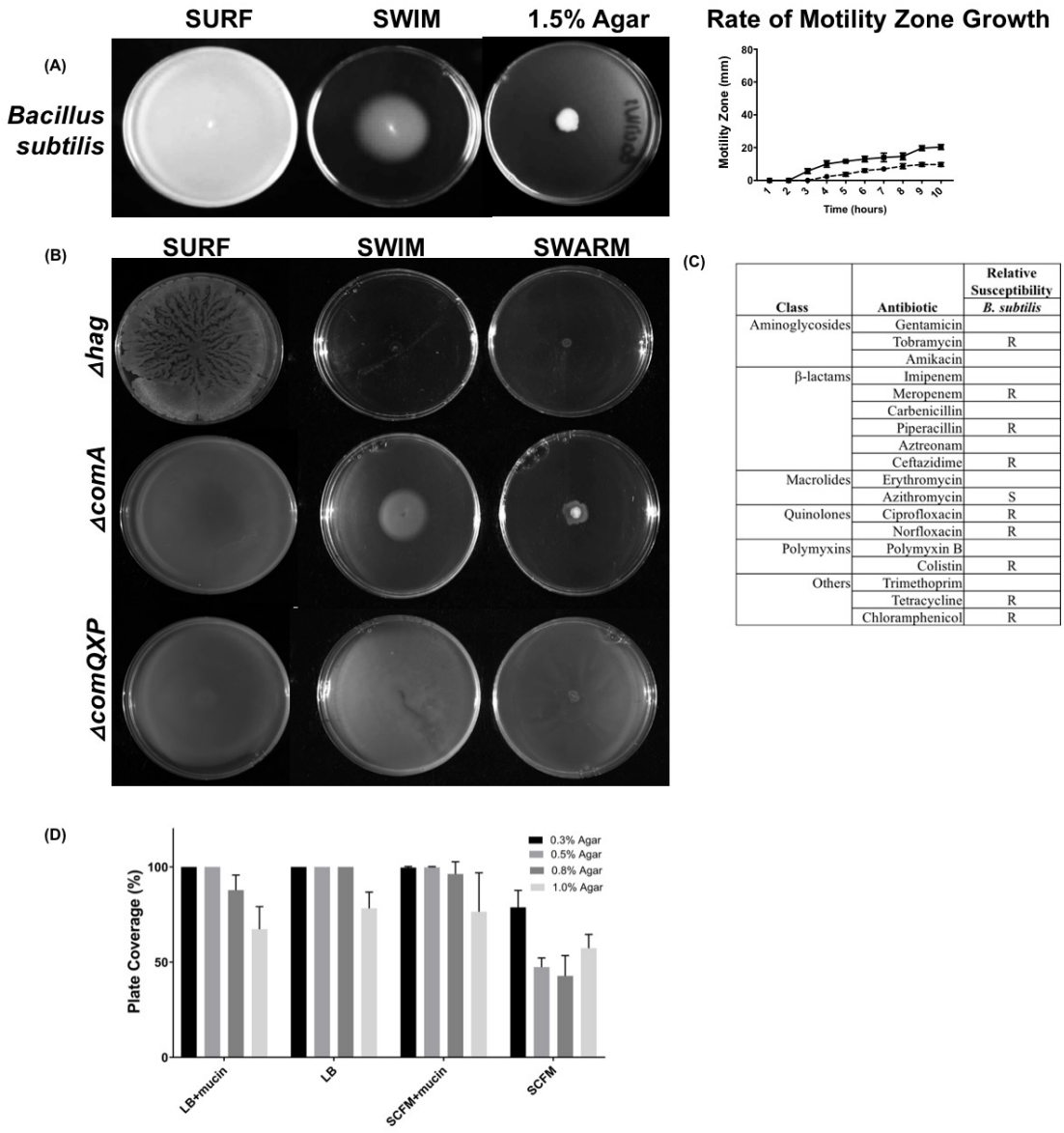


Figure A-4. Surfing motility of PA14 in rhamnolipid deficient mutants. Transposon mutants in *P. aeruginosa* PA14 of *rhlA* and *rhlB* were grown on SCFM with 0.3% agar and 0.4% mucin and incubated at 37°C for 13 hours. Both mutants still exhibited wild-type-like surfing motility.



Class	Antibiotic	Relative Susceptibility *B. subtilis*
Aminoglycosides	Gentamicin	
	Tobramycin	R
	Amikacin	
β-lactams	Imipenem	
	Meropenem	R
	Carbenicillin	
	Piperacillin	R
	Aztreonam	
	Ceftazidime	R
Macrolides	Erythromycin	
	Azithromycin	S
Quinolones	Ciprofloxacin	R
	Norfloxacin	R
Polymyxins	Polymyxin B	
	Colistin	R
Others	Trimethoprim	
	Tetracycline	R
	Chloramphenicol	R

Figure A-5. *Bacillus subtilis* exhibited rapid surface growth under high mucin conditions and surfing-mediated broad-spectrum antibiotic resistance. (A) *B. subtilis* exhibited rapid surface motility under surfing (SCFM+0.4% mucin) conditions that was slightly faster than swimming according to a 10 hour motility zone growth assay. (B) Quorum sensing mutants, Δ*comA* and Δ*comQXP*, continued to show wild-type like surfing. A flagellar mutant, Δ*hag*, exhibited swarming-like patterns under surfing conditions and no swimming or swarming under their respective conditions. The Δ*comA* mutant also exhibited attenuated swarming at 0.5% agar SCFM without ammonium. (C) *B. subtilis* exhibited broad-spectrum resistance to multiple tested antibiotics and increased susceptibility to Azithromycin relative to swimming. (D) Surface motility of *B. subtilis* in LB and SCFM at various agar concentration with and without mucin. Swarming (high agar concentration without mucin) and surfing (+ mucin) were indistinguishable and could occur at varying levels of viscosity.

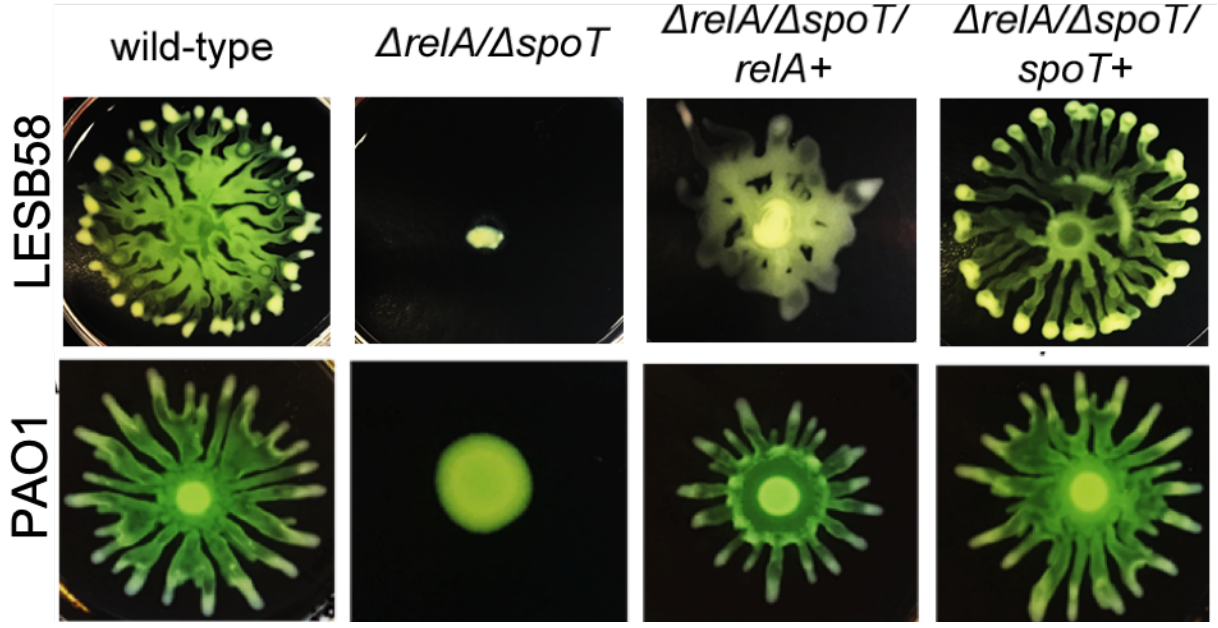


Figure A-6. Effect of stringent response knock-outs on swarming in LESB58 and PAO1. Swarming assays were performed by Daniel Pletzer on KB media with 0.5% agar grown at 37°C for 18 hours.

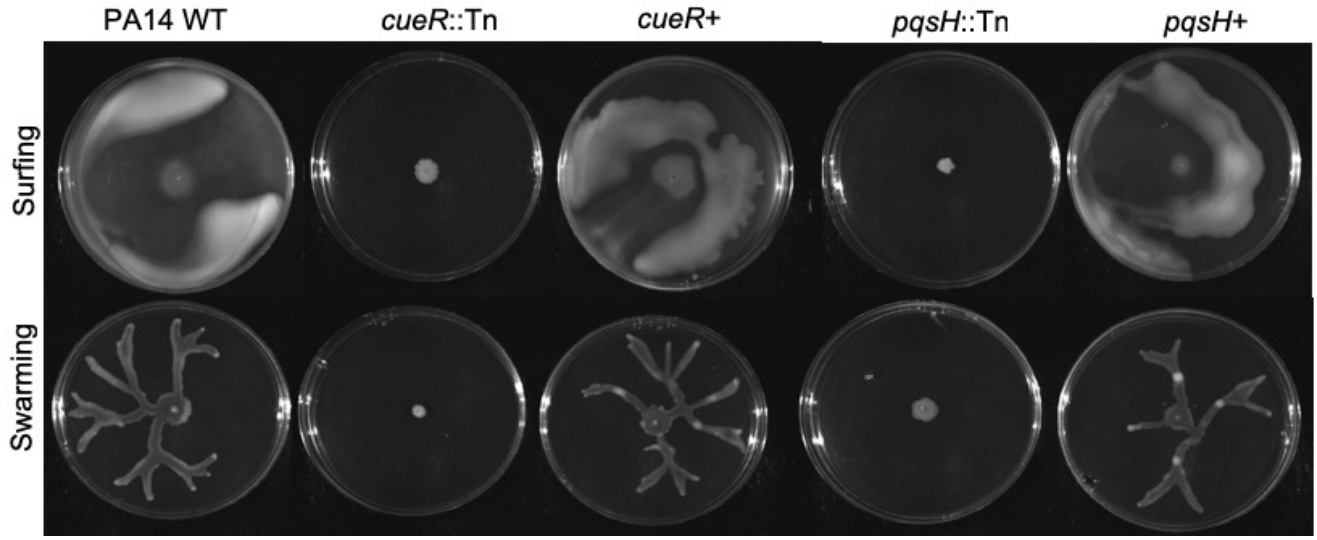


Figure A-7. Surfing and swarming dependence on *pqsH* and *cueR* in PA14. PA14 transposon mutants of *pqsH* and *cueR* were complemented with their respective genes on a high-copy plasmid, pUCp18. Surfing assays were performed in SCFM with 0.3% agar and 0.4% mucin. Swarming assays were performed on SCFM with 0.5% agar. Plates were incubated at 37°C for 18 hours.

Table A-1. Concentrations of the antibiotics in the disk diffusion assay as well as their solvents. Ten µL of each antibiotic was added per disk and dried prior to application onto agar surfaces. MeOH – methanol. DMSO – dimethyl sulfoxide.

Antibiotic	Concentration (µg/disk)	Solvent
Gentamicin	10	Water
Tobramycin	10	Water
Amikacin	5	Water
Imipenem	10	Water
Meropenem	5	Water
Ceftazidime	5	Water
Aztreonam	30	Water
Erythromycin	1000	MeOH
Clarithromycin	500	Water
Polymyxin B	10	Water
Colistin	10	Water
Norfloxacin	5	Water
Ciprofloxacin	10	Water
Trimethoprim	1000	DMSO
Tetracycline	10	MeOH
Chloramphenicol	5	Water

Table A-2. Effectors genes whose transposon mutant variants exhibited surfing deficiency (no motility, an alternative form of motility, and one-directional motility). Transposon mutants come from the PA14 transposon mutant library (Liberati et al., 2006). Gene annotations and descriptions come from www.pseudomonas.com (Winsor et al., 2016).

PAO1 Homolog	PA14 Locus Tag/Gene Name	Description
PA5015	*aceA*	Pyruvate dehydrogenase, E1 component
PA3546	*algX*	Alginate biosynthesis protein
PA4930	*alr*	Biosynthetic alanine racemase
PA5323	*argB*	Acetylglutamate kinase
PA3556	*arnT*	4-amino-4-deoxy-L-arabinose lipid A transferase
PA5555	*atpG*	ATP synthase gamma chain
PA5561	*atpI*	ATP synthase protein I
PA0420	*bioA*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase
PA0504	*bioD*	Dethiobiotin synthase
PA0501	*bioF*	8-amino-7-oxononanoate synthase
PA1073	*braD*	Branched-chain amino acid transport protein brad
PA4758	*carA*	Carbamoyl-phosphate synthase small chain
PA2904	*cobI*	Precorrin-2 methyltransferase cobi
PA2130	*cupA3*	Usher
PA1483	*cycH*	Cytochrome c-type biogenesis protein
PA1838	*cysI*	Sulfite reductase
PA1124	*dgt*	Deoxyguanosinetriphosphate triphosphohydrolase

PA0551	*epd*	D-erythrose 4-phosphate dehydrogenase
PA1982	*exaA*	PQQ-linked alcohol dehydrogenase
PA4959	*fimX*	Conserved hypothetical
PA1077	*flgB*	Flagellar basal-body and rod protein
PA1078	*flgC*	Flagellar basal-body and rod protein
PA1092	*fliC*	Flagellin type B
PA1094	*fliD*	Flagellar capping protein
PA1441	*fliK*	Flagellar hook-length control protein
PA1442	*fliL*	Flagellar basal-body associated protein
PA3583	*glpK*	Glycerol kinase
PA4724	*gltX*	Putative glutamate-tRNA synthetase
PA5203	*gshA*	Glutamate-cysteine ligase
PA2195	*hcnC*	Hydrogen cyanide synthase
PA1512	*hcpA*	Secreted protein Hcp
PA2009	*hmgA*	Homogentisate 1,2-dioxygenase
PA5193	*hslO*	Putative chaperon
PA4695	*ilvH*	Acetolactate synthase isozyme III small subunit
PA5277	*lysA*	Diaminopimelate decarboxylase
PA5025	*metY*	Homocysteine synthase
PA3244	*minD*	Cell division inhibitor minD
PA0766	*mucD*	Serine protease *mucD* precursor
PA4006	*nadD*	NadD nicotinic acid mononucleotide adenylyltransferase
PA4566	*obg*	GTP-binding protein, GTP1/Obg family
PA4208	*opmD*	Outer membrane protein
PA3280	*oprO*	Pyrophosphate-specific outer membrane porin
PA0062	PA14_00740	Putative lipoprotein
PA0066	PA14_00780	Putative carbonic anhydrases
PA0104	PA14_01270	Hypothetical
NA	PA14_03370	Hypothetical
PA0307	PA14_04020	Conserved hypothetical
PA0394	PA14_05160	Putative PLP dependent enzyme
PA0406	PA14_05300	Putative *tonB* domain protein
PA0428	PA14_05560	Putative ATP-dependent RNA helicase, DEAD box family
PA0429	PA14_05580	Conserved hypothetical protein
PA0462	PA14_06040	Hypothetical
PA0503	PA14_06540	Putative biotin synthesis protein
PA0545	PA14_07070	Putative reductase
PA0568	PA14_07380	Hypothetical
PA0583	PA14_07600	Putative 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase
PA0584	PA14_07620	TrnA nucleotidyl transferase
PA0624	PA14_08100	Conserved hypothetical
PA0663	PA14_08490	Conserved hypothetical protein
PA4233	PA14_09190	Putative MFS transporter
PA4168	PA14_09970	Putative *tonB*-dependent receptor

PA4144	PA14_10330	Putative outer membrane protein precursor
PA4137	PA14_10440	Putative porin
PA4130	PA14_10550	Putative sulfite or nitrite reductase
PA4072	PA14_11210	Putative amino acid permease
PA4069	PA14_11250	Putative dtdp-4-rhamnose reductase-related protein
PA4023	PA14_11790	Putative amino acid transporter
PA3975	PA14_12410	Possible phosphomethylpyrimidine kinase
PA3958	PA14_12670	Possible nuclease or phosphotase
PA3892	PA14_13560	Putative fusaric acid resistance protein
PA3884	PA14_13670	Hypothetical
PA3858	PA14_14100	Putative amino-acid ABC transporter binding protein
PA3836	PA14_14390	Putative ABC-type transport protein
PA3818	PA14_14680	Inositol-1-monophosphatase
PA3783	PA14_15140	Conserved hypothetical
PA3749	PA14_15920	Putative major facilitator family transporter
PA3730	PA14_16160	Hypothetical
PA3697	PA14_16580	Hypothetical
PA3649	PA14_17140	Putative membrane-associated zinc metalloprotease
PA3641	PA14_17250	Putative Na+/alanine symporter
PA3631	PA14_17370	Putative transport permease protein
PA3628	PA14_17410	Putative esterase
PA3573	PA14_18090	Putative major facilitator subfamily transporter protein
PA3526	PA14_18720	Putative outer membrane protein precursor, OmpA family
PA3489	PA14_18950	Putative NADH:ubiquinone oxidoreductase
PA3488	PA14_18960	Hypothetical protein
PA0243	PA14_19170	Putative lipoprotein
PA3342	PA14_20840	Hypothetical
PA3325	PA14_21040	Putative hydrolase
PA3324	PA14_21050	Putative short-chain dehydrogenase
NA	PA14_24360	Putative serine protease
PA3057	PA14_24570	Hypothetical
PA2936	PA14_26070	Putative cytochrome b561
PA2927	PA14_26190	Hypothetical proteins
PA2918	PA14_26310	Putative short-chain dehydrogenase
PA2779	PA14_28140	Hypothetical
PA2747	PA14_28600	Conserved hypothetical protein
PA2454	PA14_29220	Putative porin
PA2693	PA14_29290	Putative long-chain acyl-CoA thioester hydrolase
PA2685	PA14_29390	Conserved hypothetical
PA2618	PA14_30260	Putative arginyl-tRNA:protein arginylyltransferase
PA2576	PA14_30790	Putative permease
NA	PA14_30910	Hypothetical
PA2120	PA14_37150	Conserved hypothetical
PA2089	PA14_37490	Putative *tonB*-dependent receptor
PA2002	PA14_38610	Putative short-chain fatty acid transporter

PA1875	PA14_40250	Putative outer membrane protein precursor
PA1547	PA14_44460	Putative membrane protein
PA1509	PA14_44920	Conserved hypothetical
PA1442	PA14_45810	Putative flagellar protein FliL
PA1441	PA14_45830	Putative flagellar hook-length control protein FliK
NA	PA14_46610	Putative methyltransferase
PA1271	PA14_47800	Putative *tonB*-dependent receptor
PA1239	PA14_48210	Putative hydrolase
PA1210	PA14_48650	Putative porin protein
PA0718	PA14_48990	Hypothetical protein of bacteriophage Pf1
PA1187	PA14_49080	Probable acyl-coA dehydrogenase
PA1127	PA14_49800	Probable oxidoreductase
PA1120	PA14_49890	None
PA1119	PA14_49900	Probable outer membrane protein precursor
PA1045	PA14_50840	Putative DNA helicase
PA1037	PA14_50920	Conserved hypothetical protein
PA1033	PA14_50970	Probable glutathione S-transferase
PA0974	PA14_51690	Conserved hypothetical
PA0848	PA14_53300	Probable alkyl hydroperoxide reductase
PA0817	PA14_53700	Probable ring-cleaving dioxygenase
PA0794	PA14_53970	Probably aconitate hydratase
PA4333	PA14_56300	Putative fumarase
PA4431	PA14_57570	Putative cytochrome c reductase, iron-sulfur subunit
PA4455	PA14_57870	Putative toluene tolerance ABC efflux transporter
PA4471	PA14_58040	Hypothetical
PA4511	PA14_58540	Conserved hypothetical protein
PA4518	PA14_58620	Conserved hypothetical
NA	PA14_59000	Conserved hypothetical
NA	PA14_59410	Hypothetical
NA	PA14_59950	Conserved hypothetical
PA0982	PA14_59960	Putative protein-disulfide isomerase
PA1935	PA14_60080	Conserved hypothetical
PA4612	PA14_61020	Ankyrin-like protein
PA4616	PA14_61080	Putative C4-dicarboxylate-binding protein
PA4650	PA14_61520	Conserved hypothetical
PA4734	PA14_62640	Conserved hypothetical protein
PA4753	PA14_62880	Putative RNA-binding protein
PA4838	PA14_63970	Putative membrane protein
PA4975	PA14_65760	NAD(P)H quinone oxidoreductase
PA4981	PA14_65850	Putative amino acid ABC transporter, permease protein
PA5076	PA14_67050	Putative amino acid ABC transporter, periplasmic amino acid-binding protein
PA5109	PA14_67470	Conserved hypothetical
PA5174	PA14_68360	Putative beta-ketoacyl synthase
PA5376	PA14_71000	Put. lysine betaine/L-proline ABC transporter, ATP-binding subunit

PA5399	PA14_71280	Putative ferredoxin
PA4729	*panB*	3-methyl-2-oxobutanoate hydroxymethyltransferase
PA5192	*pckA*	Phosphoenolpyruvate carboxykinase
PA0773	*pdxJ*	Pyridoxal phosphate biosynthetic protein
PA4050	*pgpA*	Phosphatidylglycerophosphatase A
PA4210	*phzA1*	Probable phenazine biosynthesis protein
PA0395	*pilT*	Twitching motility protein
PA0396	*pilU*	Twitching motility protein
PA4552	*pilW*	Type 4 fimbrial biogenesis protein
PA0026	*plcB*	Phospholipase C
PA2969	*plsX*	Fatty acid/phospholipid synthesis protein
PA5368	*pstC*	Phosphate ABC transporter, permease protein
PA1013	*purC*	Phosphoribosylaminoimidazole-succinocarboxamide synthase
PA4855	*purD*	Phosphoribosylamine-glycine ligase
PA4854	*purH*	Phosphoribosylaminoimidazolecarboxamide transferase
PA3763	*purL*	Phosphoribosylformylglycinamidine synthase
PA3050	*pyrD*	Dihydroorotate dehydrogenase
PA5331	*pyrE*	Orotate phosphoribosyltransferase
PA4743	*rbfA*	Ribosome-binding factor A
PA3387	*rhlG*	Beta-ketoacyl reductase
PA1396	*RL112*	Conserved hypothetical
PA5454	*rmd*	Oxidorectase
PA4752	*rrmJ*	Cell division protein
PA0966	*ruvA*	Holliday junction DNA helicase
PA4332	*sadC*	Conserved hypothetical proteins
PA4001	*sltB1*	Soluble lytic transglycosylase B
PA0298	*spuB*	Glutamine synthetase
PA0594/ PA0595	*surA/ostA*	Peptidyl-prolyl cis-trans isomerase/organic solvent tolerance protein *ostA* precursor
PA5070	*tatC*	Sec-independent protein translocase
PA3976	*thiE*	Possible thiamin-phosphate pyrophosphorylase
PA0381	*thiG*	Thiamine biosynthesis protein, thiazole moiety
PA3735	*thrC*	Threonine synthase
PA2832	*tpm*	Thiopurine methyltransferase
PA0849	*trxB2*	Thioredoxin reductase 2

Table A-3. Genes dysregulated in PAO6609 wild-type surfing relative to swimming. RNA-Seq was performed on PAO1 WT cells collected from a surfing edge and swim colony. 499 genes were found to be dysregulated under surfing relative to swimming. A log fold-change cut-off of ± 1.5 and p-value < 0.05 was used. Gene annotations and descriptions come from www.pseudomonas.com (Winsor et al., 2016).

Gene ID	Gene Name	Description	Log FC
PA0017		Conserved Hypothetical Protein	1.68
PA0026	*plcB*	Phospholipase C, PlcB	2.25
PA0027		Hypothetical Protein	1.99
PA0028		Hypothetical Protein	2.28
PA0045		Hypothetical Protein	1.81
PA0046		Hypothetical Protein	1.75
PA0048		Probable Transcriptional Regulator	2.78
PA0049		Hypothetical Protein	4.33
PA0051	*phzH*	Potential Phenazine-Modifying Enzyme	2.68
PA0062		Hypothetical Protein	-1.53
PA0122	*rahU*	RahU	-1.66
PA0125		Hypothetical Protein	-1.65
PA0129	*bauD*	Amino Acid Permease	2.31
PA0130	*bauC*	3-Oxopropanoate Dehydrogenase	1.51
PA0132	*bauA*	Beta-Alanine:Pyruvate Transaminase	1.96
PA0144		Hypothetical Protein	2.71
PA0170		Hypothetical Protein	-1.99
PA0171		Hypothetical Protein	-1.71
PA0187		Hypothetical Protein	-2.85
PA0188		Hypothetical Protein	-3.05
PA0229	*pcaT*	Dicarboxylic Acid Transporter PcaT	-1.56
PA0234		Hypothetical Protein	-1.77
PA0241		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.20
PA0247	*pobA*	P-Hydroxybenzoate Hydroxylase	-2.04
PA0258		Hypothetical Protein	-1.76
PA0279		Probable Transcriptional Regulator	-2.51
PA0281	*cysW*	Sulfate Transport Protein CysW	1.87
PA0282	*cysT*	Sulfate Transport Protein CysT	1.97
PA0283	*sbp*	Sulfate-Binding Protein Precursor	3.10
PA0284		Hypothetical Protein	2.52
PA0349		Hypothetical Protein	-1.72
PA0417	*chpE*	Probable Chemotaxis Protein	1.79

PA0433		Hypothetical Protein	-1.90
PA0434		Hypothetical Protein	-2.56
PA0435		Hypothetical Protein	-2.17
PA0439		Probable Oxidoreductase	-2.25
PA0441	*dht*	Dihydropyrimidinase	-1.66
PA0451		Conserved Hypothetical Protein	-1.61
PA0452		Probable Stomatin-Like Protein	-2.11
PA0457		Hypothetical Protein	2.44
PA0476		Probable Permease	-2.90
PA0497		Hypothetical Protein	-2.93
PA0518	*nirM*	Cytochrome C-551 Precursor	1.83
PA0523	*norC*	Nitric-Oxide Reductase Subunit C	4.29
PA0524	*norB*	Nitric-Oxide Reductase Subunit B	3.93
PA0525		Probable Dinitrification Protein Nord	2.93
PA0531		Probable Glutamine Amidotransferase	-1.72
PA0578		Conserved Hypothetical Protein	1.55
PA0617		Probable Bacteriophage Protein	2.00
PA0618		Probable Bacteriophage Protein	1.69
PA0622		Probable Bacteriophage Protein	1.74
PA0628		Conserved Hypothetical Protein	1.54
PA0632		Hypothetical Protein	2.05
PA0634		Hypothetical Protein	1.53
PA0636		Hypothetical Protein	1.69
PA0639		Conserved Hypothetical Protein	1.67
PA0654	*speD*	S-Adenosylmethionine Decarboxylase Proenzyme	1.57
PA0686	*hxcR*	HxcR	1.79
PA0688	*lapA*	Low-Molecular-Weight Alkaline Phosphatase A, LapA	-2.49
PA0689	*lapB*	Low-Molecular-Weight Alkaline Phosphatase B, LapB	-1.52
PA0713		Hypothetical Protein	-1.79
PA0717		Hypothetical Protein Of Bacteriophage Pf1	-2.64
PA0718		Hypothetical Protein Of Bacteriophage Pf1	-3.07
PA0719		Hypothetical Protein Of Bacteriophage Pf1	-2.34
PA0720		Helix Destabilizing Protein Of Bacteriophage Pf1	-1.90
PA0723	*coaB*	Coat Protein B Of Bacteriophage Pf1	-1.80
PA0726		Hypothetical Protein Of Bacteriophage Pf1	-1.60
PA0728		Probable Bacteriophage Integrase	-1.96
PA0730		Probable Transferase	2.18
PA0737		Hypothetical Protein	-2.29
PA0781		Hypothetical Protein	-4.67

PA0790		Hypothetical Protein	-3.01
PA0844	*plcH*	Hemolytic Phospholipase C Precursor	2.16
PA0845	*cerN*	CerN	5.01
PA0850		Hypothetical Protein	-1.91
PA0851		Hypothetical Protein	1.54
PA0852	*cbpD*	Chitin-Binding Protein CbpD Precursor	2.22
PA0882		Hypothetical Protein	-2.34
PA0979		Conserved Hypothetical Protein	2.44
PA0983		Conserved Hypothetical Protein	-1.91
PA0985	*pyoS5*	Pyocin S5	1.73
PA0986		Conserved Hypothetical Protein	4.16
PA0987		Conserved Hypothetical Protein	2.63
PA1041		Probable Outer Membrane Protein Precursor	-1.82
PA1130	*rhlC*	Rhamnosyltransferase 2	-1.57
PA1151	*imm2*	Pyocin S2 Immunity Protein	-1.60
PA1168		Hypothetical Protein	4.74
PA1217		Probable 2-Isopropylmalate Synthase	-1.65
PA1221		Hypothetical Protein	-1.75
PA1224		Probable Nad(P)H Dehydrogenase	-2.06
PA1244		Hypothetical Protein	-2.05
PA1251		Probable Chemotaxis Transducer	-2.03
PA1266	*lhpE*	D-Hydroxyproline Dehydrogenase Alpha-Subunit, LphE	2.14
PA1267	*lhpB*	D-Hydroxyproline Dehydrogenase Beta-Subunit, LphB	2.17
PA1268	*lhpA*	Hydroxyproline 2-Epimerase, LhpA	3.03
PA1275	*cobD*	Cobalamin Biosynthetic Protein CobD	1.50
PA1291		Hypothetical Protein	-2.07
PA1321	*cyoE*	Cytochrome O Ubiquinol Oxidase Protein CyoE	-1.77
PA1332		Hypothetical Protein	-1.95
PA1333		Hypothetical Protein	-2.33
PA1334		Probable Oxidoreductase	1.91
PA1346		Hypothetical Protein	-2.18
PA1369		Hypothetical Protein	-1.99
PA1370		Hypothetical Protein	-2.06
PA1382		Probable Type Ii Secretion System Protein	-1.51
PA1383		Hypothetical Protein	-1.71
PA1384	*galE*	Udp-Glucose 4-Epimerase	-2.04
PA1385		Probable Glycosyl Transferase	-1.84
PA1386		Probable Atp-Binding Component Of Abc Transporter	-2.31
PA1387		Hypothetical Protein	-1.55

PA1388		Hypothetical Protein	-1.68
PA1390		Probable Glycosyl Transferase	-1.93
PA1391		Probable Glycosyl Transferase	-2.06
PA1393	*cysC*	Adenosine 5'-Phosphosulfate (Aps) Kinase	-1.78
PA1414		Hypothetical Protein	-1.97
PA1418		Probable Sodium:Solute Symport Protein	-1.82
PA1423	*bdlA*	BdlA	-1.68
PA1431	*rsaL*	Regulatory Protein RsaL	4.53
PA1471		Hypothetical Protein	-2.91
PA1499		Conserved Hypothetical Protein	-1.92
PA1503		Hypothetical Protein	-1.86
PA1507		Probable Transporter	-1.91
PA1519		Probable Transporter	-2.74
PA1525	*alkB2*	Alkane-1-Monooxygenase 2	-1.57
PA1565	*pauB2*	Fad-Dependent Oxidoreductase	2.06
PA1566	*pauA3*	Glutamylpolyamine Synthetase	1.78
PA1591		Hypothetical Protein	1.69
PA1600		Probable Cytochrome C	-1.63
PA1617		Probable Amp-Binding Enzyme	-1.57
PA1654		Probable Aminotransferase	1.73
PA1655		Probable Glutathione S-Transferase	1.51
PA1707	*pcrH*	Regulatory Protein PcrH	-2.07
PA1708	*popB*	Translocator Protein Popb	-1.92
PA1711	*exsE*	ExsE	-1.89
PA1739		Probable Oxidoreductase	-1.90
PA1771	*estX*	EstX	1.88
PA1873		Hypothetical Protein	2.21
PA1875		Probable Outer Membrane Protein Precursor	1.73
PA1877		Probable Secretion Protein	1.56
PA1887		Hypothetical Protein	-3.00
PA1888		Hypothetical Protein	-3.21
PA1892		Hypothetical Protein	1.85
PA1893		Hypothetical Protein	1.92
PA1894		Hypothetical Protein	1.76
PA1895		Hypothetical Protein	1.58
PA1897		Hypothetical Protein	1.68
PA1901	*phzC2*	Phenazine Biosynthesis Protein PhzC	-4.69
PA1905	*phzG2*	Probable Pyridoxamine 5'-Phosphate Oxidase	-5.50
PA1914		Conserved Hypothetical Protein	4.37

PA1920	*nrdD*	Class Iii (Anaerobic) Ribonucleoside-Triphosphate Reductase Subunit, Nrdd	-2.71
PA1921		Hypothetical Protein	-6.45
PA1922		Probable Tonb-Dependent Receptor	-7.63
PA1923		Hypothetical Protein	-6.78
PA1924		Hypothetical Protein	-7.49
PA1925		Hypothetical Protein	-8.59
PA1927	*metE*	5-Methyltetrahydropteroyltriglutamate-Homocysteine S-Methyltransferase	-1.80
PA1935		Hypothetical Protein	-1.63
PA1937		Conserved Hypothetical Protein	3.94
PA1938		Conserved Hypothetical Protein	1.54
PA1939		Hypothetical Protein	-1.95
PA1964		Probable ATP-Binding Component Of ABC Transporter	1.68
PA1970		Hypothetical Protein	-1.65
PA1977		Hypothetical Protein	-2.91
PA1979	*eraS*	Sensor Kinase, EraS	-1.55
PA1984	*exaC*	Nad+ Dependent Aldehyde Dehydrogenase ExaC	4.03
PA2013	*liuC*	Putative 3-Methylglutaconyl-Coa Hydratase	1.68
PA2014	*liuB*	Methylcrotonyl-Coa Carboxylase, Beta-Subunit	1.52
PA2021		Hypothetical Protein	-1.52
PA2030		Hypothetical Protein	2.19
PA2038		Hypothetical Protein	1.84
PA2073		Probable Transporter (Membrane Subunit)	-1.60
PA2074		Hypothetical Protein	-1.66
PA2089		Hypothetical Protein	-1.76
PA2096		Probable Transcriptional Regulator	-2.05
PA2099		Probable Short-Chain Dehydrogenase	-1.66
PA2103		Probable Molybdopterin Biosynthesis Protein MoeB	-1.98
PA2104		Probable Cysteine Synthase	-2.00
PA2105		Probable Acetyltransferase	-1.76
PA2106		Hypothetical Protein	-1.88
PA2109		Hypothetical Protein	2.22
PA2110		Hypothetical Protein	5.25
PA2111		Hypothetical Protein	3.83
PA2112		Conserved Hypothetical Protein	3.79
PA2113	*opdO*	Pyroglutamate Porin OpdO	4.98
PA2114		Probable Major Facilitator Superfamily (Mfs) Transporter	2.15
PA2116		Conserved Hypothetical Protein	2.38

PA2119		Alcohol Dehydrogenase (Zn-Dependent)	-1.94
PA2147	*katE*	Catalase Hpii	2.87
PA2167		Hypothetical Protein	-1.90
PA2182		Hypothetical Protein	-1.97
PA2183		Hypothetical Protein	-1.71
PA2184		Conserved Hypothetical Protein	-3.46
PA2185	*katN*	Non-Heme Catalase KatN	-2.64
PA2186		Hypothetical Protein	-2.12
PA2188		Probable Alcohol Dehydrogenase (Zn-Dependent)	-2.29
PA2190		Conserved Hypothetical Protein	-2.94
PA2192		Conserved Hypothetical Protein	-3.05
PA2204		Probable Binding Protein Component Of ABC Transporter	2.01
PA2217		Probable Aldehyde Dehydrogenase	-1.90
PA2218		Hypothetical Protein	-1.88
PA2221		Conserved Hypothetical Protein	-1.65
PA2222		Hypothetical Protein	-1.58
PA2224		Hypothetical Protein	-1.68
PA2225		Hypothetical Protein	-1.90
PA2226	*qsrO*	QsrO	-2.02
PA2229		Conserved Hypothetical Protein	-2.50
PA2231	*pslA*	PslA	-1.63
PA2232	*pslB*	PslB	-1.65
PA2233	*pslC*	PslC	-1.68
PA2234	*pslD*	PslD	-1.73
PA2235	*pslE*	PslE	-1.75
PA2260		Hypothetical Protein	1.89
PA2294		Probable Atp-Binding Component Of ABC Transporter	-2.44
PA2299		Probable Transcriptional Regulator	1.54
PA2304	*ambC*	AmbC	-1.54
PA2317		Probable Oxidoreductase	3.22
PA2318		Hypothetical Protein	2.77
PA2334		Probable Transcriptional Regulator	-2.94
PA2343	*mtlY*	Xylulose Kinase	-1.95
PA2352		Probable Glycerophosphoryl Diester Phosphodiesterase	1.52
PA2381		Hypothetical Protein	-2.48
PA2423		Hypothetical Protein	1.97
PA2429		Hypothetical Protein	-2.06
PA2434		Hypothetical Protein	-1.78
PA2437		Hypothetical Protein	-2.16

PA2438		Hypothetical Protein	-1.63
PA2439		Hypothetical Protein	-2.29
PA2441		Hypothetical Protein	-2.74
PA2458		Hypothetical Protein	1.67
PA2470	*gtdA*	Gentisate 1,2-Dioxygenase	-1.61
PA2504		Hypothetical Protein	-1.67
PA2507	*catA*	Catechol 1,2-Dioxygenase	-2.84
PA2508	*catC*	Muconolactone Delta-Isomerase	-1.98
PA2511	*antR*	AntR	-2.22
PA2512	*antA*	Anthranilate Dioxygenase Large Subunit	-3.43
PA2513	*antB*	Anthranilate Dioxygenase Small Subunit	-3.12
PA2514	*antC*	Anthranilate Dioxygenase Reductase	-2.60
PA2516	*xylZ*	Toluate 1,2-Dioxygenase Electron Transfer Component	-2.04
PA2561	*ctpH*	CtpH	-2.33
PA2562		Hypothetical Protein	-2.04
PA2567		Hypothetical Protein	-1.76
PA2580		Conserved Hypothetical Protein	-1.66
PA2587	*pqsH*	Probable Fad-Dependent Monooxygenase	1.56
PA2588		Probable Transcriptional Regulator	1.70
PA2602		3-Mercaptopropionate Dioxygenase	2.52
PA2630		Conserved Hypothetical Protein	1.88
PA2636		Hypothetical Protein	-1.63
PA2672		Probable Type Ii Secretion System Protein	-2.00
PA2673		Probable Type Ii Secretion System Protein	-1.63
PA2682		Conserved Hypothetical Protein	-1.74
PA2700	*opdB*	Proline Porin OpdB	-1.90
PA2714		Probable Molybdopterin Oxidoreductase	-2.95
PA2715		Probable Ferredoxin	-2.44
PA2736		Hypothetical Protein	-1.51
PA2747		Hypothetical Protein	-1.79
PA2754		Conserved Hypothetical Protein	-1.72
PA2759		Hypothetical Protein	-2.71
PA2771		Diguanylate Cyclase With A Self-Blocked I-Site, Dcsbis	-2.09
PA2779		Hypothetical Protein	-1.95
PA2838		Probable Transcriptional Regulator	-3.58
PA2847		Conserved Hypothetical Protein	-1.52
PA2862	*lipA*	Lactonizing Lipase Precursor	-1.74
PA2863	*lipH*	Lipase Modulator Protein	-1.63
PA2911		Probable Tonb-Dependent Receptor	-1.84

PA2912		Probable ATP-Binding Component Of ABC Transporter	-1.65
PA2913		Hypothetical Protein	-2.28
PA2914		Probable Permease Of ABC Transporter	-1.58
PA2932	*morB*	Morphinone Reductase	2.70
PA2934	*cif*	Cftr Inhibitory Factor, Cif	1.78
PA2937		Hypothetical Protein	-1.67
PA2938		Probable Transporter	-2.12
PA2939		Probable Aminopeptidase	3.40
PA3000	*aroP1*	Aromatic Amino Acid Transport Protein AroP1	1.64
PA3032	*snr1*	Cytochrome C Snr1	-3.25
PA3062	*pelC*	PelC	-2.23
PA3137		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.78
PA3142		Integrase	-1.58
PA3144		Transposase With Helix-Turn-Helix Hin Domain	-1.55
PA3160	*wzz*	O-Antigen Chain Length Regulator	-2.02
PA3181		2-Keto-3-Deoxy-6-Phosphogluconate Aldolase	2.47
PA3182	*pgl*	6-Phosphogluconolactonase	2.44
PA3183	*zwf*	Glucose-6-Phosphate 1-Dehydrogenase	2.84
PA3191	*gtrS*	Glucose Transport Sensor, GtrS	1.82
PA3192	*gltR*	Two-Component Response Regulator GltR	1.70
PA3193	*glk*	Glucokinase	1.63
PA3194	*edd*	Phosphogluconate Dehydratase	2.30
PA3195	*gapA*	Glyceraldehyde 3-Phosphate Dehydrogenase	2.71
PA3229		Hypothetical Protein	-2.46
PA3266	*capB*	Cold Acclimation Protein B	1.79
PA3281		Hypothetical Protein	-3.11
PA3282		Hypothetical Protein	-3.89
PA3283		Conserved Hypothetical Protein	-4.13
PA3284		Hypothetical Protein	-4.72
PA3315		Probable Permease Of Abc Transporter	-1.56
PA3319	*plcN*	Non-Hemolytic Phospholipase C Precursor	-1.68
PA3323		Conserved Hypothetical Protein	-1.69
PA3327		Probable Non-Ribosomal Peptide Synthetase	-2.11
PA3328		Probable FAD-Dependent Monooxygenase	-2.12
PA3329		Hypothetical Protein	-2.12
PA3330		Probable Short Chain Dehydrogenase	-2.20
PA3331		Cytochrome P450	-1.86
PA3332		Conserved Hypothetical Protein	-1.79
PA3333	*fabH2*	3-Oxoacyl-[Acyl-Carrier-Protein] Synthase III	-1.71

PA3334		Probable Acyl Carrier Protein	-1.88
PA3335		Hypothetical Protein	-1.92
PA3336		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.99
PA3359		Hypothetical Protein	-1.57
PA3360		Probable Secretion Protein	-1.78
PA3361	*lecB*	Fucose-Binding Lectin Pa-III	-2.63
PA3390		Hypothetical Protein	-1.51
PA3391	*nosR*	Regulatory Protein NosR	3.11
PA3392	*nosZ*	Nitrous-Oxide Reductase Precursor	2.60
PA3393	*nosD*	NosD Protein	2.25
PA3415		Probable Dihydrolipoamide Acetyltransferase	-1.75
PA3416		Probable Pyruvate Dehydrogenase E1 Component, Beta Chain	-1.58
PA3417		Probable Pyruvate Dehydrogenase E1 Component, Alpha Subunit	-1.67
PA3442		Probable Atp-Binding Component Of Abc Transporter	1.68
PA3445		Conserved Hypothetical Protein	1.80
PA3446		Conserved Hypothetical Protein	1.73
PA3450	*lsfA*	1-Cys Peroxiredoxin LsfA	3.39
PA3451		Hypothetical Protein	-1.78
PA3467		Probable Major Facilitator Superfamily (Mfs) Transporter	1.85
PA3497		Hypothetical Protein	-2.48
PA3498		Probable Oxidoreductase	-2.05
PA3500		Conserved Hypothetical Protein	-2.26
PA3506		Probable Decarboxylase	-1.50
PA3510		Hypothetical Protein	-1.63
PA3514		Probable ATP-Binding Component Of ABC Transporter	-1.63
PA3518		Hypothetical Protein	2.15
PA3519		Hypothetical Protein	3.17
PA3520		Hypothetical Protein	2.23
PA3532		Hypothetical Protein	2.08
PA3535		Probable Serine Protease	4.39
PA3546	*algX*	Alginate Biosynthesis Protein AlgX	-2.12
PA3572		Hypothetical Protein	-1.54
PA3577		Hypothetical Protein	1.59
PA3588		Probable Porin	-3.81
PA3593		Probable Acyl-CoA Dehydrogenase	-2.26
PA3597		Probable Amino Acid Permease	-2.44
PA3598		Conserved Hypothetical Protein	-1.73
PA3600		Conserved Hypothetical Protein	-5.90
PA3601		Conserved Hypothetical Protein	-5.13

PA3610	*potD*	Polyamine Transport Protein PotD	1.52
PA3655	*tsf*	Elongation Factor Tsf	1.60
PA3662		Hypothetical Protein	-1.68
PA3724	*lasB*	Elastase LasB	1.68
PA3741		Hypothetical Protein	1.63
PA3769	*guaA*	Gmp Synthase	1.51
PA3784		Hypothetical Protein	-1.63
PA3785		Conserved Hypothetical Protein	-2.36
PA3789		Hypothetical Protein	-1.76
PA3790	*oprC*	Putative Copper Transport Outer Membrane Porin Oprc Precursor	-2.56
PA3811	*hscB*	Heat Shock Protein Hscb	1.53
PA3819		Conserved Hypothetical Protein	-1.66
PA3841	*exoS*	Exoenzyme S	-2.00
PA3843		Hypothetical Protein	-1.76
PA3877	*narK1*	Nitrite Extrusion Protein 1	1.83
PA3901	*fecA*	Fe(Iii) Dicitrate Transport Protein FecA	2.75
PA3904		Hypothetical Protein	5.12
PA3905		Hypothetical Protein	6.43
PA3906		Hypothetical Protein	5.59
PA3907		Hypothetical Protein	5.70
PA3908		Hypothetical Protein	5.33
PA3935	*tauD*	Taurine Dioxygenase	2.00
PA3938		Probable Periplasmic Taurine-Binding Protein Precursor	1.66
PA3940		Probable Dna Binding Protein	1.90
PA3967		Hypothetical Protein	2.01
PA4028		Hypothetical Protein	-2.75
PA4062		Hypothetical Protein	-1.61
PA4063		Hypothetical Protein	-4.59
PA4065		Hypothetical Protein	-3.59
PA4066		Hypothetical Protein	-3.41
PA4071		Hypothetical Protein	3.67
PA4072		Probable Amino Acid Permease	2.77
PA4073		Probable Aldehyde Dehydrogenase	3.20
PA4100		Probable Dehydrogenase	4.78
PA4117	*bphP*	Bacterial Phytochrome, BphP	1.77
PA4133		Cytochrome C Oxidase Subunit (Cbb3-Type)	2.01
PA4134		Hypothetical Protein	2.25
PA4139		Hypothetical Protein	2.37
PA4140		Hypothetical Protein	2.41

PA4152		Probable Hydrolase	1.50
PA4170		Hypothetical Protein	-3.82
PA4175	*piv*	Protease Iv	3.72
PA4178	*eftM*	Sam-Dependent Methyltransferase , EftM	1.54
PA4181		Hypothetical Protein	2.22
PA4182		Hypothetical Protein	1.61
PA4187		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.00
PA4211	*phzB1*	Probable Phenazine Biosynthesis Protein	-1.54
PA4218	*ampP*	AmpP	2.36
PA4219	*ampO*	AmpO	2.03
PA4220		Hypothetical Protein	1.96
PA4221	*fptA*	Fe(Iii)-Pyochelin Outer Membrane Receptor Precursor	2.04
PA4222		Probable Atp-Binding Component Of Abc Transporter	2.05
PA4223		Probable Atp-Binding Component Of Abc Transporter	2.20
PA4224	*pchG*	Pyochelin Biosynthetic Protein PchG	2.16
PA4225	*pchF*	Pyochelin Synthetase	1.90
PA4226	*pchE*	Dihydroaeruginoic Acid Synthetase	1.95
PA4228	*pchD*	Pyochelin Biosynthesis Protein PchD	2.06
PA4229	*pchC*	Pyochelin Biosynthetic Protein PchC	2.38
PA4230	*pchB*	Salicylate Biosynthesis Protein PchB	2.66
PA4231	*pchA*	Salicylate Biosynthesis Isochorismate Synthase	2.22
PA4271	*rplL*	50S Ribosomal Protein L7 / L12	1.69
PA4272	*rplJ*	50S Ribosomal Protein L10	1.73
PA4273	*rplA*	50S Ribosomal Protein L1	1.50
PA4274	*rplK*	50S Ribosomal Protein L11	1.52
PA4277	*tufB*	Elongation Factor Tu	1.65
PA4298		Hypothetical Protein	-1.59
PA4300	*tadC*	TadC	-1.60
PA4303	*tadZ*	TadZ	-1.54
PA4306	*flp*	Type Ivb Pilin, Flp	-2.17
PA4341		Probable Transcriptional Regulator	-1.91
PA4355	*pyeM*	PyeM	-2.77
PA4364		Hypothetical Protein	2.69
PA4365	*lysE*	Lysine Efflux Permease	2.83
PA4442	*cysN*	ATP Sulfurylase GTP-Binding Subunit/Aps Kinase	1.79
PA4443	*cysD*	ATP Sulfurylase Small Subunit	2.12
PA4500	*dppA3*	Probable Binding Protein Component Of ABC Transporter	1.72
PA4501	*opdD*	Glycine-Glutamate Dipeptide Porin OpdP	2.08
PA4502	*dppA4*	Probable Binding Protein Component Of ABC Transporter	1.59

PA4505	*dppD*	Dipeptide ABC Transporter Atp-Binding Protein DppD	1.55
PA4549	*fimT*	Type 4 Fimbrial Biogenesis Protein Fimt	-2.42
PA4568	*rplU*	50S Ribosomal Protein L21	1.73
PA4582		Conserved Hypothetical Protein	-2.57
PA4584		Conserved Hypothetical Protein	-1.63
PA4586		Hypothetical Protein	-1.80
PA4590	*pra*	Protein Activator	2.04
PA4607		Hypothetical Protein	-2.01
PA4616		Probable C4-Dicarboxylate-Binding Protein	1.68
PA4620		Hypothetical Protein	-1.57
PA4673		Conserved Hypothetical Protein	1.66
PA4677		Hypothetical Protein	1.66
PA4714		Conserved Hypothetical Protein	1.62
PA4724		Probable Aminoacyl-Transfer RNA Synthetase (Class I)	1.84
PA4740	*pnp*	Polyribonucleotide Nucleotidyltransferase	1.66
PA4778	*cueR*	CueR	2.15
PA4800		Hypothetical Protein	1.65
PA4834		Putative Nicotianamine Synthase	-6.74
PA4835		Hypothetical Protein	-6.11
PA4836		Hypothetical Protein	-5.97
PA4837		Probable Outer Membrane Protein Precursor	-6.26
PA4838		Hypothetical Protein	-3.80
PA4869		Hypothetical Protein	2.14
PA4881		Hypothetical Protein	-1.53
PA4888	*desB*	Acyl-Coa Delta-9-Desaturase, DesB	3.56
PA4889		Probable Oxidoreductase	2.63
PA4903		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.57
PA4935	*rpsF*	30S Ribosomal Protein S6	1.53
PA4962		Conserved Hypothetical Protein	1.67
PA4973	*thiC*	Thiamin Biosynthesis Protein ThiC	1.74
PA4979		Probable Acyl-Coa Dehydrogenase	1.80
PA4980		Probable Enoyl-Coa Hydratase/Isomerase	1.82
PA5001	*ssg*	Cell Surface-Sugar Biosynthetic Glycosyltransferase, Ssg	1.55
PA5002	*dnpA*	De-N-Acetylase Involved In Persistence, DnpA	1.68
PA5024		Conserved Hypothetical Protein	1.68
PA5101		Hypothetical Protein	-1.58
PA5117	*typA*	Regulatory Protein TypA	1.76
PA5139		Hypothetical Protein	2.04
PA5180		Conserved Hypothetical Protein	-2.84

PA5181		Probable Oxidoreductase	-2.75
PA5232		Conserved Hypothetical Protein	1.56
PA5274	*rnk*	Nucleoside Diphosphate Kinase Regulator	1.66
PA5295		Hypothetical Protein	1.51
PA5325	*sphA*	SphA	3.15
PA5326	*sphD*	SphD	4.34
PA5327	*sphC*	SphC	4.11
PA5328	*sphB*	SphB	4.66
PA5352		Conserved Hypothetical Protein	-2.79
PA5353	*glcF*	Glycolate Oxidase Subunit GlcF	-2.99
PA5354	*glcE*	Glycolate Oxidase Subunit GlcE	-2.48
PA5355	*glcD*	Glycolate Oxidase Subunit GlcD	-2.47
PA5383		Conserved Hypothetical Protein	4.47
PA5392		Conserved Hypothetical Protein	1.70
PA5396		Hypothetical Protein	2.24
PA5397		Hypothetical Protein	2.41
PA5406		Hypothetical Protein	1.65
PA5407		Hypothetical Protein	1.75
PA5410	*gbcA*	GbcA	1.94
PA5415	*glyA1*	Serine Hydroxymethyltransferase	2.34
PA5416	*soxB*	Sarcosine Oxidase Beta Subunit	2.13
PA5417	*soxD*	Sarcosine Oxidase Delta Subunit	1.89
PA5418	*soxA*	Sarcosine Oxidase Alpha Subunit	1.81
PA5419	*soxG*	Sarcosine Oxidase Gamma Subunit	1.75
PA5420	*purU2*	Formyltetrahydrofolate Deformylase	2.46
PA5426	*purE*	Phosphoribosylaminoimidazole Carboxylase, Catalytic Subunit	1.59
PA5434	*mtr*	Tryptophan Permease	1.92
PA5437		Probable Transcriptional Regulator	1.86
PA5470		Probable Peptide Chain Release Factor	-2.06
PA5498	*znuA*	ZnuA	-1.53
PA5499	*zur*	Zinc Uptake Regulator, Zur	-1.55
PA5532		Hypothetical Protein	-2.32
PA5534		Hypothetical Protein	-5.48
PA5535		Conserved Hypothetical Protein	-5.69
PA5536	*dksA2*	Dksa2	-6.78
PA5537		Hypothetical Protein	-3.76
PA5538	*amiA*	N-Acetylmuramoyl-L-Alanine Amidase	-6.84
PA5539		Hypothetical Protein	-6.54
PA5540		Hypothetical Protein	-5.79

PA5541	*pyrQ*	Dihydroorotase	-5.94

Table A-4. Genes uniquely dysregulated in the Δ*pfeR* mutant but not dysregulated under wild-type surfing conditions. RNA-Seq was performed on the Δ*pfeR* mutant relative to the PAO6609 WT under surfing conditions. 1572 genes were found to be uniquely dysregulated in the mutant. A log fold-change cut-off of ± 1.5 and p-value < 0.05 was used. Gene annotations and descriptions come from www.pseudomonas.com (Winsor et al., 2016).

Gene ID	Gene Name	Description	Log FC
PA0007		Hypothetical Protein	2.07
PA0009	*glyQ*	Glycyl-Trna Synthetase Alpha Chain	1.88
PA0020	*tsaP*	T4P Secretin-Associated Protein Tsap	1.99
PA0021		Conserved Hypothetical Protein	-4.44
PA0030	*cosX*	Cosx	-2.99
PA0031	*betC*	Choline Sulfatase	-2.57
PA0037	*trpI*	Transcriptional Regulator Trpi	-1.97
PA0039		Hypothetical Protein	2.36
PA0041		Probable Hemagglutinin	-2.05
PA0043		Hypothetical Protein	-2.29
PA0044	*exoT*	Exoenzyme T	-2.03
PA0047		Hypothetical Protein	2.05
PA0050		Hypothetical Protein	3.36
PA0053		Hypothetical Protein	-2.09
PA0056		Probable Transcriptional Regulator	-2.94
PA0058	*dsbM*	Dsbm	-2.02
PA0059	*osmC*	Osmotically Inducible Protein Osmc	2.59
PA0070	*tagQ1*	Tagq1	3.72
PA0072	*tagS1*	Tags1	-2.45
PA0073	*tagT1*	Tagt1	-1.61
PA0080	*tssJ1*	Tssj1	2.26
PA0083	*tssB1*	Tssb1	3.02
PA0084	*tssC1*	Tssc1	3.11
PA0085	*hcp1*	Hcp1	3.18
PA0091	*vgrG1*	Vgrg1	2.01
PA0099		Hypothetical Protein	1.55
PA0103		Probable Sulfate Transporter	-2.49
PA0104		Hypothetical Protein	-2.52
PA0112		Hypothetical Protein	-1.59
PA0113		Probable Cytochrome C Oxidase Assembly Factor	-1.57
PA0117		Probable Short Chain Dehydrogenase	-1.87
PA0126		Hypothetical Protein	1.57
PA0134		Probable Guanine Deaminase	-2.12
PA0135		Hypothetical Protein	-3.51

PA0136		Probable Atp-Binding Component Of Abc Transporter	-3.15
PA0145		Hypothetical Protein	-1.89
PA0146		Conserved Hypothetical Protein	-2.59
PA0150		Anti-Sigma Factor	-1.77
PA0152	*pcaQ*	Transcriptional Regulator Pcaq	-2.32
PA0155	*pcaR*	Transcriptional Regulator Pcar	-2.09
PA0164		Probable Gamma-Glutamyltranspeptidase	-2.67
PA0166		Probable Transporter	-3.24
PA0172	*siaA*	Siaa	-2.03
PA0175		Probable Chemotaxis Protein Methyltransferase	2
PA0176	*aer2*	Aerotaxis Transducer Aer2	2.12
PA0177		Probable Purine-Binding Chemotaxis Protein	2.92
PA0178		Probable Two-Component Sensor	2.06
PA0181		Probable Transcriptional Regulator	-1.9
PA0182		Probable Short-Chain Dehydrogenase	-2.57
PA0183	*atsA*	Arylsulfatase	-2.83
PA0185		Probable Permease Of Abc Transporter	-4.39
PA0186		Probable Binding Protein Component Of Abc Transporter	-3.57
PA0189		Probable Porin	-3.87
PA0194		Hypothetical Protein	-2.57
PA0198	*exbB1*	Transport Protein Exbb	-2.59
PA0199	*exbD1*	Transport Protein Exbd	-1.58
PA0202		Probable Amidase	-5.47
PA0203		Probable Binding Protein Component Of Abc Transporter	-1.79
PA0206		Probable Atp-Binding Component Of Abc Transporter	-3.21
PA0207		Probable Transcriptional Regulator	-2.55
PA0213		Hypothetical Protein	-1.73
PA0218		Probable Transcriptional Regulator	-1.6
PA0222		Hypothetical Protein	-1.69
PA0226		Probable Coa Transferase, Subunit A	-2.74
PA0227		Probable Coa Transferase, Subunit B	-3.47
PA0228	*pcaF*	Beta-Ketoadipyl CoA Thiolase Pcaf	-3.21
PA0230	*pcaB*	3-Carboxy-Cis,Cis-Muconate Cycloisomerase	-2.18
PA0231	*pcaD*	Beta-Ketoadipate Enol-Lactone Hydrolase	-2.03
PA0235	*pcaK*	4-Hydroxybenzoate Transporter Pcak	-2.62
PA0237		Probable Oxidoreductase	-4.26
PA0238		Hypothetical Protein	-3.71
PA0239		Hypothetical Protein	-2.04
PA0240		Probable Porin	-2.25
PA0242		Hypothetical Protein	-4.44
PA0244		Hypothetical Protein	-4.42
PA0245	*aroQ2*	3-Dehydroquinate Dehydratase	-2.42
PA0246		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.58

PA0254	*hudA*	Huda	-2.11
PA0257		Hypothetical Protein	-2.28
PA0263	*hcpC*	Secreted Protein Hcp	1.75
PA0264		Hypothetical Protein	-2.68
PA0268		Probable Transcriptional Regulator	-2.52
PA0273		Probable Major Facilitator Superfamily (Mfs) Transporter	-7.14
PA0278		Hypothetical Protein	-2.66
PA0287	*gpuP*	3-Guanidinopropionate Transport Protein	-4.25
PA0288	*gpuA*	3-Guanidinopropionase	-3.36
PA0289	*gpuR*	Transcriptional Activator Gpur	-1.64
PA0311		Hypothetical Protein	-4.26
PA0316	*serA*	D-3-Phosphoglycerate Dehydrogenase	2.03
PA0320	*carO*	Calcium-Regulated Ob-Fold Protein Caro	-5.34
PA0322		Probable Transporter	-1.66
PA0324		Probable Permease Of Abc Transporter	-2.13
PA0325		Probable Permease Of Abc Transporter	-2.43
PA0327	*carP*	Calcium-Regulated Beta-Propeller Protein Carp	-3.58
PA0334		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.4
PA0339		Hypothetical Protein	-1.74
PA0345		Hypothetical Protein	-1.7
PA0347	*glpQ*	Glycerophosphoryl Diester Phosphodiesterase, Periplasmic	-3.25
PA0348		Hypothetical Protein	-2.79
PA0354		Conserved Hypothetical Protein	1.86
PA0355	*pfpI*	Protease Pfpi	2.91
PA0368		Conserved Hypothetical Protein	-1.73
PA0383		Conserved Hypothetical Protein	-1.56
PA0389		Hypothetical Protein	1.87
PA0403	*pyrR*	Transcriptional Regulator Pyrr	-1.83
PA0404		Conserved Hypothetical Protein	-1.69
PA0422		Conserved Hypothetical Protein	2.3
PA0423	*pasP*	Pasp	4.13
PA0432	*sahH*	S-Adenosyl-L-Homocysteine Hydrolase	1.53
PA0438	*codB*	Cytosine Permease	-2.12
PA0440		Probable Oxidoreductase	-3.59
PA0443		Probable Transporter	-1.8
PA0444		N-Carbamoyl-Beta-Alanine Amidohydrolase	-2.18
PA0446		Conserved Hypothetical Protein	1.53
PA0447	*gcdH*	Glutaryl-Coa Dehydrogenase	2.11
PA0450		Probable Phosphate Transporter	-2.31
PA0453		Hypothetical Protein	-2.63
PA0465	*creD*	Inner Membrane Protein Cred	-1.68
PA0472	*fiuI*	Fiui	2.04
PA0489		Probable Phosphoribosyl Transferase	-1.82

PA0496		Conserved Hypothetical Protein	-1.92
PA0498		Hypothetical Protein	-2.06
PA0499		Probable Pili Assembly Chaperone	-3.14
PA0504	*bioD*	Dethiobiotin Synthase	-1.57
PA0505		Hypothetical Protein	-2.02
PA0519	*nirS*	Nitrite Reductase Precursor	2.98
PA0521		Probable Cytochrome C Oxidase Subunit	-3.05
PA0526		Hypothetical Protein	1.61
PA0527	*dnr*	Transcriptional Regulator Dnr	1.75
PA0530		Probable Class Iii Pyridoxal Phosphate-Dependent Aminotransferase	-2.43
PA0534	*pauB1*	Fad-Dependent Oxidoreductase	2.08
PA0539		Hypothetical Protein	-2.14
PA0541		Hypothetical Protein	1.88
PA0545		Hypothetical Protein	-2.59
PA0546	*metK*	Methionine Adenosyltransferase	4.2
PA0547		Probable Transcriptional Regulator	3.18
PA0548	*tktA*	Transketolase	2.46
PA0551	*epd*	D-Erythrose 4-Phosphate Dehydrogenase	1.56
PA0557		Hypothetical Protein	-2.1
PA0563		Conserved Hypothetical Protein	2.37
PA0564		Probable Transcriptional Regulator	-1.58
PA0567		Conserved Hypothetical Protein	2.83
PA0573		Hypothetical Protein	-1.63
PA0576	*rpoD*	Sigma Factor RpoD	2.52
PA0579	*rpsU*	30S Ribosomal Protein S21	3.78
PA0592	*ksgA*	Rrna (Adenine-N6,N6)-Dimethyltransferase	1.90
PA0593	*pdxA*	Pyridoxal Phosphate Biosynthetic Protein PdxA	1.67
PA0594	*surA*	Peptidyl-Prolyl Cis-Trans Isomerase SurA	1.99
PA0595	*lptD*	Lps-Assembly Protein LptD	2.50
PA0603	*agtA*	AgtA	1.69
PA0607	*rpe*	Ribulose-Phosphate 3-Epimerase	2.46
PA0619		Probable Bacteriophage Protein	1.74
PA0633		Hypothetical Protein	2.14
PA0635		Hypothetical Protein	1.95
PA0637		Conserved Hypothetical Protein	-1.60
PA0642		Hypothetical Protein	-3.38
PA0643		Hypothetical Protein	-1.78
PA0644		Hypothetical Protein	-2.62
PA0645		Hypothetical Protein	-1.71
PA0649	*trpG*	Anthranilate Synthase Component II	1.71
PA0651	*trpC*	Indole-3-Glycerol-Phosphate Synthase	1.77
PA0652	*vfr*	Transcriptional Regulator Vfr	1.82

PA0663		Hypothetical Protein	1.55
PA0666		Conserved Hypothetical Protein	1.73
PA0669		Probable DNA Polymerase Alpha Chain	-1.87
PA0671		Hypothetical Protein	-2.85
PA0672	*hemO*	Heme Oxygenase	5.00
PA0673		Hypothetical Protein	-2.68
PA0675	*vreI*	Ecf Sigma Factor, VreI	-2.19
PA0676	*vreR*	Sigma Factor Regulator, VreR	-2.26
PA0677	*hxcW*	Hxcw	-3.55
PA0679	*hxcP*	Hxcp	-2.46
PA0681	*hxcT*	Hxct	-2.83
PA0685	*hxcQ*	Hxcq	-5.15
PA0690	*pdtA*	Phosphate Depletion Regulated Tps Partner A, PdtA	-3.25
PA0692	*pdtB*	Phosphate Depletion Regulated Tps Partner B, PdtB	-2.67
PA0693	*exbB2*	Transport Protein ExbB2	-5.17
PA0696		Hypothetical Protein	-3.74
PA0699		Probable Peptidyl-Prolyl Cis-Trans Isomerase	-4.91
PA0701		Probable Transcriptional Regulator	-3.97
PA0703		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.41
PA0706	*cat*	Chloramphenicol Acetyltransferase	2.23
PA0709		Hypothetical Protein	-1.57
PA0710	*gloA2*	Lactoylglutathione Lyase	-1.59
PA0714		Hypothetical Protein	-1.65
PA0715		Hypothetical Protein	-1.87
PA0716		Hypothetical Protein	-1.75
PA0724		Probable Coat Protein A Of Bacteriophage Pf1	-3.97
PA0727		Hypothetical Protein From Bacteriophage Pf1	-2.08
PA0729		Hypothetical Protein	-2.11
PA0738		Conserved Hypothetical Protein	-2.49
PA0739		Probable Transcriptional Regulator	-2.8
PA0740	*sdsA1*	Sds Hydrolase Sdsa1	-3.56
PA0744		Probable Enoyl-Coa Hydratase/Isomerase	1.80
PA0745		Probable Enoyl-Coa Hydratase/Isomerase	2.09
PA0746		Probable Acyl-Coa Dehydrogenase	2.53
PA0747		Probable Aldehyde Dehydrogenase	2.10
PA0755	*opdH*	Cis-Aconitate Porin OpdH	2.00
PA0758		Hypothetical Protein	1.84
PA0766	*mucD*	Serine Protease MucD Precursor	1.60
PA0767	*lepA*	Gtp-Binding Protein LepA	2.40
PA0768	*lepB*	Signal Peptidase I	1.98
PA0770	*rnc*	Ribonuclease Iii	1.69
PA0771	*era*	Gtp-Binding Protein Era	1.67
PA0778	*icp*	Inhibitor of Cysteine Peptidase	1.74

PA0779	*asrA*	AsrA	2.56
PA0780	*pruR*	Proline Utilization Regulator	-1.54
PA0785	*azoR1*	Fmn-Dependent NADH-Azoreductase 1, Azor1	-2.46
PA0787		Hypothetical Protein	-2.68
PA0791		Probable Transcriptional Regulator	-1.54
PA0800		Hypothetical Protein	-2.43
PA0806		Hypothetical Protein	-2.47
PA0809		Probable Transporter	-4.19
PA0811		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.29
PA0812		Hypothetical Protein	-2.99
PA0813		Hypothetical Protein	-3.07
PA0814		Conserved Hypothetical Protein	-1.56
PA0816		Probable Transcriptional Regulator	-2.00
PA0817		Probable Ring-Cleaving Dioxygenase	-2.30
PA0824		Hypothetical Protein	-3.42
PA0825		Hypothetical Protein	-1.65
PA0828		Probable Transcriptional Regulator	-2.99
PA0841		Hypothetical Protein	-1.82
PA0842		Probable Glycosyl Transferase	-3.43
PA0856		Hypothetical Protein	2.52
PA0857	*bolA*	Morphogene Protein BolA	2.50
PA0864		Probable Transcriptional Regulator	-2.37
PA0875		Conserved Hypothetical Protein	-2.48
PA0876		Probable Transcriptional Regulator	-2.05
PA0877		Probable Transcriptional Regulator	-2.78
PA0878		Hypothetical Protein	-3.64
PA0879		Probable Acyl-CoA Dehydrogenase	-1.74
PA0883		Probable Acyl-CoA Lyase Beta Chain	-3.99
PA0884		Probable C4-Dicarboxylate-Binding Periplasmic Protein	-2.07
PA0886		Probable C4-Dicarboxylate Transporter	-2.58
PA0888	*aotJ*	Arginine/Ornithine Binding Protein AotJ	1.81
PA0889	*aotQ*	Arginine/Ornithine Transport Protein AotQ	1.55
PA0892	*aotP*	Arginine/Ornithine Transport Protein AotP	1.66
PA0894		Hypothetical Protein	-2.00
PA0895	*aruC*	N2-Succinylornithine 5-Aminotransferase (Soat) = N2-Acetylornithine 5-Aminotransferase (Acoat)	2.67
PA0896	*aruF*	Subunit I Of Arginine N2-Succinyltransferase = Ornithine N2-Succinyltransferase	1.69
PA0897	*aruG*	Subunit II of Arginine N2-Succinyltransferase = Ornithine N2-Succinyltransferase	1.80
PA0907	*alpA*	Lysis Phenotype Activator, AlpA	-2.10
PA0912		Hypothetical Protein	-2.68
PA0913	*mgtE*	MgtE	1.68

PA0918		Cytochrome B561	-1.79
PA0929		Two-Component Response Regulator	2.24
PA0931	*pirA*	Ferric Enterobactin Receptor PirA	1.95
PA0938	*wzz2*	Wzz2	3.12
PA0939		Hypothetical Protein	-1.69
PA0942		Probable Transcriptional Regulator	-1.76
PA0945	*purM*	Phosphoribosylaminoimidazole Synthetase	2.15
PA0955		Hypothetical Protein	1.75
PA0963	*aspS*	Aspartyl-TrnA Synthetase	2.07
PA0964	*pmpR*	PqsR-Mediated Pqs Regulator, PmpR	1.72
PA0973	*oprL*	Peptidoglycan Associated Lipoprotein OprL Precursor	1.93
PA0977		Hypothetical Protein	-1.75
PA0980		Hypothetical Protein	-5.34
PA0981		Hypothetical Protein	-2.76
PA0996	*pqsA*	PqsA	2.43
PA0997	*pqsB*	PqsB	2.32
PA0998	*pqsC*	PqsC	2.47
PA0999	*pqsD*	3-Oxoacyl-[Acyl-Carrier-Protein] Synthase III	1.81
PA1000	*pqsE*	Quinolone Signal Response Protein	1.67
PA1001	*phnA*	Anthranilate Synthase Component I	1.72
PA1011		Hypothetical Protein	2.05
PA1016		Hypothetical Protein	-2.03
PA1017	*pauA*	Pimeloyl-CoA Synthetase	-3.62
PA1019	*mucK*	Cis,Cis-Muconate Transporter MucK	-3.76
PA1020		Probable Acyl-CoA Dehydrogenase	-3.43
PA1021		Probable Enoyl-CoA Hydratase/Isomerase	-3.48
PA1022		Probable Acyl-CoA Dehydrogenase	-2.05
PA1023		Probable Short-Chain Dehydrogenase	-2.06
PA1024		NADH:Quinone Reductase	-2.92
PA1025		Probable Porin	-1.83
PA1028	*amaA*	L-Pipecolate Oxidase	-1.99
PA1029		Hypothetical Protein	-2.23
PA1051		Probable Transporter	-1.57
PA1052		Conserved Hypothetical Protein	-3.42
PA1056	*shaC*	ShaC	-1.55
PA1065		Conserved Hypothetical Protein	-2.09
PA1067		Probable Transcriptional Regulator	-1.73
PA1077	*flgB*	Flagellar Basal-Body Rod Protein FlgB	1.91
PA1078	*flgC*	Flagellar Basal-Body Rod Protein FlgC	1.80
PA1079	*flgD*	Flagellar Basal-Body Rod Modification Protein FlgD	1.94
PA1108		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.52
PA1109		Probable Transcriptional Regulator	-1.80
PA1113		Probable ATP-Binding/Permease Fusion ABC Transporter	-2.07

PA1119	*yfiB*	YfiB	1.96
PA1122		Putative Peptide Deformylase	-1.60
PA1123		Hypothetical Protein	1.72
PA1125		Probable Cobalamin Biosynthetic Protein	-2.01
PA1129	*fosA*	Fosfomycin Resistance Protein, FosA	-1.89
PA1132		Hypothetical Protein	1.84
PA1133		Hypothetical Protein	-2.58
PA1135		Conserved Hypothetical Protein	-2.3
PA1136		Probable Transcriptional Regulator	-2.44
PA1138		Probable Transcriptional Regulator	-2.28
PA1139		Hypothetical Protein	-2.17
PA1141		Probable Transcriptional Regulator	-1.88
PA1143		Hypothetical Protein	-3.77
PA1144		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.44
PA1147		Probable Amino Acid Permease	-3.43
PA1148	*toxA*	Exotoxin A Precursor	-2.65
PA1150	*pys2*	Pyocin S2	-2.44
PA1152		Hypothetical Protein	-3.55
PA1153		Hypothetical Protein	-2.31
PA1154		Conserved Hypothetical Protein	-2.11
PA1155	*nrdB*	Nrdb, Tyrosyl Radical-Harboring Component of Class Ia Ribonucleotide Reductase	1.90
PA1156	*nrdA*	Nrda, Catalytic Component of Class Ia Ribonucleotide Reductase	1.86
PA1165	*pcpS*	PcpS	-2.55
PA1178	*oprH*	PhoP/Q and Low Mg2+ Inducible Outer Membrane Protein H1 Precursor	-1.54
PA1179	*phoP*	Two-Component Response Regulator PhoP	-1.92
PA1182		Probable Transcriptional Regulator	-2.81
PA1187		Probable Acyl-CoA Dehydrogenase	-2.17
PA1188		Hypothetical Protein	-2.10
PA1192		Conserved Hypothetical Protein	1.60
PA1193		Hypothetical Protein	1.56
PA1194		Probable Amino Acid Permease	-2.36
PA1196	*ddaR*	Transcriptional Regulator DdaR	-2.02
PA1198		Conserved Hypothetical Protein	1.73
PA1199		Probable Lipoprotein	2.12
PA1200		Conserved Hypothetical Protein	1.75
PA1205		Conserved Hypothetical Protein	-1.52
PA1209		Hypothetical Protein	-2.14
PA1211		Hypothetical Protein	-3.15
PA1212		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.29
PA1213		Hypothetical Protein	-3.33

PA1214		Hypothetical Protein	-3.31
PA1215		Hypothetical Protein	-2.81
PA1219		Hypothetical Protein	-3.64
PA1220		Hypothetical Protein	-3.11
PA1223		Probable Transcriptional Regulator	-2.12
PA1225		Probable NAD(P)H Dehydrogenase	-2.99
PA1226		Probable Transcriptional Regulator	-2.10
PA1227		Hypothetical Protein	-2.14
PA1229		Probable Transcriptional Regulator	-2.88
PA1234		Hypothetical Protein	-1.62
PA1235		Probable Transcriptional Regulator	-2.45
PA1240		Probable Enoyl-CoA Hydratase/Isomerase	-2.18
PA1249	*aprA*	Alkaline Metalloproteinase Precursor	4.64
PA1253	*lhpG*	Alpha-Ketoglutaric Semialdehyde Dehydrogenase, LhpG	-2.84
PA1257	*lhpN*	Amino Acid ABC Transporter Membrane Protein, LhpN	-2.19
PA1258	*lhpM*	Permease of ABC Transporter, LhpM	-1.59
PA1259	*lhpH*	LhpH	-1.84
PA1261	*lhpR*	Transcriptional Regulator, LhpR	-3.47
PA1262		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.01
PA1263		Hypothetical Protein	-1.71
PA1264		Probable Transcriptional Regulator	-2.61
PA1271		Probable TonB-Dependent Receptor	1.75
PA1274		Conserved Hypothetical Protein	-2.11
PA1276	*cobC*	Cobalamin Biosynthetic Protein CobC	-1.83
PA1278	*cobP*	Cobinamide Kinase	-1.63
PA1281	*cobV*	Cobalamin (5'-Phosphate) Synthase	-2.33
PA1282		Probable Major Facilitator Superfamily (Mfs) Transporter	-5.11
PA1284		Probable Acyl-CoA Dehydrogenase	-2.08
PA1288		Probable Outer Membrane Protein Precursor	2.19
PA1296		Probable 2-Hydroxyacid Dehydrogenase	-2.21
PA1300		Ecf Sigma Factor	2.13
PA1301		Probable Transmembrane Sensor	1.91
PA1310	*phnW*	2-Aminoethylphosphonate:Pyruvate Aminotransferase	-3.59
PA1311	*phnX*	2-Phosphonoacetaldehyde Hydrolase	-2.18
PA1312		Probable Transcriptional Regulator	-2.02
PA1313		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.57
PA1316		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.83
PA1318	*cyoB*	Cytochrome O Ubiquinol Oxidase Subunit I	-1.84
PA1323		Hypothetical Protein	4.48
PA1324		Hypothetical Protein	5.08
PA1328		Probable Transcriptional Regulator	-1.95
PA1330		Probable Short-Chain Dehydrogenase	-2.42
PA1347		Probable Transcriptional Regulator	-3.58

PA1351		Probable Sigma-70 Factor, Ecf Subfamily	-1.79
PA1359		Probable Transcriptional Regulator	-1.76
PA1360		Conserved Hypothetical Protein	-3.92
PA1362		Hypothetical Protein	-2.30
PA1363		Ecf Sigma Factor	1.93
PA1365		Probable Siderophore Receptor	1.61
PA1367		Hypothetical Protein	-1.72
PA1368		Hypothetical Protein	-1.65
PA1373	*fabF2*	3-Oxoacyl-Acyl Carrier Protein Synthase Ii	-2.91
PA1374		Hypothetical Protein	-1.95
PA1375	*pdxB*	Erythronate-4-Phosphate Dehydrogenase	-1.51
PA1379		Probable Short-Chain Dehydrogenase	-2.75
PA1380		Probable Transcriptional Regulator	-2.35
PA1389		Probable Glycosyl Transferase	-2.29
PA1392		Hypothetical Protein	-2.11
PA1395		Hypothetical Protein	-1.72
PA1399		Probable Transcriptional Regulator	-2.22
PA1400		Probable Pyruvate Carboxylase	-3.47
PA1402		Hypothetical Protein	-1.55
PA1403		Probable Transcriptional Regulator	-2.51
PA1404		Hypothetical Protein	1.93
PA1405		Probable Helicase	-1.8
PA1406		Hypothetical Protein	-1.94
PA1409	*aphA*	Acetylpolyamine Aminohydrolase	-1.86
PA1411		Hypothetical Protein	-2.5
PA1413		Probable Transcriptional Regulator	-2.24
PA1416		Conserved Hypothetical Protein	-3.83
PA1417		Probable Decarboxylase	-2.96
PA1419		Probable Transporter	-2.11
PA1424		Hypothetical Protein	-3.45
PA1427		Hypothetical Protein	-4.3
PA1433		Conserved Hypothetical Protein	-1.58
PA1435		Probable Resistance-Nodulation-Cell Division (RND) Efflux Membrane Fusion Protein Precursor	-3.06
PA1436		Probable Resistance-Nodulation-Cell Division (RND) Efflux Transporter	-3.06
PA1437		Probable Two-Component Response Regulator	-1.79
PA1438		Probable Two-Component Sensor	-1.66
PA1451		Conserved Hypothetical Protein	-1.60
PA1463		Hypothetical Protein	-1.51
PA1464		Probable Purine-Binding Chemotaxis Protein	-1.51
PA1467		Hypothetical Protein	-1.77
PA1469		Hypothetical Protein	-2.5

PA1479	*ccmE*	Cytochrome C-Type Biogenesis Protein CcmE	2.08
PA1485		Probable Amino Acid Permease	-1.69
PA1486	*bapF*	Beta-Peptidyl Aminopeptidase	-2.28
PA1487		Probable Carbohydrate Kinase	-2.13
PA1488		Hypothetical Protein	-2.95
PA1491		Probable Transporter	-2.05
PA1498	*pykF*	Pyruvate Kinase I	-3.15
PA1500		Probable Oxidoreductase	-3.46
PA1501		Conserved Hypothetical Protein	-3.41
PA1502	*gcl*	Glyoxylate Carboligase	-3.63
PA1504		Probable Transcriptional Regulator	1.75
PA1506		Hypothetical Protein	-2.07
PA1513		Hypothetical Protein	-3.6
PA1514		Ureidoglycolate Hydrolaseybbt	-3.42
PA1515	*alc*	Allantoicase	-3.56
PA1516		Hypothetical Protein	-4.18
PA1517		Conserved Hypothetical Protein	-3.78
PA1518		Conserved Hypothetical Protein	-3.11
PA1537		Probable Short-Chain Dehydrogenase	-1.78
PA1539		Hypothetical Protein	-2.00
PA1541		Probable Drug Efflux Transporter	-1.67
PA1545		Hypothetical Protein	-1.88
PA1546	*hemN*	Oxygen-Independent Coproporphyrinogen Iii Oxidase	2.71
PA1547		Hypothetical Protein	-2.66
PA1555	*ccoP2*	Cytochrome C Oxidase, Cbb3-Type, CcoP Subunit	3.01
PA1555.1	*ccoQ2*	Cytochrome C Oxidase, Cbb3-Type, CcoQ Subunit	2.17
PA1556	*ccoO2*	Cytochrome C Oxidase, Cbb3-Type, CcoO Subunit	3.02
PA1557	*ccoN2*	Cytochrome C Oxidase, Cbb3-Type, CcoN Subunit	2.45
PA1558		Hypothetical Protein	-2.06
PA1567		Conserved Hypothetical Protein	-2.69
PA1569		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.79
PA1572		Conserved Hypothetical Protein	-1.93
PA1573		Conserved Hypothetical Protein	-1.71
PA1577		Hypothetical Protein	-1.86
PA1578		Hypothetical Protein	-2.05
PA1595		Hypothetical Protein	-2.01
PA1596	*htpG*	Heat Shock Protein HtpG	2.25
PA1598		Conserved Hypothetical Protein	-3.25
PA1599		Probable Transcriptional Regulator	-1.8
PA1601		Probable Aldehyde Dehydrogenase	-1.91
PA1602		Probable Oxidoreductase	-1.65
PA1603		Probable Transcriptional Regulator	-1.59
PA1604		Hypothetical Protein	-1.65

PA1615		Probable Lipase	-1.53
PA1619		Probable Transcriptional Regulator	-1.63
PA1621		Probable Hydrolase	-1.97
PA1622		Probable Hydrolase	-2.13
PA1626		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.36
PA1627		Probable Transcriptional Regulator	-1.83
PA1628		Probable 3-Hydroxyacyl-Coa Dehydrogenase	-2.84
PA1629		Probable Enoyl-CoA Hydratase/Isomerase	-3.02
PA1630		Probable Transcriptional Regulator	-1.50
PA1633	*kdpA*	Potassium-Transporting ATPase, A Chain	-3.41
PA1634	*kdpB*	Potassium-Transporting ATPase, B Chain	-4.49
PA1645		Hypothetical Protein	-2.27
PA1656	*hsiA2*	HsiA2	3.3
PA1657	*hsiB2*	HsiB2	4.5
PA1658	*hsiC2*	HsiC2	4.33
PA1659	*hsiF2*	HsiF2	2.57
PA1660	*hsiG2*	HsiG2	1.74
PA1661	*hsiH2*	HsiH2	2.52
PA1664	*orfX*	OrfX	1.66
PA1667	*hsiJ2*	HsiJ2	1.51
PA1668	*dotU2*	DotU2	1.79
PA1673		Hypothetical Protein	1.56
PA1676		Hypothetical Protein	-1.70
PA1680		Hypothetical Protein	-3.91
PA1686	*alkA*	Dna-3-Methyladenine Glycosidase II	-1.52
PA1688		Hypothetical Protein	1.74
PA1690	*pscU*	Translocation Protein in Type III Secretion	-3.18
PA1693	*pscR*	Translocation Protein in Type III Secretion	-2.61
PA1695	*pscP*	Translocation Protein in Type III Secretion	-4.35
PA1697		Atp Synthase in Type III Secretion System	-2.75
PA1698	*popN*	Type Iii Secretion Outer Membrane Protein PopN Precursor	-3.16
PA1703	*pcrD*	Type Iii Secretory Apparatus Protein PcrD	-2.14
PA1705	*pcrG*	Regulator in Type III Secretion	-4.32
PA1706	*pcrV*	Type Iii Secretion Protein PcrV	-2.85
PA1709	*popD*	Translocator Outer Membrane Protein PopD Precursor	-3.06
PA1712	*exsB*	Exoenzyme S Synthesis Protein B	-3.10
PA1715	*pscB*	Type Iii Export Apparatus Protein	-2.9
PA1716	*pscC*	Type Iii Secretion Outer Membrane Protein PscC Precursor	-2.39
PA1717	*pscD*	Type Iii Export Protein PscD	-2.64
PA1722	*pscI*	Type Iii Export Protein PscI	-2.45
PA1723	*pscJ*	Type Iii Export Protein PscJ	-1.56
PA1735		Hypothetical Protein	-1.67
PA1736		Probable Acyl-CoA Thiolase	-1.66

PA1737		Probable 3-Hydroxyacyl-Coa Dehydrogenase	-1.55
PA1738		Probable Transcriptional Regulator	-1.60
PA1740		Hypothetical Protein	-3.62
PA1746		Hypothetical Protein	1.69
PA1750		Phospho-2-Dehydro-3-Deoxyheptonate Aldolase	1.61
PA1755		Hypothetical Protein	-2.29
PA1761		Hypothetical Protein	-1.76
PA1764		Hypothetical Protein	-1.77
PA1772		Probable Methyltransferase	-2.09
PA1776	*sigX*	Ecf Sigma Factor SigX	1.64
PA1778	*cobA*	Uroporphyrin-Iii C-Methyltransferase	-3.56
PA1779		Assimilatory Nitrate Reductase	-2.8
PA1781	*nirB*	Assimilatory Nitrite Reductase Large Subunit	-2.81
PA1786	*nasS*	Nass	-1.58
PA1791		Hypothetical Protein	2.41
PA1800	*tig*	Trigger Factor	2.20
PA1804	*hupB*	DNA-Binding Protein Hu	1.60
PA1812	*mltD*	Membrane-Bound Lytic Murein Transglycosylase D Precursor	1.76
PA1817		Hypothetical Protein	-1.81
PA1825		Hypothetical Protein	-1.81
PA1826		Probable Transcriptional Regulator	-2.39
PA1827		Probable Short-Chain Dehydrogenase	-3.00
PA1842		Hypothetical Protein	2.01
PA1843	*metH*	Methionine Synthase	1.77
PA1845	*tsi1*	Tsi1	1.53
PA1847	*nfuA*	Nfua	-1.64
PA1848		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.98
PA1850		Probable Transcriptional Regulator	-1.70
PA1852		Hypothetical Protein	-1.60
PA1863	*modA*	Molybdate-Binding Periplasmic Protein Precursor ModA	1.72
PA1866		Hypothetical Protein	-1.90
PA1868	*xqhA*	Secretion Protein XqhA	-3.75
PA1869		Probable Acyl Carrier Protein	5.34
PA1870		Hypothetical Protein	2.40
PA1871	*lasA*	Lasa Protease Precursor	4.48
PA1874		Hypothetical Protein	4.10
PA1876		Probable ATP-Binding/Permease Fusion ABC Transporter	2.05
PA1879		Hypothetical Protein	-2.25
PA1884		Probable Transcriptional Regulator	-1.99
PA1885		Conserved Hypothetical Protein	-1.79
PA1899	*phzA2*	Probable Phenazine Biosynthesis Protein	4.48
PA1900	*phzB2*	Probable Phenazine Biosynthesis Protein	4.69
PA1907		Hypothetical Protein	-2.25

PA1908		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.22
PA1912	*femI*	Ecf Sigma Factor, Femi	3.66
PA1913		Hypothetical Protein	2.25
PA1916		Probable Amino Acid Permease	-4.23
PA1919	*nrdG*	Class Iii (Anaerobic) Ribonucleoside-Triphosphate Reductase Activating Protein, 'Activase', Nrdg	-1.63
PA1928	*rimJ*	Ribosomal Protein Alanine Acetyltransferase	-1.95
PA1929		Hypothetical Protein	-2.05
PA1936		Hypothetical Protein	-2.91
PA1947	*rbsA*	Ribose Transport Protein RbsA	-1.56
PA1950	*rbsK*	Ribokinase	-2.19
PA1954	*fapC*	FapC	-2.12
PA1957		Hypothetical Protein	-1.68
PA1962	*azoR2*	Fmn-Dependent Nadh-Azoreductase 2, Azor2	-2.37
PA1967		Hypothetical Protein	1.72
PA1972		Conserved Hypothetical Protein	-2.35
PA1973	*pqqF*	Pyrroloquinoline Quinone Biosynthesis Protein F	-2.72
PA1974		Hypothetical Protein	-2.59
PA1975		Hypothetical Protein	-2.34
PA1976	*ercS*	ErcS	-2.75
PA1978	*erbR*	Response Regulator ErbR	-1.81
PA1980	*eraR*	Response Regulator EraR	-2.29
PA1982	*exaA*	Quinoprotein Ethanol Dehydrogenase	-1.91
PA1990	*pqqH*	PqqH	-2.55
PA1992	*ercS*	ErcS	-2.31
PA1993		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.07
PA1999	*dchA*	Dehydrocarnitine Coa Transferase, DchA	3.51
PA2000	*dchB*	Dehydrocarnitine Coa Transferase, DchB	3.02
PA2007	*maiA*	Maleylacetoacetate Isomerase	1.77
PA2009	*hmgA*	Homogentisate 1,2-Dioxygenase	2.68
PA2015	*liuA*	Putative Isovaleryl-Coa Dehydrogenase	2.72
PA2016	*liuR*	Regulator Of Liu Genes	2.42
PA2022		Probable Nucleotide Sugar Dehydrogenase	-2.36
PA2023	*galU*	Utp-Glucose-1-Phosphate Uridylyltransferase	2.73
PA2024		Probable Ring-Cleaving Dioxygenase	-1.68
PA2032		Probable Transcriptional Regulator	-2.02
PA2033		Hypothetical Protein	2.86
PA2035		Probable Decarboxylase	-2.34
PA2036		Hypothetical Protein	-4.71
PA2037		Hypothetical Protein	-1.95
PA2042		Probable Transporter (Membrane Subunit)	2.75
PA2046		Hypothetical Protein	-2.73
PA2048		Hypothetical Protein	-1.64

PA2054	*cynR*	Transcriptional Regulator CynR	-2.42
PA2056		Probable Transcriptional Regulator	-2.33
PA2057	*sppR*	Tonb-Dependent Receptor, SppR	-4.15
PA2058	*sppA*	Abc Transporter Substrate-Binding Protein, SppA	-2.99
PA2061	*sppD*	Abc Transporter Atp-Binding Protein, SppD	-4.10
PA2064	*pcoB*	Copper Resistance Protein B Precursor	-2.95
PA2065	*pcoA*	Copper Resistance Protein A Precursor	-1.72
PA2066		Hypothetical Protein	2.76
PA2069		Probable Carbamoyl Transferase	3.53
PA2070		Hypothetical Protein	-3.43
PA2071	*fusA2*	Elongation Factor G	1.54
PA2078		(7S,10S)-Hydroperoxide Diol Synthase	-5.34
PA2079		Probable Amino Acid Permease	-2.19
PA2080	*kynU*	Kynureninase KynU	-2.12
PA2084		Probable Asparagine Synthetase	-2.60
PA2093		Probable Sigma-70 Factor, Ecf Subfamily	-2.40
PA2095		Hypothetical Protein	-2.10
PA2097		Probable Flavin-Binding Monooxygenase	-2.02
PA2100		Probable Transcriptional Regulator	-2.83
PA2101		Conserved Hypothetical Protein	-2.24
PA2102		Hypothetical Protein	-2.39
PA2107		Hypothetical Protein	-2.06
PA2115		Probable Transcriptional Regulator	-1.52
PA2120		Hypothetical Protein	-2.43
PA2121		Probable Transcriptional Regulator	-1.65
PA2122		Hypothetical Protein	-1.57
PA2124		Probable Dehydrogenase	-3.79
PA2125		Probable Aldehyde Dehydrogenase	-5.48
PA2126	*cgrC*	CupA Gene Regulator C, CgrC	-1.84
PA2129	*cupA2*	Chaperone Cupa2	-4.51
PA2130	*cupA3*	Usher Cupa3	-4.35
PA2134		Hypothetical Protein	2.53
PA2135		Probable Transporter	-2.33
PA2136		Hypothetical Protein	-1.83
PA2143		Hypothetical Protein	2.03
PA2144	*glgP*	Glycogen Phosphorylase	2.58
PA2146		Conserved Hypothetical Protein	5.41
PA2149		Hypothetical Protein	1.51
PA2150		Conserved Hypothetical Protein	1.58
PA2151		Conserved Hypothetical Protein	2.01
PA2152		Probable Trehalose Synthase	1.60
PA2159		Conserved Hypothetical Protein	3.30
PA2160		Probable Glycosyl Hydrolase	2.89

PA2164		Probable Glycosyl Hydrolase	1.60
PA2165		Probable Glycogen Synthase	2.71
PA2166		Hypothetical Protein	3.34
PA2169		Hypothetical Protein	4.51
PA2170		Hypothetical Protein	3.03
PA2171		Hypothetical Protein	4.19
PA2172		Hypothetical Protein	1.93
PA2189		Hypothetical Protein	1.91
PA2193	*hcnA*	Hydrogen Cyanide Synthase HcnA	2.14
PA2195	*hcnC*	Hydrogen Cyanide Synthase HcnC	1.56
PA2206		Lysr-Type Transcriptional Regulator	-1.65
PA2207		Hypothetical Protein	-2.66
PA2209		Hypothetical Protein	-2.03
PA2210		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.49
PA2211		Conserved Hypothetical Protein	-2.24
PA2212		Conserved Hypothetical Protein	-2.86
PA2214		Putative L-Lyxonate Transporter	-5.61
PA2215	*lyxD*	L-Lyxonate Dehydratase LyxD	-2.48
PA2216		2-Keto-3-Deoxy-D-Arabinonate Dehydratase	-3.30
PA2219	*opdE*	Membrane Protein OpdE	-3.50
PA2220		Probable Transcriptional Regulator	-1.89
PA2227	*vqsM*	Arac-Type Transcriptional Regulator VqsM	-1.82
PA2228		Hypothetical Protein	-2.83
PA2241	*pslK*	PslK	-2.24
PA2243	*pslM*	Hypothetical Protein	-4.14
PA2247	*bkdA1*	2-Oxoisovalerate Dehydrogenase (Alpha Subunit)	-1.68
PA2248	*bkdA2*	2-Oxoisovalerate Dehydrogenase (Beta Subunit)	-1.57
PA2249	*bkdB*	Branched-Chain Alpha-Keto Acid Dehydrogenase (Lipoamide Component)	-1.72
PA2250	*lpdV*	Lipoamide Dehydrogenase-Val	-1.51
PA2251		Hypothetical Protein	-1.55
PA2252		Probable AgcS Sodium/Alanine/Glycine Symporter	-2.23
PA2261		Probable 2-Ketogluconate Kinase	-3.01
PA2262		Probable 2-Ketogluconate Transporter	-1.70
PA2263		Probable 2-Hydroxyacid Dehydrogenase	-2.87
PA2267		Probable Transcriptional Regulator	-1.90
PA2268		Hypothetical Protein	-1.96
PA2269		Conserved Hypothetical Protein	-2.71
PA2271		Probable Acetyltransferase	-1.62
PA2273	*soxR*	SoxR	-1.56
PA2274		Hypothetical Protein	-1.83
PA2275		Probable Alcohol Dehydrogenase (Zn-Dependent)	-2.79
PA2277	*arsR*	ArsR Protein	-1.59

PA2278	*arsB*	ArsB Protein	-3.86
PA2279	*arsC*	ArsC Protein	-1.62
PA2280		Oxidoreductase	-2.38
PA2282		Hypothetical Protein	-1.95
PA2283		Hypothetical Protein	-3.35
PA2285		Hypothetical Protein	-2.34
PA2290	*gcd*	Glucose Dehydrogenase	1.96
PA2291		Probable Glucose-Sensitive Porin	4.16
PA2295		Probable Permease of ABC Transporter	-1.77
PA2296		Hypothetical Protein	-3.26
PA2300	*chiC*	Chitinase	4.91
PA2310		Hypothetical Protein	-2.61
PA2312		Probable Transcriptional Regulator	-2.33
PA2313		Hypothetical Protein	-2.93
PA2315		Hypothetical Protein	-6.15
PA2316		Probable Transcriptional Regulator	-1.99
PA2323		Probable Glyceraldehyde-3-Phosphate Dehydrogenase	-1.60
PA2326		Hypothetical Protein	-2.01
PA2327		Probable Permease of ABC Transporter	-1.74
PA2328		Hypothetical Protein	-1.60
PA2330		Hypothetical Protein	-1.85
PA2332		Probable Transcriptional Regulator	-1.67
PA2333		Probable Sulfatase	-4.15
PA2335		Probable TonB-Dependent Receptor	-4.82
PA2336		Hypothetical Protein	-4.69
PA2337	*mtlR*	Transcriptional Regulator MtlR	-1.99
PA2338		Probable Binding Protein Component of ABC Maltose/Mannitol Transporter	-1.75
PA2339		Probable Binding-Protein-Dependent Maltose/Mannitol Transport Protein	-3.95
PA2340		Probable Binding-Protein-Dependent Maltose/Mannitol Transport Protein	-2.73
PA2341		Probable ATP-Binding Component of ABC Maltose/Mannitol Transporter	-3.04
PA2342	*mtlD*	Mannitol Dehydrogenase	-4.14
PA2344	*mtlZ*	Fructokinase	-3.42
PA2346		Conserved Hypothetical Protein	-5.17
PA2354		Probable Transcriptional Regulator	-1.9
PA2355		Probable Fmnh2-Dependent Monooxygenase	-3.48
PA2365	*hsiB3*	HsiB3	3.76
PA2366	*hsiC3*	HsiC3	4.52
PA2367	*hcp3*	Hcp3	4.01
PA2369	*hsiG3*	HsiG3	1.57
PA2371	*clpV3*	ClpV3	1.73

PA2372		Hypothetical Protein	2.33
PA2373	*vgrG3*	VgrG3	1.80
PA2377		Hypothetical Protein	-2.12
PA2384		Hypothetical Protein	3.89
PA2385	*pvdQ*	3-Oxo-C12-Homoserine Lactone Acylase PvdQ	1.96
PA2386	*pvdA*	L-Ornithine N5-Oxygenase	4.97
PA2389	*pvdR*	PvdR	1.73
PA2392	*pvdP*	PvdP	1.91
PA2393		Putative Dipeptidase	3.64
PA2394	*pvdN*	PvdN	3.73
PA2395	*pvdO*	PvdO	3.34
PA2396	*pvdF*	Pyoverdine Synthetase F	2.35
PA2397	*pvdE*	Pyoverdine Biosynthesis Protein PvdE	2.97
PA2398	*fpvA*	Ferripyoverdine Receptor	4.61
PA2399	*pvdD*	Pyoverdine Synthetase D	2.00
PA2400	*pvdJ*	PvdJ	4.57
PA2402		Probable Non-Ribosomal Peptide Synthetase	1.55
PA2403	*fpvG*	FpvG	3.49
PA2404	*fpvH*	FpvH	2.48
PA2405	*fpvJ*	FpvJ	3.44
PA2411		Probable Thioesterase	2.99
PA2412		Conserved Hypothetical Protein	4.07
PA2413	*pvdH*	L-2,4-Diaminobutyrate:2-Ketoglutarate 4-Aminotransferase, PvdH	2.19
PA2420		Probable Porin	-3.37
PA2422		Hypothetical Protein	-3.11
PA2425	*pvdG*	PvdG	3.2
PA2426	*pvdS*	Sigma Factor PvdS	5.35
PA2427		Hypothetical Protein	2.08
PA2432	*bexR*	Bistable Expression Regulator, BexR	-1.6
PA2433		Hypothetical Protein	2.06
PA2435		Probable Cation-Transporting P-Type ATPase	-1.59
PA2440		Hypothetical Protein	-2.72
PA2442	*gcvT2*	Glycine Cleavage System Protein T2	2.38
PA2443	*sdaA*	L-Serine Dehydratase	2.16
PA2444	*glyA2*	Serine Hydroxymethyltransferase	3.68
PA2445	*gcvP2*	Glycine Cleavage System Protein P2	4.10
PA2446	*gcvH2*	Glycine Cleavage System Protein H2	4.64
PA2447		Probable Transcriptional Regulator	-1.75
PA2459		Hypothetical Protein	-4.69
PA2460		Hypothetical Protein	-1.90
PA2461		Hypothetical Protein	-2.33
PA2462		Hypothetical Protein	-1.66

PA2465		Hypothetical Protein	-2.69
PA2468	*foxI*	Ecf Sigma Factor FoxI	1.84
PA2469		Probable Transcriptional Regulator	-2.28
PA2471		Conserved Hypothetical Protein	-2.37
PA2477		Probable Thiol:Disulfide Interchange Protein	-2.54
PA2478		Probable Thiol:Disulfide Interchange Protein	-2.25
PA2479		Probable Two-Component Response Regulator	-2.45
PA2480		Probable Two-Component Sensor	-2.28
PA2481		Hypothetical Protein	-1.78
PA2482		Probable Cytochrome C	-1.81
PA2483		Conserved Hypothetical Protein	-2.75
PA2487		Hypothetical Protein	-3.53
PA2488		Probable Transcriptional Regulator	-2.4
PA2489		Probable Transcriptional Regulator	-1.68
PA2490		Conserved Hypothetical Protein	-2.80
PA2493	*mexE*	Resistance-Nodulation-Cell Division (RND) Multidrug Efflux Membrane Fusion Protein MexE Precursor	-1.58
PA2495	*oprN*	Multidrug Efflux Outer Membrane Protein OprN Precursor	-1.84
PA2497		Probable Transcriptional Regulator	-2.83
PA2499		Probable Deaminase	-3.45
PA2500		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.61
PA2502		Hypothetical Protein	-1.61
PA2505	*opdT*	Tyrosine Porin OpdT	-2.84
PA2509	*catB*	Muconate Cycloisomerase I	-4.25
PA2510	*catR*	Transcriptional Regulator CatR	-2.80
PA2515	*xylL*	Cis-1,2-Dihydroxycyclohexa-3,4-Diene Carboxylate Dehydrogenase	-5.46
PA2518	*xylX*	Toluate 1,2-Dioxygenase Alpha Subunit	-4.51
PA2519	*xylS*	Transcriptional Regulator XylS	-3.23
PA2520	*czcA*	RND Divalent Metal Cation Efflux Transporter CzcA	-2.89
PA2521	*czcB*	RND Divalent Metal Cation Efflux Membrane Fusion Protein CzcB	-4.92
PA2524	*czcS*	CzcS	-2.42
PA2534		Probable Transcriptional Regulator	-2.12
PA2536		Probable Phosphatidate Cytidylyltransferase	2.21
PA2537		Probable Acyltransferase	1.68
PA2545	*xthA*	Exodeoxyribonuclease III	1.63
PA2546		Probable Ring-Cleaving Dioxygenase	-1.89
PA2548		Hypothetical Protein	-3.43
PA2552		Probable Acyl-CoA Dehydrogenase	1.65
PA2555		Probable Amp-Binding Enzyme	1.86
PA2563		Probable Sulfate Transporter	-1.89
PA2564		Hypothetical Protein	1.53

PA2565		Hypothetical Protein	2.59
PA2566		Conserved Hypothetical Protein	2.79
PA2569		Hypothetical Protein	2.07
PA2570	*lecA*	LecA	5.79
PA2574	*alkB1*	Alkane-1-Monooxygenase	-2.63
PA2576		Hypothetical Protein	-2.15
PA2589		Hypothetical Protein	-4.18
PA2592		Probable Periplasmic Spermidine/Putrescine-Binding Protein	2.28
PA2594		Conserved Hypothetical Protein	-1.64
PA2596		Conserved Hypothetical Protein	-3.70
PA2597		Hypothetical Protein	-3.49
PA2598		Hypothetical Protein	-2.82
PA2599		Conserved Hypothetical Protein	-1.53
PA2600		Hypothetical Protein	-1.78
PA2619	*infA*	Initiation Factor	1.97
PA2629	*purB*	Adenylosuccinate Lyase	1.85
PA2634	*aceA*	Isocitrate Lyase AceA	-1.75
PA2635		Hypothetical Protein	-1.61
PA2650		Conserved Hypothetical Protein	-2.24
PA2655		Hypothetical Protein	-2.84
PA2656	*carS*	Two-Component Sensor CarS	-2.3
PA2657	*carR*	Two-Component Response Regulator CarR	-2.48
PA2658		Hypothetical Protein	-3.22
PA2659		Hypothetical Protein	-3.01
PA2660		Hypothetical Protein	-1.71
PA2662		Conserved Hypothetical Protein	-2.86
PA2663	*ppyR*	Psl And Pyoverdine Operon Regulator, PpyR	-2.08
PA2664	*fhp*	Flavohemoprotein	-1.61
PA2665	*fhpR*	Transcriptional Activator of Flavohemoglobin, FhpR	-2.17
PA2669		Hypothetical Protein	-3.94
PA2670		Hypothetical Protein	-2.28
PA2675		Probable Type Ii Secretion System Protein	-2.24
PA2676		Probable Type Ii Secretion System Protein	-2.55
PA2677		Probable Type Ii Secretion Protein	-1.79
PA2678		Probable Permease of Abc-2 Transporter	-2.99
PA2680		Probable Quinone Oxidoreductase	-3.29
PA2681		Probable Transcriptional Regulator	-3.11
PA2685	*vgrG4*	VgrG4	1.59
PA2689		Hypothetical Protein	-2.39
PA2691		Conserved Hypothetical Protein	-1.98
PA2693		Conserved Hypothetical Protein	-2.18
PA2701		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.91
PA2711		Probable Periplasmic Spermidine/Putrescine-Binding Protein	-1.88

PA2712		Hypothetical Protein	-1.80
PA2719		Hypothetical Protein	-1.63
PA2723		Hypothetical Protein	-1.84
PA2724		Hypothetical Protein	-2.05
PA2750		Hypothetical Protein	-2.50
PA2758		Probable Transcriptional Regulator	-2.94
PA2760	*oprQ*	OprQ	1.71
PA2761		Hypothetical Protein	2.78
PA2762		Hypothetical Protein	-2.33
PA2764		Hypothetical Protein	-1.69
PA2772		Hypothetical Protein	-1.51
PA2775	*tsi4*	Tsi4	1.58
PA2782	*bamI*	Biofilm-Associated Metzincin Inhibitor, BamI	-1.65
PA2783	*mep72*	Mep72	-3.11
PA2791		Hypothetical Protein	-2.37
PA2792		Hypothetical Protein	1.63
PA2794		Pseudaminidase	-1.52
PA2798		Probable Two-Component Response Regulator	1.51
PA2800	*vacJ*	VacJ	1.52
PA2808	*ptrA*	Pseudomonas Type Iii Repressor A	-1.87
PA2809	*copR*	Two-Component Response Regulator, CopR	-1.60
PA2810	*copS*	Two-Component Sensor, CopS	-1.76
PA2814		Hypothetical Protein	-2.36
PA2816		Hypothetical Protein	-1.72
PA2818	*arr*	Aminoglycoside Response Regulator	-2.28
PA2819		Hypothetical Protein	-2.84
PA2827		Conserved Hypothetical Protein	-1.78
PA2833		Conserved Hypothetical Protein	-2.91
PA2835		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.13
PA2839		Conserved Hypothetical Protein	-2.63
PA2844		Conserved Hypothetical Protein	-2.38
PA2848		Probable Transcriptional Regulator	-2.65
PA2851	*efp*	Translation Elongation Factor P	2.77
PA2852	*earP*	EarP	-1.67
PA2853	*oprI*	Outer Membrane Lipoprotein OprI Precursor	1.68
PA2858		Conserved Hypothetical Protein	-1.79
PA2861	*ligT*	2'-5' RNA Ligase	-2.73
PA2874		Hypothetical Protein	-1.63
PA2877		Probable Transcriptional Regulator	-1.75
PA2878		Hypothetical Protein	-3.12
PA2879		Probable Transcriptional Regulator	-1.74
PA2880		Hypothetical Protein	-2.25
PA2890	*atuE*	Putative Isohexenylglutaconyl-CoA Hydratase	-3.60

PA2891	*atuF*	Geranyl-CoA Carboxylase, Alpha-Subunit (Biotin-Containing)	-2.38
PA2892	*atuG*	Gcase, Alpha-Subunit (Biotin-Containing)	-2.31
PA2898		Hypothetical Protein	-2.4
PA2903	*cobJ*	Precorrin-3 Methylase CobJ	-1.76
PA2907	*cobL*	Precorrin-6Y-Dependent Methyltransferase CobL	-1.84
PA2909		Hypothetical Protein	-2.61
PA2919		Hypothetical Protein	-1.57
PA2922		Probable Hydrolase	-5.75
PA2923	*hisJ*	Periplasmic Histidine-Binding Protein HisJ	-3.39
PA2926	*hisP*	Histidine Transport Protein HisP	-3.16
PA2928		Hypothetical Protein	-2.43
PA2929		Hypothetical Protein	-2.88
PA2930		Probable Transcriptional Regulator	-1.95
PA2931	*cifR*	CifR	-1.90
PA2935		Hypothetical Protein	-2.62
PA2940		Probable Acyl-Coa Thiolase	-2.52
PA2941		Hypothetical Protein	-2.62
PA2950	*pfm*	Proton Motive Force Protein, Pmf	1.84
PA2969	*plsX*	Fatty Acid Biosynthesis Protein PlsX	2.14
PA2970	*rpmF*	50S Ribosomal Protein L32	3.5
PA2971		Conserved Hypothetical Protein	3.37
PA2982		Conserved Hypothetical Protein	1.93
PA2984		Hypothetical Protein	-3.03
PA2999	*nqrA*	Na+-Translocating NADH:Ubiquinone Oxidoreductase Subunit NrqA	1.67
PA3016		Hypothetical Protein	-1.73
PA3017		Conserved Hypothetical Protein	-1.92
PA3025		Probable FAD-Dependent Glycerol-3-Phosphate Dehydrogenase	-2.54
PA3039		Probable Transporter	-2.74
PA3041		Hypothetical Protein	1.81
PA3042		Hypothetical Protein	2.13
PA3045	*rocA2*	Two-Component Response Regulator, RocA2	-1.87
PA3047		Probable D-Alanyl-D-Alanine Carboxypeptidase	1.55
PA3051		Hypothetical Protein	-1.62
PA3058	*pelG*	PelG	-2.24
PA3059	*pelF*	PelF	-2.50
PA3060	*pelE*	PelE	-3.81
PA3061	*pelD*	PelD	-2.92
PA3063	*pelB*	PelB	-4.29
PA3064	*pelA*	PelA	-3.84
PA3065		Hypothetical Protein	-7.81
PA3066		Hypothetical Protein	-8.49

PA3067		Probable Transcriptional Regulator	-7.39
PA3068	*gdhB*	NAD-Dependent Glutamate Dehydrogenase	1.66
PA3090		Hypothetical Protein	-2.64
PA3092	*fadH1*	2,4-Dienoyl-Coa Reductase FadH1	-1.63
PA3101	*xcpT*	General Secretion Pathway Protein G	1.67
PA3105	*xcpQ*	General Secretion Pathway Protein D	1.64
PA3112	*accD*	Acetyl-Coa Carboxylase Beta Subunit	1.66
PA3125		Hypothetical Protein	-3.15
PA3132		Probable Hydrolase	-3.18
PA3134	*gltX*	Glutamyl-Trna Synthetase	1.55
PA3136		Probable Secretion Protein	-1.50
PA3151	*hisF2*	Imidazoleglycerol-Phosphate Synthase, Cyclase Subunit	-1.62
PA3152	*hisH2*	Glutamine Amidotransferase	-2.01
PA3153	*wzx*	O-Antigen Translocase	-1.68
PA3161	*himD*	Integration Host Factor Beta Subunit	-1.62
PA3162	*rpsA*	30S Ribosomal Protein S1	2.61
PA3166	*pheA*	Chorismate Mutase	1.82
PA3167	*serC*	3-Phosphoserine Aminotransferase	1.76
PA3168	*gyrA*	Dna Gyrase Subunit A	1.74
PA3186	*oprB*	Glucose/Carbohydrate Outer Membrane Porin Oprb Precursor	6.52
PA3187		Probable ATP-Binding Component of ABC Transporter	5.95
PA3188		Probable Permease of ABC Sugar Transporter	6.16
PA3189		Probable Permease of ABC Sugar Transporter	5.62
PA3190		Probable Binding Protein Component of ABC Sugar Transporter	7.12
PA3207		Hypothetical Protein	-2.71
PA3215		Probable Transcriptional Regulator	-1.72
PA3216		Hypothetical Protein	-1.72
PA3219		Hypothetical Protein	-2.55
PA3231		Hypothetical Protein	1.84
PA3236	*betX*	BetX	-2.59
PA3237		Hypothetical Protein	-3.35
PA3241		Hypothetical Protein	-1.58
PA3262		Probable Peptidyl-Prolyl Cis-Trans Isomerase, FkbP-Type	2.74
PA3274		Hypothetical Protein	2.63
PA3276		Hypothetical Protein	1.75
PA3279	*oprP*	Phosphate-Specific Outer Membrane Porin OprP Precursor	-2.62
PA3287		Conserved Hypothetical Protein	-1.58
PA3291	*tli1*	Tli1	-2.18
PA3296	*phoA*	Alkaline Phosphatase	-2.71
PA3306		Hypothetical Protein	-2.62
PA3307		Hypothetical Protein	-4.54
PA3309		Conserved Hypothetical Protein	2.66

PA3312		Probable 3-Hydroxyisobutyrate Dehydrogenase	-1.69
PA3316		Probable Permease of ABC Transporter	-1.82
PA3320		Hypothetical Protein	-5.21
PA3321		Probable Transcriptional Regulator	-3.01
PA3324		Probable Short-Chain Dehydrogenase	-2.64
PA3325		Conserved Hypothetical Protein	-2.12
PA3326	*clpP2*	ClpP2	3.11
PA3358		Hypothetical Protein	-2.89
PA3362		Hypothetical Protein	-6.45
PA3363	*amiR*	Aliphatic Amidase Regulator	-6.53
PA3364	*amiC*	Aliphatic Amidase Expression-Regulating Protein	-6.28
PA3365		Probable Chaperone	-6.37
PA3366	*amiE*	Aliphatic Amidase	-6.72
PA3369		Hypothetical Protein	1.65
PA3371		Hypothetical Protein	2.47
PA3372		Conserved Hypothetical Protein	-1.72
PA3374		Conserved Hypothetical Protein	-2.91
PA3378		Conserved Hypothetical Protein	-3.75
PA3383		Binding Protein Component of ABC Phosphonate Transporter	-1.58
PA3384	*phnC*	Atp-Binding Component of ABC Phosphonate Transporter	-2.64
PA3385	*amrZ*	Alginate and Motility Regulator Z	1.68
PA3387	*rhlG*	Beta-Ketoacyl Reductase	-2.02
PA3389		Probable Ring-Cleaving Dioxygenase	-1.96
PA3397	*fprA*	FprA	3.37
PA3406	*hasD*	Transport Protein HasD	-1.67
PA3407	*hasAp*	Heme Acquisition Protein HasAp	5.96
PA3408	*hasR*	Heme Uptake Outer Membrane Receptor Hasr Precursor	1.51
PA3420		Probable Transcriptional Regulator	-3.58
PA3421		Conserved Hypothetical Protein	-2.29
PA3422		Hypothetical Protein	-2.49
PA3424		Hypothetical Protein	-2.88
PA3425		Hypothetical Protein	-3.54
PA3428		Hypothetical Protein	-1.60
PA3429		Probable Epoxide Hydrolase	-1.63
PA3433		Probable Transcriptional Regulator	-1.62
PA3436		Hypothetical Protein	-3.41
PA3444		Conserved Hypothetical Protein	-2.77
PA3456		Hypothetical Protein	-1.89
PA3457		Hypothetical Protein	-2.18
PA3459		Probable Glutamine Amidotransferase	2.19
PA3464		Hypothetical Protein	-2.47
PA3476	*rhlI*	Autoinducer Synthesis Protein RhlI	3.03
PA3477	*rhlR*	Transcriptional Regulator RhlR	2.41

PA3478	*rhlB*	Rhamnosyltransferase Chain B	3.1
PA3479	*rhlA*	Rhamnosyltransferase Chain A	4.48
PA3486	*vgrG4b*	Vgrg-4B	2.05
PA3487	*tle5*	Tle5	1.59
PA3492		Conserved Hypothetical Protein	-1.79
PA3499		Hypothetical Protein	-3.14
PA3504		Probable Aldehyde Dehydrogenase	-3.02
PA3505		Hypothetical Protein	-3.51
PA3507		Probable Short-Chain Dehydrogenase	-2.74
PA3508		Probable Transcriptional Regulator	-1.65
PA3509		Probable Hydrolase	-4.04
PA3511		Probable Short-Chain Dehydrogenase	-3.96
PA3512		Probable Permease of Abc Transporter	-1.72
PA3513		Hypothetical Protein	-2.1
PA3516		Probable Lyase	-1.97
PA3517		Probable Lyase	-1.58
PA3521	*opmE*	Opme	-4.1
PA3522	*mexQ*	Mexq	-2.78
PA3523	*mexP*	Mexp	-2.75
PA3530	*bfd*	Bacterioferritin-Associated Ferredoxin Bfd	3.39
PA3531	*bfrB*	Bacterioferritin	-3.36
PA3534		Probable Oxidoreductase	-1.89
PA3540	*algD*	Gdp-Mannose 6-Dehydrogenase Algd	-2.05
PA3541	*alg8*	Alginate Biosynthesis Protein Alg8	-2.84
PA3542	*alg44*	Alginate Biosynthesis Protein Alg44	-3.24
PA3543	*algK*	Alginate Biosynthetic Protein AlgK Precursor	-1.78
PA3544	*algE*	Alginate Production Outer Membrane Protein AlgE Precursor	-3.81
PA3545	*algG*	Alginate-C5-Mannuronan-Epimerase AlgG	-4.16
PA3548	*algI*	Alginate O-Acetyltransferase AlgI	-3.39
PA3550	*algF*	Alginate O-Acetyltransferase AlgF	-2.44
PA3551	*algA*	Phosphomannose Isomerase	-1.67
PA3552	*arnB*	ArnB	-3.66
PA3554	*arnA*	ArnA	-3.64
PA3559		Probable Nucleotide Sugar Dehydrogenase	-2.84
PA3560	*fruA*	Phosphotransferase System Transporter Fructose-Specific	-3.59
PA3561	*fruK*	1-Phosphofructokinase	-2.81
PA3562	*fruI*	Phosphotransferase System Transporter Enzyme I, Frui	-2.61
PA3564		Conserved Hypothetical Protein	-2.23
PA3565		Probable Transcriptional Regulator	-2.11
PA3569	*mmsB*	3-Hydroxyisobutyrate Dehydrogenase	1.85
PA3570	*mmsA*	Methylmalonate-Semialdehyde Dehydrogenase	3.50
PA3573		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.63
PA3584	*glpD*	Glycerol-3-Phosphate Dehydrogenase	-1.65

PA3586		Probable Hydrolase	-3.04
PA3590		Probable Hydroxyacyl-Coa Dehydrogenase	-3.24
PA3595		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.31
PA3596		Probable Methylated-DNA-Protein-Cysteine Methyltransferase	-2.12
PA3605		Hypothetical Protein	-1.65
PA3607	*potA*	Polyamine Transport Protein PotA	-2.05
PA3608	*potB*	Polyamine Transport Protein PotB	-1.63
PA3611		Hypothetical Protein	1.77
PA3615		Hypothetical Protein	-1.77
PA3635	*eno*	Enolase	2.02
PA3636	*kdsA*	2-Dehydro-3-Deoxyphosphooctonate Aldolase	1.95
PA3637	*pyrG*	Ctp Synthase	1.58
PA3640	*dnaE*	Dna Polymerase Iii, Alpha Chain	1.76
PA3652	*uppS*	Undecaprenyl Pyrophosphate Synthetase	1.61
PA3654	*pyrH*	Uridylate Kinase	2.21
PA3656	*rpsB*	30S Ribosomal Protein S2	3.66
PA3661		Hypothetical Protein	2.34
PA3669		Hypothetical Protein	-1.59
PA3681		Hypothetical Protein	-2.05
PA3686	*adk*	Adenylate Kinase	2.09
PA3691		Hypothetical Protein	4.96
PA3692	*lptF*	Lipotoxon F, LptF	5.01
PA3700	*lysS*	Lysyl-Trna Synthetase	2.28
PA3718		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.31
PA3719	*armR*	Antirepressor For MexR, ArmR	-5.23
PA3720		Hypothetical Protein	-4.34
PA3721	*nalC*	NalC	-4.39
PA3726		Conserved Hypothetical Protein	2.32
PA3727		Hypothetical Protein	2.38
PA3731		Conserved Hypothetical Protein	-2.61
PA3735	*thrC*	Threonine Synthase	1.52
PA3740		Hypothetical Protein	1.75
PA3742	*rplS*	50S Ribosomal Protein L19	2.59
PA3743	*trmD*	Trna (Guanine-N1)-Methyltransferase	3.21
PA3744	*rimM*	16S Rrna Processing Protein	2.94
PA3745	*rpsP*	30S Ribosomal Protein S16	2.78
PA3746	*ffh*	Signal Recognition Particle Protein Ffh	2.21
PA3747		Conserved Hypothetical Protein	1.84
PA3749		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.83
PA3750		Hypothetical Protein	-2.87
PA3752		Hypothetical Protein	-2.25
PA3759		Probable Aminotransferase	-2.52
PA3760		N-Acetyl-D-Glucosamine Phosphotransferase System	-1.6

		Transporter	
PA3761	*nagE*	N-Acetyl-D-Glucosamine Phosphotransferase System Transporter	-1.86
PA3765		Hypothetical Protein	-2.89
PA3770	*guaB*	Inosine-5'-Monophosphate Dehydrogenase	1.61
PA3772		Hypothetical Protein	-1.87
PA3776		Probable Transcriptional Regulator	-3.92
PA3799		Conserved Hypothetical Protein	2.22
PA3801		Conserved Hypothetical Protein	1.58
PA3802	*hisS*	Histidyl-Trna Synthetase	1.94
PA3806		Conserved Hypothetical Protein	2.10
PA3807	*ndk*	Nucleoside Diphosphate Kinase	3.09
PA3818		Extragenic Suppressor Protein Suhb	2.54
PA3820	*secF*	Secretion Protein Secf	2.05
PA3821	*secD*	Secretion Protein Secd	2.25
PA3822		Conserved Hypothetical Protein	2.30
PA3827	*lptG*	Lipopolysaccharide Export System Permease Protein Lptg	1.6
PA3829		Hypothetical Protein	-1.92
PA3830		Probable Transcriptional Regulator	-2.46
PA3835		Hypothetical Protein	-3.99
PA3851		Hypothetical Protein	-1.5
PA3863	*dauA*	Fad-Dependent Catabolic D-Arginine Dehydrogenase, DauA	-1.83
PA3868		Hypothetical Protein	-1.56
PA3870	*moaA1*	Molybdopterin Biosynthetic Protein A1	-2.77
PA3876	*narK2*	Nitrite Extrusion Protein 2	-2.34
PA3883		Probable Short-Chain Dehydrogenase	-1.63
PA3884		Hypothetical Protein	-3.15
PA3886		Hypothetical Protein	-1.53
PA3893		Conserved Hypothetical Protein	-2.88
PA3894		Probable Outer Membrane Protein Precursor	-2.34
PA3899	*fecI*	FecI	2.04
PA3903	*prfC*	Peptide Chain Release Factor 3	2.38
PA3909	*eddB*	Extracelullar Dna Degradation Protein, EddB	-1.96
PA3910	*eddA*	Extracelullar Dna Degradation Protein, EddA	-2.53
PA3911		Conserved Hypothetical Protein	-1.86
PA3914	*moeA1*	Molybdenum Cofactor Biosynthetic Protein A1	-4.32
PA3915	*moaB1*	Molybdopterin Biosynthetic Protein B1	-1.96
PA3926		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.27
PA3932		Probable Transcriptional Regulator	-1.62
PA3937		Probable Atp-Binding Component Of Abc Taurine Transporter	-3.20
PA3939		Hypothetical Protein	-3.21
PA3941		Hypothetical Protein	-1.98
PA3946	*rocS1*	Two-Component Sensor Rocs1	-2.49

PA3947	*rocR*	RocR	-1.51
PA3953		Conserved Hypothetical Protein	-1.69
PA3955		Hypothetical Protein	-1.51
PA3959		Hypothetical Protein	-1.72
PA3960		Hypothetical Protein	-1.53
PA3962		Hypothetical Protein	1.55
PA3964		Hypothetical Protein	-2.55
PA3966		Hypothetical Protein	1.59
PA3971		Hypothetical Protein	-2.59
PA3972		Probable Acyl-Coa Dehydrogenase	-2.36
PA3973		Probable Transcriptional Regulator	-1.60
PA3987	*leuS*	Leucyl-Trna Synthetase	1.67
PA3991		Hypothetical Protein	-4.46
PA3994		Probable Epoxide Hydrolase	-4.25
PA4001	*sltB1*	Soluble Lytic Transglycosylase B	1.53
PA4003	*pbpA*	Penicillin-Binding Protein 2	1.90
PA4004		Conserved Hypothetical Protein	1.50
PA4005		Conserved Hypothetical Protein	2.02
PA4006	*nadD1*	Nicotinate Mononucleotide Adenylyltransferase NadD1	1.76
PA4008		Probable Hydrolase	-3.9
PA4009		Hypothetical Protein	-2.59
PA4011		Hypothetical Protein	-1.92
PA4021		Probable Transcriptional Regulator	-1.80
PA4026		Probable Acetyltransferase	-1.60
PA4031	*ppa*	Inorganic Pyrophosphatase	2.68
PA4036		Probable Two-Component Sensor	-2.29
PA4039		Hypothetical Protein	-2.96
PA4040		Hypothetical Protein	-2.10
PA4064		Probable ATP-Binding Component of ABC Transporter	-1.55
PA4067	*oprG*	Outer Membrane Protein OprG Precursor	2.95
PA4070		Probable Transcriptional Regulator	-1.84
PA4074		Probable Transcriptional Regulator	-2.29
PA4075		Hypothetical Protein	-1.59
PA4081	*cupB6*	Fimbrial Subunit CupB6	-2.17
PA4082	*cupB5*	Adhesive Protein CupB5	-3.87
PA4084	*cupB3*	Usher CupB3	-2.78
PA4085	*cupB2*	Chaperone CupB2	-2.14
PA4087		Conserved Hypothetical Protein	-3.27
PA4090		Hypothetical Protein	1.96
PA4091	*hpaA*	4-Hydroxyphenylacetate 3-Monooxygenase Large Chain	2.02
PA4093		Hypothetical Protein	-1.92
PA4102	*bfmS*	BfmS	-1.57
PA4103		Hypothetical Protein	-2.73

PA4104		Conserved Hypothetical Protein	-2.61
PA4107	*efhP*	EfhP	-3.25
PA4111		Hypothetical Protein	-2.63
PA4113		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.96
PA4126		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.11
PA4128		Conserved Hypothetical Protein	-2.66
PA4136		Probable Major Facilitator Superfamily (Mfs) Transporter	-3.08
PA4138	*tyrS*	Tyrosyl-TrnA Synthetase	-1.58
PA4141		Hypothetical Protein	8.49
PA4142		Probable Secretion Protein	3.42
PA4144		Probable Outer Membrane Protein Precursor	-2.24
PA4146		Hypothetical Protein	-2.08
PA4149		Conserved Hypothetical Protein	-2.36
PA4158	*fepC*	Ferric Enterobactin Transport Protein FepC	-1.65
PA4160	*fepD*	Ferric Enterobactin Transport Protein FepD	-3.64
PA4161	*fepG*	Ferric Enterobactin Transport Protein FepG	-3.66
PA4162		Probable Short-Chain Dehydrogenase	-2.78
PA4165		Probable Transcriptional Regulator	-2.34
PA4166		Probable Acetyltransferase	-1.58
PA4168	*fpvB*	Second Ferric Pyoverdine Receptor FpvB	1.76
PA4171		Probable Protease	1.68
PA4174		Probable Transcriptional Regulator	-1.66
PA4179		Probable Porin	-3.60
PA4188		Conserved Hypothetical Protein	-3.40
PA4189		Probable Aldehyde Dehydrogenase	-4.15
PA4191		Probable Iron/Ascorbate Oxidoreductase	-4.69
PA4194		Probable Permease of ABC Transporter	-2.11
PA4195		Probable Binding Protein Component of ABC Transporter	-2.94
PA4197	*bfiS*	BfiS	-2.06
PA4203	*nmoR*	NmoR	-2.38
PA4209	*phzM*	Probable Phenazine-Specific Methyltransferase	3.50
PA4210	*phzA1*	Probable Phenazine Biosynthesis Protein	5.38
PA4212	*phzC1*	Phenazine Biosynthesis Protein PhzC	3.33
PA4216	*phzG1*	Probable Pyridoxamine 5'-Phosphate Oxidase	2.49
PA4217	*phzS*	Flavin-Containing Monooxygenase	4.69
PA4227	*pchR*	Transcriptional Regulator PchR	3.16
PA4237	*rplQ*	50S Ribosomal Protein L17	3.08
PA4238	*rpoA*	Dna-Directed Rna Polymerase Alpha Chain	2.74
PA4239	*rpsD*	30S Ribosomal Protein S4	2.81
PA4240	*rpsK*	30S Ribosomal Protein S11	2.95
PA4241	*rpsM*	30S Ribosomal Protein S13	2.94
PA4242	*rpmJ*	50S Ribosomal Protein L36	1.98
PA4243	*secY*	Secretion Protein Secy	3.02

PA4244	*rplO*	50S Ribosomal Protein L15	2.74
PA4245	*rpmD*	50S Ribosomal Protein L30	3.22
PA4246	*rpsE*	30S Ribosomal Protein S5	3.06
PA4247	*rplR*	50S Ribosomal Protein L18	3.47
PA4248	*rplF*	50S Ribosomal Protein L6	3.52
PA4249	*rpsH*	30S Ribosomal Protein S8	3.31
PA4250	*rpsN*	30S Ribosomal Protein S14	2.23
PA4251	*rplE*	50S Ribosomal Protein L5	2.72
PA4252	*rplX*	50S Ribosomal Protein L24	2.40
PA4253	*rplN*	50S Ribosomal Protein L14	2.06
PA4254	*rpsQ*	30S Ribosomal Protein S17	2.44
PA4255	*rpmC*	50S Ribosomal Protein L29	2.42
PA4256	*rplP*	50S Ribosomal Protein L16	2.36
PA4257	*rpsC*	30S Ribosomal Protein S3	2.39
PA4258	*rplV*	50S Ribosomal Protein L22	2.28
PA4259	*rpsS*	30S Ribosomal Protein S19	2.49
PA4260	*rplB*	50S Ribosomal Protein L2	2.62
PA4261	*rplW*	50S Ribosomal Protein L23	2.85
PA4262	*rplD*	50S Ribosomal Protein L4	2.77
PA4263	*rplC*	50S Ribosomal Protein L3	2.79
PA4264	*rpsJ*	30S Ribosomal Protein S10	2.68
PA4266	*fusA1*	Elongation Factor G	2.04
PA4267	*rpsG*	30S Ribosomal Protein S7	1.94
PA4268	*rpsL*	30S Ribosomal Protein S12	1.91
PA4276	*secE*	Secretion Protein Sece	1.93
PA4279		Hypothetical Protein	-1.74
PA4283	*recD*	Exodeoxyribonuclease V Alpha Chain	-1.65
PA4287		Hypothetical Protein	-3.13
PA4288		Probable Transcriptional Regulator	-1.78
PA4289		Probable Transporter	-3.02
PA4292		Probable Phosphate Transporter	1.55
PA4294		Hypothetical Protein	2.45
PA4302	*tadA*	Tada Atpase	2.84
PA4304	*rcpA*	RcpA	2.95
PA4305	*rcpC*	RcpC	2.62
PA4307	*pctC*	Chemotactic Transducer PctcC	1.95
PA4309	*pctA*	Chemotactic Transducer PctA	-3.44
PA4317		Hypothetical Protein	2.60
PA4342		Probable Amidase	-2.76
PA4343		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.86
PA4348		Conserved Hypothetical Protein	1.69
PA4349		Hypothetical Protein	-2.40
PA4350	*olsB*	OlsB	-3.72

PA4353		Conserved Hypothetical Protein	-1.91
PA4359		Conserved Hypothetical Protein	3.33
PA4363	*iciA*	Inhibitor of Chromosome Initiation IciA	-2.5
PA4370	*icmP*	Insulin-Cleaving Metalloproteinase Outer Membrane Protein	2.96
PA4374	*mexV*	RND Multidrug Efflux Membrane Fusion Protein MexV	1.74
PA4384		Hypothetical Protein	2.09
PA4385	*groEL*	Groel Protein	2.05
PA4390		Hypothetical Protein	1.81
PA4402	*argJ*	Glutamate N-Acetyltransferase	1.67
PA4403	*secA*	Secretion Protein Seca	2.06
PA4406	*lpxC*	Udp-3-O-Acyl-N-Acetylglucosamine Deacetylase	1.51
PA4407	*ftsZ*	Cell Division Protein FtsZ	2.06
PA4408	*ftsA*	Cell Division Protein FtsA	2.32
PA4409	*ftsQ*	Cell Division Protein FtsQ	2.14
PA4410	*ddlB*	D-Alanine-D-Alanine Ligase	2.06
PA4411	*murC*	Udp-N-Acetylmuramate-Alanine Ligase	2.06
PA4413	*ftsW*	Cell Division Protein FtsW	1.64
PA4414	*murD*	Udp-N-Acetylmuramoylalanine-D-Glutamate Ligase	2.14
PA4415	*mraY*	Phospho-N-Acetylmuramoyl-Pentapeptide-Transferase	1.94
PA4416	*murF*	Udp-N-Acetylmuramoylalanyl-D-Glutamyl-2,6-Diaminopimelate-D-Alanyl-D-Alanyl Ligase	1.57
PA4418	*ftsI*	Penicillin-Binding Protein 3	1.75
PA4419	*ftsL*	Cell Division Protein Ftsl	2.21
PA4420		Conserved Hypothetical Protein	1.87
PA4421		Conserved Hypothetical Protein	1.75
PA4432	*rpsI*	30S Ribosomal Protein S9	3.56
PA4433	*rplM*	50S Ribosomal Protein L13	3.84
PA4437		Hypothetical Protein	-1.59
PA4438		Conserved Hypothetical Protein	2.04
PA4449	*hisG*	Atp-Phosphoribosyltransferase	1.6
PA4450	*murA*	Udp-N-Acetylglucosamine 1-Carboxyvinyltransferase	1.7
PA4451		Conserved Hypothetical Protein	1.72
PA4457		Arabinose-5-Phosphate Isomerase Kdsd	1.82
PA4458		Conserved Hypothetical Protein	1.67
PA4459	*lptC*	Lipopolysaccharide Export System Protein	1.54
PA4460	*lptH*	LptH	1.68
PA4461	*lptB*	Lipopolysaccharide Export System Atp-Binding Protein	1.65
PA4467		Hypothetical Protein	3.73
PA4468	*sodM*	Superoxide Dismutase	5.59
PA4469		Hypothetical Protein	5.48
PA4470	*fumC1*	Fumarate Hydratase	5.98
PA4471		Hypothetical Protein	5.22
PA4480	*mreC*	Rod Shape-Determining Protein MreC	1.97

PA4481	*mreB*	Rod Shape-Determining Protein MreB	2.55
PA4482	*gatC*	Glu-Trna(Gln) Amidotransferase Subunit C	1.53
PA4484	*gatB*	Glu-Trna(Gln) Amidotransferase Subunit B	1.55
PA4487	*magF*	MagF	1.63
PA4495		Hypothetical Protein	2.27
PA4496	*dppA1*	Probable Binding Protein Component of ABC Transporter	3.02
PA4507		Hypothetical Protein	-2.41
PA4509		Hypothetical Protein	-2.4
PA4514		Probable Outer Membrane Receptor for Iron Transport	2.38
PA4515		Conserved Hypothetical Protein	3.03
PA4516		Hypothetical Protein	1.96
PA4519	*speC*	Ornithine Decarboxylase	2.35
PA4525	*pilA*	Type 4 Fimbrial Precursor PilA	1.99
PA4527	*pilC*	Type 4 Fimbrial Biogenesis Protein PilC (Putative Pseudogene)	-1.57
PA4540		Hypothetical Protein	-2.62
PA4541	*lepA*	Large Extracellular Protease, LepA	-1.79
PA4545	*comL*	Competence Protein ComL	2.26
PA4559	*lspA*	Prolipoprotein Signal Peptidase	1.52
PA4563	*rpsT*	30S Ribosomal Protein S20	3.98
PA4567	*rpmA*	50S Ribosomal Protein L27	1.75
PA4570		Hypothetical Protein	6.53
PA4571		Probable Cytochrome C	1.90
PA4575		Hypothetical Protein	-2.08
PA4578		Hypothetical Protein	2.02
PA4585	*rtcA*	Rna 3'-Terminal Phosphate Cyclase	-2.14
PA4587	*ccpR*	Cytochrome C551 Peroxidase Precursor	3.59
PA4593		Probable Permease of ABC Transporter	-1.77
PA4597	*oprJ*	Multidrug Efflux Outer Membrane Protein OprJ Precursor	-3.39
PA4598	*mexD*	RND Multidrug Efflux Transporter Mexd	-2.44
PA4599	*mexC*	RND Multidrug Efflux Membrane Fusion Protein Mexc Precursor	-3.41
PA4602	*glyA3*	Serine Hydroxymethyltransferase	2.82
PA4610		Hypothetical Protein	-2.45
PA4612		Conserved Hypothetical Protein	-2.27
PA4621		Probable Oxidoreductase	-2.10
PA4622		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.09
PA4624	*cdrB*	Cyclic Diguanylate-Regulated Tps Partner B, CdrB	1.76
PA4625	*cdrA*	Cyclic Diguanylate-Regulated Tps Partner A, CdrA	1.62
PA4629		Hypothetical Protein	1.55
PA4630		Hypothetical Protein	-1.53
PA4636		Hypothetical Protein	1.73
PA4648	*cupE1*	Pilin Subunit CupE1	4.72
PA4649	*cupE2*	Pilin Subunit CupE2	3.14

PA4650	*cupE3*	Pilin Subunit CupE3	2.04
PA4651	*cupE4*	Pilin Assembly Chaperone CupE4	1.86
PA4654		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.21
PA4665	*prfA*	Peptide Chain Release Factor 1	1.81
PA4669	*ipk*	Isopentenyl Monophosphate Kinase	2.37
PA4670	*prs*	Ribose-Phosphate Pyrophosphokinase	2.65
PA4671		Probable Ribosomal Protein L25	1.78
PA4672		Peptidyl-Trna Hydrolase	2.25
PA4675	*chtA*	Chta	3.20
PA4685		Hypothetical Protein	1.9
PA4686		Hypothetical Protein	1.93
PA4708	*phuT*	Heme-Transport Protein, PhuT	1.67
PA4709	*phuS*	PhuS	2.57
PA4710	*phuR*	Heme/Hemoglobin Uptake Outer Membrane Receptor PhuR	3.40
PA4723	*dksA*	Suppressor Protein DksA	2.97
PA4738		Conserved Hypothetical Protein	4.07
PA4739		Conserved Hypothetical Protein	4.64
PA4741	*rpsO*	30S Ribosomal Protein S15	2.90
PA4742	*truB*	Trna Pseudouridine 55 Synthase	2.40
PA4743	*rbfA*	Ribosome-Binding Factor A	2.91
PA4744	*infB*	Translation Initiation Factor If-2	2.52
PA4745	*nusA*	N Utilization Substance Protein A	2.51
PA4746		Conserved Hypothetical Protein	2.98
PA4748	*tpiA*	Triosephosphate Isomerase	1.89
PA4753		Conserved Hypothetical Protein	2.00
PA4765	*omlA*	Outer Membrane Lipoprotein OmlA Precursor	2.29
PA4768	*smpB*	SmpB Protein	2.43
PA4770	*lldP*	L-Lactate Permease	1.50
PA4774		Hypothetical Protein	-2.65
PA4779		Hypothetical Protein	-1.79
PA4783		Conserved Hypothetical Protein	-2.62
PA4784		Probable Transcriptional Regulator	-1.52
PA4791		Hypothetical Protein	-2.10
PA4795		Hypothetical Protein	-1.86
PA4799		Hypothetical Protein	-1.98
PA4802		Hypothetical Protein	-2.6
PA4804	*cupB3*	Potra-Like Domain-Containing Usher, CupB3	-3.27
PA4805		Probable Class III Aminotransferase	-2.16
PA4807	*selB*	Selenocysteine-Specific Elongation Factor	-1.52
PA4810	*fdnI*	Nitrate-Inducible Formate Dehydrogenase, Gamma Subunit	-1.63
PA4812	*fdnG*	Formate Dehydrogenase-O, Major Subunit	-1.58
PA4814	*fadH2*	2,4-Dienoyl-Coa Reductase FadH2	-4.95
PA4816		Hypothetical Protein	-2.07

PA4817		Hypothetical Protein	-1.86
PA4821		Probable Transporter	-3.60
PA4822		Hypothetical Protein	-1.72
PA4828		Conserved Hypothetical Protein	-2.12
PA4830		Hypothetical Protein	-3.14
PA4832		Probable Short-Chain Dehydrogenase	-2.64
PA4843	*gcbA*	GcbA	1.70
PA4844	*ctpL*	CtpL	-2.42
PA4846	*aroQ1*	3-Dehydroquinate Dehydratase	1.86
PA4848	*accC*	Biotin Carboxylase	1.63
PA4850	*prmA*	Ribosomal Protein L11 Methyltransferase	1.52
PA4853	*fis*	Dna-Binding Protein Fis	2.04
PA4858		Conserved Hypothetical Protein	-3.12
PA4864	*ureD*	Urease Accessory Protein	-2.06
PA4871		Hypothetical Protein	-3.21
PA4876	*osmE*	Osmotically Inducible Lipoprotein OsmE	3.49
PA4877		Hypothetical Protein	2.19
PA4880		Probable Bacterioferritin	1.88
PA4882		Hypothetical Protein	-1.71
PA4885	*irlR*	Two-Component Response Regulator	-2.41
PA4886		Probable Two-Component Sensor	-2.78
PA4896		Probable Sigma-70 Factor, Ecf Subfamily	2.73
PA4898	*opdK*	Histidine Porin OpdK	-2.86
PA4899		Probable Aldehyde Dehydrogenase	-2.14
PA4900		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.06
PA4901	*mdlC*	Benzoylformate Decarboxylase	-4.47
PA4904	*vanA*	Vanillate O-Demethylase Oxygenase Subunit	-4.57
PA4905	*vanB*	Vanillate O-Demethylase Oxidoreductase	-2.99
PA4908		Hypothetical Protein	-1.69
PA4921	*choE*	Cholinesterase, ChoE	-1.99
PA4925		Conserved Hypothetical Protein	1.66
PA4927		Conserved Hypothetical Protein	-1.86
PA4928		Conserved Hypothetical Protein	1.8
PA4932	*rplI*	50S Ribosomal Protein L9	3.41
PA4933		Hypothetical Protein	3.48
PA4934	*rpsR*	30S Ribosomal Protein S18	4.34
PA4938	*purA*	Adenylosuccinate Synthetase	1.69
PA4940		Conserved Hypothetical Protein	2.35
PA4941	*hflC*	Protease Subunit HflC	1.73
PA4967	*parE*	Topoisomerase Iv Subunit B	1.55
PA4968		Conserved Hypothetical Protein	1.72
PA4972		Hypothetical Protein	1.74
PA4976	*aruH*	Arginine:Pyruvate Transaminas, AruH	-1.57

PA4977	*aruI*	2-Ketoarginine Decarboxylase, AruI	-2.32
PA4978		Hypothetical Protein	-2.88
PA4981	*lysP*	Lysine-Specific Permease	-2.39
PA4982		Probable Two-Component Sensor	-2.31
PA4985		Uncharacterized Protein	-2.02
PA4986		Probable Oxidoreductase	-1.51
PA4988	*waaA*	3-Deoxy-D-Manno-Octulosonic-Acid (Kdo) Transferase	-2.00
PA4989		Probable Transcriptional Regulator	-3.06
PA4994		Probable Acyl-Coa Dehydrogenase	-2.1
PA4995		Probable Acyl-Coa Dehydrogenase	-1.68
PA5005		Probable Carbamoyl Transferase	1.80
PA5010	*waaG*	Udp-Glucose:(Heptosyl) Lps Alpha 1,3-Glucosyltransferase WaaG	2.10
PA5015	*aceE*	Pyruvate Dehydrogenase	1.71
PA5016	*aceF*	Dihydrolipoamide Acetyltransferase	1.60
PA5027		Hypothetical Protein	2.06
PA5029		Probable Transcriptional Regulator	-1.87
PA5031		Probable Short Chain Dehydrogenase	-3.45
PA5032		Probable Transcriptional Regulator	-3.70
PA5034	*hemE*	Uroporphyrinogen Decarboxylase	1.68
PA5041	*pilP*	Type 4 Fimbrial Biogenesis Protein PilP	1.83
PA5042	*pilO*	Type 4 Fimbrial Biogenesis Protein PilO	1.96
PA5043	*pilN*	Type 4 Fimbrial Biogenesis Protein PilN	1.76
PA5045	*ponA*	Penicillin-Binding Protein 1A	1.67
PA5046		Malic Enzyme	1.77
PA5049	*rpmE*	50S Ribosomal Protein L31	3.67
PA5053	*hslV*	Heat Shock Protein HslV	2.33
PA5054	*hslU*	Heat Shock Protein HslU	2.38
PA5058	*phaC2*	Poly(3-Hydroxyalkanoic Acid) Synthase 2	2.31
PA5059		Probable Transcriptional Regulator	2.00
PA5075		Probable Permease Of Abc Transporter	1.52
PA5078	*opgG*	OpgG	1.70
PA5083	*dguB*	DguB	-2.30
PA5084	*dguA*	DguA	-3.82
PA5085	*dguR*	DguR	-1.73
PA5100	*hutU*	Urocanase	2.09
PA5102		Hypothetical Protein	-2.51
PA5103	*puuR*	PuuR	1.66
PA5110	*fbp*	Fructose-1,6-Bisphosphatase	2.08
PA5115		Conserved Hypothetical Protein	-1.80
PA5118	*thiI*	Thiazole Biosynthesis Protein ThiI	2.78
PA5128	*secB*	Secretion Protein SecB	3.25
PA5129	*grxC*	GrxC	2.36

PA5130		Conserved Hypothetical Protein	1.78
PA5131	*pgm*	Phosphoglycerate Mutase	1.67
PA5132		Hypothetical Protein	-2.80
PA5136		Hypothetical Protein	1.54
PA5145		Hypothetical Protein	-1.90
PA5158		Probable Outer Membrane Protein Precursor	-2.02
PA5161	*rmlB*	Dtdp-D-Glucose 4,6-Dehydratase	2.55
PA5163	*rmlA*	Glucose-1-Phosphate Thymidylyltransferase	2.33
PA5164	*rmlC*	Dtdp-4-Dehydrorhamnose 3,5-Epimerase	2.58
PA5170	*arcD*	Arginine/Ornithine Antiporter	2.56
PA5171	*arcA*	Arginine Deiminase	2.94
PA5172	*arcB*	Ornithine Carbamoyltransferase, Catabolic	2.99
PA5173	*arcC*	Carbamate Kinase	2.44
PA5178		Conserved Hypothetical Protein	2.45
PA5179		Probable Transcriptional Regulator	-1.55
PA5183		Hypothetical Protein	-1.57
PA5186		Probable Iron-Containing Alcohol Dehydrogenase	-2.73
PA5188		Probable 3-Hydroxyacyl-Coa Dehydrogenase	-2.15
PA5189		Probable Transcriptional Regulator	-2.21
PA5192	*pckA*	Phosphoenolpyruvate Carboxykinase	1.78
PA5204	*argA*	N-Acetylglutamate Synthase	1.78
PA5211		Conserved Hypothetical Protein	-2.91
PA5217		Probable Binding Protein Component of ABC Iron Transporter	3.14
PA5218		Probable Transcriptional Regulator	-1.87
PA5220		Hypothetical Protein	4.46
PA5230		Probable Permease of ABC Transporter	1.75
PA5231		Probable ATP-Binding/Permease Fusion ABC Transporter	1.70
PA5238		Probable O-Antigen Acetylase	-1.57
PA5239	*rho*	Transcription Termination Factor Rho	3.31
PA5264		Hypothetical Protein	-1.95
PA5265		Hypothetical Protein	-1.61
PA5267	*hcpB*	Secreted Protein Hcp	2.29
PA5282		Probable Major Facilitator Superfamily (Mfs) Transporter	-4.08
PA5286		Conserved Hypothetical Protein	1.71
PA5290		Conserved Hypothetical Protein	-1.74
PA5294		Putative Multidrug Efflux Pump	-4.03
PA5296	*rep*	Atp-Dependent Dna Helicase Rep	1.78
PA5298		Xanthine Phosphoribosyltransferase	2.46
PA5303		Conserved Hypothetical Protein	1.64
PA5304	*dadA*	D-Amino Acid Dehydrogenase, Small Subunit	1.54
PA5311		Probable Major Facilitator Superfamily (Mfs) Transporter	-1.99
PA5315	*rpmG*	50S Ribosomal Protein L33	3.63
PA5316	*rpmB*	50S Ribosomal Protein L28	3.72

PA5321	*dut*	Deoxyuridine 5'-Triphosphate Nucleotidohydrolase	1.71
PA5322	*algC*	Phosphomannomutase AlgC	2.15
PA5323	*argB*	Acetylglutamate Kinase	2.48
PA5324	*sphR*	Sphingosine-Responsive Regulator, SphR	-1.71
PA5369	*pstS*	Periplasmic Phosphate-Binding Protein ABC Transporter, PstS	1.81
PA5379	*sdaB*	L-Serine Dehydratase	-2.28
PA5381		Hypothetical Protein	-2.16
PA5382		Probable Transcriptional Regulator	-2.51
PA5387	*cdhC*	Cdhc, Carnitine Dehydrogenase-Related Gene C	-3.19
PA5389	*cdhR*	Cdhr, Transcriptional Regulator	-2.05
PA5390		Probable Peptidic Bond Hydrolase	-2.89
PA5398	*dgcA*	Dgca, Dimethylglycine Catabolism	-2.2
PA5399	*dgcB*	Dgcb, Dimethylglycine Catabolism	-2.93
PA5400		Probable Electron Transfer Flavoprotein Alpha Subunit	-3.71
PA5401		Hypothetical Protein	-3.24
PA5408		Hypothetical Protein	-1.79
PA5409		Hypothetical Protein	-1.82
PA5411	*gbcB*	GbcB	-1.61
PA5412		Hypothetical Protein	-1.58
PA5421	*fdhA*	Glutathione-Independent Formaldehyde Dehydrogenase	-1.93
PA5427	*adhA*	Alcohol Dehydrogenase	1.90
PA5431		Probable Transcriptional Regulator	-3.26
PA5432		Probable Acetyltransferase	-1.74
PA5433		Conserved Hypothetical Protein	-2.11
PA5446		Hypothetical Protein	-2.41
PA5465		Hypothetical Protein	-2.14
PA5466		Hypothetical Protein	-3.4
PA5468		Probable Citrate Transporter	-2.11
PA5469		Conserved Hypothetical Protein	-1.87
PA5471.1		Pa5471 Leader Peptide	1.72
PA5475		Hypothetical Protein	2.84
PA5479	*gltP*	Proton-Glutamate Symporter	2.36
PA5480		Hypothetical Protein	-1.98
PA5481		Hypothetical Protein	4.87
PA5482		Hypothetical Protein	4.05
PA5507		Hypothetical Protein	1.74
PA5515		Hypothetical Protein	-1.52
PA5521		Probable Short-Chain Dehydrogenase	-1.73
PA5522	*pauA6*	Glutamylpolyamine Synthetase	-2.61
PA5523		Probable Aminotransferase	-3.24
PA5524		Probable Short-Chain Dehydrogenase	-2.85
PA5525		Probable Transcriptional Regulator	-1.86
PA5531	*tonB1*	TonB1	2.43

PA5543		Hypothetical Protein	-1.53
PA5548		Probable Major Facilitator Superfamily (Mfs) Transporter	-2.00
PA5549	*glmS*	Glucosamine-Fructose-6-Phosphate Aminotransferase	1.76
PA5550	*glmR*	Glmr Transcriptional Regulator	2.16
PA5554	*atpD*	ATP Synthase Beta Chain	1.95
PA5555	*atpG*	ATP Synthase Gamma Chain	1.92
PA5556	*atpA*	ATP Synthase Alpha Chain	1.9
PA5557	*atpH*	ATP Synthase Delta Chain	2.27
PA5558	*atpF*	ATP Synthase B Chain	1.96
PA5559	*atpE*	ATP Synthase C Chain	1.59
PA5560	*atpB*	ATP Synthase A Chain	2.23
PA5564	*gidB*	Glucose Inhibited Division Protein B	2.26
PA5565	*gidA*	Glucose-Inhibited Division Protein A	1.56
PA5568		Conserved Hypothetical Protein	2.65
PA5569	*rnpA*	Ribonuclease P Protein Component	3.04
PA5570	*rpmH*	50S Ribosomal Protein L34	3.03
PA1291		Hypothetical protein	-2.27
PA1321	*cyoE*	Cytochrome o ubiquinol oxidase protein CyoE	-1.59
PA1334		Probable oxidoreductase	-3.30
PA1346		Hypothetical protein	-6.48
PA1382		Probable type II secretion system protein	-3.53
PA1383		Hypothetical protein	-2.76
PA1384	*galE*	UDP-glucose 4-epimerase	-4.32
PA1385		Probable glycosyl transferase	-2.57
PA1386		Probable ATP-binding component of ABC transporter	-5.00
PA1387		Hypothetical protein	-2.52
PA1388		Hypothetical protein	-3.31
PA1390		Probable glycosyl transferase	-3.18
PA1391		Probable glycosyl transferase	-3.70
PA1393	*cysC*	Adenosine 5'-phosphosulfate (APS) kinase	-2.92
PA1418		Probable sodium:solute symport protein	-2.93
PA1499		Conserved hypothetical protein	-5.44
PA1503		Hypothetical protein	-4.24
PA1507		Probable transporter	-1.68
PA1519		Probable transporter	-4.29
PA1525	*alkB2*	Alkane-1-monooxygenase 2	-2.41
PA1566	*pauA3*	Glutamylpolyamine synthetase	-2.54
PA1600		Probable cytochrome c	-5.88
PA1707	*pcrH*	Regulatory protein PcrH	-3.55
PA1708	*popB*	Translocator protein PopB	-3.70
PA1711	*exsE*	ExsE	-2.83
PA1739		Probable oxidoreductase	-4.01

PA1875		Probable outer membrane protein precursor	4.30
PA1877		Probable secretion protein	3.42
PA1887		Hypothetical protein	2.57
PA1888		Hypothetical protein	2.05
PA1901	*phzC2*	Phenazine biosynthesis protein PhzC	-3.09
PA1905	*phzG2*	Probable pyridoxamine 5'-phosphate oxidase	-3.98
PA1914		Conserved hypothetical protein	4.73
PA1921		Hypothetical protein	-3.99
PA1922		Probable tonB-dependent receptor	-4.35
PA1923		Hypothetical protein	-5.63
PA1924		Hypothetical protein	-6.63
PA1925		Hypothetical protein	-4.21
PA1927	*metE*	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	3.70
PA1935		Hypothetical protein	-2.55
PA1937		Conserved hypothetical protein	1.88
PA1977		Hypothetical protein	-8.03
PA1979	*eraS*	Sensor kinase, EraS	-4.74
PA2013	*liuC*	Putative 3-methylglutaconyl-CoA hydratase	1.82
PA2014	*liuB*	Methylcrotonyl-coa carboxylase, beta-subunit	1.71
PA2073		Probable transporter (membrane subunit)	-1.76
PA2074		Hypothetical protein	-4.14
PA2089		Hypothetical protein	-4.26
PA2096		Probable transcriptional regulator	-4.17
PA2099		Probable short-chain dehydrogenase	-4.56
PA2103	*moeB*	Probable molybdopterin biosynthesis protein MoeB	-1.93
PA2104		Probable cysteine synthase	-1.98
PA2105		Probable acetyltransferase	-1.9
PA2106		Hypothetical protein	-2.36
PA2110		Hypothetical protein	-1.61
PA2113	*opdO*	Pyroglutamate porin OpdO	1.87
PA2114		Probable major facilitator superfamily (MFS) transporter	2.35
PA2147	*katE*	Catalase HPII	2.48
PA2182		Hypothetical protein	-1.79
PA2185	*katN*	Non-heme catalase KatN	-2.95
PA2186		Hypothetical protein	-3.4
PA2188		Probable alcohol dehydrogenase (Zn-dependent)	-3.07
PA2192		Conserved hypothetical protein	-2.13
PA2204		Probable binding protein component of ABC transporter	2.38
PA2217		Probable aldehyde dehydrogenase	-5.59
PA2218		Hypothetical protein	-2.49
PA2221		Conserved hypothetical protein	-2.66

PA2222		Hypothetical protein	-1.52
PA2224		Hypothetical protein	-2.05
PA2225		Hypothetical protein	-2.42
PA2226	*qsrO*	QsrO	-1.76
PA2229		Conserved hypothetical protein	-3.36
PA2260		Hypothetical protein	-3.15
PA2294		Probable ATP-binding component of ABC transporter	-6.17
PA2317		Probable oxidoreductase	1.68
PA2318		Hypothetical protein	1.93
PA2334		Probable transcriptional regulator	-6.19
PA2343	*mtlY*	Xylulose kinase	-7.46
PA2381		Hypothetical protein	-2.57
PA2423		Hypothetical protein	1.56
PA2429		Hypothetical protein	-3.15
PA2434		Hypothetical protein	-1.98
PA2437		Hypothetical protein	-3.76
PA2438		Hypothetical protein	-2.00
PA2439		Hypothetical protein	-3.38
PA2441		Hypothetical protein	-5.48
PA2470	*gtdA*	Gentisate 1,2-dioxygenase	-3.68
PA2507	*catA*	Catechol 1,2-dioxygenase	-6.31
PA2508	*catC*	Muconolactone delta-isomerase	-5.06
PA2511	*antR*	AntR	-2.71
PA2512	*antA*	Anthranilate dioxygenase large subunit	-5.30
PA2513	*antB*	Anthranilate dioxygenase small subunit	-5.11
PA2514	*antC*	Anthranilate dioxygenase reductase	-4.52
PA2516	*xylZ*	Toluate 1,2-dioxygenase electron transfer component	-5.88
PA2580		Conserved hypothetical protein	-2.42
PA2588		Probable transcriptional regulator	2.15
PA2630		Conserved hypothetical protein	2.45
PA2636		Hypothetical protein	-5.57
PA2672		Probable type II secretion system protein	-4.53
PA2673		Probable type II secretion system protein	-3.08
PA2682		Conserved hypothetical protein	-2.76
PA2700	*opdB*	Proline porin OpdB	-1.94
PA2714		Probable molybdopterin oxidoreductase	-3.42
PA2715		Probable ferredoxin	-2.50
PA2736		Hypothetical protein	-1.62
PA2754		Conserved hypothetical protein	1.92
PA2759		Hypothetical protein	-1.76
PA2838		Probable transcriptional regulator	-6.44
PA2847		Conserved hypothetical protein	-5.53

PA2863	*lipH*	Lipase modulator protein	-2.14
PA2912		Probable ATP-binding component of ABC transporter	-1.65
PA2913		Hypothetical protein	-2.02
PA2914		Probable permease of ABC transporter	-3.48
PA2934	*cif*	CFTR inhibitory factor, Cif	-1.62
PA2937		Hypothetical protein	-3.67
PA2938		Probable transporter	-4.54
PA2939		Probable aminopeptidase	4.54
PA3062	*pelC*	Pelc	-4.85
PA3137		Probable major facilitator superfamily (MFS) transporter	-2.10
PA3142		Integrase	-1.77
PA3160	*wzz*	O-antigen chain length regulator	-2.65
PA3181		2-keto-3-deoxy-6-phosphogluconate aldolase	2.08
PA3183	*zwf*	Glucose-6-phosphate 1-dehydrogenase	3.74
PA3192	*gltR*	Two-component response regulator GltR	1.58
PA3194	*edd*	Phosphogluconate dehydratase	2.19
PA3195	*gapA*	Glyceraldehyde 3-phosphate dehydrogenase	3.46
PA3266	*capB*	Cold acclimation protein B	2.83
PA3281		Hypothetical protein	-2.49
PA3282		Hypothetical protein	-2.3
PA3315		Probable permease of ABC transporter	-2.36
PA3319	*plcN*	Non-hemolytic phospholipase C precursor	-3.17
PA3323		Conserved hypothetical protein	-2.56
PA3331		Cytochrome P450	1.69
PA3332		Conserved hypothetical protein	2.82
PA3334		Probable acyl carrier protein	2.00
PA3335		Hypothetical protein	1.71
PA3359		Hypothetical protein	-4.85
PA3360		Probable secretion protein	-5.66
PA3361	*lecB*	Fucose-binding lectin PA-IIL	5.78
PA3390		Hypothetical protein	-2.39
PA3393	*nosD*	NosD protein	-1.82
PA3415		Probable dihydrolipoamide acetyltransferase	-2.23
PA3416		Probable pyruvate dehydrogenase E1 component, β chain	-1.98
PA3417		Probable pyruvate dehydrogenase E1 component, α chain	-2.44
PA3442		Probable ATP-binding component of ABC transporter	-2.77
PA3450	*lsfA*	1-Cys peroxiredoxin LsfA	1.93
PA3467		Probable major facilitator superfamily (MFS) transporter	-3.32
PA3497		Hypothetical protein	-4.74
PA3498		Probable oxidoreductase	-2.93
PA3500		Conserved hypothetical protein	-3.26
PA3506		Probable decarboxylase	-3.18

PA3520		Hypothetical protein	2.74
PA3546	*algX*	Alginate biosynthesis protein AlgX	-4.73
PA3588		Probable porin	-6.87
PA3593		Probable acyl-coa dehydrogenase	-6.03
PA3597		Probable amino acid permease	-2.49
PA3655	*tsf*	Elongation factor Ts	3.86
PA3662		Hypothetical protein	-1.77
PA3724	*lasB*	Elastase LasB	6.73
PA3769	*guaA*	GMP synthase	2.62
PA3789		Hypothetical protein	-1.74
PA3841	*exoS*	Exoenzyme S	-2.84
PA3843		Hypothetical protein	-2.00
PA3901	*fecA*	Fe(III) dicitrate transport protein	1.99
PA3906		Hypothetical protein	3.60
PA3907		Hypothetical protein	1.81
PA3908		Hypothetical protein	3.00
PA3935	*tauD*	Taurine dioxygenase	-3.01
PA3940		Probable DNA binding protein	4.02
PA3967		Hypothetical protein	2.28
PA4028		Hypothetical protein	-6.42
PA4062		Hypothetical protein	-2.68
PA4139		Hypothetical protein	4.11
PA4170		Hypothetical protein	-2.33
PA4181		Hypothetical protein	-2.00
PA4182		Hypothetical protein	-2.00
PA4187		Probable major facilitator superfamily (MFS) transporter	-5.84
PA4211	*phzB1*	Probable phenazine biosynthesis protein	6.31
PA4218	*ampP*	AmpP	1.92
PA4220		Hypothetical protein	2.26
PA4221	*fptA*	Fe(III)-pyochelin outer membrane receptor precursor	4.58
PA4223		Probable ATP-binding component of ABC transporter	1.76
PA4224	*pchG*	Pyochelin biosynthetic protein	1.63
PA4225	*pchF*	Pyochelin synthetase	2.01
PA4226	*pchE*	Dihydroaeruginoic acid synthetase	2.10
PA4228	*pchD*	Pyochelin biosynthesis protein	3.94
PA4229	*pchC*	Pyochelin biosynthetic protein	2.22
PA4230	*pchB*	Salicylate biosynthesis protein	5.09
PA4231	*pchA*	Salicylate biosynthesis isochorismate synthase	4.11
PA4271	*rplL*	50S ribosomal protein L7 / L12	3.09
PA4272	*rplJ*	50S ribosomal protein L10	3.13
PA4273	*rplA*	50S ribosomal protein L1	1.83
PA4274	*rplK*	50S ribosomal protein L11	1.90

PA4277	*tufB*	Elongation factor Tu	1.94
PA4300	*tadC*	TadC	2.15
PA4303	*tadZ*	TadZ	1.65
PA4306	*flp*	Type IVb pilin, Flp	6.21
PA4355	*pyeM*	PyeM	-4.78
PA4364		Hypothetical protein	-1.73
PA4365	*lysE*	Lysine efflux permease	-1.83
PA4443	*cysD*	ATP sulfurylase small subunit	2.08
PA4549	*fimT*	Type 4 fimbrial biogenesis protein FimT	-3.99
PA4568	*rplU*	50S ribosomal protein L21	2.10
PA4586		Hypothetical protein	-3.11
PA4590	*pra*	Protein activator	3.78
PA4607		Hypothetical protein	1.80
PA4620		Hypothetical protein	-1.88
PA4673		Conserved hypothetical protein	2.08
PA4714		Conserved hypothetical protein	2.38
PA4740	*pnp*	Polyribonucleotide nucleotidyltransferase	2.65
PA4834		Putative nicotianamine synthase	-1.88
PA4835		Hypothetical protein	-2.15
PA4903		Probable major facilitator superfamily (MFS) transporter	-7.58
PA4935	*rpsF*	30S ribosomal protein S6	4.34
PA4973	*thiC*	Thiamin biosynthesis protein	1.57
PA4980		Probable enoyl-coA hydratase/isomerase	-1.91
PA5001	*ssg*	Cell surface-sugar biosynthetic glycosyltransferase, Ssg	1.66
PA5002	*dnpA*	De-N-acetylase involved in persistence	1.53
PA5117	*typA*	Regulatory protein TypA	2.89
PA5139		Hypothetical protein	1.98
PA5232		Conserved hypothetical protein	2.40
PA5274	*rnk*	Nucleoside diphosphate kinase regulator	1.95
PA5354	*glcE*	Glycolate oxidase subunit	-1.55
PA5396		Hypothetical protein	-1.83
PA5397		Hypothetical protein	-1.96
PA5419	*soxG*	Sarcosine oxidase gamma subunit	-1.52
PA5426	*purE*	Phosphoribosylaminoimidazole carboxylase, catalytic SU	2.18
PA5470		Probable peptide chain release factor	-3.07
PA5537		Hypothetical protein	-2.30
PA5538	*amiA*	N-acetylmuramoyl-L-alanine amidase	-2.82
PA5539		Hypothetical protein	-2.86
PA0688	*lapA*	Low-molecular-weight alkaline phosphatase A, LapA	-5.12
PA0689	*lapB*	Low-molecular-weight alkaline phosphatase B, LapB	-2.01
PA0717		Hypothetical protein of bacteriophage Pf1	-2.43
PA0718		Hypothetical protein of bacteriophage Pf1	-2.44

PA0719		Hypothetical protein of bacteriophage Pf1	-2.45
PA0726		Hypothetical protein of bacteriophage Pf1	-4.14
PA0728		Probable bacteriophage integrase	-3.28
PA0730		Probable transferase	2.68
PA0737		Hypothetical protein	-3.20
PA0790		Hypothetical protein	-6.54
PA0850		Hypothetical protein	-1.61
PA0852	*cbpD*	Chitin-binding protein CbpD precursor	5.64
PA0882		Hypothetical protein	-4.24
PA0986		Conserved hypothetical protein	2.39
PA1151	*imm2*	Pyocin S2 immunity protein	-2.24
PA1217		Probable 2-isopropylmalate synthase	-1.68
PA1221		Hypothetical protein	-2.36
PA1224		Probable NAD(P)H dehydrogenase	-3.90
PA1251		Probable chemotaxis transducer	-3.62
PA0187		Hypothetical protein	-2.57
PA0188		Hypothetical protein	-4.72
PA0229	*pcaT*	Dicarboxylic acid transporter PcaT	-2.10
PA0234		Hypothetical protein	-2.2
PA0241		Probable major facilitator superfamily (MFS) transporter	-5.31
PA0247	*pobA*	P-hydroxybenzoate hydroxylase	-3.59
PA0258		Hypothetical protein	-2.49
PA0279		Probable transcriptional regulator	-2.90
PA0283	*sbp*	Sulfate-binding protein precursor	2.25
PA0284		Hypothetical protein	1.56
PA0349		Hypothetical protein	-4.56
PA0417	*chpE*	Probable chemotaxis protein	-2.30
PA0434		Hypothetical protein	-1.54
PA0435		Hypothetical protein	-2.78
PA0439		Probable oxidoreductase	-3.35
PA0476		Probable permease	-3.72
PA0497		Hypothetical protein	-3.57
PA0518	*nirM*	Cytochrome c-551 precursor	3.53
PA0523	*norC*	Nitric-oxide reductase subunit C	2.86
PA0524	*norB*	Nitric-oxide reductase subunit B	2.41
PA0531		Probable glutamine amidotransferase	-3.92
PA0578		Conserved hypothetical protein	3.90
PA0617		Probable bacteriophage protein	2.09
PA0634		Hypothetical protein	2.67
PA0654	*speD*	S-adenosylmethionine decarboxylase proenzyme	2.19
PA0045		Hypothetical protein	4.14

PA0046		Hypothetical protein	3.91
PA0051	*phzH*	Potential phenazine-modifying enzyme	2.82

Table A-5. Dysregulated genes in the ΔPA1463o mutant not dysregulated in PA14 wild-type surfing relative to swimming. RNA-Seq was performed on the surfing deficient ΔPA1463o mutant and PA14 WT surfing on SCFM with 0.4% mucin. The ΔPA1463o mutant log fold change was determined relative to wild-type surfing. PA14WT surfing was compared to a swim control. 653 genes were identified as uniquely dysregulated in the mutant relative to the wild-type under surfing conditions that were not found in wild-type surfing relative to swimming. Log fold-change cut-off of ± 1.5 and p-value < 0.05 was used. Gene annotations and descriptions come from www.pseudomonas.com (Winsor et al., 2016). Genes with no PAO1 gene ID were provided with their PA14 gene locus tag instead.

PA01 Gene ID/locus	Gene Name	Description	Log FC
PA0043		Hypothetical Protein	-1.86
PA0051	*phzH*	Potential Phenazine-Modifying Enzyme	3.55
PA0057		Hypothetical Protein	2.22
PA0059	*osmC*	Osmotically Inducible Protein OsmC	2.36
PA0060		Hypothetical Protein	1.78
PA0062		Lipoprotein	1.95
PA0071		Hypothetical Protein	-2.08
PA0102		Carbonic Anhydrase	-2.61
PA0103		Sulfate Transporter	-2.50
PA0122		Hemolysin	1.64
PA0127		Lipoprotein	-1.79
PA0143	*nuh*	Nonspecific Ribonucleoside Hydrolase	1.80
PA0147		Oxidoreductase	-1.61
PA0151		TonB-Dependent Receptor	-1.74
PA0155	*pcaR*	Transcriptional Regulator PcaR	-1.75
PA0157		RND Efflux Membrane Fusion Protein	1.55
PA0173	*cheB*	Chemotaxis-Specific Methylesterase	1.81
PA0174		Hypothetical Protein	2.16
PA0175		Chemotaxis Protein Methyltransferase	2.03
PA0178	*cheA*	Two-Component Sensor	-2.20
PA0197	*tonB2*	Hypothetical Protein	3.69
PA0201		Hypothetical Protein	1.56
PA0218		LysR Family Transcriptional Regulator	-1.52
PA0226		CoA Transferase, Subunit A	-4.27
PA0227		CoA Transferase Subunit B	-4.03
PA0228	*pcaF*	Beta-Ketoadipyl CoA Thiolase	-3.85
PA0235	*pcaK*	4-Hydroxybenzoate Transporter PcaK	-3.29
PA0236		IclR Family Transcriptional Regulator	1.72
PA0241		Mfs Transporter	-2.79
PA0242		Hypothetical Protein	-1.85
PA0247	*pobA*	4-Hydroxybenzoate 3-Monooxygenase	-2.60

PA0263	*hcpC*	Secreted Protein Hcp	3.31
PA0263	*hcp2*	Secreted Protein Hcp	3.39
PA0263	*hcpB*	Secreted Protein Hcp	3.60
PA0264		Hypothetical Protein	-1.89
PA0267		Hypothetical Protein	1.56
PA0277		Zn-Dependent Protease With Chaperone Function	-1.53
PA0280	*cysA*	Sulfate Transport Protein CysA	1.70
PA0281	*cysW*	Sulfate Transport Protein CysW	2.25
PA0282	*cysT*	Sulfate Transport Protein CysT	2.42
PA0283	*sbp*	Sulfate-Binding Protein	3.26
PA0284		Hypothetical Protein	2.67
PA0289		Transcriptional Regulator	-1.82
PA0296		Glutamine Synthetase	-1.61
PA0297	*spuA*	Glutamine Amidotransferase	-1.81
PA0298	*spuB*	Glutamine Synthetase	-2.07
PA0299	*spuC*	Aminotransferase	-1.66
PA0320		Hypothetical Protein	-5.78
PA0327		Transcriptional Regulator	-3.71
PA0336	*ygdP*	Dinucleoside Polyphosphate Hydrolase	-1.87
PA0337	*ptsP*	Phosphoenolpyruvate-Protein Phosphotransferase PtsP	-1.65
PA0354		Hypothetical Protein	2.55
PA0355	*pfpI*	Protease Pfpi	3.00
PA0359		Hypothetical Protein	-1.77
PA0368		Hypothetical Protein	-1.53
PA0377		Hypothetical Protein	1.71
PA0402	*pyrB*	Aspartate Carbamoyltransferase	-1.64
PA0403	*pyrR*	Bifunctional Pyrimidine Regulatory Protein PyrR	-1.80
PA0404	*yqgF*	Holliday Junction Resolvase-Like Protein	-2.11
PA0405		Hypothetical Protein	-1.73
PA0433		Hypothetical Protein	1.79
PA0460		Hypothetical Protein	1.86
PA0469		Hypothetical Protein	-1.83
PA0476		Permease	-1.66
PA0489		Phosphoribosyl Transferase	1.63
PA0490		Hypothetical Protein	1.80
PA0496		Hydrolase	-2.01
PA0532		Hypothetical Protein	-2.25
PA0545		Hypothetical Protein	-1.75
PA0553		Hypothetical Protein	1.70
PA0554		Hypothetical Protein	2.13
PA0567		Hypothetical Protein	2.01
PA0573		Hypothetical Protein	-1.72
PA0578		Hypothetical Protein	1.80
PA0589	*glpE*	Thiosulfate Sulfurtransferase	-1.56
PA0593	*pdxA*	4-Hydroxythreonine-4-Phosphate Dehydrogenase	1.54

PA0602		ABC Transporter Substrate-Binding Protein	-1.56
PA0612		Hypothetical Protein	1.81
PA0613		Hypothetical Protein	2.19
PA0632		Hypothetical Protein	3.36
PA0673		Hypothetical Protein	-1.73
PA0677	*hxcW*	HxcW	-1.67
PA0679	*hxcP*	HxcP	-3.02
PA0693	*exbB2*	Transport Protein Exbb2	-1.85
PA0707	*toxR*	Transcriptional Regulator ToxR	2.00
PA0713		Hypothetical Protein	-1.56
PA0730		Transferase	1.82
PA0756		Two-Component Response Regulator	1.56
PA0761	*nadB*	L-Aspartate Oxidase	-1.76
PA0766	*mucD*	Serine Protease MucD	1.73
PA0775		Hypothetical Protein	1.83
PA0781		Hypothetical Protein	2.70
PA0807		Hypothetical Protein	1.83
PA0841		Hypothetical Protein	-2.09
PA0845		Hypothetical Protein	-3.17
PA0852	*cpbD*	Chitin-Binding Protein Cbpd	3.71
PA0875		Hypothetical Protein	-1.62
PA0887	*acsA*	Acetyl-Coa Synthetase	-1.58
PA0895	*argD*	BifunctionalN-Succinyldiaminopimelate-Aminotransferase/	1.63
PA0907		Hypothetical Protein	-1.58
PA0922		Hypothetical Protein	-1.61
PA0938		Hypothetical Protein	2.14
PA0942		Transcriptional Regulator	-2.15
PA0952		Hypothetical Protein	-3.02
PA0979		Hypothetical Protein	1.60
PA0990		Hypothetical Protein	1.86
PA0996	*pqsA*	PqsA	1.72
PA0997	*pqsB*	PqsB	2.19
PA0998	*pqsC*	PqsC	2.47
PA0999	*pqsD*	3-Oxoacyl-Acp Synthase	2.36
PA1000	*pqsE*	Quinolone Signal Response Protein	2.36
PA1001	*phnA*	Anthranilate Synthase Component I	2.93
PA1017	*pauA*	Pimeloyl-CoA Synthetase	-1.97
PA1029		Hypothetical Protein	-2.71
PA1030		Hypothetical Protein	-1.68
PA1068		Hsp90 Family Protein	-2.00
PA1108		Mfs Family Transporter	-1.96
PA1111		Hypothetical Protein	1.78
PA1122		Peptide Deformylase	-1.54
PA1132		Hypothetical Protein	2.12

PA1134		Hypothetical Protein	2.00
PA1135	*hchA*	Chaperone Protein HchA	-2.25
PA1136		Transcriptional Regulator	-2.92
PA1139		Hypothetical Protein	-1.59
PA1160		Hypothetical Protein	-1.54
PA1169		Lipoxygenase	1.71
PA1170		Hypothetical Protein	1.88
PA1178	*oprH*	PhoP/Q And Low Mg^{2+} Inducible Outer Membrane Protein	-2.67
PA1188		Hypothetical Protein	-2.11
PA1190		Hypothetical Protein	-2.11
PA1193		Hypothetical Protein	1.59
PA1209		Hypothetical Protein	-1.51
PA1226		Transcriptional Regulator	-1.75
PA1227		Hypothetical Protein	-1.59
PA1242		Hypothetical Protein	2.54
PA1243		Sensor/Response Regulator Hybrid	2.39
PA1244		Hypothetical Protein	-3.08
PA1245		Hypothetical Protein	2.75
PA1246	*aprD*	Alkaline Protease Secretion Protein AprD	2.49
PA1247	*aprE*	Alkaline Protease Secretion Protein AprE	2.70
PA1248	*aprF*	Alkaline Protease Secretion Outer Membrane Protein AprF	3.03
PA1249	*aprA*	Alkaline Metalloproteinase	5.43
PA1284		Acyl-CoA Dehydrogenase	-1.81
PA1296		2-Hydroxyacid Dehydrogenase	-1.67
PA1309		LysR Family Transcriptional Regulator	-2.15
PA1315		Transcriptional Regulator	-1.71
PA1317	*cyoA*	Cytochrome O Ubiquinol Oxidase Subunit Ii	1.62
PA1323		Hypothetical Protein	2.67
PA1324		Hypothetical Protein	2.60
PA1332		Hypothetical Protein	-1.76
PA1373	*fabF2*	3-Oxoacyl-Acp Synthase	-2.12
PA1377		Acetyltransferase	-1.59
PA14_00410		Dioxygenase	-1.63
PA14_03166		Hypothetical Protein	1.64
PA14_03320		Hypothetical Protein	1.51
PA14_03330		Hypothetical Protein	1.58
PA14_03370		Hypothetical Protein	2.32
PA14_04830		Acetyltransferase	-1.77
PA14_07460		Hypothetical Protein	-1.54
PA14_13210		Hypothetical Protein	3.27
PA14_13630		Hypothetical Protein	2.03
PA14_13920		Hypothetical Protein	1.51
PA14_13950		Hypothetical Protein	-1.55
PA14_14320		Hypothetical Protein	2.18
PA14_14420		Hypothetical Protein	1.52

PA14_14550		Hypothetical Protein	1.94
PA14_14560		Hypothetical Protein	6.67
PA14_15490		Hypothetical Protein	1.59
PA14_15570		Hypothetical Protein	-2.12
PA14_16110		Hypothetical Protein	2.02
PA14_18070		Periplasmic Metal-Binding Protein	-1.7
PA14_20060		Hypothetical Protein	2.98
PA14_21830		Hypothetical Protein	1.85
PA14_22080		Resolvase	-2.67
PA14_22180		Hypothetical Protein	1.84
PA14_22210		Hypothetical Protein	1.86
PA14_22240		Hypothetical Protein	1.78
PA14_22270		Recombinase	1.72
PA14_22500		Protein-Disulfide Isomerase	1.76
PA14_23350	*orfA*	Hypothetical Protein	-2.32
PA14_23420		Zinc-Binding Dehydrogenase	1.56
PA14_23430		Heparinase	2.28
PA14_23440	*orfL*	Group 1 Glycosyl Transferase	2.28
PA14_28240		Hypothetical Protein	1.51
PA14_28250		Secreted Acid Phosphatase	2.81
PA14_28360		Hypothetical Protein	1.81
PA14_28470		Hypothetical Protein	-2.15
PA14_28520		Hypothetical Protein	2.89
PA14_28820		Hypothetical Protein	2.02
PA14_28830		Hypothetical Protein	2.33
PA14_28840		Helicase	1.98
PA14_29330		Hypothetical Protein	2.80
PA14_31060		Hypothetical Protein	-1.53
PA14_31150		Hypothetical Protein	-3.83
PA14_31430		Hypothetical Protein	3.04
PA14_33300		Hypothetical Protein	2.29
PA14_33310		Hypothetical Protein	2.39
PA14_33320		Hypothetical Protein	2.23
PA14_33330		Hypothetical Protein	1.64
PA14_33340		Helicase	2.32
PA14_33350		Hypothetical Protein	2.69
PA14_33970		Hypothetical Protein	3.72
PA14_33980		Hypothetical Protein	2.75
PA14_35740		Transposase	1.88
PA14_35760		Hypothetical Protein	2.22
PA14_35770		Hypothetical Protein	2.71
PA14_35780		Hypothetical Protein	3.10
PA14_35890		Diaminobutyrate-2-Oxoglutarate Aminotransferase	1.61
PA14_35920		Acetate Permease	-1.72
PA14_36480		Hypothetical Protein	2.71

PA14_36790		Hypothetical Protein	2.89
PA14_36900		Hypothetical Protein	3.32
PA14_36940		Hypothetical Protein	1.82
PA14_39470		Hypothetical Protein	2.03
PA14_39480		Hypothetical Protein	2.40
PA14_39880	*phzG2*	Pyridoxamine 5'-Phosphate Oxidase	2.76
PA14_40740		Hypothetical Protein	-2.77
PA14_46460		Hypothetical Protein	2.91
PA14_46510		Hypothetical Protein	2.13
PA14_46520		Hypothetical Protein	2.84
PA14_46530		Hypothetical Protein	5.38
PA14_46540		Hypothetical Protein	2.56
PA14_46550		Ribonuclease	2.85
PA14_49480		Hypothetical Protein	3.77
PA14_49990		Hypothetical Protein	-2.16
PA14_51560		Acetyltransferase	1.55
PA14_51590		Hypothetical Protein	-2.37
PA14_53450		Hypothetical Protein	-1.92
PA14_53580		Hypothetical Protein	1.66
PA14_53590		Hypothetical Protein	2.83
PA14_53600		Hypothetical Protein	2.88
PA14_53610		Hypothetical Protein	2.87
PA14_54850		Hypothetical Protein	2.84
PA14_55080		Hypothetical Protein	2.00
PA14_55090		Hypothetical Protein	1.52
PA14_58730	*pilA*	Type Iv Pilin Structural Subunit	2.31
PA14_59150		Single-Stranded DNA-Binding Protein	1.57
PA14_59240	*pilL2*	Type Iv B Pilus Protein	-1.96
PA14_59340	*pilT2*	Type Iv B Pilus Protein	2.50
PA14_59350	*pilV2*	Type Iv B Pilus Protein	-2.08
PA14_59390		Hypothetical Protein	-1.72
PA14_59845		Hypothetical Protein	-2.33
PA14_60030		Hypothetical Protein	-1.5
PA14_60040		Hypothetical Protein	-1.72
PA14_60050		Plasmid Stabilization Protein	-1.92
PA14_60100	*dtd*	Deoxycytidine Triphosphate Deaminase	2.77
PA14_60110		Hypothetical Protein	1.51
PA14_60120	*dcd2*	Deoxycytidine Deaminase	1.61
PA14_60140		XerD-Like Integrase	1.60
PA14_61110		Hypothetical Protein	-1.67
PA14_64430		Hypothetical Protein	2.74
PA14_72370		Hypothetical Protein	2.49
PA1402		Hypothetical Protein	-2.08
PA1405		Helicase	-1.68
PA1413		LysR Family Transcriptional Regulator	-1.98

PA1435		RND Efflux Membrane Fusion Protein	-2.20
PA1436		RND Efflux Transporter	-2.10
PA1437		Two-Component Response Regulator	-1.88
PA1498	*pykF*	Pyruvate Kinase	-1.56
PA1500		Oxidoreductase	-2.37
PA1501		Hydroxypyruvate Isomerase	-3.70
PA1502	*gcl*	Glyoxylate Carboligase	-2.20
PA1505	*moaA2*	Molybdenum Cofactor Biosynthesis Protein A	-1.54
PA1507		Transporter	-1.52
PA1513		Hypothetical Protein	-1.59
PA1514		Ureidoglycolate Hydrolase	-3.83
PA1515	*alc*	Allantoicase	-1.68
PA1516		Hypothetical Protein	-2.17
PA1517		Hypothetical Protein	-2.00
PA1518		Transthyretin Family Protein	-2.08
PA1519		Transporter	-2.12
PA1525	*alkB2*	Alkane-1 Monooxygenase	-1.69
PA1542		Hypothetical Protein	-2.31
PA1545	*pemB*	PemB	-1.69
PA1569		Sugar Mfs Transporter	-3.29
PA1572		Atp-Nad Kinase	-2.65
PA1573		Hypothetical Protein	-2.34
PA1597		Hypothetical Protein	2.65
PA1606		Hypothetical Protein	1.59
PA1607		Hypothetical Protein	-1.66
PA1621		Hydrolase	-1.55
PA1622		Hydrolase	-1.56
PA1625		Hypothetical Protein	1.83
PA1627		GntR Family Transcriptional Regulator	-2.07
PA1628		3-Hydroxyacyl-Coa Dehydrogenase	-1.73
PA1629		Enoyl-CoA Hydratase	-1.75
PA1656	*hsiA2*	Hsia2	1.70
PA1657	*hsiB2*	Hsib2	2.27
PA1658	*hsiC2*	Hsic2	2.60
PA1659		Hypothetical Protein	2.44
PA1660	*hsiG2*	Hsig2	2.86
PA1661	*hsiH2*	Hsih2	3.14
PA1663	*sfa2*	Sfa2	2.97
PA1664	*lip2.2*	Lip2.2	3.12
PA1665	*fha2*	Fha2	3.22
PA1668	*dotU2*	Dotu2	3.12
PA1736		Acetyl-CoA Acetyltransferase	-1.91
PA1737		3-Hydroxyacyl-Coa Dehydrogenase	-2.11
PA1755		Hypothetical Protein	-2.26
PA1761		Hypothetical Protein	-2.02

PA1769		Hypothetical Protein	-1.84
PA1772	*rraA*	Ribonuclease Activity Regulator Protein RraA	-1.92
PA1779		Assimilatory Nitrate Reductase	-1.77
PA1793	*ppiB*	Peptidyl-Prolyl Cis-Trans Isomerase B	-1.65
PA1825		Hypothetical Protein	-1.51
PA1827		Short-Chain Dehydrogenase	-1.74
PA1838	*cysI*	Sulfite Reductase	1.53
PA1845		Hypothetical Protein	-1.77
PA1847		Hypothetical Protein	-1.53
PA1852		Hypothetical Protein	-1.52
PA1864		Tetr Family Transcriptional Regulator	1.63
PA1865		Hypothetical Protein	-1.66
PA1866		Hypothetical Protein	-1.64
PA1869		Acyl Carrier Protein	2.73
PA1870		Hypothetical Protein	3.07
PA1871	*lasA*	LasA Protease	2.64
PA1873		Cation Transporter	2.68
PA1877		Secretion Protein	1.65
PA1887		Hypothetical Protein	1.64
PA1888		Hypothetical Protein	1.73
PA1889		Hypothetical Protein	2.23
PA1899	*phzA2*	Phenazine Biosynthesis Protein	2.85
PA1900	*phzB2*	Phenazine Biosynthesis Protein	2.73
PA1913		Hypothetical Protein	3.94
PA1914		Hypothetical Protein	5.38
PA1921		Hypothetical Protein	4.07
PA1922		TonB-Dependent Receptor	4.01
PA1923	*cobN*	Cobaltochelatase Subunit CobN	3.39
PA1925		Hypothetical Protein	-2.97
PA1928	*rimJ*	Ribosomal Protein Alanine Acetyltransferase	-2.25
PA1929		Hypothetical Protein	-1.83
PA1932		Hydroxylase Molybdopterin-Containing Subunit	2.02
PA1942		Hypothetical Protein	-1.95
PA1968		Hypothetical Protein	-1.91
PA1984		NAD+ Dependent Acetaldehyde Dehydrogenase	-2.3
PA1991		Iron-Containing Alcohol Dehydrogenase	-1.87
PA1992		Two-Component Sensor	-2.17
PA1997		Acetoacetyl-CoA Synthetase	-1.52
PA1998		LysR Family Transcriptional Regulator	-2.16
PA2017		Hypothetical Protein	-1.52
PA2019		Periplasmic Multidrug Efflux Lipoprotein	-1.55
PA2021		Hypothetical Protein	2.67
PA2023	*galU*	Utp-Glucose-1-Phosphate Uridylyltransferase	1.53
PA2024		Ring-Cleaving Dioxygenase	-2.72
PA2035		Thiamine Pyrophosphate Protein	-2.13

PA2041		Amino Acid Permease	-1.62
PA2046		Hypothetical Protein	4.45
PA2062		Pyridoxal-Phosphate Dependent Protein	2.34
PA2066		Hypothetical Protein	2.78
PA2067		Hydrolase	2.68
PA2068		Mfs Transporter	2.35
PA2069		Carbamoyl Transferase	2.67
PA2086		Hydrolase	2.40
PA2090		Flavin-Dependent Oxidoreductase	2.91
PA2092		Mfs Transporter	2.50
PA2095		Hypothetical Protein	1.69
PA2096		AraC Family Transcriptional Regulator	-1.65
PA2107		Hypothetical Protein	2.74
PA2108		Thiamine Pyrophosphate Protein	2.80
PA2109		Hypothetical Protein	1.85
PA2120		Hypothetical Protein	-2.29
PA2134		Hypothetical Protein	2.32
PA2135		Transporter	2.51
PA2136		Hypothetical Protein	1.92
PA2140		Metallothionein	2.57
PA2141		Ompetence-Damaged Protein	2.88
PA2142		Short-Chain Dehydrogenase	3.09
PA2143		Hypothetical Protein	2.42
PA2144	*glgP*	Glycogen Phosphorylase	2.43
PA2146		Hypothetical Protein	-1.95
PA2147	*katE*	Hydroperoxidase	3.21
PA2148		Hypothetical Protein	2.99
PA2149		Hypothetical Protein	2.38
PA2150		Ku Domain-Containing Protein	2.31
PA2151		Hypothetical Protein	2.80
PA2152		Trehalose Synthase	2.85
PA2153	*glgB*	Glycogen Branching Protein	2.57
PA2154		Hypothetical Protein	4.06
PA2155		Cardiolipin Synthase 2	2.49
PA2156		Hypothetical Protein	3.53
PA2157		Hypothetical Protein	3.00
PA2158		Alcohol Dehydrogenase	2.59
PA2159		Hypothetical Protein	2.55
PA2160		Glycosyl Hydrolase	2.28
PA2161		Hypothetical Protein	3.23
PA2162		Maltooligosyl Trehalose Synthase	2.88
PA2163		4-Alpha-Glucanotransferase	3.00
PA2164		Glycosyl Hydrolase	2.89
PA2165	*glgA*	Glycogen Synthase	2.59
PA2167		Hypothetical Protein	2.09

PA2168		Hypothetical Protein	1.76
PA2169		Hypothetical Protein	2.09
PA2171		Hypothetical Protein	-1.76
PA2172		Hypothetical Protein	-1.59
PA2173		Hypothetical Protein	2.17
PA2175		Hypothetical Protein	2.26
PA2176		Hypothetical Protein	2.32
PA2178		Hypothetical Protein	-1.65
PA2179		Hypothetical Protein	2.93
PA2180		Hypothetical Protein	2.67
PA2181		Carboxylate-Amine Ligase	-1.65
PA2187		Hypothetical Protein	-2.13
PA2189		Hypothetical Protein	2.10
PA2204		ABC Transporter Substrate-Binding Protein	1.87
PA2210		Mfs Transporter	-2.19
PA2212	*pdxA*	4-Hydroxythreonine-4-Phosphate Dehydrogenase	-2.61
PA2235	*pslE*	Hypothetical Protein	-3.53
PA2236	*pslF*	Hypothetical Protein	-2.49
PA2244	*pslN*	Hypothetical Protein	2.98
PA2245	*pslO*	Hypothetical Protein	2.17
PA2261		2-Ketogluconate Kinase	-2.09
PA2262		2-Ketogluconate Transporter	-1.72
PA2263		2-Hydroxyacid Dehydrogenase	-2.09
PA2281		AraC Family Transcriptional Regulator	1.60
PA2282		Hypothetical Protein	-1.52
PA2291		Glucose-Sensitive Porin	-1.90
PA2299		GntR Family Transcriptional Regulator	2.58
PA2300	*chiC*	Chitinase	3.64
PA2309		ABC Transporter Substrate-Binding Protein	2.27
PA2311		Hypothetical Protein	1.94
PA2312		Xre Family Transcriptional Regulator	2.8
PA2338		Maltose/Mannitol ABC Transporter Substrate-Binding Protein	-1.75
PA2345		Hypothetical Protein	1.58
PA2359	*sfa3*	Sfa3	1.56
PA2381		Hypothetical Protein	-2.22
PA2382	*lldA*	L-Lactate Dehydrogenase	1.79
PA2384		Hypothetical Protein	2.52
PA2385	*pvdQ*	Penicillin Acylase-Related Protein	2.41
PA2386	*pvdA*	L-Ornithine N5-Oxygenase	2.06
PA2392	*pvdP*	Protein PvdP	3.43
PA2394	*pvdN*	Protein PvdN	2.49
PA2397	*pvdE*	Pyoverdine Biosynthesis Protein PvdE	2.26
PA2399	*pvdD*	Pyoverdine Synthetase D	2.52
PA2400	*pvdJ*	Protein PvdJ	2.54

PA2403		Hypothetical Protein	2.23
PA2404		Hypothetical Protein	2.10
PA2405		Hypothetical Protein	2.01
PA2406		Hypothetical Protein	2.52
PA2407		Adhesion Protein	2.06
PA2408		ABC Transporter ATP-Binding Protein	1.68
PA2409		ABC Transporter Permease	1.66
PA2410		Hypothetical Protein	1.87
PA2412		Hypothetical Protein	3.06
PA2413	*pvdH*	Diaminobutyrate-2-Oxoglutarate Aminotransferase	2.42
PA2414	*sndH*	L-Sorbosone Dehydrogenase	3.46
PA2415		Hypothetical Protein	3.53
PA2416	*treA*	Trehalase	3.15
PA2424	*pvdL*	Peptide Synthase	2.18
PA2425	*pvdG*	Protein PvdG	3.13
PA2427		Hypothetical Protein	2.04
PA2432		Transcriptional Regulator	-1.66
PA2437		Hypothetical Protein	-1.72
PA2439		Hypothetical Protein	-1.85
PA2440		Hypothetical Protein	3.79
PA2448		Hypothetical Protein	1.93
PA2467		Transmembrane Sensor	-1.54
PA2469		Transcriptional Regulator	-1.78
PA2481		Hypothetical Protein	-1.95
PA2482		Cytochrome C	-2.02
PA2483		Hypothetical Protein	-1.57
PA2493	*mexE*	Rnd Multidrug Efflux Membrane Fusion Protein MexE	-1.70
PA2494	*mexF*	Rnd Multidrug Efflux Transporter MexF	-1.80
PA2495	*oprN*	Multidrug Efflux Outer Membrane Protein OprN Precursor	-1.73
PA2507	*catA*	Catechol 1,2-Dioxygenase	-6.22
PA2508	*catC*	Muconolactone Delta-Isomerase	-6.54
PA2509	*catB*	Muconate Cycloisomerase I	-2.23
PA2510	*catR*	Transcriptional Regulator CatR	-1.67
PA2511		Transcriptional Regulator	-3.12
PA2512	*antA*	Anthranilate Dioxygenase Large Subunit	-2.39
PA2513	*antB*	Anthranilate Dioxygenase Small Subunit	-7.09
PA2514	*antC*	Anthranilate Dioxygenase Reductase	-7.8
PA2518	*xylX*	Toluate 1,2-Dioxygenase Subunit Alpha	-2.2
PA2519	*xylS*	Transcriptional Regulator XylA	-3.32
PA2531		Aminotransferase	-1.69
PA2536		Phosphatidate Cytidylyltransferase	1.57
PA2540		Hypothetical Protein	1.79
PA2551		LysR Family Transcriptional Regulator	-1.96
PA2569		Hypothetical Protein	3.29
PA2570	*pa1L*	Pa-I Galactophilic Lectin	-1.98

PA2582		Osmoprotectant Transporter Activator Protein	-1.94
PA2587	*pqsH*	Fad-Dependent Monooxygenase	1.70
PA2601		Lysr Family Transcriptional Regulator	-2.15
PA2602		Hypothetical Protein	-2.32
PA2603		Thiosulfate Sulfurtransferase	-1.86
PA2604		Hypothetical Protein	-1.66
PA2631		Acetyl Transferase	-1.96
PA2634		Isocitrate Lyase	-2.60
PA2656		Two-Component Sensor	-1.91
PA2657		Two-Component Response Regulator	-2.30
PA2658		Hypothetical Protein	-3.25
PA2659		Hypothetical Protein	-2.99
PA2675		Type Ii Secretion System Protein	-2.03
PA2681		LysR Family Transcriptional Regulator	-1.73
PA2682		Hypothetical Protein	-1.76
PA2688	*pfeA*	Outer Membrane Receptor PfeA	-2.32
PA2693		Hypothetical Protein	-1.77
PA2694		Thioredoxin	1.86
PA2696		Transcriptional Regulator	-1.74
PA2708		Hypothetical Protein	2.04
PA2711		Periplasmic Spermidine/Putrescine-Binding Protein	-3.57
PA2737		Transcriptional Regulator	-1.64
PA2738	*ihfA*	Integration Host Factor Subunit Alpha	-1.65
PA2751		Hypothetical Protein	1.53
PA2754		Hypothetical Protein	2.54
PA2758		LysR Family Transcriptional Regulator	-1.77
PA2759		Hypothetical Protein	-2.90
PA2762		Hypothetical Protein	-3.67
PA2764		Hypothetical Protein	-1.63
PA2773		Hypothetical Protein	1.96
PA2776		Hypothetical Protein	-1.82
PA2782		Hypothetical Protein	-1.88
PA2786		Hypothetical Protein	2.03
PA2814		Hypothetical Protein	-2.14
PA2833		Hypothetical Protein	-1.67
PA2846		LysR Family Transcriptional Regulator	-1.75
PA2886		Hypothetical Protein	1.57
PA2893		Long-Chain-Acyl-CoA Synthetase	-1.71
PA2895	*sbrR*	SbrR	1.69
PA2896	*sbrI*	SbrI	1.55
PA2927		Hypothetical Protein	1.82
PA2934		Hydrolase	1.92
PA2937		Hypothetical Protein	-2.46
PA2938		Transporter	-1.66
PA2939		Aminopeptidase	2.41

PA2985		Hypothetical Protein	-1.5
PA3041		Hypothetical Protein	1.64
PA3042		Hypothetical Protein	1.69
PA3049	*rmf*	Ribosome Modulation Factor	-1.90
PA3053		Hydrolytic Enzyme	-1.73
PA3069		Lipoprotein	2.08
PA3126	*ibpA*	Heat-Shock Protein IbpA	-1.93
PA3146	*orfM*	NAD Dependent Epimerase/Dehydratase	2.75
PA3161	*ihfB*	Integration Host Factor Subunit Beta	-2.96
PA3191		Two-Component Sensor	1.62
PA3195	*gapA*	Glyceraldehyde-3-Phosphate Dehydrogenase	2.02
PA3205		Hypothetical Protein	-1.91
PA3215		AraC Family Transcriptional Regulator	-1.75
PA3222		Permease	-1.5
PA3229		Hypothetical Protein	-2.5
PA3230		Hypothetical Protein	1.66
PA3231		Hypothetical Protein	1.56
PA3234	*actP*	Acetate Permease	-1.67
PA3235		Hypothetical Protein	-2.16
PA3236		Glycine Betaine-Binding Protein	-3.18
PA3273		Hypothetical Protein	2.57
PA3274		Hypothetical Protein	2.47
PA3276		Hypothetical Protein	1.51
PA3293		Hypothetical Protein	2.45
PA3294		Hypothetical Protein	2.94
PA3306	*alkB*	Hypothetical Protein	-1.57
PA3307		Hypothetical Protein	-1.99
PA3321		LysR Family Transcriptional Activator	-2.90
PA3323		Hypothetical Protein	-1.91
PA3324		Short Chain Dehydrogenase	-1.51
PA3338		Hypothetical Protein	-1.72
PA3369		Hypothetical Protein	1.94
PA3370		Hypothetical Protein	2.20
PA3371		Hypothetical Protein	1.75
PA3384	*phnC*	ABC Phosphonate Transporter ATP-Binding Protein	1.56
PA3389		Ring-Cleaving Dioxygenase	-1.52
PA3390		Hypothetical Protein	-2.31
PA3397	*fpr*	Ferredoxin-NADP+ Reductase	1.54
PA3406	*hasD*	Transport Protein HasD	-1.53
PA3417		Pyruvate Dehydrogenase E1 Component Subunit α	-3.36
PA3420		Transcriptional Regulator	-2.07
PA3422		Hypothetical Protein	-1.79
PA3424		Hypothetical Protein	-4.02
PA3425		Hypothetical Protein	-5.08
PA3427		Oxidoreductase	-2.89

PA3441		Molybdopterin-Binding Protein	4.36
PA3442	*ssuB*	Aliphatic Sulfonates Transport ATP-Binding Subunit	4.11
PA3444	*ssuD*	Alkanesulfonate Monooxygenase	4.39
PA3446		NAD(P)H-Dependent FMN Reductase	3.71
PA3449		Hypothetical Protein	4.47
PA3450	*lsfA*	1-Cys Peroxiredoxin LsfA	4.02
PA3459		Asparagine Synthetase	2.29
PA3460		Gnat Family Acetyltransferase	2.14
PA3461		Hypothetical Protein	1.99
PA3467		Mfs Transporter	-2.01
PA3473		Hypothetical Protein	-1.85
PA3478	*rhlB*	Rhamnosyltransferase Chain B	2.35
PA3479	*rhlA*	Rhamnosyltransferase Chain A	2.23
PA3529		Peroxidase	-1.90
PA3531	*bfrB*	Bacterioferritin	-2.67
PA3554		Bifunctional UDP-Glucuronic Acid Decarboxylase	-1.63
PA3566		Hypothetical Protein	-1.61
PA3567		Oxidoreductase	-1.62
PA3577		Hypothetical Protein	-1.55
PA3584	*glpD*	Glycerol-3-Phosphate Dehydrogenase	-2.61
PA3588		Porin	-1.53
PA3598		Hypothetical Protein	2.29
PA3600	*rpmJ*	50S Ribosomal Protein L36	-5.11
PA3601	*rpmE2*	50S Ribosomal Protein L31	-4.56
PA3617	*recA*	Recombinase A	-1.62
PA3623		Hypothetical Protein	-1.74
PA3677		Efflux Transmembrane Protein	-1.61
PA3687	*ppc*	Phosphoenolpyruvate Carboxylase	-1.56
PA3689	*cadR*	Transcriptional Regulator CadR	-2.57
PA3691		Lipoprotein	2.60
PA3692	*ompA*	Outer Membrane Protein, OmpA	2.52
PA3709		MFS Transporter	1.51
PA3720		Hypothetical Protein	-2.21
PA3721		Transcriptional Regulator	-1.81
PA3729		Hypothetical Protein	1.63
PA3730		Hypothetical Protein	-2.04
PA3731		Hypothetical Protein	-3.77
PA3732		Hypothetical Protein	-3.02
PA3741		Hypothetical Protein	1.92
PA3752		Hypothetical Protein	-2.14
PA3753		Hypothetical Protein	-1.96
PA3754		Hypothetical Protein	-2.13
PA3765		Hypothetical Protein	-1.75
PA3769	*guaA*	GMP Synthase	1.63
PA3795		Oxidoreductase	1.59

PA3813		Scaffold Protein	-1.62
PA3814	*iscS*	Cysteine Desulfurase	-1.84
PA3835		Hypothetical Protein	-1.71
PA3865		Amino Acid ABC Transporter	-2.48
PA3888		ABC Transporter Permease	2.51
PA3889		ABC Transporter Substrate-Binding Protein	1.59
PA3890		Abc Transporter Permease	2.05
PA3891		Abc Transporter ATP-Binding Protein	1.88
PA3895		LysR Family Transcriptional Regulator	-1.99
PA3901	*fecA*	Fe(III) Dicitrate Transport Protein FecA	-2.11
PA3904		Hypothetical Protein	1.71
PA3905		Hypothetical Protein	2.06
PA3906		Hypothetical Protein	2.89
PA3907		Hypothetical Protein	3.20
PA3908		Hypothetical Protein	2.82
PA3931		Hypothetical Protein	3.17
PA3937		Taurine ABC Transporter ATP-Binding Protein	2.34
PA3938		Taurine ABC Transporter Periplasmic Protein	3.83
PA3952		Hypothetical Protein	1.78
PA3962		Hypothetical Protein	1.83
PA3969		Hypothetical Protein	-1.61
PA4026		Acetyltransferase	-1.83
PA4063		Hypothetical Protein	-3.31
PA4065		Permease	-2.61
PA4066		Hypothetical Protein	-1.89
PA4070		DNA-Binding Transcriptional Activator Fear	-2.59
PA4078		Nonribosomal Peptide Synthetase	1.56
PA4082	*cupB5*	Adhesive Protein CupB5	-1.67
PA4094		AraC Family Transcriptional Regulator	-1.69
PA4111		Hypothetical Protein	-1.72
PA4127	*hpcG*	2-Oxo-Hepta-3-Ene-1,7-Dioic Acid Hydratase	-1.74
PA4139		Hypothetical Protein	-1.93
PA4140		Hypothetical Protein	-1.67
PA4141		Hypothetical Protein	1.87
PA4142		Secretion Protein	2.14
PA4143		Toxin Transporter	1.93
PA4144		Outer Membrane Protein	1.99
PA4148		Short-Chain Dehydrogenase	-1.8
PA4149		Hypothetical Protein	-1.59
PA4150		Dehydrogenase E1 Component	-1.76
PA4152		Branched-Chain Alpha-Keto Acid Dehydrogenase Subunit E2	-2.07
PA4153	*adh*	2,3-Butanediol Dehydrogenase	-1.67
PA4154		Sh3 Domain-Containing Protein	1.90
PA4159	*fepB*	Iron-Enterobactin Transporter Periplasmic Binding Protein	-1.74

PA4163		Amidase	-1.70
PA4164		Hypothetical Protein	-1.64
PA4171		Protease	1.80
PA4172		Exonuclease Iii	2.93
PA4175	*prpL*	PvdS-Regulated Endoprotease, Lysyl Class	1.62
PA4185		GntR Family Transcriptional Regulator	-1.87
PA4204		Hypothetical Protein	1.89
PA4222		ABC Transporter ATP-Binding Protein	1.75
PA4223		ABC Transporter ATP-Binding Protein	1.74
PA4224	*pchG*	Pyochelin Biosynthetic Protein PchG	2.33
PA4225	*pchF*	Pyochelin Synthetase	1.99
PA4226	*pchE*	Dihydroaeruginoic Acid Synthetase	1.86
PA4229	*pchC*	Pyochelin Biosynthetic Protein PchC	1.60
PA4230	*pchB*	Isochorismate-Pyruvate Lyase	2.19
PA4231	*pchA*	Salicylate Biosynthesis Isochorismate Synthase	1.94
PA4304		Type Ii Secretion System Protein	1.64
PA4305		Pilus Assembly Protein	-2.12
PA4306		Hypothetical Protein	-3.59
PA4309	*pctA*	Chemotactic Transducer PctA	-2.15
PA4324		Hypothetical Protein	-2.11
PA4344		Hydrolase	1.63
PA4345		Hypothetical Protein	1.83
PA4349		Hypothetical Protein	-1.56
PA4352		Hypothetical Protein	-1.53
PA4354		Hypothetical Protein	-1.97
PA4357		Hypothetical Protein	-1.95
PA4359	*feoA*	Ferrous Iron Transport Protein A	-2.52
PA4384		Hypothetical Protein	2.33
PA4390		Hypothetical Protein	2.65
PA4394		Hypothetical Protein	2.44
PA4442	*cysN*	Bifunctional Sulfate Adenylyltransferase Subunit 1	1.74
PA4443	*cysD*	Sulfate Adenylyltransferase Subunit 2	2.12
PA4463		Hypothetical Protein	-1.61
PA4500	*dppA3*	Dipeptide ABC Transporter Substrate-Binding Protein Dppa3	-1.57
PA4521		Hypothetical Protein	-1.51
PA4541		Large Exoprotein	-2.07
PA4552	*pilW*	Type 4 Fimbrial Biogenesis Protein PilW	2.11
PA4553	*pilX*	Type 4 Fimbrial Biogenesis Protein PilX	1.88
PA4554	*pilY1*	Type 4 Fimbrial Biogenesis Protein PilY1	1.74
PA4556	*pilE*	Type 4 Fimbrial Biogenesis Protein PilE	1.92
PA4598	*mexD*	Multidrug Efflux RND Transporter MexD	-1.91
PA4611		Hypothetical Protein	-1.73
PA4616		C4-Dicarboxylate-Binding Protein	-1.66
PA4618		Hypothetical Protein	-1.74

PA4624		Hypothetical Protein	-2.11
PA4625		Hypothetical Protein	-1.77
PA4630		Hypothetical Protein	-3.36
PA4697		Hypothetical Protein	-1.58
PA4738		Hypothetical Protein	2.24
PA4739		Hypothetical Protein	1.90
PA4763	*recN*	DNA Repair Protein RecN	-1.96
PA4791		Hypothetical Protein	-1.64
PA4799		Adenylate Kinase	1.63
PA4803		Methyltransferase	-1.90
PA4810	*fdnI*	Nitrate-Inducible Formate Dehydrogenase Subunit Gamma	-1.79
PA4811	*fdnH*	Nitrate-Inducible Formate Dehydrogenase Subunit Beta	-2.07
PA4812	*fdnG*	Formate Dehydrogenase-O, Major Subunit	-1.69
PA4825	*mgtA*	Mg(2+) Transport ATPase, P-Type 2	-1.71
PA4834		Hypothetical Protein	-4.18
PA4835		Hypothetical Protein	-3.48
PA4836		Hypothetical Protein	-5.30
PA4837		Outer Membrane Protein	-5.04
PA4838		Hypothetical Protein	-3.12
PA4876	*osmE*	Dna-Binding Transcriptional Activator OsmE	2.59
PA4877		Hypothetical Protein	2.13
PA4879		Hypothetical Protein	1.76
PA4880		Bacterioferritin	2.22
PA4881		Hypothetical Protein	-1.93
PA4885	*irlR*	Two-Component Response Regulator	-1.63
PA4899		Aldehyde Dehydrogenase	-1.74
PA4900		Mfs Transporter	-2.43
PA4904	*vanA*	Vanillate O-Demethylase Oxygenase	-2.05
PA4916		Hypothetical Protein	1.57
PA4917		Hypothetical Protein	1.79
PA4921		Hypothetical Protein	-1.95
PA4929		Hypothetical Protein	-2.38
PA4980		Enoyl-CoA Hydratase/Isomerase	-1.96
PA4984		TetR Family Transcriptional Regulator	-1.82
PA4989		Transcriptional Regulator	-1.90
PA4995		Acyl-CoA Dehydrogenase	-1.71
PA5020		Acyl-CoA Dehydrogenase	-2.41
PA5023		Hypothetical Protein	-1.87
PA5055		Hypothetical Protein	-1.70
PA5058	*phaC2*	Poly(3-Hydroxyalkanoic Acid) Synthase 2	1.64
PA5059		TetR Family Transcriptional Regulator	1.89
PA5085		LysR Family Transcriptional Regulator	-2.13
PA5099		Cytosine/Purines Uracil Thiamine Allantoin Permease	1.55
PA5109		Hypothetical Protein	1.99
PA5111	*gloA3*	Lactoylglutathione Lyase	1.85

PA5131	*pgm*	Phosphoglyceromutase	1.54
PA5161	*rmlB*	DtdP-D-Glucose 4,6-Dehydratase	1.66
PA5162	*rmlD*	DtdP-4-Dehydrorhamnose Reductase	1.59
PA5163	*rmlA*	Glucose-1-Phosphate Thymidylyltransferase	1.60
PA5164	*rmlC*	Dtdp-4-Dehydrorhamnose 3,5-Epimerase	1.70
PA5177		Hydrolase	-1.64
PA5179		LysR Family Transcriptional Regulator	-1.60
PA5206	*argE*	Acetylornithine Deacetylase	-1.63
PA5209		Hypothetical Protein	1.57
PA5218		LysR Family Transcriptional Regulator	-1.54
PA5228		5-Formyltetrahydrofolate Cyclo-Ligase	-2.63
PA5230		ABC Transporter Permease	1.57
PA5231		ABC Transporter ATP-Binding Protein/Permease	1.80
PA5266	*vgrG14*	VgrG14	2.31
PA5269		Hypothetical Protein	-1.52
PA5284		Fimbrial Protein	-2.53
PA5291		Choline Transporter	1.73
PA5297	*poxB*	Pyruvate Dehydrogenase (Cytochrome)	1.57
PA5308	*lrp*	Leucine-Responsive Regulatory Protein	-1.92
PA5312		Aldehyde Dehydrogenase	-1.55
PA5313		Omega Amino Acid-Pyruvate Transaminase	-1.73
PA5314		Hypothetical Protein	-1.86
PA5323	*argB*	Acetylglutamate Kinase	1.64
PA5324	*sphR*	Sphingosine-Responsive Regulator, SphR	-1.89
PA5325	*sphA*	SphA	-3.23
PA5326	*sphD*	SphD	1.60
PA5327	*sphC*	SphC	-2.92
PA5328	*sphB*	SphB	-2.97
PA5332	*crc*	Catabolite Repression Control Protein	-1.52
PA5379	*sdaB*	L-Serine Dehydratase	-2.89
PA5381		Hypothetical Protein	-1.75
PA5383		Hypothetical Protein	4.15
PA5388		Hypothetical Protein	-1.51
PA5389		AraC Family Transcriptional Regulator	-1.77
PA5396		Hypothetical Protein	1.59
PA5397		Hypothetical Protein	-3.78
PA5398		FMN Oxidoreductase	-3.34
PA5399		Ferredoxin	-3.49
PA5400		Electron Transfer Flavoprotein Alpha Subunit	-3.08
PA5401		Hypothetical Protein	-2.33
PA5408		Hypothetical Protein	-1.93
PA5409		Hypothetical Protein	-1.94
PA5410		Ring Hydroxylating Dioxygenase, Alpha-Subunit	-2.89
PA5411		Ferredoxin	-2.22
PA5415	*glyA1*	Serine Hydroxymethyltransferase	-2.65

PA5416	*soxB*	Sarcosine Oxidase Beta Subunit	-2.84
PA5417	*soxD*	Sarcosine Oxidase Delta Subunit	-2.69
PA5418	*soxA*	Sarcosine Oxidase Alpha Subunit	-2.69
PA5419	*soxG*	Sarcosine Oxidase Gamma Subunit	-2.71
PA5420	*purU2*	Formyltetrahydrofolate Deformylase	-2.49
PA5421	*fdhA*	Glutathione-Independent Formaldehyde Dehydrogenase	-3.21
PA5425	*purK*	Phosphoribosylaminoimidazole Carboxylase ATPase Subunit	1.77
PA5432		GnaT Family Acetyltransferase	-2.05
PA5433		GnaT Family Acetyltransferase	-2.27
PA5435		Pyruvate Carboxylase Subunit B	-2.27
PA5436		Pyruvate Carboxylase Subunit A	-2.19
PA5446		Hypothetical Protein	-3.02
PA5465		Hypothetical Protein	-1.83
PA5481		Hypothetical Protein	2.58
PA5484		Two-Component Sensor	2.00
PA5514		Beta-Lactamase	2.11
PA5518		Potassium Efflux Transporter	-1.58
PA5523		Aminotransferase	-1.8
PA5524		Short-Chain Dehydrogenase	-1.79
PA5526		Lipoprotein	1.61
PA5534		Hypothetical Protein	-5.97
PA5535		Hypothetical Protein	-5.84
PA5536		DksA/TraR Family C4-Type Zinc Finger Protein	-5.04
PA5538	*amiA*	N-Acetylmuramoyl-L-Alanine Amidase	3.16
PA5539		GTP Cyclohydrolase	-3.36
PA5540		Hypothetical Protein	-3.85
PA5541	*pyrQ*	Dihydroorotase	5.40

Table A-6. Influence of mucin on MIC. Liquid MIC results done in SCFM with 0.4% mucin and without mucin grown at 37°C overnight with an inoculum size of 2-7 x 10^5cells (n=3-5).

	MIC (μg/ml)	
Antibiotic	**+ mucin**	**- mucin**
Gentamicin	4	1
Tobramycin	2	2
Amikacin	16	4
Imipenem	0.325	0.625
Meropenem	0.125	0.125
Ceftazidime	31.25	31.25
Aztreonam	4	8
Piperacillin	4	4
Erythromycin	500	250
Clarithromycin	2000	2000
Polymyxin B	16	16
Colistin	16	2

Norfloxacin	16	16
Ciprofloxacin	1	0.5
Trimethoprim	128	128
Tetracycline	64	256
Chloramphenicol	32	16

Table A-7. Resistome mutant susceptibility under surfing conditions - raw data. Average zone of inhibition measurements for resistome mutants tested for five selected antibiotics. Mutants of up-regulated resistome genes were tested against 10 μg/disk of antibiotic and down-regulated against 100 μg/disk. Statistical significance relative to wild-type was determined using two-way ANOVA. (n=3 resistome mutants; n=6 wild-type) * $p<0.5$, ** $p<0.01$, *** $p< 10^{-3}$, **** $p<10^{-4}$. Standard deviations range from 0 to 2.5mm.

Mutant	Zone of Inhibition (mm)				
	Imipenem	**Tetracycline**	**Polymyxin B**	**Tobramycin**	**Norfloxacin**
10 μg/disk antibiotic concentration					
Wild-type	5.7	5.0	5.6	3.3	1.0
ΔrecG	7.3	8.7*	9.7**	12.5****	7.3****
ΔddaH	9.0*	0***	5.3	3.0	2.3
ΔPA5130	10**	0***	0****	0*	6.5****
cycH	4.5	4.3	11****	9.7****	0
100 μg/disk antibiotic concentration					
Wild-type	12.3	6.7	8	12	14.7
ΔarmR	0****	0****	1****	6.3****	0****
ΔPA3576	12.0	3.0*	6.0	8.3*	10.7*
ΔPA1428	12.7	7.7	8.0	7.0***	0.0****
ΔPA2047	12.3	7.0	5.7	7.3**	9.7***
ΔPA1553	9.0	7.3	7.7	8.0*	10.5*
*ΔatpB**	9.7	4.0	8.0	8.3*	9.7***
ΔPA4292	7.7**	5.3	17****	5****	10**
ΔclpS	8.3*	6.3	15****	6.7***	8.3****
ΔnuoB	10.7	0****	18****	6.3****	10.3**
ΔPA3721	10	2**	14.5****	0****	10**
ΔPA4429	11	8.7	20****	0****	7.7****
ΔetfA	12.3	9	15****	7.3**	10**
ΔnuoG	9.7	6.5	6	0****	7.3****
ΔPA4781	12.3	10	10.3	6.7***	9****
ΔserA	14.7	8.7	12.7**	7.3**	11*
ΔccmF	14	8.7	13.3***	7.3**	8.7****
ΔPA3667	15.7	0.0****	7.7	10.0	12.0
ΔPA1513	10.7	0.0****	7.3	10.7	14.0
ΔpchF	9.7	5.0	7.3	1.1****	13.3
Δrph	13.0	9.0	6.0	5.0****	15.0
ΔPA2566	11.0	4.0	7.0	9.3	9.7***
ΔgidA	9.7	7.3	9.0	10.5	10.0**

ΔmutS	16.0	9.3	4.3*	6.3****	11.5
ΔthiG	6.3****	6.7	7.0	8.7	10.3**
ΔnuoF	11.0	5.3	6.7	7.0***	14.7
ΔpckA	9.7	4.8	7.0	6.3****	12.0
ΔPA2571	12.7	6.7	7.0	7.3**	11.7
ΔPA4766	13.7	6.0	6.5	7.0***	13.7
ΔPA1348	16.7**	5.5	8.3	10.7	10.7*
ΔbraB	11.0	6.7	5.7	10.0	10.7*
ΔhtpX	12.7	5.7	8.3	11.0	9.5**
ΔspeA	11.3	4.5	5.3	10.3	12.0
ΔadhA	12.3	7.0	6.7	10.3	13.3

Table A-8. RT-qPCR results confirmed the dysregulation of resistome genes shown in RNA-Seq. The relative fold-change of expression of select resistome genes under surfing conditions (SCFM + 0.4% mucin) relative to swimming (SCFM 0.3% agar) from both the RNA-Seq experiment and RT-qPCR of cells collected from the centre and edge of a surfing colony relative to swimming cells. (FC cut-off of RNA-Seq is ± 1.5).

	Gene Expression (FC)			
	RT-qPCR		**RNA-Seq**	
Gene	**Centre**	**Edge**	**Centre**	**Edge**
recG	16.4	8.1	1.9	2.1
PA5130	2.2	1.5	NC	2.4
ddaH	2.8	3.2	4.9	2.4
PA1428	-2.2	-3.3	-3.4	NC
PA2047	3.1	-1.4	NC	-2.1
thiG	-2.5	1.8	-2.9	NC
PA3667	-1.2	-3.4	-1.7	-2.5
PA3576	2.3	-4.4	NC	-2.9
atpB	-1.5	1.1	-2.1	NC
PA4292	-2.3	-2.4	-6.7	NC
nuoB	-1.0	-1.2	-2.8	NC
PA3721	-2.0	2.1	-5.3	-2.7
clpS	5.0	-3.8	NC	-2.3
armR	-4.4	2.9	-3.2	-5.1
cycH	5.7	1.4	NC	2.2