PEPTIDE 1018 INHIBITS SWARMING MOTILITY AND DYSREGULATES TRANSCRIPTIONAL REGULATORS OF SWARMING IN *PSEUDOMONAS AERUGINOSA*

by

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Abstract

Pseudomonas aeruginosa is a Gram-negative environmental pathogen responsible for considerable human morbidity and mortality, especially in vulnerable hospital populations and individuals with cystic fibrosis. Much of this impact stems from its enormous capacity to adapt, colonize, and thrive in a broad variety of host and environmental niches. In *P. aeruginosa,* adaptive behaviours like biofilm formation and swarming motility can confer significant but conditionally reversible multiple antibiotic resistance, and considerably reduce the efficacy of many clinical antibiotics. Swarming motility is a transitory adaptive behaviour that is induced under stringent conditions (e.g., nutrient limitation, medium viscosity) and has been linked to both in vivo virulence and acute infection in *P. aeruginosa*. A small, synthetic host defense peptide, 1018, with weak bactericidal activity inhibits the adaptive behaviour biofilm formation at low concentrations in a broad spectrum of Gram-negative and Gram-positive pathogenic bacteria. It also shows synergy with a number of conventional antibiotics. This study aimed to investigate the effect of this peptide on swarming motility.

Peptide 1018 inhibited swarming motility at low concentrations in *P. aeruginosa* and disrupted the expression of seventy-four regulatory genes, including ten of the thirty-five genes identified as swarming regulators. Peptide treatment of bacteria also induced a gene expression profile with significant similarity (67.7%) to cells with a stationary, biofilm-like phenotype. A moderate number of *P. aeruginosa* mutants with single gene interruptions showed weak tolerance to peptide 1018, and the majority of these interrupted genes were linked to adaptation and survival under stringent conditions. The tolerance phenotype associated with two of these genes, *rhlB* and *anr*, was confirmed by complementation. Enhancing the bacterial stringent response through induced amino acid starvation appeared to improve the tolerance of *P. aeruginosa* to peptide 1018 in a swarming environment. Under these conditions, the wild-type strain and the peptide-tolerant mutants showed respective rescued and enhanced swarming motility when treated with peptide 1018. This study thus supports a link between the mechanism of action of peptide 1018 and the stringent response and demonstrates that peptide 1018 inhibits and broadly dysregulates swarming motility, an adaptive behaviour promoting enhanced antibiotic resistance.

Lay Summary

Pseudomonas aeruginosa is a bacterium that commonly causes serious hospital-acquired infections and eventually fatal lung infections in people with cystic fibrosis. It is challenging to treat because of its innate antibiotic resistance and adaptive ability. Most clinical antibiotics are ineffective against *P. aeruginosa* infections. This study examined the effects of a synthetic peptide on swarming motility, an adaptive behaviour linked to acute infection and adaptive antibiotic resistance. The peptide inhibited swarming motility and dysregulated several key regulatory genes that control adaptive behaviours in *P. aeruginosa*. The anti-swarming effect of this peptide was reduced when bacteria were treated with a chemical that induced the bacterial stringent (stress) response. The stringent response regulates adaptive behaviour in *P. aeruginosa*, and peptide 1018 binds to and promotes degradation of the two central stringent response signalling molecules. Peptide 1018 may inhibit swarming motility, at least in part, by dysregulating both the stringent response and several regulatory genes that control adaptive behaviour and antibiotic resistance.

Preface

The majority of the experimental work in this thesis was conducted by the author, L. Wilkinson. No part of this text was taken directly from previously published work.

In Chapter 2, Catherine (Bing) Wu, who at the time was a University of British Columbia (UBC) undergraduate student, assisted in conducting additional replicates of the broad and confirmatory swarming screens. Bradford Ross (UBC Bioimaging facility) performed the transmission electron microscopy imaging described in this chapter. I prepared all of the samples for imaging, selected the images to capture from each sample and analyzed these images. Manjeet Bains and Dr. Daniel Pletzer advised me regarding the experimental design for the complementation assay of the *anr* and *rhlB* PA14 transposon insertion mutants. I performed all other experimental work in this chapter. Dr. Bob Hancock provided input on experimental design and the results of this research.

In Chapter 3, Reza Falsafi performed the purity assessment, enrichment, indexing, and RNA-Seq on the RNA that I isolated from my biological samples. Dr. Amy Lee assisted with the conducting principal component analysis, generating read count tables, and estimating the fold-change in gene expression between different sample groups. She also provided feedback on my interpretation of the data. I performed the rest of the experimental work and analysis in this chapter. Dr. Bob Hancock provided advice and guidance through the interpretation of the RNA-Seq data.

In chapter 4, Dr. Daniel Pletzer provided the clean deletion stringent response mutants. I performed all of the experimental work in this chapter. Dr. Bob Hancock provided input on my experimental design and gave feedback on my interpretation of the results.

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List of Abbreviations

BHI	Brain heart infusion medium
BM2	Basal Medium 2
bp	Base pair
cAMP	Cyclic adenosine monophosphate
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
C_4-HSL	N-butyryl-homoserine lactone
DE	Differentially expressed
EPS	Extracellular polymeric substance
ev	Empty vector
Fmoc	Fluorenylmethoxy carbonyl
gDNA	Genomic DNA
Gen	Gentamycin
HAP	Hospital-acquired pneumonia
HAA	3-(3-hydroxyalkanoyloxy) alkanoic acid
HDP	Host defense peptide
HPLC	High-performance liquid chromatography
ICU	Intensive care unit
IDR	Immune defense regulator
Kan	Kanamycin
LESB58	Liverpool epidemic strain
LPS	Lipopolysaccharide
LB	Luria-Bertani medium
MBL	Metallo-β-lactamases
PCA	Principal component analysis
(p)ppGpp	Guanosine pentaphosphate and guanosine tetraphosphate
PQS	*Pseudomonas* quinolone signal
QS	Quorum sensing
SBC	UBC Sequencing and Bioinformatics Consortium
SHX	L-serine hydroxamate
T2SS	Type II secretion system
T3SS	Type III secretion system
TCR	Two-component response regulator
TCS	Two-component system
TEM	Transmission electron microscope
-Tn	Transposon
VAP	Ventilator-associated pneumonia
WHO	World Health Organization

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Dr. Erin Gaynor and Dr. John Smit, my committee members, have been both patient and encouraging as I have pursued this degree. Their constructive feedback has shaped this thesis. I thank them for their support and their mentorship.

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I cannot quantify my gratitude for my loving and patient family. Mom, Dad, Jerome, Michael and Cam, thank you for your support, patience, love, and fine coffee during this degree. Your conviction in my ability to see this master's thesis through kept me progressing through my most challenging moments.

Chapter 1: Introduction

1.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a ubiquitous and highly adaptable Gram-negative bacterium of critical importance to public health. It is a leading cause of health care-associated infections and accounts for significant mortality and morbidity in immunocompromised individuals. It is also the primary bacterium associated with eventually fatal chronic lung infections in individuals with Cystic Fibrosis (CF) (1). The World Health Organization (WHO) recently named *P. aeruginosa* as one of the three most critical antibiotic-resistant bacteria threatening public health on a global scale (2). *P. aeruginosa* has steadily become resistant to a broad range of antibiotics, including last-resort drugs such as polymyxin B and carbapenems (3). Novel and effective therapeutics are desperately needed, especially those developed with an understanding of the resistance mechanisms and lifestyle choices that *P. aeruginosa* employs to colonize and survive in the host environment.

1.1.1 *P. aeruginosa* as a nosocomial pathogen

P. aeruginosa displays remarkable levels of genomic complexity and flexibility. It has one of the largest genomes among sequenced bacteria (5.5-7Mbp), and approximately one-tenth of its genes function as regulators (4,5). *P. aeruginosa* can rapidly sense its environment, modulate its gene expression, and ultimately colonize a diverse range of niches. This adaptive ability presents significant challenges in the context of human health. *P. aeruginosa* is a major nosocomial pathogen; it rarely infects healthy individuals with intact physical barriers and robust immune systems but instead poses a serious threat in hospitals which contain a large number of vulnerable individuals (6).

P. aeruginosa is a leading cause of acute and chronic pulmonary infections (7). It is responsible for an estimated 13.2-22.6% of infections in intensive care units (ICUs) and is the most common Gram-negative bacterium associated with hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) (7–9). It also frequently responsible for central line-associated bloodstream infections, urinary catheter-related infections, surgical infections and transplantation and implant-associated infections (10,11). As a consequence, *P. aeruginosa* infections burden health care systems with significant levels of patient morbidity and mortality and contribute to longer stays for hospitalized individuals (12).

P. aeruginosa has also been isolated from nearly every conceivable hospital surface,

including soaps, sinks, medical equipment, and clothing, and each of these areas is a potential reservoir for nosocomial *P. aeruginosa* infections (13,14). *P. aeruginosa* is highly resilient and difficult to eradicate from surfaces, further highlighting why its inherent genomic flexibility and adaptability pose such a challenge in health care settings (15,16).

1.1.2 *P. aeruginosa* in cystic fibrosis

P. aeruginosa infections are particularly devastating in individuals with CF, the most common eventually fatal genetic disease among individuals of Northern European descent (17). CF is typified by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), a gene that encodes a transmembrane chloride-conducting channel. This dysfunction has systemic consequences for affected individuals, but most critically, it results in poor mucocilliary clearance and chronic retention of a thick layer of mucus in the lungs. Normal mucocilliary clearance functions to remove foreign material, including bacteria, from the lungs (18).

Individuals with CF are prone to pulmonary infections, and an estimated 37-46.4% of patients are colonized with *P. aeruginosa* (19,20). Between 80-95% of CF patients ultimately perish from respiratory failure brought on by chronic, progressive lung infection. *P. aeruginosa* is the predominant Gram-negative pathogen in the majority of these infections (19,20). The high level of genomic complexity and flexibility that enables *P. aeruginosa* to colonize a broad array of niches presents extraordinary challenges during attempts to treat these infected individuals. *P. aeruginosa* can resist clearance by antimicrobials and the host immune system and persists as a chronic infection in the lungs of CF patients (21). Over the course a chronic infection, *P. aeruginosa* routinely acquires resistance to each antibiotic used in treatment, cumulating in an intractable infection and respiratory failure. At this point, the lungs are typically so damaged that lung transplantation is the only viable intervention (22,23).

1.2 Mechanisms of resistance in *P. aeruginosa*

P. aeruginosa employs multiple mechanisms to evade and resist the effects of conventional antibiotics and the pressures of the host immune system. These mechanisms can be grouped into three broad categories: intrinsic, acquired, and adaptive resistance. The cumulative effect of these resistance mechanisms gives rise to *P. aeruginosa* strains that are effectively resistant to all currently available therapeutics, including last-resort drugs such as polymyxin-B and carbapenems (24).

1.2.1 Intrinsic resistance

Intrinsic resistance refers to features that are naturally chromosomally present in all *P. aeruginosa* strains. These features are independent of horizontal gene transfer or antibiotic exposure and are constitutively expressed. Intrinsic resistance features such as low outer membrane permeability (due to limited porin mediated permeation), inducible chromosomal β-lactamase (e.g., AmpC), and multidrug efflux pumps (e.g., MexAB-OprM) are inherent to *P. aeruginosa* and contribute to low levels of susceptibility to multiple classes of drugs including β-lactams, macrolides, aminoglycosides, tetracyclines, sulfonamides, and most fluoroquinolones (25–27).

1.2.2 Acquired resistance

P. aeruginosa can acquire resistance through mutation under selective pressure from antibiotic treatment and the host immune response, as well as through horizontal transfer of mobile genetic elements from other bacteria. Single mutations can confer breakthrough resistance (minimal inhibitory concentrations – MICs – higher than the clinical breakpoint) but can also confer modest levels of drug resistance; however, the cumulative effect of several mutations can dramatically reduce antibiotic susceptibility. This stepwise acquisition of resistance may only become apparent in a clinical setting once several mutations are accumulated, a gradual process that is referred to as 'creeping baseline resistance' (28). The efficacy of small molecule antibiotics like β-lactams is already limited due to the intrinsic low outer membrane permeability of *P. aeruginosa*. Mutational loss of porins such as OprF and OprD and mutations that increase levels of β-lactamases, such as the disruption of *ampR*, or dysregulate efflux systems, can synergistically increase antibiotic resistance (29,30).

P. aeruginosa can also rapidly acquire resistance and multidrug resistance through resistance cassettes obtained through horizontal transfer. Plasmid-acquired resistance to last resort drugs like carbapenems has been documented in clinical strains of *P. aeruginosa* (31). Acquired or transferrable resistance cassettes containing metallo-β-lactamases (MBL) can confer resistance to penicillins, cephalosporins, and carbapenems. The majority of these MBLs are plasmid encoded and have circulated among *Enterobacteriaciae*, as well as P. aeruginosa (32,33). These plasmids spread resistance traits between a variety of bacterial species and present a serious threat to public health.

1.2.3 Adaptive resistance

The large genome and huge regulatory capacity of *P. aeruginosa* enable it to colonize diverse niches and enhance its survival in the hostile conditions present during antibiotic treatment. Adaptive resistance often influences susceptibility to multiple antibiotics and involves collective and completely reversible changes in bacterial gene expression in response to specific environmental stimuli. Factors such as nutrient limitation, oxidative stress, shearing forces, heat, and changes in substrate viscosity can induce the complex community behaviours which include adaptive resistance (34). Cooperative behaviours such as biofilm formation and swarming motility promote broad phenotypic changes in cell physiology and morphology and enable *P. aeruginosa* to resist antibiotics at concentrations hundreds to thousands of times higher than those that eradicate planktonic cells (35,36). Outside of the lab, bacteria are faced with a wide array of environmental conditions. It is useful to consider adaptive behaviours like biofilm formation and swarming motility as part of a gradient of flexible community phenotypes. They permit bacteria to rapidly switch between different lifestyle modes as conditions warrant, in order to optimize survival and to colonize new niches.

1.2.3.1 Biofilms

Biofilms are surface-associated, sessile, highly structured and differentiated bacterial communities encased in a self-produced extracellular polymeric substance (EPS) matrix (37). They play a central role in bacterial persistence and chronic infection and are estimated to be involved in upwards of two-thirds of all bacterial infections (38). The biofilm state also confers considerable levels of adaptive resistance against antimicrobial agents and protects bacterial communities from clearance by the host immune system (39). Biofilm characteristics such as differentiated cell populations, reduced cell growth and metabolism, and structural features like EPS exhibit contribute to their intractable phenotype. Biofilms exhibit a 10-1000 fold decrease in susceptibility to most conventional antibiotics in contrast with planktonic cells. Conversely, biofilms exclude neutrophils while the rhamnolipids produced by biofilms cause cellular necrosis and eliminate neutrophils, thereby diminishing the efficacy of the innate immune response to infection (40–42).

P. aeruginosa readily forms biofilms on a broad range of biotic and abiotic surfaces, at liquid-surface interfaces, and under shearing forces like liquid flow (15). Biofilms can be comprised of a single species or multiple species of organisms, and they are thought to be the

predominant state of existence in Nature of most bacteria (43–45). Like other adaptive behaviours, the biofilm state is, in its essence, a finely-tuned survival response to environmental stressors. Cell-cell chemical communication, or quorum sensing (QS), is vital for the coordinated execution of adaptive behaviours like biofilm formation. Three QS systems have been identified in P. aeruginosa, two that are homoserine lactone-based (LasRI, RhlRI) and the Pseudomonas quinolone signal (PQS). These communication mechanisms, triggered by bacterial population density (the quorum), aid bacteria in regulating virulence and in adaptive lifestyles like biofilm and swarming states (46,47).

1.2.3.2 Swarming motility

Swarming is a reversible and highly regulated adaptive state that enables bacteria to rapidly move across semisolid surfaces and colonize new niches in a coordinated manner (15). In P. aeruginosa, swarming occurs on viscous surfaces (typically 0.5-0.7% agar), under nitrogen-limited conditions (e.g., amino acids as a nitrogen source) and in the presence of specific amino acids and carbon sources (48). Swarming colony phenotypes vary between different P. aeruginosa strains and under different nutrient conditions (Fig. 1).

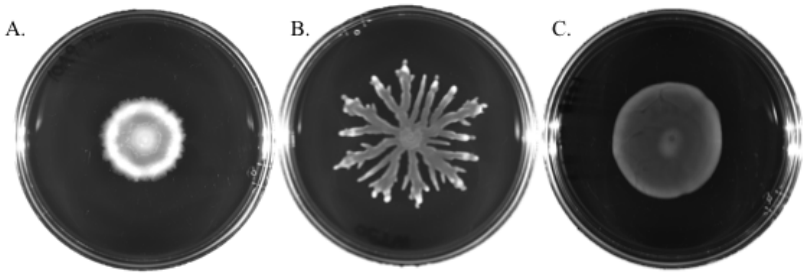

Figure 1. Colonial morphology of swarming cells (A) PAO1 is the most commonly used research strain and was derived from an original PAO wound isolate in Australia (4). It is moderately virulent and swarms in a solar flare-like pattern on Basal Medium 2 (BM2). **(B)** PA14 is hyper-virulent burn wound isolate and a common laboratory reference strain (5). It swarms in a radiating, dendritic pattern on BM2 **(C)** The Liverpool epidemic strain (LESB58) is a hypervirulent CF lung isolate (49). After a significant lag phase, it swarms in a uniform, expanding circular shape in 20% brain heart infusion (BHI) medium.

Actively swarming cells are elongated, typically possess two polar flagella (as opposed to the single polar flagellum of swimming *Pseudomonas*), and in some instances, require the presence of type IV pili (36,50,51). A wetting agent is also essential in order for *P. aeruginosa* to overcome surface tension (48). *P. aeruginosa* produces rhamnolipids that act as biosurfactants,

and the rhamnolipid precursor, 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA), is the absolute minimal surfactant required for swarming motility (52). Rhamnolipids are a QS-dependent virulence factor in *P. aeruginosa* and in addition to their role as surface-wetting agents in swarming motility, they function as signalling molecules for both biofilms and swarming colonies (51,53).

Swarming motility is a complex lifestyle adaptation. Colonies of swarming bacteria undergo major shifts in gene expression beyond those required merely for motility. Approximately 7.5% of the *P. aeruginosa* genome is dysregulated under swarming conditions when compared to swimming bacteria, including genes involved in cellular metabolism, stress response, and virulence (54). A previous comprehensive study of *P. aeruginosa* PA14 transposon insertion mutants revealed thirty-five transcriptional regulators that significantly modulate swarming motility, and supports the notion that swarming motility is a highly regulated and specialized adaptive behaviour (36). Swarming colonies are differentiated, with two main cell phenotypes; the actively swarming cells at the leading edge of the colony, and the higher density, more sessile cells in the centre of the colony (55). These cell phenotypes have distinct gene expression profiles, with many virulence, metabolic, and stress response genes showing inverse regulation (55). When compared to planktonic bacteria, swarming cells show enhanced resistance to antimicrobials and overexpress virulence factors such as toxins and extracellular proteases (e.g., *lasB, ladS*) secreted by the Type II secretion system (T2SS) and the Type III secretion system (T3SS), which can promote pulmonary colonization and tissue damage (54,56).

1.3 The stringent response

The stringent response is a highly conserved environmental stress response that is activated under conditions such as nutrient limitation, heat shock and antibiotic treatment. Under stressful conditions, *P. aeruginosa* synthesizes guanosine pentaphosphate and guanosine tetraphosphate (referred to collectively as (p)ppGpp), small nucleotide second messengers which act as alarmones and instigate broad transcriptional changes in bacteria (57). Two genes, *relA* and *spoT,* mediate the stringent response through the production and degradation of (p)ppGpp. While *spoT* can both synthesize and degrade (p)ppGpp and is induced in response to environmental stressors like phosphate and iron starvation, *relA* can only synthesize (p)ppGpp and is induced by ribosomal binding of uncharged tRNA during amino acid starvation (58). When activated, the stringent response modulates transcriptional expression and bacterial phenotypes to enable

bacteria to overcome stringent conditions (e.g., altering cell metabolic and energy requirements) (57,58). Adaptive resistance is mediated by the stringent response which in turn, is regulated by expression of (p)ppGpp (58,59). Bacteria that are (p)ppGpp-deficient experience reduced in vivo pathogenicity and reduced biofilm formation and swarming motility (57,59,60).

1.4 Adaptive behaviour as a lifestyle choice in cystic fibrosis

During chronic infections of the CF lung, *P. aeruginosa* cells typically show a reduction in virulence factors such as the siderophores pyocyanin and pyoverdine and typically convert to mucoid (alginate-producing), and LPS O-antigen lacking (rough) phenotypes (61,62). High levels of alginate production have been (controversially) linked to the biofilm state and provide a physical and chemical barrier to help protect bacteria from the host immune system. Alginate has also been shown to limit the effects of free radicals from activated macrophages, neutrophil chemotaxis, and phagocytosis (7,63). In contrast, actively motile cells are highly immunogenic and are associated with greater disease severity and acute infection (64).

While biofilms are considered to have a role in chronic CF lung infections, swarming may factor in during the colonization of lung microenvironments (65). The CF lung is a complex environment, with concentration gradients of many factors relevant in both biofilm formation and swarming motility. *P. aeruginosa* swarms on semisolid surfaces, conditions which may resemble the environment of viscous sputum and mucous coated epithelial surfaces found in the CF lung (22,54,66). Other important factors for *P. aeruginosa* swarming such as the presence of a poor nitrogen source (amino acids) and production of rhamnolipids have also been described in the CF lung (67,68).

The characteristic accumulation of thick mucus in the CF lung paradoxically creates a largely anoxic host environment. *P. aeruginosa* is a facultative anaerobe and favours forming robust biofilms under these conditions, although it can also swarm (69–71). The CF lung is rich with regional niches that drive heterogeneity in bacterial populations (72). Although the predominant adaptive phenotype in chronic CF lung infections is thought to be the biofilm state, swarming motility may factor in the colonization of regional niches and bacterial dispersal, especially during acute exacerbations of pulmonary infections (21,73).

There is a growing body of research on the mechanisms leading to adaptive resistance during biofilm formation, however much less is understood about similar mechanisms at play during swarming motility (25). There are currently no effective, clinically available anti-biofilm or anti-

swarming drugs. Mature biofilms resist eradication by conventional antibiotics at concentrations that could grievously harm patients so, at present, antibiotic treatment during chronic infection serves more to treat the intermittent exacerbations of infections and prevent cell dispersal, rather than to eradicate mature colonies and provide a 'cure' (37,74).

It is challenging to generate lab conditions that mimic the dynamic environments that bacteria experience in vivo, but research into therapeutics that address the adaptive response is critical. Drugs can have drastically different efficacies depending on the bacterial phenotypes present at the time of treatment. The current array of clinically available treatments does not adequately address the challenges presented by adaptive resistance during infections (75).

1.5 Host defense peptides as inhibitors of adaptive behaviour

Host defense peptides (HDP) are an ancient and fundamental feature of the immune system and along with their synthetic derivatives have great potential as therapeutics (76). HDPs are typically short (10-50 amino acids), cationic (net positive charge +2 to +9), and amphipathic, containing a high proportion of hydrophobic residues (76,77). They possess incredible diversity in both sequence and biological activity across species. While some cationic antimicrobial peptides like the polymyxins act through direct and potent killing mechanisms, many HDPs have weak antimicrobial activity (76). Polymyxins have been in clinical use since the 1950s and act in part by binding to the lipopolysaccharide (LPS) of the bacterial outer membrane, promoting their access to internal targets which are not well understood but might include disruption of cellular respiration and ultimately cell lysis (76,78). However, bacteria, including *P. aeruginosa* are increasingly developing resistance to this last resort drug class (3). HDPs with indirect mechanisms of action or diverse microbial targets might have greater clinical longevity than conventional bactericidal drugs that target a limited number of essential bacterial proteins (79).

Even though bacteria share an ancient history of exposure to HDPs, these peptides remain an effective and essential part of the innate immune system and do not encounter widespread bacterial resistance (79,80). Furthermore, synthetic peptides can be designed to have specific clinical functions by altering physiochemical and structural features such as residue composition, hydrophobicity, and charge (81,82). A recent study by Haney et al. demonstrated that peptides could be computationally modelled and screened in-silico to identify and select for specific therapeutic activities (immune modulating, anti-biofilm, antimicrobial) in vitro and in vivo (83). While therapeutics with direct killing mechanisms remain clinically significant, synthetic

peptides designed with immunomodulatory activities or anti-biofilm or anti-swarming activity may prove invaluable as novel therapeutics.

1.6 Innate defense regulator (IDR-) peptide 1018

Peptide IDR-1018 (VRLIVAVRIWRR-NH2) is a broad-spectrum synthetic peptide loosely derived from bactenecin, a bovine host defense peptide (84,85). This peptide has relatively weak antimicrobial activity against planktonic cells (85). Conversely, it has shown anti-biofilm activity against a range of multidrug-resistant, clinical strains of bacteria at low to moderate concentrations (as low as 0.8μg/mL), potent anti-infective immunomodulatory effects, and up to 64-fold synergy with several conventional antibiotics against biofilms (85,86).

There is evidence that under biofilm and in vivo conditions, peptide 1018 binds and promotes the degradation of the stringent response alarmones, (p)ppGpp (85,87,88). Given that (p)ppGpp and the stringent response are critical for both the chronic (biofilm) and acute (swarming) adaptive lifestyles, and an impaired stress response can induce bacterial sensitivity to currently available antimicrobials, peptide 1018 has potential as a representative of a new and finessed class of therapeutics (88). This potential stems from peptide 1018's ability to prevent and inhibit adaptive resistance behaviours in a broad range of multidrug-resistant, clinical strains of bacteria, its synergy with several conventional antibiotics, and its anti-infective immunomodulatory effects (8,11).

1.7 Goals of this study

Peptide 1018 inhibits biofilm formation and induces the dispersal of mature biofilms, however, its effect on actively swarming bacteria has not-as-yet been documented. Swarming motility is a complex and transitory adaptive behaviour and likely reflects only a small portion of the life history of a bacterial community. These characteristics present challenges for in vivo studies. However, given the association between swarming motility, antibiotic resistance, and acute virulence, research into this adaptive behaviour could add to the collective understanding of bacterial lifestyle choices and also yield clinically significant insights. There has been little research into novel methods of inhibiting adaptive resistance during acute infection, and in fact, research into the clinical significance of transitory adaptive behaviours like swarming motility during infection is still in its nascence (58,89).

Swarming motility is associated with acute infections, and relatively few studies have

investigated novel methods of targeting this adaptive behaviour. This study aimed to investigate the effects of peptide 1018 in the context of swarming motility in *P. aeruginosa*.

Hypotheses:

1) Peptide 1018 dysregulates many of the transcriptional regulators important for swarming motility and induces broad changes in global gene expression.

2) Resistance to peptide 1018 will be minimal in single gene transposon insertion mutants, as this peptide likely acts in an indirect and multigenic/complex manner. Resistance will thus require multiple mechanisms of action.

3) The stringent response is involved in the mechanism of action for peptide 1018 under swarming conditions.

Chapter 2: Peptide 1018 inhibits swarming motility in PA14 with minor tolerance phenotypes observed in transposon insertion mutants linked to the stringent response

2.1 Introduction

Peptide 1018 inhibits biofilm formation and induces biofilm dispersal in a broad spectrum of bacteria, including *Staphylococcus aureus*, and *Klebsiella pneumoniae. E. coli,* and *P. aeruginosa* (87). Both the biofilm state and swarming motility are complex community behaviours that confer substantial levels of adaptive antibiotic resistance, thus limiting antibiotic efficacy (54,90,91). The resistance associated with swarming and biofilm phenotypes is reversible and is lost upon transfer to a liquid medium and cell dispersal (35,92).

Biofilm formation and swarming motility share large networks of regulatory genes, including ones involved in quorum sensing, expression of virulence factors, flagella and pili production, and the stringent response (15,36). Both typically occur when bacteria are exposed to specific and stressful environmental stimuli, but while the biofilm state is a sessile adaptation linked with chronic infection, swarming motility is associated with immunogenicity and acute virulence (86,93). Swarming is thought to be important for the initial attachment and eventual dispersal of cells in biofilms, and there is evidence that the two behaviours are inversely regulated (36,94,95). Bacteria that are deficient in swarming motility form flat, unstructured biofilms and the expression of several regulatory systems, including *sbrIR*, *sadBC,* and *algRZ*, direct the switch between these two lifestyle adaptations (51,95,96). Unlike swimming motility, which is entirely dependent on a functional flagellum, the adaptive swarming phenotype requires complex regulatory elements (e.g., QS, stringent response) and is only induced in the presence of specific environmental stimuli (15).

Peptide 1018 exhibits relatively weak antimicrobial activity against planktonic cells and instead has robust anti-biofilm and immunomodulatory effects (85). There is evidence that peptide 1018 inhibits the stringent response by binding to and stimulating the degradation of the stringent response alarmone, (p)ppGpp, which effectively inhibits biofilm formation and induces the dispersal of pre-formed biofilms (85,87,88). Reduced levels of (p)ppGpp also inhibit swarming motility (59). Stringent response-deficient mutants (Δ*relA*Δ*spoT*) are defective in swarming motility and show reduced expression of swarming features like T3SS and rhamnolipid and elastase production(59,97,98). The impact of peptide 1018 on actively swarming bacteria had not yet been documented.

2.2 Materials and methods

2.2.1 Bacterial strains and plasmids

The PA14NR Library consists of 5,850 transposon insertion mutants corresponding to 4,596 predicted PA14 genes and was used to screen for swarming mutants that were tolerant to the inhibitory effects of peptide 1018 (99). All other bacterial strains and the plasmids used in this chapter are listed in Table 1. *Escherichia coli* TOP 10 cells were used for standard genetic manipulations such as cloning and plasmid construction.

Table 1. List of Strains and Plasmids

Strain or Plasmid	Description	Reference
E. coli		
E. coli TOP10	DH5α parent; F^- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*_M15 Δ*lacX*74 *rec*A1 *ara*D139 Δ(*ara*-leu)7697 *gal*U *gal*K *rps*L (STR^R) *end*A1 *nup*G	Invitrogen
P. aeruginosa		
PA14 WT	Laboratory wild type *P. aeruginosa* strain PA14	(100)
PA14 *rhlB::*MAR2xT7	*rhlB* transposon mutant; Gen^R	(99)
PA14 *anr::MAR2xT7*	*anr* transposon mutant; Gen^R	(99)
*PA*14 WT (pBBR2)	Wild type *P. aeruginosa*, with pBBR1MCS-2, Kan^R (empty vector)	This study
PA14 *rhlB::*MAR2xT7 (pBBR2)	PA14 *rhlB*::MrT7; Gen^R, with pBBR1MCS-2, Kan^R (empty vector)	This study
PA14 *anr::MAR2xT7* (pBBR2)	PA14 *anr*::MrT7; Gen^R, with pBBR1MCS-2, Kan^R (empty vector)	This study
PA14 *rhlB*::MAR2xT7 (pBBR2.*rhlB*)	PA14 *rhlB*::MrT7; Gen^R, with pBBR1MCS-2 containing *rhlB,* Kan^R	This study
PA14 *anr*::MAR2xT7 (pBBR2.*anr*)	PA14 *anr*::MrT7; Gen^R, with pBBR1MCS-2 containing *anr*, Kan^R	This study
PA14 *anr*::MAR2xT7 (pBBR2.*anr-apt*)	PA14 *anr-apt*::MrT7; Gen^R, with pBBR1MCS-2 containing *anr-apt*, Kan^R	This study
Plasmids		
pCR-Blunt II-TOPO	PCR cloning vector; Kan^r	Invitrogen
pCR-*rhlB*	pCR-Blunt II-TOPO containing 1.4kb *rhlB* gene	This study
pCR-*anr*	pCR-Blunt II-TOPO containing 1kb anr gene	This study
pCR-*anr-apt*	pCR-Blunt II-TOPO containing 1.6kb *anr-apt* operon	This study

Strain or Plasmid	Description	Reference
pBBR1MCS-2	Broad-host-range cloning vector, Km^R*lacZα*, mob^+, P*T3*	(101)
pBBR2.*rhlB*	pBBR1MCS-2 containing 1.4kb *rhlB* fragment from pCR-*rhlB*	This study
pBBR2.*anr*	pBBR1MCS-2 containing 1kb *anr* fragment from pCR-*anr*	This study
pBBR2.*anr-apt*	pBBR1MCS-2 containing 1.6kb *anr-apt* fragment from pCR-*anr-apt*	This study

2.2.2 Growth conditions

Bacterial strains were grown overnight in Luria-Bertani (LB) broth at 37°C under shaking conditions. Unless specified, overnight cultures were sub-cultured into either LB broth or BM2 minimal glucose medium [62 mM potassium phosphate buffer (pH 7), 7 mM $(NH4)_2SO_4$, 2 mM MgSO4, 10 μM $FeSO_4$, 0.4% (wt./vol) glucose] at a normalized optical density at 600nm (OD_{600}) of 0.1 and grown to mid-log phase (OD_{600} of 0.4-0.6) at 37°C under shaking conditions. When required for transposon or plasmid selection and maintenance, antibiotics were used in the following concentrations: 15μg/mL gentamicin (Gen) for MAR2xT7, 50μg/mL kanamycin (Kan) for pCR-Blunt II-TOPO and pBBR1mcs-2 *E.coli*, and 300μg/mL Kan for pBBR1mcs-2.

2.2.3 Swarming assays

2.2.3.1 Broad library swarming screen

A high throughput swarming screen was modified from Overhage et al. (102), to examine the inhibition of swarming motility by peptide 1018 in the P14NR transposon insertion mutant library. Bacteria were grown in LB overnight in 96-well plates. A custom-made 98-pin stamp was used to transfer approximately 1μL of overnight culture onto agar plates containing 50% (wt./vol) brain heart infusion (BHI) or BM2 swarming medium [62 mM potassium phosphate buffer (pH 7), 2 mM $MgSO_4$, 10 μM $FeSO_4$, 0.4% (wt./vol) glucose, 0.1% (wt./vol) Casamino Acids (CAA), and 0.5% (wt./vol) Difco agar] (102). BHI and BM2 plates were examined under peptide-treated conditions, but only the BHI plate was assessed without the peptide as untreated BM2 plates swarmed too vigorously to be readable. All swarming plates were incubated at 37°C for 18-20 hrs. When required, peptide 1018 was incorporated directly into the agar. Colonies were visually assessed for their ability to swarm in the presence of the peptide. Two to three replicates were conducted for each plate in the PA14NR library.

2.2.3.2 Standard swarming assay

The standard swarming assay for *P. aeruginosa* involved inoculating 1μL of mid-log phase sub-culture into the centre of a polystyrene plate containing BM2 swarming agar. When necessary, peptide 1018 or antibiotics were incorporated directly into the agar. Plates were incubated at 37°C for 20 hrs. This assay was used to verify the results of the broad library screen and for all subsequent experiments. At 20 hrs, an image of each plate was capture and the surface area of each swarming colony was quantified using ImageJ software and assessed as a percentage relative to controls. Each swarming assay was carried out three to five times. A two-sample Student's t-test was used to evaluate the significance of test conditions on swarming motility.

2.2.4 Peptide

Peptide 1018 was aliquotted into distilled water and was stored at -20°C until used. Initially, slight differences in peptide efficacy were observed between different peptide batches (± 0.1 to 0.3μg/mL). All subsequent experiments were performed using batch CL-03-00140 of peptide 1018 (>95% purity), synthesized by CPC Scientific using solid-phase 9-fluorenylmethoxy carbonyl (Fmoc) chemistry and purified using reverse-phase high-performance liquid chromatography (HPLC).

2.2.5 Minimum inhibitory concentration of peptide 1018 for swarming motility

MICs for peptide 1018 in *P. aeruginosa* reference strains, PA14 and PA01, have been previously described by Fuente-Núñez et al. (87). The minimal concentration of peptide necessary to inhibit swarming motility in strains PA14 and PA01 was determined by performing a series of swarming assays with increasingly diluted peptide concentrations. Inhibition of swarming or tolerance to the peptide was readily visible as non-swarming colonies remained as a small dot at the point of inoculation while swarming bacteria rapidly spread across the plate (Fig. 2).

2.2.6 Microscopy

Wet mounts and swarming assays conducted on thin layers of BM2 swarming agar grown over slides and were examined using light microscopy to assess motility and observe *P. aeruginosa* PA14 WT swarming phenotypes with and without treatment with peptide 1018. A transmission electron microscope (TEM) was used to investigate the potential differentiation of

cellular morphotypes between untreated cells from the swarming edge and swarm centre, and peptide treated cells. The TEM protocol was modified from Köhler et al. (48). In brief, cells were picked with a sterile pipette tip and gently re-suspended in 10µL of water. Formvar and carbon coated copper TEM grids (200-mesh) were placed on top of the suspension for 30s to allow for cell adherence. Excess liquid was removed using filter paper. The grids were stained with 5µL of 2% aqueous uranyl acetate solution for 30s and then washed for 5s in 10 µL water. Excess liquid was removed from the grids with filter paper, and they were allowed to air dry. Images from multiple grid sections were taken with a Hitachi H7600 TEM at the UBC Bioimaging facility.

2.2.7 Complementation of *rhlB* and *anr* transposon insertion mutants

Two *P. aeruginosa* PA14NR transposon insertion mutants that swarmed in the presence of peptide 1018 were selected for complementation. The *rhlB* gene was PCR amplified from *P. aeruginosa* PA14 WT genomic DNA (gDNA) using primers rhlB_F and rhlB_R, and the *anr* gene and *anr-apt* operon were amplified using primers anr_F, anr_R, anr-apt_F and anr-aptR (Table 2). PCR products were gel purified and cloned into the pCR-Blunt II TOPO vector using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen) and transformed into TOP10 *E. coli* competent cells. The sequencing for both genes was verified using the UBC Sequencing and Bioinformatics Consortium services. TOPO vectors were digested using enzymes BamHI and HindIII (ThermoFisher) and ligated into the low copy plasmid, pBBR1mcs-2, which contains a lac promoter. pBBR1MCS-2 was a gift from Kenneth Peterson (Addgene plasmid # 85168) (101). The resulting plasmids were transformed into TOP10 *E. coli* and were electroporated into the respective *anr* and *rhlB* PA14NR transposon mutants. An empty pBBR1MCS-2 vector was also electroporated into both transposon mutants and the PA14 WT strain for controls.

Table 2. Primer sequences used during complementation

Primer	Forward Sequence (5'-3')	Reverse Sequence (3'-5')
rhlB	CCAAGCTTAACCCTTGACCTGCGAAG	CCGGATCCTATCTGTTATGCCAGCAC
anr	CCAAGCTTTTCCAGTCACTCCGGGAA	CCGGATCCGTCGAAAATCATGGAAGA
anr-apt	CCAAGCTTTTCCAGTCACTCCGGGAA	CCGGATCCTATCAACGCTCGTCGAGA

2.3 Results

2.3.1 Peptide 1018 inhibited swarming motility in *P. aeruginosa* at low concentrations

P. aeruginosa was cultured on BM2 swarming agar plates, with and without peptide 1018, using three to five replicates for each condition. As shown in Fig. 2, peptide 1018 inhibited swarming motility in *P. aeruginosa* strain PA14 at low concentrations. It also inhibited swarming motility in PAO1, at slightly higher concentrations (1.0 µg/mL) (Appendix Fig. A1). Although concentrations of both 0.67µg/mL and 0.70µg/mL of peptide 1018 inhibited swarming in PA14 WT, the slightly higher concentration of 0.75µg/mL was used in all standard swarming assays to guard against random error (e.g., agar volume precision, uneven drying between large batches of plates), such that each swarming assay always had a non-swarming PA14 WT control under peptide treated conditions.

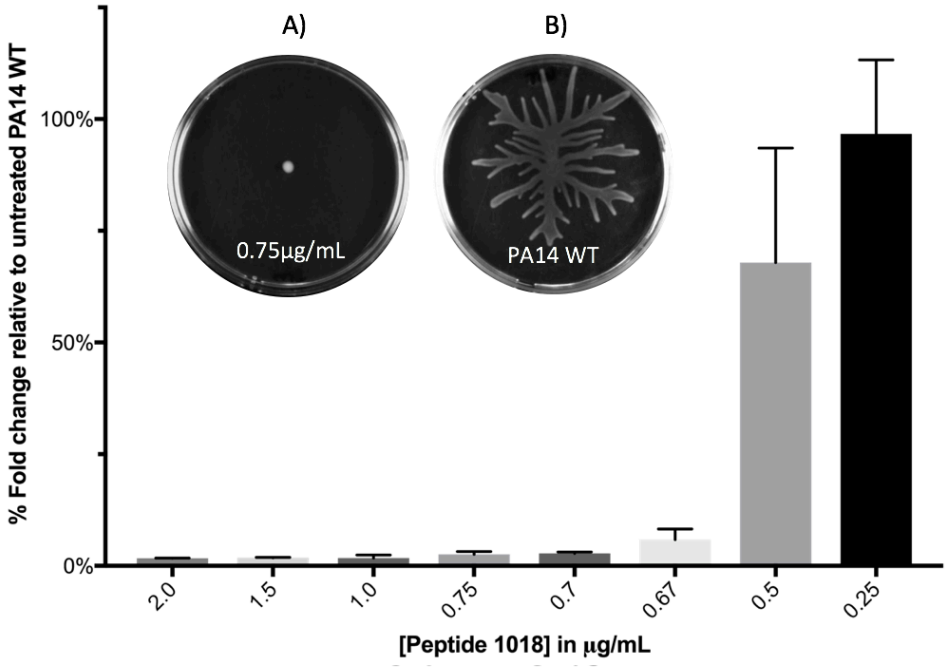

Figure 2. Peptide 1018 inhibited swarming motility in PA14 at low concentrations. Inset: **(A)** PA14 WT swarming motility was inhibited by 0.75µg/mL of peptide 1018. **(B)** The classic dendritic swarming pattern of a PA14 WT colony on BM2 agar.

Although they were unable to swarm, bacteria from peptide 1018-treated swarming plates were motile when examined by wet mount. Previous work has shown that swarming deficient *P. aeruginosa* mutants (e.g., *sbrR*, *rhlR, cbrA*) are still capable of swimming, and unlike swimming motility, swarming motility is regulated and sustained by elements beyond a functional flagellum (e.g., quorum sensing, type IV pili, etc.) (36,96). Peptide 1018-treated bacteria showed actively

dividing cells when examined by light microscopy (Appendix Fig. A2).

TEM revealed that peptide 1018-treated bacteria were flagellated and showed a morphological resemblance to cells taken from the centre of untreated swarming colonies (Fig. 3). Both sets of cells were flagellated and had a similar range of lengths (≤ 2µm). Actively swarming cells from untreated plates were also flagellated but were longer (2-4 µm) when compared to both peptide-1018 treated cells and cells from the centre of swarming colonies. Cell elongation and the presence of flagella are both phenotypes associated with actively swarming bacteria (15).

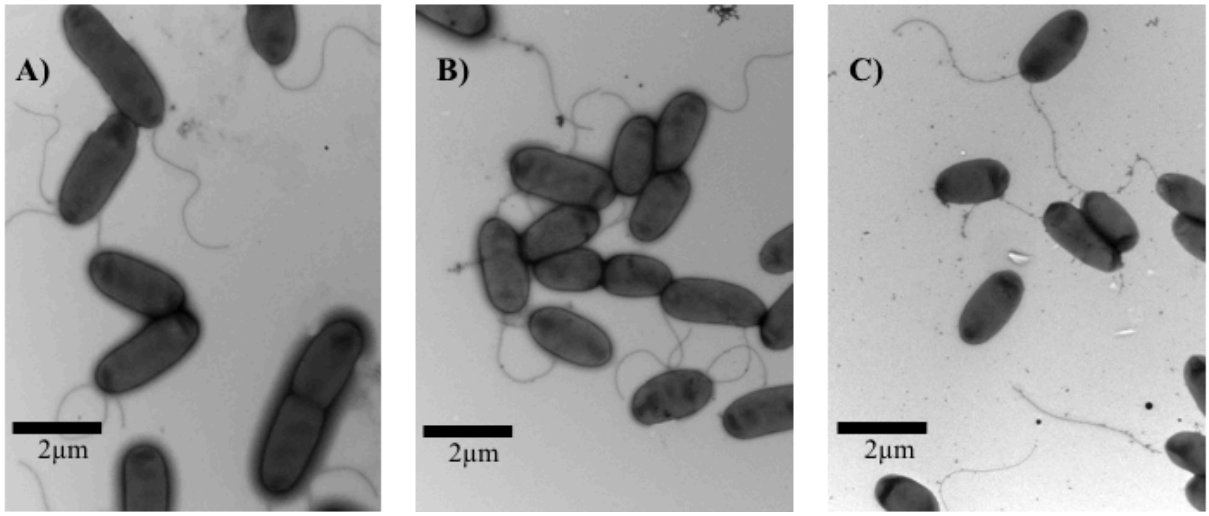

Figure 3. Representative images selected from TEM of PA14 WT (A) Actively swarming cells from the edge of a swarming colony **(B)** Cells taken from the centre of a swarming colony. These cells do not actively swarm **(C)** Flagellated cells from a swarming plate treated with 0.75µg/mL of peptide 1018.

2.3.2 Thirty transposon insertion mutants showed minor tolerance to peptide 1018

A series of increasingly discriminative screening assays were conducted with the PA14 NR transposon insertion mutant library to investigate whether single gene interruptions could confer tolerance to peptide 1018 in *P. aeruginosa* under swarming conditions. The preliminary broad screen allowed for a high volume of mutants to be rapidly screened, but this gain in efficiency came at the cost of reduced screening sensitivity. At the conclusion of the broad screen, several hundred mutants were identified as potentially tolerant to peptide 1018 under swarming conditions. False positives were eliminated during follow up assays using the more sensitive standard swarming motility screen on BM2 swarming agar (Fig. 2). Following a series of confirmatory assays, 30 transposon insertion mutants were found to be partially tolerant to

peptide 1018 under swarming conditions (Table 3). A minimum of 3 biological replicates was used to confirm the peptide 1018-tolerant phenotype for each transposon insertion mutant.

Table 3. Thirty transposon insertion mutants that demonstrated partial tolerance to peptide 1018 under swarming conditions

PA gene number and gene name	Gene Product
Regulatory Genes	
PA0464 (*creC)*	Alkaline metalloproteinase
PA3233 (*orfK*)	Signal-transduction protein
PA3045	Two-component response regulator
PA1544 (*anr*)	Transcriptional regulator Anr
Cell and Energy Metabolism	
PA4131	Iron-sulfur cluster-binding protein
PA2644 (*nuoI*)	NADH Dehydrogenase I chain I
PA3695	Hydrolase, alpha/beta family
PA5357 (*ubiC*)	Chorismate-pyruvate lyase
PA2110$^{\nabla}$	Putative allophanate hydrolase subunit 2
Protein synthesis, DNA repair, and cell division	
PA4272 (*rplJ*)$^{\nabla}$	50S ribosomal protein L10
PA4001 (*sltB1*)	Soluble lytic transglycosylase B
PA2138 (*ligD*)$^{\Delta}$	ATP-dependent DNA ligase
PA4400	Putative pyrophosphohydrolase
PA14_28800	Putative cell filamentation protein Fic
Iron/sulfur metabolism and transport	
PA3983	Putative Mg2+ and Co2+ transporter CorC
PA2862 (*lipA)*	Lactonizing lipase precursor
PA2057 (*sppR*)	TonB-dependent receptor
PA4225 (*pchF*)	Pyochelin synthetase
PA2596	Periplasmic aliphatic sulfonate-binding protein
Quorum sensing	
PA3478 (*rhlB*)$^{\Delta}$	Rhamnosyltransferase chain B
Adaption/Stress response	
PA4613 (*katB*)	Catalase
PA0725	Bacteriophage protein
PA1880$^{\Delta}$	Oxidoreductase
PA1828	Short-chain dehydrogenase
Hypothetical function	
PA3855$^{\nabla}$	Hypothetical protein
PA3342	Hypothetical protein
PA2864$^{\nabla}$	Hypothetical protein
PA2814	Hypothetical protein
PA0822	Hypothetical protein

$^{\nabla}$: Genes down-regulated in peptide-treated cells

$^{\Delta}$: Genes up-regulated in peptide-treated cells

Under swarming conditions and in the presence of peptide 1018, each of these mutants displayed a swarming phenotype and had a larger colony size than the PA14 WT control, which did not swarm in the presence of this peptide (Fig. 4). However, none of the peptide 1018-tolerant transposon mutants were able to swarm to the same degree as their equivalent, untreated swarming phenotype (Fig. 4 A, B). Each peptide 1018-treated tolerant colony showed at least a 75% reduction in swarm colony area compared to untreated conditions (Fig. 4). Furthermore, increasing the concentration of peptide 1018 in the swarming assay (>2µg/mL) eliminated the tolerance phenotype in all of these transposon insertion mutants (data not shown).

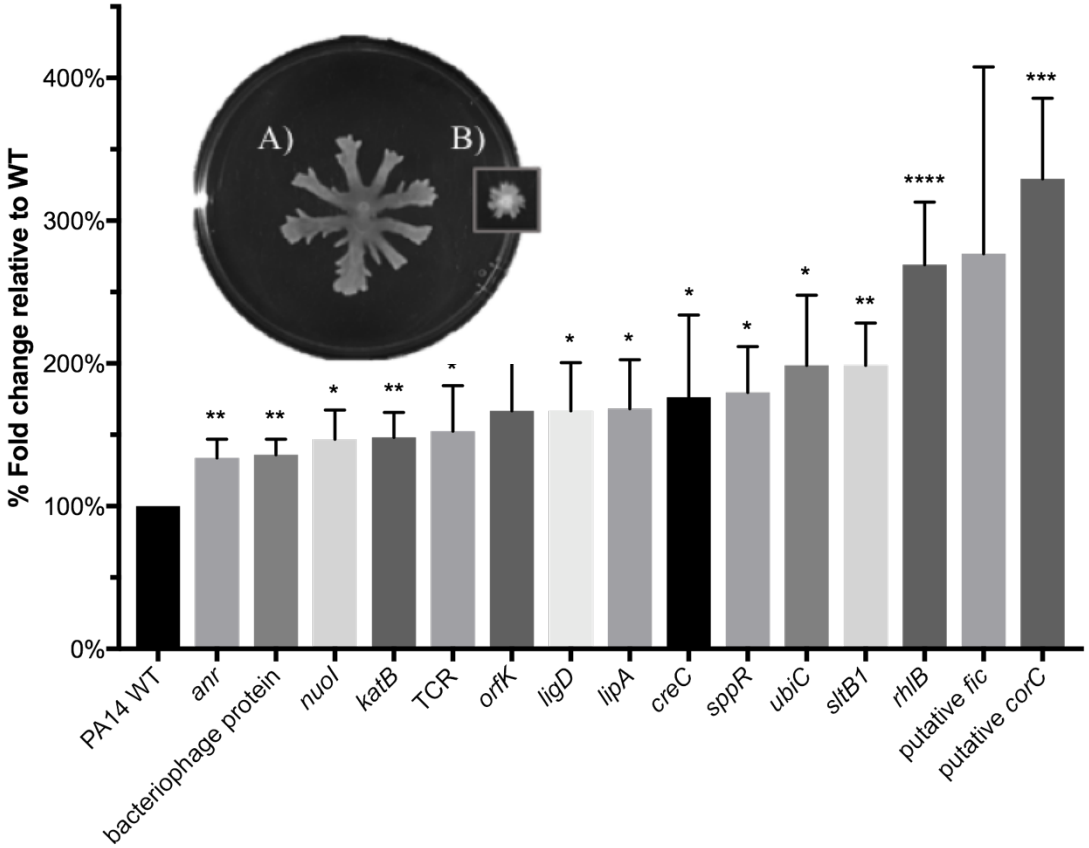

Figure 4. A selection of PA14 NR library mutants that were tolerant to peptide 1018 under swarming conditions (+0.75µg/mL peptide 1018). The size of swarming colonies relative to that of PA14 WT in the presence of peptide is given as % fold change relative to PA14 WT. At least three biological replicates for each transposon mutant were examined. Transposon mutagenesis is an efficient but less rigorous technique for library screening, than creating individual clean gene deletion mutants. Genes may only be partially interrupted in transposon insertion mutants, so transposon phenotypes require additional confirmation (e.g. complementation assay). Inset: **(A)** *rhlB*::Tn mutant swarming under untreated conditions **(B)** *rhlB*::Tn mutant swarming under peptide treated conditions. The *rhlB*::Tn mutant swarmed on average 86.7% less under peptide 1018-treated conditions than the WT under untreated conditions

Twenty-five of the 30 transposon insertion mutants that were identified as tolerant to peptide 1018 were genes with stringent response-associated functions and the remaining five represented genes with undefined functions and products (Table 3). Several of these tolerant transposon mutants genes, including *creC*, *anr*, *katB*, *sppR*, *lipA*, and *rhlB* have been experimentally linked to both the stringent response and adaptive behaviours like biofilm formation and swarming motility (88,103–107).

2.3.3 Genetic complementation of enhanced tolerance to peptide 1018 in transposon mutants in two adaptation genes, *anr* and *rhlB*

Two of the 30 peptide 1018-tolerant transposon insertion mutants were selected for confirmation by complementation. The first gene in the two gene *anr-apt* operon, *anr,* as well as *rhlB*, which is described as the second gene in the two gene *rhlAB* operon, were selected for analysis by complement testing. Both *anr* and *rhlB* were of particular interest because of their roles in stress regulation, QS, and adaptive behaviour. Anr is an oxygen-sensing global regulator that amongst other actions is involved in the induction of the *spoT*-mediated biosynthesis of (p)ppGpp under oxygen-limiting conditions (104). RhlB is a catalytic subunit of rhamnosyltransferase and is integral to rhamnolipid biosynthesis. Rhamnolipid production is QS-regulated, and beyond acting as surfactants, rhamnolipids have roles in swarm colony and biofilm structure (maintaining cell-free regions) and have been implicated in in vivo virulence and stringent conditions (51,67,107,108).

The *anr* and *rhlB* transposon insertion mutants lost their peptide 1018-tolerant phenotypes but maintained their untreated swarming phenotype when complemented with their respective PA14 WT *anr*, *anr-apt*, and *rhlB* genes (Fig. 5). Introducing the pBBR1MCS-2 empty vector into both the PA14 WT and *anr* and *rhlB* transposon insertion mutants had no significant impact on the untreated swarming phenotype (Fig. 5). These results support the validity of the peptide 1018-tolerant phenotype observed in both the *rhlB* and the *anr* transposon insertion mutants. It is thus likely that the observed peptide 1018 tolerance phenotype was due to the interruption of the *anr* and *rhlB* genes.

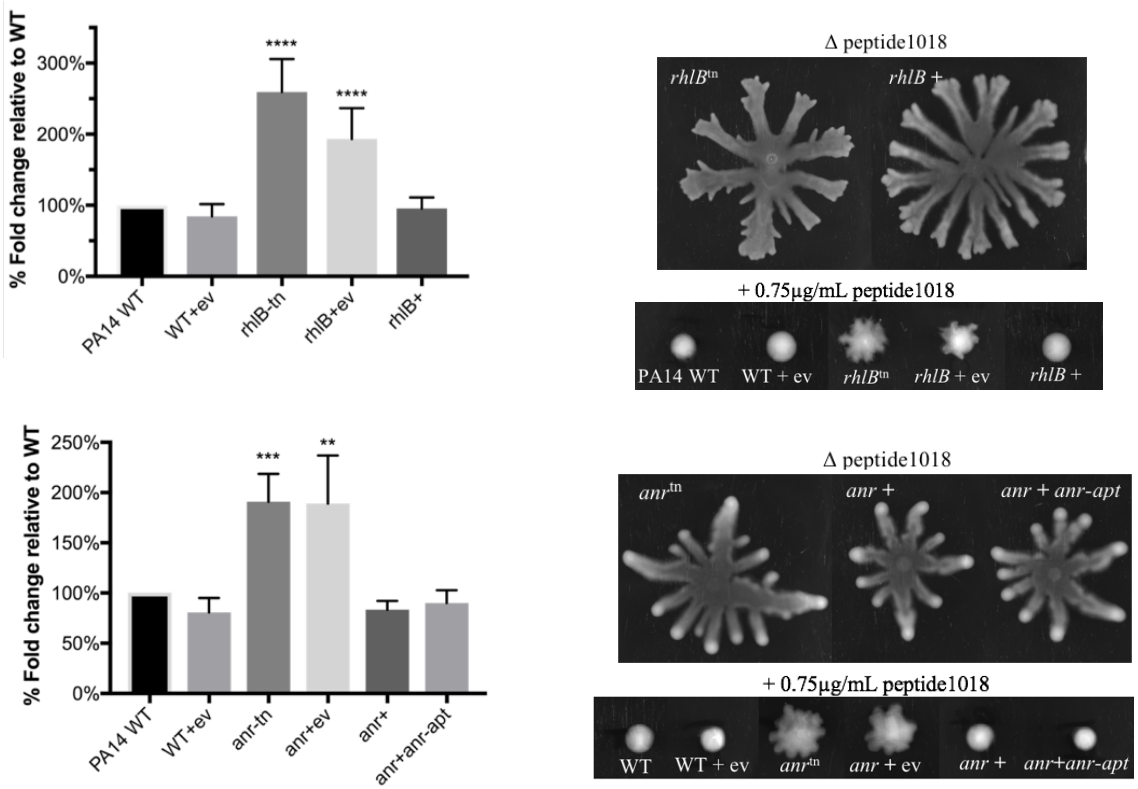

Figure 5. **The peptide 1018 tolerant phenotypes of two transposon (-tn) mutants, *anr* and *rhlB*, were eliminated by complementation by the PA14 WT *anr*, *anr-apt*, and *rhlB* genetic regions (A,B).** Complement testing with the *rhlB*-Tn and *anr*-Tn mutants confirmed that interrupting these genes induced peptide 1018 tolerance under swarming conditions **(C,D)** Empty vector (ev) and complemented transposon mutant swarmed normally in untreated conditions (shown as Δ peptide 1018). The PA14 WT non-swarming phenotype was restored in both mutants upon complementation (shown as mutant^{+} and $^{+}$*anr-apt* for the operon complemented *anr*-Tn mutant) and treatment with 0.75µg/mL peptide 1018.

2.4 Discussion

The results in this Chapter demonstrated that in addition to its previously described biofilm inhibition and immunomodulatory effects, peptide 1018 inhibits swarming motility at very low concentrations. Swarming motility is an adaption linked to both virulence expression and the acute infection state (54,58). It has also been characterized as a stringent response regulated behaviour, based on the non-swarming behaviour of a stringent response double mutant (Δ*relA*Δ*spoT*) (58).

Several transposon insertion mutants in genes associated with functions such as metal ion metabolism and transport, transcriptional regulation, quorum sensing, DNA repair, cell wall metabolism, and two-component systems were able to swarm to some extent in the presence of

peptide 1018. Many of these genes are associated with the stringent response, as well as with adaptive resistance behaviours (swarming motility, biofilm formation) and in vivo virulence.

Only a small percentage of interrupted *P. aeruginosa* genes (<0.5%) displayed a peptide 1018-tolerant phenotype under swarming condition. Moreover, none of these mutants had swarming phenotypes under untreated conditions. All showed at least a 75% reduction in surface coverage in the presence of the peptide and the tolerance phenotype was lost at increased concentrations of peptide. It is possible that bacteria might accumulate several of these mutations and display a more vigorous tolerance phenotype; however, most of these transposon insertion mutants were in genes that express proteins with functions related to stringent survival, adaptive behaviour, and in vivo virulence (Table 3). Conversely altered expression of several of these proteins in the wild-type (as marked in Table 1 based on data presented in the following Chapter) might collectively contribute to susceptibility to peptide 1018. Thus, the tolerance phenotype of these mutants might provide insights into the types of swarming-specific bacterial targets and processes affected by treatment with peptide 1018. This genomic library screen, in concert with analysis of the expression signature associated with peptide 1018 treatment under swarming conditions (Chapter 3), might thus illuminate the complex modes of action and targets of peptide 1018.

A number of the genes that were identified in the peptide tolerance screen have been implicated in *spoT*-mediated activation of the stringent response. LipA is a lipase that is involved in both fatty acid metabolism and the regulation of the iron-starvation σ factor, PvdS. Indeed, *lipA* expression is essential for full pyoverdine production under iron-limited conditions and shows reduced expression in stringent response mutants (88,109). Likewise, *creC,* which encodes a catabolite regulatory sensor, has been shown to activate PhoB which regulates adaptation to phosphate starvation in response to low levels of inorganic phosphate through a *spoT* starvation-induced pathway (103). Inorganic phosphate is required for swarming motility in *P. aeruginosa*, and reduced levels are associated with the switch to biofilm formation and decreased T3SS expression (110,111). KatB, a catalase induced by exogenous hydrogen peroxide, is an important enzyme for stationary cell survival and antibiotic tolerance and is mediated by the stringent response (105). All of these genes enable bacteria to recognize, adapt, and survive in adverse environmental conditions and all are regulated, to some degree under stringent conditions.

The two genes that were confirmed to have roles in peptide 1018 tolerance through complement testing were also associated with adaptive survival and the stringent response. Anr is a global oxygen sensing regulator and is induced under anoxic conditions which are known to occur, for example in CF lung infections (112,113). It controls the expression of universal stress proteins and was required for in vivo virulence in a murine pneumonia model (104,114,115). Anr can also increase virulence in oxic environments in a phosphatidylcholine degradation-dependent manner (114). Anr can induce the *spoT*-mediated stringent response and regulates stationary phase gene expression, enhances biofilm formation, and is required for growth under anaerobic conditions (104,114,115).

As a central gene in rhamnolipid biosynthesis, *rhlB* is intimately linked to both swarming motility, biofilm formation, and in vivo virulence (116,117). Interrupting the expression of *rhlB* interrupts rhamnolipid biosynthesis by preventing the formation of mono-rhamnolipids from 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs). RhlB catalyzes this reaction, which is an intermediary step in the process of synthesizing di-rhamnolipids (95). The rhamnolipid precursors, HAAs, are the minimum necessary surfactant required for swarming motility in *P. aeruginosa*, but bacteria display a significantly different swarm colony morphology with HAAs as the sole surfactant (51,52). QS and stringent response-associated genes regulate rhamnolipid biosynthesis, and in turn, *rhlB* expression. When bound to the QS autoinducer, N-butyryl-homoserine lactone (C_4-HSL), the QS regulator *rhlR* promotes the expression of the rhamnolipid biosynthetic pathway, including *rhlB*, while unbound rhlR can repress its transcription (118,119). The stress-associated stationary σ factor, RpoS, has also been shown to mediate rhamnolipid biosynthesis by inducing expression under stationary (nutrient-limited) conditions (120,121). Thus the two mutants studied in detail strongly reflect the concept that adaptation processes are related to the action of peptide 1018 vs. swarming cells.

Chapter 3: Peptide 1018 broadly dysregulated the transcriptome of swarming *P. aeruginosa* inducing a gene expression signature analogous to cells from the centre of untreated swarming colonies

3.1 Introduction

Swarming motility is a tightly regulated and complex adaptive behaviour that involves differential expression of a significant proportion of the *P. aeruginosa* genome (36,54,55) (See also Appendix Table A1). As an IDR peptide with weak antibiotic activity, peptide 1018 has been predicted to have a complex and indirect mechanism for inhibiting adaptive resistance behaviours like biofilm formation (and swarming motility) (85). While the outer membrane lipid bilayer of bacteria has previously been described as a target for cationic antimicrobial peptides, disruption of this layer does not lead to cell killing per se and both membrane-associated (cell wall, cytoplasmic membrane integrity and cell division) and intracellular targets such as RNA, DNA, and protein synthesis have been observed (122). Using whole transcriptome-based analysis, the changes in bacterial gene expression that occur upon cationic peptide treatment can be examined by RNA-Seq-based comparative transcriptome analysis and used to infer potential drug targets and mechanisms of action. Transcriptome-based analysis can also yield insights into drug targets that are not involved in direct killing, represent resistance mechanisms triggered in response to potentially lethal stress (the peptide) or reflect subtler mechanisms of action, all of which can be challenging to identify and/or characterize efficiently solely by lab-based analysis. Peptide 1018 has previously been described as a stringent response-targeting therapeutic in the context of its broad-spectrum action on biofilms (86). Together with confirmatory lab assays, the global transcription profile of peptide 1018-treated *P. aeruginosa* under swarming conditions was investigated here to assist in revealing how this therapeutic worked and the impact that it had on bacterial cells.

3.2 Materials and methods

3.2.1 Bacterial strains and swarming motility growth conditions

P. aeruginosa PA14 WT was grown overnight in LB broth at 37°C under shaking conditions. Overnight cultures were sub-cultured into BM2 glucose broth at a normalized OD_{600} of 0.1 and grown to mid-log phase (OD_{600} of 0.4-0.6) at 37°C, also under shaking conditions. One μL of mid-log phase sub-culture was inoculated into the centre of each BM2 glucose

swarming agar plate, with or without 1μg/mL peptide 1018. Plates were incubated for 20 hrs at 37°C, after which cells were harvested for RNA isolation and RNA-Seq analysis.

3.2.2 RNA isolation and RNA-Seq

P. aeruginosa PA14 WT cells were collected with sterile swabs from the leading swarming edge (2-3 mm of the swarming edge) and centre of swarming colonies grown on standard BM2 swarming plates. The entire colony was collected from plates treated with peptide 1018. Three independent biological replicates were performed for each set of edge, centre, and peptide-treated cells, each with more than 10 technical replicates. With the technical assistance of Reza Falsafi from our lab, RNA was extracted from the cells using an RNeasy Mini kit (Qiagen) and underwent deoxyribonuclease (DNase) treatment with the TURBO DNA-free kit (ThermoFisher) to remove contaminating chromosomal DNA. RNA purity was assessed using the Agilent 2100 Bioanalyzer. Coding sequences (mRNA) were enriched by rRNA depletion with the RiboZero Bacteria kit (Illumina), followed by the Kapa stranded Total RNA kit (Kapa Biosystems) and the indexing kit (Bioo Scientific, USA) to construct multiplexed cDNA libraries for sequencing. These were sequenced on an Illumina HiSeq 2500 platform in one lane of a high-output flowcell to generate 100 base pair (bp) single-end reads at the UBC Sequencing and Bioinformatics Consortium (SBC). Subsequently, with the assistance of bioinformaticist Dr. Amy Lee from our lab, FastQC v0.11.5 and MulitQC v0.8.dev were used to obtain the quality score for FASTQ files (123). STAR aligner was used to align reads to the UCBPP-PA14 genome (GenBank database gene annotations) (124,125). Read count tables were then generated with HTseq-count v0.6.1p1 (126). DESeq2 was used to estimate the fold-change in gene expression between the untreated edge and centre cells, and peptide treated cells under swarming conditions (127).

3.3 Results

3.3.1 RNA-Seq transcriptomic profiling of *P. aeruginosa* grown on BM2 swarming plates was generally consistent with previous studies

The PA14 WT transcriptome was examined under three conditions. The gene expression profiles of actively swarming bacteria from the leading edge of swarming colonies, cells from the centre of swarming colonies (not actively swarming), and cells that were grown on peptide 1018-treated swarming plates were examined and compared. Principal component analysis (PCA) of these data showed distinct clustering for each sample type ($P<0.05$ when adjusted for

multiple testing), indicating that each of these three cell conditions was associated with a unique transcriptome (Appendix Fig. A3). Only genes that exhibited ≥1.5-fold changes in expression ($P < 0.05$) were included for analysis in this study.

Two previous studies had conducted a transcriptional analysis of *P. aeruginosa* gene expression under swarming conditions; one with PAO1 WT in actively swarming cells compared to cells grown in broth conditions, and the other comparing actively swarming PA14 WT from the leading edge of swarming colonies to cells from swarm colony centres (54,55). Both of these studies used microarray analysis, and both found substantial significant differentially expressed (DE) gene expression in actively swarming *P. aeruginosa* cells (54,55). One PAO1-based dataset (55) was excluded due to the use of different growth substrates for control and experimental conditions (broth vs. minimal medium based swarming agar) and consequent potential for substrate-associated differential gene expression. The dataset from the microarray analysis comparing the expression of the PA14 swarming edge and swarming centre (55) was compared with the results of RNA-Seq analysis conducted here. Interestingly, more than half (50.7%) of the differentially expressed (DE) genes identified by microarray were also DE in the PA14 RNA-Seq swarming dataset.

The PA14 RNA-Seq swarming analysis revealed more than 16-fold more DE genes in actively swarming cells than did the microarray (2369 vs. 142 DE genes). This large difference in DE genes is due to variations in hybridization efficiency across a microarray and the need in microarrays to perform background correction which can obscure differential expression. One potentially important difference between the two data sets was observed in the differential expression of *pvd* iron-acquisition genes. Iron depletion is an important environmental cue for inducing swarming motility (128,129), and *pvd* iron-acquisition genes constituted six of the top ten most up-regulated genes in the untreated swarming edge (fold change of 68-140FC), with 13 *pvd* genes in total up-regulated in the RNA-Seq dataset (Appendix Table A1). Of these genes, only *pvdS* was down-regulated. These results varied significantly from those observed by Tremblay et al., (55) who found that most *pvd* genes were down-regulated in actively swarming cells.

3.3.2 Peptide 1018 induced a complex differential gene expression signature in almost 20% of PA14 genes under swarming conditions

Under swarming conditions, treatment with peptide 1018 dysregulated nearly a fifth (19.9%) of the annotated PA14 genome, with an overall trend towards increased gene expression (Table 4). Even at a 2-fold cut-off, there were a total of 768 genes dysregulated (576 up-regulated and 192 down-regulated).

Table 4. Treatment with peptide 1018 under swarming conditions resulted in broad transcriptional changes in PA14 WT cf. untreated swarming edge cells:

Up-regulated genes	755
Down-regulated genes	435
Total	1190
Percentage of differentially expressed PA14 genes	19.9%

Genes spanning twenty-five predicted functional categories, including adaptation and protection, transport of small molecules, energy metabolism, and membrane proteins were DE under peptide treated conditions (Fig. 6). However, 24% of all the DE genes in this dataset had no currently defined function (hypothetical or conserved hypothetical functions).

Of the 1190 genes that were DE in peptide 1018-treated cells when compared to the leading edge of untreated swarming colonies, 806 showed the same direction of dysregulation as cells at the centre of untreated swarming colonies (cf. actively swarming edge cells) (Appendix Table A1). Conversely, a total of 384 genes showed unique differential expression (cf. swarming centre cells) when compared to actively swarming edge cells. Of the 2369 total genes that were DE between untreated actively swarming (leading edge) cells and swarm centre cells, 1510 were not dysregulated under peptide 1018-treated conditions (Appendix Table A1).

3.3.2.1 Down-regulated genes under peptide 1018-treated swarming conditions

A total of 435 genes, including genes from three main functional categories, showed down-regulation under peptide 1018-treated conditions when compared to untreated swarming cells from the leading edge (Table 4). Thus genes in the functional categories transcription, RNA-associated processes (processing, degradation, translation, and post-translational modification), and non-coding RNA genes, which are all integral to protein synthesis, showed an overall decrease in expression in the presence of peptide 1018 (Fig. 6). In particular, 62% of all currently annotated genomic PA14 tRNAs were down-regulated. Likewise, genes encoding 41% of all

annotated PA14 30S ribosomal subunit proteins, and 43% of all PA14 50S ribosomal subunit proteins were down-regulated after peptide 1018 treatment.

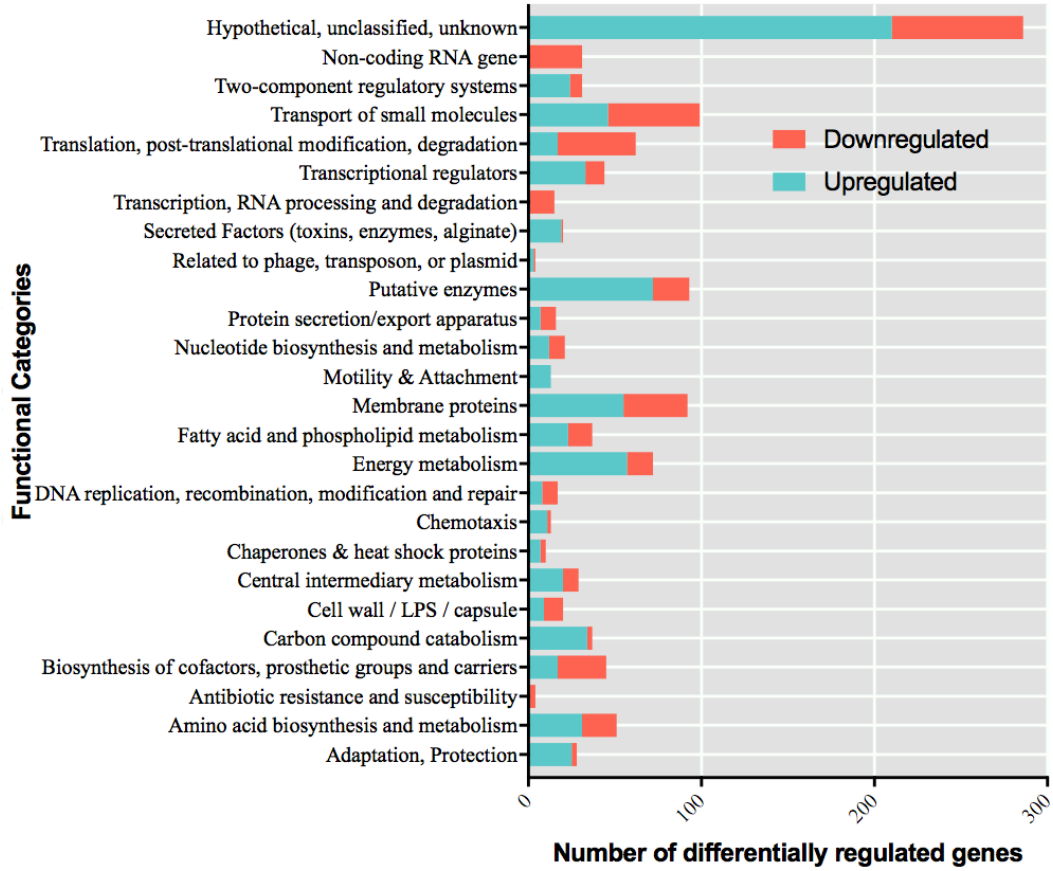

Figure 6. Dysregulated PA14 genes under peptide 1018-treated conditions when compared to untreated leading-edge swarming cells. Genes were categorized by their primary PseudoCAP functional class according to the *Pseudomonas* Genome Database (www.pseudomonas.com) (130). For genes that had multiple predicted functional categories, the categories other than "hypothetical" were selected.

Genes predicted to be involved in the transport and acquisition of small molecules accounted for almost half of the thirty-five most down-regulated genes under peptide-treated conditions in the dataset (Appendix Table A1). In particular, genes with functions in metal ion, sulfate, amino acid, taurine and polyamine transport were down-regulated in the presence of peptide 1018. These genes were also down-regulated in untreated cells from swarm colony centres cf. actively swarming cells from the colony edge (Appendix Table A1). Among these genes were an outer membrane ferric siderophore receptor (PA4514), a taurine ABC transporter periplasmic protein (PA3938), and the complete *potAD* operon. Intriguingly, PA4514, PA3938, and *potA* were also among the most repressed transcripts in untreated swarm centres cf. actively swarming cells. Cysteine biosynthesis and sulfate transport showed an overall downregulation under peptide

treated conditions and were also repressed in swarm centres.

Of the 435 genes that were down-regulated under treatment with peptide 1018, 78 were uniquely expressed, meaning their dysregulation was unique to treatment with peptide 1018 (Table 5). These genes were either DE only in peptide 1018-treated conditions or showed an opposite direction of differential gene expression in swarm centre cells when both conditions were compared with actively swarming leading edge cells. In particular, 8 of these uniquely down-regulated genes were regulators (e.g., *cbrA, exsA, pilS*).

Table 5. Down-regulated genes uniquely DE under peptide 1018-treated conditions. Hypothetical genes with no currently defined function were excluded from this table but can be found in Appendix Table A1.

PA gene number and gene name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4850 (*prmA*)	50S ribosomal protein L11 methyltransferase - homologue of RpoE	-1.7	0
PA2666	6-pyruvoyl-tetrahydropterin synthase	-2.2	0
PA5074	ABC transporter ATP-binding protein	-1.6	0
PA5075	ABC transporter permease	-1.6	0
PA5138	ABC-type amino acid transport protein. periplasmic component	-2	0
PA5458	acyltransferase	-1.8	0
PA3247	aminopeptidase 2	-1.6	0
PA3798	aminotransferase	-1.5	0
PA2512 (*antA*)	anthranilate dioxygenase large subunit	-2.3	1.8
PA2514 (*antC)*	anthranilate dioxygenase reductase	-3	0
PA0866 (*aroP2*)	aromatic amino acid transport protein	-1.8	0
PA1779	assimilatory nitrate reductase	-1.7	1.7
PA1780 (*nirD*)	assimilatory nitrite reductase small subunit	-1.8	1.8
PA3387 (*rhlG*)	beta-ketoacyl reductase	-2.4	0
PA5320 (*coaC*)	bifunctional phosphopantothenoylcysteine decarboxylase/phosphopantothenate synthase	-1.6	0
PA4561 (*ribF*)	bifunctional riboflavin kinase/FMN adenylyltransferase	-1.5	0
PA4133	cbb3-type cytochrome c oxidase subunit I	-2	0
PA3365	chaperone	-1.7	3.4
PA4307 (*pctC*)	chemotactic transducer PctC	-1.7	0
PA0706 (*cat*)	chloramphenicol acetyltransferase	-1.8	0

PA gene number and gene name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5491	cytochrome	-1.6	0
PA1816 (*dnaQi)*	DNA polymerase III subunit epsilon	-1.5	0
PA4631	epimerase	-1.6	0
PA3710	GMC-type oxidoreductase	-1.9	2.4
PA1674 (*folE*)	GTP cyclohydrolase I	-1.7	0
PA5177	hydrolase	-1.6	0
PA4645	hypoxanthine-guanine phosphoribosyltransferase	-1.9	0
PA2112	LamB/YcsF family protein	-1.8	0
PA3604	LuxR family transcriptional regulator	-1.5	0
PA1993	major facilitator superfamily transporter	-2.1	0
PA2114	major facilitator transporter	-1.7	0
PA3452 (*mqoA*)	malate:quinone oxidoreductase	-2.1	0
PA0412 (*pilK*)	methyltransferase PilK	-1.5	0
PA3709	MFS transporter	-2.4	2.9
PA5274 (*rnk*)	nucleoside diphosphate kinase regulator - substrate (1/3) of serine-tRNA ligase	-1.6	0
PA0608	phosphoglycolate phosphatase	-1.7	0
PA3975 (*thiD*)	phosphomethylpyrimidine kinase	-1.6	0
PA2113	porin	-2.1	0
PA4055 (*ribC*)	riboflavin synthase subunit alpha	-1.8	0
PA1815 (*rnhA*)	ribonuclease H	-1.6	0
PA0770 (*rnc*)	ribonuclease III	-1.5	0
PA1161 (*rrmA*)	rRNA methyltransferase	-1.7	0
PA0342 (*thyA*)	thymidylate synthase	-1.6	0
PA14_55050	TonB-dependent receptor	-1.5	0
PA0797	transcriptional regulator	-1.9	0
PA1713 (*exsA*)	transcriptional regulator ExsA	-1.7	0
PA14_20500	tRNA-Arg	-2.3	0
PA1396	two-component sensor	-1.8	0
PA4725 (*cbrA*)	two-component sensor CbrA	-1.5	0
PA4546 (*pilS*)	two-component sensor PilS	-1.7	0
PA1778 (*cobA*)	uroporphyrin-III C-methyltransferase	-1.6	1.9
PA4562	virulence factor/membrane protein	-1.7	0

3.3.2.2 Up-regulated genes under peptide 1018-treated swarming conditions

DE genes involved in regulation (transcriptional regulators, two-component regulatory systems), secreted factors, metabolic processes (nucleotide biosynthesis and metabolism, energy metabolism, and central intermediary metabolism), adaptation and protection, and motility and attachment showed a bias for upregulation under peptide treated conditions (Fig. 6). In particular, two genes responsible for encoding non-homologous end joining DNA double-stranded break repair proteins, *ligD* and *Ku* (131), were strongly induced in the presence of peptide 1018 but also in the centre of swarming colonies under untreated conditions. Genes involved glycogen, trehalose, and glycolate biosynthesis and metabolism that are activated under nutrient-limited conditions, were associated with stationary growth and highly induced under peptide 1018-treated conditions (132–134). These genes were also up-regulated in untreated swarm centre cells.

Of the 384 uniquely DE genes in peptide 1018-treated conditions, 306 were up-regulated. Several genes associated with improved bacterial survival under oxidative stress and heat shock conditions were among these uniquely expressed genes. These included *osmC, ibpA, htpG, grpE, dnaK,* and *groEL*, some of which are virulence factors associated with the biofilm state (135,136). A number of genes encoding transcriptional regulators were also uniquely up-regulated by peptide 1018. These included the RNA binding protein/translational regulator, *rsmA*, the sigma (σ) and anti-σ factor pair, *sbrIR*, and the alternative σ factor *algU* (Table 6).

Table 6. Up-regulated genes uniquely DE in peptide 1018-treated conditions. Hypothetical genes with no currently defined function were excluded from this table but can be found in Appendix Table A1.

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3092 (*fadH1*)	2.4-dienoyl-CoA reductase	1.9	0
PA0230 (*pcaB*)	3-carboxy-cis.cis-muconate cycloisomerase	2.9	0
PA3013 (*fadA*)	3-ketoacyl-CoA thiolase	1.8	0
PA3384 (*phnC*)	ABC phosphonate transporter ATP-binding protein	2.9	0
PA3891	ABC transporter ATP-binding protein	5.5	0
PA3228	ABC transporter ATP-binding protein/permease	1.6	0
PA0138	ABC transporter permease	2	0
PA3888	ABC transporter permease	5.2	0
PA3890	ABC transporter permease	5.6	0
PA0604	ABC transporter substrate-binding protein	1.6	0

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1260	ABC transporter substrate-binding protein	2.7	0
PA3889	ABC transporter substrate-binding protein	5.5	0
PA0836	acetate kinase	1.9	0
PA1409 (*aphA*)	acetylpolyamine aminohydrolase	2.1	0
PA2889	acyl-CoA dehydrogenase	2.1	0
PA3972	acyl-CoA dehydrogenase	2.6	0
PA3337 (*rfaD*)	ADP-L-glycero-D-manno-heptose-6-epimerase	2	-5.4
PA1561 (*aer*)	aerotaxis receptor	2.4	-1.5
PA5427 (*adhA*)	alcohol dehydrogenase	5.8	-1.8
PA4189	aldehyde dehydrogenase	2.6	0
PA4899	aldehyde dehydrogenase	3.2	0
PA3549 (*algJ*)	alginate o-acetyltransferase	3.4	0
PA1515 (*alc*)	allantoicase	2.3	0
PA1617	AMP-binding protein	1.6	0
PA1920	anaerobic ribonucleoside triphosphate reductase	2.4	-9.9
PA0763 (*mucA*)	anti-σ factor	3.1	0
PA5171 (*arcA*)	arginine deiminase	6.8	0
PA5170 (*arcD*)	arginine/ornithine antiporter	2.7	0
PA3272	ATP-dependent DNA helicase	2.5	0
PA0779	ATP-dependent protease	3.6	0
PA5054 (*hslU*)	ATP-dependent protease ATP-binding subunit	3.4	0
PA1432 (*lasI*)	autoinducer synthesis protein	2.3	0
PA0231 (*pcaD*)	beta-ketoadipate enol-lactone hydrolase	2.7	0
PA0228 (*pcaF*)	beta-ketoadipyl CoA thiolase	3.4	0
PA5514	beta-lactamase	2.1	0
PA2891	biotin carboxylase	1.9	0
PA2888	biotin-dependent carboxylase	2.3	0
PA1183 (*dctA*)	C4-dicarboxylate transporter	2.9	0
PA5173 (*arcC*)	carbamate kinase	4.6	0
PA0905 (*rsmA*)	RNA binding protein translational regulator	1.6	0
PA4236 (*katA*)	catalase	2.9	0
PA1429	cation-transporting P-type ATPase	3.9	-3
PA1557	cbb3-type cytochrome c oxidase subunit I	3.5	-5.5
PA1556	cbb3-type cytochrome c oxidase subunit II	4.4	-8
PA4760 (*dnaJ*)	chaperone protein	2.4	0
PA4385 (*groEL*)	chaperonin	2.9	-1.8
PA4310 (*pctB*)	chemotactic transducer	1.7	0
PA1423	chemotaxis transducer	4.6	0
PA2788	chemotaxis transducer	2.9	0
PA4633	chemotaxis transducer	2.1	0
PA0852 (*cpbD*)	chitin-binding protein CbpD	1.8	0

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5291	choline transporter	2.4	0
PA4542 (*clpB*)	clpB protein	5	0
PA0227	CoA transferase subunit B	3.4	0
PA0226	CoA transferase. subunit A	2.9	0
PA14_30900	conjugal transfer protein TrbJ	4.5	0
PA1546 (*hemN*)	coproporphyrinogen III oxidase	1.9	-4.8
PA1555	cytochrome c oxidase. cbb3-type subunit III	3.8	-6.3
PA4587 (*ccpR*)	cytochrome c551 peroxidase	3.5	-17
PA3043	deoxyguanosinetriphosphate triphosphohydrolase-like protein	2	0
PA0387	deoxyribonucleotide triphosphate pyrophosphatase	1.6	0
PA0229 (*pcaT*)	dicarboxylic acid transporter	4.2	0
PA4759 (*dapB*)	dihydrodipicolinate reductase	2.2	0
PA1254	dihydrodipicolinate synthetase	4.4	0
PA5016 (*aceF*)	dihydrolipoamide acetyltransferase	1.5	-2
PA1587 (*lpdG*)	dihydrolipoamide dehydrogenase	1.7	0
PA0525 (*norD*)	dinitrification protein	5.9	-6.9
PA0962	DNA-binding stress protein	2.3	0
PA4876 (*osmE*)	DNA-binding transcriptional activator	5.7	0
PA5257	enzyme of heme biosynthesis	1.6	0
PA3909	extracellular nuclease	1.9	0
PA5399	ferredoxin	2.2	0
PA1083 (*flgH*)	flagellar basal body L-ring protein	1.5	0
PA1094 (*fliD*)	flagellar capping protein	1.9	0
PA1080 (*flgE*)	flagellar hook protein	1.8	0
PA1086 (*flgK*)	flagellar hook-associated protein	1.8	0
PA1087 (flgL)	flagellar hook-associated protein	1.9	0
PA1084 (*flgI*)	flagellar P-ring protein precursor	1.6	0
PA1085 (*flgJ*)	flagellar rod assembly protein/muramidase	1.8	0
PA1092 (*fliC*)	flagellin type B	2.5	0
PA4217 (*phzS*)	flavin-containing monooxygenase	5.6	-4.7
PA2777	formate/nitrate transporter	4.9	0
PA5420 (*purU2*)	formyltetrahydrofolate deformylase	2.8	0
PA0854 (*fumC2*)	fumarate hydratase	2.5	0
PA0232 (*pcaC*)	gamma-carboxymuconolactone decarboxylase	3.4	0
PA5439	glucose-6-phosphate 1-dehydrogenase	1.6	0
PA2826	glutathione peroxidase	2.2	0
PA2299	GntR family transcriptional regulator	1.7	0
PA1596 (*htpG*)	heat shock protein 90	2.8	-2.3
PA4762 (*grpE*)	heat shock protein	2.4	0
PA3126 (*ibpA*)	heat-shock protein	9.3	0

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0516 (*nirF*)	heme d1 biosynthesis protein	3	-7.8
PA0511 (*nirJ*)	heme d1 biosynthesis protein	3.8	-13
PA0514 (*nirL*)	heme d1 biosynthesis protein	3.2	-9.8
PA2194 (*hcnB*)	hydrogen cyanide synthase	1.8	-5.6
PA2195 (*hcnC*)	hydrogen cyanide synthase	2.2	-4.9
PA0480	hydrolase	4.1	0
PA2934	hydrolase	8.7	0
PA5245	isoprenoid biosynthesis protein with amidotransferase-like domain	2.4	0
PA14_59780 (*rcsC*)	kinase sensor protein	1.6	0
PA2414 (*sndH*)	L-sorbosone dehydrogenase	6.4	0
PA5111 (*gloA3*)	lactoylglutathione lyase	3	0
PA4661 (*pagL*)	Lipid A 3-O-deacylase	3.1	0
PA0062	lipoprotein	2.5	0
PA3031	lipoprotein	1.6	0
PA3691	lipoprotein	5.6	0
PA14_10830	LysR family transcriptional regulator	1.9	0
PA3630 (*gfnR*)	LysR family transcriptional regulator / glutathione-dependent formaldehyde neutralization regulator	2.3	0
PA0482 (*glcB*)	malate synthase G	1.6	0
PA2825	MarR family transcriptional regulator	2.2	0
PA3690	metal-transporting P-type ATPase	2.1	0
PA4205 (*mexG*)	Aminoglycoside efflux protein MexG	1.9	0
PA3718	Major facilitator superfamily permease	2.9	0
PA2933	Major facilitator superfamily transporter	8.5	0
PA4761 (*dnaK*)	molecular chaperone	4.7	0
PA2932 (*morB*)	morphinone reductase	12	0
PA4920 (*nadE*)	NAD synthetase	1.9	0
PA3068 (*gdhB*)	NAD-dependent glutamate dehydrogenase	1.5	0
PA0764 (*mucB*)	negative regulator for alginate biosynthesis	2.1	0
PA4919 (*pcnB1*)	nicotinate phosphoribosyltransferase	2	0
PA0512 (*nirH*)	Denitrification protein NirH	3.3	-11
PA3392 (*nosZ*)	nitrous-oxide reductase	3.7	0
PA2022	nucleotide sugar dehydrogenase	2.9	0
PA14_18720	OmpA family membrane protein	1.9	0
PA5172 (*arcB*)	ornithine carbamoyltransferase	5.6	0
PA0059 (*osmC*)	osmotically inducible protein	6.2	0
PA4208 (*opmD*)	outer membrane protein	2	0
PA14_16630	outer membrane protein, OmpA-family	6.5	0
PA0853	oxidoreductase	2.6	0

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1137	oxidoreductase	3.1	0
PA1833	oxidoreductase	1.9	0
PA3795	oxidoreductase	3.3	0
PA4155	oxidoreductase	2.6	0
PA4621	oxidoreductase	2.1	0
PA3871	peptidyl-prolyl cis-trans isomerase. PpiC-type	3.1	0
PA14_48490	peptidylarginine deiminase	2.7	0
PA3529	peroxidase	1.8	0
PA1900 *phzB2*	phenazine biosynthesis protein	2	0
PA1901 (*phzC2*)	phenazine biosynthesis protein	3.7	0
phzC1	phenazine biosynthesis protein	5.8	-5.9
PA4213 (*phzD1*)	phenazine biosynthesis protein	3.4	0
PA4214 (*phzE1*)	phenazine biosynthesis proteinE	3.1	0
PA0835 (*pta*)	phosphate acetyltransferase	1.6	0
PA0843 (*plcR*)	phospholipase accessory protein	3.1	0
PA3383 (*phnD*)	phosphonate ABC transporter substrate-binding protein	3	0
PA0489	phosphoribosyl transferase	2.2	0
PA0765 (*mucC*)	positive regulator for alginate biosynthesis	1.8	0
prpR	propionate catabolism operon regulator	1.8	0
PA0355 (*pfpI*)	ATP-dependent protease	6	0
PA1545 (*PemB*)	protein secretion by the type III secretion system	2.4	0
PA2244 (*pslN*)	PslN	7.5	0
PA2245 (*pslO*)	PslO	8.3	0
phzG2	pyridoxamine 5'-phosphate oxidase	2.7	0
PA1905 (*phzG1*)	pyrodoxamine 5'-phosphate oxidase	5.2	-2.7
PA5297 *(poxB*)	pyruvate dehydrogenase (cytochrome)	4	0
PA5015 (*aceE)*	pyruvate dehydrogenase subunit E1	1.6	-1.8
PA1919	radical SAM protein	2.2	-9.5
PA2273	redox-sensing activator of SoxS	2.4	0
PA0520 (*nirQ*)	regulatory protein NirQ	3.2	0
PA3875 (*narG*)	respiratory nitrate reductase alpha subunit	4.9	0
PA3874 (*narH*)	respiratory nitrate reductase beta subunit	3.6	0
PA2896 (s*brI*)	RNA polymerase σ factor	2.5	0
PA0762 (*algU*)	RNA polymerase σ factor	2.1	0
PA0156	RND efflux membrane fusion protein	2.2	0
PA0157	RND efflux membrane fusion protein	1.8	0
PA4206 (*mexH*)	RND efflux membrane fusion protein	2.1	0
PA0158	RND efflux transporter	1.7	0
PA4207 (*mexI*)	RND efflux transporter	1.8	0
PA5418 (*soxA*)	sarcosine oxidase alpha subunit	3.7	0

PA gene number and gene name	Gene Product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5416 (*soxB*)	sarcosine oxidase beta subunit	3.7	0
PA2895 (*sbrR*)	Anti-σ factor SbrR	2.6	0
PA1243	sensor/response regulator hybrid	5.6	0
PA3177	sensory box GGDEF domain-containing protein	2.2	0
PA5415 (*glyA1*)	serine hydroxymethyltransferase	3.9	0
PA0766 (*mucD*)	serine protease	2	0
PA3330	short chain dehydrogenas	1.6	-1.6
PA1537	short-chain dehydrogenase	2.8	0
PA4691	sulfite oxidase subunit YedZ	1.9	0
PA2549	TerC family protein	2.2	0
PA2694	thioredoxin	2	0
PA0233	transcriptional regulator	1.6	0
PA0515	transcriptional regulator	3.4	-10
PA0791	transcriptional regulator	2	0
PA1196	transcriptional regulator	2.1	0
PA1290	transcriptional regulator	3.3	0
PA2931	transcriptional regulator	5.1	0
PA0610 (*prtN*)	transcriptional regulator	2.6	0
PA1519	transporter	2.7	0
PA2416 (*treA*)	trehalase	3	0
rcsB	two component response regulator	1.7	-1.6
PA5483 (*algB)*	two-component response regulator AlgB	3.6	0
PA1458	two-component sensor	1.5	0
PA1976	two-component sensor	2.5	0
PA5484	two-component sensor	3.6	0
PA0510 (*nirE*)	uroporphyrin-III c-methyltransferase	5	-5.6
PA2023 (*galU*)	UTP-glucose-1-phosphate uridylyltransferase	2	0
PA1522	xanthine dehydrogenase accessory factor X	1.9	0

3.3.3 Peptide 1018 dysregulated the expression of 74 regulatory genes under swarming conditions

As an opportunistic and ubiquitous pathogen with a broad host range, *P. aeruginosa* possesses an extensive network of regulatory genes which enable it to adapt to and colonize diverse hosts and environmental niches (4). Swarming is a highly regulated adaptive behaviour, and many regulatory genes are involved in swarming motility (36). In the current study, 6.3% (74) of the DE genes identified under peptide 1018-treated conditions were regulators (transcriptional or translational regulators or two-component system genes) (Table 7). Ten of

these genes had been identified as swarming regulators in a previous screen of the PA14 NR transposon insertion library by Yeung et al., with the mutants of two genes, *fis* and *cbrA*, observed to be swarming deficient (36). Both *fis* and *cbrA* were down-regulated under peptide 1018-treated conditions, with *cbrA* uniquely DE by 1018. Overall, nineteen of the total regulatory genes that were dysregulated under peptide treated conditions have functions associated with swarming motility. Of these genes, thirteen were uniquely DE with peptide 1018 treatment, and five were among the swarming regulators identified by Yeung et al. (Table 7) (36).

A number of the genes that were uniquely DE under peptide 1018-treated conditions also have roles in mediating antimicrobial resistance. These genes included regulators *cbrA*, *rcsB*, *rsmA*, *mucA*, *mucB*, *mucC algB*, *algR*, *algU*, *algZ* and *glmR* (137–142). Several of these genes are involved in regulating alginate expression and the conversion a mucoid phenotype, which can enhance antimicrobial tolerance and has clinical significance for infections of the cystic fibrosis lung (141,143). Likewise, *cbrA* and *rsmA* modulate antibiotic resistance in addition to positively regulating motility and acute in vivo virulence (138,144).

Table 7. Select DE regulator genes during treatment with peptide 1018 compared to actively swarming edge cells

PA gene number and gene name	Product	Fold Change
PA0652 (*vfr*)	cAMP-regulatory protein (virulence factor regulator)	-1.7
PA5550 (*glmR*)[d]	Transcriptional regulator (regulation of polysaccharide biosynthetic process)	-2
PA2426 (*pvdS*)	extracytoplasmic-function σ-70 factor	-2
PA3879 (*narL*)	transcriptional regulator	1.7
PA5483 (*algB*)*	two-component response regulator	3.6
PA0610 (*prtN*)*	transcriptional regulator	2.6
PA3630 (*gfnR*)*	glutathione-dependent formaldehyde neutralization regulator–sarcosine metabolism	2.3
PA5274 (*rnk*)*	nucleoside diphosphate kinase regulator	-1.6
prpR *	propionate catabolism operon regulator	1.8
*rcsB**	Two-component response regulator, CupD activation	-1.6
Regulatory genes associated with swarming motility		
PA4853 (*fis*)[1d]	DNA-binding protein Fis	-1.8
PA0905 (*rsmA*) *[1d]	RNA binding protein translational regulator	1.6
PA4725 (*cbrA*) *[1d]	two-component sensor CbrA	-1.5
PA5261 (*algR*)[1d]	alginate biosynthesis regulatory protein AlgR	2.1
PA0762 (*algU*)*	RNA polymerase σ factor AlgU	2.1

PA gene number and gene name	Product	Fold Change
PA2895 (*sbrR*)*[d]	SbrR, anti-σ factor	2.6
PA2896 (*sbrI*)*	RNA polymerase σ factor (inhibits swarming)	2.5
PA1713 (*exsA*)*[d]	transcriptional regulator	-1.7
PA4546 (*pilS)*[d]	two component sensor pilS (twitiching, biofilm, swarming)	-1.7
PA0763 (*mucA*)*	anti-σ factor	3.1
PA0764 (*mucB*)*	negative regulator for alginate biosynthesis	2.1
PA0765 (*mucC*)*	positive regulator for alginate biosynthesis	1.8
PA5262 (*algZ*)	Alginate biosynthesis protein (virulence, twitching, biofilm, swarming)	2
PA0479[1d]	LysR family transcriptional regulator	2.1
PA2072[1d]	sensory box protein	2
PA2571[1d]	signal transduction histidine kinase	1.7
PA1976*[1d]	two-component sensor	2.5
PA1196*[1d]	transcriptional regulator	2.1
PA1458*[1d]	two-component sensor	1.5

*: Genes uniquely expressed under peptide 1018-treated conditions
[1]: Transcriptional regulators controlling swarming motility identified by Yeung et al. (36)
[d]: Swarming deficient phenotype when mutated (36,96,102,137)

Notably, the expression of the global virulence regulator Vfr and the T3SS transcriptional activator ExsA were down-regulated in peptide-1018 treated bacteria. Vfr and the intracellular second messenger, cyclic adenosine monophosphate (cAMP), regulate the expression of acute virulence factors in *P. aeruginosa* such as T3SS, elastase, exotoxin A, and type IV pili (145,146). Vfr also directly activates *exsA* expression by binding with an upstream promoter and stimulating alginate production through the *algR-algZ(fimS)* two-component system (TCS) (146,147). A number of *alg* genes, including the *algR-algZ*, *algB*-PA14_72390, and *algU-mucABCD* operons, and *alg-ADFGJK8* were also DE in peptide 1018-treated conditions (Table 7). These genes are involved in alginate biosynthesis and regulation and were either up-regulated or not DE in cells from the swarm colony centre cf. actively swarming colony edge cells (Appendix Table A1) (61).

The rhamnolipid biosynthesis genes *rhlABC* were induced under peptide 1018-treated conditions, and their expression was also up-regulated in swarm centre cells relative to actively swarming bacteria (Appendix Table A1). Although *algR* is a repressor of rhamnolipid production, it has been hypothesized that it represses rhamnolipids in a biofilm-specific manner, which may explain why *algR* and *rhlABC* were all up-regulated in peptide 1018-treated conditions (132–134).

3.3.4 Several adaptation and virulence factors associated with stationary growth were dysregulated under peptide 1018-treated conditions

Certain genes associated with cell protection during stringent conditions were relatively up-regulated in the swarm centre cf. the leading edge of actively swarming bacteria, and some also showed induction under peptide 1018-treated conditions. Several of these genes are involved in heat shock, nutrient, oxidative and nitrosative stress protection and are associated with non-motile communities of bacteria (e.g., *cioAB*, *cysTWA*, *ibpA*, and *dnaK*) (135,136,148). Two catalase genes, *katA* and *katE* were up-regulated in peptide-treated conditions (Table 8). While *katE* was up-regulated in swarm centre cells cf. actively swarming cells, *katA* was uniquely dysregulated under treatment with peptide 1018. These catalases are peroxide-scavenging enzymes associated with stationary growth and peroxide resistance in biofilms (149). The majority of these genes were DE in both untreated and treated conditions, with DE in peptide 1018-treated genes showing a similar expression pattern to cells in the centre of untreated swarming colonies (Appendix Table A1). However, the heat shock genes, *ibpA, dnaK*, *htpG* and *grpE* were uniquely DE in peptide 1018-treated cells (Table 6)

A number of conventional secreted factors, including *lasA*, *lasB*, and *rhlB* were up-regulated under peptide 1018-treated conditions while genes involved in T3SS and iron acquisition were down-regulated (Table 8). This expression pattern resembled that of untreated swarm centre cells cf. swarm edge cells (Appendix Table A1) (55). Five genes involved in phenazine biosynthesis and the phenazine-associated MexGHI-OpmD efflux pump were up-regulated under peptide treated conditions (Table 8) (150,151). These genes were uniquely expressed under peptide 1018 treatment and had been previously described as up-regulated in actively swarming bacteria when compared to broth conditions (54). Phenazine is a QS-regulated secondary metabolite and precursor to pyocyanin with virulence properties and involvement in transport, carbon metabolism, and redox homeostasis in biofilms (150,152,153). It is induced under anoxic conditions and mediates DNA charge transport in biofilms and increases DNA viscosity, contributing to the biofilm structural matrix (154). Interrupting phenazine biosynthesis genes can enhance swarming motility (155). Only a few genes associated with iron acquisition were down-regulated under peptide 1018-treated conditions, and untreated swarm centre cells strongly repressed *pvd* iron-acquisition genes cf. actively swarming edge cells (Appendix Table A1). Except for a slight down-regulation in *pvdS*, no other *pvd* genes were DE under peptide 1018

treated conditions. It seems unlikely that downregulation of iron uptake under the minimal medium conditions of this swarming assay would be advantageous for cells, and iron-limited conditions typically enhance swarming motility (156,157).

Table 8. Selected DE adaptation and virulence factor genes during treatment with peptide 1018 compared to actively swarming edge cells.

PA gene number and gene name	Product	Fold Change
Secreted Factors		
PA1249 (*aprA*)	alkaline metalloproteinase	2.4
PA3724 (*lasB)*	elastase	2.2
PA1871 (*lasA*)	LasA protease	4.3
PA3478 (*rhlB*)	rhamnosyltransferase chain B	2.4
Type T3SS		
PA1698 (*popN*)	T3SS outer membrane protein	-2.3
PA1703 (*pcrD*)	type III secretory apparatus protein	-2.2
PA1713 (*exsA*)*	transcriptional regulator	-1.7
PA1724 (*pscK*)	PscK type III export protein	-2.2
PA1690 (*pscU*)	translocation protein in type III secretion	-2.4
PA4853 (*fis*)	DNA-binding protein	-1.8
PA1715 (*pscB*)	type III export apparatus protein	-1.8
Iron Acquisition		
PA2426 (*pvdS*)	extracytoplasmic-function σ-70 factor	-2
PA4710	heme/hemoglobin uptake outer membrane receptor	-2
PA14_55050*	TonB-dependent receptor	-1.5
PA4675	TonB-dependent receptor	-1.8
PA1271	TonB-dependent receptor	-1.9
PA3268	TonB-dependent receptor	-4.3
Phenazine Biosynthesis (Pyocyanin)		
PA4214 (*phzE1*)*	phenazine biosynthesis protein	3.1
PA4213 (*phzD1*)*	phenazine biosynthesis protein	3.4
PA1901 (*phzC2*)*	phenazine biosynthesis protein	3.7
phzC1 *	phenazine biosynthesis protein	5.8
PA1900 (*phzB2*)*	phenazine biosynthesis protein	2
Adaption/Stress response		
PA4236 (*katA*)*	Global cAMP regulator of virulence factors	2.9
PA2147 (*katE*)	Global cAMP regulator of virulence factors	14
PA3361 (*lecB*)	fucose-binding lectin PA-IIL	1.8
PA2570 (*pa1L*)	PA-I galactophilic lectin	2.5

*: Genes uniquely expressed under peptide 1018- treated conditions

3.3.5 The gene expression signature of peptide 1018-treated swarming bacteria shared homology with the gene expression signature of cells in the centre of untreated swarming colonies

Although peptide 1018 induced the upregulation of a number of virulence factors that are down-regulated in untreated, actively swarming cells, the majority of these genes were also up-regulated in the center of untreated swarming colonies (Fig. 7). In fact, 67.7% of all DE genes under peptide 1018-treated conditions had a similar expression pattern to cells from swarm colony centres (Fig. 7). Tremblay et al. observed that certain virulence and stress response factors are induced, and metabolic activity is reduced in cells at the centre of swarming colonies and described these cells as 'biofilm-like' (55). This same trend was seen in peptide 1018-treated bacteria. Cells in the centre of swarming colonies appeared to exist in a relatively non-motile state while the more metabolically active swarming cells from the leading edge of swarming colonies act as scouts and rapidly colonize new niches (55).

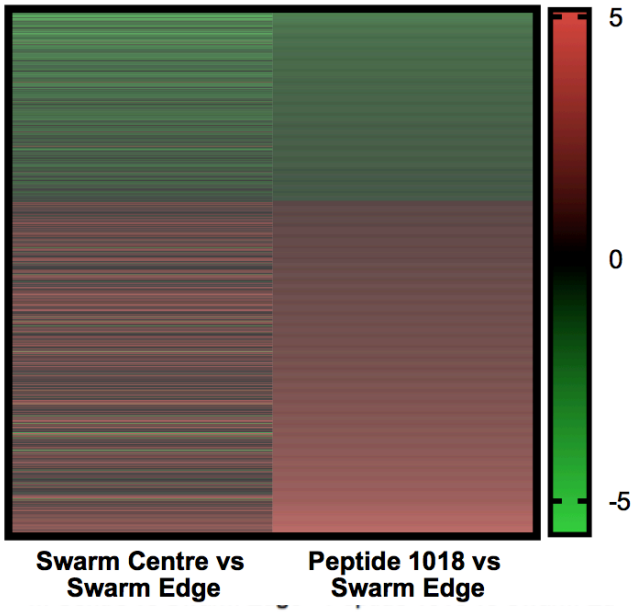

Figure 7. **DE genes under peptide treated conditions showed a similar expression pattern to cells from swarm colony centres.** Red indicates upregulation and green, down-regulation. 67.7% of all DE genes under peptide 1018-treated conditions were expressed in the same direction in cells from swarm colony centres.

3.4 Discussion

Whole transcriptome analysis was used to examine the global impact that treatment with peptide 1018 had on *P. aeruginosa* under swarming conditions. Although the transcriptomic profile of the control group in this study (swarming edge cells vs. swarm centre) was generally

consistent with previous microarray analysis, there were differences in the total number of genes expressed and the expression levels of specific genes. In particular, *pvd* iron acquisition-associated genes were among the most highly induced genes in actively swarming cells in my dataset (cf. swarm centre) but were described as down-regulated in a microarray study by Tremblay et al. (55). Swarming is a nutritionally sensitive adaptive behaviour, and iron depletion is a key environmental cue that induces swarming motility in *P. aeruginosa* (128,129). The previous study used a different swarming medium (M9DCAA) than this study, which used BM2 minimal medium, and this (e.g. levels of iron) might account, in part, for the discrepancy seen in *pvd* gene expression. Interestingly the *pvd* locus was up-regulated in actively swarming cells in the PAO1 microarray dataset which used BM2 minimal medium and compared actively swarming cells to those grown in broth culture (54).

The difference between the total number of DE genes identified by Tremblay et al., who used microarray analysis and this study, which used RNA-Seq, likely resulted from intrinsic differences between these two experimental techniques. Microarray, a hybridization-based technique, is limited by gene probe specificity and especially by the need to determine the background (non-specific hybridization to the glass slide) which varies across the slide. Conversely, RNA-Seq captures absolute transcript levels across the entire bacterial genome and does not require background correction and is thus considered much more accurate (158).

Seven of the genes identified in the transposon insertion mutant screen of peptide 1018-tolerance were also DE under peptide-treated conditions (Appendix Table A1). These genes included *rplJ*, *ligD*, PA1880 (an oxidoreductase), and *rhlB*, which had its peptide tolerant phenotype confirmed through complement testing. The expression of *rhlB* is up-regulated under peptide treated conditions and in the untreated swarm centre cf. actively swarming cells (Appendix A1). I hypothesize that the *rhlB* transposon insertion mutant was able to rescue the swarming phenotype in peptide 1018-treated conditions by preventing the peptide-induced upregulation of *rhlB*. Disrupting *rhlB* would interrupt rhamnolipid biosynthesis and leave HAAs as the primary wetting agents for swarming motility. Rhamnolipids can also act as signalling molecules, which may have significance for the tolerance phenotype observed in the *rhlB* transposon insertion mutant (51,52).

Peptide 1018 has relatively weak direct antimicrobial activity and instead can act as an anti-infective immune enhancer and broad-spectrum inhibitor of adaptive resistance behaviours like

swarming motility and biofilm formation in bacteria. As such, its expression signature differs considerably from those seen in bacteria treated with bactericidal drugs with more direct mechanisms of action. Under peptide 1018-treated conditions, DE gene expression patterns showed a number of cellular features and processes analogous to those seen in stationary phase cells or biofilm colonies. Thus swarm colony centers contain high densities of cells that are not actively swarming. These cells have previously been described as showing stationary or even biofilm-like characteristics (55,159). Intriguingly, the expression signature of peptide 1018-treated swarming colonies showed significant homology (67.7%) to the DE gene expression profile of untreated cells from the swarm colony centre. This might reflect inhibition by 1018 or the fact that these swarm centre cells would remain under peptide treated conditions. TEM images of these two groups of cells also showed similar cell morphologies which were distinct from actively swarming cells (Fig. 3). This switch to a more sessile lifestyle despite conditions that favour motility was also highlighted by the differential expression under peptide treated conditions of genes with products that are involved in protein synthesis, nutrient acquisition, regulation and stress responses.

Examining just those genes that were uniquely dysregulated after peptide treatment revealed several that, when mutated, lead to the inhibition of swarming motility (36). These included the regulatory genes *exsA*, *cbrA*, *pilS*, *narL*, and *sbrI* (Table 7). The repression of these genes could, at least in part, explain the inhibition of swarming motility by peptide 1018.

ExsA is a master regulator of T3SS, and through interactions with the DNA binding protein, Fis, has a variety of roles in optimizing bacterial adaptation and virulence (160,161). T3SS is an important virulence factor in acute in vivo infections, and its expression is positively associated with swarming motility but not the biofilm phenotype (93,162). Both *exsA* and *fis* were down-regulated in the swarm centre cf. actively swarming cells and were also repressed in peptide 1018-treated conditions (Appendix Table A1). Mutants of these regulatory genes are swarming deficient (36).

Like *exsA* and *fis*, the global regulator gene, *cbrA*, is involved in the regulation of swarming motility and biofilm formation. A null deletion mutant of *cbrA* completely inhibited swarming motility but showed enhanced biofilm formation, human lung cell cytotoxicity, and antibiotic resistance in vitro assays (138). In acute in vivo lung infection models, moreover, the expression of *cbrA* was found to be necessary for full virulence (138,144). Upon further investigation, *cbrA*

expression also had a protective effect against neutrophil and macrophage phagocytosis in vitro (144). The CbrA sensor kinase enables *P. aeruginosa* to tune virulence, QS, carbon compound catabolism, and other factors to specific environmental conditions, all of which can be advantageous in variable and complex in vivo environment. The expression of *cbrA* was uniquely down-regulated under peptide 1018-treated conditions.

Similarly, the unique upregulation of specific genes may also contribute to a swarming deficient phenotype during treatment with peptide 1018. Expression of both the extracytoplasmic function σ factor, *sbrI,* and its anti-σ factor, *sbrR*, is associated with inhibited swarming motility and enhanced biofilm formation (96). The expression of these genes was uniquely induced in cells treated with peptide 1018 (Table 7). Although SbrR directly inhibits the expression of *sbrI*, in the presence of peptide 1018, *sbrI* and its associated gene PA1494 (potential *muiA*) (96), were both up-regulated (Appendix Table A1). Increased expression of *sbrI* and PA1494 can inhibit swarming motility in *P.aeruginosa* when they are derepressed by *sbrR* (96).

Genes involved in protein synthesis and several genes involved in DNA synthesis and repair (e.g., *dnaE, dnaQ, efp, nusA, tsf,* and *tufB*) were all down-regulated under peptide 1018-treated conditions. Protein synthesis is necessary for initial surface colonization and, ultimately, in vivo virulence (163). Protein synthesis is typically up-regulated in actively swarming cells and can regulate, and in turn be modulated by, the stringent response, potentially as an energy conservation tactic under nutrient-limited conditions (55,164,165). Along with down-regulated protein synthesis, a number of protein secretion genes including several T3SS-associated genes (e.g., *vfr, exsA, pscBCAU, pcrD,* and *popN*) and T2SS genes (e.g., *secBDEFGY* and *yajC*) were down-regulated in the presence of peptide 1018. Except for *exsA* and *pemB,* which were uniquely expressed with peptide 1018 treatment, most of these genes were down-regulated at the centre of swarming colonies (Table 6, Appendix Table A1). Previous studies have described a positive association between swarming motility, T3SS, T2SS, and in vivo virulence (54,55,93). Although T3SS expression has been linked to the active swarming phenotype, T3SS expression and flagellar biosynthesis are thought to be inversely regulated (54,93,166). This inverse pattern of expression was also observed under peptide 1018-treated conditions, and T3SS associated genes were downregulated while several genes involved in flagellar biosynthesis, including *fliEHIJKLCD,* were up-regulated. All of these flagellar biosynthesis genes except *fliD* and fliC were also up-regulated at the swarm centre. The up-regulated gene expression seen in *fliD* and

fliC was, however, unique to peptide 1018-treated bacteria.

Swarming motility is a finely tuned, nutrient-regulated adaptive behaviour. The swarming phenotype can be induced, modified, or abrogated depending on the source and relative availability of factors like nitrogen, carbon, and metal ions (48,128). Genes involved in metal ion, sulfate, amino acid, taurine and polyamine transport were down-regulated in the presence of peptide 1018, and they were strongly down-regulated at the swarm centre (Appendix Table A1). A number of these transport genes are induced under nutrient limitation and in conditions linked to in-vivo growth (167–169). For instance, sulfate limitation induces the cysteine biosynthetic pathway and sulfated-mucin found in the cystic fibrosis lung is thought to activate sulfate transport (170). A number of genes involved in cysteine biosynthesis and sulfate transport were dysregulated under peptide 1018-treated conditions. Given that swarming motility is a nutrient-sensitive adaptive behaviour, this inverse expression pattern indicates that dysregulating nutrient acquisition might be an important effect of peptide 1018 to provoke a more sessile phenotype (58).

Glycogen biosynthesis and metabolism genes showed high levels of induction under peptide 1018-treated conditions and in the swarm centre relative to actively swarming bacteria. *P. aeruginosa* accumulates glycogen reserves under non-carbon nutrient limitation, and these stores can be used as a future source of carbon and energy, enabling bacteria to cope with starvation conditions (132,171). Glycogen biosynthesis and catabolism are critical for biofilm formation, and glycogen is typically synthesized in the early stationary phase of cell growth (172,173), and genes involved in this process were highly (8.4 to 10 fold) up-regulated in peptide 1018-treated conditions. The biosynthesis and metabolism of other carbon sources such as glycolate and trehalose, a dimer of glucose that can be used a carbon reserve, were also up-regulated in the presence of the peptide. Trehalose can have a protective effect against environmental stresses (osmotic, oxidative, etc.) and may promote nitrogen acquisition (132–134). The carbon storage DE gene expression pattern that was observed under peptide 1018-treated conditions is consistent with sessile adaptations to stringent conditions, but not with active motility (172–174).

Although genes associated with bacterial adaptation and protection showed an overall trend towards upregulation under peptide 1018-treated conditions, several genes involved in the response to DNA damage were among the top 35 most down-regulated genes. The spermidine synthesis gene, *speD,* which is induced in response to DNA damage and magnesium limitation

(175) was the twelfth most down-regulated gene in the dataset with unique DE expression in peptide 1018-treated bacteria. It has previously shown to demonstrate elevated expression in aminoglycoside-resistant small colony variants and may have a role in modulating surface associated behaviours and biofilm formation (175–177). *P. aeruginosa* small colony variants are associated with enhanced biofilm formation, persistence, antibiotic resistance, and in vivo infection during CF (176,178). Alternatively, the two non-homologous end joining DNA double-stranded break repair protein genes, *ku* and *ligD*, were strongly induced by peptide 1018-treated conditions. These genes are hypothesized to mediate genetic variation in *P. aeruginosa* biofilms by repairing mutagenic DNA double-stranded breaks and were down-regulated in actively swarming cells (179,180). Mutations in genes involved in DNA repair can also lead to small colony variants, are frequently observed in biofilms, and are common features of CF infections (181). For the most part, the differential expression of DNA repair genes under peptide 1018-treated conditions was analogous to that observed at the swarm centre cf. actively swarming cells, and shared similarity with the profile of cells engaged in sessile lifestyles.

The *algR-algZ(fimS)* TCS regulates alginate biosynthesis, LPS and hydrogen cyanide production, biofilm formation, and surface motilities (twitching and swarming) (102,182). Transcription of this TCS was up-regulated at the swarm centre cf. actively swarming cells, and was likewise induced by treatment with peptide 1018. Alginate production is an important virulence factor in chronic cystic fibrosis lung infections, and the alginate (mucoid) phenotype is positively associated with the biofilm state and higher rates of morbidity and mortality (61,183). AlgR can influence swarming motility in part by repressing the expression of the *rhl* quorum sensing system and inhibiting rhamnolipid production (184).

While 67.7% of DE genes in peptide 1018-treated bacteria shared significant homology with the DE gene expression seen in the more sessile cells in the centre of swarm colony, the remaining the remaining 37.3% of DE genes might provide specific insights into the effect of peptide 1018 on swarming bacteria. For these genes, the DE gene expression was unique to peptide treatment. In particular, of the 74 regulatory genes dysregulated with peptide treatment, 38 were uniquely expressed (Tables 5, 6). Fourteen of these genes uniquely DE genes have been linked with the regulation of swarming motility and biofilm formation in *P. aeruginosa* and several of these genes, including *sbrI, sbrR*, *rsmA*, *exsA*, *pilS*, and *cbrA* can regulate the switch between these motile and sessile adaptive behaviours (96,144,162,185,186). For five of these

regulatory genes, transposon insertion resulted in a reduced or abolished swarming phenotype (36). Therefore, disrupting the expression of key regulators of motile and sessile adaptive behaviour may be a way in which peptide 1018 inhibits swarming motility.

Chapter 4: Peptide 1018 might inhibit swarming motility by dysregulating the stringent response

4.1 Introduction

Recent studies have provided evidence that peptide 1018 in its action on bacterial biofilms targets the cellular stress response by binding to and stimulating the degradation of (p)ppGpp (85,87,88). Peptide 1018 has anti-biofilm activity across a diverse range of clinically important bacterial species, including both Gram-negative and Gram-positive pathogens (86). The stringent response is highly conserved across bacterial species and has been proposed as a common target for peptide 1018 (85,86). The stringent response and its associated nucleotide alarmones (p)ppGpp (59), have been implicated in adaptive resistance behaviours like biofilm formation (85,87,88) and, to some extent, swarming motility (60). In *P. aeruginosa,* biofilm formation is inhibited in stringent response deficient (*ΔrelAΔspoT*) mutants (87). Furthermore, stringent response mutants are less virulent and are more susceptible to antibiotic treatment (58,88,187). Here I tested its importance in another adaptive behaviour, swarming motility

Swarming motility and the stringent response are both tightly regulated and produce broad transcriptional changes with complex gene expression signatures (54,55,188). A number of the DE gene changes observed in this study support the notion that, as for biofilms, peptide 1018 might disrupt the stringent response of actively swarming bacteria. To probe this link, in vitro assays were conducted with L-serine hydroxamate (SHX), a structural analogue of L-serine that induces the stringent response in *P. aeruginosa*. SHX competitively inhibits the charging of seryl-tRNA synthetase, which induces the stringent response in *P. aeruginosa* through amino acid starvation and activates the RelA-driven synthesis of (p)ppGpp (189). If peptide 1018 acts by binding and degrading (p)ppGpp, then increasing the levels of (p)ppGpp should reduce the efficacy of peptide 1018 as shown for peptide 1018 inhibition of *P. aeruginosa* biofilm formation (87). The effect that raising (p)ppGpp levels had on PA14 swarming motility as well as on peptide 1018 tolerance was examined in this study.

4.2 Materials and methods

4.2.1 Bacterial strains and growth conditions

Several PA14NR transposon insertion mutants and PA14 WT were used in this study (99). PAO1 WT and the PAO1 stringent response and null deletion and complemented mutants were

kindly provided by Dr. Daniel Pletzer (88). Bacterial strains were grown overnight in LB broth at 37°C under shaking conditions. Overnight cultures were sub-cultured into BM2 broth at a normalized optical density at 600nm (OD_{600}) of 0.1 and grown to mid-log phase (0.4-0.6 OD_{600}) at 37°C under shaking conditions.

Table 9. List of *P. aeruginosa* strains

Strain or Plasmid	Description	Reference
P. aeruginosa		
PA14 WT	Laboratory wild type *P. aeruginosa* strain PA14	(100)
PAO1 WT	Wild-type P. aeruginosa PAO1; strain H103	(4)
PAO1 *ΔrelAΔspoT*	Double deletion mutant of *relA* and *spoT*	(88)
PAO1 *ΔrelAΔspoT/relA*$^{+}$	Double deletion mutant of *relA* and *spoT* chromosomally complemented with *relA*	(88)
PAO1 *ΔrelAΔspoT/spoT*$^{+}$	Double deletion mutant of *relA* and *spoT* chromosomally complemented with *spoT*	(88)

4.2.2 Serine hydroxamate swarming assay

As in the standard swarming assay for *P. aeruginosa*, 1μL of mid-log phase sub-culture was inoculated in the centre of plates containing BM2 swarming agar. PAO1 swarming assays were conducted on BM2 swarming agar with 0.5% CAA instead of 0.1% CAA, which was used for PA14. When required, peptide 1018 and/or SHX were incorporated directly into the agar. Plates were incubated at 37°C for 20 hrs. At 20 hrs, each plate was imaged, and the surface area of each swarming colony was quantified using ImageJ software and assessed as a percentage relative to controls. Unless noted, each swarming assay was carried out three to five times. Two-sample Student t-tests were used to evaluate the significance of test conditions on swarming motility.

4.3 Results

4.3.1 Serine hydroxamate inhibited swarming motility at concentrations that enhanced biofilm formation

Previous studies have shown that the mediating genes of the stringent response (*ΔrelAΔspoT*) are involved biofilm formation and, to a certain degree, swarming motility but not in non-adaptive behaviours like swimming motility, which rely on only structural features like a functional flagellum (60,90). In contrast to the minor (~35%) changes observed previously (60), I observed nearly complete inhibition of swarming in the *ΔrelAΔspoT* double mutant (Appendix

Fig. A5).

SHX-driven activation of the stringent response can enhance biofilm formation, and one study demonstrated that while 10 μM increased biofilm formation by two-fold, 320 μM SHX increased biofilm formation by nearly four-fold (86). Swarming experiments performed with increasing concentrations of SHX showed an opposite trend (Fig. 8). Increasing the amount of SHX in PA14 swarming assays inhibited the swarming phenotype in a concentration-dependent manner.

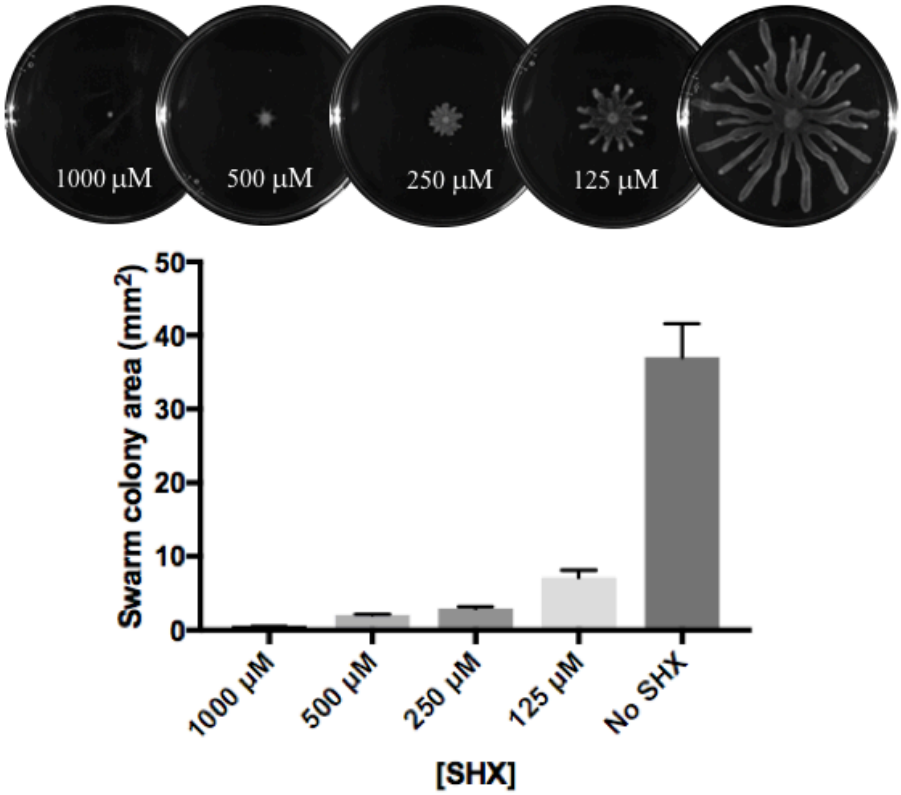

Figure 8. SHX inhibited the swarming phenotype in PA14 WT with increasing concentrations. The swarming phenotype was completely abolished at 1000 μM SHX.

4.3.2 Serine hydroxamate enhanced the swarming phenotype in peptide 1018-tolerant transposon insertion mutants

The effect of raising (p)ppGpp levels on the efficacy of peptide 1018 on swarming bacteria was examined. If peptide 1018 inhibited swarming in a fashion similar to biofilms, by binding to and degrading (p)ppGpp, then modulating the level of (p)ppGpp under peptide 1018-treated conditions should have affected the efficacy of the peptide. At 125 μM SHX, PA14 WT showed minor swarming with 0.75 μg/mL peptide 1018, a concentration of peptide that normally

inhibited swarming motility (Appendix Fig. A6). This swarming phenotype was enhanced at reduced levels of (p)ppGpp (lower SHX concentrations) and disappeared with rising levels of (p)ppGpp (higher SHX concentrations) (Appendix Fig. A6). At a concentration of 250 μM SHX, PA14 WT did not swarm, but intriguingly several peptide-tolerant transposon mutants showed substantially enhanced swarming (Fig. 9).

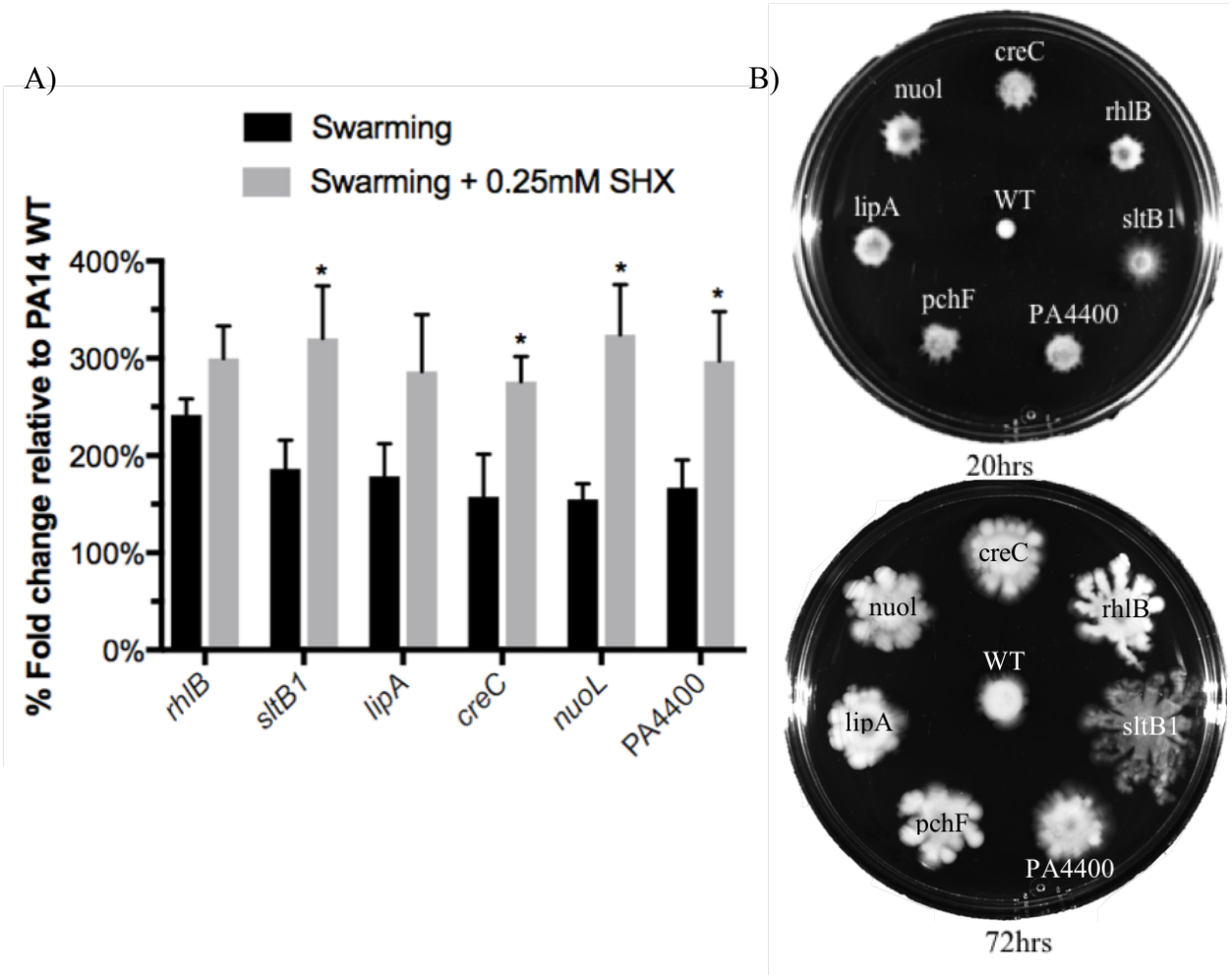

Figure 9. SHX enhanced the swarming phenotype of peptide 1018-tolerant transposon insertion mutants. (A) There was no significant difference between the colony size of PA14WT with and without 250 μM SHX in the presence of 0.75 μg/mL peptide 1018. However, peptide-tolerant mutants showed enhanced swarming. **(B)** Increasing the plate incubation time (cf lower and upper plate) further enhanced the peptide-tolerant phenotype (both plates contained 250 μM SHX and 0.75 μg/mL peptide 1018).

4.4 Discussion

Although RNA-Seq transcriptomic analysis can provide insight into drug targets and

mechanisms of action, whole genome DE analysis also exposes the inherent complexity of drug-pathogen interactions. Theoretically, patterns of change in bacterial gene expression, or expression signatures, will be unique to the drug used and the concentration at which it is applied (190). Bacteria treated with peptide 1018 should show a peptide 1018-specific gene expression signature among their DE genes. However, in reality, compounds can have multiple targets, indirect effects, secondary effects, and bystander effects, all of which complicate the process of isolating specific mechanisms of action from the global gene expression signature (191). Further investigation was needed to corroborate the trends observed in the RNA-Seq data that indicated that peptide 1018 was dysregulating aspects of the stringent response under swarming conditions.

While there is a clear link between (p)ppGpp levels, peptide 1018, and biofilm formation, further research was required to confirm this link in swarming motility (86). A functional stringent response assists to some extent in mediating the swarming phenotype (60; Appendix Fig. A5), and peptide 1018 was found to inhibit swarming motility (Fig. 2). In contrast to the situation for biofilms, raising the level of (p)ppGpp through SHX-induced amino acid starvation appeared to inhibit, rather than enhance the swarming phenotype. This might indeed reflect the importance of protein synthesis in swarming as implicated from gene expression studies. It is also possible that swarming might be enhanced at a lower threshold of SHX, and further experimental work is required to examine the SHX-peptide 1018 interaction in relation to swarming conditions.

Several transposon insertion mutants with tolerance to peptide 1018 showed enhanced swarming in the presence of SHX which may indicate that these genes contribute to the switch between motile and non-motile phenotypes under stringent conditions. Peptide 1018 reduces (p)ppGpp levels by binding to and triggering degradation of these alarmones, and SHX stimulates the production of (p)ppGpp by inducing an amino acid starvation response (86,189). At the extremes of both conditions, PA14 WT does not swarm. However, transposon insertions in specific genes were able to restore the swarming phenotype at (p)ppGpp levels that should be both lower (peptide 1018-treatment) and higher (SHX treatment) in WT strain PA14. It is possible that these mutants are less sensitive to fluctuating levels of (p)ppGpp under swarming conditions and perhaps, that their interrupted genes have roles in modulating motility when (p)ppGpp levels exceed certain thresholds.

Concluding remarks

Swarming motility and biofilm formation are adaptive behaviours that enable bacterial communities to finely tune their phenotypes to better survive challenging environmental conditions. The stringent response is a major regulator of these adaptive behaviours. Bacteria are able tocan gauge the levels of the stringent response alarmones, (p)ppGpp, and combine this information with environmental data from other sensing systems (e.g., surface viscosity, N metabolism, anoxic conditions) to modulate their gene and phenotype expression accordingly (192,193). Removing the ability of bacteria to adapt to their environment can make them vulnerable to antibiotic treatment and clearance by the immune system (85,144,194). It is promising to note that under peptide treated conditions, *P. aeruginosa* displays a non-swarming phenotype and a gene expression signature with characteristics of a sedentary lifestyle when treated with peptide 1018. Peptide-treated bacteria expressed a DE gene profile typical of sedentary cells for a variety of cellular processes such as secretion, protein synthesis, and nutrient acquisition and metabolism. A number of transcriptional regulators, some with direct roles in mediating the switch between sessile and motile lifestyles including *sbrIR, algZ-U*, *exsA,* and *cbrA,* were also dysregulated upon treatment with peptide 1018, and around half of these regulatory genes were uniquely expressed with peptide treatment. The dysregulation of these transcriptional regulators may indicate that in the presence of peptide 1018, *P. aeruginosa* is unable to coordinate the appropriate adaptive behavioural response (swarming motility) for conditions that favour motility.

As a complex and tightly regulated lifestyle adaptation, swarming motility can confer considerable fitness advantages in specific circumstances. For instance, active swarming motility can enhance surface colonization, cell survival in the presence of bacteriophages, and antibiotic resistance (56,58,195). Swarming motility has also been implicated in acute in vivo infection (58,93). In vivo studies with peptide 1018 have shown positive immunomodulatory effects and significant protective effects, including faster wound healing and reduced morbidity and mortality (85,88). Peptide 1018 induces a sedentary phenotype similar to the one observed at the high cell density center of swarming colonies. The dysregulation of swarming motility in conditions that normally favour this adaptive behaviour could have fitness consequences for *P. aeruginosa*, especially in stringent environments.

Peptide 1018 inhibits both biofilm formation and swarming motility at low concentrations,

shows synergy with clinical antibiotics, positively modulates the immune response and is proposed to act by targeting bacterial levels of (p)ppGpp, conserved alarmones in bacterial cells that are not present in mammalian cells (85,196,197). All of these features indicate that IDRs like peptide 1018 have potential as a new type of therapeutic. This study showed that peptide 1018 inhibits swarming motility in *P. aeruginosa*, potentially by dysregulating transcriptional regulators of adaptive behaviour and the bacterial stringent response in conditions that favour motility. However, more research is required to illuminate the specifics of its mechanism of action. Future work could involve an in-depth examination of the effects of the peptide 1018-SHX interaction on swarming motility, and ideally, the impact of (p)ppGpp levels on swarming motility should be clarified. Constructing clean deletion and complemented stringent response mutants for PA14 would also be useful for investigating the impact of peptide 1018, both on actively swarming bacteria and on biofilm formation. The phenotypes and DE gene expression of these mutants under peptide 1018-treated and untreated conditions could be analyzed in both in vitro and in vivo models. Comparing DE gene expression between several species of bacteria that have disrupted swarming motility and biofilm formation under peptide 1018-treated conditions could reveal specifics of a peptide 1018-specific gene expression signature. Ideally, a stronger link between swarming motility, in vivo infection, and acute infection should also be confirmed.

References

1. Govan JR. Infection control in cystic fibrosis: methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex. J R Soc Med. 2000;(Suppl 38):40–5.
2. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27.
3. Rodríguez-Martínez J-M, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2009;53(11):4783–8.
4. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature. 2000;406(6799):959–64.
5. Lee DG, Urbach JM, Wu G, Liberati NT, Feinbaum RL, Miyata S, et al. Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. Genome Biol. 2006;7(10):R90.
6. Cao B, Wang H, Sun H, Zhu Y, Chen M. Risk factors and clinical outcomes of nosocomial multi-drug resistant *Pseudomonas aeruginosa* infections. J Hosp Infect. 2004;57(2):112–8.
7. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs. 2007;67(3):351–68.
8. Barbier F, Andremont A, Wolff M, Bouadma L. Hospital-acquired pneumonia and ventilator-associated pneumonia. Curr Opin Pulm Med. 2013;19(3):216–28.
9. Montravers P, Harpan A, Guivarch E. Current and future considerations for the treatment of hospital-acquired pneumonia. Adv Ther. 2016;33(2):151–66.
10. Nathwani D, Raman G, Sulham K, Gavaghan M, Menon V. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections: a systematic review and meta-analysis. Antimicrob Resist Infect Control. 2014;3(1):32.
11. Trubiano JA, Padiglione AA. Nosocomial infections in the intensive care unit. Anaesthesia and Intensive Care Medicine. 2015;16:598–602.
12. Walkty A, Lagace-Wiens P, Adam H, Baxter M, Karlowsky J, Mulvey MR, et al. Antimicrobial susceptibility of 2906 *Pseudomonas aeruginosa* clinical isolates obtained from patients in Canadian hospitals over a period of 8 years: Results of the Canadian Ward surveillance study (CANWARD), 2008-2015. Diagn Microbiol Infect Dis. 2017;87(1):60–3.
13. Thom KA, Md DE, Boutin Mph MA, Zhan Phd M, Harris AD, Kristie J, et al. Frequent contamination of nursing scrubs is associated with specific care activities. Am J Infect Control. 2018;46:503–6.
14. Kanamori H, Rutala WA, Weber DJ. The role of patient care items as a fomite in healthcare-associated outbreaks and infection prevention. Clin Infect Dis. 2017;65(8):1412–9.
15. Verstraeten N, Braeken K, Debkumari B, Fauvart M, Fransaer J, Vermant J, et al. Living on a surface: swarming and biofilm formation. Trends in Microbiology. 2008;16:496–506.
16. Smith K, Hunter IS. Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. J Med Microbiol. 2008;57(8):966–73.

17. Elborn JS. Cystic fibrosis. Lancet. 2016;388(10059):2519–31.
18. Campodónico VL, Gadjeva M, Paradis-Bleau C, Uluer A, Pier GB. Airway epithelial control of *Pseudomonas aeruginosa* infection in cystic fibrosis. Trends Mol Med. 2008;14(3):120–33.
19. Barley M, Mcnally J, Marshall B, Faro A, Elbert A, Fink A, et al. Annual data report 2016 cystic fibrosis foundation patient registry. Cyst Fibros Found Patient Regist. 2016;1–94.
20. Cystic Fibrosis Canada. The Canadian CF registry 2016 annual data report. 2017;44.
21. Staudinger BJ, Muller JF, Halldórsson S, Boles B, Angermeyer A, Nguyen D, et al. Conditions associated with the cystic fibrosis defect promote chronic *Pseudomonas aeruginosa* infection. Am J Respir Crit Care Med. 2014;189(7):812–24.
22. Folkesson A, Jelsbak L, Yang L, Johansen HK, Ciofu O, Høiby N, et al. Adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis airway: an evolutionary perspective. Nat Rev Microbiol. 2012;10(12):841–51.
23. Ratjen F, Bell SC, Rowe SM, Goss CH, Quittner AL, Bush A. Cystic fibrosis. Nat Rev Dis Prim. 2015;1:15010.
24. The 10 × '20 Initiative: Pursuing a global commitment to develop 10 new antibacterial drugs by 2020. Clin Infect Dis. 2010;50(8):1081–3.
25. Breidenstein EBM, de la Fuente-Núñez C, Hancock REW. *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol. 2011;19(8):419–26.
26. Nicas TI, Hancock REW. *Pseudomonas aeruginosa* outer membrane permeability: Isolation of a porin protein F-deficient mutant. J Bacteriol. 1983;153(1):281–5.
27. Hancock REW, Speert DP. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and impact on treatment. Drug Resistance Updates. 2000;3:247–55.
28. Fernández L, Breidenstein EBM, Hancock REW. Creeping baselines and adaptive resistance to antibiotics. Drug Resist Updat. 2011;14(1):1–21.
29. Kong K-F, Jayawardena SR, Indulkar SD, del Puerto A, Koh C-L, Hoiby N, et al. *Pseudomonas aeruginosa* AmpR Is a global transcriptional factor that regulates expression of AmpC and PoxB -lactamases, proteases, quorum sensing, and other virulence factors. Antimicrob Agents Chemother. 2005;49(11):4567–75.
30. Hancock REW. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clin Infect Dis. 1998;27(s1):S93–9.
31. San Millan A, Toll-Riera M, Escudero JA, Cantón R, Coque TM, MacLean RC. Sequencing of plasmids pAMBL1 and pAMBL2 from *Pseudomonas aeruginosa* reveals a *bla* $_{\text{VIM-1}}$ amplification causing high-level carbapenem resistance. J Antimicrob Chemother. 2015;70(11):3000–3.
32. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends in Molecular Medicine. Elsevier Current Trends; 2012;18: 263–72.
33. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Current Opinion in Microbiology. 2010;13:558–64.
34. Rodesney CA, Roman B, Dhamani N, Cooley BJ, Touhami A, Gordon VD. Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. Proc Natl Acad Sci. 2017;114(23):5906–11.
35. Costerton JW, Stewart PS. Battling biofilms. Sci Am. 2001;285(1):74-81.
36. Yeung ATY, Torfs ECW, Jamshidi F, Bains M, Wiegand I, Hancock REW, et al. Swarming of *Pseudomonas aeruginosa* is controlled by a broad spectrum of

transcriptional regulators, including MetR. J Bacteriol. 2009;191(18):5592–602.
37. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167–93.
38. Sanchez CJ, Mende K, Beckius ML, Akers KS, Romano DR, Wenke JC, et al. Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infect Dis. 2013;13(1):47.
39. Wu H, Moser C, Wang HZ, Høiby N, Song ZJ. Strategies for combating bacterial biofilm infections. International Journal of Oral Science. 2015; 7(1): 1–7.
40. Jensen PØ, Givskov M, Bjarnsholt T, Moser C. The immune system vs. *Pseudomonas aeruginosa* biofilms. FEMS Immunology and Medical Microbiology. 2010;59(3):292-305.
41. Dumas J-L, Delden C, Perron K, Khöler T. Analysis of antibiotic resistance gene expression in *Pseudomonas aeruginosa* by quantitative real-time-PCR. FEMS Microbiol Lett. 2006;254(2):217–25.
42. Nickel JC, Wright JB, Ruseska I, Marrie TJ, Whitfield C, Costerton JW. Antibiotic resistance of *Pseudomonas aeruginosa* colonizing a urinary catheter in vitro. Eur J Clin Microbiol. 1985;4(2):213–8.
43. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318–22.
44. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: From the natural environment to infectious diseases. Nature Reviews Microbiology. 2004;2(2):95-108.
45. Elias S, Banin E. Multi-species biofilms: living with friendly neighbors. FEMS Microbiol Rev. 2012;36(5):990–1004.
46. Shrout JD, Chopp DL, Just CL, Hentzer M, Givskov M, Parsek MR. The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. Mol Microbiol. 2006;62(5):1264–77.
47. Guo Q, Kong W, Jin S, Chen L, Xu Y, Duan K. PqsR-dependent and PqsR-independent regulation of motility and biofilm formation by PQS in *Pseudomonas aeruginosa* PAO1. J Basic Microbiol. 2014;54(7):633–43.
48. Köhler TK, Curty LK, Barja F, Delden C Van, Pech Re J-C. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J Bacteriol. 2000;182(21):5990–6.
49. McCallum SJ, Gallagher MJ, Corkill JE, Hart CA, Ledson MJ, Walshaw MJ. Spread of an epidemic *Pseudomonas aeruginosa* strain from a patient with cystic fibrosis (CF) to non-CF relatives. Thorax. 2002;57(6):559–60.
50. Anyan ME, Amiri A, Harvey CW, Tierra G, Morales-Soto N, Driscoll CM, et al. Type IV pili interactions promote intercellular association and moderate swarming of *Pseudomonas aeruginosa*. Proc Natl Acad Sci. 2014;111(50):18013–8.
51. Caiazza NC, Shanks RMQ, O'Toole GA. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. J Bacteriol. 2005;187(21):7351–61.
52. Déziel E, Lépine F, Milot S, Villemur R. *rhlA* is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology. 2003;149:2005–13.
53. Bhattacharjee A, Nusca TD, Hochbaum AI. Rhamnolipids mediate an interspecies biofilm dispersal signaling pathway. ACS Chem Biol. 2016;11(11):3068–76.

54. Overhage J, Bains M, Brazas MD, Hancock REW. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J Bacteriol. 2008;190(8):2671–9.
55. Tremblay J, Déziel E. Gene expression in *Pseudomonas aeruginosa* swarming motility. BMC Genomics. 2010;11(1):587.
56. Butler MT, Wang Q, Harshey RM. Cell density and mobility protect swarming bacteria against antibiotics. Proc Natl Acad Sci. 2010;107(8):3776–81.
57. Potrykus K, Cashel M. (p)ppGpp: Still Magical? Annu Rev Microbiol. 2008;62(1):35–51.
58. Vogt SL, Green C, Stevens KM, Day B, Erickson DL, Woods DE, et al. The stringent response is essential for *Pseudomonas aeruginosa* virulence in the rat lung agar bead and drosophila melanogaster feeding models of infection. Infect Immun. 2011;79(10):4094–104.
59. Xu X, Yu H, Zhang D, Xiong J, Qiu J, Xin R, et al. Role of ppGpp in *Pseudomonas aeruginosa* acute pulmonary infection and virulence regulation. Microbiol Res. 2016;192:84–95.
60. Braeken K, Moris M, Daniels R, Vanderleyden J, Michiels J. New horizons for (p)ppGpp in bacterial and plant physiology. Trends Microbiol. 2006;14(1):45–54.
61. Ramsey DM, Wozniak DJ. Understanding the control of *Pseudomonas aeruginosa* alginate synthesis and the prospects for management of chronic infections in cystic fibrosis. Molecular Microbiology 2005;56(2):309–22.
62. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, et al. Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. J Infect Dis. 2001;183(12):1767–74.
63. Simpson JA, Smith SE, Dean RT. Scavenging by alginate of free radicals released by macrophages. Free Radic Biol Med. 1989;6(4):347–53.
64. Amiel E, Lovewell RR, O'Toole GA, Hogan DA, Berwin B. *Pseudomonas aeruginosa* evasion of phagocytosis is mediated by loss of swimming motility and is independent of flagellum expression. Infect Immun. 2010;78(7):2937–45.
65. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000;407(6805):762–4.
66. Barth AL, Pitt TL. The high amino-acid content of sputum from cystic fibrosis patients promotes growth of auxotrophic *Pseudomonas aeruginosa*. J Med Microbiol. 1996;45(2):110–9.
67. Kownatzki R, Tümmler B, Döring G. Rhamnolipid of *Pseudomonas aeruginosa* in sputum of cystic fibrosis patients. The Lancet. Elsevier; 1987;329:1026–7.
68. Palmer KL, Aye LM, Whiteley M. Nutritional cues control *Pseudomonas aeruginosa* multicellular behavior in cystic fibrosis sputum. J Bacteriol. 2007;189(22):8079–87.
69. O'May CY, Reid DW, Kirov SM. Anaerobic culture conditions favor biofilm-like phenotypes in *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. FEMS Immunol Med Microbiol. 2006;48(3):373–80.
70. Sun Yoon S, Hennigan RF, Hilliard GM, Ochsner UA, Parvatiyar K, Kamani MC, et al. *Pseudomonas aeruginosa* Anaerobic Respiration in Biofilms: Relationships to Cystic Fibrosis Pathogenesis of toxic NO, a byproduct of anaerobic respiration. Pro- teomic analyses identified an outer membrane protein. Dev Cell. 2002;3(4):593–603.
71. Quinn RA, Lim YW, Maughan H, Conrad D, Rohwer F, Whiteson KL. Biogeochemical

forces shape the composition and physiology of polymicrobial communities in the cystic fibrosis lung. MBio. 2014;5(2):e00956-13.
72. Jorth P, Staudinger BJ, Wu X, Hisert KB, Hayden H, Garudathri J, et al. Regional isolation drives bacterial diversification within cystic fibrosis lungs. Cell Host Microbe. 2015;18(3):307–19.
73. Goss CH, Burns JL. Exacerbations in cystic fibrosis???: Epidemiology and pathogenesis. Thorax. 2007;62(4):360-7.
74. Gupta K, Marques CNH, Petrova OE, Sauer K. Antimicrobial tolerance of *Pseudomonas aeruginosa* biofilms is activated during an early developmental stage and requires the two-component hybrid SagS. J Bacteriol. 2013;195(21):4975–87.
75. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1–12.
76. Zasloff M. Antimicrobial peptides of multicellular organisms. Vol. 415, Nature.2002;415:389-395.
77. Hancock RE., Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol. 1998;16(2):82–8.
78. Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. Vol. 21, Clinical Microbiology Reviews. 2008;21(3):449-65.
79. Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnl. 2006;24(12):1551–7.
80. Peschel A, Sahl H-G. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat Rev Microbiol. 2006;4(7):529–36.
81. Fjell CD, Hiss JA, Hancock REW, Schneider G. Designing antimicrobial peptides: form follows function. Nature Reviews Drug Discovery. 16;11(1):37-51.
82. Haney EF, Hancock REW. Peptide design for antimicrobial and immunomodulatory applications. Biopolymers. 2013;100(6):572-83.
83. Haney EF, Brito-Sánchez Y, Trimble MJ, Mansour SC, Cherkasov A, Hancock REW. Computer-aided discovery of peptides that specifically attack bacterial biofilms. Sci Rep. 2018;8(1):1871.
84. Hilpert K, Volkmer-Engert R, Walter T, Hancock REW. High-throughput generation of small antibacterial peptides with improved activity. Nat Biotechnol. 2005;23(8):1008–12.
85. Mansour SC, De La Fuente-Núñez C, Hancock REWW. Peptide IDR-1018: modulating the immune system and targeting bacterial biofilms to treat antibiotic-resistant bacterial infections. J Pept Sci. 2015;21(5):323–9.
86. de la Fuente-Núñez C, Korolik V, Bains M, Nguyen U, Breidenstein EBM, Horsman S, et al. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob Agents Chemother. 2012;56(5):2696–704.
87. de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock REW. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog. 2014;10(5):e1004152.
88. Pletzer D, Wolfmeier H, Bains M, Hancock REW. Synthetic peptides to target stringent response-controlled virulence in a *Pseudomonas aeruginosa* murine cutaneous infection model. Front Microbiol. 2017;8:1867.
89. O'May C, Ciobanu A, Lam H, Tufenkji N. Tannin derived materials can block swarming motility and enhance biofilm formation in *Pseudomonas aeruginosa*. Biofouling.

2012;28(10):1063–76.
90. Kim W, Surette MG. Swarming populations of *Salmonella* represent a unique physiological state coupled to multiple mechanisms of antibiotic resistance. Biol Proced Online. 2003;5(1):189–96.
91. Lai S, Tremblay J, Déziel E. Swarming motility: A multicellular behaviour conferring antimicrobial resistance. Environ Microbiol. 2009;11(1):126–36.
92. Harshey RM. Bacterial motility on a surface: many ways to a common goal. Annu Rev Microbiol. 2003;57:249–73.
93. Murray TS, Ledizet M, Kazmierczak BI. Swarming motility, secretion of type 3 effectors and biofilm formation phenotypes exhibited within a large cohort of *Pseudomonas aeruginosa* clinical isolates. J Med Microbiol. 2010;59(5):511–20.
94. Kuchma SL, Brothers KM, Merritt JH, Liberati NT, Ausubel FM, O'Toole GA. BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol. 2007;189(22):8165–78.
95. Caiazza NC, Merritt JH, Brothers KM, O'Toole GA. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol. 2007;189(9):3603–12.
96. McGuffie BA, Vallet-Gely I, Dove SL. σ Factor and Anti-σ Factor That Control Swarming Motility and Biofilm Formation in *Pseudomonas aeruginosa*. J Bacteriol. 2015;198(5):755–65.
97. Schafhauser J, Lepine F, McKay G, Ahlgren HG, Khakimova M, Nguyen D. The stringent response modulates 4-hydroxy-2-alkylquinoline biosynthesis and quorum-sensing hierarchy in *Pseudomonas aeruginosa*. J Bacteriol. 2014;196(9):1641–50.
98. Van Delden C, Comte R, Bally M. Stringent response activates quorum sensing and modulates cell density-dependent gene expression in *Pseudomonas aeruginosa*. J Bacteriol. 2001;183(18):5376–84.
99. Liberati NT, Urbach JM, Miyata S, Lee DG, Drenkard E, Wu G, et al. An ordered, nonredundant library of *Pseudomonas aeruginosa* strain PA14 transposon insertion mutants. Proc Natl Acad Sci U S A. 2006;103(8):2833–8.
100. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. Common virulence factors for bacterial pathogenicity in plants and animals. Science. 1995;268(5219):1899–902.
101. Kovach ME, Elzer PH, Steven Hill D, Robertson GT, Farris MA, Roop RM, et al. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene. 1995;166(1):175–6.
102. Overhage J, Lewenza S, Marr AK, Hancock REW. Identification of genes involved in swarming motility using a *Pseudomonas aeruginosa* PAO1 mini-Tn5-lux mutant library. J Bacteriol. 2007;189(5):2164–9.
103. Rao NN, Liu S, Kornberg A. Inorganic polyphosphate in Escherichia coli: The phosphate regulon and the stringent response. J Bacteriol. 1998;180(8):2186–93.
104. Boes N, Schreiber K, Schobert M. SpoT-triggered stringent response controls usp gene expression in *Pseudomonas aeruginosa*. J Bacteriol. 2008;190(21):7189–99.
105. Khakimova M, Ahlgren HG, Harrison JJ, English AM, Nguyen D. The stringent response controls catalases in *Pseudomonas aeruginosa* and is required for hydrogen peroxide and antibiotic tolerance. J Bacteriol. 2013;195(9):2011–20.
106. Pletzer D, Braun Y, Weingart H. Swarming motility is modulated by expression of the

putative xenosiderophore transporter SppR-SppABCD in *Pseudomonas aeruginosa* PA14. Antonie Van Leeuwenhoek. 2016;109(6):737–53.
107. Mellbye B, Schuster M. Physiological framework for the regulation of quorum sensing-dependent public goods in *Pseudomonas aeruginosa*. J Bacteriol. 2014;196(6):1155–64.
108. Davey ME, Caiazza NC, O'Toole GA. Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. J Bacteriol. 2003;185(3):1027–36.
109. Funken H, Knapp A, Vasil ML, Wilhelm S, Jaeger KE, Rosenau F. The lipase lipA (PA2862) but not lipC (PA4813) from *Pseudomonas aeruginosa* influences regulation of pyoverdine production and expression of the sigma factor PvdS. Journal of Bacteriology. American Society for Microbiology. 2011;193(20):5858-60.
110. Haddad A, Jensen V, Becker T, Häussler S. The Pho regulon influences biofilm formation and type three secretion in *Pseudomonas aeruginosa*. Environ Microbiol Rep. 2009;1(6):488–94.
111. Rashid MH, Kornberg A. Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. Proc Natl Acad Sci. 2000;97(9):4885–90.
112. Hammond JH, Dolben EF, Smith TJ, Bhuju S, Hogan DA. Links between Anr and quorum sensing in *Pseudomonas aeruginosa* biofilms. J Bacteriol. 2015;197(17):2810–20.
113. Schobert M, Jahn D. Anaerobic physiology of *Pseudomonas aeruginosa* in the cystic fibrosis lung. International Journal of Medical Microbiology. 2010;300(8):549-56
114. Jackson AA, Gross MJ, Daniels EF, Hampton TH, Hammond JH, Vallet-Gely I, et al. Anr and its activation by PlcH activity in *Pseudomonas aeruginosa* host colonization and virulence. J Bacteriol. 2013;195(13):3093–104.
115. Tribelli PM, Hay AG, López NI. The Global Anaerobic Regulator Anr, Is Involved in Cell Attachment and Aggregation Influencing the First Stages of Biofilm Development in Pseudomonas extremaustralis. PLoS One. 2013;8(10):e76685.
116. Boles BR, Thoendel M, Singh PK. Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. Mol Microbiol. 2005;57(5):1210–23.
117. Zulianello L, Canard C, Köhler T, Caille D, Lacroix J-S, Meda P. Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by *Pseudomonas aeruginosa*. Infect Immun. 2006;74(6):3134–47.
118. Ochsner UA, Reiser J. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. Proc Natl Acad Sci. 1995;92(14):6424–8.
119. Medina G, Juárez K, Valderrama B, Soberón-Chávez G. Mechanism of *Pseudomonas aeruginosa* RhlR transcriptional regulation of the rhlAB promoter. J Bacteriol. 2003;185(20):5976–83.
120. Medina G, Juárez K, Díaz R, Soberón-Chávez G. Transcriptional regulation of *Pseudomonas aeruginosa* rhlR, encoding a quorum-sensing regulatory protein. Microbiology. 2003;149(11):3073–81.
121. Soberón-Chávez G, Lépine F, Déziel E. Production of rhamnolipids by *Pseudomonas aeruginosa*. Applied Microbiology and Biotechnology Springer-Verlag;2005;68(6):718-25.
122. Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock REW. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. Antimicrob Agents Chemother. 2002;46(3):605–14.
123. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: Summarize analysis results for

multiple tools and samples in a single report. Bioinformatics. 2016;32(19):3047–8.
124. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, et al. GenBank. Nucleic Acids Res. 2012;41(D1):D36–42.
125. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15–21.
126. Anders S, Pyl PT, Huber W. HTSeq-A Python framework to work with high-throughput sequencing data. Bioinformatics. 2015;31(2):166–9.
127. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
128. Lin CS, Tsai YH, Chang CJ, Tseng SF, Wu TR, Lu CC, et al. An iron detection system determines bacterial swarming initiation and biofilm formation. Sci Rep. 2016;6(1):36747.
129. Imperi F, Tiburzi F, Fimia GM, Visca P. Transcriptional control of the *pvdS* iron starvation sigma factor gene by the master regulator of sulfur metabolism CysB in *Pseudomonas aeruginosa*. Environ Microbiol. 2010;12(6):1630–42.
130. Winsor GL, Griffiths EJ, Lo R, Dhillon BK, Shay JA, Brinkman FSL. Enhanced annotations and features for comparing thousands of Pseudomonas genomes in the Pseudomonas genome database. Nucleic Acids Res. ; 2016;44(D1):D646–53.
131. Zhu H, Shuman S. Gap filling activities of *Pseudomonas* DNA ligase D (LigD) polymerase and functional interactions of LigD with the DNA end-binding Ku protein. J Biol Chem. 2010;285(7):4815–25.
132. Chandra G, Chater KF, Bornemann S. Unexpected and widespread connections between bacterial glycogen and trehalose metabolism. Microbiology. 2011;157(Pt 6):1565-72.
133. Strom AR, Kaasen I. Trehalose metabolism in Escherichia coli: stress protection and stress regulation of gene expression. Vol. 8, Molecular Microbiology. 1993;8(2):205-10.
134. Djonović S, Urbach JM, Drenkard E, Bush J, Feinbaum R, Ausubel JL, et al. Trehalose biosynthesis promotes *Pseudomonas aeruginosa* pathogenicity in plants. PLoS Pathog. 2013;9(3):e1003217.
135. Gophna U, Ron EZ. Virulence and the heat shock response. Int J Med Microbiol. 2003;292(7–8):453–61.
136. Torres-Barceló C, Cabot G, Oliver A, Buckling A, Maclean C. A trade-off between oxidative stress resistance and DNA repair plays a role in the evolution of elevated mutation rates in bacteria. Proc R Soc B Biol Sci. 2013;280(1757):20130007.
137. Ramos-Aires J, Plésiat P, Kocjancic-Curty L, Köhler T. Selection of an antibiotic-hypersusceptible mutant of *Pseudomonas aeruginosa*: identification of the GlmR transcriptional regulator. Antimicrob Agents Chemother. 2004;48(3):843–51.
138. Yeung ATY, Bains M, Hancock REW. The sensor kinase CbrA is a global regulator that modulates metabolism, virulence, and antibiotic resistance in *Pseudomonas aeruginosa*. J Bacteriol. 2011;193(4):918–31.
139. Alvarez-Ortega C, Wiegand I, Olivares J, Hancock REW, Martínez JL. Genetic determinants involved in the susceptibility of *Pseudomonas aeruginosa* to beta-lactam antibiotics. Antimicrob Agents Chemother. 2010;54(10):4159–67.
140. Boucher JC, Yu H, Mudd MH, Deretic V. Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: characterization of muc mutations in clinical isolates and analysis of clearance in a mouse model of respiratory infection. Infect Immun. 1997;65(9):3838–46.
141. Goltermann L, Tolker-Nielsen T. Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. Antimicrob Agents

Chemother. 2017;61(4):e02696-16.
142. Mulcahy H, O'Callaghan J, O'Grady EP, Maciá MD, Borrell N, Gómez C, et al. *Pseudomonas aeruginosa* RsmA plays an important role during murine infection by influencing colonization, virulence, persistence, and pulmonary inflammation. Infect Immun. 2008;76(2):632–8.
143. Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiol Rev. 1996;60(3):539–74.
144. Yeung ATY, Janot L, Pena OM, Neidig A, Kukavica-Ibrulj I, Hilchie A, et al. Requirement of the *Pseudomonas aeruginosa* CbrA sensor kinase for full virulence in a murine acute lung infection model. Infect Immun. 2014;82(3):1256–67.
145. Fuchs EL, Brutinel ED, Jones AK, Fulcher NB, Urbanowski ML, Yahr TL, et al. The *Pseudomonas aeruginosa* Vfr regulator controls global virulence factor expression through cyclic AMP-dependent and -independent mechanisms. J Bacteriol. 2010;15;192(14):3553–64.
146. Marsden AE, Intile PJ, Schulmeyer KH, Simmons-Patterson ER, Urbanowski ML, Wolfgang MC, et al. Vfr Directly activates *exsA* transcription to regulate expression of the *Pseudomonas aeruginosa* type III secretion system. J Bacteriol. 2016 1;198(9):1442–50.
147. Chang C-Y. Surface Sensing for Biofilm Formation in *Pseudomonas aeruginosa*. Front Microbiol. 2017;8:2671.
148. Arai H, Igarashi Y, Kodama T. Expression of the *nir* and *nor* genes for denitrification of *Pseudomonas aeruginosa* requires a novel CRP/FNR-related transcriptional regulator, DNR, in addition to ANR. FEBS Lett. 1995;371(1):73–6.
149. Shin D-H, Choi Y-S, Cho Y-H. Unusual properties of catalase A (KatA) of *Pseudomonas aeruginosa* PA14 are associated with its biofilm peroxide resistance. J Bacteriol. 2008;190(8):2663–70.
150. Dietrich LEP, Price-Whelan A, Petersen A, Whiteley M, Newman DK. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. Mol Microbiol. 2006;61(5):1308–21.
151. Price-Whelan A, Dietrich LEP, Newman DK. Pyocyanin Alters Redox Homeostasis and Carbon Flux through Central Metabolic Pathways in *Pseudomonas aeruginosa* PA14. J Bacteriol. 2007;189(17):6372–81.
152. Rada B, Leto TL. Pyocyanin effects on respiratory epithelium: relevance in *Pseudomonas aeruginosa* airway infections. Trends Microbiol. 2013;21(2):73–81.
153. Lin YC, Sekedat MD, Cornell WC, Silva GM, Okegbe C, Price-Whelan A, et al. Phenazines regulate Napdependent denitrification in *Pseudomonas aeruginosa* biofilms. J Bacteriol. 2018;200(9):e00031-18.
154. Das T, Kutty SK, Tavallaie R, Ibugo AI, Panchompoo J, Sehar S, et al. Phenazine virulence factor binding to extracellular DNA is important for *Pseudomonas aeruginosa* biofilm formation. Sci Rep. 2015;5(1):8398.
155. Ramos I, Dietrich LEP, Price-Whelan A, Newman DK. Phenazines affect biofilm formation by *Pseudomonas aeruginosa* in similar ways at various scales. Res Microbiol. 2010;161(3):187–91.
156. Nadal Jimenez P, Koch G, Papaioannou E, Wahjudi M, Krzeslak J, Coenye T, et al. Role of PvdQ in *Pseudomonas aeruginosa* virulence under iron-limiting conditions. Front Microbiol. 2010 ;156(Pt 1):49-59..
157. Leoni L, Orsi N, de Lorenzo V, Visca P. Functional analysis of PvdS, an iron starvation

sigma factor of *Pseudomonas aeruginosa*. J Bacteriol. 2000;182(6):1481–91.
158. Finotello F, Di Camillo B. Measuring differential gene expression with RNA-seq: challenges and strategies for data analysis. Brief Funct Genomics. 2015;14(2):130–42.
159. Darnton NC, Turner L, Rojevsky S, Berg HC. Dynamics of bacterial swarming. Biophys J. 2010;98(10):2082–90.
160. Hauser AR. The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. Nat Rev Microbiol. 2009;7(9):654–65.
161. Deng X, Li M, Pan X, Zheng R, Liu C, Chen F, et al. Fis regulates type III secretion system by influencing the transcription of exsA in *Pseudomonas aeruginosa* Strain PA14. Front Microbiol. 2017;8:669.
162. Zhu M, Zhao J, Kang H, Kong W, Liang H, Wu M, et al. Modulation of type III secretion system in *Pseudomonas aeruginosa*: involvement of the PA4857 gene product. Front Microbiol. 2016;7:7.
163. Crouzet M, Claverol S, Lomenech A-M, Le Sénéchal C, Costaglioli P, Barthe C, et al. *Pseudomonas aeruginosa* cells attached to a surface display a typical proteome early as 20 minutes of incubation. Cascales E, editor. PLoS One. 2017;12(7):e0180341.
164. Nogales J, Dominguez-Ferreras A, Amaya-Gomez C V, van Dillewijn P, Cuellar V, Sanjuan J, et al. Transcriptome profiling of a *Sinorhizobium meliloti fadD* mutant reveals the role of rhizobactin 1021 biosynthesis and regulation genes in the control of swarming. BMC Genomics. 2010;11(1):157.
165. Svitils AL, Cashel M, Zyskindsll JW. Guanosine tetraphosphate inhibits protein synthesis in vivo. J Biol Chem. 1993;268(4):2307–11.
166. Yahr TL, Wolfgang MC. Transcriptional regulation of the *Pseudomonas aeruginosa* type III secretion system. Mol Microbiol. 2006;62(3):631–40.
167. Bielecki P, Puchałka J, Wos-Oxley ML, Loessner H, Glik J, Kawecki M, et al. In-Vivo Expression Profiling of *Pseudomonas aeruginosa* Infections reveals niche-specific and strain-independent transcriptional programs. PLoS One. 2011;6(9):e24235.
168. Chace K V., Leahy DS, Martin R, Carubelli R, Flux M, Sachdev GP. Respiratory mucous secretions in patients with cystic fibrosis: relationship between levels of highly sulfated mucin component and severity of the disease. Clin Chim Acta. 1983;132(2):143–55.
169. Frangipani E, Slaveykova VI, Reimmann C, Haas D. Adaptation of aerobically growing *Pseudomonas aeruginosa* to copper starvation. J Bacteriol. 2008;190(20):6706–17.
170. Xia B, Royall JA, Damera G, Sachdev GP, Cummings RD. Altered O-glycosylation and sulfation of airway mucins associated with cystic fibrosis. Glycobiology. 2005;15(8):747–75.
171. Henrissat B, Deleury E, Coutinho PM. Glycogen metabolism loss: A common marker of parasitic behaviour in bacteria? Trends Genet. 2002;18(9):437–40.
172. Jackson DW, Suzuki K, Oakford L, Simecka JW, Hart ME, Romeo T. Biofilm formation and dispersal under the influence of the global regulator CsrA of *Escherichia coli*. J Bacteriol. 2002;184(1):290–301.
173. Sauer K, Cullen MC, Rickard AH, Zeef LAH, Davies DG, Gilbert P. Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. J Bacteriol. 2004;186(21):7312–26.
174. Baker CS, Morozov I, Suzuki K, Romeo T, Babitzke P. CsrA regulates glycogen biosynthesis by preventing translation of glgC in Escherichia coli. Mol Microbiol. 2002;44(6):1599–610.

175. Johnson L, Mulcahy H, Kanevets U, Shi Y, Lewenza S. Surface-localized spermidine protects the *Pseudomonas aeruginosa*: Outer membrane from antibiotic treatment and oxidative stress. J Bacteriol. 2012;194(4):813–26.
176. Schniederjans M, Koska M, Häusslera S. Transcriptional and mutational profiling of an aminoglycoside-resistant *Pseudomonas aeruginosa* small-colony variant. Antimicrob Agents Chemother. 2017;61(11):e01178-17.
177. Karatan E, Watnick P. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev. 2009 Jun 1;73(2):310–47.
178. Evans TJ. Small colony variants of *Pseudomonas aeruginosa* in chronic bacterial infection of the lung in cystic fibrosis. Future Microbiology. 2015;10(2):231-9.
179. Kivisaar M. Mechanisms of stationary-phase mutagenesis in bacteria: mutational processes in pseudomonads. FEMS Microbiol Lett. 2010;312(1):1–14.
180. Boles BR, Singh PK. Endogenous oxidative stress produces diversity and adaptability in biofilm communities. 2008;105(34).
181. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science. 2000;288(5469):1251-4.
182. Gooderham WJ, Hancock REW. Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*. FEMS Microbiol Rev. 2009;33(2):279–94.
183. Pritt B, O'Brien L, Winn W. Mucoid Pseudomonas in cystic fibrosis. Am J Clin Pathol. 2007;128(1):32–4.
184. Morici LA, Carterson AJ, Wagner VE, Frisk A, Schurr JR, Höner zu Bentrup K, et al. *Pseudomonas aeruginosa* AlgR represses the Rhl quorum-sensing system in a biofilm-specific manner. J Bacteriol. 2007;189(21):7752–64.
185. Heurlier K, Williams F, Heeb S, Dormond C, Pessi G, Singer D, et al. Positive control of swarming, rhamnolipid synthesis, and lipase production by the posttranscriptional RsmA/RsmZ system in *Pseudomonas aeruginosa* PAO1. J Bacteriol. 2004;186(10):2936–45.
186. Kilmury SLN, Burrows LL. The *Pseudomonas aeruginosa* PilSR two-component system regulates both twitching and swimming motilities. MBio. 2018 Jul 24;9(4):e01310-18.
187. Poole K. Bacterial stress responses as determinants of antimicrobial resistance. J Antimicrob Chemother. 2012;67(9):2069–89.
188. Durfee T, Hansen A-M, Zhi H, Blattner FR, Jin DJ. Transcription profiling of the stringent response in Escherichia coli. J Bacteriol. 2008;190(3):1084–96.
189. Tosa T, Pizer LI. Biochemical bases for the antimetabolite action of L-serine hydroxamate. J Bacteriol. 1971;106(3):972–82.
190. Howden BP, Beaume M, Harrison PF, Hernandez D, Schrenzel J, Seemann T, et al. Analysis of the small RNA transcriptional response in multidrug-resistant *Staphylococcus aureus* after antimicrobial exposure. Antimicrob Agents Chemother. 2013;57(8):3864–74.
191. Brazas MD, Hancock REW. Using microarray gene signatures to elucidate mechanisms of antibiotic action and resistance. Drug Discovery Today. Elsevier Current Trends; 2005;10(18):1245–52.
192. Schuster M, Greenberg EP. A network of networks: Quorum-sensing gene regulation in *Pseudomonas aeruginosa*. International Journal of Medical Microbiology. 2006;296(2-3):73-81.

193. Lee J, Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell. 2014;6(1):26–41.
194. Reffuveille F, De La Fuente-Nũnez C, Mansour S, Hancock REW. A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob Agents Chemother. 2014;58(9):5363–71.
195. Taylor TB, Buckling A. Bacterial motility confers fitness advantage in the presence of phages. J Evol Biol. 2013;26(10):2154–60.
196. Mittenhuber G. Comparative genomics and evolution of genes encoding bacterial (p)ppGpp synthetases/hydrolases (the Rel, RelA and SpoT proteins) JMMB Research Article. J Mol Microbiol Biotechnol. 2001;3(4):585–600.
197. Pollard JW, Lam T, Stanners CP. Mammalian cells do not have a stringent response. J Cell Physiol. 1980;105(2):313–25.

Appendix

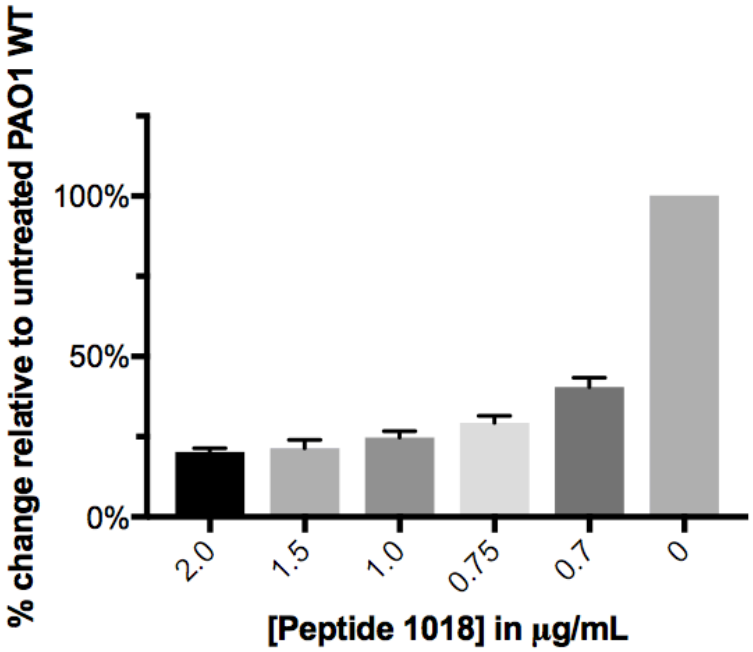

Figure A1. Peptide 1018 inhibited swarming motility in PAO1 at low concentrations. A minimum of three biological replicates was analyzed for each condition.

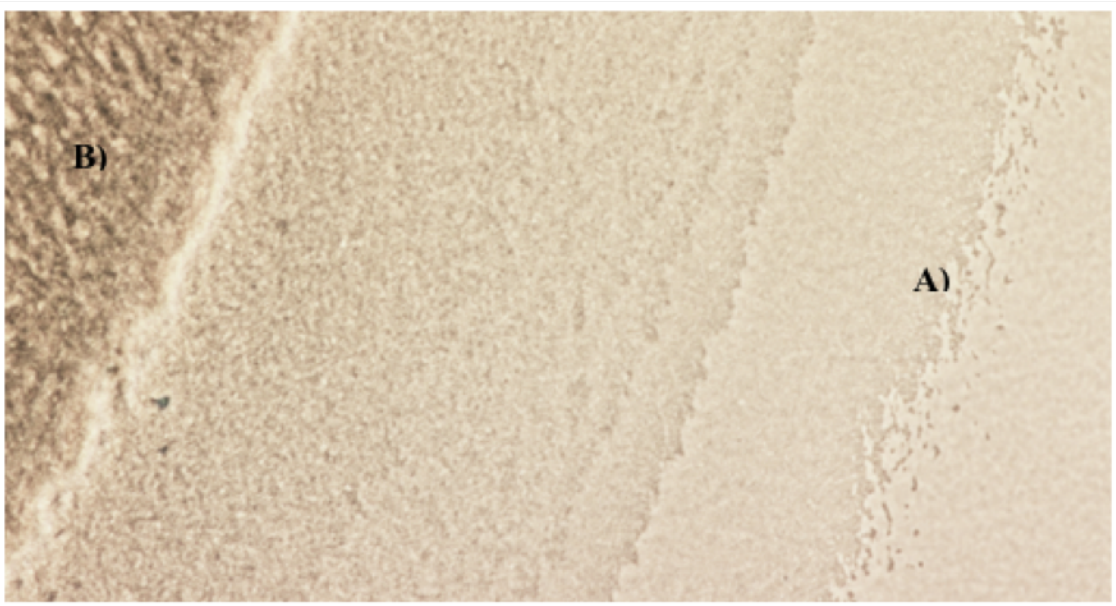

Figure A2. PA14 WT growth zones on a peptide 1018-treated BM2 swarming agar at 20hrs incubation at 37°C (A) Outer edge of the colony. **(B)** High cell density centre of the colony. A Light microscope video showing active cell division and slight motility in PA14 WT in peptide 1018-treated BM2 swarming agar after 20 hrs incubation at 37°C is shown in a YouTube video at https://youtu.be/bUo-a-Wl_8g.

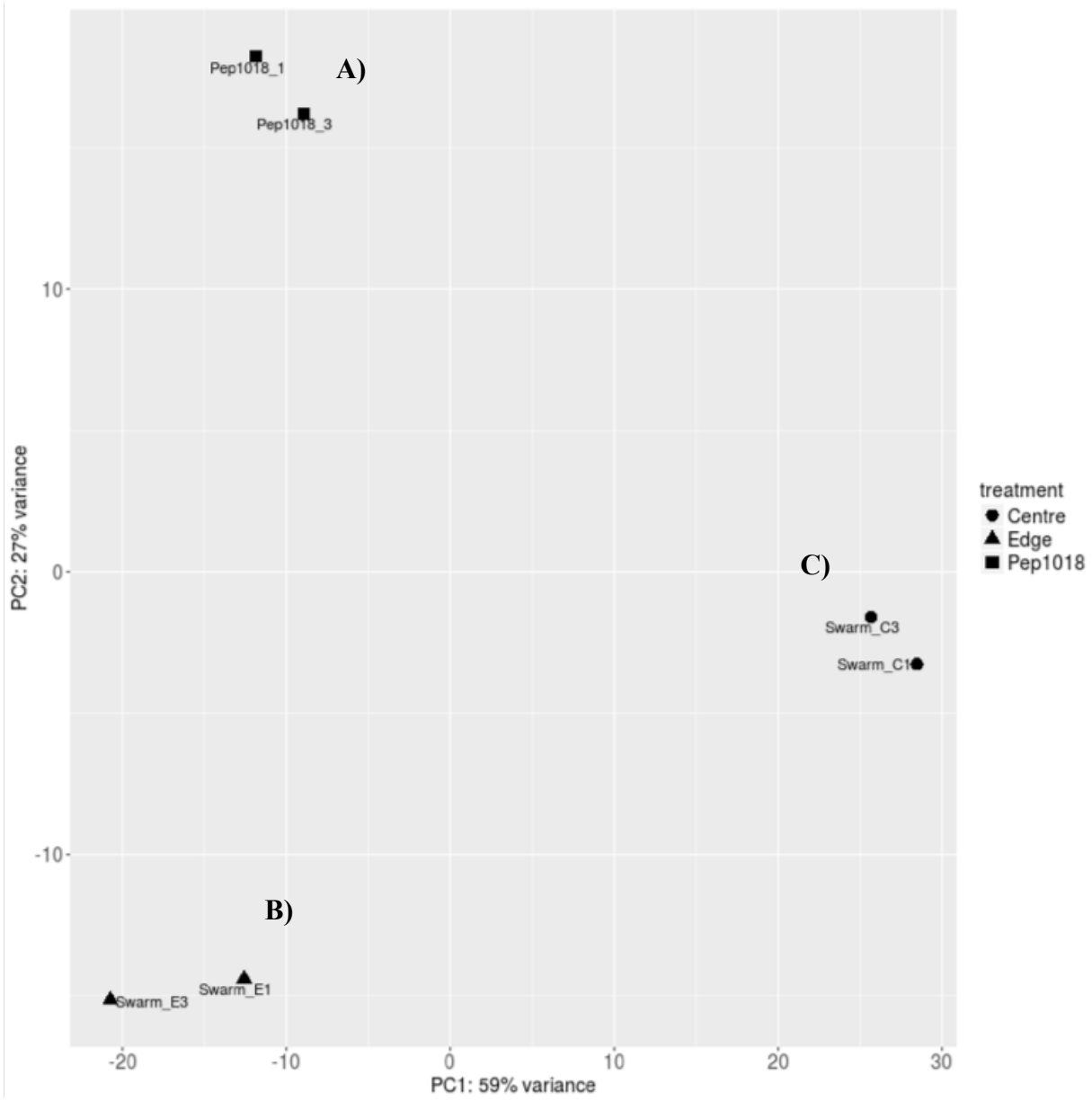

Figure A3. Principal Component Analysis (PCA) of variance and gene clustering between samples (DESeq2). The Benjamin-Hochberg multiple-test correction was used (padj<0.05). **(A)** Cells treated with 1μg/mL peptide 1018 under swarming conditions. **(B)** Untreated cells collected from the leading edge of dendritic PA14 swarming colonies. These cells are considered to be actively swarming and display high levels of motility and an elongated cell phenotype (48). **(C)** Untreated cells collected from the centre of a swarming colony. These cells are differentiated from actively swarming edge cells by a lower percentage of motile cells, shorter cell length, decreased metabolic activity, and an increased expression of stringent and virulence factors (48,55).

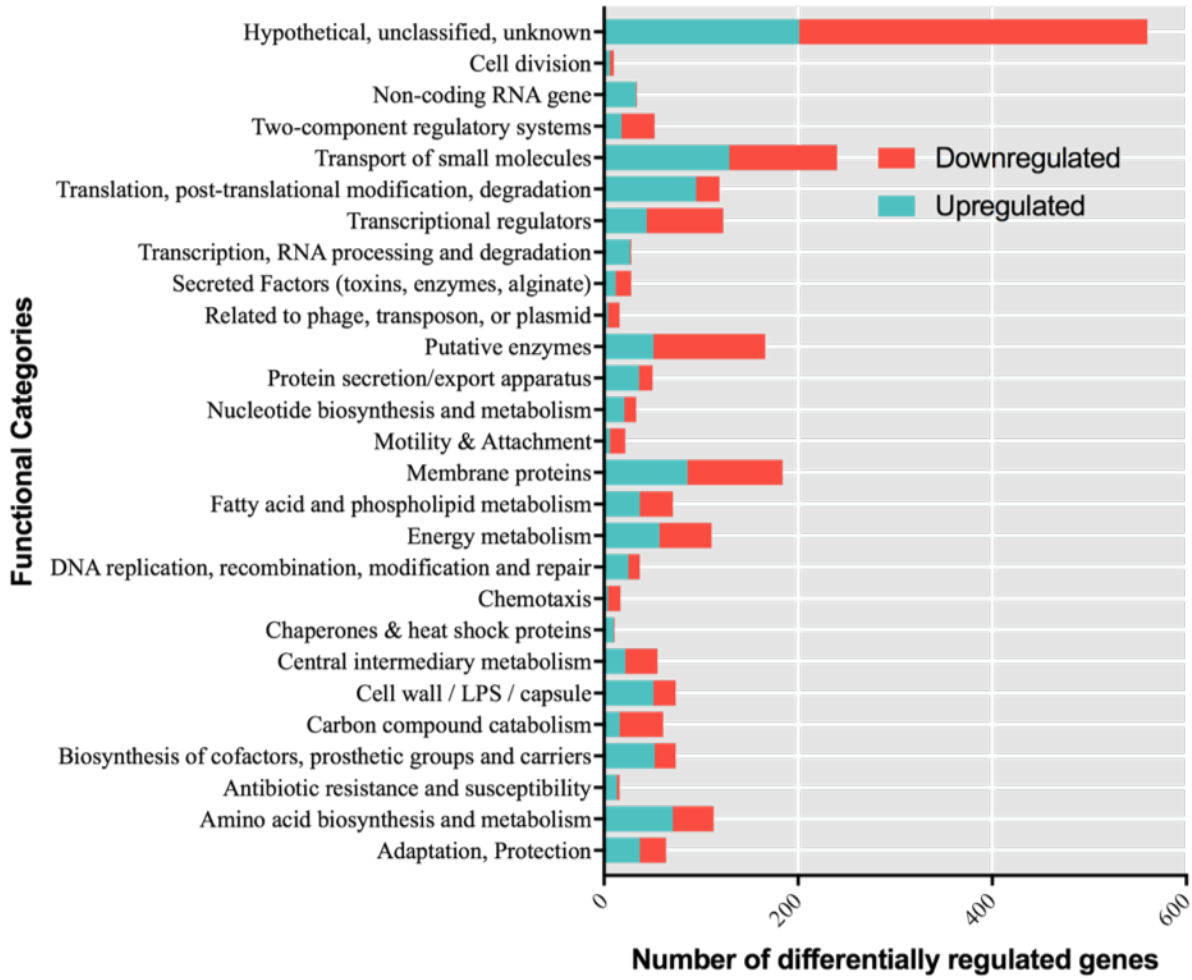

Figure A4. Dysregulated PA14 genes under untreated swarming conditions (leading swarm edge vs. swarm centre) were categorized by their primary PseudoCAP functional class according to the *Pseudomonas* Genome Database (www.pseudomonas.com) (130). When genes had multiple predicted functional categories, the first non-hypothetical category was selected.

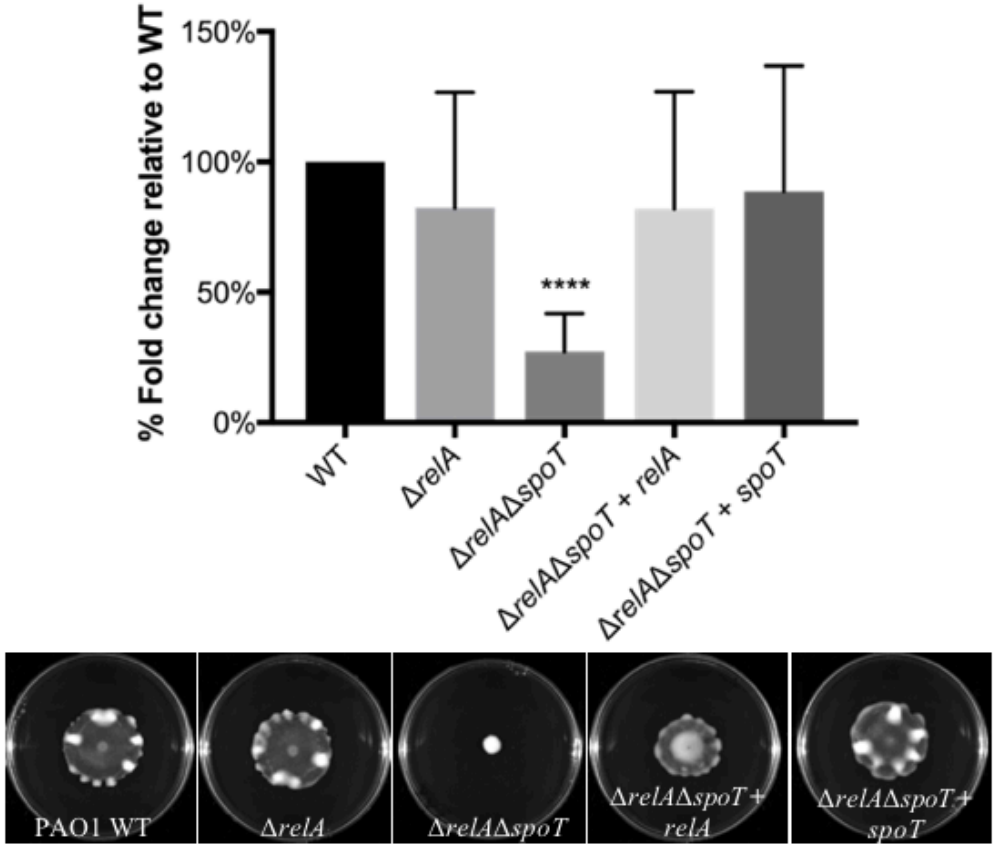

Figure A5. The central mediators of the stringent response, relA and spoT were critical for swarming motility in PAO1. A double deletion of *relA* and *spoT* genes completely inhibited swarming motility in PAO1 under swarming conditions.

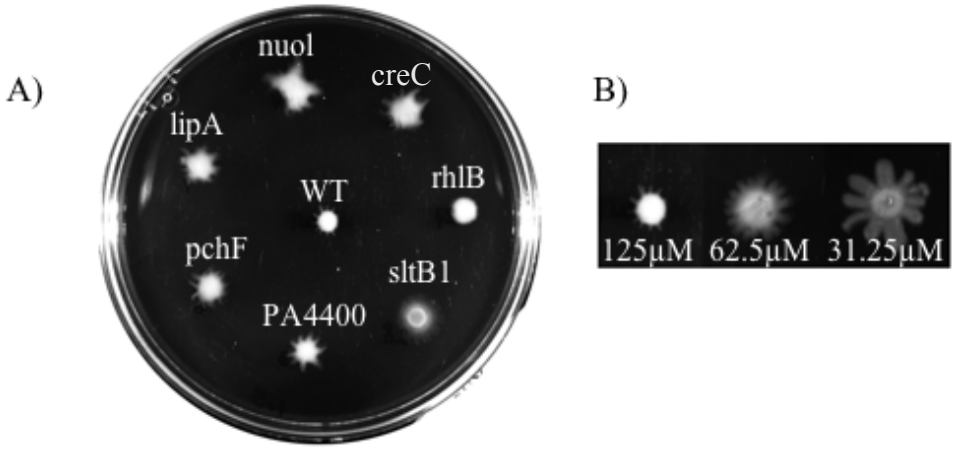

Figure A6. Peptide 1018 had a reduced impact on swarming motility at low levels of SHX that induced the stringent response. (A) Peptide 1018-tolerant transposon mutants and PA14 WT swarmed in the presence of 0.75μg/mL 1018 in the presence of 125 μM SHX. **(B)** PA14 WT appeared to swarm at increasing levels in the presence of 0.75μg/mL 1018, with 125 μM, 62.5 μM, and 31.25 μM SHX respectively.

Table A1. Complete list of DE gene expression for peptide 1018-treated conditions and the untreated swarm colony centre, compared to untreated actively swarming cells from the swarm colony edge

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4514		outer membrane ferric siderophore receptor	-9.9	-18
PA3938		taurine ABC transporter periplasmic protein	-6.4	-16
PA0252		hypothetical protein	-6.3	-4.8
PA3449		hypothetical protein	-4.7	-11
PA5024		hypothetical protein	-4.6	-15
PA14_27190		tRNA-Ser	-4.4	-4.5
PA5406		hypothetical protein	-4.4	-6.6
PA3268		TonB-dependent receptor	-4.3	-8.8
PA4599	*mexC*	multidrug efflux RND membrane fusion protein	-4.3	-3.8
PA4629		hypothetical protein	-4.1	-4.3
PA0654	*speD*	S-adenosylmethionine decarboxylase	-3.8	-5.6
PA14_14570		tRNA-Leu	-3.8	-5.3
PA14_36030		paraquat-inducible protein A	-3.8	-3.7
PA5407		hypothetical protein	-3.8	-3.5
PA2202		amino acid ABC transporter permease	-3.7	-19
PA2761		hypothetical protein	-3.7	-12
PA3607	*potA*	polyamine transport protein PotA	-3.7	-8.7
PA14_14560		hypothetical protein	-3.6	-12
PA2204		ABC transporter substrate-binding protein	-3.6	-19
PA3610	*potD*	polyamine ABC transporter	-3.6	-4.1
PA4826		hypothetical protein	-3.4	0
PA2203		amino acid permease	-3.3	-18
PA4513		oxidoreductase	-3.3	-8.4
PA14_60150		tRNA-Lys	-3.1	-5.6
PA0045		lipoprotein	-3.1	-8.4
PA2653		transporter	-3.1	-6.3
PA4443	*cysD*	sulfate adenylyltransferase subunit 2	-3.1	-11
PA4825	*mgtA*	Mg(2+) transport ATPase. P-type 2	-3.1	-3.8
PA2514	*antC*	anthranilate dioxygenase reductase	-3	0
PA3641		amino acid permease	-3	-4.2
PA4178	*eftM*	SAM-dependent methyltransferase	-3	-2.7
PA4365		transporter	-3	-3.1
PA0320		hypothetical protein	-2.9	-2.1
PA0914		hypothetical protein	-2.9	0
PA4873		heat-shock protein	-2.9	-4.4
PA14_60180		tRNA-Asn	-2.8	-5
PA3741		hypothetical protein	-2.8	-6.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5139		ABC-type amino acid transporter	-2.8	-3.1
PA14_21810		tRNA-Asp	-2.7	-3.9
PA14_08660		tRNA-Gly	-2.7	-3.8
PA14_04710		hypothetical protein	-2.7	-2.1
PA0282	*cysT*	sulfate transport protein CysT	-2.7	-8.5
PA1116		hypothetical protein	-2.7	-2.8
PA2840		ATP-dependent RNA helicase	-2.7	-5.3
PA4177		hypothetical protein	-2.7	0
PA14_23580		tRNA-Glu	-2.6	-6.1
PA14_08650		tRNA-Tyr	-2.6	-5.2
PA14_08670		tRNA-Thr	-2.6	-5
PA14_07460		hypothetical protein	-2.6	-1.9
PA0975		radical activating enzyme	-2.6	-3.7
PA2328		hypothetical protein	-2.6	-2.2
PA2331		hypothetical protein	-2.6	-2.1
PA2913		hypothetical protein	-2.6	-7.3
PA4671		50S ribosomal protein L25	-2.6	-2.9
PA14_41320		tRNA-Leu	-2.5	-4
PA14_65210		tRNA-Leu	-2.5	-4
PA14_23570		tRNA-Ala	-2.5	-2.9
PA0277		Zn-dependent protease with chaperone function	-2.5	-4.4
PA0281	*cysW*	sulfate transport protein CysW	-2.5	-8.1
PA0390	*metX*	homoserine O-acetyltransferase	-2.5	-4.4
PA1838	*cysI*	sulfite reductase	-2.5	-9.3
PA2760		outer membrane OprD family porin	-2.5	-2.8
PA4442	*cysN*	bifunctional sulfate adenylyltransferase subunit 1/adenylylsulfate kinase - cellular response to sulfate starvation	-2.5	-11
PA4934	*rpsR*	30S ribosomal protein S18	-2.5	-4.5
PA14_27610		tRNA-Gly	-2.4	-5.5
PA14_30680		tRNA-Gly	-2.4	-2.7
PA0046		lipoprotein	-2.4	-6.5
PA0283	*sbp*	sulfate-binding protein	-2.4	-9
PA1299		hypothetical protein	-2.4	-3
PA1690	*pscU*	translocation protein in type III secretion	-2.4	-5.1
PA1824		hypothetical protein	-2.4	-3
PA2829		hypothetical protein	-2.4	-3.2
PA2971		hypothetical protein	-2.4	-3.6
PA3387	*rhlG*	beta-ketoacyl reductase	-2.4	0
PA3709		MFS transporter	-2.4	2.9
PA4219		hypothetical protein	-2.4	-6.5
PA4438		hypothetical protein	-2.4	-5.1
PA4935	*rpsF*	30S ribosomal protein S6	-2.4	-4.2

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5316	*rpmB*	50S ribosomal protein L28	-2.4	-4.3
PA14_51660		tRNA-Lys	-2.3	-3.9
PA14_41340		tRNA-Arg	-2.3	-3.8
PA14_20500		tRNA-Arg	-2.3	0
PA0386		coproporphyrinogen III oxidase	-2.3	-4
PA0915		hypothetical protein	-2.3	0
PA1698	*popN*	Type III secretion outer membrane protein PopN precursor	-2.3	-5.2
PA1839		ribosomal RNA large subunit methyltransferase N	-2.3	-2.4
PA2329		ABC transporter ATP-binding protein	-2.3	-2.4
PA2444	*glyA2*	serine hydroxymethyltransferase	-2.3	-16
PA2512	*antA*	anthranilate dioxygenase large subunit	-2.3	1.8
PA2775		hypothetical protein	-2.3	-2.2
PA2970	*rpmF*	50S ribosomal protein L32	-2.3	-3.8
PA3743	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	-2.3	-4.5
PA4272	*rplJ*	50S ribosomal protein L10	-2.3	-4.2
PA4433	*rplM*	50S ribosomal protein L13	-2.3	-3.9
PA4616		c4-dicarboxylate-binding protein	-2.3	-5.6
PA4821		transporter	-2.3	-3.5
PA5315	*rpmG*	50S ribosomal protein L33	-2.3	-3.9
PA14_62790		tRNA-Met	-2.2	-2.8
PA0089	*tssG1*	tssG1	-2.2	-2.4
PA0293	*aguB*	N-carbamoylputrescine amidohydrolase	-2.2	-2.8
PA0547		ArsR family transcriptional regulator	-2.2	-3.3
PA0579	*rpsU*	30S ribosomal protein S21	-2.2	-3
PA0976		hypothetical protein	-2.2	-2.1
PA1703	*pcrD*	type III secretory apparatus protein PcrD	-2.2	-3.8
PA1724	*pscK*	PscK type III export protein	-2.2	-3.2
PA1837		hypothetical protein	-2.2	-7.2
PA2666		6-pyruvoyl-tetrahydropterin synthase	-2.2	0
PA3397	*fpr*	ferredoxin--NADP+ reductase	-2.2	-3
PA3656	*rpsB*	30S ribosomal protein S2	-2.2	-4.2
PA3744	*rimM*	16S rRNA-processing protein RimM	-2.2	-3.5
PA3967		hypothetical protein	-2.2	-2.1
PA3990		hypothetical protein	-2.2	0
PA4134		hypothetical protein	-2.2	0
PA4271	*rplL*	50S ribosomal protein L7/L12	-2.2	-4.2
PA4277	*tufB*	elongation factor Tu	-2.2	-3.9
PA4321		hypothetical protein	-2.2	-2.3

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4432	*rpsI*	30S ribosomal protein S9	-2.2	-3.2
PA4563	*rpsT*	30S ribosomal protein S20	-2.2	-3.4
PA4568	*rplU*	50S ribosomal protein L21	-2.2	-3.7
PA4669	*ipk*	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	-2.2	-3.2
PA4747	*secG*	preprotein translocase subunit SecG	-2.2	-3.2
PA4933		hypothetical protein	-2.2	-4.2
PA5049	*rpmE*	50S ribosomal protein L31	-2.2	-3.6
PA14_27620		tRNA-Gly	-2.1	-4.4
PA14_30720		tRNA-Cys	-2.1	-3.9
PA14_41330		tRNA-His	-2.1	-3.6
PA14_65220		tRNA-Leu	-2.1	-3.6
PA14_24120		tRNA-Asp	-2.1	-3
PA14_07470		tRNA-Met	-2.1	-2
PA14_61760		tRNA-Gln	-2.1	-2
PA0284		hypothetical protein	-2.1	-8
PA0352		transporter	-2.1	-3.7
PA0389		methionine biosynthesis protein	-2.1	-3.7
PA0921		hypothetical protein	-2.1	-2.3
PA1716	*pscC*	Type III secretion outer membrane protein PscC precursor	-2.1	-2.8
PA1959	*bacA*	UDP pyrophosphate phosphatase	-2.1	-3.3
PA1993		major facilitator superfamily transporter	-2.1	0
PA2002		hypothetical protein	-2.1	0
PA2113		porin	-2.1	0
PA2619	*infA*	translation initiation factor IF-1	-2.1	-2.9
PA2969	*plsX*	glycerol-3-phosphate acyltransferase PlsX	-2.1	-3.3
PA3452	*mqoA*	malate:quinone oxidoreductase	-2.1	0
PA3742	*rplS*	50S ribosomal protein L19	-2.1	-3.8
PA4319		hypothetical protein	-2.1	-2.3
PA4479	*mreD*	rod shape-determining protein MreD	-2.1	-4.1
PA4567	*rpmA*	50S ribosomal protein L27	-2.1	-3.5
PA4672	*pth*	peptidyl-tRNA hydrolase	-2.1	-2.3
PA4731	*panD*	aspartate alpha-decarboxylase	-2.1	-2.7
PA4746		hypothetical protein	-2.1	-2.2
PA5118	*thiI*	thiamine biosynthesis protein ThiI	-2.1	-2.8
PA5426	*purE*	phosphoribosylaminoimidazole carboxylase catalytic subunit	-2.1	-1.9
PA14_44090		Fe-S-cluster oxidoreductase	-2	-2.6
PA14_51230		tRNA-Ser	-2	-1.8
PA0280	*cysA*	sulfate transport protein CysA	-2	-5.9
PA0968		hypothetical protein	-2	-2.7
PA1275	*cobD*	cobalamin biosynthesis protein	-2	-3.3
PA1277	*cobQ*	cobyric acid synthase	-2	-3.5

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1591		hypothetical protein	-2	-1.8
PA1894		hypothetical protein	-2	3.1
PA2042		serine/threonine transporter SstT	-2	-1.9
PA2110		hypothetical protein	-2	0
PA2111		hypothetical protein	-2	0
PA2330		hypothetical protein	-2	-2.2
PA2352		glycerophosphoryl diester phosphodiesterase	-2	-3.6
PA2426	*pvdS*	extracytoplasmic-function sigma-70 factor	-2	-52
PA2502		kinase	-2	-2.5
PA2503		hypothetical protein	-2	-2.7
PA2982		hypothetical protein	-2	-2.4
PA3246	*rluA*	pseudouridine synthase	-2	-2.2
PA3650	*dxr*	1-deoxy-D-xylulose 5-phosphate reductoisomerase	-2	-2.6
PA3651	*cdsA*	phosphatidate cytidylyltransferase	-2	-2.8
PA3655	*tsf*	elongation factor Ts	-2	-4.1
PA3725	*recJ*	single-stranded-DNA-specific exonuclease RecJ	-2	-2
PA3726		hypothetical protein	-2	-2.2
PA3818		extragenic suppressor protein SuhB	-2	-2.6
PA3822	*yajC*	preprotein translocase subunit YajC	-2	-3.2
PA3905		hypothetical protein	-2	-2.2
PA3906		hypothetical protein	-2	-2
PA4031	*ppa*	inorganic pyrophosphatase	-2	-2.7
PA4050	*pgpA*	phosphatidylglycerophosphatase A	-2	-1.6
PA4133		cbb3-type cytochrome c oxidase subunit I	-2	0
PA4181		hypothetical protein	-2	-3.1
PA4280	*birA*	biotin--protein ligase	-2	-2.8
PA4628	*lysP*	APC family lysine-specific permease	-2	-3.1
PA4644		hypothetical protein	-2	-2
PA4670	*prs*	ribose-phosphate pyrophosphokinase	-2	-2.2
PA4710		heme/hemoglobin uptake outer membrane receptor PhuR	-2	-9.1
PA4743	*rbfA*	ribosome-binding factor A	-2	-2.2
PA4932	*rplI*	50S ribosomal protein L9	-2	-4.7
PA5138		ABC-type amino acid transport protein. periplasmic component	-2	0
PA5550	*glmR*	GlmR transcriptional regulator - egulation of polysaccharide biosynthetic process	-2	-2.3
PA5557	*atpH*	F0F1 ATP synthase subunit delta	-2	-3.4
PA14_52540		tRNA-Arg	-1.9	-3.3

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA14_62800		tRNA-Leu	-1.9	-2.3
PA0013		hypothetical protein	-1.9	0
PA0022		hypothetical protein	-1.9	-1.7
PA0091	*vgrG1a*	vgrG1a	-1.9	-2.6
PA0382	*trmB*	tRNA (guanine-N(7)-)-methyltransferase	-1.9	-2.5
PA0559		hypothetical protein	-1.9	-2.7
PA0578		hypothetical protein	-1.9	-3
PA0775		hypothetical protein	-1.9	-2.9
PA0797		transcriptional regulator	-1.9	0
PA1132		hypothetical protein	-1.9	-2.2
PA1271		tonB-dependent receptor	-1.9	-3.2
PA1276	*cobC*	threonine-phosphate decarboxylase	-1.9	-3
PA1612		hypothetical protein	-1.9	-1.8
PA2043		pseudouridylate synthase	-1.9	-2
PA2063		hypothetical protein	-1.9	-3.4
PA2109		hypothetical protein	-1.9	0
PA2446	*gcvH2*	glycine cleavage system protein H	-1.9	-5.9
PA2947		hypothetical protein	-1.9	-1.9
PA3019		ABC transporter ATP-binding protein	-1.9	-2.4
PA3294		hypothetical protein	-1.9	-2.8
PA3710		GMC-type oxidoreductase	-1.9	2.4
PA3716		hypothetical protein	-1.9	-2.2
PA3745	*rpsP*	30S ribosomal protein S16	-1.9	-3
PA3820	*secF*	preprotein translocase subunit SecF	-1.9	-3.7
PA3823	*tgt*	queuine tRNA-ribosyltransferase	-1.9	-3.2
PA3824	*queA*	S-adenosylmethionine--tRNA ribosyltransferase-isomerase	-1.9	-2.5
PA3907		hypothetical protein	-1.9	-2.1
PA3908		hypothetical protein	-1.9	-1.9
PA3979		hypothetical protein	-1.9	-2.9
PA4275	*nusG*	transcription antitermination protein NusG	-1.9	-2.2
PA4276	*secE*	preprotein translocase subunit SecE	-1.9	-2.5
PA4292		phosphate transporter	-1.9	-1.9
PA4323		hypothetical protein	-1.9	-2.2
PA4569	*ispB*	octaprenyl-diphosphate synthase	-1.9	-2.6
PA4589		outer membrane protein	-1.9	-1.9
PA4645		hypoxanthine-guanine phosphoribosyltransferase	-1.9	0
PA4673		GTP-dependent nucleic acid-binding protein EngD	-1.9	-2.3
PA4720	*trmA*	tRNA (uracil-5-)-methyltransferase	-1.9	-1.9
PA4741	*rpsO*	30S ribosomal protein S15	-1.9	-2.3
PA4742	*truB*	tRNA pseudouridine synthase B	-1.9	-1.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4745	*nusA*	transcription elongation factor NusA	-1.9	-2
PA4748	*tpiA*	triosephosphate isomerase	-1.9	-3.2
PA4753		hypothetical protein	-1.9	0
PA4800		hypothetical protein	-1.9	-2
PA4928		hypothetical protein	-1.9	-2.7
PA5136		hypothetical protein	-1.9	-3.2
PA5194		hypothetical protein	-1.9	-2.8
PA5202		hypothetical protein	-1.9	-2.9
PA5286		hypothetical protein	-1.9	-3.2
PA5296	*rep*	ATP-dependent DNA helicase Rep	-1.9	-2.6
PA5434	*mtr*	tryptophan permease	-1.9	-2.3
PA5437		LysR family transcriptional regulator	-1.9	-2
PA5472		hypothetical protein	-1.9	-2.2
PA5503	*metN*	DL-methionine transporter ATP-binding subunit	-1.9	-2.6
PA5504		ABC transporter permease	-1.9	-2.7
PA5512		two-component sensor	-1.9	-1.9
PA5558	*atpF*	F0F1 ATP synthase subunit B	-1.9	-2.9
PA5559	*atpE*	F0F1 ATP synthase subunit C	-1.9	-2.6
PA5560	*atpB*	F0F1 ATP synthase subunit A	-1.9	-2.7
PA5561	*atpI*	F0F1 ATP synthase subunit I	-1.9	-1.8
PA14_08690		tRNA-Trp	-1.8	-2.3
PA14_54850		hypothetical protein	-1.8	-2
PA0380		sulfur carrier protein ThiS	-1.8	-2.2
PA0580	*gcp*	DNA-binding/iron metalloprotein/AP endonuclease	-1.8	-1.7
PA0706	*cat*	chloramphenicol acetyltransferase	-1.8	0
PA0866	*aroP2*	aromatic amino acid transport protein AroP2	-1.8	0
PA1278	*cobU*	adenosylcobinamide kinase/adenosylcobinamide-phosphate guanylyltransferase	-1.8	-3.3
PA1396		two-component sensor	-1.8	0
PA1639		hypothetical protein	-1.8	-1.7
PA1715	*pscB*	type III export apparatus protein	-1.8	-2.4
PA1780	*nirD*	assimilatory nitrite reductase small subunit	-1.8	1.8
PA2112		LamB/YcsF family protein	-1.8	0
PA2682		hypothetical protein	-1.8	2.3
PA2769		hypothetical protein	-1.8	0
PA2806	*queF*	7-cyano-7-deazaguanine reductase	-1.8	-1.9
PA2851	*efp*	elongation factor P	-1.8	-2.9
PA2948	*cobM*	precorrin-3 methylase	-1.8	-1.7
PA2981	*lpxK*	tetraacyldisaccharide 4'-kinase - lipid A biosynthetic process	-1.8	-2

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3000	*aroP1*	aromatic amino acid transport protein AroP1	-1.8	-2.5
PA3046		hypothetical protein	-1.8	0
PA3652	*uppS*	UDP pyrophosphate synthetase	-1.8	-2.2
PA3654	*pyrH*	uridylate kinase	-1.8	-2.4
PA3685		hypothetical protein	-1.8	-1.9
PA3713		hypothetical protein	-1.8	-2.1
PA3821	*secD*	preprotein translocase subunit SecD	-1.8	-3.4
PA3850		hypothetical protein	-1.8	-1.6
PA4051	*thiL*	thiamine monophosphate kinase	-1.8	-2
PA4055	*ribC*	riboflavin synthase subunit alpha	-1.8	0
PA4237	*rplQ*	50S ribosomal protein L17	-1.8	-2.6
PA4278		hypothetical protein	-1.8	-1.9
PA4317		hypothetical protein	-1.8	-2.1
PA4318		hypothetical protein	-1.8	-1.6
PA4320		hypothetical protein	-1.8	-2.2
PA4454		hypothetical protein	-1.8	-1.6
PA4480	*mreC*	rod shape-determining protein MreC	-1.8	-3.8
PA4675		TonB-dependent receptor	-1.8	-5.1
PA4768	*smpB*	SsrA-binding protein	-1.8	-2.2
PA4853	*fis*	DNA-binding protein Fis	-1.8	-2.1
PA5128	*secB*	preprotein translocase subunit SecB	-1.8	-2.2
PA5129	*grx*	glutaredoxin	-1.8	-2.4
PA5201		hypothetical protein	-1.8	-3.5
PA5425	*purK*	phosphoribosylaminoimidazole carboxylase ATPase subunit	-1.8	-2.1
PA5458		acyltransferase	-1.8	0
PA5555	*atpG*	F0F1 ATP synthase subunit gamma	-1.8	-3.3
PA14_24130		tRNA-Asp	-1.7	-2.8
PA14_52550		tRNA-Arg	-1.7	-2.4
PA0608		phosphoglycolate phosphatase	-1.7	0
PA0652	*vfr*	cAMP-regulatory protein -loss of Vfr contributes to the reduced peak cyanide production in mucA22 mutants	-1.7	-1.6
PA0774		hypothetical protein	-1.7	-2.7
PA0896	*aruF*	arginine/ornithine succinyltransferase AI subunit	-1.7	-1.8
PA0955		hypothetical protein	-1.7	-2.2
PA0965	*ruvC*	Holliday junction resolvase	-1.7	-1.9
PA0979		hypothetical protein	-1.7	-1.5
PA1007		hypothetical protein	-1.7	-2.6
PA1161	*rrmA*	rRNA methyltransferase	-1.7	0
PA1162	*dapE*	succinyl-diaminopimelate desuccinylase	-1.7	-1.7
PA1281	*cobS*	cobalamin synthase	-1.7	-2.6

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PA1574		hypothetical protein	-1.7	-1.6
PA1674	*folE*	GTP cyclohydrolase I	-1.7	0
PA1713	*exsA*	transcriptional regulator ExsA	-1.7	0
PA1756	*cysH*	phosphoadenosine phosphosulfate reductase	-1.7	-2
PA1771		esterase	-1.7	-5.9
PA1779		assimilatory nitrate reductase	-1.7	1.7
PA1791		hypothetical protein	-1.7	-2.9
PA2114		major facilitator transporter	-1.7	0
PA2557		AMP-binding protein	-1.7	-2.3
PA2983		tolQ-type transport protein	-1.7	-2.2
PA3009		hypothetical protein	-1.7	0
PA3114	*truA*	tRNA pseudouridine synthase A	-1.7	-1.9
PA3165	*hisC2*	histidinol-phosphate aminotransferase	-1.7	-1.7
PA3308	*hepA*	ATP-dependent helicase HepA	-1.7	-2.8
PA3365		chaperone	-1.7	3.4
PA3453		hypothetical protein	-1.7	-1.7
PA3633	*ispD*	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	-1.7	-1.5
PA3645	*fabZ*	(3R)-hydroxymyristoyl-ACP dehydratase	-1.7	-2
PA3679		hypothetical protein	-1.7	0
PA3747		hypothetical protein	-1.7	-2.1
PA3807	*ndk*	nucleoside diphosphate kinase	-1.7	-2.5
PA3817		methyltransferase	-1.7	-2
PA3904		hypothetical protein	-1.7	0
PA4003	*pbpA*	penicillin-binding protein 2	-1.7	-2.3
PA4004		rRNA large subunit methyltransferase	-1.7	-1.6
PA4132		GntR family transcriptional regulator	-1.7	-1.8
PA4242	*rpmJ*	50S ribosomal protein L36	-1.7	-2
PA4243	*secY*	preprotein translocase subunit SecY	-1.7	-2.5
PA4307	*pctC*	chemotactic transducer PctC	-1.7	0
PA4372		lipoprotein	-1.7	-3.8
PA4391		hypothetical protein	-1.7	-2.3
PA4455		ABC transporter permease	-1.7	-2.2
PA4456		ABC transporter ATP-binding protein	-1.7	-2.1
PA4546	*pilS*	two-component sensor PilS	-1.7	0
PA4562		virulence factor. membrane protein	-1.7	0
PA4684		hypothetical protein	-1.7	-2.7
PA4850	*prmA*	50S ribosomal protein L11 methyltransferase - homologue of RpoE	-1.7	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4940		hypothetical protein	-1.7	0
PA4997	*msbA*	transport protein MsbA	-1.7	-2.2
PA4998		hypothetical protein	-1.7	-2.2
PA5002	*dnpA*	de-N-acetylase involved in persistence, DnpA	-1.7	-2.1
PA5034	*hemE*	uroporphyrinogen decarboxylase	-1.7	-2.7
PA5046	*maeB*	malic enzyme	-1.7	-2.5
PA5090	*vgrG5*	vgrG5	-1.7	-1.6
PA5143	*hisB*	imidazoleglycerol-phosphate dehydratase	-1.7	-1.6
PA5192	*pckA*	phosphoenolpyruvate carboxykinase	-1.7	-3.2
PA5235	*glpT*	sn-glycerol-3-phosphate transporter	-1.7	-1.6
PA5295		hypothetical protein	-1.7	0
PA5569	*rnpA*	ribonuclease P	-1.7	-2.5
PA0342	*thyA*	thymidylate synthase	-1.6	0
PA0563		hypothetical protein	-1.6	0
PA0664		hypothetical protein	-1.6	-1.5
PA0897	*aruG*	arginine/ornithine succinyltransferase AII subunit	-1.6	-1.9
PA0904	*lysC*	aspartate kinase	-1.6	-2
PA0926		hypothetical protein	-1.6	0
PA0932	*cysM*	cysteine synthase B	-1.6	-1.6
PA0944	*purN*	phosphoribosylglycinamide formyltransferase	-1.6	-2
PA0947		DNA replication initiation factor	-1.6	-2.4
PA0973	*oprL*	peptidoglycan associated lipoprotein OprL precursor	-1.6	-2.1
PA1012		hypothetical protein	-1.6	-2
PA1014		glycosyl transferase family protein	-1.6	-1.9
PA1045	*dinG*	ATP-dependent DNA helicase DinG	-1.6	-1.9
PA1273	*cobB*	cobyrinic acid a.c-diamide synthase	-1.6	-2.8
PA1778	*cobA*	uroporphyrin-III C-methyltransferase	-1.6	1.9
PA1815	*rnhA*	ribonuclease H	-1.6	0
PA1926		hypothetical protein	-1.6	-2.2
PA2741	*rplT*	50S ribosomal protein L20	-1.6	-1.8
PA2742	*rpmI*	50S ribosomal protein L35	-1.6	-1.8
PA2795		tRNA-dihydrouridine synthase A	-1.6	-1.7
PA2828		aminotransferase	-1.6	-2.1
PA2944	*cobN*	cobaltochelatase subunit CobN	-1.6	-2.7
PA3116		aspartate-semialdehyde dehydrogenase	-1.6	-2
PA3247		aminopeptidase 2	-1.6	0
PA3640	*dnaE*	DNA polymerase III subunit alpha	-1.6	-1.7
PA3643	*lpxB*	lipid-A-disaccharide synthase	-1.6	-2.4

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3644	*lpxA*	UDP-N-acetylglucosamine acyltransferase	-1.6	-1.8
PA3732		hypothetical protein	-1.6	-2.2
PA3736	*hom*	homoserine dehydrogenase	-1.6	-1.6
PA3975	*thiD*	phosphomethylpyrimidine kinase	-1.6	0
PA3980		(dimethylallyl)adenosine tRNA methylthiotransferase	-1.6	-1.7
PA4002	*rodA*	rod shape-determining protein	-1.6	-1.7
PA4045		hypothetical protein	-1.6	0
PA4157		transcriptional regulator	-1.6	-1.9
PA4322		hypothetical protein	-1.6	-2
PA4451		hypothetical protein	-1.6	-1.7
PA4544	*rluD*	pseudouridine synthase	-1.6	-1.7
PA4545	*comL*	competence protein ComL	-1.6	-1.7
PA4592		outer membrane protein	-1.6	-2.1
PA4627		hypothetical protein	-1.6	-1.6
PA4631		epimerase	-1.6	0
PA4632		lipoprotein	-1.6	-2.2
PA4643		hypothetical protein	-1.6	-1.6
PA4664	*PrmC*	S-adenosylmethionine-dependent methyltransferase, PrmC	-1.6	-2.4
PA4700	*mrcB*	penicillin-binding protein 1B	-1.6	-2.2
PA4715		aminotransferase	-1.6	-2.6
PA4724		glutamyl-Q tRNA(Asp) synthetase	-1.6	-2.1
PA4851		hypothetical protein	-1.6	-1.6
PA4960	*serB*	phosphoserine phosphatase	-1.6	-1.7
PA5074		ABC transporter ATP-binding protein	-1.6	0
PA5075		ABC transporter permease	-1.6	0
PA5130		rhodanese-like domain-containing protein	-1.6	-2.2
PA5140	*hisF1*	imidazole glycerol phosphate synthase subunit HisF	-1.6	-1.7
PA5141	*hisA*	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase	-1.6	-1.9
PA5177		hydrolase	-1.6	0
PA5274	*rnk*	nucleoside diphosphate kinase regulator - substrate (1/3) of serine-tRNA ligase	-1.6	0
PA5320	*coaC*	bifunctional phosphopantothenoylcysteine decarboxylase/phosphopantothenate synthase	-1.6	0
PA5442		hypothetical protein	-1.6	-1.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5478		hypothetical protein	-1.6	-1.9
PA5491		cytochrome	-1.6	0
PA5515		hypothetical protein	-1.6	0
PA5567	*trmE*	tRNA modification GTPase TrmE	-1.6	-1.7
PA14_55050		TonB-dependent receptor	-1.5	0
PA0412	*pilK*	methyltransferase PilK	-1.5	0
PA0770	*rnc*	ribonuclease III	-1.5	0
PA0799		helicase	-1.5	-1.7
PA0961		cold-shock protein	-1.5	-3.1
PA1816	*dnaQ*	DNA polymerase III subunit epsilon	-1.5	0
PA2965	*fabF1*	3-oxoacyl-ACP synthase	-1.5	-1.9
PA3050	*pyrD*	dihydroorotate dehydrogenase 2	-1.5	-1.6
PA3604		LuxR family transcriptional regulator	-1.5	0
PA3798		aminotransferase	-1.5	0
PA4481	*mreB*	rod shape-determining protein MreB	-1.5	-2.8
PA4561	*ribF*	bifunctional riboflavin kinase/FMN adenylyltransferase	-1.5	0
PA4725	*cbrA*	two-component sensor CbrA	-1.5	0
PA4968		hypothetical protein	-1.5	0
PA14_13920		hypothetical protein	0	-7.6
PA14_21260		hypothetical protein	0	-7
PA14_62050		5S ribosomal RNA	0	-4.6
PA14_50750		hypothetical protein	0	-3.5
PA14_23420		zinc-binding dehydrogenase	0	-3.1
PA14_23440	*orfL*	group 1 glycosyl transferase	0	-3
PA14_23430		heparinase	0	-3
PA14_59560		transposase	0	-3
PA14_23410	*orfJ*	glycosyl transferase family protein	0	-2.8
PA14_24360		hypothetical protein	0	-2.8
PA14_15350		integrase	0	-2.7
PA14_36010		hypothetical protein	0	-2.4
PA14_23390	*orfE*	polysaccharide biosynthesis protein	0	-2.3
PA14_48500		transcriptional regulator	0	-2.3
PA14_34610		hypothetical protein	0	-2.2
PA14_23400		hypothetical protein	0	-2.1
PA14_24665		hypothetical protein	0	-2.1
PA14_67900		hypothetical protein	0	-2
PA14_13950		hypothetical protein	0	-2
PA14_36020		paraquat-inducible protein B	0	-2
PA14_59710	*cupD1*	fimbrial protein	0	-1.9
PA14_43070		secreted protein Hcp	0	-1.9
PA14_59790	*pvrR*	two component response regulator	0	-1.8
PA14_52560		tRNA-Ser	0	-1.8
PA14_15570		hypothetical protein	0	-1.7
PA14_07480		reverse transcriptase	0	-1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA14_15580		Type II restriction enzyme. methylase subunit	0	-1.6
PA14_45260		hypothetical protein	0	-1.6
PA14_28740		tRNA-Pro	0	-1.5
PA14_03285		hypothetical protein	0	-1.5
PA14_03265		hypothetical protein	0	-1.5
PA14_19600		hypothetical protein	0	1.5
PA14_59390		hypothetical protein	0	1.6
PA14_28360		hypothetical protein	0	1.6
PA14_42710		hypothetical protein	0	1.6
PA14_55070		hypothetical protein	0	1.7
PA14_04010		hypothetical protein	0	1.7
PA14_59380		hypothetical protein	0	1.7
PA14_60030		hypothetical protein	0	1.7
PA14_35820	*tnpS*	cointegrate resolution protein S	0	1.8
PA14_35800		hypothetical protein	0	1.8
PA14_59400		hypothetical protein	0	1.8
PA14_51550		transposase	0	1.9
PA14_05910		periplasmic transport system	0	1.9
PA14_14550		hypothetical protein	0	1.9
PA14_13200		hypothetical protein	0	1.9
PA14_61110		hypothetical protein	0	1.9
PA14_54860		hypothetical protein	0	1.9
PA14_72830		hypothetical protein	0	1.9
PA14_35740		transposase	0	2
PA14_15435		hypothetical protein	0	2
PA14_35750		tpnA repressor protein	0	2.1
PA14_35850		hypothetical protein	0	2.1
PA14_30690		hypothetical protein	0	2.1
PA14_59200		hypothetical protein	0	2.1
PA14_54070		hypothetical protein	0	2.1
PA14_15630		hypothetical protein	0	2.1
PA14_54880		serine acetyltransferase	0	2.2
PA14_35920		acetate permease	0	2.3
PA14_54050		hypothetical protein	0	2.3
PA14_01770		nucleoside-binding outer membrane protein	0	2.4
PA14_59190		hypothetical protein	0	2.4
PA14_55080		hypothetical protein	0	2.4
PA14_61410		hypothetical protein	0	2.4
PA14_15475	*merT*	mercuric transport protein	0	2.4
PA14_59340	*pilT2*	type IV B pilus protein	0	2.5
PA14_59480		hypothetical protein	0	2.5
PA14_71400		hypothetical protein	0	2.6
PA14_37170		hypothetical protein	0	2.7
PA14_35860		amino acid permease	0	2.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA14_46510		hypothetical protein	0	2.8
PA14_55090		hypothetical protein	0	2.9
PA14_44230		hypothetical protein	0	2.9
PA14_06875		rsmYregulatoryRNA	0	3
PA14_46550		ribonuclease	0	3.2
PA14_11730		protein kinase	0	3.2
PA14_28260		hypothetical protein	0	3.2
PA14_40820		hydrolase	0	3.5
PA14_46530		hypothetical protein	0	3.5
PA14_27640		protein associated with synthesis and assembly of refractile inclusion bodies	0	3.5
PA14_13220		protein-tyrosine-phosphatase	0	3.6
PA14_37670		hypothetical protein	0	3.7
PA14_27630		protein associated with synthesis and assembly of refractile inclusion bodies	0	3.9
PA14_36860		hypothetical protein	0	4.7
PA14_33970		hypothetical protein	0	5.8
PA14_33980		hypothetical protein	0	7.2
PA14_61380		hypothetical protein	0	12
PA0008	*glyS*	glycyl-tRNA synthetase subunit beta	0	-1.9
PA0009	*glyQ*	glycyl-tRNA synthetase subunit alpha	0	-1.9
PA0010	*tag*	DNA-3-methyladenine glycosidase I	0	1.9
PA0017		tRNA and rRNA cytosine-C5-methylases	0	-1.6
PA0018	*fmt*	methionyl-tRNA formyltransferase	0	-2
PA0024	*hemF*	coproporphyrinogen III oxidase	0	-2
PA0025	*aroE*	shikimate 5-dehydrogenase	0	-1.5
PA0035	*trpA*	tryptophan synthase subunit alpha	0	-1.8
PA0036	*trpB*	tryptophan synthase subunit beta	0	-2.1
PA0047		lipoprotein	0	-3.7
PA0048		transcriptional regulator	0	3
PA0049		hypothetical protein	0	3.5
PA0050		hypothetical protein	0	2.1
PA0061		hypothetical protein	0	2
PA0070	*tagQ1*	TagQ1	0	-2
PA0074	*ppkA*	serine/threonine protein kinase PpkA	0	-1.6
PA0081	*fha1*	Fha1	0	1.8
PA0087		hypothetical protein	0	-2.1
PA0090	*clpV1*	ClpA/B-type chaperone	0	-1.7
PA0093		hypothetical protein	0	-3
PA0095		hypothetical protein	0	1.9
PA0102		carbonic anhydrase	0	1.7
PA0104		hypothetical protein	0	3.4

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0106	*coxA*	cytochrome c oxidase subunit I	0	13
PA0110		hypothetical protein	0	11
PA0114		hypothetical protein	0	2
PA0131		hypothetical protein	0	2.5
PA0132	*aptA*	beta alanine--pyruvate transaminase	0	3.3
PA0140	*ahpF*	alkyl hydroperoxide reductase	0	-1.9
PA0144		nucleoside 2-deoxyribosyltransferase	0	3.2
PA0148		adenosine deaminase	0	1.6
PA0149		RNA polymerase ECF-subfamily sigma-70 factor	0	-4.6
PA0150		transmembrane sensor	0	-5.2
PA0155	*pcaR*	transcriptional regulator PcaR	0	-1.8
PA0161		hypothetical protein	0	-2.1
PA0162		outer membrane porin	0	-3.9
PA0174		hypothetical protein	0	4.3
PA0175		chemotaxis protein methyltransferase	0	4.7
PA0180		chemotaxis transducer	0	3.1
PA0191		transcriptional regulator	0	-2.7
PA0201		hypothetical protein	0	-12
PA0208	*mdcA*	malonate decarboxylase subunit alpha	0	7.4
PA0209		triphosphoribosyl-dephospho-CoA synthase	0	9.9
PA0210	*mdcC*	malonate decarboxylase subunit delta	0	13
PA0213		phosphoribosyl-dephospho-CoA transferase	0	9.1
PA0214		epsilon subunit of malonate decarboxylase	0	8.2
PA0215		malonate carrier protein	0	6.9
PA0216		malonate transporter subunit MadM	0	4
PA0218		LysR family transcriptional regulator	0	2
PA0219		aldehyde dehydrogenase	0	2.3
PA0220		amino acid permease	0	3.7
PA0222		hypothetical protein	0	4.2
PA0249		acetyltransferase	0	1.9
PA0250		hypothetical protein	0	2.6
PA0256		hypothetical protein	0	3.6
PA0263	*hcpB*	secreted protein Hcp	0	-2
PA0274		hypothetical protein	0	3.3
PA0275		transcriptional regulator	0	2.1
PA0285		sensory box GGDEF domain-containing protein	0	-1.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0287		sodium:solute symporter	0	2.5
PA0288	*speB1*	agmatinase	0	3.3
PA0289		transcriptional regulator	0	1.8
PA0290		sensory box GGDEF domain-containing protein	0	2.9
PA0291	*oprE*	anaerobically-induced outer membrane porin OprE precursor	0	-4
PA0296		glutamine synthetase	0	1.7
PA0297	*spuA*	glutamine amidotransferase	0	1.8
PA0300	*spuD*	polyamine transport protein	0	2.1
PA0301	*spuE*	polyamine transport protein	0	1.7
PA0302	*spuF*	polyamine transport protein PotG	0	1.6
PA0303	*spuG*	polyamine transport protein PotH	0	1.6
PA0304	*spuH*	polyamine transport protein PotI	0	1.6
PA0313		ABC transporter permease	0	1.7
PA0314		ABC transporter substrate-binding protein	0	1.7
PA0315		hypothetical protein	0	1.7
PA0316	*serA*	D-3-phosphoglycerate dehydrogenase	0	-2
PA0319		transcriptional regulator	0	-1.6
PA0321		acetylpolyamine aminohydrolase	0	2.4
PA0323		ABC transporter substrate-binding protein	0	4.4
PA0324		ABC transporter permease	0	4.4
PA0325		ABC transporter permease	0	3.7
PA0327		transcriptional regulator	0	2.1
PA0328		hypothetical protein	0	1.6
PA0329		hypothetical protein	0	1.7
PA0332		hypothetical protein	0	2.1
PA0350	*folA*	dihydrofolate reductase	0	-2
PA0356		hypothetical protein	0	-1.7
PA0358		hypothetical protein	0	-1.9
PA0363	*coaD*	phosphopantetheine adenylyltransferase	0	-1.6
PA0364		oxidoreductase	0	1.6
PA0365		hypothetical protein	0	2.1
PA0367		TetR family transcriptional regulator	0	1.7
PA0381	*thiG*	thiazole synthase	0	-1.9
PA0383		hypothetical protein	0	-1.9
PA0384		hypothetical protein	0	4.4
PA0396	*pilU*	twitching motility protein PilU	0	1.5
PA0397		cation efflux system protein	0	2.8
PA0399		cystathionine beta-synthase	0	-1.6
PA0400		cystathionine gamma-lyase	0	-2.1
PA0408	*pilG*	twitching motility protein PilG	0	-1.7

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PA0410	*pilI*	twitching motility protein PilI	0	-1.5
PA0411	*pilJ*	twitching motility protein PilJ	0	-1.7
PA0417		chemotaxis protein	0	3.7
PA0419		16S ribosomal RNA methyltransferase RsmE	0	-1.8
PA0422		hypothetical protein	0	1.7
PA0430	*metF*	5.10-methylenetetrahydrofolate reductase	0	-2.2
PA0431		hypothetical protein	0	-2.2
PA0432	*sahH*	S-adenosyl-L-homocysteine hydrolase	0	-2.4
PA0438	*codB*	cytosine permease	0	1.9
PA0448		LysR family transcriptional regulator	0	-1.8
PA0457		hypothetical protein	0	-1.7
PA0464	*creC*	sensory histidine kinase CreC	0	-1.6
PA0467		hypothetical protein	0	1.7
PA0468		hypothetical protein	0	1.8
PA0471		transmembrane sensor	0	-9.5
PA0472		RNA polymerase sigma factor	0	-9.7
PA0483		GNAT family acetyltransferase	0	3.6
PA0485		hypothetical protein	0	2
PA0488		hypothetical protein	0	2.3
PA0496		hydrolase	0	1.6
PA0501	*bioF*	8-amino-7-oxononanoate synthase	0	-1.6
PA0505		hypothetical protein	0	4.6
PA0508		acyl-CoA dehydrogenase	0	-1.5
PA0509	*nirN*	c-type cytochrome	0	-6.1
PA0513	*nirG*	transcriptional regulator	0	-12
PA0517	*nirC*	c-type cytochrome	0	-46
PA0518	*nirM*	cytochrome c-551	0	-15
PA0519	*nirS*	nitrite reductase	0	-18
PA0523	*norC*	nitric-oxide reductase subunit C	0	-8.9
PA0524	*norB*	nitric-oxide reductase subunit B	0	-9.4
PA0526		hypothetical protein	0	-5.6
PA0527	*dnr*	transcriptional regulator Dnr	0	-4.8
PA0531		glutamine amidotransferase	0	2.5
PA0540		hypothetical protein	0	1.8
PA0542		hypothetical protein	0	-2
PA0546	*metK*	S-adenosylmethionine synthetase	0	-2.9
PA0548	*tktA*	transketolase	0	-1.5
PA0551	*epd*	D-erythrose 4-phosphate dehydrogenase	0	-2.6
PA0552	*pgk*	phosphoglycerate kinase	0	-2.4
PA0556		hypothetical protein	0	-2.2
PA0557		hypothetical protein	0	3.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0558		hypothetical protein	0	1.5
PA0565		hypothetical protein	0	2.7
PA0574		hypothetical protein	0	-2
PA0575		hypothetical protein	0	2.6
PA0581		glycerol-3-phosphate acyltransferase PlsY	0	1.5
PA0585		hypothetical protein	0	5
PA0593	*pdxA*	4-hydroxythreonine-4-phosphate dehydrogenase	0	-1.6
PA0595	*ostA*	organic solvent tolerance protein OstA	0	-1.6
PA0599		hypothetical protein	0	1.6
PA0607	*rpe*	ribulose-phosphate 3-epimerase	0	-2.3
PA0613		hypothetical protein	0	1.8
PA0614		hypothetical protein	0	2
PA0618		phage baseplate assembly protein	0	2
PA0628		phage late control gene D protein	0	2.1
PA0629		lytic enzyme	0	2.6
PA0630		hypothetical protein	0	2.5
PA0635		hypothetical protein	0	2.5
PA0637		hypothetical protein	0	2.3
PA0639		hypothetical protein	0	2.3
PA0641		phage-related protein. tail component	0	2.1
PA0659		hypothetical protein	0	-1.6
PA0663		hypothetical protein	0	-1.6
PA0672	*nemO*	heme oxygenase	0	-17
PA0673		hypothetical protein	0	2.4
PA0679	*hxcP*	HcxP	0	3.1
PA0690		hypothetical protein	0	2.1
PA0703		MFS family transporter	0	1.7
PA0707	*toxR*	transcriptional regulator ToxR	0	-13
PA0730		transferase	0	8
PA0734		hypothetical protein	0	2
PA0741		hypothetical protein	0	3
PA0742		hypothetical protein	0	5.4
PA0750	*ung*	uracil-DNA glycosylase	0	-1.6
PA0767	*lepA*	GTP-binding protein LepA	0	-1.8
PA0781		hypothetical protein	0	-57
PA0782	*putA*	bifunctional proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase	0	-3.7
PA0783	*putP*	sodium/proline symporter PutP	0	-3.5
PA0789		amino acid permease	0	-3
PA0792	*prpD*	2-methylcitrate dehydratase	0	1.8
PA0798	*pmtA*	phospholipid methyltransferase	0	3.1

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0800		hypothetical protein	0	-7.2
PA0801		hypothetical protein	0	-7.6
PA0802		hypothetical protein	0	-14
PA0803		hypothetical protein	0	2.7
PA0804		oxidoreductase	0	2.9
PA0808		hypothetical protein	0	2.3
PA0809	*mntH2*	manganese transport protein MntH	0	2.8
PA0810		haloacid dehalogenase	0	2.7
PA0811		MFS family transporter	0	2.4
PA0812		hypothetical protein	0	4.1
PA0814		hypothetical protein	0	3.8
PA0826		hypothetical protein	0	2.7
PA0831	*oruR*	transcriptional regulator OruR	0	-1.8
PA0834		acyltransferase	0	-1.9
PA0838		glutathione peroxidase	0	2
PA0844	*plcH*	hemolytic phospholipase C	0	2.9
PA0847		hypothetical protein	0	2.3
PA0849	*trxB2*	thioredoxin reductase 2	0	2.2
PA0850		hypothetical protein	0	2.7
PA0851		hypothetical protein	0	3.2
PA0855		hypothetical protein	0	1.5
PA0861		hypothetical protein	0	3
PA0862		hypothetical protein	0	2.2
PA0863		oxidoreductase	0	2.2
PA0865	*hpd*	4-hydroxyphenylpyruvate dioxygenase	0	1.9
PA0875		hypothetical protein	0	2.6
PA0878		hypothetical protein	0	16
PA0879		acyl-CoA dehydrogenase	0	22
PA0880		ring-cleaving dioxygenase	0	30
PA0881		hypothetical protein	0	37
PA0882		hypothetical protein	0	12
PA0883		acyl-CoA lyase subunit beta	0	18
PA0885		C4-dicarboxylate transporter	0	4.5
PA0886		C4-dicarboxylate transporter	0	3.8
PA0887	*acsA*	acetyl-CoA synthetase	0	1.6
PA0894		hypothetical protein	0	2.7
PA0895	*argD*	bifunctional N-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase	0	-1.9
PA0898	*astD*	succinylglutamic semialdehyde dehydrogenase	0	-1.8
PA0903	*alaS*	alanyl-tRNA synthetase	0	-2
PA0910		hypothetical protein	0	2.2
PA0913	*mgtE*	Mg transporter MgtE	0	-1.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0917	*kup*	potassium uptake protein Kup	0	1.8
PA0918		cytochrome b561	0	2.7
PA0925		hypothetical protein	0	-1.6
PA0928		sensor/response regulator hybrid	0	1.6
PA0929		two-component response regulator	0	-11
PA0945	*purM*	phosphoribosylaminoimidazole synthetase	0	-1.9
PA0948		hypothetical protein	0	-2
PA0956	*proS*	prolyl-tRNA synthetase	0	-1.6
PA0958	*oprD*	basic amino acid. basic peptide and imipenem outer membrane porin OprD precursor	0	1.9
PA0959		hypothetical protein	0	2.2
PA0960		hypothetical protein	0	2.1
PA0963	*aspS*	aspartyl-tRNA synthetase	0	-1.8
PA0964		hypothetical protein	0	-2
PA0967	*ruvB*	Holliday junction DNA helicase RuvB	0	-1.6
PA0969	*tolQ*	TolQ protein	0	-2.2
PA0970	*tolR*	TolR protein	0	-2.5
PA0971	*tolA*	TolA protein	0	-1.9
PA0972	*tolB*	translocation protein TolB	0	-1.9
PA0974		hypothetical protein	0	-1.7
PA0996	*pqsA*	coenzyme A ligase	0	-1.9
PA0997	*pqsB*	PqsB	0	-2
PA0998	*pqsC*	PqsC	0	-2
PA0999	*pqsD*	3-oxoacyl-ACP synthase	0	-2
PA1000	*pqsE*	quinolone signal response protein	0	-2.2
PA1001	*phnA*	anthranilate synthase component I	0	-2.1
PA1002	*phnB*	anthranilate synthase component II	0	-1.8
PA1006		hypothetical protein	0	-1.7
PA1010	*dapA*	dihydrodipicolinate synthase	0	-1.7
PA1011		hypothetical protein	0	-2
PA1028		oxidoreductase	0	2.2
PA1035		hypothetical protein	0	-2
PA1036		hypothetical protein	0	1.8
PA1038		hypothetical protein	0	1.8
PA1042		hypothetical protein	0	2.2
PA1048		hypothetical protein	0	1.7
PA1060		hypothetical protein	0	2.1
PA1061		hypothetical protein	0	1.6
PA1062		hypothetical protein	0	2
PA1063		hypothetical protein	0	2.1
PA1065		hypothetical protein	0	2.7
PA1066		short chain dehydrogenase	0	2.1
PA1098	*fleS*	two-component sensor	0	-2.1

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1099	*fleR*	two-component response regulator	0	-2.1
PA1107		hypothetical protein	0	6.1
PA1117		hypothetical protein	0	2.4
PA1119		hypothetical protein	0	1.5
PA1120	*tpbB*	TpbB	0	1.8
PA1121		hypothetical protein	0	2.4
PA1122		peptide deformylase	0	1.8
PA1126		hypothetical protein	0	1.9
PA1127		oxidoreductase	0	2.5
PA1131		MFS family transporter	0	4
PA1133		hypothetical protein	0	2.2
PA1134		hypothetical protein	0	-8.8
PA1157		two-component response regulator	0	-1.9
PA1163		glucosyl transferase	0	2.6
PA1167		hypothetical protein	0	2.4
PA1168		hypothetical protein	0	35
PA1175	*napD*	NapD protein of periplasmic nitrate reductase	0	4.8
PA1178	*oprH*	PhoP/Q and low Mg2+ inducible outer membrane prote	0	-2.4
PA1179	*phoP*	two-component response regulator PhoP	0	-2.2
PA1180	*phoQ*	two-component sensor PhoQ	0	-1.9
PA1181		sensor protein	0	1.9
PA1188		hypothetical protein	0	1.6
PA1191		hypothetical protein	0	2.4
PA1192		C32 tRNA thiolase	0	-1.6
PA1193		hypothetical protein	0	-1.9
PA1203		hypothetical protein	0	2
PA1204		hypothetical protein	0	1.6
PA1205		hypothetical protein	0	1.5
PA1208		hypothetical protein	0	2.2
PA1210		hypothetical protein	0	2.4
PA1222		membrane-bound lytic murein transglycosylase A	0	-1.5
PA1228		hypothetical protein	0	-7.8
PA1232		hypothetical protein	0	2.3
PA1244		hypothetical protein	0	-2.1
PA1265		hypothetical protein	0	4.4
PA1268		hypothetical protein	0	2.6
PA1272	*cobO*	cob(I)yrinic acid a.c-diamide adenosyltransferase	0	-2.2
PA1274		hypothetical protein	0	-2.7
PA1279	*cobT*	nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase	0	-2.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1280		hypothetical protein	0	-2.9
PA1282		major facilitator transporter	0	-3.9
PA1285		MarR family transcriptional regulator	0	2
PA1287		glutathione peroxidase	0	2.2
PA1288		outer membrane protein	0	-2
PA1296		2-hydroxyacid dehydrogenase	0	2.3
PA1300		RNA polymerase ECF-subfamily sigma-70 factor	0	-28
PA1301		transmembrane sensor	0	-16
PA1302		heme utilization protein	0	-4.6
PA1325		hypothetical protein	0	2.6
PA1326	*ilvA2*	threonine dehydratase	0	4.2
PA1338	*ggt*	gamma-glutamyltranspeptidase	0	1.6
PA1339		ABC transporter ATP-binding protein	0	1.6
PA1340		ABC transporter permease	0	1.5
PA1341		ABC transporter permease	0	1.9
PA1342		ABC transporter substrate-binding protein	0	2
PA1343		hypothetical protein	0	-2.3
PA1346		lysine decarboxylase	0	2.8
PA1347		transcriptional regulator	0	3.9
PA1353		hypothetical protein	0	5
PA1354		hypothetical protein	0	2.8
PA1358		hypothetical protein	0	3.4
PA1360		hypothetical protein	0	-2
PA1361		transporter	0	-1.9
PA1363		RNA polymerase ECF-subfamily sigma-70 factor	0	-2.8
PA1364		transmembrane sensor	0	-2.3
PA1365		siderophore receptor	0	-2.8
PA1401		hypothetical protein	0	1.7
PA1405		helicase	0	-2
PA1406		hypothetical protein	0	2
PA1419		transporter	0	2.5
PA1430	*lasR*	transcriptional regulator LasR	0	1.9
PA1440		hypothetical protein	0	1.7
PA1452	*flhA*	flagellar biosynthesis protein FlhA	0	-1.7
PA1462		plasmid partitioning protein	0	1.5
PA1463		CheW domain-containing protein	0	1.6
PA1473		FlhB domain-containing protein	0	1.8
PA1475	*ccmA*	cytochrome c biogenesis protein CcmA	0	-2
PA1479	*ccmE*	cytochrome c-type biogenesis protein CcmE	0	-1.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1482	*ccmH*	cytochrome C-type biogenesis protein CcmH	0	-1.7
PA1483	*cycH*	cytochrome c-type biogenesis protein	0	-2
PA1484		transcriptional regulator	0	1.6
PA1485		amino acid permease	0	4
PA1491		transporter	0	2.9
PA1492		hypothetical protein	0	2.2
PA1505	*moaA2*	molybdenum cofactor biosynthesis protein A	0	2.1
PA1507		transporter	0	1.8
PA1532	*dnaX*	DNA polymerase III subunits gamma and tau	0	-1.6
PA1533		hypothetical protein	0	-1.8
PA1534	*recR*	recombination protein RecR	0	-1.8
PA1535		acyl-CoA dehydrogenase	0	-1.9
PA1544	*anr*	transcriptional regulator Anr	0	1.5
PA1549		cation-transporting P-type ATPase	0	-1.8
PA1563		RNA 2'-O-ribose methyltransferase	0	-1.7
PA1565		oxidoreductase	0	2.5
PA1575		hypothetical protein	0	2.9
PA1580	*gltA*	type II citrate synthase	0	-2
PA1600		cytochrome c	0	2.5
PA1603		transcriptional regulator	0	1.8
PA1613		hypothetical protein	0	-1.8
PA1619		transcriptional regulator	0	4.9
PA1620		hypothetical protein	0	4.8
PA1621		hydrolase	0	1.7
PA1631		acyl-CoA dehydrogenase	0	2
PA1636	*kdpD*	two-component sensor KdpD	0	1.5
PA1638		glutaminase	0	-1.8
PA1641		lipoprotein	0	1.8
PA1651		transporter	0	2.8
PA1654		aminotransferase	0	-2.1
PA1655		glutathione S-transferase	0	-1.8
PA1656		hypothetical protein	0	-2.5
PA1657		hypothetical protein	0	-3.3
PA1658		hypothetical protein	0	-3.2
PA1659		hypothetical protein	0	-2.4
PA1660		hypothetical protein	0	-3.3
PA1661		hypothetical protein	0	-2.2
PA1662		ClpA/B-type protease	0	-2.9
PA1663		transcriptional regulator	0	-3.4
PA1664		hypothetical protein	0	-3
PA1665		hypothetical protein	0	-3.1
PA1666		lipoprotein	0	-2.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1667		hypothetical protein	0	-2.1
PA1668		hypothetical protein	0	-2.2
PA1669		hypothetical protein	0	-2.7
PA1670	*stp1*	serine/threonine phosphoprotein phosphatase Stp1	0	-2.2
PA1671	*stk1*	serine-threonine kinase Stk1	0	-2
PA1672		hypothetical protein	0	1.5
PA1676		hypothetical protein	0	2.2
PA1687	*speE*	spermidine synthase	0	-2
PA1688		hypothetical protein	0	-1.6
PA1689		hypothetical protein	0	-1.7
PA1693	*pscR*	type III secretion system protein	0	-5.1
PA1694	*pscQ*	type III secretion system protein	0	-6.7
PA1695	*pscP*	translocation protein in type III secretion	0	-7.5
PA1696	*pscO*	translocation protein in type III secretion	0	-5.6
PA1697	*pscN*	type III secretion system ATPase	0	-6.6
PA1699		protein in type III secretion	0	-5.9
PA1700		type III secretion protein	0	-5.3
PA1701		hypothetical protein	0	-8.5
PA1702		hypothetical protein	0	-5.8
PA1704	*pcrR*	transcriptional regulator protein PcrR	0	-3
PA1714		hypothetical protein	0	-1.8
PA1717	*pscD*	type III export protein PscD	0	-2.8
PA1721	*pscH*	type III export protein PscH	0	-2.4
PA1723	*pscJ*	pscJ type III export protein	0	-2.4
PA1725	*pscL*	type III secretion system protein	0	-3.2
PA1726	*bglX*	beta-glucosidase	0	-1.9
PA1729		hypothetical protein	0	3
PA1741		hypothetical protein	0	1.5
PA1742		amidotransferase	0	1.6
PA1750	*aroF-1*	phospho-2-dehydro-3-deoxyheptonate aldolase	0	-1.6
PA1753		universal stress protein	0	2.2
PA1754	*cysB*	transcriptional regulator CysB	0	1.5
PA1758	*pabB*	para-aminobenzoate synthase component I	0	-1.8
PA1759		transcriptional regulator	0	1.8
PA1760		transcriptional regulator	0	2.1
PA1761		hypothetical protein	0	3.1
PA1762		hypothetical protein	0	3.2
PA1763		hypothetical protein	0	2.4
PA1766		hypothetical protein	0	-1.8
PA1767		hypothetical protein	0	-1.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1768		hypothetical protein	0	-1.6
PA1781	*nirB*	assimilatory nitrite reductase large subunit	0	1.6
PA1782		serine/threonine-protein kinase	0	2.4
PA1783	*nasA*	nitrate transporter	0	2.4
PA1797		beta-lactamase	0	-2.1
PA1800	*tig*	trigger factor	0	-2.5
PA1805	*ppiD*	peptidyl-prolyl cis-trans isomerase D	0	-2.3
PA1806	*fabI*	NADH-dependent enoyl-ACP reductase	0	-1.5
PA1807		ABC transporter ATP-binding protein	0	-1.7
PA1810	*nppA2*	ABC transporter substrate-binding protein NppA2	0	-1.5
PA1813		hydroxyacylglutathione hydrolase	0	1.7
PA1814		hypothetical protein	0	1.7
PA1817		hypothetical protein	0	1.8
PA1835		hypothetical protein	0	2.6
PA1836		transcriptional regulator	0	1.9
PA1841		hypothetical protein	0	-2
PA1844		hypothetical protein	0	-4.6
PA1845		hypothetical protein	0	-3.2
PA1846	*cti*	cis/trans isomerase	0	-2
PA1847		hypothetical protein	0	1.5
PA1851		two-component response regulator	0	3.9
PA1852		hypothetical protein	0	2.1
PA1856	*ccoN-2*	cbb3-type cytochrome c oxidase subunit I	0	-6.4
PA1859		LysR family transcriptional regulator	0	1.8
PA1863	*modA*	molybdate-binding periplasmic protein precursor modA	0	2.2
PA1864		TetR family transcriptional regulator	0	3.6
PA1875		outer membrane protein	0	1.9
PA1876		ABC transporter ATP-binding protein/permease	0	2
PA1877		secretion protein	0	1.9
PA1881		oxidoreductase	0	4
PA1891		hypothetical protein	0	3.6
PA1892		hypothetical protein	0	3.5
PA1893		hypothetical protein	0	2.8
PA1895		hypothetical protein	0	3.9
PA1896		hypothetical protein	0	3.8
PA1897		hypothetical protein	0	4.6
PA1898	*qscR*	transcriptional regulator	0	3
PA1911		transmembrane sensor	0	-9.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1912		ECF subfamily RNA polymerase sigma-70 factor	0	-5.4
PA1915		regulatory protein	0	2
PA1922		TonB-dependent receptor	0	-6
PA1925		hypothetical protein	0	-7.2
PA1934		hypothetical protein	0	-2.6
PA1943		hypothetical protein	0	2.2
PA1945		sigma-54 dependent transcriptional regulator	0	3.3
PA1946	*rbsB*	ribose ABC transporter substrate-binding protein	0	1.6
PA1947	*rbsA*	ribose transporter	0	2.7
PA1954		hypothetical protein	0	4.1
PA1963		hypothetical protein	0	2.4
PA1964		ABC transporter ATP-binding protein	0	-1.9
PA1971	*braZ*	branched-chain amino acid transport carrier	0	-2.2
PA1980		two-component response regulator	0	3.9
PA1994		hypothetical protein	0	2
PA1995		hypothetical protein	0	1.7
PA1998		LysR family transcriptional regulator	0	2.3
PA1999		CoA transferase. subunit A	0	-1.7
PA2000		CoA transferase subunit B	0	-2
PA2011	*gnyL*	hydroxymethylglutaryl-CoA lyase	0	-2
PA2012	*gnyA*	alpha subunit of geranoyl-CoA carboxylase. GnyA	0	-2.2
PA2013	*gnyH*	gamma-carboxygeranoyl-CoA hydratase	0	-2
PA2014	*gnyB*	acyl-CoA carboxyltransferase subunit beta	0	-2.3
PA2015	*gnyD*	citronelloyl-CoA dehydrogenase. GnyD	0	-2.1
PA2016	*gnyR*	regulatory gene of gnyRDBHAL cluster. GnyR	0	-2.5
PA2018	*amrB*	multidrug efflux protein	0	-3.1
PA2019		periplasmic multidrug efflux lipoprotein	0	-3.5
PA2020		transcriptional regulator	0	-1.7
PA2026		bile acid/Na+ symporter family transporter	0	2.6
PA2027		hypothetical protein	0	5.9
PA2033		hypothetical protein	0	-3.5
PA2035		thiamine pyrophosphate protein	0	2.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2047	*cmrA*	AraC family transcriptional regulator	0	1.8
PA2048		Antibiotic biosynthesis monooxygenase	0	2.2
PA2056		LysR family transcriptional regulator	0	-1.9
PA2062		pyridoxal-phosphate dependent protein	0	-3.1
PA2066		hypothetical protein	0	1.5
PA2067		hydrolase	0	1.5
PA2070		TonB dependent receptor	0	2.3
PA2075		hypothetical protein	0	3.3
PA2080		kynureninase	0	-1.8
PA2083		ring-hydroxylating dioxygenase. large terminal subunit	0	-2.4
PA2084		asparagine synthetase. glutamine-hydrolysing	0	-4.3
PA2122		hypothetical protein	0	2.8
PA2123		LysR family transcriptional regulator	0	1.9
PA2193	*hcnA*	hydrogen cyanide synthase HcnA	0	-4.8
PA2235	*pslE*	hypothetical protein	0	2.7
PA2236	*pslF*	hypothetical protein	0	3.2
PA2237	*pslG*	glycosyl hydrolase	0	4.3
PA2238	*pslH*	hypothetical protein	0	3
PA2239	*pslI*	transferase	0	3.8
PA2240	*pslJ*	hypothetical protein	0	3
PA2241	*pslK*	hypothetical protein	0	3.1
PA2242	*pslL*	hypothetical protein	0	3.4
PA2247	*bkdA1*	2-oxoisovalerate dehydrogenase subunit alpha	0	-3.5
PA2248	*bkdA2*	2-oxoisovalerate dehydrogenase subunit beta	0	-3
PA2249	*bkdB*	branched-chain alpha-keto acid dehydrogenase subunit E2	0	-3
PA2250	*lpdV*	dihydrolipoamide dehydrogenase	0	-3.1
PA2259	*ptxS*	transcriptional regulator PtxS	0	2.1
PA2260		hypothetical protein	0	2
PA2264		hypothetical protein	0	2
PA2265	*gnd*	gluconate dehydrogenase	0	1.9
PA2275		alcohol dehydrogenase	0	2.5
PA2281		AraC family transcriptional regulator	0	-1.8
PA2289		TonB-dependent receptor	0	1.6
PA2291		glucose-sensitive porin	0	2
PA2306		amino acid transporter LysE	0	4.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2311		hypothetical protein	0	-5.1
PA2312		XRE family transcriptional regulator	0	-10
PA2314		major facilitator transporter	0	2.7
PA2320	*gntR*	transcriptional regulator GntR	0	1.6
PA2322	*gnuT*	gluconate permease	0	-2.3
PA2327		ABC transporter permease	0	-2.6
PA2337	*mtlR*	transcriptional regulator MtlR	0	1.7
PA2338		maltose/mannitol ABC transporter substrate-binding protein	0	2.4
PA2345		hypothetical protein	0	2.6
PA2355		FMNH2-dependent monooxygenase	0	2
PA2360	*hsiA3*	HsiA3 (tssA3)	0	4.3
PA2361	*icmF3*	IcmF3 (tssM3)	0	4.7
PA2362		hypothetical protein	0	4.9
PA2363	*hsiJ3*	HsiJ3 (tssK3)	0	3.6
PA2364	*lip3*	Lip3 (tssJ3)	0	4.4
PA2365	*hsiB3*	HsiB3 (tssB3)	0	10
PA2367	*hcp3*	Hcp3	0	8.4
PA2368	*hsiF3*	HsiF3 (tssE3)	0	9.5
PA2369	*hsiG3*	HsiG3 (tssF3)	0	8.7
PA2370	*hsiH3*	HsiH3 (tssG3)	0	9.6
PA2371	*clpV3*	ClpV3	0	7.4
PA2373	*vgrG3*	VgrG3	0	5.9
PA2374	*tseF*	TseF	0	3.7
PA2375		hypothetical protein	0	5.6
PA2376		transcriptional regulator	0	2.4
PA2378		aldehyde dehydrogenase	0	2.4
PA2379		oxidoreductase	0	2.4
PA2380		hypothetical protein	0	1.6
PA2384		hypothetical protein	0	-19
PA2385	*pvdQ*	penicillin acylase-related protein	0	-130
PA2386	*pvdA*	L-ornithine N5-oxygenase	0	-68
PA2389		hypothetical protein	0	-9.9
PA2390		ABC transporter ATP-binding protein/permease	0	-10
PA2391		outer membrane protein	0	-11
PA2392	*pvdP*	protein PvdP	0	-31
PA2393		dipeptidase	0	-81
PA2394	*pvdN*	protein PvdN	0	-130
PA2395	*pvdO*	protein PvdO	0	-140
PA2396	*pvdF*	pyoverdine synthetase F	0	-8.1
PA2397	*pvdE*	pyoverdine biosynthesis protein PvdE	0	-37
PA2398	*fpvA*	ferripyoverdine receptor	0	-24
PA2399	*pvdD*	pyoverdine synthetase D	0	-12
PA2400	*pvdJ*	protein PvdJ	0	-19

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2402		peptide synthase	0	-22
PA2409		ABC transporter permease	0	-18
PA2411		thioesterase	0	-31
PA2412		hypothetical protein	0	-50
PA2413	*pvdH*	diaminobutyrate--2-oxoglutarate aminotransferase	0	-11
PA2418		pirin-related protein	0	2.8
PA2420		porin	0	2.9
PA2423		hypothetical protein	0	3.5
PA2424	*pvdL*	peptide synthase	0	-80
PA2425	*pvdG*	protein PvdG	0	-81
PA2427		hypothetical protein	0	-39
PA2430		hypothetical protein	0	3.7
PA2431		hypothetical protein	0	2.7
PA2436		hypothetical protein	0	-1.6
PA2437		hypothetical protein	0	-2.3
PA2438		hypothetical protein	0	-2.1
PA2439		hypothetical protein	0	-1.9
PA2440		hypothetical protein	0	13
PA2442	*gcvT2*	glycine cleavage system protein T2	0	1.5
PA2443	*sdaA*	L-serine dehydratase	0	1.6
PA2445	*gcvP2*	glycine dehydrogenase	0	-7.2
PA2453		hypothetical protein	0	-4.1
PA2454		hypothetical protein	0	-2
PA2463		hypothetical protein	0	-2.5
PA2466		TonB-dependent receptor	0	-4.8
PA2467		transmembrane sensor	0	-6.5
PA2468		ECF subfamily RNA polymerase sigma-70 factor	0	-5.4
PA2475		cytochrome P450	0	3.4
PA2484		TetR family transcriptional regulator	0	1.5
PA2489		AraC family transcriptional regulator	0	1.8
PA2490		hypothetical protein	0	3
PA2491		oxidoreductase	0	1.6
PA2500		cyanate permease	0	1.8
PA2507	*catA*	catechol 1.2-dioxygenase	0	4.5
PA2508	*catC*	muconolactone delta-isomerase	0	4.3
PA2509	*catB*	muconate cycloisomerase I	0	5.1
PA2511		transcriptional regulator	0	4.9
PA2518	*xylX*	toluate 1.2-dioxygenase subunit alpha	0	2.9
PA2519	*xylS*	transcriptional regulator XylS	0	3.3
PA2525		outer membrane protein	0	-1.8
PA2526		efflux transporter	0	-1.9
PA2531		aminotransferase	0	-5.4

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2541		CDP-alcohol phosphatidyltransferase	0	1.8
PA2544		hypothetical protein	0	3
PA2555		AMP-binding protein	0	-1.6
PA2560		hypothetical protein	0	2.2
PA2568		hypothetical protein	0	1.7
PA2581		hypothetical protein	0	-1.8
PA2590		outer membrane receptor protein	0	2.2
PA2591		LuxR family transcriptional regulator	0	2.2
PA2592		periplasmic spermidine/putrescine-binding protein	0	1.6
PA2604		hypothetical protein	0	2
PA2605		sulfur transfer complex subunit TusD	0	1.8
PA2606		sulfur relay protein TusC	0	2.2
PA2607		hypothetical protein	0	1.9
PA2608		hypothetical protein	0	1.6
PA2609		hypothetical protein	0	1.7
PA2610		hypothetical protein	0	2.1
PA2621	*clpS*	ATP-dependent Clp protease adaptor protein ClpS	0	1.8
PA2622	*cspD*	cold-shock protein CspD	0	4.6
PA2623	*icd*	isocitrate dehydrogenase	0	1.8
PA2626	*mnmA*	tRNA-specific 2-thiouridylase MnmA	0	-1.7
PA2627		hypothetical protein	0	-1.7
PA2629	*purB*	adenylosuccinate lyase	0	-2.8
PA2630		hypothetical protein	0	-3.1
PA2633		hypothetical protein	0	2.2
PA2635		hypothetical protein	0	1.9
PA2659		hypothetical protein	0	1.5
PA2684		hypothetical protein	0	-2
PA2685		hypothetical protein	0	-1.6
PA2687	*pfeS*	two-component sensor PfeS	0	-7.9
PA2696		transcriptional regulator	0	1.9
PA2700		porin	0	4.1
PA2709	*cysK*	cysteine synthase A	0	1.7
PA2710		hypothetical protein	0	2.2
PA2718		MerR family transcriptional regulator	0	2.5
PA2719		hypothetical protein	0	2
PA2722		hypothetical protein	0	6.1
PA2723		hypothetical protein	0	2.8
PA2724		hypothetical protein	0	1.9
PA2727		hypothetical protein	0	-1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2739	*pheT*	phenylalanyl-tRNA synthetase subunit beta	0	-1.6
PA2756		hypothetical protein	0	1.5
PA2764		hypothetical protein	0	2.8
PA2765		hypothetical protein	0	-2.5
PA2774		hypothetical protein	0	-1.8
PA2780		hypothetical protein	0	4.6
PA2781		hypothetical protein	0	4.8
PA2787	*cpg2*	glutamate carboxypeptidase	0	5.1
PA2790		hypothetical protein	0	3.1
PA2792		hypothetical protein	0	-2.2
PA2793		hypothetical protein	0	-2
PA2796	*tal*	transaldolase B	0	1.6
PA2811		ABC transporter permease	0	-1.5
PA2821		glutathione S-transferase	0	1.6
PA2823		hypothetical protein	0	-1.5
PA2827		methionine sulfoxide reductase B	0	2
PA2830	*htpX*	heat shock protein HtpX	0	-2
PA2839		hypothetical protein	0	2.2
PA2841		enoyl-CoA hydratase	0	2.1
PA2842		hypothetical protein	0	-1.6
PA2850	*ohr*	organic hydroperoxide resistance protein	0	-2.3
PA2864		hypothetical protein	0	2.4
PA2867		chemotaxis transducer	0	-1.6
PA2868		hypothetical protein	0	2
PA2869		hypothetical protein	0	2.8
PA2870		hypothetical protein	0	3.8
PA2872		hypothetical protein	0	1.5
PA2876	*pyrF*	orotidine 5'-phosphate decarboxylase	0	-1.9
PA2893		long-chain-acyl-CoA synthetase	0	2.4
PA2897		GntR family transcriptional regulator	0	2.7
PA2901		lipoprotein	0	-2
PA2904	*cobI*	precorrin-2 C(20)-methyltransferase	0	-1.8
PA2911		TonB-dependent receptor	0	-7.3
PA2912		ABC transporter ATP-binding protein	0	-5
PA2914		ABC transporter permease	0	-4.2
PA2918		short chain dehydrogenase	0	5.7
PA2919		hypothetical protein	0	4.8
PA2943	*aroF*	phospho-2-dehydro-3-deoxyheptonate aldolase	0	-3.6
PA2945		cobalamin biosynthesis protein cobW	0	-2.1
PA2949		lipase	0	1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2950		trans-2-enoyl-CoA reductase	0	-1.8
PA2951	*etfA*	electron transfer flavoprotein subunit alpha	0	-1.5
PA2952	*etfB*	electron transfer flavoprotein subunit beta	0	-1.7
PA2956		hypothetical protein	0	-1.6
PA2959		TatD family deoxyribonuclease	0	-1.7
PA2961	*holB*	DNA polymerase III subunit delta'	0	-2.1
PA2962	*tmk*	thymidylate kinase	0	-1.8
PA2963		hypothetical protein	0	-2
PA2964	*pabC*	4-amino-4-deoxychorismate lyase	0	-1.7
PA2966	*acpP*	acyl carrier protein	0	-1.6
PA2967	*fabG*	3-ketoacyl-ACP reductase	0	-1.5
PA2968	*fabD*	malonyl-CoA-ACP transacylase	0	-1.6
PA2974		hydrolase	0	-1.5
PA2975	*rluC*	ribosomal large subunit pseudouridine synthase C	0	-1.7
PA2976	*rne*	ribonuclease E	0	-2
PA2977	*murB*	UDP-N-acetylenolpyruvoylglucosamine reductase	0	-1.7
PA2978	*ptpA*	phosphotyrosine protein phosphatase	0	-1.6
PA2986		hypothetical protein	0	-3.1
PA2987		lipoprotein releasing system. ATP-binding protein	0	-2.1
PA2988		hypothetical protein	0	-2.5
PA2989		hypothetical protein	0	1.6
PA2993		thiamine biosynthesis lipoprotein	0	-1.8
PA2994	*nqrF*	Na(+)-translocating NADH-quinone reductase subunit F	0	-1.8
PA2995	*nqrE*	Na(+)-translocating NADH-quinone reductase subunit E	0	-1.8
PA2996	*nqrD*	Na(+)-translocating NADH-quinone reductase subunit D	0	-1.6
PA2997	*nqrC*	Na(+)-translocating NADH-quinone reductase subunit C	0	-1.9
PA2998	*nqrB*	Na(+)-translocating NADH-quinone reductase subunit B	0	-1.9
PA2999	*nqrA*	Na(+)-translocating NADH-quinone reductase subunit A	0	-1.7
PA3001		glyceraldehyde-3-phosphate dehydrogenase	0	-1.9
PA3004		5'-methylthioadenosine phosphorylase	0	-1.5
PA3011	*topA*	DNA topoisomerase I	0	-1.6
PA3018		hypothetical protein	0	-3.4

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3048		hypothetical protein	0	-2.5
PA3058	*pelG*	hypothetical protein	0	3.2
PA3059	*pelF*	hypothetical protein	0	2.8
PA3060	*pelE*	hypothetical protein	0	6.2
PA3061	*pelD*	hypothetical protein	0	4.1
PA3062	*pelC*	lipoprotein	0	3.6
PA3063	*pelB*	hypothetical protein	0	2.9
PA3064	*pelA*	hypothetical protein	0	4.7
PA3069		lipoprotein	0	-1.9
PA3076		hypothetical protein	0	1.7
PA3077		two-component response regulator	0	-1.9
PA3081		hypothetical protein	0	-1.9
PA3082	*gbt*	glycine betaine transmethylase	0	-2.2
PA3089		hypothetical protein	0	3
PA3093		hypothetical protein	0	-2
PA3098	*xcpW*	general secretion pathway protein J	0	-1.5
PA3105	*xcpQ*	general secretion pathway protein D	0	-1.8
PA3106		oxidoreductase	0	-1.6
PA3112	*accD*	acetyl-CoA carboxylase subunit beta	0	-1.5
PA3115	*fimV*	pilus assembly protein	0	-1.5
PA3129		hypothetical protein	0	-1.8
PA3136		secretion protein	0	-2.4
PA3137		MFS transporter	0	-2.3
PA3145	*orfN*	group 4 glycosyl transferase	0	-2.8
PA3146	*orfM*	NAD dependent epimerase/dehydratase	0	-2.8
PA3148	*orfK*	UDP-N-acetylglucosamine 2-epimerase	0	-2.2
PA3159	*orfH*	UDP-N-acetyl-D-mannosaminuronate dehydrogenase	0	-2.2
PA3160	*wzz*	O-antigen chain length regulator	0	-1.7
PA3162	*rpsA*	30S ribosomal protein S1	0	-1.9
PA3169	*mtnA*	methylthioribose-1-phosphate isomerase	0	-2.2
PA3180		hypothetical protein	0	3.1
PA3186	*oprB*	glucose/carbohydrate outer membrane porin OprB precursor	0	-1.7
PA3187	*gltK*	ABC transporter ATP-binding protein	0	-1.9
PA3188		ABC sugar transporter permease	0	-1.8
PA3195	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	0	2.1
PA3198		hypothetical protein	0	1.7
PA3199		SUA5/yciO/yrdC family:Sua5/YciO/YrdC/YwlC family protein	0	1.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3200		PHP domain-containing protein	0	1.7
PA3205		hypothetical protein	0	1.9
PA3209		ferredoxin	0	-2.6
PA3220		AraC family transcriptional regulator	0	1.5
PA3224		hypothetical protein	0	1.6
PA3232		DNA polymerase III subunit epsilon	0	2.8
PA3233		hypothetical protein	0	2.6
PA3234	*actP*	acetate permease	0	4.1
PA3235		hypothetical protein	0	5
PA3238		hypothetical protein	0	1.6
PA3242	*htrB*	lipid A biosynthesis lauroyl acyltransferase	0	-1.5
PA3244	*minD*	cell division inhibitor MinD	0	-1.6
PA3245	*minE*	cell division topological specificity factor MinE	0	-1.6
PA3249		transcriptional regulator	0	3.1
PA3250		hypothetical protein	0	5.4
PA3251		hypothetical protein	0	4.2
PA3252		ABC transporter permease	0	4.4
PA3253		ABC transporter permease	0	4
PA3254		ABC transporter ATP-binding protein	0	2.5
PA3261		hypothetical protein	0	2.7
PA3262		peptidyl-prolyl cis-trans isomerase. FkbP-type	0	-1.9
PA3263	*rdgC*	recombination associated protein	0	-2.7
PA3276		hypothetical protein	0	-2.9
PA3279	*oprP*	phosphate-specific outer membrane porin OprP precursor	0	4.5
PA3287		ankyrin domain-containing protein	0	2.7
PA3289		hypothetical protein	0	3.5
PA3292		hypothetical protein	0	-2.7
PA3297	*hrpA*	ATP-dependent helicase	0	-1.7
PA3300	*fadD2*	long-chain-fatty-acid--CoA ligase	0	1.6
PA3304		hypothetical protein	0	2.1
PA3306	*alkB*	hypothetical protein	0	2
PA3310		hypothetical protein	0	-1.8
PA3311		hypothetical protein	0	6.5
PA3319	*plcN*	non-hemolytic phospholipase C	0	2.2
PA3326		ATP-dependent Clp protease proteolytic subunit	0	1.7
PA3327		non-ribosomal peptide synthetase	0	-1.9
PA3328		FAD-dependent monooxygenase	0	-2.1
PA3331	*cyp23*	cytochrome P450	0	-1.7
PA3332		isomerase	0	-1.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3333	*fabH2*	3-oxoacyl-ACP synthase	0	-1.7
PA3344	*recQ*	ATP-dependent DNA helicase RecQ	0	-1.6
PA3347		hypothetical protein	0	3.5
PA3348		chemotaxis protein methyltransferase	0	1.7
PA3350	*flgA*	flagellar basal body P-ring biosynthesis protein FlgA	0	1.5
PA3351	*flgM*	hypothetical protein	0	1.5
PA3352	*flgN*	hypothetical protein	0	1.6
PA3353		glycosyltransferase	0	2
PA3355		MFS transporter	0	2.1
PA3363	*amiR*	aliphatic amidase regulator	0	2.7
PA3364	*amiC*	aliphatic amidase expression-regulating protein	0	2.4
PA3366	*amiE*	acylamide amidohydrolase	0	3.7
PA3385	*amrZ*	DNA binding-protein amrZ	0	1.5
PA3391	*nosR*	regulatory protein NosR	0	-4.4
PA3399		hypothetical protein	0	1.8
PA3403		hypothetical protein	0	3
PA3407	*hasAp*	heme acquisition protein HasAp	0	-13
PA3408	*hasR*	heme uptake outer membrane receptor HasR	0	-8.1
PA3410		RNA polymerase ECF-subfamily sigma-70 factor	0	-14
PA3419		hypothetical protein	0	2
PA3421		hypothetical protein	0	3
PA3426		enoyl-CoA hydratase	0	2.8
PA3428		hypothetical protein	0	5.3
PA3429		alpha/beta hydrolase	0	2.4
PA3430		aldolase	0	2
PA3442	*ssuB*	aliphatic sulfonates transport ATP-binding subunit	0	-8.5
PA3443		ABC transporter permease	0	-6.9
PA3444	*ssuD*	alkanesulfonate monooxygenase	0	-15
PA3445		hypothetical protein	0	-6.2
PA3446		NAD(P)H-dependent FMN reductase	0	-13
PA3448		ABC transporter permease	0	-4.3
PA3450		antioxidant protein	0	-9.3
PA3455		hypothetical protein	0	1.9
PA3456	*mnmC*	5-methylaminomethyl-2-thiouridine methyltransferase	0	-1.5
PA3458		transcriptional regulator	0	-1.8
PA3462		sensor/response regulator hybrid	0	2.3
PA3475	*pheC*	cyclohexadienyl dehydratase	0	1.5
PA3476	*rhlI*	autoinducer synthesis protein RhlI	0	1.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3477	*rhlR*	transcriptional regulator RhlR	0	3.3
PA3487	*pldA*	phospholipase D	0	-1.6
PA3490		electron transport complex protein RnfB	0	-1.9
PA3491		electron transport complex protein RnfC	0	-1.7
PA3493		hypothetical protein	0	-1.9
PA3520		hypothetical protein	0	2.6
PA3525	*argG*	argininosuccinate synthase	0	-2
PA3530		hypothetical protein	0	-13
PA3534		oxidoreductase	0	1.9
PA3537	*argF*	ornithine carbamoyltransferase	0	-1.5
PA3542	*alg44*	alginate biosynthesis protein Alg44	0	4
PA3544	*algE*	alginate production outer membrane protein AlgE	0	4.3
PA3548	*algI*	alginate o-acetyltransferase AlgI	0	2.6
PA3552		UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase	0	-1.6
PA3553		glycosyl transferase family protein	0	-1.6
PA3554		bifunctional UDP-glucuronic acid decarboxylase/UDP-4-amino-4-deoxy-L-arabinose formyltransferase	0	-1.8
PA3555		hypothetical protein	0	-1.8
PA3556	*arnT*	4-amino-4-deoxy-L-arabinose transferase	0	-1.9
PA3557		hypothetical protein	0	-2
PA3558		hypothetical protein	0	-1.8
PA3568		acetyl-coa synthetase	0	1.7
PA3569	*mmsB*	3-hydroxyisobutyrate dehydrogenase	0	1.7
PA3573		major facilitator subfamily transporter protein	0	-1.6
PA3576		hypothetical protein	0	2.5
PA3577		hypothetical protein	0	1.6
PA3578		PhzF family phenazine biosynthesis protein	0	1.5
PA3581	*glpF*	glycerol uptake facilitator protein	0	3.2
PA3582	*glpK*	glycerol kinase	0	2.5
PA3584	*glpD*	glycerol-3-phosphate dehydrogenase	0	3
PA3598		hypothetical protein	0	-2.4
PA3600	*rpmJ*	50S ribosomal protein L36	0	-23
PA3601	*rpmE2*	50S ribosomal protein L31	0	-26
PA3605		hypothetical protein	0	-2
PA3608	*potB*	polyamine transport protein PotB	0	-4
PA3619		hypothetical protein	0	1.7
PA3620	*mutS*	DNA mismatch repair protein MutS	0	-1.6
PA3622	*rpoS*	RNA polymerase sigma factor RpoS	0	2.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3623		hypothetical protein	0	2.9
PA3625	*surE*	stationary phase survival protein SurE	0	-1.6
PA3628		esterase	0	2
PA3629	*adhC*	alcohol dehydrogenase	0	2
PA3642	*rnhB*	ribonuclease HII	0	-1.9
PA3646	*lpxD*	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	0	-1.9
PA3647		hypothetical protein	0	-1.6
PA3648		outer membrane antigen	0	-1.9
PA3653	*frr*	ribosome recycling factor	0	-1.9
PA3662		hypothetical protein	0	2.7
PA3667		pyridoxal-phosphate dependent protein	0	1.7
PA3668		hypothetical protein	0	2.3
PA3673	*plsB*	glycerol-3-phosphate acyltransferase	0	-1.8
PA3675		hypothetical protein	0	-2
PA3676		efflux transmembrane protein	0	-3.1
PA3677		efflux transmembrane protein	0	-3.3
PA3678		TetR family transcriptional regulator	0	-2.3
PA3682		hypothetical protein	0	2
PA3686	*adk*	adenylate kinase	0	-1.8
PA3697		hypothetical protein	0	1.5
PA3698		lipoprotein	0	1.6
PA3714		two-component response regulator	0	2.1
PA3715		hypothetical protein	0	1.7
PA3720		hypothetical protein	0	-2.1
PA3722		hypothetical protein	0	1.7
PA3727		hypothetical protein	0	-3.1
PA3728		hypothetical protein	0	-2.8
PA3729		hypothetical protein	0	-2.1
PA3730		hypothetical protein	0	-2
PA3731		hypothetical protein	0	-2
PA3735	*thrC*	threonine synthase	0	-1.7
PA3756		hypothetical protein	0	1.6
PA3758		N-acetylglucosamine-6-phosphate deacetylase	0	1.8
PA3759		aminotransferase	0	1.9
PA3760		phosphoenolpyruvate-protein phosphotransferase	0	1.6
PA3761		PTS system N-acetylglucosamine-specific IIBC component	0	1.8
PA3762		hypothetical protein	0	-2
PA3768		metallo-oxidoreductase	0	-4.4
PA3769	*guaA*	GMP synthase	0	-2

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3770	*guaB*	inosine 5'-monophosphate dehydrogenase	0	-1.8
PA3771		transcriptional regulator	0	2.6
PA3772		hypothetical protein	0	3.2
PA3787		hypothetical protein	0	2.7
PA3796		hypothetical protein	0	1.8
PA3797		hypothetical protein	0	1.6
PA3800		hypothetical protein	0	-1.7
PA3801		hypothetical protein	0	-1.6
PA3802	*hisS*	histidyl-tRNA synthetase	0	-1.8
PA3806		hypothetical protein	0	-1.8
PA3809	*fdx2*	ferredoxin 2Fe-2S	0	-1.8
PA3810	*hscA*	chaperone protein HscA	0	-1.8
PA3816	*cysE*	serine O-acetyltransferase	0	-1.6
PA3825		hypothetical protein	0	2.6
PA3826		hypothetical protein	0	-2
PA3833		hypothetical protein	0	2.4
PA3838		ABC-transporter ATP-binding component	0	-1.7
PA3840		SAM-dependent methyltransferase	0	-2.1
PA3847		hypothetical protein	0	2
PA3848		hypothetical protein	0	2.4
PA3851		hypothetical protein	0	1.9
PA3852		hypothetical protein	0	1.8
PA3855		hypothetical protein	0	1.7
PA3858		amino acid-binding protein	0	2.6
PA3861	*rhl*	ATP-dependent RNA helicase RhlB	0	-1.6
PA3862		hypothetical protein	0	-1.7
PA3864		hypothetical protein	0	-1.8
PA3878	*narX*	two-component sensor NarX	0	2.3
PA3885	*tpbA*	protein tyrosine phosphatase TpbA	0	2.4
PA3887	*nhaP*	Na+/H+ antiporter NhaP	0	-2.1
PA3893		outer membrane protein	0	-2.1
PA3894		outer membrane protein	0	-1.9
PA3897		hypothetical protein	0	7.7
PA3898		AraC family transcriptional regulator	0	4
PA3900		transmembrane sensor	0	-3.7
PA3901	*fecA*	Fe(III) dicitrate transport protein FecA	0	-3.3
PA3903	*prfC*	peptide chain release factor 3	0	-2.4
PA3911		hypothetical protein	0	-4.6
PA3912		hypothetical protein	0	-9.2
PA3913		protease	0	-14
PA3919		hypothetical protein	0	2.4
PA3920		metal transporting P-type ATPase	0	1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3921		transcriptional regulator	0	2
PA3922		hypothetical protein	0	2.7
PA3923		hypothetical protein	0	3.1
PA3924		long-chain-fatty-acid--CoA ligase	0	3.8
PA3928		hypothetical protein	0	2.8
PA3931		hypothetical protein	0	-2.2
PA3933		choline transporter	0	-1.9
PA3934		hypothetical protein	0	-1.5
PA3935	*tauD*	taurine dioxygenase	0	-7.5
PA3936		taurine ABC transporter permease	0	-12
PA3937		taurine ABC transporter ATP-binding protein	0	-9.5
PA3949		hypothetical protein	0	-1.6
PA3968		pseudouridine synthase	0	-1.8
PA3969		hypothetical protein	0	1.8
PA3973		AcrR family transcriptional regulator	0	1.9
PA3982		metalloprotease	0	-1.9
PA3984	*lnt*	apolipoprotein N-acyltransferase	0	-1.9
PA3985		hypothetical protein	0	1.9
PA3987	*leuS*	leucyl-tRNA synthetase	0	-1.7
PA3992		murein transglycosylase	0	-1.6
PA3995		transcriptional regulator	0	2.2
PA4001	*sltB1*	soluble lytic transglycosylase B	0	-1.5
PA4005		hypothetical protein	0	-1.6
PA4012		hypothetical protein	0	1.6
PA4013		hypothetical protein	0	-1.7
PA4015		hypothetical protein	0	2.3
PA4021		transcriptional regulator	0	1.9
PA4022		aldehyde dehydrogenase	0	1.6
PA4023		amino acid transporter	0	2.9
PA4024	*eutB*	ethanolamine ammonia-lyase large subunit	0	2.7
PA4025		ethanolamine ammonia-lyase small subunit	0	2.7
PA4030		hypothetical protein	0	-2.1
PA4041		hypothetical protein	0	3.1
PA4052	*nusB*	transcription antitermination protein NusB	0	-1.6
PA4053	*ribH*	6.7-dimethyl-8-ribityllumazine synthase	0	-1.8
PA4063		hypothetical protein	0	-12
PA4064		ABC transporter ATP-binding protein	0	-5.3
PA4065		permease	0	-5.9
PA4066		hypothetical protein	0	-4.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4067	*oprG*	outer membrane protein OprG precursor	0	-3.8
PA4070		DNA-binding transcriptional activator FeaR	0	2.2
PA4074		transcriptional regulator	0	2.4
PA4084	*cupB3*	usher CupB3	0	2.6
PA4090		hypothetical protein	0	-1.8
PA4094		AraC family transcriptional regulator	0	1.7
PA4108		HDIG domain-containing protein	0	3.7
PA4111		hypothetical protein	0	2.1
PA4112		sensor/response regulator hybrid	0	3.1
PA4116		hypothetical protein	0	1.9
PA4117		bacteriophytochrome	0	1.8
PA4119	*aph*	aminoglycoside 3'-phosphotransferase type IIB	0	-1.9
PA4147	*acoR*	transcriptional regulator AcoR	0	8.6
PA4148		short-chain dehydrogenase	0	18
PA4149		hypothetical protein	0	17
PA4150		dehydrogenase E1 component	0	3.6
PA4151	*acoB*	acetoin catabolism protein AcoB	0	5.9
PA4152		branched-chain alpha-keto acid dehydrogenase subunit E2	0	3.7
PA4153	*adh*	2.3-butanediol dehydrogenase	0	3.7
PA4154		SH3 domain-containing protein	0	-2.7
PA4156		TonB-dependent receptor protein	0	-3.1
PA4158	*fepC*	ferric enterobactin transport protein FepC	0	-4.8
PA4159	*fepB*	iron-enterobactin transporter periplasmic binding protein	0	-4.9
PA4162		short chain dehydrogenase	0	1.9
PA4168	*fpvB*	type I ferripyoverdine receptor. FpvB	0	-2.2
PA4170		oxidoreductase	0	-2.8
PA4175	*prpL*	Pvds-regulated endoprotease. lysyl class	0	1.9
PA4176	*ppiC2*	peptidyl-prolyl cis-trans isomerase C2	0	2.2
PA4180		acetolactate synthase	0	2.7
PA4182		transcriptional regulator	0	-1.6
PA4200		hypothetical protein	0	1.9
PA4209	*phzM*	phenazine-specific methyltransferase	0	-2
PA4210	*phzA1*	phenazine biosynthesis protein	0	-27
PA4211	*phzB1*	phenazine biosynthesis protein	0	-8.3
PA4218		transporter	0	-4.9
PA4220		hypothetical protein	0	-7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4221	*fptA*	Fe(III)-pyochelin outer membrane receptor	0	-7.1
PA4222		ABC transporter ATP-binding protein	0	-10
PA4223		ABC transporter ATP-binding protein	0	-11
PA4224	*pchG*	pyochelin biosynthetic protein PchG	0	-8.8
PA4225	*pchF*	pyochelin synthetase	0	-11
PA4226	*pchE*	dihydroaeruginoic acid synthetase	0	-10
PA4228	*pchD*	pyochelin biosynthesis protein PchD	0	-6.2
PA4229	*pchC*	pyochelin biosynthetic protein PchC	0	-5.8
PA4230	*pchB*	isochorismate-pyruvate lyase	0	-7.4
PA4231	*pchA*	salicylate biosynthesis isochorismate synthase	0	-7
PA4234	*uvrA*	excinuclease ABC subunit A	0	-1.9
PA4235	*bfrA*	bacterioferritin	0	-2.3
PA4238	*rpoA*	DNA-directed RNA polymerase subunit alpha	0	-2.2
PA4239	*rpsD*	30S ribosomal protein S4	0	-2.2
PA4240	*rpsK*	30S ribosomal protein S11	0	-2.3
PA4241	*rpsM*	30S ribosomal protein S13	0	-2.3
PA4244	*rplO*	50S ribosomal protein L15	0	-2.3
PA4245	*rpmD*	50S ribosomal protein L30	0	-2.6
PA4246	*rpsE*	30S ribosomal protein S5	0	-2.5
PA4247	*rplR*	50S ribosomal protein L18	0	-2.6
PA4248	*rplF*	50S ribosomal protein L6	0	-2.4
PA4249	*rpsH*	30S ribosomal protein S8	0	-2.2
PA4250	*rpsN*	30S ribosomal protein S14	0	-2.7
PA4251	*rplE*	50S ribosomal protein L5	0	-3.1
PA4252	*rplX*	50S ribosomal protein L24	0	-3.1
PA4253	*rplN*	50S ribosomal protein L14	0	-2.9
PA4254	*rpsQ*	30S ribosomal protein S17	0	-3.1
PA4255	*rpmC*	50S ribosomal protein L29	0	-2.8
PA4256	*rplP*	50S ribosomal protein L16	0	-2.6
PA4257	*rpsC*	30S ribosomal protein S3	0	-2.7
PA4258	*rplV*	50S ribosomal protein L22	0	-2.7
PA4259	*rpsS*	30S ribosomal protein S19	0	-2.9
PA4260	*rplB*	50S ribosomal protein L2	0	-2.8
PA4261	*rplW*	50S ribosomal protein L23	0	-3.3
PA4262	*rplD*	50S ribosomal protein L4	0	-3.1
PA4263	*rplC*	50S ribosomal protein L3	0	-2.7
PA4264	*rpsJ*	30S ribosomal protein S10	0	-2.7
PA4265	*tufA*	elongation factor Tu	0	-2.5
PA4266	*fusA1*	elongation factor G	0	-2.7
PA4267	*rpsG*	30S ribosomal protein S7	0	-2.1
PA4268	*rpsL*	30S ribosomal protein S12	0	-2.1

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4269	*rpoC*	DNA-directed RNA polymerase subunit beta'	0	-2
PA4270	*rpoB*	DNA-directed RNA polymerase subunit beta	0	-2
PA4273	*rplA*	50S ribosomal protein L1	0	-2.6
PA4274	*rplK*	50S ribosomal protein L11	0	-2.7
PA4279		pantothenate kinase	0	-2
PA4290		chemotaxis transducer	0	2.6
PA4300		hypothetical protein	0	3.2
PA4301		hypothetical protein	0	3.2
PA4302		type II secretion system protein	0	2.9
PA4304		type II secretion system protein	0	2.6
PA4314	*purU*	formyltetrahydrofolate deformylase	0	-1.9
PA4324		hypothetical protein	0	1.6
PA4325		hypothetical protein	0	1.8
PA4326		lipoprotein	0	1.6
PA4327		hypothetical protein	0	1.6
PA4330		enoyl-CoA hydratase/isomerase	0	1.6
PA4333		fumarase	0	-2.4
PA4341		transcriptional regulator	0	3.3
PA4342		amidase	0	2.2
PA4343		MFS transporter	0	2.5
PA4348		hypothetical protein	0	-5.5
PA4356		xenobiotic reductase	0	-2.7
PA4357		hypothetical protein	0	-9.1
PA4358	*feoB*	ferrous iron transport protein B	0	-12
PA4359	*feoA*	ferrous iron transport protein A	0	-12
PA4364		hypothetical protein	0	-4.5
PA4367		diguanylate cyclase	0	1.7
PA4370	*icmP*	metalloproteinase outer membrane	0	-7
PA4371		hypothetical protein	0	-16
PA4373		hypothetical protein	0	-3.8
PA4377		hypothetical protein	0	2.9
PA4378	*inaA*	InaA protein	0	2.1
PA4379		hypothetical protein	0	2.2
PA4383		camphor resistance protein CrcB	0	2
PA4390		hypothetical protein	0	-2
PA4392		hypothetical protein	0	1.9
PA4397	*apbA*	2-dehydropantoate 2-reductase	0	2.8
PA4399		hypothetical protein	0	1.6
PA4403	*secA*	preprotein translocase subunit SecA	0	-1.5
PA4404		hypothetical protein	0	-1.9
PA4405		hypothetical protein	0	1.8
PA4408	*ftsA*	cell division protein FtsA	0	-1.6
PA4410	*ddl*	D-alanine--D-alanine ligase	0	-1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4411	*murC*	UDP-N-acetylmuramate--L-alanine ligase	0	-1.6
PA4412	*murG*	UDPdiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	0	-1.6
PA4414	*murD*	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	0	-1.5
PA4415	*mraY*	phospho-N-acetylmuramoyl-pentapeptide- transferase	0	-1.6
PA4417	*murE*	UDP-N-acetylmuramoylalanyl-D-glutamate--2. 6-diaminopimelate ligase	0	-1.6
PA4420	*mraW*	S-adenosyl-methyltransferase MraW	0	-1.6
PA4421		cell division protein MraZ	0	-1.7
PA4428	*sspA*	stringent starvation protein A	0	-2.1
PA4429		cytochrome c1	0	-1.8
PA4430		cytochrome b	0	-1.8
PA4431		cytochrome c reductase. iron-sulfur subun	0	-1.8
PA4434		oxidoreductase	0	1.6
PA4439	*trpS*	tryptophanyl-tRNA synthetase	0	-1.7
PA4445		hypothetical protein	0	-2
PA4447	*hisC1*	histidinol-phosphate aminotransferase	0	-1.6
PA4448	*hisD*	histidinol dehydrogenase	0	-2
PA4449	*hisG*	ATP phosphoribosyltransferase	0	-1.8
PA4450	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	0	-1.7
PA4457		hypothetical protein	0	-2
PA4458		hypothetical protein	0	-1.7
PA4459		hypothetical protein	0	-1.9
PA4460		hypothetical protein	0	-1.6
PA4463		hypothetical protein	0	1.6
PA4464	*ptsN*	nitrogen regulatory IIA protein	0	1.6
PA4467		hypothetical protein	0	-7.8
PA4468	*sodM*	superoxide dismutase	0	-11
PA4469		hypothetical protein	0	-14
PA4470	*fumC*	fumarate hydratase	0	-14
PA4471		hypothetical protein	0	-7.1
PA4475		hypothetical protein	0	1.7
PA4485		hypothetical protein	0	-1.7
PA4496	*dppA1*	ABC transporter substrate-binding protein DppA1	0	3
PA4497	*dppA2*	ABC transporter substrate-binding protein DppA2	0	3.3

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4500	*dppA3*	dipeptide ABC transporter substrate-binding protein DppA3	0	1.8
PA4501	*opdP*	glycine-glutamate dipeptide porin OpdP	0	4.1
PA4502	*dppA4*	dipeptide ABC transporter substrate-binding protein DppA4	0	3.9
PA4503	*dppB*	dipeptide ABC transporter permease DppB	0	3.6
PA4504	*dppC*	ABC transporter permease	0	3.7
PA4505	*dppD*	ABC transporter ATP-binding protein	0	3.6
PA4506	*dppF*	dipeptide transporter ATP-binding subunit	0	3.7
PA4507		hypothetical protein	0	5.2
PA4508		AsnC family transcriptional regulator	0	2.8
PA4515		hydroxylase	0	-8.2
PA4516		hypothetical protein	0	-4.8
PA4517		hypothetical protein	0	1.7
PA4535		hypothetical protein	0	1.5
PA4543		hypothetical protein	0	-1.6
PA4558		peptidyl-prolyl cis-trans isomerase. FkbP-type	0	-1.7
PA4559	*lspA*	lipoprotein signal peptidase	0	-1.5
PA4560	*ileS*	isoleucyl-tRNA synthetase	0	-1.6
PA4565	*proB*	gamma-glutamyl kinase	0	-2.2
PA4566	*obgE*	GTPase ObgE	0	-2.4
PA4570		hypothetical protein	0	-7.5
PA4571		cytochrome c	0	-8.3
PA4572	*fklB*	peptidyl-prolyl cis-trans isomerase FklB	0	1.7
PA4574		hypothetical protein	0	-2.2
PA4578		hypothetical protein	0	-1.5
PA4579		hypothetical protein	0	-2.1
PA4582		hypothetical protein	0	1.8
PA4588	*gdhA*	glutamate dehydrogenase	0	-5.2
PA4591		hypothetical protein	0	-1.8
PA4593		ABC transporter permease	0	-1.9
PA4594		ABC transporter ATP-binding protein	0	1.6
PA4600	*nfxB*	transcriptional regulator NfxB	0	-2.1
PA4601	*morA*	motility regulator	0	1.5
PA4602	*glyA*	serine hydroxymethyltransferase	0	-1.7
PA4603		hypothetical protein	0	1.8
PA4604		hypothetical protein	0	1.5
PA4605		hypothetical protein	0	1.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4606		hypothetical protein	0	1.8
PA4608		hypothetical protein	0	3.1
PA4610		hypothetical protein	0	-3.3
PA4614	*mscL*	large-conductance mechanosensitive channel	0	2.1
PA4622		transmembrane protein	0	-2.1
PA4630		hypothetical protein	0	2.1
PA4635		magnesium transporter. MgtC family	0	-3.3
PA4636		hypothetical protein	0	-1.9
PA4649		hypothetical protein	0	2.7
PA4651		pili assembly chaperone	0	2
PA4665	*prfA*	peptide chain release factor 1	0	-2.3
PA4666	*hemA*	glutamyl-tRNA reductase	0	-1.6
PA4668	*lolB*	outer membrane lipoprotein LolB	0	-1.5
PA4676		carbonic anhydrase	0	-1.9
PA4680		hypothetical protein	0	2.4
PA4681		hypothetical protein	0	2.2
PA4685		hypothetical protein	0	-3.5
PA4686		hypothetical protein	0	-1.9
PA4687	*hitA*	ferric iron-binding periplasmic protein HitA	0	-2.9
PA4688	*hitB*	iron ABC transporter. permease	0	-8
PA4694	*ilvC*	ketol-acid reductoisomerase	0	-2
PA4695	*ilvH*	acetolactate synthase 3 regulatory subunit	0	-2
PA4696	*ilvI*	acetolactate synthase 3 catalytic subunit	0	-1.9
PA4698		hypothetical protein	0	-1.6
PA4703		hypothetical protein	0	5.3
PA4704		hypothetical protein	0	2.4
PA4705		hypothetical protein	0	-2
PA4706	*hmuV*	hemin importer ATP-binding subunit	0	-2.8
PA4707		ABC transporter permease	0	-2.8
PA4708		hypothetical protein	0	-3.2
PA4709		hemin degrading factor	0	-11
PA4711		Rieske family iron-sulfur cluster-binding protein	0	-2.2
PA4712		hypothetical protein	0	1.8
PA4719		transporter	0	-1.7
PA4723	*dksA*	suppressor protein DksA	0	-1.7
PA4726	*cbrB*	two-component response regulator CbrB	0	1.8
PA4729	*panB*	3-methyl-2-oxobutanoate hydroxymethyltransferase	0	-1.6
PA4730	*panC*	pantoate--beta-alanine ligase	0	-1.8

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4735		hypothetical protein	0	1.9
PA4744	*infB*	translation initiation factor IF-2	0	-2.2
PA4751	*ftsH*	cell division protein FtsH	0	1.7
PA4770	*lldP*	L-lactate permease	0	-3.8
PA4778		transcriptional regulator	0	1.7
PA4779		hypothetical protein	0	2.3
PA4780		hypothetical protein	0	1.7
PA4787		transcriptional regulator	0	1.6
PA4793		lipoprotein	0	1.7
PA4803		methyltransferase	0	2.1
PA4810	*fdnI*	nitrate-inducible formate dehydrogenase subunit gamma	0	1.8
PA4811	*fdnH*	nitrate-inducible formate dehydrogenase subunit beta	0	1.9
PA4812	*fdnG*	formate dehydrogenase-O. major subunit	0	2.1
PA4827		N-hydroxyarylamine O-acetyltransferase	0	1.7
PA4828		hypothetical protein	0	3.3
PA4830		hypothetical protein	0	3.7
PA4834		hypothetical protein	0	-27
PA4835		hypothetical protein	0	-30
PA4836		hypothetical protein	0	-37
PA4837		uter membrane protein	0	-54
PA4838		hypothetical protein	0	-5.6
PA4840		translation initiation factor Sui1	0	-1.7
PA4843		two-component response regulator	0	1.6
PA4845	*dipZ*	thiol:disulfide interchange protein	0	-2.1
PA4849		hypothetical protein	0	-2.1
PA4852		hypothetical protein	0	-1.6
PA4854	*purH*	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	0	-2
PA4855	*purD*	phosphoribosylamine--glycine ligase	0	-2.4
PA4869		hypothetical protein	0	-2.1
PA4870		hypothetical protein	0	3.8
PA4874		hypothetical protein	0	3.2
PA4875		hypothetical protein	0	2.2
PA4884		hypothetical protein	0	5.3
PA4887		MFS transporter	0	-1.8
PA4888		hypothetical protein	0	-8.4
PA4889		oxidoreductase	0	-7.3
PA4890		TetR family transcriptional regulator	0	-1.9
PA4891	*ureE*	urease accessory protein UreE	0	2.1
PA4893	*ureG*	urease accessory protein UreG	0	1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4894	*ureJ*	hypothetical protein	0	1.6
PA4895		transmembrane sensor	0	-4.6
PA4896		RNA polymerase sigma factor	0	-5.7
PA4901	*mdlC*	benzoylformate decarboxylase	0	2.8
PA4905	*vanB*	vanillate O-demethylase	0	2.9
PA4907		short-chain dehydrogenase	0	2.1
PA4908		ornithine cyclodeaminase	0	3
PA4909		ABC transporter ATP-binding protein	0	2.9
PA4910		ABC transporter ATP-binding protein	0	2.6
PA4912		branched chain amino acid ABC transporter permease	0	3
PA4913		ABC transporter substrate-binding protein	0	3.5
PA4914		LysR family transcriptional regulator	0	2.3
PA4923		hypothetical protein	0	-1.8
PA4926		hypothetical protein	0	2.7
PA4927		hypothetical protein	0	2.2
PA4929		hypothetical protein	0	5.7
PA4930	*alr*	biosynthetic alanine racemase	0	-1.6
PA4931	*dnaB*	replicative DNA helicase	0	-2.2
PA4947	*amiB*	N-acetylmuramoyl-L-alanine amidase	0	1.5
PA4951	*orn*	oligoribonuclease	0	1.6
PA4957	*psd*	phosphatidylserine decarboxylase	0	-1.5
PA4958		hypothetical protein	0	1.8
PA4965		hypothetical protein	0	-2.3
PA4966		hypothetical protein	0	-1.8
PA4967	*parE*	DNA topoisomerase IV subunit B	0	-2.1
PA4969		hypothetical protein	0	-1.7
PA4973	*thiC*	thiamine biosynthesis protein ThiC	0	-1.7
PA4974		outer membrane efflux protein	0	-2
PA4975		NAD(P)H quinone oxidoreductase	0	1.9
PA4993		hypothetical protein	0	2.4
PA4994		acyl-CoA dehydrogenase	0	2.1
PA4996	*rfaE*	bifunctional heptose 7-phosphate kinase/heptose 1-phosphate adenyltransferase	0	-1.6
PA4999		hypothetical protein	0	-2.2
PA5001		hypothetical protein	0	-1.8
PA5003		hypothetical protein	0	-1.8
PA5004		glycosyl transferase family protein	0	-1.7
PA5005		carbamoyl transferase	0	-1.6
PA5006		hypothetical protein	0	-1.6

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5007		hypothetical protein	0	-1.6
PA5009	*waaP*	lipopolysaccharide kinase WaaP	0	-1.7
PA5010	*waaG*	UDP-glucose:(heptosyl) LPS alpha 1.3-glucosyltransferase WaaG	0	-1.7
PA5011	*waaC*	lipopolysaccharide heptosyltransferase I	0	-1.5
PA5025	*metY*	O-acetylhomoserine aminocarboxypropyltransferase	0	-3.7
PA5028		hypothetical protein	0	1.6
PA5030		MFS transporter	0	-2.7
PA5050	*priA*	primosome assembly protein PriA	0	-1.6
PA5051	*argS*	arginyl-tRNA synthetase	0	-2.3
PA5052		hypothetical protein	0	-1.8
PA5056	*phaC1*	poly(3-hydroxyalkanoic acid) synthase 1	0	1.9
PA5057	*phaD*	poly(3-hydroxyalkanoic acid) depolymerase	0	2.2
PA5059		TetR family transcriptional regulator	0	3.5
PA5071		16S ribosomal RNA methyltransferase RsmE	0	-1.6
PA5072		chemotaxis transducer	0	-2.2
PA5077	*mdoH*	glucosyltransferase MdoH	0	-1.6
PA5078	*mdoG*	glucan biosynthesis protein G	0	-1.6
PA5091	*hutG*	N-formylglutamate amidohydrolase	0	2.2
PA5092	*hutI*	imidazolonepropionase	0	2
PA5093		histidine/phenylalanine ammonia-lyase	0	2.1
PA5094		ABC transporter ATP-binding protein	0	2.2
PA5095		ABC transporter permease	0	3.2
PA5096		ABC transporter substrate-binding protein	0	4.2
PA5097		amino acid permease	0	2.4
PA5098	*hutH*	histidine ammonia-lyase	0	1.7
PA5099		cytosine/purines uracil thiamine allantoin permease	0	2.2
PA5101		hypothetical protein	0	2.5
PA5103		ABC transporter substrate-binding protein	0	2.3
PA5105	*hutC*	histidine utilization genes repressor protein	0	-1.7
PA5106		N-formimino-L-glutamate deiminase	0	-3.1
PA5112	*estA*	esterase EstA	0	1.6
PA5117	*typA*	GTP-binding protein TypA	0	-2.1
PA5126		hypothetical protein	0	-1.9
PA5131	*pgm*	phosphoglyceromutase	0	-1.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5142	*hisH*	imidazole glycerol phosphate synthase subunit HisH	0	-1.6
PA5147	*mutY*	A/G-specific adenine glycosylase	0	-1.6
PA5148		hypothetical protein	0	-1.7
PA5153		periplasmic binding protein	0	2.1
PA5154		ABC transporter permease	0	1.7
PA5157		transcriptional regulator	0	-2
PA5158		outer membrane protein	0	-1.7
PA5161	*rmlB*	dTDP-D-glucose 4.6-dehydratase	0	1.8
PA5162	*rmlD*	dTDP-4-dehydrorhamnose reductase	0	1.8
PA5163	*rmlA*	glucose-1-phosphate thymidylyltransferase	0	1.7
PA5164	*rmlC*	dTDP-4-dehydrorhamnose 3.5-epimerase	0	1.9
PA5167		c4-dicarboxylate-binding protein	0	1.9
PA5168		dicarboxylate transporter	0	2.2
PA5169		C4-dicarboxylate transporter	0	1.8
PA5185		hypothetical protein	0	2.5
PA5187		acyl-CoA dehydrogenase	0	2.4
PA5191		hypothetical protein	0	2.3
PA5193	*hslO*	Hsp33-like chaperonin	0	-2.1
PA5214	*gcvH1*	glycine cleavage system protein H	0	1.6
PA5216		iron ABC transporter. permease	0	-2.4
PA5217		iron ABC transporter substrate-binding protein	0	-3.5
PA5222		hypothetical protein	0	-1.5
PA5229		hypothetical protein	0	1.6
PA5230		ABC transporter permease	0	-1.7
PA5231		ABC transporter ATP-binding protein/permease	0	-1.7
PA5248		hypothetical protein	0	-3.7
PA5249		LysE family efflux protein	0	1.9
PA5252		ABC transporter ATP-binding protein	0	-1.7
PA5253	*algP*	alginate regulatory protein AlgP	0	1.9
PA5255	*algQ*	anti-RNA polymerase sigma 70 factor	0	2
PA5266		hypothetical protein	0	-4.3
PA5271		hypothetical protein	0	2.9
PA5283		transcriptional regulator	0	1.8
PA5298	*xpt*	xanthine phosphoribosyltransferase	0	-1.7
PA5302	*dadX*	alanine racemase	0	-1.5
PA5312		aldehyde dehydrogenase	0	1.7
PA5313		omega amino acid--pyruvate transaminase	0	2.6
PA5314		hypothetical protein	0	2.3

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5329		hypothetical protein	0	2.7
PA5334	*rph*	ribonuclease PH	0	-1.6
PA5343		hypothetical protein	0	-1.5
PA5345	*recG*	ATP-dependent DNA helicase RecG	0	-1.6
PA5348		HU family DNA-binding protein	0	2.3
PA5357	*ubiC*	hypothetical protein	0	-2
PA5363		hypothetical protein	0	2
PA5379	*sdaB*	L-serine dehydratase	0	3
PA5383		hypothetical protein	0	-39
PA5395		hypothetical protein	0	2.3
PA5408		hypothetical protein	0	2.4
PA5409		hypothetical protein	0	2
PA5436		pyruvate carboxylase subunit A	0	-1.7
PA5440		peptidase	0	-7.4
PA5444		hypothetical protein	0	1.9
PA5461		hypothetical protein	0	2.2
PA5463		hypothetical protein	0	-2.1
PA5464		hypothetical protein	0	-2.7
PA5471		hypothetical protein	0	-2.1
PA5476	*citA*	citrate transporter	0	4.5
PA5479	*gltP*	glutamate/aspartate:proton symporter	0	-3.2
PA5498		adhesin	0	-1.7
PA5500	*znuC*	zinc transporter	0	-1.6
PA5501	*znuB*	ABC zinc transporter permease ZnuB	0	-2
PA5505		TonB-dependent receptor	0	-1.8
PA5506		hypothetical protein	0	-2.2
PA5507		hypothetical protein	0	-2.5
PA5508		glutamine synthetase	0	-2.4
PA5511		two-component response regulator	0	-1.6
PA5520		hypothetical protein	0	2.4
PA5522		glutamine synthetase	0	2.1
PA5523		aminotransferase	0	1.8
PA5524		short-chain dehydrogenase	0	1.8
PA5525		transcriptional regulator	0	2.3
PA5527		hypothetical protein	0	3.4
PA5530		MFS dicarboxylate transporter	0	-10
PA5531	*tonB*	TonB protein	0	-3.1
PA5532		G3E family GTPase	0	-2.7
PA5534		hypothetical protein	0	-22
PA5535		hypothetical protein	0	-17
PA5536		DksA/TraR family C4-type zinc finger protein	0	-31
PA5538	*amiA*	N-acetylmuramoyl-L-alanine amidase	0	-18

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5539		GTP cyclohydrolase	0	-44
PA5540		hypothetical protein	0	-18
PA5541	*pyrQ*	dihydroorotase	0	-18
PA5545		hypothetical protein	0	2.2
PA5549	*glmS*	glucosamine--fructose-6-phosphate aminotransferase	0	-2.8
PA5553	*atpC*	F0F1 ATP synthase subunit epsilon	0	-3.1
PA5554	*atpD*	F0F1 ATP synthase subunit beta	0	-3.2
PA5556	*atpA*	F0F1 ATP synthase subunit alpha	0	-3.1
PA5564	*gidB*	16S rRNA methyltransferase GidB	0	-1.9
PA5568		inner membrane protein translocase component YidC	0	-3.4
PA14_35810		hypothetical protein	1.5	1.8
PA0178	*cheA*	two-component sensor	1.5	3.8
PA0506		acyl-CoA dehydrogenase	1.5	2.2
PA1083	*flgH*	flagellar basal body L-ring protein	1.5	0
PA1458		two-component sensor	1.5	0
PA2262		2-ketogluconate transporter	1.5	1.9
PA3068	*gdhB*	NAD-dependent glutamate dehydrogenase	1.5	0
PA4026		acetyltransferase	1.5	3.5
PA4472	*pmbA*	proteolysis/ metallopeptidase activity	1.5	2.1
PA5016	*aceF*	dihydrolipoamide acetyltransferase	1.5	-2
PA5305		hypothetical protein	1.5	0
PA5378		hypothetical protein	1.5	2.2
PA14_59780	*rcsC*	kinase sensor protein	1.6	0
PA14_40740		hypothetical protein	1.6	1.9
PA0122		hemolysin	1.6	4.2
PA0177	*cheW*	purine-binding chemotaxis protein	1.6	4.2
PA0179		two-component response regulator	1.6	3.9
PA0233		transcriptional regulator	1.6	0
PA0299	*spuC*	aminotransferase	1.6	1.6
PA0387		deoxyribonucleotide triphosphate pyrophosphatase	1.6	0
PA0482	*glcB*	malate synthase G	1.6	0
PA0604		ABC transporter substrate-binding protein	1.6	0
PA0815		transcriptional regulator	1.6	2.2
PA0835	*pta*	phosphate acetyltransferase	1.6	0
PA0905	*rsmA*	RNA binding protein translational regulator	1.6	0
PA1084	*flgI*	flagellar basal body P-ring protein	1.6	0
PA1106		hypothetical protein	1.6	3.2
PA1441		hypothetical protein	1.6	0
PA1617		AMP-binding protein	1.6	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1679		hypothetical protein	1.6	0
PA1944		30S ribosomal protein S6 modification protein	1.6	2.6
PA1951		hypothetical protein	1.6	4.8
PA2261		2-ketogluconate kinase	1.6	2
PA2290	*gcd*	glucose dehydrogenase	1.6	2.2
PA2679		hypothetical protein	1.6	2.3
PA2704		AraC family transcriptional regulator	1.6	2.6
PA3031		lipoprotein	1.6	0
PA3228		ABC transporter ATP-binding protein/permease	1.6	0
PA3307		hypothetical protein	1.6	2.8
PA3330		short chain dehydrogenas	1.6	-1.6
PA3346		two-component response regulator	1.6	3.6
PA3740		hypothetical protein	1.6	5
PA3786		hypothetical protein	1.6	2.8
PA3856		hypothetical protein	1.6	1.7
PA3951		hypothetical protein	1.6	0
PA4296		two-component response regulator	1.6	2.3
PA4297		hypothetical protein	1.6	3.4
PA4532		hypothetical protein	1.6	0
PA4648		hypothetical protein	1.6	2.7
PA4674		Antitoxin HigA - virulence-associated protein	1.6	1.8
PA4733	*acsB*	acetyl-CoA synthetase	1.6	1.7
PA4782		hypothetical protein	1.6	1.7
PA4841		hypothetical protein	1.6	0
PA4842		hypothetical protein	1.6	0
PA4911		branched chain amino acid ABC transporter permease	1.6	3.1
PA5015	*aceE*	pyruvate dehydrogenase subunit E1	1.6	-1.8
PA5100	*hutU*	urocanate hydratase	1.6	2
PA5257		enzyme of heme biosynthesis	1.6	0
PA5439		glucose-6-phosphate 1-dehydrogenase	1.6	0
PA14_59770	*rcsB*	two component response regulator	1.7	-1.6
PA14_33320		hypothetical protein	1.7	0
PA14_54920		non-ribosomal peptide synthetase	1.7	1.6
PA14_15460	*merA*	mercuric reductase	1.7	1.7
PA14_54940		siderophore biosynthesis enzyme	1.7	1.8
PA14_28460		hypothetical protein	1.7	2.2
PA14_51590		hypothetical protein	1.7	3.1
PA14_29330		hypothetical protein	1.7	3.3
PA14_46540		hypothetical protein	1.7	3.7
PA14_43250		hypothetical protein	1.7	4.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0023	*qor*	quinone oxidoreductase	1.7	1.6
PA0158		RND efflux transporter	1.7	0
PA0326		ABC transporter ATP-binding protein	1.7	4.2
PA0388		hypothetical protein	1.7	0
PA0656		HIT family protein	1.7	3.4
PA0704		amidase	1.7	3.8
PA0920		hypothetical protein	1.7	0
PA1474		hypothetical protein	1.7	1.9
PA1566		glutamine synthetase	1.7	3.6
PA1587	*lpdG*	dihydrolipoamide dehydrogenase	1.7	0
PA1601		aldehyde dehydrogenase	1.7	2.1
PA1786	*nasS*	hypothetical protein	1.7	3
PA2032		transcriptional regulator	1.7	1.9
PA2266		cytochrome c precursor	1.7	2
PA2299		GntR family transcriptional regulator	1.7	0
PA2483		hypothetical protein	1.7	0
PA2571		signal transduction histidine kinase	1.7	4.2
PA2705		hypothetical protein	1.7	0
PA2746		hypothetical protein	1.7	11
PA2958		hypothetical protein	1.7	1.9
PA3017		hypothetical protein	1.7	3
PA3230		hypothetical protein	1.7	0
PA3340		Tfp pilus assembly protein FimV	1.7	3.9
PA3857	*pcs*	phosphatidylserine synthase	1.7	1.9
PA3879	*narL*	transcriptional regulator NarL	1.7	1.6
PA4294		hypothetical protein	1.7	2.3
PA4310	*pctB*	chemotactic transducer PctB	1.7	0
PA4474		hypothetical protein	1.7	2
PA4717		hypothetical protein	1.7	2.4
PA4781		two-component response regulator	1.7	2.2
PA5058	*phaC2*	poly(3-hydroxyalkanoic acid) synthase 2	1.7	4
PA5209		hypothetical protein	1.7	0
PA5258		hypothetical protein	1.7	0
PA14_36000	*prpR*	propionate catabolism operon regulator	1.8	0
PA14_54930		non-ribosomal peptide synthetase	1.8	1.8
PA14_13210		hypothetical protein	1.8	7.2
PA0007		hypothetical protein	1.8	4.6
PA0157		RND efflux membrane fusion protein	1.8	0
PA0765	*mucC*	positive regulator for alginate biosynthesis MucC	1.8	0
PA0852	*cpbD*	chitin-binding protein CbpD	1.8	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1027		aldehyde dehydrogenase	1.8	2
PA1080	*flgE*	flagellar hook protein FlgE	1.8	0
PA1085	*flgJ*	flagellar rod assembly protein/muramidase FlgJ	1.8	0
PA1086	*flgK*	flagellar hook-associated protein FlgK	1.8	0
PA1093		hypothetical protein	1.8	0
PA1123		hypothetical protein	1.8	0
PA1130	*rhlC*	rhamnosyltransferase 2	1.8	5
PA1252		L-malate dehydrogenase	1.8	2.2
PA1337	*ansB*	glutaminase-asparaginase	1.8	1.8
PA1345		glutathione synthase	1.8	2.9
PA1785	*nasT*	hypothetical protein - regulation of nitrate assimilation	1.8	3.4
PA2194	*hcnB*	hydrogen cyanide synthase HcnB	1.8	-5.6
PA2263		2-hydroxyacid dehydrogenase	1.8	2.4
PA2572		two-component response regulator	1.8	3.9
PA2721		hypothetical protein	1.8	6.1
PA2778		hypothetical protein	1.8	3.2
PA2939		aminopeptidase	1.8	3.3
PA3013	*fadA*	3-ketoacyl-CoA thiolase	1.8	0
PA3216		hypothetical protein	1.8	2.9
PA3349		chemotaxis protein	1.8	1.7
PA3354		hypothetical protein	1.8	3.1
PA3361	*lecB*	fucose-binding lectin PA-IIL - biofilm formation	1.8	5.3
PA3431		hypothetical protein	1.8	-2.2
PA3529		peroxidase	1.8	0
PA3846		isochorismatase family hydrolase	1.8	3.3
PA4027		hypothetical protein	1.8	3.8
PA4207	*mexI*	RND efflux transporter	1.8	0
PA4575		hypothetical protein	1.8	1.6
PA4682		hypothetical protein	1.8	3.1
PA5186		iron-containing alcohol dehydrogenase	1.8	3.1
PA5377		BC-type proline/glycine betaine transport system. permease component	1.8	2.1
PA5423		hypothetical protein	1.8	1.6
PA14_68450		hypothetical protein	1.9	0
PA14_10830		LysR family transcriptional regulator	1.9	0
PA14_46520		hypothetical protein	1.9	3.2
PA0221		aminotransferase	1.9	3.7
PA0322		transporter	1.9	2.8
PA0366		aldehyde dehydrogenase	1.9	2.2

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0545		hypothetical protein	1.9	0
PA0836		acetate kinase	1.9	0
PA1087	*flgL*	flagellar hook-associated protein FlgL	1.9	0
PA1094	*fliD*	flagellar capping protein FliD	1.9	0
PA1166		hypothetical protein	1.9	3.5
PA1327		protease	1.9	4.2
PA1351		RNA polymerase ECF-subfamily sigma-70 factor	1.9	5
PA1415		hypothetical protein	1.9	2.8
PA1522		xanthine dehydrogenase accessory factor X	1.9	0
PA1546	*hemN*	coproporphyrinogen III oxidase	1.9	-4.8
PA1731		hypothetical protein	1.9	3.2
PA1733		hypothetical protein	1.9	3.2
PA1833		oxidoreductase	1.9	0
PA2127		hypothetical protein	1.9	-3.4
PA2366	*hsiC3*	hsiC3	1.9	8.4
PA2891		biotin carboxylase	1.9	0
PA2927		hypothetical protein	1.9	3.4
PA3092	*fadH1*	2.4-dienoyl-CoA reductase	1.9	0
PA3526		OmpA family membrane protein	1.9	0
PA3687	*ppc*	phosphoenolpyruvate carboxylase	1.9	1.7
PA3909		extracellular nuclease	1.9	0
PA3945		acetyltransferase	1.9	3
PA4205	*mexG*	hypothetical protein	1.9	0
PA4299		pilus assembly protein	1.9	3.2
PA4303		hypothetical protein	1.9	2.9
PA4305		pilus assembly protein	1.9	3
PA4362		hypothetical protein	1.9	3.2
PA4607		hypothetical protein	1.9	3.5
PA4691		sulfite oxidase subunit YedZ	1.9	0
PA4915		methyl-accepting chemotaxis protein	1.9	3.9
PA4920	*nadE*	NAD synthetase	1.9	0
PA4976	*aspC*	aspartate transaminase	1.9	2.1
PA14_33300		hypothetical protein	2	0
PA14_31280		integrase	2	1.7
PA14_61350		hypothetical protein	2	2
PA14_31450		hypothetical protein	2	7.3
PA0138		ABC transporter permease	2	0
PA0345		hypothetical protein	2	0
PA0476		permease	2	3
PA0766	*mucD*	serine protease MucD	2	0
PA0791		transcriptional regulator	2	0
PA1195		hypothetical protein	2	0
PA1202		hydrolase	2	3.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1289		hypothetical protein	2	2.9
PA1470		short chain dehydrogenase	2	2.3
PA1730		hypothetical protein	2	3.3
PA1784		lyase	2	5.9
PA1826		LysR family transcriptional regulatory protein	2	1.9
PA1900	*phzB2*	phenazine biosynthesis protein	2	0
PA2023	*galU*	UTP-glucose-1-phosphate uridylyltransferase	2	0
PA2072		sensory box protein	2	4.6
PA2301		tRNA synthase	2	2
PA2441		hypothetical protein	2	14
PA2575		hypothetical protein	2	0
PA2694		thioredoxin	2	0
PA2716		FMN oxidoreductase	2	3.9
PA3043		deoxyguanosinetriphosphate triphosphohydrolase-like protein	2	0
PA3337	*rfaD*	ADP-L-glycero-D-manno-heptose-6-epimerase	2	-5.4
PA3451		hypothetical protein	2	6
PA3465		hypothetical protein	2	2.4
PA3880		hypothetical protein	2	0
PA3930	*cioA*	CioA. cyanide insensitive terminal oxidase	2	2.5
PA4198		acyl-CoA synthetase	2	2.3
PA4199		acyl-CoA dehydrogenase	2	2.1
PA4208	*opmD*	outer membrane protein	2	0
PA4298		hypothetical protein	2	3.8
PA4495		hypothetical protein	2	1.7
PA4533		hypothetical protein	2	0
PA4573		hypothetical protein	2	6.6
PA4919	*pncB1*	nicotinate phosphoribosyltransferase	2	0
PA5262	*algZ*	alginate biosynthesis protein AlgZ/FimS	2	1.8
PA5359		hypothetical protein	2	5.4
PA5376		lycine betaine/L-proline ABC transporter. ATP-binding subunit	2	2
PA14_39700		hypothetical protein	2.1	-6.3
PA14_35900		dehydrogenase	2.1	2.6
PA14_35890		diaminobutyrate--2-oxoglutarate aminotransferase	2.1	2.7
PA0109		hypothetical protein	2.1	4
PA0134		guanine deaminase	2.1	1.7
PA0147		oxidoreductase	2.1	1.9
PA0212	*mdcE*	malonate decarboxylase subunit gamma	2.1	9.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0344		hypothetical protein	2.1	0
PA0479		LysR family transcriptional regulator	2.1	1.9
PA0484		ACT domain-containing protein	2.1	4.8
PA0743		3-hydroxyisobutyrate dehydrogenase	2.1	3.2
PA0762	*algU*	RNA polymerase sigma factor AlgU	2.1	0
PA0764	*mucB*	negative regulator for alginate biosynthesis MucB	2.1	0
PA1169		lipoxygenase	2.1	25
PA1172	*napc*	cytochrome c-type protein NapC	2.1	5.9
PA1177	*napE*	periplasmic nitrate reductase NapE	2.1	6.7
PA1196		transcriptional regulator	2.1	0
PA1409	*aphA*	acetylpolyamine aminohydrolase	2.1	0
PA1513		hypothetical protein	2.1	0
PA1567		glycine/D-amino acid oxidase	2.1	3.4
PA1732		transglutaminase	2.1	3.2
PA1860		hypothetical protein	2.1	4.6
PA1874		hypothetical protein	2.1	2.1
PA1880		oxidoreductase	2.1	3.6
PA2003	*bdhA*	3-hydroxybutyrate dehydrogenase	2.1	1.7
PA2562		hypothetical protein	2.1	3.9
PA2573		methyl-accepting chemotaxis transducer	2.1	3.3
PA2889		acyl-CoA dehydrogenase	2.1	0
PA3436		hypothetical protein	2.1	5.4
PA3690		metal-transporting P-type ATPase	2.1	0
PA3712		hypothetical protein	2.1	0
PA3739		sodium/hydrogen antiporter	2.1	1.9
PA3929	*cioB*	CioB. cyanide insensitive terminal oxidase	2.1	2.5
PA3957		short chain dehydrogenase	2.1	4.5
PA3986		hypothetical protein	2.1	7.1
PA4206	*mexH*	RND efflux membrane fusion protein	2.1	0
PA4293		two-component sensor	2.1	2.1
PA4384		hypothetical protein	2.1	2.6
PA4611		hypothetical protein	2.1	2.4
PA4621		oxidoreductase	2.1	0
PA4633		chemotaxis transducer	2.1	0
PA4702		hypothetical protein	2.1	4.1
PA5178		LysM domain/BON superfamily protein	2.1	2.5
PA5261	*algR*	alginate biosynthesis regulatory protein AlgR	2.1	2.3
PA5380		AraC family transcriptional regulator	2.1	4.7

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5422		hypothetical protein	2.1	0
PA5514		beta-lactamase	2.1	0
PA5521		short-chain dehydrogenase	2.1	1.8
PA14_61340		hypothetical protein	2.2	2.2
PA14_54910		thioesterase	2.2	2.6
PA14_35880		gamma-aminobutyraldehyde dehydrogenase	2.2	3.1
PA0103		sulfate transporter	2.2	4.8
PA0156		RND efflux membrane fusion protein	2.2	0
PA0173	*cheB*	chemotaxis-specific methylesterase	2.2	5.6
PA0276		hypothetical protein	2.2	0
PA0354		hypothetical protein	2.2	-1.8
PA0462		hypothetical protein	2.2	0
PA0489		phosphoribosyl transferase	2.2	0
PA1041		hypothetical protein	2.2	3.5
PA1176	*napF*	ferredoxin protein NapF	2.2	6.3
PA1330		short chain dehydrogenase	2.2	2
PA1494		hypothetical protein	2.2	0
PA1728		hypothetical protein	2.2	6
PA1919		radical SAM protein	2.2	-9.5
PA1997		acetoacetyl-CoA synthetase	2.2	2.3
PA2195	*hcnC*	hydrogen cyanide synthase HcnC	2.2	-4.9
PA2300	*chiC*	chitinase	2.2	1.7
PA2549		TerC family protein	2.2	0
PA2565		hypothetical protein	2.2	3.1
PA2620	*clpA*	ATP-dependent Clp protease. ATP-binding subunit ClpA	2.2	2
PA2664	*fhp*	nitric oxide dioxygenase	2.2	2.2
PA2799		hypothetical protein	2.2	4
PA2825		MarR family transcriptional regulator	2.2	0
PA2826		glutathione peroxidase	2.2	0
PA2920		chemotaxis transducer	2.2	3.7
PA3049	*rmf*	ribosome modulation factor - negative regulation of translation	2.2	8.1
PA3123		translation initiation inhibitor	2.2	3.3
PA3177		sensory box GGDEF domain-containing protein	2.2	0
PA3229		hypothetical protein	2.2	2.7
PA3374	*phnM*	phosphonate metabolism protein	2.2	2.1
PA3614		hypothetical protein	2.2	0
PA3723		FMN oxidoreductase	2.2	3.3
PA3724	*lasB*	elastase LasB	2.2	4.7
PA3971		hypothetical protein	2.2	0
PA4394		hypothetical protein	2.2	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4657		hypothetical protein	2.2	2.9
PA4759	*dapB*	dihydrodipicolinate reductase	2.2	0
PA4829	*lpd3*	dihydrolipoamide dehydrogenase	2.2	2.7
PA5299		hypothetical protein	2.2	1.7
PA5396		hypothetical protein	2.2	2.2
PA5399		ferredoxin	2.2	0
PA14_49860		hypothetical protein	2.3	2.3
PA14_40750		hypothetical protein	2.3	5.5
PA0211	*mdcD*	malonate decarboxylase subunit beta	2.3	9
PA0962		DNA-binding stress protein	2.3	0
PA1344		short-chain dehydrogenase	2.3	2.9
PA1356		hypothetical protein	2.3	6.9
PA1432	*lasI*	autoinducer synthesis protein LasI	2.3	0
PA1515	*alc*	allantoicase	2.3	0
PA1745		hypothetical protein	2.3	4.2
PA1889		hypothetical protein	2.3	0
PA2303		regulatory protein	2.3	2
PA2779		hypothetical protein	2.3	3.4
PA2888		biotin-dependent carboxylase	2.3	0
PA2915		hypothetical protein	2.3	2.2
PA3630	*gfnR*	LysR family transcriptional regulator / glutathione-dependent formaldehyde neutralization regulator GfnR	2.3	0
PA3688		hypothetical protein	2.3	5.9
PA4313		hypothetical protein	2.3	1.8
PA4925		hypothetical protein	2.3	4.8
PA5424		hypothetical protein	2.3	2
PA14_33310		hypothetical protein	2.4	0
PA14_43900		hypothetical protein	2.4	0
PA14_31270		hypothetical protein	2.4	2.6
PA0052		hypothetical protein	2.4	4.5
PA0269		hypothetical protein	2.4	0
PA0270		hypothetical protein	2.4	0
PA1249	*aprA*	alkaline metalloproteinase	2.4	2.1
PA1255		hypothetical protein	2.4	2.4
PA1349		hypothetical protein	2.4	6.3
PA1350		hypothetical protein	2.4	5.3
PA1517		hypothetical protein	2.4	0
PA1545	*PemB*	protein secretion by the type III secretion system	2.4	0
PA1561	*aer*	aerotaxis receptor Aer	2.4	-1.5
PA1737		3-hydroxyacyl-CoA dehydrogenase	2.4	1.5
PA1914		hypothetical protein	2.4	3.8
PA1920		anaerobic ribonucleoside triphosphate reductase	2.4	-9.9

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2126		hypothetical protein	2.4	-1.9
PA2174		hypothetical protein	2.4	5.9
PA2273		redox-sensing activator of soxS	2.4	0
PA2448		hypothetical protein	2.4	1.7
PA2564		hypothetical protein	2.4	4.3
PA2663		hypothetical protein	2.4	2
PA3283		hypothetical protein	2.4	2.2
PA3284		hypothetical protein	2.4	2.5
PA3323		hypothetical protein	2.4	2.6
PA3478	*rhlB*	rhamnosyltransferase chain B	2.4	6.1
PA3479	*rhlA*	rhamnosyltransferase chain A	2.4	5.7
PA3962		hypothetical protein	2.4	0
PA4140		hypothetical protein	2.4	31
PA4309	*pctA*	chemotactic transducer PctA	2.4	1.6
PA4349		hypothetical protein	2.4	0
PA4624		hypothetical protein	2.4	10
PA4760	*dnaJ*	chaperone protein DnaJ	2.4	0
PA4762	*grpE*	heat shock protein GrpE	2.4	0
PA4900		MFS transporter	2.4	2.6
PA4918	*pncA*	hypothetical protein	2.4	2.3
PA5061		hypothetical protein	2.4	4
PA5245		isoprenoid biosynthesis protein with amidotransferase-like domain	2.4	0
PA5291		choline transporter	2.4	0
PA14_35940		acyl-CoA synthetase	2.5	1.9
PA0062		lipoprotein	2.5	0
PA0146		hypothetical protein	2.5	2.1
PA0166		transporter	2.5	1.8
PA0271		hypothetical protein	2.5	0
PA0553		hypothetical protein	2.5	0
PA0586		SpoVR family protein	2.5	3.9
PA0813		hypothetical protein	2.5	4.5
PA0854	*fumC2*	fumarate hydratase	2.5	0
PA1092	*fliC*	flagellin type B	2.5	0
PA1115		hypothetical protein	2.5	1.5
PA1118		hypothetical protein	2.5	4.1
PA1173	*napB*	cytochrome c-type protein NapB precursor	2.5	6.5
PA1174	*napA*	nitrate reductase catalytic subunit	2.5	5.3
PA1516		hypothetical protein	2.5	0
PA1976		two-component sensor	2.5	0
PA1988	*pqqD*	pyrroloquinoline quinone biosynthesis protein PqqD	2.5	2.8
PA2570	*pa1L*	PA-I galactophilic lectin	2.5	6.7
PA2896	*SbrI*	RNA polymerase sigma factor	2.5	0
PA3272		ATP-dependent DNA helicase	2.5	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3615		hypothetical protein	2.5	1.6
PA4306		hypothetical protein	2.5	6.7
PA4337		hypothetical protein	2.5	0
PA14_35970		acyl-CoA dehydrogenase	2.6	2.9
PA0176		chemotaxis transducer	2.6	4.7
PA0610	*prtN*	transcriptional regulator PrtN	2.6	0
PA0853		oxidoreductase	2.6	0
PA1355		hypothetical protein	2.6	5.8
PA1604		hypothetical protein	2.6	0
PA1967		hypothetical protein	2.6	0
PA1990		peptidase	2.6	2.5
PA2274		hypothetical protein	2.6	0
PA2304		regulatory protein	2.6	2
PA2381		hypothetical protein	2.6	0
PA2419		hydrolase	2.6	4.2
PA2895	*sbrR*	sbrR	2.6	0
PA3324		short chain dehydrogenase	2.6	1.9
PA3545	*algG*	alginate-c5-mannuronan-epimerase AlgG	2.6	2.4
PA3972		acyl-CoA dehydrogenase	2.6	0
PA4017		hypothetical protein	2.6	2.2
PA4155		oxidoreductase	2.6	0
PA4189		aldehyde dehydrogenase	2.6	0
PA5027		hypothetical protein	2.6	-2.8
PA5060	*phaF*	polyhydroxyalkanoate synthesis protein PhaF	2.6	2.9
PA14_48490		peptidylarginine deiminase	2.7	0
PA14_39880	*phzG2*	pyridoxamine 5'-phosphate oxidase	2.7	0
PA14_20060		hypothetical protein	2.7	5.2
PA0231	*pcaD*	beta-ketoadipate enol-lactone hydrolase	2.7	0
PA0490		hypothetical protein	2.7	1.8
PA1114		hypothetical protein	2.7	0
PA1190		hypothetical protein	2.7	9.9
PA1260		ABC transporter substrate-binding protein	2.7	0
PA1348		hypothetical protein	2.7	4.4
PA1362		hypothetical protein	2.7	0
PA1519		transporter	2.7	0
PA1524	*xdhA*	xanthine dehydrogenase	2.7	2.2
PA1789		hypothetical protein	2.7	1.7
PA1870		hypothetical protein	2.7	0
PA5170	*arcD*	arginine/ornithine antiporter	2.7	0
PA1523	*xdhB*	xanthine dehydrogenase	2.8	2.1
PA1537		short-chain dehydrogenase	2.8	0
PA1596	*htpG*	heat shock protein 90	2.8	-2.3

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2566		hypothetical protein	2.8	4.3
PA2701		MFS transporter	2.8	3.6
PA3032	*snr1*	cytochrome c Snr1	2.8	7.4
PA4312		hypothetical protein	2.8	2.3
PA5115		hypothetical protein	2.8	0
PA5420	*purU2*	formyltetrahydrofolate deformylase	2.8	0
PA0226		CoA transferase. subunit A	2.9	0
PA0230	*pcaB*	3-carboxy-cis.cis-muconate cycloisomerase	2.9	0
PA0587		hypothetical protein	2.9	3.9
PA0884		C4-dicarboxylate-binding periplasmic protein	2.9	22
PA1183	*dctA*	C4-dicarboxylate transporter DctA	2.9	0
PA1930		chemotaxis transducer	2.9	6.9
PA1987	*pqqC*	pyrroloquinoline quinone biosynthesis protein PqqC	2.9	2.5
PA2022		nucleotide sugar dehydrogenase	2.9	0
PA2788		chemotaxis transducer	2.9	0
PA3384	*phnC*	ABC phosphonate transporter ATP-binding protein	2.9	0
PA3718		MFS permease	2.9	0
PA3734		hypothetical protein	2.9	4.9
PA4236	*katA*	catalase	2.9	0
PA4338		hypothetical protein	2.9	1.9
PA4385	*groEL*	chaperonin GroEL	2.9	-1.8
PA4788	*phaJ3*	hypothetical protein	2.9	3.1
PA5213	*gcvP1*	glycine dehydrogenase	2.9	3.6
PA0105	*coxB*	cytochrome c oxidase subunit II	3	11
PA0444	*amaB*	allantoate amidohydrolase	3	3.1
PA0460		hypothetical protein	3	1.6
PA0516	*nirF*	heme d1 biosynthesis protein NirF	3	-7.8
PA0572		hypothetical protein	3	1.8
PA0732		hypothetical protein	3	2.4
PA1989	*pqqE*	pyrroloquinoline quinone biosynthesis protein PqqE	3	2.5
PA2416	*treA*	trehalase	3	0
PA2773		hypothetical protein	3	0
PA3236		glycine betaine-binding protein	3	2.5
PA3282		hypothetical protein	3	0
PA3383	*phnD*	phosphonate ABC transporter substrate-binding protein	3	0
PA4625		hypothetical protein	3	16
PA4879		hypothetical protein	3	1.7
PA5111	*gloA3*	lactoylglutathione lyase	3	0
PA5353	*glcF*	glycolate oxidase iron-sulfur subunit	3	25
PA14_13630		hypothetical protein	3.1	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0763	*mucA*	anti-sigma factor MucA	3.1	0
PA0788		hypothetical protein	3.1	3.6
PA0843	*plcR*	phospholipase accessory protein PlcR	3.1	0
PA1137		oxidoreductase	3.1	0
PA2071	*fusA2*	elongation factor G	3.1	3.2
PA2618		arginyl-tRNA-protein transferase	3.1	3.8
PA2883		hypothetical protein	3.1	2.1
PA3309		hypothetical protein	3.1	-1.7
PA3572		hypothetical protein	3.1	0
PA3871		peptidyl-prolyl cis-trans isomerase. PpiC-type	3.1	0
PA4214	*phzE1*	phenazine biosynthesis protein PhzE	3.1	0
PA4328		hypothetical protein - response to stress	3.1	0
PA4661	*pagL*	Lipid A 3-O-deacylase	3.1	0
PA5352		GlcG protein	3.1	24
PA5421	*fdhA*	glutathione-independent formaldehyde dehydrogenase	3.1	2.1
PA5473		hypothetical protein	3.1	1.6
PA14_16110		hypothetical protein	3.2	1.8
PA0451		membrane-bound protease	3.2	3.3
PA0514	*nirL*	heme d1 biosynthesis protein NirL	3.2	-9.8
PA0520	*nirQ*	regulatory protein NirQ	3.2	0
PA2030		hypothetical protein	3.2	20
PA2302		non-ribosomal peptide synthetase	3.2	1.7
PA2504		hypothetical protein	3.2	4.1
PA2569		hypothetical protein	3.2	2
PA3613		hypothetical protein	3.2	-2.5
PA4139		hypothetical protein	3.2	35
PA4899		aldehyde dehydrogenase	3.2	0
PA14_35950		dehydrogenase	3.3	3.1
PA0200		hypothetical protein	3.3	-2.2
PA0247	*pobA*	4-hydroxybenzoate 3-monooxygenase	3.3	3.6
PA0443		transporter	3.3	4.4
PA0512	*nirH*	hypothetical protein	3.3	-11
PA0776		hypothetical protein	3.3	8.8
PA1112		dehydrogenase	3.3	1.7
PA1290		transcriptional regulator	3.3	0
PA1985	*pqqA*	coenzyme PQQ synthesis protein PqqA	3.3	4.3
PA2024		ring-cleaving dioxygenase	3.3	4.9
PA2031		hypothetical protein	3.3	20
PA2717	*cpo*	chloroperoxidase	3.3	6.9
PA2937		hypothetical protein	3.3	5

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA3541	*alg8*	alginate biosynthesis protein Alg8	3.3	4
PA3795		oxidoreductase	3.3	0
PA4204		hypothetical protein	3.3	0
PA5400		electron transfer flavoprotein alpha subunit	3.3	3.6
PA0227		CoA transferase subunit B	3.4	0
PA0228	*pcaF*	beta-ketoadipyl CoA thiolase	3.4	0
PA0232	*pcaC*	gamma-carboxymuconolactone decarboxylase	3.4	0
PA0515		transcriptional regulator	3.4	-10
PA1211		hypothetical protein	3.4	4.2
PA1605		hypothetical protein	3.4	0
PA1673		hypothetical protein	3.4	-5.5
PA2662		hypothetical protein	3.4	2.2
PA3549	*algJ*	alginate o-acetyltransferase AlgJ	3.4	0
PA4213	*phzD1*	phenazine biosynthesis protein PhzD	3.4	0
PA5054	*hslU*	ATP-dependent protease ATP-binding subunit HslU	3.4	0
PA5398		FMN oxidoreductase	3.4	2
PA5460		hypothetical protein	3.4	18
PA0039		hypothetical protein	3.5	2.8
PA0107		cytochrome C oxidase assembly protein	3.5	12
PA0141		hypothetical protein	3.5	-2.6
PA0440		oxidoreductase	3.5	3.5
PA1557		cbb3-type cytochrome c oxidase subunit I	3.5	-5.5
PA1746		hypothetical protein	3.5	-3.8
PA2197		hypothetical protein	3.5	4.9
PA4346		hypothetical protein	3.5	0
PA4587	*ccpR*	cytochrome c551 peroxidase	3.5	-17
PA4785		acetyl-CoA acetyltransferase	3.5	2.4
PA4786	*fabG*	3-ketoacyl-ACP reductase	3.5	2
PA0038		hypothetical protein	3.6	2.2
PA0112		hypothetical protein	3.6	12
PA0779		ATP-dependent protease	3.6	0
PA1887		hypothetical protein	3.6	3.4
PA2816		hypothetical protein	3.6	2.6
PA3040		hypothetical protein	3.6	0
PA3788		hypothetical protein	3.6	2.4
PA3819		hypothetical protein	3.6	0
PA3874	*narH*	respiratory nitrate reductase beta subuni	3.6	0
PA3952		hypothetical protein	3.6	0
PA4881		hypothetical protein	3.6	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA5483	*algB*	two-component response regulator AlgB - positive regulation of transcription, DNA-templated	3.6	0
PA5484		two-component sensor	3.6	0
PA0439		dihydropyrimidine dehydrogenase	3.7	3.5
PA0737		hypothetical protein	3.7	0
PA1212		MFS transporter	3.7	3.5
PA1571		hypothetical protein	3.7	3.3
PA1901	*phzC2*	phenazine biosynthesis protein PhzC	3.7	0
PA1986	*pqqB*	pyrroloquinoline quinone biosynthesis protein PqqB	3.7	3.7
PA3023		lipid kinase	3.7	1.8
PA3392	*nosZ*	nitrous-oxide reductase	3.7	0
PA3547	*algL*	poly(beta-D-mannuronate) lyase	3.7	4.5
PA5416	*soxB*	sarcosine oxidase beta subunit	3.7	0
PA5418	*soxA*	sarcosine oxidase alpha subunit	3.7	0
PA0441	*dhT*	phenylhydantoinase	3.8	3.4
PA0511	*nirJ*	heme d1 biosynthesis protein NirJ	3.8	-13
PA0543		hypothetical protein	3.8	3
PA1414		hypothetical protein	3.8	1.6
PA1555		cytochrome c oxidase. cbb3-type subunit III	3.8	-6.3
PA5475		hypothetical protein	3.8	-2.5
PA0452		stomatin-like protein	3.9	4.1
PA1429		cation-transporting P-type ATPase	3.9	-3
PA3540	*algD*	GDP-mannose 6-dehydrogenase AlgD	3.9	8.5
PA5410		ring hydroxylating dioxygenase. alpha-subunit	3.9	2.1
PA5415	*glyA1*	serine hydroxymethyltransferase	3.9	0
PA14_28520		hypothetical protein	4	0
PA0108	*coIII*	cytochrome c oxidase subunit III	4	12
PA0588		hypothetical protein	4	3.7
PA1332		hypothetical protein	4	0
PA1408		hypothetical protein	4	2.9
PA2137		hypothetical protein	4	3.4
PA3041		hypothetical protein	4	1.6
PA3042		hypothetical protein	4	0
PA4311		hypothetical protein	4	2.9
PA4344		hydrolase	4	2.2
PA5297	*poxB*	pyruvate dehydrogenase (cytochrome)	4	0
PA5546		hypothetical protein	4	2.7
PA14_21830		hypothetical protein	4.1	0
PA0111		hypothetical protein	4.1	12
PA0113		protoheme IX farnesyltransferase	4.1	12

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA0480		hydrolase	4.1	0
PA2422		hypothetical protein	4.1	0
PA14_28610		hypothetical protein	4.2	6
PA0229	*pcaT*	dicarboxylic acid transporter PcaT	4.2	0
PA2699		hypothetical protein	4.2	3.1
PA4577		hypothetical protein	4.2	0
PA5355	*glcD*	glycolate oxidase subunit GlcD	4.2	25
PA0990		hypothetical protein	4.3	0
PA1221		AMP-binding protein	4.3	4.1
PA1597		hypothetical protein	4.3	0
PA1606		hypothetical protein	4.3	0
PA1871	*lasA*	LasA protease	4.3	2.2
PA2138	*ligD*	ATP-dependent DNA ligase - DNA repair	4.3	3.5
PA2145		hypothetical protein	4.3	3.2
PA2177		sensor/response regulator hybrid	4.3	2.7
PA2708		hypothetical protein	4.3	1.7
PA3273		hypothetical protein	4.3	0
PA3550	*algF*	alginate o-acetyltransferase AlgF	4.3	3.3
PA0242		hypothetical protein	4.4	0
PA1218		hypothetical protein	4.4	4
PA1219		hypothetical protein	4.4	4.1
PA1254		dihydrodipicolinate synthetase	4.4	0
PA1556		cbb3-type cytochrome c oxidase subunit II	4.4	-8
PA2747		hypothetical protein	4.4	2.3
PA2753		hypothetical protein	4.4	-3.3
PA3418	*ldh*	leucine dehydrogenase	4.4	5.1
PA14_30900		conjugal transfer protein TrbJ	4.5	0
PA14_72370		hypothetical protein	4.5	2.6
PA5212		hypothetical protein	4.5	0
PA14_36940		hypothetical protein	4.6	2.4
PA1242		hypothetical protein	4.6	0
PA1423		chemotaxis transducer	4.6	0
PA2268		hypothetical protein	4.6	0
PA5173	*arcC*	carbamate kinase	4.6	0
PA5481		hypothetical protein	4.6	2.4
PA14_54750		hypothetical protein	4.7	2.2
PA4738		hypothetical protein	4.7	1.8
PA4739		hypothetical protein	4.7	1.8
PA4761	*dnaK*	molecular chaperone DnaK	4.7	0
PA0060		hypothetical protein	4.8	0
PA2751		hypothetical protein	4.8	0
PA2754		hypothetical protein	4.8	0
PA4345		hypothetical protein	4.8	0

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA4352		hypothetical protein - response to stress	4.8	-3.3
PA1562	*acnA*	aconitate hydratase	4.9	2.2
PA2485		hypothetical protein	4.9	0
PA2777		formate/nitrate transporter	4.9	0
PA3875	*narG*	respiratory nitrate reductase alpha subun	4.9	0
PA14_36400		hypothetical protein	5	3.5
PA0510	*nirE*	uroporphyrin-III c-methyltransferase	5	-5.6
PA1213		clavaminic acid synthetase	5	3.9
PA2486		hypothetical protein	5	1.9
PA4542	*clpB*	clpB protein	5	0
PA0567		hypothetical protein	5.1	0
PA2931		transcriptional regulator	5.1	0
PA3369		hypothetical protein	5.1	2.3
PA3543	*algK*	alginate biosynthetic protein AlgK	5.1	4.7
PA1216		hypothetical protein	5.2	3.5
PA1220		hypothetical protein	5.2	3.9
PA1905	*phzG1*	pyrodoxamine 5'-phosphate oxidase	5.2	-2.7
PA3888		ABC transporter permease	5.2	0
PA0459		ClpA/B protease ATP binding subunit	5.3	1.9
PA1921		hypothetical protein	5.3	0
PA2046		hypothetical protein	5.3	0
PA1324		hypothetical protein	5.4	0
PA2815	*fadE*	acyl-CoA dehydrogenase	5.4	1.8
PA3459		asparagine synthetase	5.5	2.7
PA3889		ABC transporter substrate-binding protein	5.5	0
PA3891		ABC transporter ATP-binding protein	5.5	0
PA1111		hypothetical protein	5.6	0
PA1243		sensor/response regulator hybrid	5.6	0
PA3461		hypothetical protein	5.6	2.2
PA3691		lipoprotein	5.6	0
PA3890		ABC transporter permease	5.6	0
PA4217	*phzS*	hypothetical protein	5.6	-4.7
PA5172	*arcB*	ornithine carbamoyltransferase	5.6	0
PA1931		ferredoxin	5.7	6.7
PA2146		hypothetical protein	5.7	19
PA2166		hypothetical protein	5.7	3.9
PA3460		GNAT family acetyltransferase	5.7	2.2
PA4876	*osmE*	DNA-binding transcriptional activator OsmE	5.7	0
PA4877		hypothetical protein	5.7	2.1
PA14_09460	*phzC1*	phenazine biosynthesis protein PhzC	5.8	-5.9

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PA1214		sparagine synthase	5.8	3.7
PA1217		2-isopropylmalate synthase	5.8	3.8
PA1323		hypothetical protein	5.8	0
PA5354	*glcE*	glycolate oxidase FAD binding subunit	5.8	33
PA5427	*adhA*	alcohol dehydrogenase	5.8	-1.8
PA0525		dinitrification protein NorD	5.9	-6.9
PA3551	*algA*	mannose-1-phosphate guanylyltransferase	5.9	5.4
PA0355	*pfpI*	protease PfpI	6	0
PA1215		AMP-binding protein	6	3.5
PA3370		hypothetical protein	6	2.6
PA1404		hypothetical protein	6.1	2.2
PA0059	*osmC*	osmotically inducible protein OsmC / response to oxidative stress	6.2	0
PA1888		hypothetical protein	6.3	3.1
PA3371		hypothetical protein	6.3	2.7
PA2414	*sndH*	L-sorbosone dehydrogenase	6.4	0
PA2167		hypothetical protein	6.5	2.7
PA3692		outer membrane protein. OmpA	6.5	0
PA4078		nonribosomal peptide synthetase	6.7	2.4
PA5171	*arcA*	arginine deiminase	6.8	0
PA2175		hypothetical protein	6.9	0
PA2168		hypothetical protein	7	3.1
PA2189		hypothetical protein	7	3.3
PA4880		bacterioferritin	7	1.8
PA3231		hypothetical protein	7.1	3.3
PA2433		hypothetical protein	7.4	2.2
PA2143		hypothetical protein	7.5	2.4
PA2244	*pslN*	hypothetical protein	7.5	0
PA2415		hypothetical protein	7.5	2.6
PA2154		hypothetical protein	7.6	3.6
PA4171		protease	8	3.1
PA3415		branched-chain alpha-keto acid dehydrogenase subunit E2	8.1	7.5
PA2245	*pslO*	hypothetical protein	8.3	0
PA2144	*glgP*	glycogen phosphorylase	8.4	2.6
PA2933		MFS transporter	8.5	0
PA4172		exonuclease III	8.6	3
PA2021		hypothetical protein	8.7	1.9
PA2107		hypothetical protein	8.7	2.8
PA2149		hypothetical protein	8.7	2.5
PA2934		hydrolase	8.7	0
PA3416		pyruvate dehydrogenase E1 component. beta chain	8.7	6.6
PA2178		hypothetical protein	9	3.5

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA1932		hydroxylase molybdopterin-containing subunit	9.1	3.7
PA1933		hydroxylase large subunit - part of the hutUHIG operon involved in histidine catabolism	9.1	3.1
PA2176		hypothetical protein	9.1	2.4
PA2165	*glgA*	glycogen (starch) synthase	9.3	3.1
PA3126	*ibpA*	heat-shock protein IbpA	9.3	0
PA3274		hypothetical protein	9.3	3.3
PA2155		cardiolipin synthase 2	9.5	3.9
PA2162		maltooligosyl trehalose synthase	9.5	2.6
PA2164		glycosyl hydrolase	9.6	2.9
PA2108		thiamine pyrophosphate protein	9.7	3.3
PA2159		hypothetical protein	9.7	2.9
PA2187		hypothetical protein	9.7	3.6
PA2181		carboxylate-amine ligase	9.8	4.1
PA3417		pyruvate dehydrogenase E1 component subunit alpha	9.8	5.6
PA14_36900		hypothetical protein	10	6.5
PA2135		transporter	10	3.7
PA2136		hypothetical protein	10	5.1
PA2142		short-chain dehydrogenase	10	3.8
PA2151		hypothetical protein	10	2.9
PA2153	*glgB*	glycogen branching protein	10	2.5
PA2160		glycosyl hydrolase	10	2.8
PA2161		hypothetical protein	10	2.8
PA2163		4-alpha-glucanotransferase	10	2.7
PA2169		hypothetical protein	10	4.5
PA2141		ompetence-damaged protein	11	4.5
PA2148		hypothetical protein	11	3.6
PA2150		KU domain-containing protein	11	4
PA2152		trehalose synthase	11	2.7
PA2156		hypothetical protein	11	3.7
PA2157		hypothetical protein	11	3.7
PA2158		alcohol dehydrogenase	11	3.6
PA2179		hypothetical protein	11	3.8
PA2180		hypothetical protein	11	3.5
PA14_36480		hypothetical protein	12	3.1
PA2134		hypothetical protein	12	4.3
PA2171		hypothetical protein	12	4.1
PA2932	*morB*	morphinone reductase	12	0
PA14_36790		hypothetical protein	13	3.7
PA2147	*katE*	hydroperoxidase II - heme binding/response to oxidative stress	14	3.9
PA2172		hypothetical protein	14	3.7
PA2173		hypothetical protein	14	3.5

PA gene locus	Gene Name	Gene product	Peptide 1018 vs. Edge Fold Change	Swarm Centre vs. Edge Fold Change
PA2140		metallothionein	17	6.6