THE ROLE OF MULTIDRUG EFFLUX PUMPS IN THE STRESS RESPONSE OF *PSEUDOMONAS AERUGINOSA* TO ORGANIC CONTAMINATION

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ABSTRACT

Natural microbial communities are the ultimate drivers of change in any ecosystem. Through chemical contamination of natural environments, these communities are exposed to many different types of chemical stressors; however, research on whole genome responses to this contaminant stress is limited. This research examined the stress response of a common soil bacterium, *Pseudomonas aeruginosa,* to a common environmental pollutant, pentachlorophenol (PCP). In the first part of the research, it was revealed that nutrient-limited *P. aeruginosa* is able to respond to PCP with minimal physiological damage due to the upregulation of multidrug efflux pumps. Further study of this PCP-mediated induction of efflux pumps revealed a simultaneous increase in antibiotic resistance. It was discovered that the resistance nodulation-cell division (RND) efflux pump, MexAB-OprM, in particular is responsible for the PCP-induced increase in antibiotic resistance.

Both whole cell physiological indicators and whole genome analysis were used to examine the stress response of *P. aeruginosa* to PCP. Cells were grown in a chemostat at a low growth rate to simulate nutrient-limiting growth in the natural environment. Whole cell acetate uptake rates (WAUR) and viable cell counts as colony forming units (CFU) were determined as cells were exposed to increasing concentration of PCP. At the same time, changes in gene expression were examined by Affymetrix microarray technology. Results showed little change in whole-cell physiology, with no difference in WAUR and only a slight reduction in CFU. However, the microarrays revealed that over 100 genes either increased or decreased expression greater than two-fold due to the PCP exposure. In particular, multiple multidrug efflux genes were upregulated in response to the PCP. The results were validated by real time reverse transcription polymerase chain reaction (RT-PCR) for one of these genes. Further analysis of the effects of MexAB-OprM showed that this particular efflux pump is essential for the response of *P. aeruginosa* to the toxin PCP.

Induction of multidrug efflux pumps is responsible for the development of antibiotic resistance in strains of *P. aeruginosa*. Therefore, it was investigated whether PCP might induce resistance to a variety of antibiotics. The research was further extended to examine the effect of a variety of organic contaminants on MexAB-OprM efflux and antibiotic resistance development. PCP, 2,4-dinitrophenol, benzoate and Roundup® all induced antibiotic resistance. However, although MexAB-OprM is required for optimal growth in the presence of all chemicals, this particular efflux pump is only involved in increased resistance with PCP. This was confirmed using RT-PCR as *mexB* expression was induced by PCP, but not by the other three chemicals. A long term generational

study on the effects of PCP did not result in a stable antibiotic-resistant phenotype; however, RT-PCR showed that *mexB* induction is a direct result of PCP exposure and can be reversed by removal of PCP.

Together, these results demonstrate the necessity to understand functional responses to contaminant stress. Discovery of direct induction of multidrug efflux pumps and the resulting increase in antibiotic resistance has significant implications for environmental microbiology and public health. This research suggests that organic contamination may result in antibiotic resistance and that antibiotic resistant strains may have a survival advantage in contaminated environments.

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an uncharted land, or opened a new heaven to the human spirit"
- Helen Keller

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Dr. Johanna Craig assisted with the data analysis of microarray-generated gene expression in Chapter 3.

Chapter 1. EXECUTIVE SUMMARY

Introduction

Bacteria are the most abundant life forms on earth (Whitman et al. 1998). Influencing all processes required for life (Newman and Banfield 2002), healthy microbial communities are essential to the proper functioning of all ecosystems, including: sedimentation of carbon in the oceans, mineralization and weathering of trace metals and nutrients in the soils, degradation of detritus, cycling of nutrients in the environment, and maintaining the health of crop species. Bacteria are also now well-recognized as the major contributors to remediation of chemical contamination. Through their interaction and response to chemical contamination in soil and water environments, they initiate changes in the micro-scale ecosystem that drive changes seen at the macro-scale. Past research has shown that the population structure of microbial communities is dramatically affected by chemical contamination. As microbial functional changes are the ultimate drivers of ecosystem change, it is now essential to understand these changes during the microbial response to contamination.

In individual species, the functional responses to chemical stress are taking place at the molecular level. Yet, very little research has been done to determine the underlying molecular mechanisms involved in their ability to adapt to the contamination. Furthermore, the studies that have examined these responses have rarely done so under environmentally-relevant conditions (Santos et al. 2004; Dominguez-Cuevas et al. 2006; Velazquez et al. 2006). As the responses will be different depending upon the physiological status of the cells and the manner in which they are exposed to the toxin

(Veeranagouda et al. 2006), a complete understanding requires investigations that closely mimic environmental conditions.

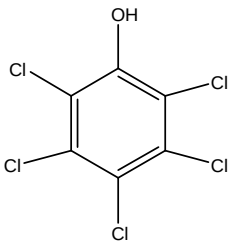
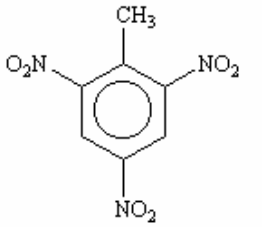
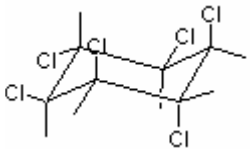
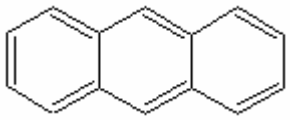
To determine how cells adapt at the cellular level to chemical stressors, the first part of this research examined the stress response of a common soil bacterium, *Pseudomonas aeruginosa*, to a common environmental contaminant, pentachlorophenol. A whole-genome approach allowed the investigation of changes in gene expression with no bias towards specific stress responses. Cells were grown using a chemostat to achieve a nutrient-limited, balanced growth state. The use of the chemostat allowed a unique look at the stress response of *P. aeruginosa* under conditions similar to those experienced in the natural environment. Results were unexpected, and showed that *P. aeruginosa* is able to adapt to the PCP not by utilizing common stress response mechanisms, but by increasing the expression of multiple multi-drug efflux pumps. As multi-drug efflux pumps are responsible for the development of antibiotic resistance in *P. aeruginosa*, the second part of this research investigated the effects of this PCP-mediated efflux response on antibiotic resistance. Exposure to PCP directly induced expression of the MexAB-OprM multi-drug efflux pump, resulting in resistance to a variety of antibiotics. The results presented here are the first to demonstrate induction of the MexAB-OprM pump by a toxic anthropogenic chemical, raising questions that have significant implications not only for chemical remediation but also for public health: does organic contamination select for antibiotic resistance in the environment? Does organic contamination selectively allow survival of antibiotic resistant strains in the natural environment?

Pentachlorophenol and *Pseudomonas aeruginosa*: model contaminant and model organism

Pentachlorophenol (CAS# 87-86-5) is a restricted-use pesticide and an industrial wood preservative. It is found at 20% of the contaminated sites listed on the National Priority List by the U.S. EPA. Toxicity of this contaminat has been studied widely on eukaryotes and to a lesser extent on prokaryotes. PCP causes oxidative stress and cytotoxicity in eukaryotes (Wang et al. 2001; Yang et al. 2005), and is a known metabolic uncoupler (Weinbach and Garbus 1965; Escher and Schwarzenbach 1996) and suspected endocrine disrupter (Weinbach and Garbus 1965; Escher and Schwarzenbach 1996; Gerhard et al. 1999). The main toxic effect of PCP is on the cell membrane (cytoplasmic in bacteria, mitochondrial in eukaryotes) where it embeds as a monomer and act as an indiscriminant proton shuttle (Escher et al. 1999). This uncouples oxidative phosphorylation, dissipates the proton motive force, and cells are unable to generate sufficient ATP for cellular metabolic processes. In *E. coli*, PCP has been shown to induce expression of proteins similar to starvation-induced stress proteins (Blom et al. 1992), and in Kocuria varians it induces changes in membrane lipid structure (Dercova et al. 2004).

P. aeruginosa is a common soil bacterium able to respond to and survive in many chemically contaminated environments. Isolates with the ability to degrade different types of organic contaminants, including PCP, have been isolated from a variety of sources (Table 1.1). As shown in the recent study examining diversity within a river, a diversity of strains are highly adaptive to survive in natural and contaminated waters (Pirnay et al. 2005). In fact, this same study revealed that the highest numbers of *P. aeruginosa* were found in the most polluted stretch of the river.

Table 1.1. Compounds degraded by *Pseudomonas aeruginosa* isolated from various environmental sources

Compound name	Structure	Environment	Reference
Pentachlorophenol		Aquifer containing wood treatment waste fluid	(Schmidt et al. 1999)
2,4,6-Trinitrotoluene	 2,4,6-Trinitrotoluene	Munitions-contaminated soil	(Oh et al. 2003)
Complex hydrocarbon mixture – gasoline, kerosene and diesel	Various hydrocarbons; 15 to 5 carbon atoms	Water in used kerosene tank	(Wongsa et al. 2004)
Hexachlorocyclohexane	 (γ-isomer)	Contaminated soil surrounding a HCH manufacturing plant	(Kumar et al. 2005)
Anthracene		Contaminated soil at a petrochemical landfarming site	(Jacques et al. 2005)
Crude oil	Various hydrocarbons, complex mixture	Contaminated soil at an oil field	(Song et al. 2006)

P. aeruginosa is also well-studied as an opportunistic pathogen. Genetic profiling of isolates from a wide variety of settings show that *P. aeruginosa* strains throughout the world display an epidemic population structure, suggesting that the same clones of *P. aeruginosa* are widespread throughout both the natural environment and clinical settings (Pirnay et al. 2002; Pirnay et al. 2005). Therefore, environmental strains are considered potential pathogens, and clinical strains survive when released to the environment. *P. aeruginosa* PAO1 is a strain isolated from a clinical setting, and due to the availability of the genome sequence, it has been widely studied at the molecular level (Stover et al. 2000). This strain is also unable to degrade PCP, which ensured that study of the stress response would not be complicated by changes due to catabolism. Due to the ubiquitous nature of *P. aeruginosa*, the large number of research studies, and a commercially available whole-genome microarray, PAO1 was chosen as the model organism for this work.

The stress response of *Pseudomonas aeruginosa* to pentachlorophenol: a transcriptome study (Chapter 3)

The objective of the first part of this research was to link whole-cell physiological changes to genetic-level changes and to identify specific genes which were highly regulated in response to PCP. These studies were undertaken in chemostat-grown *P. aeruginosa* to achieve nutrient-limited, balanced growth conditions, similar to those found in the natural environment. From the results of previous studies, it was expected that common "stress" response genes would be highly upregulated in response to the toxic chemical (Blom et al. 1992; Dominguez-Cuevas et al. 2006). Examining whole-cell changes, we determined that *P. aeruginosa* was not physiologically stressed by the

addition of the contaminant. However, this resistance was not due to the upregulation of common stress genes. Instead, results demonstrate that *P. aeruginosa* is able to respond quite readily to the toxic chemical addition by strong upregulation of multiple multi-drug efflux pumps. Further, we show that one specific efflux pump, MexAB-OprM, is required for optimal growth in the presence of PCP.

Chemical contaminant induction of antibiotic resistance (Chapter 4)

To extend findings from the first portion of this research, we sought to understand the effect of PCP-induced upregulation of MexAB-OprM on the physiology of *P. aeruginosa*. The MexAB-OprM multidrug efflux system is one of the most highly studied efflux systems of *P. aeruginosa*, because it is involved in resistance to a wide variety of antibiotics. Therefore, we hypothesized that PCP induces a MexAB-OprM-dependent increase in antibiotic resistance in *P. aeruginosa*. Broadening the research, we postulated that this efflux pump is required for optimal growth in a variety of contaminants, and that these contaminants cause a similar increase in antibiotic resistance. The results showed that MexAB-OprM is required for optimal growth in various concentrations of benzoate, catechol, 2,4-dinitrophenol, and the herbicide Roundup ®. Although these chemicals induced slight antibiotic resistance, only PCP induced clinically-relevant resistance in a MexAB-OprM dependent manner.

Hence, this research has uncovered a previously unknown functional response to the contaminant, PCP, and shown induction of MexAB-OprM by anthropogenic chemical addition for the first time. Upregulation of efflux pumps by organic contamination has significant implications for environmental microbiology: (1) organic contamination may

play a role in the development of antibiotic resistance in the environment; (2) organic contamination may select for the survival of multi-drug resistant strains in the environment.

Concluding remarks and the need for future research

The majority of research on antibiotic resistance development within the natural environment is still focused on relating the over-use of clinical and veterinary/agricultural antibiotics to the development of antibiotic resistance (Kummerer and Henninger 2003; Levy and Marshall 2004). However, this work demonstrates the importance of studying the effects of organic contamination on the development of resistant strains. As multidrug efflux is a mechanism to survive and thrive in solvent contaminated environments, any multidrug resistant mutant may have a greater chance of survival if released to these environments (Alonso et al. 2001). Further analysis of this phenomenon is crucial to understanding the development of antibiotic resistance and recognizing bacteria in the environment as potential and probable pathogens.

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Chapter 2. Literature Review: Microbial Stress Responses to Environmental Organic Chemical Contamination and the Development of Antibiotic Resistance

Bacterial stress responses have long been studied by microbiologists. Understanding these stress responses is essential to understanding bacterial survival and adaptation. Although there are specific mechanisms that bacteria employ to deal with specific stressors, there are also general mechanisms that bacteria utilize to deal with a wide range of stressors. This review focuses on bacteria in natural water or soil environments: environmental conditions contributing to the stress response; stress responses to organic contamination that are commonly stimulated by other specific stressors; and stress responses that are unique to organic contamination. It is the latter, more unique responses that lead to the possibility of increasing antibiotic resistance due to organic contamination.

Environmental growth conditions induce stress-resistant physiology

The ability of bacteria to respond to any stressor depends upon the physiological state of the organism (Mason et al. 1999). In the natural environment, bacteria are, for the majority of their life cycle, in a physiological state of nutrient-limited growth (Morita 1997). Within an uncontaminated soil, sediment, or aqueous environment, the nutrients limiting growth can be any essential nutrient; however, low bioavailable carbon, nitrogen or phosphorus are usually the primary limiting nutrients (Morita 1993; van Elsas and Overbeek 1993). Due to both this nutrient limitation, and other environmental factors, the average generation time can vary widely: from 20 hours in a nutrient-rich environment (such as an intestinal tract), to 20 days in soil or 210 days in seawater

(Matin et al. 1989). Although these generation times vary, they are all within the range of growth which physiologists considered "starved" (Ferenci 2001).

With a few exceptions, the majority of research to date studying starvation in bacteria is done during the stationary phase of growth. Stationary phase is the period during batch growth that is characterized by a rapid decrease in growth as cells become starved for an essential nutrient. During this phase bacteria have an unbalanced macromolecular composition from the rapidly changing conditions, which is drastically different from the nutrient limitation experienced in the environment (Tempest and Neijssel 1978). In natural soils and waters, bacteria are commonly limited by the slow hydrolysis of particulate and colloidal organic carbon (Morita 1993). In contrast to stationary phase this slow, constant release of nutrients can be simulated by a low dilution rate chemostat; therefore, many researchers describe the chemostat to more accurately describe the physiological conditions experienced by bacteria in the environment (Matin 1979; Roszak and Colwell 1987; Kovárová-Kovar and Egli 1998; Whiteley et al. 2001; Esteve-Nunez et al. 2005). With the chemostat, the growth rate of the culture is held constant, allowing the cells to reach a nutrient-limited balanced growth state, or a steady-state macromolecular composition equilibrium (Tempest and Neijssel 1978).

Physiological changes studied in nutrient-limited chemostats and natural soils and waters show that there are distinct changes in bacterial structure and macromolecular composition. With this physiological state, bacteria become smaller, and their membrane structure changes. In fact, some researchers refer to the "normal" morphology in the environment as "ultramicrocells" (Roszak and Colwell 1987; van Elsas and Overbeek

1993). Decreases in respiring cell counts and in culturability are also seen, although the number of bacteria may remain unchanged (Morita 1997). Bacteria switch to endogenous metabolism (using internal energy sources from the breakdown of materials such as RNA and proteins), have been shown to uptake a variety of substrates more quickly, and will utilize different carbon compounds simultaneously (Lendenmann et al. 1996; Kovárová-Kovar and Egli 1998).

Many studies of cellular changes induced by starvation and nutrient limiting-conditions also show that bacteria are more stress-resistant in this state than under conditions of active, exponential growth (Hengge-Aronis 2000). These changes were identified by studying the transition between the exponential phase and stationary phase of batch growth, and revealed that the sigma factor, RpoS (σ^s), plays a major role in the stress response. Although first discovered in *Escherichia coli*, this regulator is also found in many other bacterial species (Ramos-González and Molin 1998; Elias et al. 2000; Kojic and Ventrui 2001; Nunez et al. 2006). The increase in σ^s during stationary phase affects the expression of greater than 100 genes both positively and negatively (Hengge-Aronis 1996; Van Dyk et al. 1998; Patten et al. 2004; Schuster et al. 2004; Nunez et al. 2006). Genes that are known to be upregulated due to this general stress response are involved in various other specific stress response mechanisms: oxidative stress, heat shock, osmotic shock, acidic pH, and ethanol stress (Hengge-Aronis 1996; Hengge-Aronis 2000; Hengge-Aronis 2002; Schuster et al. 2004). Due to these changes, bacteria are not only protected against starvation stress, but they also have increased resistance to a variety of other potentially lethal stressors.

Stress response mechanisms to environmental organic contaminants

In an uncontaminated environment, bacteria are subject to numerous stressors such as nutrient deprivation, pH changes, osmotic pressure, desiccation, temperature fluctuations, and limited oxygen availability (Paul and Clark 1996). Organic chemical contamination adds an entirely different set of stressors on bacterial communities, and currently it is unclear how bacteria regulate their response to these stresses at the molecular level. It is clear, however, that chemical stressors trigger distinct physiological changes in bacteria (Blom et al. 1992; Lupi et al. 1995; Mirpuri et al. 1997; Ferguson et al. 1998; Carmel-Harel and Storz 2000; Cho et al. 2000). Recent studies utilizing proteomics and genomics have shown that even in bacteria capable of degradation of a toxic chemical, the primary functional response is due to the effects of the chemical as a stressor (Santos et al. 2004; Dominguez-Cuevas et al. 2006; Velazquez et al. 2006).

Organic contaminant stress responses common to "natural" environmental stressors

Organic chemical contaminant stress can lead to responses that are also commonly stimulated by a variety of natural stresses. For example, characteristic oxidative stress response mechanisms have been shown to be induced by polychlorinated biphenyls (PCBs) (Chavez et al. 2006), phenol (Santos et al. 2004) and toluene (Dominguez-Cuevas et al. 2006). In addition to the oxidative stress response, both phenol and toluene exhibit stress responses similar to the general carbon-starvation stress response and upregulate genes involved in heat shock, fatty acid and membrane biosynthesis and transport of small molecules (Santos et al. 2004; Dominguez-Cuevas et al. 2006). Oxidative and electrophilic stressors cause an increase in glutathione-S-tranferase levels and glutathione conjugation (Carmel-Harel and Storz 2000; Riccillo et al. 2000; Bott and Love 2002;

Santos et al. 2002). In studies investigating protein levels, organic contaminants have resulted in protein profiles both similar to other specific stress responses and yet unique to the contaminant stress response (Blom et al. 1992; Keith and Bender 1999; Cho et al. 2000). Independent studies have shown that some protein changes due to contaminant stress are the same as the RpoS-regulated carbon starvation response. This response is seen even at levels of contaminant that are not inhibitory to the growth rate or are even growth-supporting (Blom et al. 1992).

Stress responses specific to organic contaminants. Hydrophobic organic chemicals induce stress responses that are targeted towards the cell membrane. The cell membrane is the target of toxicity due to the lipophilicity of the compounds, as compounds with a higher log K_{ow} (octanol:water partition coefficient), between 1-5, have an increased toxicity towards the membrane. Bacteria have multiple ways to deal specifically with these compounds: changes in cytoplasmic membrane structure, changes in outer membrane structure, and active efflux of the contaminant.

Organic solvent contamination often results in changes in cytoplasmic membrane structure through changes in lipid composition, by both biosynthesis and by immediate *cis*-to-*trans* isomerizations (Heipieper et al. 1992; Ramos et al. 1997; Norman et al. 2002). Earlier studies on the solvent-tolerant *Pseudomonas putida* DOT-T1 show that this organism uses both molecular-level mechanisms to create a more rigid membrane in response to various solvent stressors (Ramos et al. 1997). The contribution of each response, however, depended upon the log K_{ow} of each solvent. The short term response, happening within minutes, is the *cis*-to-*trans* isomerization of fatty acids in the phospholipid membrane. The long term response involves both changes in fatty acid

composition and changes in the polar head groups associated with the phospholipids. Specifically, phosphatidylethanolamine decreases and cardiolipin increases. The lower the K_{ow} (the more polar the compound), the greater these changes in polar head groups are. Overall the effect is to create a more rigid membrane to inhibit solvent influx into the cell.

Solvent exposure has also led to changes in the porin composition within the outer membrane of Pseudomonads (Kieboom and de Bont 2001; Dominguez-Cuevas et al. 2006). Outer membrane porins allow the passage of different chemicals through the outer membrane. *Pseudomonas* have different types of porins: general, which allow compounds through based on size, and specific which allow passage of classes of chemicals, such as amino acids, sugars, and small aromatics (Hancock and Brinkman 2002; Tamber et al. 2006). Changes in porin composition in *P. putida* result in changes in solvent tolerance. The loss of the peptidoglycan-associated lipoprotein OprL resulted in an inability to grow with toluene, although the degradative capacity was still functional (Ramos et al. 1997). In a recent genome-wide study three specific (OprD-type) porins were differentially regulated in response to toluene stress (Dominguez-Cuevas et al. 2006).

Efflux of contaminants outside the cell is an active response to organic contamination, and usually couples efflux of a compound with influx of sodium, protons, or the hydrolysis of ATP (Nikaido 1996). In multiple species of Pseudomonads and in *E. coli*, it has been shown that efflux pumps are required for growth in and tolerance of organic solvents and polycyclic aromatic hydrocarbons (Kieboom et al. 1998; Bugg et al. 2000; Tsukagoshi and Aono 2000; Rojas et al. 2001). These efflux pumps are all within

the resistance-nodulation-cell-division (RND) family of multi-drug efflux pumps, and recently Meguro et al. were able to describe a diverse group of these transporters in petroleum-contaminated soil (Meguro et al. 2005). Therefore, it appears that this efflux mechanism is a common way for bacteria to avoid the toxic effects of organic chemical contamination.

Response to organic contaminant stress and increasing antibiotic resistance

Antibiotic resistance is a pressing concern throughout the world community (Williams 2002). Concern is growing particularly with respect to *P. aeruginosa*, which is considered an emerging pathogen due to its extremely rapid development of resistance to antibiotics (Sharma et al. 2003). As the recent examination of diversity of *P. aeruginosa* shows, most strains are widespread in both the environment and clinical settings, indicating that the pathogenic strains are derived from environmental strains (Pirnay et al. 2005). Therefore, it is extremely important to understand the effects of organic contamination on adaptation of *P. aeruginosa*, especially with multi-drug resistant strains. Antibiotic resistance in *P. aeruginosa* can occur by a variety of mechanisms. Two of these mechanisms are common to the bacterial response to organic contaminants: active efflux and changing outer membrane porin composition. Pump systems that efflux solvents have also been shown to efflux antibiotics; however, to date there are no quantitative studies that link the development of antibiotic resistance with solvent tolerance (Fernandes et al. 2003). Studies on solvent-induced porin changes and resulting antibiotic resistance are completely lacking, as no study to date has examined this potential link.

Outer membrane compositional changes and antibiotic resistance. Changes in the outer membrane porin composition are known to cause increased antibiotic resistance in *P. aeruginosa* (Hancock and Brinkman 2002; Kumar and Schweizer 2005). The elimination of the general porin, OprF, in clinical isolates has been shown to contribute marginally to antibiotic resistance (Woodruff and Hancock 1988). However, decreases in the specific porins, OprD and OprE, contribute substantially to resistance of imipenem and meropenem, and quinolones and cephalosporin, respectively, antibiotics all commonly used to treat *P. aeruginosa* infections (Yamano et al. 1990; Ochs et al. 1999, Bodmann 2005). Although the effect of organic solvents on porin levels in *P. aeruginosa* has not yet been investigated, the research cited above with *P. putida* implies that changes may also occur in *P. aeruginosa*. Research *in P. aeruginosa* does show that exposure to the aromatic compound benzoate results in reduced OprD levels and an increased resistance to imipenem (Ochs et al. 1999). At present, the mechanism of OprD reduction is unknown, but this limited research suggests that organic contamination can induce porin-mediated antibiotic resistance.

Multidrug efflux pumps. The RND family of efflux pumps is part of a larger division of efflux mechanisms which result in multidrug resistance of bacteria. The importance of *P. aeruginosa* as an opportunistic pathogen is due in part to its relatively large number of putative RND pumps within the genome. It has twelve potential RND pumps, seven of which have been well-characterized (Poole 2004). These seven characterized efflux pumps are both constitutively expressed and inducible, and can render *P. aeruginosa* resistant to a wide variety of antibiotics and antimicrobials. RND pumps consist of three

proteins whose encoding genes are usually found as an operon: a membrane efflux transporter (ET) which spans the cytoplasmic membrane but has large loops extending into the periplasm, an outer membrane protein (OMP) which spans the outer membrane but also extends into the periplasm, and a membrane fusion protein (MFP) that seems to link the outer membrane protein and the efflux transporter in the periplasm (Figure 2.1) (Higgins et al. 2004; Elkins and Beenken 2005). It is thought that the substrate recognition site(s) lies within the efflux transporter, at sites both within the cytoplasmic membrane and the periplasmic loops (Middlemiss and Poole 2004). Recognition sites in both areas is useful for effluxing compounds found both within the cytoplasmic membrane and periplasm, thereby eliminating some toxins before they even reach the site of damage.

Only one RND pump in *P. aeruginosa*, MexAB-OprM, has been shown to efflux organic solvents (Li et al. 1998). This pump is considered a constitutive pump that is only induced to levels above basal expression under conditions of iron starvation (Poole et al. 1993; Morita et al. 2001). Growth in the organic solvents hexane and p-xylene results in a stable mutation of *mexR*, the gene coding for the transcriptional repressor, MexR (Li et al. 1998; Li and Poole 1999). These mutations then lead to increased levels of MexAB-OprM through derepression and therefore, increased antibiotic resistance. The same mutations are also readily found in clinical isolates of *P. aeruginosa* strains

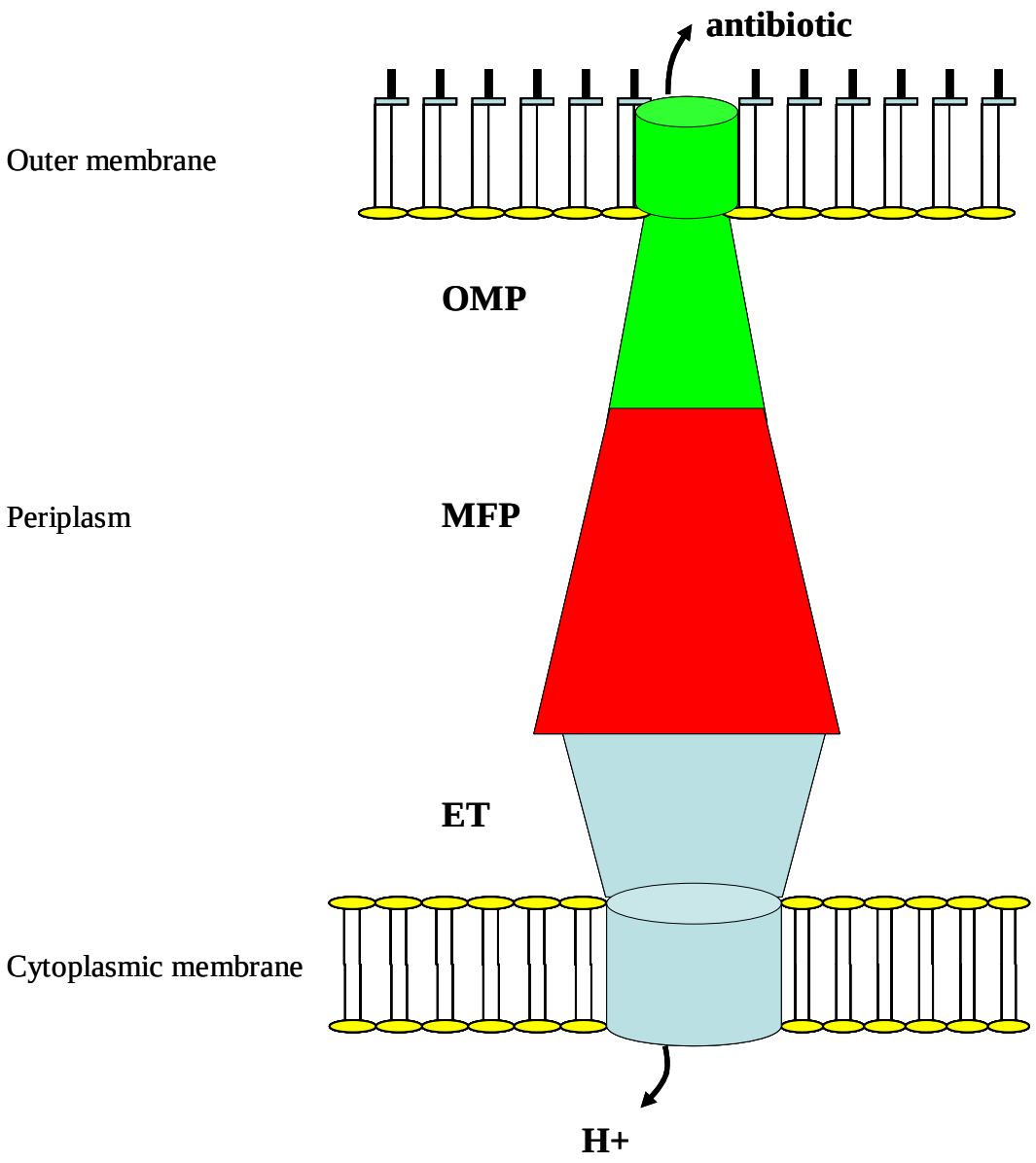

Figure 2.1. A depiction of the current model of RND efflux pumps in Gram negative bacteria. OMP, outer membrane protein, MFP, membrane fusion protein, ET, efflux transporter. Compounds are recognized by the ET and conformational changes in MFP and OMP allow extrusion outside the cell. Most RND pumps are antiporters, coupling proton influx into the cytoplasm with antibiotic or drug efflux.

that have been exposed to treatments of antibiotics. Furthermore, antibiotic treatment leads to a mutation in PA3721, a transcriptional repressor of the operon PA3719-PA3720 (Cao et al. 2004). Although there is no known homology in any genomic database, PA3719 is known to positively regulate expression of MexAB-OprM. Interestingly, no antibiotic or organic contaminant has been shown to increase expression of MexAB-OprM in *P. aeruginosa* directly, without a mutation in a transcription factor.

Direct induction of efflux pumps by organic solvents has been shown in *P. putida*, and certain pumps have been shown to efflux antibiotics as well as organic solvents. Two RND pumps, TtgABC and TtgGHI, cause increased antibiotic resistance if induced by prior treatment of toluene or xylene in *P. putida* DOT-T1E. However, they are not induced by the antibiotic itself (Rojas et al. 2001). Genes coding for TtgABC are also induced by toluene in *P. putida* KT2440, along with genes coding for an unidentified RND efflux system (Dominguez-Cuevas et al. 2006). Other efflux pumps in *P. putida* S12 are specific only for the solvents, and are not involved in antibiotic resistance (Isken and De Bont 2000).

A comparison of the known solvent-induced pumps of *P. putida* with the genome of *P. aeruginosa* might reveal insights into the potential for RND pump induction by organic contaminants in *P. aeruginosa* (Table 2.1) (Winsor et al. 2005). Table 2.1 is the result of a Blast-P search of *P. aeruginosa* using *P. putida* protein sequences. The pump components listed are those in the *P. aeruginosa* genome that showed the highest homology with the *P. putida* component. Interestingly, Table 2.1 shows that although the described *P. putida* pumps are different from each other (with the exception of TtgGHI and SprABC which, at 98% homology, may be the same pump), each is most similar

Table 2.1. Homology[a] of known solvent-inducible *P. putida* RND components to *P. aeruginosa*.

		***P. putida* components**				***P. aeruginosa* components** (% identity)				
Compound(s) inducing efflux	Strain[b]	MFP	ET	OMP	Antibiotic resistance	MFP	ET	OMP	Antibiotic resistance	References
toluene, propylbenzene ethylbenzene m-xylene styrene	*P. putida* DOT-T1E, *P. putida*KT2440	TtgA	TtgB	TtgC	carbenicillin, naladixic acid, chloramphenicol, tetracycline, ampicillin	MexA (65%)	MexB (75%)	OprM (65%)	B-lactams, fluoroquinolones, chloramphenicol, tetracycline, triclosan, p-xylene, hexane, ethidium bromide, SDS, crystal violet, sulphonamides and other various antimicrobials.	(Rojas et al. 2001; Poole 2004; Dominguez-Cuevas et al. 2006)
Same as previous	*P. putida* DOT-T1E (AF299253)	TtgG	TtgH	TtgI	carbencillin, tetetracycline, ampicillin	MexA (58%)	MexB (64%)	OprM (59%)		
toluene styrene	*P. putida* DOT-T1E	TtgD	TtgE	TtgF	None	MexA (56%)	MexB (63%)	OprM (60%)		
aromatic solvents aliphatic solvents alcohols	*P. putida* S12 (AF029405)	SrpA	SrpB	SrpC	None	MexA (58%)	MexB (65%)	OprM (60%)		(Kieboom et al. 1998)
toluene o-xylene	*P. putida* KT2440	PP 1516	PP 1517	--	Not tested	MexJ (59%)	MexK (78%)	--	triclosan, erythromycin, tetracyline, ciprofloxacin	(Poole 2004; Dominguez-Cuevas et al. 2006)

[a]Blast-P search was done to determine homology as percent identity. The components reported for *P. aeruginosa* showed the highest homology to input *P. putida* sequence.

[b]The strains used are as given. For KT2440, the Blast-P search was done using sequence in PseudoCAP (Winsor et al. 2005). For other strains, the sequence of the given GenBank accession number was used for Blast-P search through NCBI (Altschul et al. 1997).

to the MexAB-OprM pump in *P. aeruginosa*. The components of the final, un-described putative RND efflux pump, PP1516-PP1517, are most closely related to the MexJK efflux system of *P. aeruginosa*. For this pump in both organisms, the MFP and ET-encoding genes are not found next to an OMP-encoding gene. This may give the pump the flexibility of using multiple OMPs depending upon the substrate effluxed (Chuanchuen et al. 2005). This comparison of RND proteins suggests that the MexAB-OprM and MexJK pumps of *P. aeruginosa* may be among those induced by organic contamination. An understanding of chemicals or chemical classes that induce expression of these multidrug efflux pumps will greatly increase understanding of the development of antibacterial resistance in this opportunistic pathogen.

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Chapter 3. TRANSCRIPTOME ANALYSIS REVEALS THAT MULTIDRUG EFFLUX GENES ARE UPREGULATED TO PROTECT *PSEUDOMONAS AERUGINOSA* FROM PENTACHLOROPHENOL STRESS

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ABSTRACT

Through chemical contamination of natural environments, microbial communities are exposed to many different types of chemical stressors; however, research on whole genome responses to this contaminant stress is limited. This study examined the transcriptome response of a common soil bacterium, *Pseudomonas aeruginosa*, to the common environmental contaminant pentachlorophenol (PCP). Cells were grown in chemostats at a low growth rate to obtain substrate-limited, steady-state, balanced-growth conditions. The PCP stress was administered as a continuous increase in concentration, and samples taken over time were examined for physiological function changes with whole cell acetate uptake rates (WAUR) and cell viability, and for gene expression changes using Affymetrix GeneChip technology and RT-PCR. Cell viability, measured by heterotrophic plate counts, showed a moderately steady decrease after exposure to the stressor, but WAURs did not change in response to PCP. In contrast to the physiological data, the microarray data showed significant changes in the expression of several genes. In particular, genes coding for multi-drug efflux pumps, including MexAB-OprM, were strongly upregulated. The upregulation of these efflux pumps protected the cells from the potentially toxic effects of PCP, allowing the physiological whole-cell function to remain constant.

INTRODUCTION

Functional, genetic-level responses of bacteria to chemical stress drive ecosystem-level changes in both structure and function within contaminated environments. Therefore, the focus of much research has shifted to understanding the relationship between microbial community structure and bacterial function (Gray and Head 2001; Jaspers and Overmann 2004; Yu and Chu 2005). As a result, there are a few studies that have identified specific functional stress responses to chemical contamination within a mixed community, such as heat shock protein induction (Bott and Love 2001) and upregulation of various cellular transporters (Meguro et al. 2005). Although these studies give valuable information, they are limited in scope by the specific responses chosen for analysis. Recently, more comprehensive approaches such as genomics and proteomics have contributed greatly to understanding chemical stress responses.

The pseudomonads are species of microorganisms that are found in many different contaminated environments, and are known to adapt well to chemical contamination. Recent studies have examined mechanisms responsible for the adaptive ability of *Pseudomonas putida*, a common soil organism that is able to degrade a range of organic contaminants (Santos et al. 2004; Dominguez-Cuevas et al. 2006; Velazquez et al. 2006). These studies, looking at proteome and transcriptome responses to aromatic hydrocarbons easily degraded by *P. putida*, showed a trade-off in regulation between stress responses and metabolic capabilities. In each of these cases, during exponential growth, multiple stress genes were upregulated prior to or in addition to degradation genes.

Pseudomonas aeruginosa is also a soil organism that is ubiquitous throughout the environment, and, like *P. putida*, is able to survive in many different chemically-contaminated ecosystems (Oh et al. 2003; Wongsa et al. 2004; Jacques et al. 2005; Kumar et al. 2005). Unlike *P. putida*, it is an opportunistic pathogen, with an epidemic population structure (Pirnay et al. 2002). For this reason, the effects of environmental contamination on functional responses of this bacterium are of great interest. The stress response of bacteria depends upon physiological growth conditions; therefore it was important to study the response under conditions most closely resembling the natural environment. In the environment, bacteria are often found in low-nutrient, low-growth rate conditions. Although the stationary phase of batch growth has been used in the past to simulate "starvation" conditions, this unbalanced growth phase is most likely not similar to growth experienced in the natural environment (Roszak and Colwell 1987; Egli 1995). The chemostat more closely simulates growth in the natural environment by achieving balanced-growth, low growth-rate conditions.

The objectives of this study were to investigate expression changes over the entire genome as cells were exposed to PCP and to correlate these changes with whole-cell physiological changes. Affymetrix GeneChips were used to study gene expression changes in *P. aeruginosa* as it responded to pentachlorophenol (PCP). PCP was chosen as the chemical of study due to its widespread contamination and recalcitrance in the environment. A chemostat was used to achieve substrate-limited conditions, and PCP was added to mimic a groundwater contaminant plume. With many transcriptome studies published to date, this is the first to examine the stress response to contamination under ecologically-relevant conditions.

MATERIALS AND METHODS

Chemicals. All chemicals used for media preparation were laboratory grade or higher (Fisher Scientific, Atlanta, GA). Technical grade PCP was purchased from Sigma (Sigma-Aldrich, Inc., St. Louis, MO). Hexane solvent for PCP analysis was analytical grade quality (Fisher Scientific).

Strains and batch culture conditions. Wild type *Pseudomonas aeruginosa* PAO1 (WT1) and an RpoS-deficient mutant ($RpoS^-$), obtained from E. P. Greenberg (Schuster et al. 2004), were used in chemostat experiments and minimum inhibitory concentration (MIC) assays with PCP. A second WT strain of PAO1 (WT2) and the MexAB-OprM mutant (PAO200) of this strain were obtained from H. Schweizer for batch growth experiments (Schweizer 1998). Batch cultures were grown at 37^{o}C in a minimal medium (MM) consisting of the following: (in g/L) KH_2PO_4 (3.4), K_2HPO_4 (4.35), NH_4Cl (1.0), CH_3COONa (1.65); (in mg/L) EDTA (186), $MgSO_4 \cdot 7H_2O$ (150), $MnSO_4 \cdot 4H_2O$ (4.5), $NaMoO_4 \cdot 2H_2O$ (0.5), H_3BO_3 (0.15), $CaCl_2$ (20), $ZnCl_2$ (1.5), $CuCl_2 \cdot 2H_2O$ (0.5), $CoCl_2 \cdot 6H_2O$ (1.5), $FeCl_2 \cdot 4H_2O$ (11). The final pH of this medium was 6.9. Cultures were grown in 250 mL Erlenmeyer flasks in a volume of 100 mL. The chemostat growth conditions are described below.

Determining minimum inhibitory concentration (MIC) of PCP. Cells were grown in MM overnight at 37^{o}C, and diluted to 10^6 cells/mL in LB broth. PCP in LB broth was 2-fold serially diluted in 96-well polystyrene plates (Costar, Corning, NY) to a final volume of 50 μL. LB was used in place of MM to be consistent with MIC assays for chemicals

reported in the literature. Fifty μL of diluted cells were added to the wells containing the PCP dilution series, and the plate was incubated at 37°C. After 18 hours, growth in the wells was recorded qualitatively by eye and confirmed by measuring absorbance (600 nm) using a microplate reader. The MIC was the lowest concentration of PCP that did not permit growth.

Chemostat growth startup and conditions. Chemostat cultures were grown in a 5 L vessel, with a 4.8 L working volume, stirring at 300 rpm (B. Braun Biotech International, Allentown, PA). Starting cultures for each chemostat were taken from frozen stocks that had been prepared by sequentially transferring batch-grown cultures (MM medium, 20°C) every day for 7 days. These stocks were prepared to ensure the physiological state of the cultures were adapted to the MM and 20°C growth conditions. The frozen culture was plated out, a single colony transferred to MM batch medium, and grown at 20°C for two consecutive transfers. One hundred mL of batch culture was inoculated into the 4.8 L volume of chemostat MM medium. The chemostat growth medium contained one half the concentration of nutrients used in the batch culture without EDTA in order to maintain a yield of approximately 2×10^8 cells/mL, because greater cell numbers caused problems with foaming and biofilm growth in the reactor. The culture was allowed to grow to 80% of maximum optical density in batch growth before the pumps were turned on and chemostat growth was started. MM was fed to the reactor using a peristaltic pump set at 0.29 +/-0.01 L/hour, which kept the dilution rate (and therefore the growth rate) at 0.06 hr^{-1} and the generation time at 11.5 hours. Effluent was controlled by a peristaltic pump connected to a Y-tube within the reactor, so that the effluent was only pumped out when the volume in the reactor was at or above the 4.8 L volume. The MM

was acidified (20 mM H_2SO_4) to keep metals in solution; therefore, pH was monitored using an autoclaveable pH probe coupled with a pH-controller (05997-20, Cole-Parmer Instrument Company, Vernon Hills, IL) linked to a peristaltic pump for addition of NaOH to maintain the pH between 6.8 and 6.9. Oxygen levels were maintained at approximately 6 mg/L using a controlled low flow of pure oxygen. Dissolved oxygen levels in the chemostat were monitored daily with an external D.O. probe (YSI 5905 BOD Probe, YSI Model 58 Dissolved Oxygen Meter, Yellow Springs Instrument Co., Yellow Springs, OH).

PCP stress conditions in chemostat cultures. PCP was added to the reactor as a continuous, slow increase in concentration, as might be experienced by organisms being exposed to a moving contaminated plume. PCP stock solution (feed) was made to a final concentration of 3.5 g/L in deionized sterile water and solubilized by the addition of 36 mM NaOH. PCP was added to the chemostat using a separate peristaltic pump at a flow rate of 4.75 mL/hour. Viton and Tygon tubing were used for the PCP feed to minimize adsorption to the tubing walls. Samples were collected to measure the chemostat PCP concentrations over time as explained below.

Sample timing was designed to capture significant changes in expression as a result of the changing PCP concentrations. In order to determine sample timing, preliminary experiments examined the timing of catechol-1,2-dioxygenase (C12O) induction when the sole carbon source was switched from acetate to an equal concentration of benzoate to induce C120. C120 activity was determined from cell free extracts of samples removed from the reactor over time. Briefly, 75 mL samples were removed from the chemostat, and cells were pelleted, washed and resuspended in 10 mL

of cold 0.01 M sodium phosphate buffer (pH 7.0, 4^{o}C). The final suspension was placed on ice and lysed with six rounds of 30 second sonication (40% output) using a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, CT). Cell free extracts were collected as supernatant of centrifuged lysed cells (37,500 x g, 10 minutes, 4^{o}C). These extracts were immediately tested for C120 activity using a previously described assay (Nakazawa and Nakazawa 1970). Protein levels were determined using the Pierce BCA assay kit (Pierce Biotechnology, Inc., Rockford, IL). Timepoints of 6.5, 13 and 26 hours after addition of benzoate captured 50-fold, 500-fold and 1,000-fold increases in C12O expression levels over the background.

To choose effective PCP concentrations, preliminary short-term (10 minute) batch experiments were performed on chemostat-grown cells to determine the effect of PCP on respiratory inhibition as previously described (Bott and Love 2002). Using this data, the chemostat experiments were designed to target 15, 30 and 40 mg/L PCP at timepoints 6.5, 13 and 26 hours, respectively. These concentrations, which correspond to approximately 10, 25 and 30% respiration inhibition, reflect sublethal chemical stress conditions.

Samples were taken from the reactor to measure the stress response just prior to PCP addition, and 6.5, 13 and 26 hours after PCP addition was started. Sterile samples were removed from the reactor for RNA isolation, cell counts, whole cell acetate uptake rate (WAUR), and PCP concentration. The WT1 chemostat experiment was performed four times with RNA from two experiments used for microarray studies, and RNA from the other two experiments used for real time polymearase chain reaction (RT-PCR)

analysis. The RpoS$^-$ mutant chemostat experiment was run in triplicate, with samples from two experiments used for RT-PCR and one for microarray analysis.

Physiological stress analysis. The physiological state of cells was measured using optical density, cell counts and substrate uptake rates. Optical density was measured at 600 nm (OD_{600}); heterotrophic plate counts (HPCs) and direct microscopic counts were done to determine cell number. The HPCs were determined at each timepoint using duplicate dilution series to 10^{-5} in 0.01 M potassium phosphate buffer and triplicate platings of 50 µL on LB agar for each series. HPCs are reported as viable cell concentrations. Substrate uptake rates were determined by removing samples from the chemostat and performing short-term batch experiments in triplicate. Briefly, cells were taken from the chemostat and immediately mixed with 200 mg/L chloramphenicol to inhibit protein synthesis (Vazquez 1974). The sample was split into 3 sterile flasks, and 100 mg/L acetate was added. Every 15 minutes, a sub-sample was taken from each flask, filtered into a sterile sample vial, and the filtrate was analyzed for acetate concentrations. A DX-120 ion chromatograph with an AS9-HC IonPac 4x250 mm column and an AG9-HC guard column was used to measure the acetate (Dionex Corporation, Sunnyvale, CA). The eluent consisted of 12 mM Na_2CO_3 and 5 mM $NaHCO_3$, at a flow rate of 1.0 mL/minute. By this method, the linear range of acetate detection was between 25 and 150 mg/L acetate. Duplicate acetate measurements were taken for technical replication. The uptake rate was determined by the average of the triplicate biological samples and normalized to viable cell number to determine the WAUR. Initial experiments determined that steady state, based on optical density and WAUR, was reached in the chemostat after approximately 70 hours and remained constant until at least 120 hours.

The initial, unstressed timepoint of all experiments was taken at approximately 80 hours of chemostat growth in all experiments. To determine significant changes in cell viability and WAUR, t-tests were performed. When comparing timepoints within an experiment and within a strain, a 2-sided, paired t-test was performed. When comparing between the WT1 and RpoS$^-$ mutant experiments, a 2-sided, unpaired t-test (student's t-test) was performed. Stated statistical significance indicates a p-value of less than 0.05.

Gene expression analysis. Cells were removed from the chemostat in two aliquots of 75 mL and immediately centrifuged at 4^{o}C, 37,500 x g, for 10 minutes. The large volume was necessary to obtain sufficient quantities of RNA. Each pellet was resuspended in 1 mL of a mixture of 0.5 mM phosphate buffer and RNAprotect Bacteria Reagent (Qiagen, Valencia, CA) as directed by the supplier. After the cells were pelleted a second time, all supernatant was removed and pellets were stored at -50^{o}C until they were analyzed. For extraction, the cells were thawed on ice and RNA was extracted using the RNeasy Kit (Qiagen), with on-column DNase-digestion. For cell lysis, 1 mg/mL of lysozyme was incubated with the cells for 10 minutes at room temperature. Following the addition of Buffer RLT, an additional mechanical homogenization with a sterile syringe and 23-gauge needle was performed to ensure sufficient lysis. RNA was used for either microarray analysis or RT-PCR.

Microarray analysis was done using Affymetrix *P. aeruginosa* GeneChips (Affymetrix, Santa Clara, CA) at the Virginia Bioinformatics Institute Core Laboratory Facility (Virginia Tech, Blacksburg, VA). RNA quality, quantity and DNA contamination were determined using an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). cDNA preparation and hybridization were carried out as

described in the Affymetrix GeneChip Expression Analysis manual for *P. aeruginosa* RNA samples. The GeneChip Operating System (GCOS) was used for control of hybridization and scanning of fluorescent intensities. Intensities of hybridized RNA at each timepoint were globally scaled to 100. This number was chosen after examining the average intensity of each chip throughout an experiment. For the *P. aeruginosa* array, 13 probe sets were used to determine expression of one gene. GeneSpring software (Agilent Technologies) was used to organize the data and find genes whose expression was changing due to the PCP exposure. Fold-change was then determined using the Comparison Analysis function of GCOS, with the pre-PCP (time 0) RNA sample as the baseline GeneChip. GCOS directly compares results between two GeneChips for each of the 13 probe sets and performs a signed rank analysis to determine change in expression. Changes are positive (increasing) if $p \leq 0.0045$ and negative (decreasing) if $p \geq 0.9955$. We report here average 2-fold or greater increases or decreases at any timepoint compared to time zero.

RT-PCR was used to validate microarray results of the *mexAB-oprM* operon using *mexB*. Two housekeeping genes were used as controls, *proC* (Sayli et al. 2003) and *nadB* (unpublished results, Ann M. Stevens), and expression levels were normalized to each control. cDNA was made from 1 μg of total RNA using random hexamer primers and SuperScript II Reverse Transciptase (Invitrogen Life Technologies, Carlsbad, CA). The resulting cDNA was diluted 1:10 and used as template in RT-PCR with SYBR Green detection using a BioRad iCycle iQ Real-Time PCR Detection System (BioRad, Hercules, CA). The starting quantity of template for each sample was determined using a six point standard curve. Melting curves were analyzed at the end of each PCR run, and

controls included PCR reactions without total RNA and without the SuperScript II Reverse Transciptase. Table 1 shows primers used for RT-PCR reactions.

Table 3.1. Primers used for RT-PCR

Gene	Forward primer	Reverse primer	Product Size (bp)	Reference:
mexA	GTGTTCGGCTCGCAGTACTC	AACCGTCGGGATTGACCTTG	244	(Yoneda et al. 2005)
proC	CAGGCCGGGCAGTTGCTGTC	GGTCAGGCGCGAGGCTGTCT	180	(Sayli et al. 2003)
nadB	CTTCACCGTGGAGCATAGC	GCCTTCCTCGTGGTTGTG	94	This study

Pentachlorophenol analysis. Pentachlorophenol was measured using gas chromatography with electron capture detection (ECD) after acetylation and extraction with hexanes (Langwaldt et al. 1998). Briefly, in triplicate at each timepoint, 400 µl samples were mixed with 100 mL acetic anhydride, vortexed for one minute, and allowed to react at room temperature for one hour. The acetylated PCP was then solvent-extracted overnight at 20^{o}C in 3 mL hexanes. Samples were centrifuged at 1,000 x g and 4^{o}C to separate the solvent and aqueous layers, and the hexane layer was removed into GC vials crimped with Teflon-lined septa. PCP was analyzed using a Hewlett Packard 5890 GC (Agilent Technologies) with a DB-1701 column (30 m x 25 mm I.D., and 0.25 µm film thickness) (J+W Scientific, Agilent Technologies). One µL was injected using a Hewlett Packard 7673 GC/SFC autosampler. The column was held at 60^{o}C for 1 min, ramped to 240^{o}C at 25.7^{o}C/min and held at 240^{o}C for 8 minutes. Helium, the carrier gas,

flowed at 1.2 mL/min and make-up nitrogen gas flowed at 20 mL/min. The inlet and ECD temperatures were 225^{o}C and 350^{o}C, respectively.

RESULTS

Whole cell physiological tests show *P. aeruginosa* responds to PCP with minimal stress.

In slow-growing chemostat cultures, *P. aeruginosa* was able to respond to PCP with minimal physiological stress. This experiment was designed to mimic contamination of an oligotrophic groundwater sediment or soil. Therefore, cells were grown under steady-state nutrient-limited conditions in a chemostat and the PCP was added gradually (Figure 3.1A) to mimic concentration increases in a contaminant plume. The WAUR, which indicated catabolic potential, did not change in response to PCP for WT1, and only increased slightly at the last timepoint for the RpoS^{-} mutant (p-value = 0.02, relative to initial timepoint) (Figure 3.1A). In contrast to the WAUR results, cell viability decreased slightly in the WT1 strain as they were exposed to the PCP (Figure 3.1B). This decrease, although slight, was significant for the 13 and 26 hour timepoints during each experiment (p-values $\leq$ 0.004, relative to initial timepoint). Interestingly, the RpoS- mutant showed only a slight decrease in cell viability with exposure to PCP at the 26 hour timepoint (12% reduction, p-value=0.0009, relative to initial timepoint). Chemostats without PCP exposure were run for the same length of time, and did not show any changes in WAUR or cell viability (data not shown, p-values $\geq$ 0.26). These data indicate that *P. aeruginosa* is fairly resistant to PCP stress, and the absence of RpoS does not compromise this resistance.

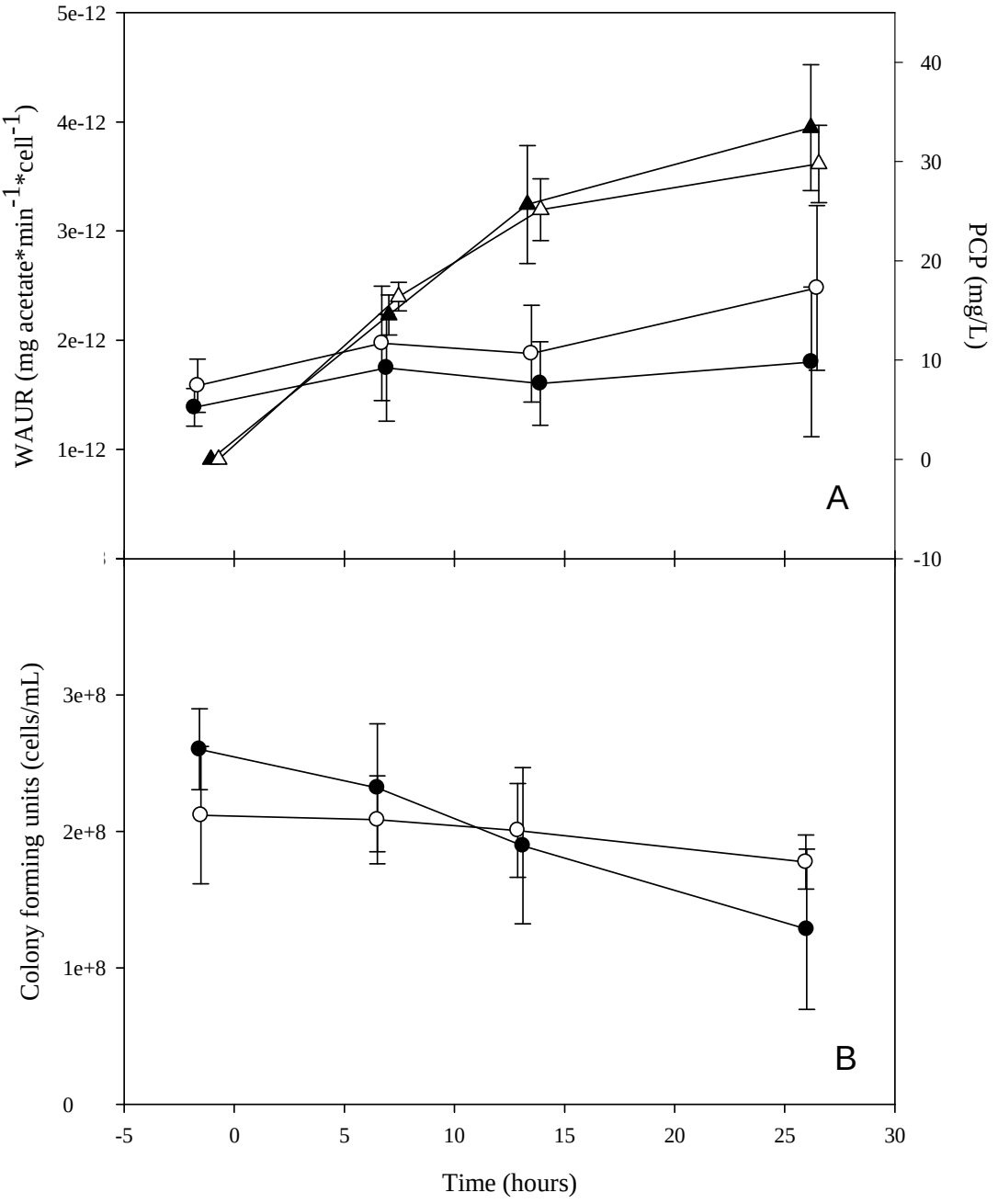

Figure 3.1. Physiological changes as cells are exposed to increasing concentrations of pentachlorophenol. A. Whole cell acetate uptake rate (WAUR) does not change as cells are exposed to increasing PCP concentrations. B. Cell viability of *P. aeruginosa* in chemostat culture exposed to pentachlorophenol at each timepoint. PCP: ▲WT1, △ RpoS$^-$ mutant. WAUR (A), CFU (B): ● WT1, ○ RpoS- mutant. Error bars indicate standard deviations for triplicate samples and at least duplicate reactor runs for each strain.

Transcriptome response to pentachlorophenol.

In contrast to results from the whole cell tests, the gene expression data show distinct physiological changes as the cells respond to PCP. In total, 60 genes showed a ≥ two-fold increase and 53 genes showed a ≥ two-fold decrease at any post-PCP timepoint in comparison with the pre-PCP timepoint. Summarizing the data based on functional categories shows distinct differences in up- and downregulated genes (Figure 3.2). Genes coding for transport and membrane proteins comprise over thirty percent of upregulated transcripts, while metabolism genes compromise greater than twenty percent of downregulated transcripts. Interestingly, genes coding for adaptation and protection proteins represent a large percentage of downregulated transcripts.

Multiple multidrug efflux transporters are upregulated in response to PCP.

Examination of the transport and membrane genes that changed in response to the PCP stress shows that multiple multidrug efflux operons increased (Table 3.2). Genes coding for multiple resistance-nodulation-cell-division (RND) efflux proteins were upregulated strongly at all timepoints in response to PCP. *mexAB-oprM* (PA0425-PA0427) are genes which code for the well-characterized MexAB-OprM RND transporter. This efflux pump is responsible for resistance to a wide variety of antibiotics and has also been shown to efflux solvents (Li and Poole 1999). The increase in this operon was verified with RT-PCR showing increased *mexB* expression during independent chemostat reactor experiments for both the WT and RpoS$^-$ strains (Figure 3.3). The operon PA3676-PA3678 has been described as the RND efflux operon *mexJKL*, and encodes the system responsible for efflux of a few different antibiotics and the antimicrobial compound

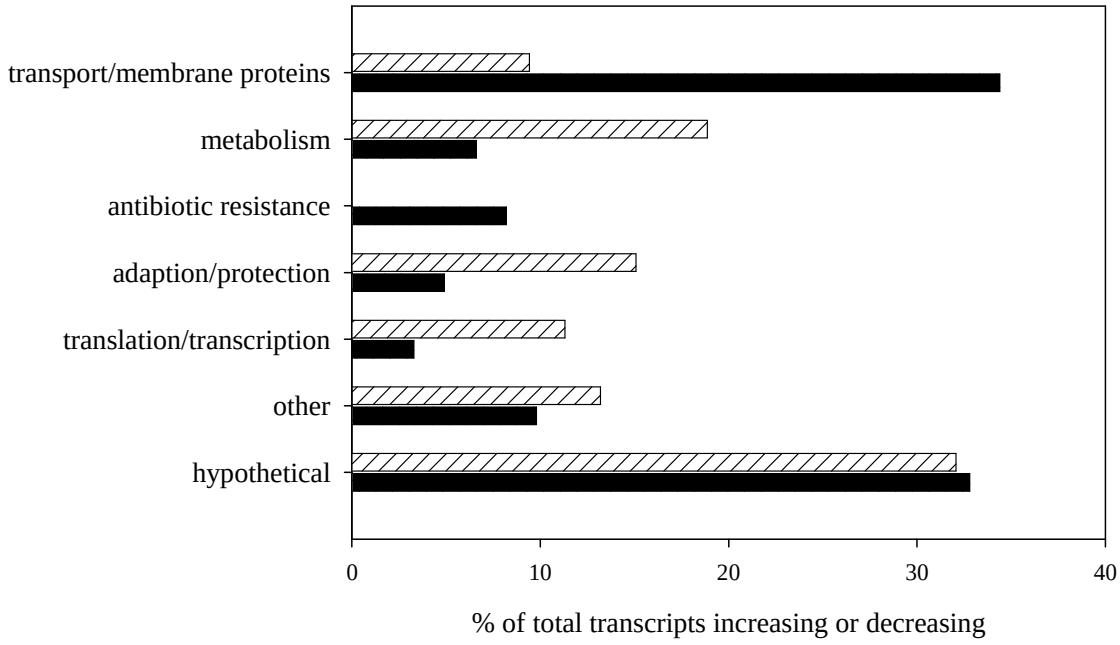

Figure 3.2. Transcripts increasing or decreasing by at least 2-fold at any timepoint after PCP stress, grouped by functional category. The categories as described in the *Pseudomonas aeruginosa* Community Annotation Project (www.pseudomonas.com) (Winsor et al. 2005) have been grouped to clarify differences between increasing and decreasing transcripts. Increasing transcripts ■, decreasing transcripts ▨.

triclosan (Chuanchuen et al. 2002; Chuanchuen et al. 2005). Interestingly, the operon PA3719-PA3720 was also upregulated strongly. Although the functions of these genes are unknown, the increased expression of PA3719 has been reported to increase expression of *mexAB-oprM* (Cao et al. 2004).

Transcripts coding for probable major facilitator superfamily (MFS) efflux proteins were also upregulated strongly at 13 and 26 hours post-PCP stress (PA3136-PA3137 and PA5157-PA5160) (Table 3.2). These genes have not been previously described in the literature, but are similar to the *emrA* and *emrB* genes of *Escherichia coli*, which code for components of a MFS pump that is able to efflux, among other compounds, hydrophobic uncouplers (Lomovskaya and Lewis 1992; Poole 2004). MFS pumps have been shown to function with only the drug efflux transporter or as a three-component pump, coupling the transporter with a membrane fusion protein and outer membrane protein (Kumar and Schweizer 2005). Both operons induced by PCP contain genes coding for the membrane fusion protein and drug efflux transporter; however, one operon, PA5157-PA5160, also contains genes coding for the outer membrane protein and putative regulator. A third gene coding for a putative MFS transporter, PA0246, increased only at the first timepoint.

Other transcripts increasing in response to PCP include those involved in different transport mechanisms and iron uptake (Table 3.2, Table 3.3). The transcripts PA0204, PA4037 and PA5216 code for probable components of different ATP-binding cassette (ABC) transporters. Transporters belonging to this ABC family use the hydrolysis of ATP to drive transport of a compound and are involved in both uptake and efflux (Kumar and Schweizer 2005). Genes associated with iron uptake, *pvdN*, *pvdE* and *fpvA* (Table

Table 3.2. Efflux, transport, and membrane transcripts increasing[a] in response to PCP.

PA number[d] (*gene*)	product name	Fold Change[b] PAO1 WT[c] 6.5 hours	13 hours	26 hours	PAO1 *rpoS* mutant 6.5 hours	13 hours	26 hours
increasing at all timepoints							
PA0424 (*mexR*)	multidrug resistance operon repressor MexR	**2.5**	**3.1**	**3.0**		**2.5**	**3.0**
PA0425 (*mexA*)	RND multidrug efflux protein MexA precursor	**2.0**	**4.3**	**3.8**		**7.5**	**6.5**
PA0426 (*mexB*)	RND multidrug efflux transporter MexB	**2.8**	**3.7**	**3.7**		**6.5**	**5.3**
PA0427 (*oprM*)	efflux outer membrane protein OprM precursor	**3.5**	**4.4**	**4.0**		**6.1**	**5.3**
PA3369	hypothetical protein	**2.2**	**3.6**	**10.3**			
PA3677	probable RND membrane protein precursor	**2.4**	**3.4**	**4.4**		**2.3**	**2.0**
PA3678	probable transcriptional regulator	**1.7**	**2.1**	**2.3**			
PA3719	hypothetical protein	**5.6**	**9.2**	**9.5**	**2.0**	**13.0**	**14.9**
PA3720	hypothetical protein	**7.2**	**15.5**	**15.0**		**16.0**	**16.0**
PA3721	probable transcriptional regulator	**6.3**	**11.8**	**8.9**	**2.3**	**9.2**	**8.0**
PA5157	probable transcriptional regulator	**12.3**	**8.5**	**11.2**		**3.5**	**2.6**
PA5158	probable outer membrane protein precursor	**2.1**	**3.9**	**3.3**		**4.0**	**2.8**
PA5159	multidrug resistance protein	**5.2**	**7.7**	**6.6**		**7.0**	**4.6**
PA5160	drug efflux transporter	**3.2**	**3.5**	**3.9**		**3.2**	**2.3**
increasing at 6.5 hours							
PA0204	probable permease of ABC transporter	**2.9**					*-2.0*
PA0246	probable MFS transporter	**2.3**					*-2.0*
PA2397 (*pvdE*)	pyoverdine biosynthesis protein	**2.7**			**2.1**		
PA2398 (*fpvA*)	ferripyoverdine receptor	**3.5**					
PA3278	hypothetical protein	**3.7**		**2.7**		*-3.7*	*-3.5*
PA3676	probable RND efflux transporter	**2.6**	2.6	3.0		**2.0**	
PA4037	probable component of ABC transporter	**8.0**		2.2		*-2.0*	*-2.8*
PA5216	probable permease of ABC iron transporter	**2.1**	4.2	8.2			

Table 3.2. cont.

PA number[d] (*gene*)	product name	Fold Change[b] PAO1 WT[c] 6.5 hours	13 hours	26 hours	PAO1 *rpoS* mutant 6.5 hours	13 hours	26 hours
increasing at 6.5 hours							
PA5469	conserved hypothetical protein	**4.9**	2.0	2.0	**2.1**	*-2.5*	*-2.8*
increasing at 13 and 26 hours							
PA3136	probable secretion protein	3.0	**2.4**	**2.6**	**2.1**		**2.1**
PA3137	probable MFS transporter	3.5	**3.0**	**2.9**			
increasing at 26 hours							
PA1230	hypothetical protein	4.9		**4.3**	2.1	*-2.6*	*-2.5*
PA3370	hypothetical protein	2.1	2.8	**9.1**			
PA3679	hypothetical protein	2.3		**2.3**			
PA4981	probable amino acid permease	4.0		**2.3**		*-2.0*	*-2.1*

[a] Genes are categorized based on response within the WT chemostat runs. The response in the RpoS$^-$ strain may be different.
[b] Fold-change determination, as described in Materials and Methods, is based on comparison of 13 probe-sets for each gene to the pre-PCP (0 hour) microarray. Increasing genes ($p<0.0045$) are shown in bold and decreasing genes ($p>0.9955$) are shown in italics. Number shown in normal type do not meet the significance criteria for duplicate microarrays.
[c] WT numbers are the average fold change for duplicate chemostat reactor runs. The RpoS$^-$ data are from a single microarray experiment.
[d] PA numbers in italics signify that the gene was absent at the pre-PCP timepoint.

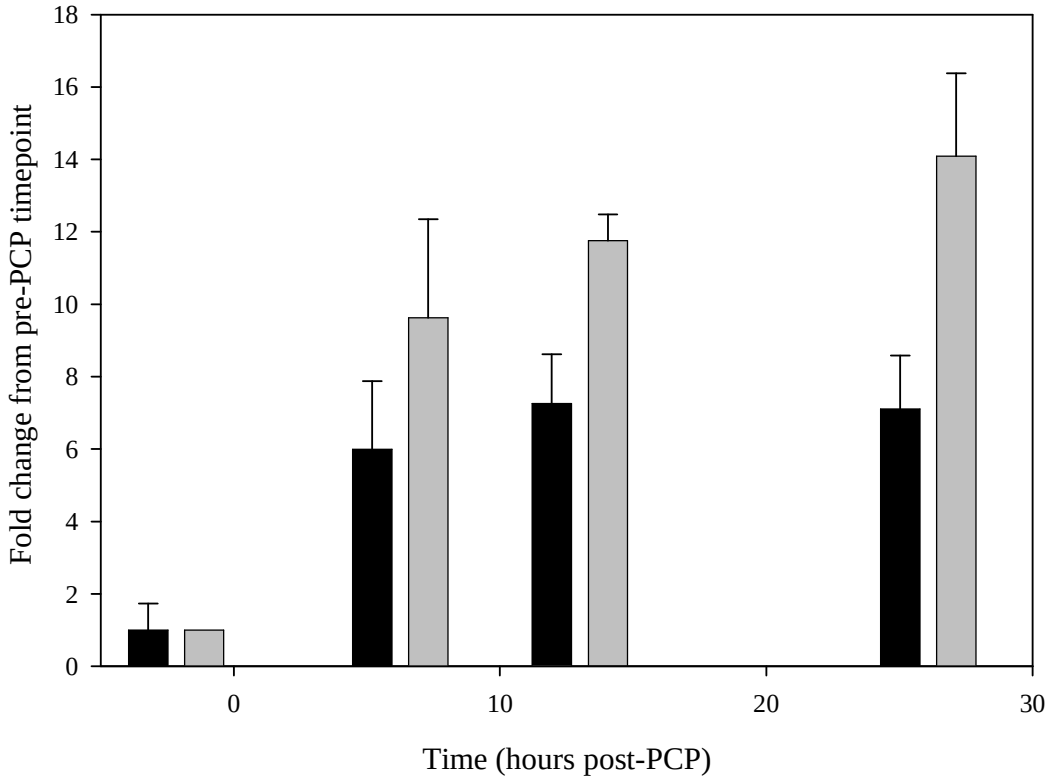

Figure 3.3. Microarray results were validated by quantitative RT-PCR of *mexB*. Shown is the average fold change after normalized to each housekeeping geneWT1 ■, RpoS$^-$ mutant □. Results are reported as average and standard error.

3.2, Table 3.3), code for proteins involved in pyoverdine and ferripyoverdine receptor synthesis (McMorran et al. 1996; Shen et al. 2002; Lamont and Martin 2003). Interestingly, pyoverdine production and ferripyoverdine synthesis are known to be co-regulated with the *mexAB-oprM* efflux system (Poole et al. 1993).

Metabolism genes show slight, transient downregulation in response to PCP. Transcripts downregulated (Table 3.4) in response to PCP include genes associated with central metabolism (*cysD, rpsT, tpiA, rpmH*), fatty acid metabolism (*accB*), and energy metabolism (*sucC, etfA*). With no change in whole cell metabolism as measured by WAUR, this may be partly unexpected, but a closer examination of the timing of gene expression shows that this decrease in metabolism genes is transient and only occurs at the first timepoint. After at least 13 hours of exposure to PCP, metabolic gene expression was back to unstressed levels.

***PCP exposure caused decrease in adaptation and protection genes*.** Interestingly, the second most represented functional category for downregulated transcripts was adaptation/protection (Figure 3.2, Table 3.4). A few of these adaptation/protection genes, *ohr* and *oprH*, are downregulated consistently in response to PCP. The transcript *ohr* codes for the organic hydroperoxide resistance protein known to protect the cell from oxidative damage induced by reactive oxygen species (Lesniak et al. 2002). The product of *oprH* is a low magnesium-inducible PhoPQ-regulated outer membrane porin, OprH (Macfarlane et al. 1999). Porin changes in the cell suggest a change in membrane structure, a common stress response to organic chemical contaminants (Isken and de Bont 1998). As with the metabolism genes, some of these adaptation/protection genes decrease only transiently at the first timepoint, and are back to pre- stress levels

Table 3.3[a]. Other transcripts increasing in response to PCP.

PA number (*gene*)	product name	Fold Change					
		PAO1 WT			PAO1 *rpoS* mutant		
		6.5	13 hours	26	6.5	13 hours	26
PA0355 (pfpI)	protease PfpI	**8.7**	2.0	7.2	**2.0**		*-2.0*
PA0417 (*chpE*)	probable chemotaxis protein	2.0		**2.4**			*-2.0*
PA0445	probable transposase	**2.9**				*-2.1*	*-2.5*
PA0526	hypothetical protein	**5.1**		3.1		*-2.8*	*-3.2*
PA0633	hypothetical protein	**2.5**					
PA0726	hypothetical protein of bacteriophage Pf1	**6.6**	3.6	**7.5**		*-2.5*	*-3.5*
PA0868 (RF-1)	conserved hypothetical protein	**4.4**	2.9	5.8			
PA1281 (cobV)	cobalamin (5'-phosphate) synthase	**1.9**					
PA1393 (cysC)	adenosine 5'-phosphosulfate (APS) kinase	**10.9**	7.5	6.5	**2.3**	*-2.3*	*-2.3*
PA1705 (*pcrG*)	regulator in type III secretion	**2.7**		2.0			*-2.8*
PA1780 (nirD)	assimilatory nitrite reductase small subunit	**4.3**	2.1	4.9			
PA1982 (exaA)	quinoprotein alcohol dehydrogenase	**3.8**					*-2.1*
PA2166	hypothetical protein	**2.3**		**2.8**			*-2.6*
PA2394 (pvdN)	PvdN	**7.2**	2.8	2.6			*-2.5*
PA2669	hypothetical protein	**7.7**		3.4	**2.1**		*-3.5*
PA2906	probable oxidoreductase	**2.9**		2.4			
PA2941	hypothetical protein	**2.1**					
PA3371	hypothetical protein	**2.8**		**5.9**			*-2.1*
PA3547 (algL)	poly(beta-d-mannuronate) lyase precursor AlgL	**4.0**	5.9	**14.8**	**2.0**		*-2.1*
PA3954	hypothetical protein	**9.1**	2.7	4.6	**2.1**		*-2.3*
PA4033	hypothetical protein	**3.1**		**2.4**	**2.3**		
PA4178	hypothetical protein	**2.2**		2.1	**2.1**		
PA4217 (phzS)	flavin-containing monooxygenase	**2.6**					
PA4344	probable hydrolase	**3.4**		2.9			

Table 3.3.cont.

PA number (*gene*)	product name	Fold Change PAO1 WT 6.5 hours	13 hours	26 hours	PAO1 *rpoS* mutant 6.5 hours	13 hours	26 hours
PA4738	conserved hypothetical protein	**2.3**	**2.9**	**11.8**			
PA4739	conserved hypothetical protein	**2.8**	**3.8**	**17.4**			
PA4816	hypothetical protein	**2.1**					
PA5326	hypothetical protein	2.0		**2.6**	**2.5**		*-2.6*
PA5390	probable peptidic bond hydrolase	5.2	3.9	**6.1**			
PA5407	hypothetical protein	**2.0**					*-2.0*
PA5481	hypothetical protein	**5.5**	3.7	**9.9**	**2.3**	*-3.7*	*-3.0*
PA5482	hypothetical protein	**3.9**	**4.8**	**16.2**	**2.0**		*-2.5*

[a] See Table 2 footnotes for complete explanation.

Table 3.4. Transcripts decreasing[a] in response to pentachlorophenol, grouped by functional category.

PA number (*gene*)	product name	Fold Change[b] PAO1 WT[c] 6.5 hours	13 hours	26 hours	PAO1 *rpoS* mutant 6.5 hours	13 hours	26 hours
Metabolism							
PA1588 (*sucC*)	succinyl-CoA synthetase beta chain	**-2.1**			**-5.3**	*2.0*	
PA2951 (*etfA*)	electron transfer flavoprotein alpha-subunit	**-2.2**			**-4.6**	*2.0*	
PA3814 (*iscS*)	L-cysteine desulfurase	**-2.3**			**-4.6**		
PA4443 (*cysD*)	ATP sulfurylase small subunit	**-2.3**					*1.9*
PA4563 (*rpsT*)	30S ribosomal protein S20	**-2.0**					*2.5*
PA4748 (*tpiA*)	triosephosphate isomerase	**-2.1**				*2.1*	*2.1*
PA4847 (*accB*)	biotin carboxyl carrier protein (BCCP)	**-2.0**			**-2.1**		
PA5013 (*ilvE*)	branched-chain amino acid transferase	**-2.0**			**-3.0**		
PA5570 (*rpmH*)	50S ribosomal protein L34	**-2.3**			**-2.0**	*1.9*	*2.5*
Adaptation, protection							
PA0848	probable alkyl hydroperoxide reductase	-2.8	**-2.2**	**-2.5**		**-4.0**	**-3.7**
PA0962	probable dna-binding stress protein	**-2.2**			**-4.9**		
PA1178 (*oprH*)	outer membrane protein H1 precursor	**-4.1**	**-4.5**	**-4.6**	**-6.1**	**-5.3**	**-4.3**
PA1793 (*ppiB*)	peptidyl-prolyl cis-trans isomerase B	**-2.0**			**-2.3**		
PA2850 (*ohr*)	organic hydroperoxide resistance protein	**-2.5**	**-2.4**	**-2.2**	**-3.2**	**-4.6**	**-4.9**
PA3266 (*capB*)	cold acclimation protein B	**-2.6**			**-2.1**		
PA3450	probable antioxidant protein	**-2.1**	**-2.1**	-1.9	-1.9		
PA4386 (*groES*)	GroES protein	**-2.2**			**-4.9**		
Membrane proteins							
PA3690	probable metal-transporting P-type ATPase	**-2.7**			**-8.0**		
PA3819	conserved hypothetical protein	**-2.4**			**-4.9**	**-2.5**	
PA5182	hypothetical protein	**-2.0**			**-2.0**	**-2.3**	**-1.9**
Putative enzymes							
PA0224	probable aldolase	**-1.9**		**-2.3**	**-2.1**		**-2.6**

Table 3.4. cont.

PA number (*gene*)	product name	Fold Change					
		PAO1 WT			PAO1 *rpoS* mutant		
		6.5	13 hours	26	6.5	13 hours	26
PA2062	pyridoxal-phosphate dependent enzyme			**-2.0**			
PA3328	probable FAD-dependent monooxygenase			**-2.2**	**-4.0**		
PA5312	probable aldehyde dehydrogenase	**-2.0**			**-3.2**		
Transcriptional regulators							
PA0762 (*algU*)	sigma factor AlgU	**-2.5**			**-2.5**		
PA1544 (*anr*)	transcriptional regulator Anr			**-1.9**			
PA5253 (*algP*)	alginate regulatory protein AlgP	**-2.3**			**-5.3**		
Translation, post-translational modification, degradation							
PA0579 (*rspU*)	30S ribosomal protein S21	**-2.3**					*2.5*
PA3162 (*rspA*)	30S ribosomal protein S1	**-2.5**			**-4.0**	*1.9*	*1.9*
PA4568 (*rplU*)	50S ribosomal protein L21	**-2.7**			**-3.7**		*2.0*
Hypothetical							
PA0284	hypothetical protein	**-3.2**	**-2.1**	**-2.6**	**-3.5**		
PA1198	conserved hypothetical protein	**-2.1**			**-2.1**		*2.3*
PA1656	hypothetical protein			**-2.3**	-1.4		
PA1657	conserved hypothetical protein	**-1.9**		**-3.4**	**-4.6**		
PA1658	conserved hypothetical protein	**-2.0**		**-3.8**	**-3.7**		
PA1660	hypothetical protein		-2.2	**-1.9**			
PA1664	hypothetical protein			**-2.6**			
PA2562	hypothetical protein	**-2.3**			**-2.0**		
PA2658	hypothetical protein			**-2.4**			
PA2808	hypothetical protein	**-2.2**			**-6.5**		
PA3287	conserved hypothetical protein		**-1.9**	**-3.0**		**-6.5**	**-7.0**
PA3552	conserved hypothetical protein			**-2.1**		**-1.9**	**-2.3**
PA3815	conserved hypothetical protein	**-2.2**			**-2.8**		

Table 3.4. cont.

PA number (*gene*)	product name	Fold Change					
		PAO1 WT			PAO1 *rpoS* mutant		
		6.5	13 hours	26	6.5	13 hours	26
Hypothetical							
PA3904	hypothetical protein	**-2.1**			**-2.3**		
PA3905	hypothetical protein	**-2.0**					
PA5212	hypothetical protein		**-1.9**		**-2.6**	**-3.0**	**-3.2**
PA5369	hypothetical protein			**-2.1**			
Other							
PA0263 (*hcpC*)	secreted protein Hcp			**-3.6**	**-4.3**		
PA1493 (*cysP*)	sulfate-binding protein of ABC transporter	**-1.9**			**-2.8**		
PA2760	probable outer membrane protein precursor	**-2.1**			**-4.9**		
PA3159 (*wbpA*)	probable dehydrogenase WbpA	**-2.3**			**-2.8**		
PA4053 (*ribE*)	6,7-dimethyl-8-ribityllumazine synthase	**-1.9**			**-2.3**		

[a] Genes are categorized based on response within the WT chemostat runs. The response in the RpoS$^-$ strain may be different.
[b] Fold-change determination, as described in Materials and Methods, is based on comparison of 13 probe-sets for each gene to the pre-PCP (0 hour) microarray. Decreasing genes ($p>0.9955$) are shown in bold and increasing genes ($p<0.0045$) are shown in italics. Number shown in normal type do not meet the significance criteria for duplicate microarrays.
[c] WT numbers are the average fold change for duplicate chemostat reactor runs. The RpoS$^-$ data are from a single microarray experiment.

Three of these genes are involved in general stress resistance mechanisms: *groES* codes for a stress-induced chaperone, *capB* codes for cold acclimation protein B, and PA0962 codes for a probably DNA-binding stress protein.

The RND pump MexAB-OprM is required for optimal growth in pentachlorophenol.

To validate the results from the microarray experiments, which indicated that efflux is the major response of *P. aeruginosa* to PCP stress, the effect of the MexAB-OprM efflux pump on growth in the presence of PCP was examined. Using a MexAB-OprM mutant (PAO200) along with its WT strain (WT2), cells were grown in batch cultures using MM with acetate as the sole carbon source. The cultures were grown at 37^{o}C with and without 30 mg/L PCP added. PAO200 grew normally in medium without PCP; however, growth was significantly reduced in the presence of PCP (Figure 3.4). To further investigate the effect of MexAB-OprM, an MIC assay was performed on these cultures to assess their ability to grow on PCP. The MIC for the WT was 1,000 mg/L PCP, whereas the MIC for the mutant was four-fold lower at 250 mg/L. These results validate the microarray data, showing that MexAB-OprM-mediated efflux of PCP is required for *P. aeruginosa* to maintain normal physiological status when grown in the presence of PCP.

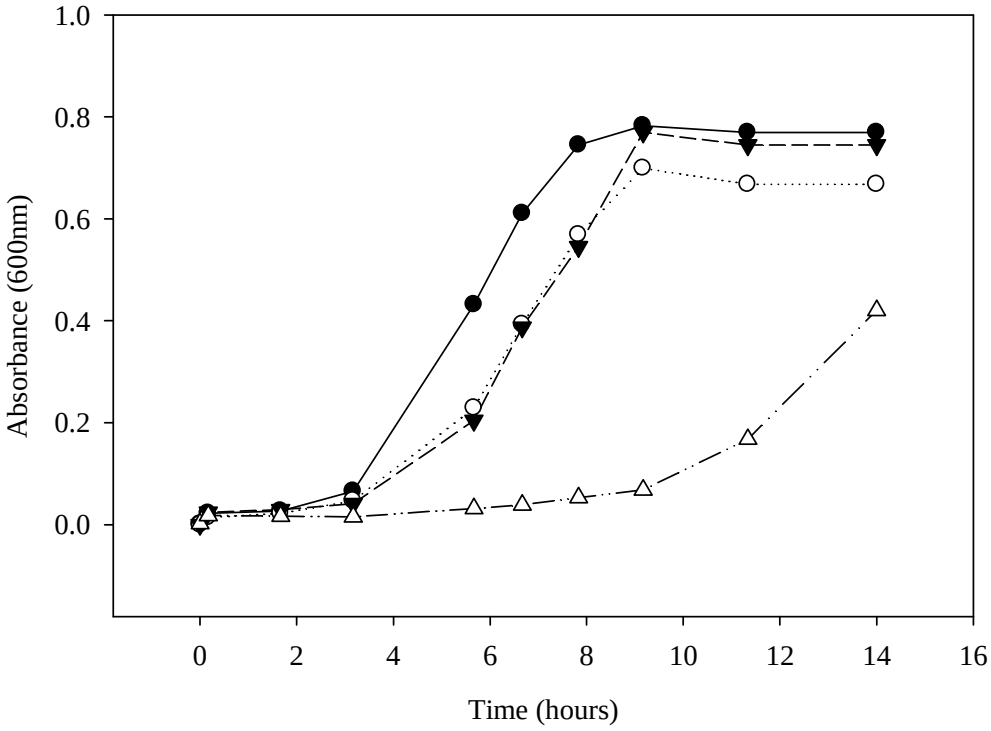

Figure 3.4. Growth of MexAB-OprM mutant (PAO200) is reduced in 30 mg/L PCP in comparison with the WT *Pseudomonas aeruginosa* (PAO1) strain. WT2 ●, WT2 + PCP ○, PAO200 ▲, PAO200 + PCP △. The experiment was repeated three times, and a single representative experiment is shown.

DISCUSSION

Populations of slow-growing *P. aeruginosa* actively respond to and resist the potentially toxic effects of PCP. This resistance was shown by examining both whole-cell physiological changes and changes in gene expression as cells were exposed to increasing concentrations of the contaminant under substrate-limited growth conditions. While whole-cell assays demonstrated no significant stress response, the transcriptome analysis revealed this resistance was due to upregulation of multiple multi-drug efflux pumps. Further analysis revealed that MexAB-OprM in particular is upregulated in response to the PCP and is necessary for optimal growth in the presence of the compound. This is the first study to show induced expression of MexAB-OprM in *P. aeruginosa* due to exposure to an anthropogenic chemical, and the first study to show induction of multi-drug efflux systems by a chlorinated aromatic contaminant.

P. aeruginosa is resistant to PCP stress. The ability of *P. aeruginosa* to respond to PCP on the whole-cell level was examined with whole-cell substrate uptake rates and cell viability. It was expected that cells would respond by changing their substrate uptake rate to shift cellular resources away from growth towards protective, stress-response mechanisms. In eukaryotes, PCP has been shown to cause oxidative stress, cytotoxicity (Wang et al. 2001; Yang et al. 2005), and is a known metabolic uncoupler (Weinbach and Garbus 1965; Escher and Schwarzenbach 1996), and suspected endocrine disrupter (Gerhard et al. 1999). In prokaryotes, it has been shown to induce expression of general stress proteins (Blom et al. 1992), decrease bioluminescence (Repetto et al. 2001), and cause changes in membrane composition (Dercova et al. 2004). The main toxic effect of

PCP is thought to be on the cell membrane where it embeds as a monomer and acts as an indiscriminant proton shuttle, uncoupling oxidative phosphorylation (Escher et al. 1999). Expected cellular stress response with an uncoupler is an increase of the substrate uptake rate to compensate for disruption of the proton motive force. However, with these experiments, the acetate uptake rate did not change substantially as *P. aeruginosa* was exposed to the increasing concentrations of PCP (Figure 3.1A). The only indication of a slight stress response came from a moderate decrease in cell viability (Figure 3.1B). In the WT population, viability decreased to 78% and 54% of pre-stress levels at 13 and 26 hours, respectively (p-value < 0.05). In the RpoS$^-$ population, this decrease was only to 88% of pre-stress levels at the 26 hour timepoint (p-value < 0.05).

RpoS is not involved in the protective mechanism. As the sigma factor RpoS has been shown to regulate increased resistance to different types of stressors in *P. aeruginosa* and many other species under conditions of starvation (Ramos-González and Molin 1998; Suh et al. 1999; Elias et al. 2000; Hengge-Aronis 2000; Nunez et al. 2006), the effects of an RpoS$^-$ mutation on stress resistance to PCP were also examined. Comparison of the whole-cell data between the WT and its RpoS$^-$ mutant shows that RpoS does not play a major role in protecting *P. aeruginosa* from PCP stress. Although starvation growth is different from the nutrient-limited balanced growth state achieved here, the dilution rate of the chemostat was kept low to maintain a physiology approaching a starvation state (Ferenci 2001). Interestingly, the cell viability of the RpoS$^-$ mutant was affected to a lesser extent than WT1 (Figure 3.1B), indicating that the loss of RpoS$^-$ results in greater protection of the cells. This phenotype has also been shown by other researchers

whereby RpoS$^-$ mutants survive either as well as or better than their WT counterparts in stress-induced environments (Jorgensen et al. 1999; Whiteley et al. 2001).

P. aeruginosa protects itself from PCP stress by upregulating multidrug efflux pumps. Analysis of the transcriptome response of *P. aeruginosa* to PCP reveals that multiple multidrug efflux operons are strongly upregulated to protect the cells from the stress (Figure 3.2, Table 3.2). Specifically, two operons coding for RND pumps, one operon (PA3720-PA3719) coding for proteins that positively regulate the RND pump MexAB-OprM, and two operons coding for putative MFS transporters are upregulated strongly at all timepoints in response to PCP. This increase in expression was verified with independent chemostat experiments measuring expression levels of *mexB*. Figure 3.3 shows that levels of *mexB* are significantly upregulated to their full extent at 13 and 26 hours (p-values < 0.05). Consistent with the whole cell data which indicated that the RpoS$^-$ strain had slightly greater protection from PCP, the levels of *mexB* are expressed higher at these timepoints in the mutant when compared with the WT (p-values = 0.04 at both 13 and 26 hours). Although this research does not demonstrate a mechanism by which PCP upregulates *mexAB-oprM*, growth of a MexAB-OprM mutant was impaired in the presence of PCP demonstrating the importance of this efflux pump in the stress response of *P. aeruginosa* to PCP.

Multidrug efflux systems in *P. aeruginosa* are known to efflux a wide variety of compounds and are involved in the increased antibiotic resistance of this organism (Zgurskaya and Nikaido 2000; Poole 2004). Within the RND family itself, there are seven characterized and, from analysis of the genome (Winsor et al. 2005), five more

putative pump systems. RND pumps consist of three components: a membrane transport protein, a membrane fusion protein, and an outer membrane protein (Kumar and Schweizer 2005). MexAB-OprM is one of the most highly studied RND efflux systems in *P. aeruginosa* (Nikaido 1996; Poole 2004). It has been reported to efflux 19 different chemicals or chemical classes, including the solvents hexane and p-xylene (Li et al. 1998; Poole 2004). The MexAB-OprM system is expressed at constitutive basal levels in WT strains, and over-produced in cells containing mutations in either MexR or PA3721, transcriptional repressors of *mexAB-oprM* and PA3720-PA3719 transcription, respectively (Srikumar et al. 2000; Cao et al. 2004). Low-iron growth conditions are the only conditions reported to induce increased levels of MexAB-OprM (Poole et al. 1993; Morita et al. 2001). MexJK, the second characterized RND pump whose genes are upregulated in response to PCP, is known to use either OpmH or OprM as outer membrane protein depending upon what compound is being effluxed (Chuanchuen et al. 2005). It is possible that MexJK is partially responsible for the PCP resistance, using OprM for the outer membrane protein, as the gene coding for probable OpmH (PA4974) was not upregulated.

Although the MexAB-OprM mutant is deficient in growth in the presence of PCP, it is not completely inhibited (Figure 3.4). Therefore, the genes coding for MFS efflux pumps may also play a significant role in the ability of *P. aeruginosa* to resist PCP stress. The transcripts coding for the membrane fusion protein and drug efflux transporter, PA3136, PA5159 and PA3137, PA5160, are homologous with EmrA and EmrB, proteins in *E. coli* which are known to function with an outer membrane protein, TolC in the export of CCCP, FCCP, and 2,4 dinitrophenol, other hydrophobic uncouplers (Borges-

Walmsley et al. 2003). Therefore, it is entirely possible that both putative MFS systems upregulated here function to export the uncoupler, PCP.

General metabolism decreases transiently in response to PCP stress. The functional category with the greatest representation in decreasing transcripts upon exposure to PCP is metabolism (Figure 3.2). This decrease is transient, and genes are decreased only at the first timepoint (Table 3.4). A decrease in metabolism genes may seem to be contrary to the data, which show no change in metabolic physiology at the whole cell level (WAURs, Figure 3.1A). However, it may be a way for the cells to temporarily shift metabolism away from less-essential metabolisms and focus the energy from sustained acetate uptake towards the response to PCP. Two of the most recent studies looking at the response of *P. putida* to phenol and toluene suggest that this is the result from organic solvent stress as well (Santos et al. 2004; Dominguez-Cuevas et al. 2006). In the case of the present study with PCP, the stress response, or increase in efflux pumps, was sufficiently upregulated after 13 hours (one generation) for *P. aeruginosa* to resume the pre-shock metabolic state.

Downregulated genes suggest that slow-growing cells were primed for adaptation to PCP stress. A surprising finding of this study was that *P. aeruginosa* downregulated genes coding for stress proteins in response to PCP. Genes coding for a probable DNA-binding stress protein (PA0962), a probable antioxidant protein (PA3450), the heat shock protein GroES, and the cold-shock response protein CapB were all downregulated at the first timepoint after PCP addition. However, this downregulation might be expected if

the cultures were initially stressed due to the low-growth rate conditions. Upon exposure to the chemical stressor a re-organization of cellular metabolism may have caused a temporary decrease in these stress response genes, ensuring that sufficient metabolic resources were available to allow a quick response to the PCP.

Starvation conditions and nutrient limitation during balanced growth in a chemostat are both known to induce a more resistant cellular physiology, with a general upregulation of protective stress mechanisms (Matin 1979; Hengge-Aronis 1996; Jorgensen et al. 1999; Ferenci 2001). This stress-resistant physiology is similar to that found in the natural environment, due to the nutrient limitation and low growth rates experienced in soils and waters (Gu and Mazzola 2001). Comparison with results from other transcriptome studies examining the stress response of *P. aeruginosa* to various stressors indicates that the cultures in the present study were initially stressed. Genes that have been shown by others to be strongly upregulated to oxidative or osmotic stress were downregulated in response to PCP in this study, and vice versa, indicating that the chemostat-grown *P. aeruginosa* was already demonstrating a stressed state before PCP shcok. The gene coding for organic hydroperoxide resistance protein *ohr* was upregulated in response to hydrogen peroxide stress (Salunkhe et al. 2005), but downregulated strongly at all timepoints in response to PCP. The genes PA3278 and *fpvA* (PA2398) were both downregulated upon oxidative stress (Palma et al. 2004; Chang et al. 2005) and upregulated in response to PCP. When *P. aeruginosa* is exposed to osmotic stress, *algU* and PA0962 are induced (Aspedon et al. 2006). In the present study, these two transcripts initially decreased in response to PCP, but then returned to pre-stress levels after 13 hours.

Previous research on the response of *E. coli* to PCP indicated that general stress response proteins were upregulated as part of the stress response (Blom et al. 1992). Similarly, recent transcriptome studies of the response of *P. putida* to aromatic solvent contaminants show that genes upregulated include those coding for proteins involved in oxidative stress response, glutathione-mediated stress response, and the general protective heat shock response (Santos et al. 2004; Dominguez-Cuevas et al. 2006; Velazquez et al. 2006). These studies all examined stress responses during batch growth conditions. The present study examined the transcriptome-level response to a contaminant under steady-state, low growth rate conditions, and suggests that stress response patterns may be very different from those experienced during batch growth. As similar nutrient-limiting conditions are far more prevalent in the natural environment, future research should focus on understanding this difference in stress responses.

Efflux-mediated stress response is an essential response to contamination. In studies with both pure cultures and soil communities, efflux pumps have been shown to play a role in tolerance to hydrocarbon contamination (Bugg et al. 2000; Rojas et al. 2001; Meguro et al. 2005). In the present study multiple multidrug efflux pumps are upregulated in response to PCP. Using an efflux-deficient mutant, it was shown that the MexAB-OprM system, in particular, is required for this resistance. This is not entirely surprising, as the MexAB-OprM system has been shown to efflux many different compounds, including a wide variety of antibiotics and a few different organic solvents (Nikaido 1996; Fernandes et al. 2003; Poole 2004). Upregulation of this pump by an anthropogenic chemical inducer, however, has not been demonstrated before now.

Revealed through the analysis of genome-wide expression changes relative to whole cell physiological function, this study emphasizes the great importance efflux expression plays in the metabolic capability and resistance properties of *P. aeruginosa*.

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Chapter 4. ANTIBIOTIC RESISTANCE IS INDUCED IN *PSEUDOMONAS AERUGINOSA* BY PENTACHLOROPHENOL

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen with the ability to develop multidrug resistance (MDR) rapidly with selective pressure**.** Previous research showed that upon exposure to pentachlorophenol (PCP), *P. aeruginosa* PAO1 increases expression of multidrug efflux pumps, especially the MexAB-OprM pump. The research described here investigated whether PCP-induced expression resulted in increased antibiotic resistance, and if long-term PCP exposure resulted in a stable antibiotic resistant phenotype. Results showed that PCP does induce multidrug resistance, similar to the *nalC* phenotype. However, there was no selection of a stable MDR population after 600 generations of batch growth under PCP selection. The use of RT-PCR to examine *mexB* expression showed that PCP induction of MexAB-OprM is reversible. This research was extended further to examine the effect of MexAB-OprM on growth with different organic contaminants. Results demonstrated that MexAB-OprM is required for optimal growth of *P. aeruginosa* in 2,4-dinitrophenol, benzoate, catechol and Roundup®. The effect of these organic contaminants on antibiotic resistance in *P. aeruginosa* was then investigated. Disk diffusion and minimum inhibitory concentration assays showed that resistance increases in the presence of these chemicals; however, unlike with PCP, the resistance is independent of MexAB-OprM. This is the first research to show chemical induction of MexAB-OprM resulting in antibiotic resistance, and the first research to show induction of an RND pump by a chlorinated compound.

This adds strength to the literature that suggests organic contamination is a significant contributor to the evolution of antibiotic resistance in the environment.

INTRODUCTION

The development of antibiotic resistance in the environment is a growing and pressing concern to the global community (Williams 2002). Selective pressure in the environment is thought to come from widespread use of antibiotics in medicine and agriculture (Salyers et al. 2002), industrial metal pollution (Baker-Austin et al. 2006), and, as recently shown, from competition within natural microbial communities (D'Costa et al. 2006). Evidence is also emerging for another possible environmental selective pressure: organic chemical contamination. Limited research indicates that organic contamination also selects for bacteria with increased resistance to a wide variety of antibiotics (Li et al. 1998; Rojas et al. 2001; Hearn et al. 2003).

Antibiotic resistance in bacteria can occur by a variety of mechanisms. One of these mechanisms, the active efflux of compounds across the cell membrane or cell envelope, is found in a variety of bacterial species, both Gram positive and Gram negative. There are five different types of multidrug efflux pumps that function in slightly different manners, but all work to expel compounds either outside the cytoplasmic membrane into the periplasm or expel the compound completely outside of the cell envelope (Poole 2004; Kumar and Schweizer 2005). Pumps in all five families are known to confer resistance to a variety of compounds, although resistance-nodulation-cell-division (RND) family pumps are the most non-specific, recognizing the widest variety of substrates.

RND pumps are also the most prevalent in Gram negative bacteria, and are increasingly recognized as the pumps responsible for antibiotic resistance in clinical isolates of various species of bacteria (Poole 2004). This is true especially for the opportunistic pathogen, *Pseudomonas aeruginosa*. *P. aeruginosa* has seven characterized RND efflux pumps that each recognize various compounds, and from analysis of the genome sequence (Stover et al. 2000), five more previously uncharacterized RND pumps can be found. The MexAB-OprM RND efflux pump alone recognizes and effluxes 19 different chemicals or antibiotics (Poole 2004; Kumar and Schweizer 2005).

Previous limited research has shown that compounds other than antibiotics can be substrates for these efflux pumps. Of special interest are anthropogenic contaminants. Efflux of polycyclic aromatic hydrocarbons (PAHs) was found in three different species of soil bacteria, and the specific RND pump utilized was identified for one of the organisms (Hearn et al. 2003; Hearn et al. 2006). Although this new pump was identified in *P. fluorescens* based on its ability to efflux PAHs, the authors also demonstrated its ability to efflux various antibiotics. The common soil Pseudomonad *P. putida* upregulates an efflux pump in response to toluene that results in antibiotic resistance (Rojas et al. 2001; Dominguez-Cuevas et al. 2006), and the solvents *p*-xylene and hexane were shown to cause mutations in *mexR*, increasing expression of MexAB-OprM in *P. aeruginosa* (Li et al. 1998). These solvent-induced mutations were directly linked to antibiotic resistance, as the mutant strains were more antibiotic resistant than the wild type.

It is extremely important to understand the effects of contamination on these RND pumps and their interplay with antibiotic resistance. The majority of research on this has examined the common soil organisms, *P. putida* and *P. fluorescens* (Fernandes et al. 2003). However, very little work has been done with *P. aeruginosa*. As *P. aeruginosa* is the only human pathogen of these common soil Pseudomonads, this lack of research hinders understanding on the development, transmission and survivability of pathogens in the environment.

Previous research has shown that genes coding for the RND family pump MexAB-OprM were upregulated in *P. aeruginosa* by the common pollutant, pentachlorophenol (PCP) (Fraga Muller et al. 2006). As this was the first report of contaminant facilitated induction of *mexAB-oprM*, it was important to examine if it resulted in antibiotic resistance as well. The research reported here shows that this upregulation does result in multi-drug resistance (MDR). Long-term exposure to PCP, however, did not result in a stable antibiotic resistant phenotype. This may be due to a direct result of PCP exposure on expression of the pumps as PCP-induced expression of *mexB* was reversible upon the removal of PCP. To expand the research to organic contamination in general, the effect of three different types of organic contaminants on antibiotic resistance was investigated. Antibiotic resistance did increase; however, it occurred to a lesser extent than with PCP and was not dependent upon the MexAB-OprM pump. These results are discussed with the suggestion that organic contaminants may enhance the survivability of MDR strains in the natural environment.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Wild type (PAO1) and a MexAB-OprM deficient mutant (PAO200) *P. aeruginosa* strains were obtained from the lab of H. P. Schweizer (Schweizer 1998). Prior to every experiment, cells were re-constituted from frozen stock onto Luria-Burtani agar plates (LB, Lennox; Fisher Scientific, Atlanta, GA) and grown up at 37^{o}C. Colonies were selected and grown in either LB broth or a previously described minimal medium (MM) (Sayli et al. 2003), depending on the experiment.

Chemicals. All chemicals used for media preparation were laboratory grade or higher (Fisher Scientific, Atlanta, GA). Technical grade pentachlorophenol was purchased from Sigma (Sigma-Aldrich, Inc., St. Louis, MO). A stock concentration of 3.5 g/L was made in sterile nanopure water. PCP was solubilized by the addition of 36 mM NaOH. 2,4-dinitrophenol stock was made to a 50 mM concentration, solubilized by the addition of 4.8 mM NaOH and filter sterilized. 1 M benzoate and catechol stocks (laboratory grade, Fisher Scientific) were made weekly, filter sterilized, and stored in the dark. Roundup® (Ready-to-use Plus, Monsanto Chemical, St. Louis, MO) was purchased from Heavener Hardware, Blacksburg, VA, and filter sterilized. Ready-to-use Plus Roundup® contains 2 % glypohosate, 2 % pelargonic acid and fatty acids, and 96 % other ingredients. All antibiotics except carbenicillin were purchased as powders (Biotech grade, Fisher Scientific), made into aqueous stocks, filter sterilized, divided into single use aliquots, and frozen until just prior to use. Naladixic acid was solubilized with the addition of 2.5 % sodium hydroxide, and chloramphenicol was solubilized in 100 % methanol. Carbenicillin was purchased as a 50 mg/mL solution (K-D Medical, Inc., Columbia,

MD). For the disk diffusion assays, the following antibiotic disks were purchased from Becton-Dickson (BBL Sensi-Disc Antimicrobial Susceptibility Test Disks, Becton-Dickson, Franklin Lakes, NJ): Amikacin AN-30, Carbenicillin CB-100, Chloramphenicol C-30, Ciprofloxacin CIP-5, Erythromycin E-15, Gentamicin GM-100, Imipenem IPM-10, Piperacillin PIP-100, Tetracycline Te-30, and Tobramycin NN-10.

Antibiotic susceptibility testing. The resistance of strains to antibiotics was tested by the common MIC assay and disk diffusion assay. Briefly, for the MIC assay, 96-well polystyrene plates (Corning Life Sciences, Corning, NY) were set-up to include triplicate wells of eight concentrations of antibiotics (two-fold serial dilutions) in 50 μL LB broth. Cells in mid-log phase growth were added in an additional 50 μL of LB broth (with or without chemical contaminant addition) to a final concentration of approximately 5×10^5 cells/mL. Plates were then incubated at 37^{o}C without shaking for 18 hours, and the optical density read at 600 nm. The minimum concentration of antibiotic to inhibit growth (MIC) was recorded.

For the disk diffusion assay the protocol followed manufacturer's instructions closely. Overnight cultures of cells were re-inoculated in batch medium and grown to an optical density of 0.8 at 600 nm, corresponding to approximately a 0.5 McFarland Standard. Cultures were swabbed onto Mueller Hinton agar plates (Oxoid, Ltd., Hampshire, UK) that were prepared either with or without chemical contaminant additions. Within 10 minutes of the culture swab, antibiotic disks were applied. The plates were incubated for 24 hours at 37^{o}C, and zones of inhibition (diameter surrounding the disk) were measured in mm at 18 and 24 hours. To achieve an optimal lawn of growth in the presence of chemical contaminants, the 24-hour measurement was chosen

to represent the zone of inhibition. Student's t-tests were performed to test differences in antibiotic resistance between cells exposed to each chemical and the control. Differences were considered significant with a p-value ≤ 0.05.

Determination of antibiotic resistance with generational exposure to PCP. An experiment was designed to examine for adaptation to a stable MDR phenotype with long-term exposure to PCP. PAO1 was grown on MM in 50 mL liquid cultures at 20^{o}C (150 mL Erlenmeyer flasks), and aerated using 0.5 inch magnetic stir bars on a multi-position stir plate set to 650 rpm (Variomag, P15). The experiment was repeated twice with two control flasks (no chemical addition) and two PCP flasks (with 30 mg/L PCP) for each duplicate trial (n=4 control, n=4 PCP). Cells were transferred every 24 hours into fresh medium, and antibiotic resistance was determined approximately every five days (30 generations) by MIC assay. Cells were diluted 10^{-4} in LB during exponential phase to a concentration of approximately 5×10^{5} cells/mL. The MIC assay was performed as described above both with (MIC-PCP) and without (MIC) 30 mg/L PCP added to the assay for each culture. At various frequent intervals, cultures were streaked onto LB plates to ensure cultures remained pure. Cells were grown for approximately 650 generations (100 days) in PCP, after which the selection was removed for 10 days. MIC analysis was done twice in this 10 day period, and the selection was added back to the culture for another 10 days of growth in PCP before termination of the experiment. The non-parametric Wilcoxon rank sum analysis was performed to determine differences between the control and PCP samples.

Growth in presence of organic contaminants. Chemicals were chosen based on their environmental significance and their varied chemical nature. 2,4 Dinitrophenol (2,4-DNP), like PCP, is an uncoupler of oxidative phosphorylation, and is used in a variety of different industrially relevant processes, including manufacturing of wood preservative, dyes and insecticides. It is an aromatic compound with a lower lipophilicity than PCP ($\log K_{ow} = 1.67$). Benzoate and catechol are common metabolites of soil processes, from the breakdown of plant exudates, lignin, and aromatic amino acids. Benzoate is also used as a food preservative in high concentrations. Although both are aromatic compounds, they are highly soluble in water, unlike PCP and 2,4-DNP. Roundup® is a herbicide that is widely used in both commercial agriculture and at the household level. The active ingredient is glyphosate, a straight chain organic compound, similar in structure to the amino acid glycine. Common formulations used contain many other proprietary chemicals, including surfactants and fatty acids. The concentrations of Roundup® tested were approximated given instructions of use as recommended by the manufacturer (apply 22 fluid ounces/acre of a 48% glyphosate solution). Assuming the top one cm of a soil with 20% water content was saturated with the herbicide, the amount of Ready-to-use Roundup® was calculated as 2 μL/mL. To account for errors in assumptions, the concentrations tested ranged from 0.1 to 10 μL/mL.

To examine the effect of MexAB-OprM on growth in the presence of a variety of chemicals, PAO1 and PAO200 were grown in 4 mL cultures (10 mL cuvette) of MM at 37^{o}C, 200 rpm shaking. Growth was followed by measuring optical density at 600 nm. The growth rate was determined over the log phase of growth as the change in OD over change in time (Microsoft Excel, linear regression of OD_{600} vs. time plots, $R^2 > 0.9$).

Chemicals were screened over a range of concentrations, and differences in growth rates between PAO1 and PAO200 in duplicate cultures indicated an effect of MexAB-OprM on optimal growth in the presence of the contaminant.

Induction of *mexB* in the presence of organic contaminants. Batch cultures of PAO1 were grown in 100 mL of MM (250 mL Erlenmeyer flasks) at 30^{o}C, stirring at 650 rpm (Variomag, P15) with and without the following chemicals: 35 mg/L PCP, 5 mM 2,4-DNP, 25 mM benzoate, 10 μL/mL Roundup®. Triplicate experiments were done for each growth condition. Growth was monitored by measuring optical density at 600 nm. At early-log and late-log phases of growth (approximately 0.2 and 0.6 optical density at 600 nm, respectively), triplicate samples were taken and immediately treated with RNAprotect Bacterial Reagent (Qiagen Inc.-USA, Valencia, CA), and stored at -80^{o}C for later RNA extraction.

To determine if PCP-induced *mexB* expression is reversible, after reaching late-log phase, the cells grown in the PCP medium were pelleted (5000 x g, 10 minutes, 25^{o}C), washed with a 50 mM potassium phosphate buffer, and re-suspended in PCP-free medium to an optical density of 0.2. After approximately one-half hour of acclimation to the PCP-free medium, the early-log phase timepoint was taken for RNA analysis. The cells grew to late-log phase, samples were taken, the cells were re-pelleted, and then resuspended in fresh PCP-containing medium. Samples were again processed for RNA extraction at the early and late-log phases of growth.

The expression of *mexB* was determined by quantitative reverse trancription polymerase chain reaction (RT-PCR). Unpaired t-tests were performed to test differences

in expression between cells exposed to each chemical and the control. Student's t-tests were performed to test expression differences within the PCP exposed samples. Differences were considered significant with a p-value ≤ 0.05.

RT-PCR analysis of the *mexB* gene. RNA was extracted using the RNeasy kit with on-column DNase digestion (Qiagen Inc.-USA) from the pelleted cells within 12 hours of sample collection. Extracted RNA, eluted with 50 μL RNase-free water was immediately aliquoted and frozen at -80^{o}C until cDNA was ready to be prepared. All cDNA preparation and RT-PCR was done in the Core Laboratory Facility of the Virginia Bioinformatics Institute at Virginia Tech as previously described (Fraga Muller et al. 2006). Briefly, cDNA was made from 1 μg of the isolated total RNA using random hexamer primers and SuperScript II Reverse Transciptase (Invitrogen Company, Carlsbad, CA). The resulting cDNA was diluted 1:10 and used as template in RT-PCR with SYBR Green detection using a BioRad iCycle iQ Real-Time PCR Detection System (BioRad Laboratories, Hercules, CA). Triplicate RT-PCR reactions were performed for each cDNA, with the primers described previously (Fraga Muller et al. 2006). The starting quantity of template for each sample was determined using a six point standard curve. *mexB* expression was determined after normalization to the housekeeping gene *proC* (Sayli et al. 2003). Melting curves were analyzed at the end of each PCR run, and controls included PCR reactions without RNA and without the SuperScript II Reverse Transciptase.

RESULTS

MexAB-OprM-dependent antibiotic resistance increases in the presence of PCP.

To study the effects of PCP on antibiotic resistance, minimum inhibitory concentration (MIC) assays were done in the presence and absence of the chemical. Cells were grown in PCP-free medium prior to the assay, so any antibiotic resistance was due to upregulation within the assay. Table 4.1 shows a two- to eight-fold increase in resistance to a variety of different antibiotics for PAO1 in the presence of 30 mg/L PCP. The fold-increases in tetracycline, chloramphenicol, naladixic acid and carbenicillin are characteristic of a *nalC* strain (Cao et al. 2004). Gentamycin was used a negative control, as it is not effluxed by MexAB-OprM. Furthermore, the *mexAB-oprM* deletion mutant strain did not show any increase in antibiotic resistance (Table 4.1), confirming that this pump is responsible for the PCP-induced resistance enhancement.

Table 4.1. Antibiotic resistance increases in the presence of PCP.

Antibiotic	PAO1			PAO200		
	MIC (μg/mL)		fold change	MIC (μg/mL)		fold change
	no addn	+ PCP	with PCP	no addn	+ PCP	with PCP
Tetracycline	8	32	**4**	0.25-0.5	0.25-0.5	**-**
Chloramphenicol	8-16	64	**4-8**	0.5	0.5	**-**
Naladixic acid	32	256	**8**	2-4	4	**0-2**
Ciprofloxacin	0.05-0.1	0.2	**2-4**	0.00625	0.00625	**-**
Carbenicllin	32	128	**4**	0.5	0.5	**-**
Gentamycin	4	2-4	**-2-0**	1	0.5-1	**-2-0**

Continuous growth in the presence of PCP does not result in a stable MDR phenotype.

To determine the environmental relevance of PCP-induced antibiotic resistance, cells were grown for over 600 generations in the presence and absence of 30 mg/L (113 μM) PCP. Although the experiment was done with batch flasks, not in continuous culture, cells were grown in a minimal medium and at 20^{o}C to keep growth conditions closer to those that might be found in a natural soil or water. Antibiotic resistance was assayed approximately every 30 generations by MIC analysis in the presence (MIC-PCP) and absence (MIC) of PCP. It was expected that cultures grown in the presence of PCP would show stable increased antibiotic resistance, similar to a *nalC* phenotype. However, results show (Table 4.2) that there was no significant difference in the MICs of populations exposed to PCP when compared with populations that were grown without any chemical addition. For clarity, only MIC results for assays done in the absence of PCP are shown. The MIC-PCP results also do not show any difference between cultures grown without selection and cultures selectively grown on PCP (data not shown). The PCP selection was removed after approximately 620 generations, then applied again at approximately 685 generations (65 generations after removal) to determine if removal of the selection changed the resistance phenotype (Table 4.2). There was no discernable effect seen, and it can be concluded that, under these selective conditions, PCP exposure does not result in a stable antibiotic resistant phenotype.

PCP-induced expression of *mexB* is reversible.

The absence of a stable antibiotic resistant phenotype after more than 600 generations in selection was surprising. In an attempt to explain this, an experiment was designed to

Table 4.2. Generational exposure to PCP does not result in adaptation of an antibiotic resistant phenotype.

	MIC[c] (μg/mL)											
antibiotic[a]:	**TET**		**CAM**		**NAL**		**CIP**		**CAR**		**GM**	
Number of generations	NA[b]	PCP	NA	PCP	NA	PCP	NA	PCP	NA	PCP	NA	PCP
0	8	8	16	16	32	32	0.1	0.1	32-64	32-64	4	4
120	8-16	8	16	16	32	32	0.05-0.1	0.05	32	32	4	4
224	8-16	8	8-16	8-16	32	32	0.05	0.05	32	16-32	2-4	2-4
306	4-8	4-8	16-32	8-16	16-32	16-32	0.05-0.1	0.05	32	32	2	2
413	8	8	16	16	32-64	64	0.05-0.1	0.05	32	32-64	1-2	2
592	8-16	8-32	16-32	16-64	32-64	32-64	0.05	0.05	32-64	32-64	2	1-2
PCP removed from the growth medium												
650	8-16	4-8	16-64	16-32	32-64	32-128	0.05	0.05	32-64	32-64	2	2
683	4-16	4	8-16	8-16	32-64	32-64	0.05	0.05	16-32	32	2	2
PCP added back to the growth medium												
709	8	8	8-16	8-16	32-64	64	0.05	0.05	16-32	32-128	1-2	2
731	8-16	8-16	8	8	32-64	64	0.05	0.05	32	32-64	2	2

[a]TET, tetracycline; CAM, chloramphenicol; NAL, naladixic acid; CIP, ciprofloxacin; CAR, carbenicillin; GM, gentamycin.
[b]Indicating addition to growth medium; NA= no additions, PCP = 30 mg/L pentachlorophenol. [c]The numbers reported are from four biological experiments, MICs were done in triplicate for each trial at each generation time (n=12).

examine whether the PCP-induced expression of *mexAB-oprM* can be reversed. Cultures were grown in minimal medium in the presence and absence of PCP and RT-PCR targeting *mexB* was performed on RNA isolated at the early- and late-log phases of growth. As expected, PCP induced expression of *mexB* (Figure 4.1A) four fold over cells grown without PCP (p-values 0.0003, 0.015 at early- and late-log phase, respectively). When PCP was removed from the environment (Figure 4.1B), the expression was reduced to levels approximately four-fold lower than the cultures that were initially free of PCP (p-values 0.01, 0.003 at early- and late-log phase). The levels increased again when PCP was added back to the cells, this time ten-fold at early-log phase and three-fold at late-log phase (p-values 0.003, 0.02).

MexAB-OprM is required for optimal growth in the presence of organic pollutants. MexAB-OprM is known to efflux many different types of compounds. To investigate the potential role of MexAB-OprM in survival of *P. aeruginosa* in contaminated environments, growth was examined in the presence of different concentrations of various organic chemicals. Growth of PAO1 and PAO200 was examined in five different concentrations of these chemicals by tracking the optical density (600 nm) over time. Growth rates were determined during the log phase of growth as a change in optical density over time (linear regression of OD_{600} vs. time plots, $r^2 > 0.9$). Figure 4.2 shows the growth rates of PAO1 and PAO200 on the highest concentration of each chemical tested. With all compounds, MexAB-OprM was required for optimal growth in at least the two highest concentrations (data not shown, Figure 4.2). In fact, PAO200 was unable to grow in 5 mM catechol.

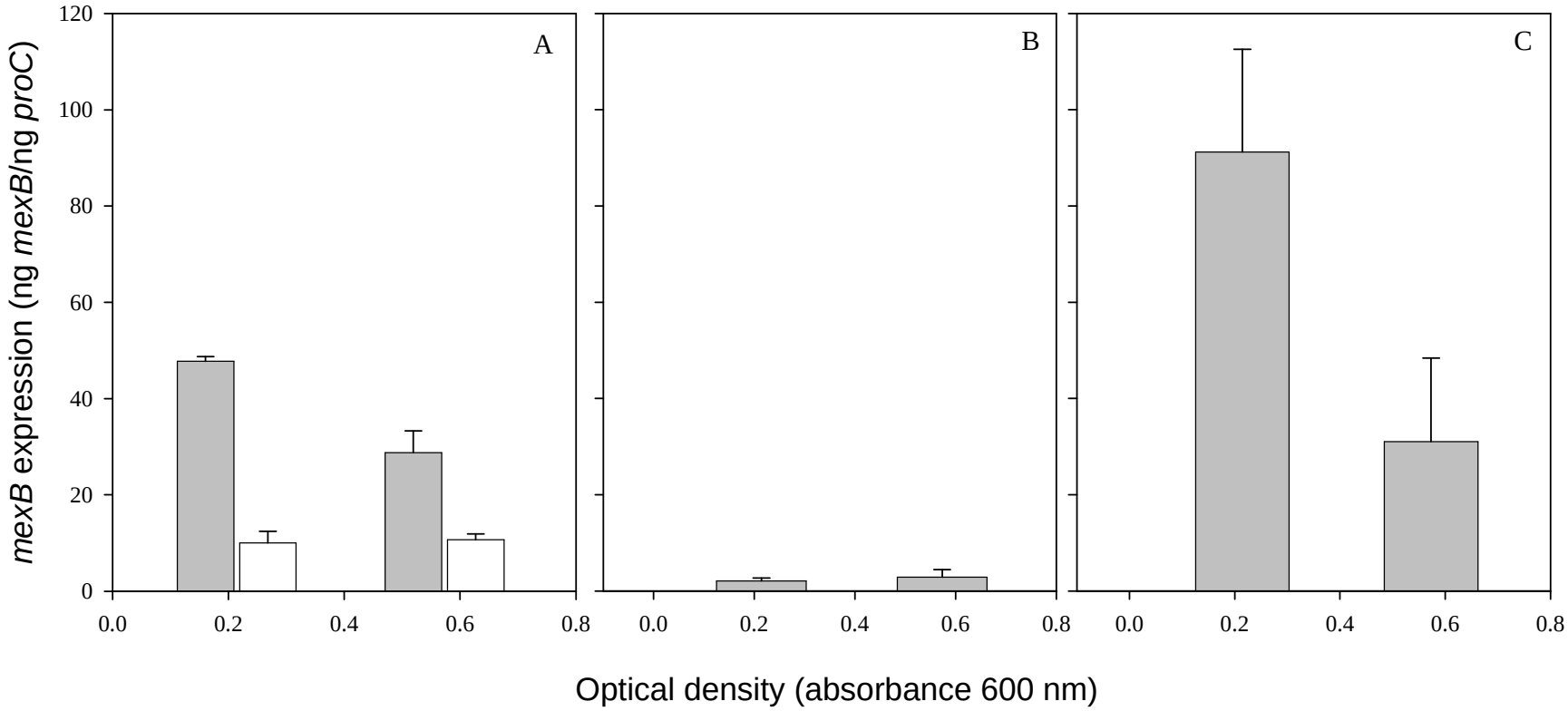

Figure 4.1. Induction of *mexB* by pentachlorophenol. A . cells grown in the presence of PCP; cells grown without PCP; B. PCP-grown cells washed and re-suspended in PCP-free medium; C. cells re-suspended in PCP medium. The experiment was repeated in triplicate and triplicate RT-PCR reactions were done at each timepoint. The average and standard deviation of normalized *mexB* expression is shown at each timepoint.

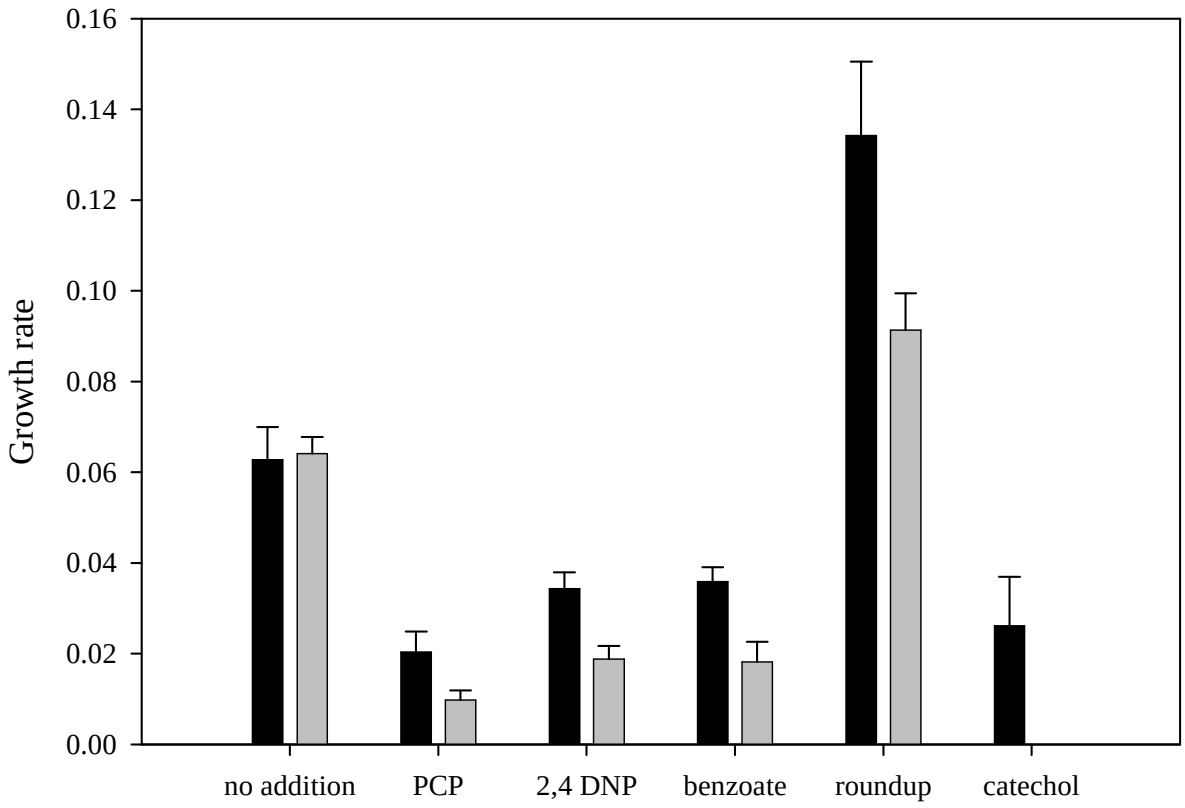

Figure 4.2. Growth rates of PAO1 ■ and PAO200 ▒ in the presence of various organic chemicals. Concentrations are 120 μM PCP, 5 mM 2,4-DNP, 25 mM benzoate, 10 μL/mL roundup, 5 mM catechol. The average of duplicate biological experiments is shown with error bars representing the maximum growth rate.

MexAB-OprM-independent antibiotic resistance increases in the presence of organic contaminants.

As MexAB-OprM was required for optimal growth in the presence of these compounds, it was hypothesized that they may also actively stimulate expression of the pump and result in increased antibiotic resistance. This was examined using both the MIC assay in the presence/absence of the potentially inducing chemical contaminant and disk diffusion assays. Catechol was not tested because cultures could not grow in the rich assay medium with catechol addition. With the disk diffusion assay (Table 4.4), a significant difference in antibiotic resistance was seen for at least three antibiotics with each chemical treatment. In particular, all tested chemicals showed practically no zone of clearing around both tetracycline and chloramphenicol (note that 6 mm is the diameter of the disk). When PAO200 was plated for the disk diffusion assay, the only significant difference was with benzoate grown cells and imipenem resistance (data not shown). This is consistent with other research which suggests that benzoate-induced imipenem resistance is due to downregulation of the membrane porin OprD, and not to efflux (Ochs et al. 1999).

Table 4.4. Zone of inhibition decrease for PAO1 in the presence of organic contaminants

	Zone of inhibition in mm (*p-value*)[a]				
chemical / **antibiotic**	na[b]	35 mg/L PCP	5 mM 2,4 DNP	50 mM Benzoate	10 µL/mL Roundup
Tetracycline	12.4	6.0 *(0.0004)*	7.8 *(0.0004)*	6.0 *(0.0004)*	6.0 *(0.0004)*
Chloramphenicol	13.0	6.0 *(0.0004)*	6.0 *(0.0004)*	6.0 *(0.0004)*	6.0 *(0.0004)*
Ciprofloxacin	32.0	27.3 *(0.011)*	28.5 *(0.0383)*	29.1 *(0.0431)*	
Carbenicillin	21.3	16.3 *(0.003)*			
Piperacillin	27.6	24.3 *(0.002)*			
Gentamycin	17.1	19.0 *(0.0006)*	19.8 *(0.0376)*		19.3 *(0.001)*
Imipenem	24.8			11.4 *(0.0003)*	

[a]For chemical additions, the mm values are followed by Student's t-test significance p-value in parentheses reflecting the difference from control (n=4). Disk diameter is 6 mm.
[b]control, na=no chemical addition.

While the disk diffusion results suggest that MexAB-OprM might be involved in antibiotic resistance, the MIC results do not support this conclusion (Table 4.5). Although there was a slight increase in antibiotic resistance with the chemicals for a few different antibiotics, the MIC results show that it was not dependent upon MexAB-OprM. With 2,4-DNP, the increase in resistance to tetracycline and naladixic acid is the same for both PAO1 and PAO200. For benzoate, there are slight increases in all antibiotics tested except carbenicillin. The increase for chloramphenicol was the same in both strains, and the increase in naladixic acid was higher in PAO200, indicating that the MexAB-OprM pump is not involved in the resistance to these antibiotics. Also, resistance to gentamicin, which is not effluxed by this particular pump, increased with benzoate in the WT relative to the *mexAB-oprM* mutant. Roundup® exposure caused a slight but repeatable increase in antibiotic resistance to both chloramphenicol and naladixic acid, although the increased resistance to naladixic acid was also seen in PAO200. Altogether, these results suggest that the antibiotic resistance increase observed in PAO1 when grown on solid medium in the presence of these contaminants is not dependent upon MexAB-OprM.

mexB expression is not induced by 2,4-dinitrophenol, benzoate, or Roundup®.

In order to verify that MexAB-OprM expression was not induced by 2,4-dinitrophenol, benzoate or Roundup®, a final study was done to examine *mexB* expression with *P. aeruginosa* grown in the different contaminants. RNA was extracted at the early- and late-log phases of growth from cells grown in the presence of 5 mM 2,4-DNP, 50 mM benzoate, and 10 μL/mL Roundup®. Using RT-PCR to examine *mexB* expression,

Table 4.5. MIC increase in the presence of organic contaminants

Antibiotic	PAO1 MIC (μg/mL)	PAO1 fold increase	PAO200 MIC (μg/mL)	PAO200 fold increase
		Control (no contaminant)		
Tetracycline	4-16		0.25-0.5	
Chloramphenicol	8		0.25-0.5	
Naladixic acid	32-64		2	
Ciprofloxacin	0.05		0.005-0.01	
Carbenicllin	32		0.5	
Gentamycin	2-4		0.5-1	
		5mM 2,4 Dinitrophenol		
Tetracycline	16	2-4	0.5-1	2
Chloramphenicol	8		0.5-1	2
Naladixic acid	128-256	4-8	8	4
Ciprofloxacin	0.05-0.1		0.005-0.01	
Carbenicllin	32		0.5	
Gentamycin	0.25	-4 to -8	0.0625	-16
		50mM benzoate		
Tetracycline	8-16	0-2[a]	0.25-0.5	
Chloramphenicol	16	2	0.25-0.5	0-2
Naladixic acid	64-128	2	16	8
Ciprofloxacin	0.1	2	0.005	
Carbenicllin	32		0.5	
Gentamycin	4	2	1	
		10μL/mL Roundup®		
Tetracycline	8		0.125-0.25	-2 to 0
Chloramphenicol	16	2	0.125-0.25	-2
Naladixic acid	64	0-2[b]	4-8	2-4
Ciprofloxacin	0.05		0.005	
Carbenicllin	32		<0.125-0.25	-2 to -4
Gentamycin	2		0.5-1	

[a] In 0-fold change MIC assays, a 2-fold increase in OD_{600} was seen at highest growth concentration.
[b] In 0-fold change MIC assays, a 10-fold increase in OD_{600} was seen.

results showed that there was a slight decrease in expression when comparing the cells exposed to each chemical with the control cells (Figure 4.3). With 2,4-DNP, expression was increased at early log phase (p-value 0.03) and decreased at late log phase (p-value 0.02) in comparison with the control cells grown without chemical addition. Cells grown in benzoate and Roundup® showed decreased expression at late-log phase growth (p-value, 0.013 and 0.009, respectively).

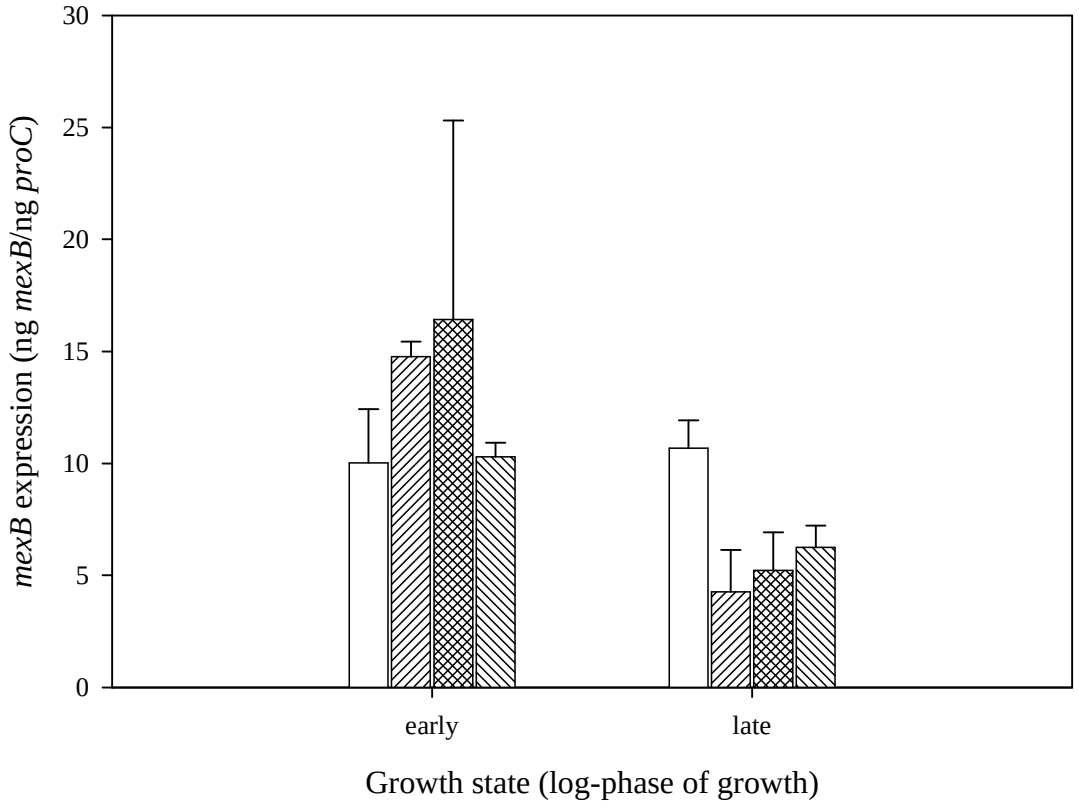

Figure 4.3. Expression of *mexB* in PAO1 grown with different organic contaminants. Expression levels are shown at different stages of log phase for control ☐, 5 mM 2, 4-DNP ▨, 50 mM benzoate ▩, and 10 µL/mL Roundup ▧. Triplicate biological experiments were done with triplicate RT-PCR at each timepoint. Shown are the average levels of expression normalized to *proC*, along with standard deviation.

DISCUSSION

A recent study by Fraga-Muller et al. revealed an increase in gene expression of multidrug efflux systems when chemostat-grown *P. aeruginosa* was exposed to PCP (Fraga Muller et al. 2006). Here, it is demonstrated in batch cultures that this increase in MexAB-OprM causes a simultaneous increase in antibiotic resistance. Furthermore, it is shown that the effects of PCP directly induce expression of the MexAB-OprM pump. Although other RND pumps are known to be induced by antibiotics and/or organic solvents, this is the first report of anthropogenic chemical induction of MexAB-OprM-mediated antibiotic resistance.

The multidrug resistance pattern shown by PCP induction is similar to that of a *nalC* phenotype (Srikumar et al. 2000). Previously, this phenotype was shown to be due to a mutation in PA3721, a probable repressor of the PA3720-PA3719 operon (Cao et al. 2004). In fact, it was the increased expression of PA3719 that caused over-expression of MexAB-OprM and the *nalC* multidrug resistant phenotype. In a microarray study on the effects of PCP stress on *P. aeruginosa*, PA3721-PA3719 was strongly upregulated upon exposure to the chemical (Fraga Muller et al. 2006). Although this was not further investigated, it is possible that the similarity in PCP-induced antibiotic resistance profile with the *nalC* mutant is due to increased expression of *mexAB-oprM* via increased expression of PA3719. Interestingly, a BlastP search reveals that PA3719 is found only in *P. aeruginosa* strains, and has no known homology with any protein (as of July 15, 2006).

MexAB-OprM was previously shown to play a role in organic contaminant efflux, when it was demonstrated to be responsible for the efflux of *p*-xylene and hexane (Li et al. 1998). Increased expression of the efflux pump in a solvent-tolerant mutant was necessary for growth on *p*-xylene; however, growth in hexane was achieved to a similar extent in both the wild type and solvent-tolerant mutants (Li et al. 1998; Li and Poole 1999). In these studies, *P. aeruginosa* transferred consecutively in medium containing increasing concentrations of the solvents consistently evolved MexAB-OprM over-expressing mutants. The mutation was found to be in *mexR*, the gene coding for the repressor of *mexAB-oprM* transcription. The authors suggested that environmental contamination plays a role in the development of MDR strains. Indeed, antibiotic resistance was increased in the solvent-tolerant mutants after selection.

PCP is also a relevant environmental contaminant; therefore, it was hypothesized that selection on PCP would result in a stable MDR phenotype. The experiment described here investigated generational exposure to a single sublethal concentration of PCP in a minimal medium with acetate as the sole carbon source. After more than 600 generations, selection on PCP did not result in adaptation to a MDR phenotype. This result was very surprising, given the length of the experiment. However, if the mechanism of PCP-induced expression of MexAB-OprM is closer to the intended function of this pump, it is not unexpected. To investigate this further, an experiment was designed to determine whether PCP-induced expression of *mexAB-oprM* is easily reversible. RT-PCR targeting a portion of *mexB* showed that the increased expression of cells induced with PCP is quickly reversed, and *mexB* expression is reduced to even below basal levels if PCP selection is removed. This expression was then again easily

induced when the cells were put back into fresh medium containing PCP. These results conclusively demonstrate that PCP, either directly or indirectly, induces expression of MexAB-OprM.

This research clarifies the first report of induction of MexAB-OprM by an organic contaminant, and the first report of induction of any efflux pump by a chlorinated aromatic compound (Fraga Muller et al. 2006). Similar induction of RND efflux pumps by solvents has been shown in strains of *P. putida* (Kieboom et al. 1998; Rojas et al. 2001; Dominguez-Cuevas et al. 2006). The aromatic solvents toluene, *m*-xylene, styrene, ethylbenzene and propylbenzene, in particular, are known to induce expression of two RND pumps that participate in the efflux of antibiotics (Rojas et al. 2001; Dominguez-Cuevas et al. 2006). With a log K_{ow} (octanol:water partition coefficient) between 2.7 and 3.9, the toxic effect of these solvents is targeted to the cell membrane; therefore, a system within the membrane to recognize and efflux these compounds is an effective way to detoxify the cell. PCP is also an aromatic compound with a high log K_{ow} (5.15) thought to exert its toxic effect on the cell membrane (Weinbach and Garbus 1965; Escher and Schwarzenbach 1996). In *P. putida*, the pump TtgABC is important for efflux and tolerance to all solvents listed above, and it is the RND pump in this organism that shows the most homology with MexAB-OprM. TtgA, TtgB, and TtgC show 68%, 77% and 63% identity with MexA, MexB and OprM, respectively.

With the similarities of the two pump systems and the chemical actions on the cells, it is reasonable to assume that more hydrophobic or aromatic contaminants may induce expression of MexAB-OprM in *P. aeruginosa*. This was tested by looking at chemicals with a broad range of characteristics. 2,4-DNP is an uncoupler of oxidative

phosphorylation, and like PCP and aromatic solvents, exerts its toxic effect by embedding into the cell membrane. Benzoate and catechol are aromatic compounds that are toxic to cells in high concentrations. Both are intermediates in the degradation of many aromatic amino acids and plant exudates and are therefore important compounds in natural biodegradation processes. Roundup® was chosen as a contaminant due to its widespread use as an herbicide throughout the world. Although its active ingredient, glyphosate, is not an aromatic compound, it was of interest to examine the effects of a common aliphatic herbicide on antibiotic resistance.

As this study was particularly focused on the MexAB-OprM efflux pump, these chemicals were first screened for their ability to affect growth of a MexAB-OprM mutant strain. All chemicals tested did show a reduction in growth when comparing the wild type with the mutant, demonstrating that the constitutive expression of this pump is indeed necessary for growth in the presence of these diverse contaminants. However, tests on the effects of these chemicals on antibiotic resistance, along with levels of *mexB* during growth in the presence of the chemicals, showed that MexAB-OprM is not induced. The results of the disk diffusion assay were slightly contradictory to this conclusion as wild type cells did increase antibiotic resistance in the presence of the chemicals, and the mutant strain did not. However, these results can be explained by the reduced growth of the mutant that is not expressing the constitutive, wild type, levels of MexAB-OprM. Indeed, PAO200, with the majority of combinations of antibiotics and chemicals showed substantial growth of additional mutant colonies inside the original zone of inhibition within 48 hours (data not shown). It is possible that these additional mutants grew more readily in the MIC liquid assay, resulting in no difference between

PAO1 and PAO200 in the fold change of antibiotic resistance to the presence of the contaminants. However, this cannot be confirmed from the results of this study.

Although MexAB-OprM-induced MDR in *P. aeruginosa* was shown only with PCP, the increased resistance with the other contaminants could be due to multiple factors, including changes in membrane porin expression and changes in other efflux pumps (Kumar and Schweizer 2005). For example, it has been shown previously that benzoate induces imipenem resistance through downregulation of the porin OprD (Ochs et al. 1999). Interestingly, the stretch of the genome containing benzoate and catechol dioxygenases (PA2507-PA2519) is adjacent to three RND operons (*mexEF-oprN*, *czrABC*, and PA2525-PA2528) and an OprD-family porin, OpdT (PA2505). Increased expression of either *mexEF-oprN* or *czrABC* result in decreased expression of OprD (Ochs et al. 1999; Perron et al. 2004). It is known that the benzoate-induced repression of OprD is not related to *mexEF-oprN* expression (Ochs et al. 1999), and it is thought that there is some unknown regulator affected by benzoate. Of further interest is the recent finding by Sobel et al. that an oxidoreductase (PA2491) just upstream of *mexT* (the positive regulator of *mexEF-oprN*) is involved in mediating expression of this pump (Sobel et al. 2005). The authors note that the regulation of efflux genes by an oxidoreductase suggests that efflux and detoxification of cellular metabolites are highly integrated. This seems like a very plausible analysis, as it is well known that metabolites produced by the benzoate degradation pathway, catechol in particular, can create toxic intermediates. The involvement of a heavy metal efflux pump (*czrABC*) may be an evolved mechanism to deal with the production of reactive oxygen species by catechol in the presence of heavy metals (Schweigert et al. 2001).

In general, the increased antibiotic resistance shown when *P. aeruginosa* is exposed to various organic contaminants is a result that has great importance to understanding the survival of this pathogen in the environment. Recent studies examining the diversity of a wide variety of isolates of *P. aeruginosa* found that clinical strains are distributed evenly throughout the environment (Pirnay et al. 2002; Pirnay et al. 2005). With this, the authors caution that any strain of *P. aeruginosa* should be considered a potential pathogen. As organic contaminant-assisted changes in cell physiology, including upregulation of efflux pumps and changes in cell membrane structure, show increased antibiotic resistance, it is entirely possible that this same contamination can assist in the development of MDR *P. aeruginosa* in the environment. Alternatively, and maybe of even greater relevance, is the probability that organic contamination can assist in the survival of MDR strains of *P. aeruginosa* that are released to the environment.

Antibiotic resistance is one of the most pressing public health problems worldwide. As economically-developing countries strive to deal with the high death rate from water-borne pathogens, industrial waste treatment may be seen as a lesser relevant goal. In economically developed countries, chemical contamination and public health water issues are seen as separate problems. Health effects of chemical contamination aside, this research adds to the growing amount of scientific evidence that the microbial ecology of chemically contaminated environments has real and significant implications for public health microbiology. To combat this growing problem of antibiotic resistance, it is extremely imperative to recognize and understand this relationship. Future research

needs to focus on examining the contribution of organic contamination to survival of MDR strains of *P. aeruginosa* and other important pathogens in the environment.

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Chapter 5. ENGINEERING SIGNIFICANCE

Natural soils, sediments and waters throughout the world are contaminated with toxic anthropogenic chemicals. For the protection of public and ecosystem health, remediation of these environments is essential. Engineering solutions rely on the actions of native microbial communities, the first responders to this contamination. Therefore, an understanding of the functional responses to contamination is required for engineering the most effective and efficient remediation solutions. This research examined genetic level functional responses of a common soil bacterium, *P. aeruginosa*, to a common environmental pollutant, PCP. The techniques used in this research allowed us to examine the stress response over the entire genome under nutrient-limiting environmentally relevant conditions. Through the use of these techniques, we found upregulation of multidrug efflux pumps in response to the PCP contamination. Results show that this upregulation is sufficient for maintenance of physiological state in *P. aeruginosa* and is the main protective mechanism under these nutrient-limiting conditions.

Multidrug efflux pumps are found in many different bacterial species with the ability to efflux many different toxic compounds such as metals, organic solvents and antibiotics. The results in the research show that if we are able to understand the regulation of these efflux mechanisms, we may be able to engineer suitable conditions where these pumps are upregulated and protecting the bacteria. With the efflux pumps upregulated, the bacteria are then free to perform normal metabolic functions. This could be especially relevant in bioreactors treating industrial toxic waste, in municipal

wastewater treatment plants treating industrial waste, or in engineering bioremediation systems.

Most importantly, this is the first finding of an anthropogenic chemical induction of the multidrug efflux pump, MexAB-OprM. Further investigation of this phenomenon showed that the upregulation also results in increased antibiotic resistance. This is a direct link between environmental microbiology and public health microbiology and implies that organic contamination may result in either the development of antibiotic resistant strains or allow survival of multidrug resistant strains when released to the environment.

Given the widespread abundance of multidrug efflux pumps in bacteria, future research into the significance of this phenomenon is essential. Specifically, it is important to look at geographical instances of multidrug efflux expression and antibiotic resistance. These questions link public health microbiology with environmental microbiology, and deal directly with the current push to understand transmission of infectious diseases:

- Do efflux-mediated multidrug resistant (MDR) isolates of *P. aeruginosa* and other strains show increased survival in contaminated environments over their wild type counterparts?
- Is efflux gene expression upregulated in contaminated environments and do bacteria from these environments have increased antibiotic resistance?
- Can profiles of antibiotic resistance and multidrug efflux expression from clinical isolates be correlated geographically with profiles of isolates from contaminated water sources?

Understanding pathogen transport and survival in the natural environment is currently a major research need throughout the world. Each year in developing countries, more than 1.5 million children under the age of five die from diarrhea illnesses caused by

contaminated water and unsanitary living conditions. Although multiple epidemiological studies attribute a heterogeneous distribution of pathogens in the environment to these diseases, there is little knowledge about the presence, diversity and transmission of pathogenic bacteria in natural waters. This research contributes significantly to our understanding of the effect of contamination on antibiotic resistance, and demonstrates that public health relies on future studies investigating the link between microbial response to organic contamination and development of antibiotic resistance.

Appendix A

Data for Chapter 3

Figure 3.1

Data compiled in Excel file: *replicate PCP shock data_may_2_200*5

PCP concentration (mg/L), WT chemostat samples

	June 22, 2004		July 8, 2004		Sept 15, 2005		Nov 10, 2005	
hours post-PCP[a]	mg/L	S.D.[b]	mg/L	S.D.	mg/L	S.D.	mg/L	S.D.
7.02	16.45	1.03	13.64	0.49	16.08	0.60	11.97	0.86
13.31	34.11	2.20	26.24	1.27	24.02	1.63	18.37	0.39
26.19	39.25	1.89	38.71	1.48	30.96	2.16	24.72	1.63

[a]Hours are average of time for all experiments
[b]Standard deviation of triplicate biological samples from the chemostat at each timepoint. Data points in Figure 3.1 were generated as the average and standard deviation of all reactors runs.

PCP concentration (mg/L), RpoS$^-$ chemostat samples

	June 19, 2005		Sept 5, 2005	
hours post-PCP	mg/L	S.D.	mg/L	S.D.
7.46	16.45	1.03	13.64	0.49
13.92	34.11	2.20	26.24	1.27
26.55	39.25	1.89	38.71	1.48

Colony forming units (cells/mL), WT chemostats

July 8, 2004				June 22, 2004			
hours[a]	cells/mL[b]	S.D.	p-value[c]	hours	cells/mL	S.D.	p-value
-67.58	2.20E+08	3.27E+07		-62.5	2.61E+08	1.75E+07	
-42.33	2.10E+08	7.21E+06		-39.5	2.93E+08	1.29E+07	
-13.58	3.42E+08	6.81E+07		-12.75	3.57E+08	2.40E+07	
-2.33	2.93E+08	1.68E+07		-2	2.79E+08	1.14E+07	
6.5	1.75E+08	1.03E+07	0.0079	6.5	2.81E+08	3.78E+07	0.9630
12.92	1.89E+08	7.02E+06	0.0102	13.25	2.69E+08	2.60E+07	0.5739
26.17	2.06E+08	2.43E+07	0.0675	26	1.97E+08	6.43E+06	0.0147

[a]Hours post-PCP stress initiation
[b]Average of six plates counting CFU, with standard deviation (S.D.)
[c]Student's t-test p-value with comparison to timepoint just prior to PCP stress.

Colony forming units (cells/mL), WT chemostats

Sept 19, 2005				Nov 10, 2005			
hours	cells/mL	S.D.	p-value	hours	cells/mL	S.D.	p-value
-74.75	3.53E+08	3.36E+07		-68.25	5.00E+08	1.34E+08	
-45.5	3.23E+08	3.08E+07		-41.5	5.06E+08	4.62E+07	
-24.5	3.73E+08	1.92E+07		-18.25	3.91E+08	2.53E+07	
-1	2.30E+08	2.59E+07		-1	2.64E+08	1.44E+07	
6.5	2.14E+08	3.08E+07	0.3689	6.5	2.54E+08	4.00E+07	0.6389
13	1.23E+08	9.35E+06	0.0001	13.25	2.16E+08	2.89E+07	0.0125
25.5	7.96E+07	1.44E+07	0.0003	26.33	9.57E+07	2.27E+07	0.0000

Colony forming units (cells/mL), RpoS$^-$ chemostats

June 19, 2005				Sept 5, 2005			
hours	cells/mL	S.D.	p-value	hours	cells/mL	S.D.	p-value
-75	2.59E+08	2.53E+07		-65.25	3.06E+08	5.05E+07	
-46.25	1.83E+08	3.70E+07		-46.75	2.6E+08	2.71E+07	
-22.25	1.34E+08	2.19E+07		-22.5	2.21E+08	2.52E+07	
-2.08	1.42E+08	2.27E+07		-0.95	2.02E+08	4.72E+07	
6.5	1.35E+08	2.36E+07	0.4216	6.5	2.26E+08	3.14E+07	0.9270
12.75	1.5E+08	2.63E+07	0.2108	13	1.84E+08	3.60E+07	0.2030
25.92	1.2E+08	1.77E+07	0.0009	26	1.85E+08	1.99E+07	0.3690

Data for WAUR determination in WT chemostats

June 22, 2004

hours post-PCP	0		6.5		13		26	
	AUR[a]	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.3977	0.9476	0.4004	0.9939	0.4145	0.9937	0.2924	0.8345
B	0.3896	0.997	0.4423	0.9577	0.374	0.9878		
C	0.3504	0.9195	0.4631	0.9964	0.3388	0.9867	0.3561	0.9405
avg. CFU	2.79E+08		2.81E+08		2.69E+08		1.97E+08	
WAUR	1.36E-12		1.55E-12		1.40E-12		1.64E-12	
S.D.	9.06E-14		1.14E-13		1.41E-13			
p-value[b]			0.2309		0.4369		0.4331	

July 8, 2004

hours post-PCP	0		6.5		13		26	
	AUR	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.3402	0.978	0.4278	0.9512	0.3718	0.8532	0.2259	0.9052
B	0.3943	0.902	0.3536	0.8412	0.356	0.9766	0.2781	0.8484
C	0.4733	0.9904	0.3977	0.8987	0.3908	0.8811	0.3878	0.9753
avg. CFU	2.93E+08		1.75E+08		1.89E+08		2.06E+08	
WAUR	1.38E-12		2.24E-12		1.98E-12		1.44E-12	
S.D.	2.29E-13		2.13E-13		9.24E-14		4.01E-13	
p-value			0.0521		0.0301		0.5729	

Sept 19, 2005

hours post-PCP	0		6.5		13		26	
	AUR	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.3322	0.9868	0.3213	0.8636	0.1092	0.7809	0.205	0.902
B	0.3834	0.9596	0.269	0.8496	0.2404	0.8942	0.213	0.9744
C	0.31	0.984	0.3768	0.8302	0.1642	0.9672	0.1138	0.6285
avg. CFU	2.30E+08		2.14E+08		1.23E+08		7.96E+07	
WAUR	1.48E-12		1.50E-12		1.39E-12		2.23E-12	
S.D.	1.63E-13		2.52E-13		5.34E-13		6.92E-13	
p-value			0.9412		0.7354		0.1543	

Data for WAUR determination in WT chemostats (continued)

Nov 10, 2005

hours post-PCP	0		6.5		13		26	
	AUR	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.2949	0.6636	0.1891	0.7561	0.3073	0.8824	0.1405	0.9278
B	0.4165	0.9631	0.5322	0.9837	0.3128	0.8896	0.0934	0.6338
C	0.3364	0.8195	0.5663	0.9474	0.4526	0.9263	0.2937	0.9159
avg. CFU	2.64E+08		2.54E+08		2.16E+08		9.57E+07	
WAUR	1.32E-12		1.69E-12		1.65E-12		1.84E-12	
S.D.	2.34E-13		8.22E-13		3.81E-13		1.09E-12	
p-value			0.4435		0.3504		0.5354	

[a]Acetate uptake rate, mg/L acetate/minute. A, B, and C are replicate biological samples for each timepoint.
[b]Student's t-test p-value in comparison to time 0.

Data for WAUR determination in RpoS$^-$ chemostats

June 19, 2005

hours post-PCP	0		6.5		13		26	
	AUR	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.2658	0.9896	0.3343	0.9744	0.3645	0.9522	0.3638	0.6567
B	0.2444	0.9173	0.3072	0.9777	0.3016	0.9604	0.422	0.8489
C	0.2446	0.6915	0.3371	0.93	0.1997	0.9922	0.3404	0.9299
avg. CFU	1.42E+08		1.35E+08		1.50E+08		1.20E+08	
WAUR	1.78E-12		2.41E-12		1.93E-12		3.12E-12	
S.D.	8.69E-14		1.22E-13		5.55E-13		3.49E-13	
p-value			0.0108		0.6504		0.0257	

Sept 5, 2005

hours post-PCP	0		6.5		13		26	
	AUR	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**	**AUR**	**R^2**
A	0.3159	0.9522	0.3124	0.7303	0.4192	0.9702	0.3928	0.9625
B	0.2791	0.9903	0.4231	0.8756	0.3244	0.9631	0.3079	0.9317
C	0.2473	0.9464	0.3003	0.722	0.2668	0.9091	0.32	0.9511
avg. CFU	2.02E+08		2.26E+08		1.84E+08		1.85E+08	
WAUR	1.39E-12		1.53E-12		1.83E-12		1.83E-12	
S.D.	1.70E-13		3.00E-13		4.18E-13		2.48E-13	
p-value			0.5444		0.0928		0.0342	

Figure 3.2, Tables 3.2, 3.3, 3.4 (data found in Excel file: *GCOSs100_final_datab*)

Affymetrix GCOS data for genes increasing in response to PCP

Time 0 hours

	WTA[a] 0 hours						WTB 0 hours					
	Detection[b]			Change[c]			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0204_at	22.6	P	0.0054				20.8	A	0.4034			
PA0246_at	25.3	P	0.0139				18.5	A	0.2315			
PA0355_pfpI_at	23.9	A	0.0665				3.6	A	0.7892			
PA0417_at	27.4	A	0.0865				16.1	A	0.4861			
PA0424_mexR_at	89	P	0.0320				142.4	A	0.2108			
PA0425_mexA_at	225.4	P	0.0007				269	P	0.0036			
PA0426_mexB_at	233.7	P	0.0007				251.2	P	0.0029			
PA0427_oprM_at	222.3	P	0.0007				140.8	P	0.0015			
PA0445_s_at	55.3	P	0.0015				80.9	P	0.0232			
PA0526_at	14.4	P	0.0007				8.4	A	0.3001			
PA0633_at	71.7	P	0.0007				27.7	A	0.0760			
PA0726_at	49.2	P	0.0054				5	A	0.6999			
PA0868_at	47.6	P	0.0273				9.8	A	0.5139			
PA1230_at	18.4	P	0.0434				7.8	A	0.5417			
PA1281_cobV_at	41.9	P	0.0015				27.3	A	0.0760			
PA1393_cysC_at	15.4	P	0.0232				3.2	A	0.7466			
PA1705_pcrG_i_at	35.9	P	0.0012				97.2	P	0.0239			
PA1780_nirD_at	20	P	0.0036				4.6	A	0.5139			
PA1982_exaA_at	44.1	P	0.0139				14.4	A	0.5693			
PA2166_at	41.4	P	0.0096				23.7	M	0.0503			
PA2394_at	15.2	A	0.1912				2.8	A	0.7685			
PA2397_pvdE_at	15.9	P	0.0012				40.7	A	0.0760			
PA2398_fpvA_at	15	P	0.0054				44.3	A	0.0759			
PA2669_at	10	P	0.0374				4	A	0.8606			
PA2906_at	35.1	P	0.0080				14.8	A	0.3504			
PA2941_at	56.6	P	0.0320				22.5	A	0.3001			
PA3136_at	20.1	A	0.0980				51.5	M	0.0503			
PA3137_at	16.7	A	0.1107				27.6	A	0.4583			
PA3278_at	18.8	P	0.0012				14	A	0.5417			
PA3369_at	117	P	0.0009				157.3	P	0.0023			
PA3370_at	34.7	P	0.0320				36.8	A	0.4583			
PA3371_at	35.6	P	0.0320				34.8	A	0.3001			
PA3547_algL_at	20.2	A	0.0760				1.7	A	0.8274			
PA3676_at	47.1	P	0.0139				64.2	A	0.0760			
PA3677_at	25.7	A	0.1244				57.4	A	0.3766			
PA3678_at	39.5	P	0.0007				62.9	M	0.0579			
PA3679_at	55	P	0.0007				17.9	A	0.1554			
PA3719_at	62.3	P	0.0374				54.8	A	0.3766			
PA3720_at	61.9	P	0.0007				78	P	0.0029			
PA3721_at	31	P	0.0015				44.1	A	0.1727			
PA3954_at	15	P	0.0165				8.7	A	0.4861			
PA4033_at	27.5	P	0.0116				22.8	A	0.1912			

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	WTA 0 hours						WTB 0 hours					
	Detection[b]			Change[c]			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA4037_at	12.3	P	0.0320				3.8	A	0.8273			
PA4178_at	17.3	P	0.0374				22.8	A	0.2762			
PA4217_at	29	P	0.0036				19	A	0.3504			
PA4344_at	27.6	P	0.0066				7.5	A	0.5693			
PA4738_at	72.1	P	0.0116				145.5	A	0.0865			
PA4739_at	140	P	0.0007				85.9	P	0.0012			
PA4816_at	30.9	P	0.0044				27.9	A	0.1912			
PA4981_at	24.2	P	0.0434				26.7	A	0.1912			
PA5157_at	37.3	P	0.0139				10.2	A	0.6752			
PA5158_at	46.3	P	0.0029				73.3	P	0.0196			
PA5159_at	25.2	P	0.0116				62.8	P	0.0374			
PA5160_at	44.1	P	0.0374				60.7	P	0.0165			
PA5216_at	27.9	A	0.0760				4.5	A	0.7685			
PA5326_at	18	A	0.0665				19.4	A	0.5417			
PA5390_at	19.9	A	0.0980				2.6	A	0.8273			
PA5407_at	30.1	P	0.0007				24.9	A	0.1912			
PA5469_at	15.9	P	0.0273				2.5	A	0.9240			
PA5481_at	27.1	P	0.0374				14.7	A	0.6752			
PA5482_at	77.9	P	0.0023				28.7	A	0.3766			

Time 6.5 hours

	WTA 6.5 hours						WTB 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0204_at	93.9	P	0.0009	1.7	I	0.0000	66.5	P	0.0434	1.4	I	0.0025
PA0246_at	73.6	P	0.0015	1.5	I	0.0001	43.2	P	0.0374	0.9	NC	0.3376
PA0355_pfpI_at	74.4	P	0.0007	1.8	I	0.0000	68.4	P	0.0434	3.8	I	0.0001
PA0417_at	105.9	P	0.0066	1.4	I	0.0000	29.8	A	0.3766	0.5	NC	0.1292
PA0424_mexR_at	146.6	P	0.0019	1.1	I	0.0000	315.3	P	0.0096	1.5	I	0.0000
PA0425_mexA_at	387.2	P	0.0007	0.7	I	0.0000	578.5	P	0.0007	1.2	I	0.0000
PA0426_mexB_at	439.8	P	0.0007	0.9	I	0.0002	608.6	P	0.0007	1.9	I	0.0000
PA0427_oprM_at	513.9	P	0.0009	0.8	I	0.0000	914.6	P	0.0007	2.4	I	0.0000
PA0445_s_at	257.3	P	0.0007	2.1	I	0.0000	110.1	P	0.0139	0.6	I	0.0020
PA0526_at	83.1	P	0.0015	2.7	I	0.0000	58.6	P	0.0054	1.9	I	0.0007
PA0633_at	155.9	P	0.0007	1.1	I	0.0000	65.7	P	0.0054	1.5	MI	0.0053
PA0726_at	199.5	P	0.0007	2	I	0.0000	79.9	P	0.0165	3.2	I	0.0002
PA0868_at	58.3	P	0.0007	0.5	I	0.0000	101.6	P	0.0116	2.9	I	0.0003
PA1230_at	117.5	P	0.0015	2.3	I	0.0000	55.6	A	0.1107	3.1	I	0.0009
PA1281_cobV_at	87.9	P	0.0009	1	I	0.0000	87.3	P	0.0029	0.9	I	0.0005
PA1393_cysC_at	76.7	P	0.0007	2.8	I	0.0000	53.2	P	0.0320	3.9	I	0.0001
PA1705_pcrG_i_at	160.4	P	0.0007	1.9	I	0.0000	151.4	P	0.0007	0.8	I	0.0045
PA1780_nirD_at	83.5	P	0.0007	2	I	0.0000	60.2	P	0.0139	2.2	I	0.0006
PA1982_exaA_at	85.3	P	0.0029	0.6	I	0.0002	191.3	P	0.0320	2.6	I	0.0001
PA2166_at	107.2	P	0.0012	1.4	I	0.0000	76.9	P	0.0029	0.9	NC	0.0481
PA2394_at	105.1	P	0.0015	2.8	I	0.0000	38.7	P	0.0273	2.9	I	0.0001

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	WTA 6.5 hours						WTB 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA2397_pvdE_at	67.1	P	0.0009	2.1	I	0.0000	64.1	P	0.0012	0.2	I	0.0008
PA2398_fpvA_at	94.4	P	0.0007	2.5	I	0.0000	58.4	P	0.0116	0.4	I	0.0012
PA2669_at	69.5	P	0.0009	2.3	I	0.0002	44.1	P	0.0273	3.4	I	0.0004
PA2906_at	100.4	P	0.0007	1.2	I	0.0000	48.8	P	0.0096	1.8	I	0.0007
PA2941_at	88.7	P	0.0012	0.8	I	0.0000	65.2	P	0.0434	1.3	I	0.0007
PA3136_at	110.7	P	0.0009	1.6	I	0.0000	48.5	A	0.1912	-0.1	NC	0.5000
PA3137_at	67.2	P	0.0019	1.8	I	0.0000	58.1	A	0.1727	1.3	I	0.0001
PA3278_at	83.5	P	0.0007	2.2	I	0.0000	33.9	P	0.0007	1.5	I	0.0004
PA3369_at	157.1	P	0.0007	0.5	I	0.0001	523.3	P	0.0009	1.6	I	0.0000
PA3370_at	88.4	P	0.0029	1.1	I	0.0000	95.3	M	0.0579	1.5	I	0.0006
PA3371_at	96.9	P	0.0015	1.4	I	0.0000	107.4	M	0.0503	1.6	I	0.0006
PA3547_algL_at	91.4	P	0.0009	1.9	I	0.0000	24.6	A	0.3001	2.1	I	0.0007
PA3676_at	155.3	P	0.0116	1.5	I	0.0000	156.2	M	0.0579	1.3	I	0.0005
PA3677_at	102.2	P	0.0029	1.3	I	0.0002	91.5	P	0.0374	1.2	NC	0.0140
PA3678_at	111.2	P	0.0007	1	I	0.0000	101.1	P	0.0116	0.4	I	0.0029
PA3679_at	104.7	P	0.0007	1.2	I	0.0000	70.8	P	0.0165	1.8	NC	0.0998
PA3719_at	190.5	P	0.0036	1.9	I	0.0000	418.7	P	0.0196	2.9	I	0.0000
PA3720_at	300.7	P	0.0007	2.2	I	0.0000	1003	P	0.0019	3.3	I	0.0000
PA3721_at	179.7	P	0.0007	2.6	I	0.0000	313.5	P	0.0009	2.7	I	0.0000
PA3954_at	111.1	P	0.0007	2.4	I	0.0000	57.6	P	0.0196	3.7	I	0.0021
PA4033_at	78.9	P	0.0007	1.8	I	0.0000	51.7	P	0.0165	1.4	I	0.0002
PA4037_at	94	P	0.0007	3	I	0.0000	38.1	P	0.0165	3.1	I	0.0021
PA4178_at	51.8	P	0.0066	1.2	I	0.0023	71.7	P	0.0320	1.1	MI	0.0050
PA4217_at	86.4	P	0.0012	1.5	I	0.0000	55.9	P	0.0196	1.3	I	0.0025
PA4344_at	91.3	P	0.0009	1.9	I	0.0020	49.8	P	0.0036	1.6	I	0.0013
PA4738_at	190.4	P	0.0009	0.8	I	0.0000	336.4	P	0.0036	1.5	I	0.0000
PA4739_at	197	P	0.0007	0.6	I	0.0000	348.9	P	0.0009	2	I	0.0000
PA4816_at	79.5	P	0.0009	1.2	I	0.0009	47.8	P	0.0044	0.9	I	0.0032
PA4981_at	109.6	P	0.0007	2	I	0.0000	58.8	M	0.0579	1.1	NC	0.0622
PA5157_at	85.2	P	0.0012	1.8	I	0.0000	141.4	P	0.0273	4.4	I	0.0009
PA5158_at	126	P	0.0007	1.4	I	0.0000	183.3	P	0.0007	0.7	I	0.0000
PA5159_at	129.4	P	0.0015	2	I	0.0000	242.3	P	0.0044	2.7	I	0.0000
PA5160_at	104.3	P	0.0015	1.7	I	0.0000	274.8	P	0.0015	1.9	I	0.0001
PA5216_at	77.6	P	0.0096	1.1	I	0.0001	56.2	P	0.0165	2.4	I	0.0001
PA5326_at	80.7	P	0.0015	1.7	I	0.0000	7.3	A	0.5693	-0.4	NC	0.5000
PA5390_at	64.3	P	0.0012	1.8	I	0.0000	23	A	0.3248	2.8	NC	0.0367
PA5407_at	118.5	P	0.0007	1.3	I	0.0000	48.3	P	0.0036	0.6	I	0.0025
PA5469_at	69.1	P	0.0012	2.3	I	0.0000	32.7	P	0.0273	3.5	I	0.0000
PA5481_at	126	P	0.0080	1.6	I	0.0000	128.3	P	0.0273	3	I	0.0000
PA5482_at	180.5	P	0.0009	1.1	I	0.0000	198	P	0.0196	2.5	I	0.0000

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

Time 13 hours

	WTA 13 hours						WTB 13 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0204_at	31.3	P	0.0232	0.1	NC	0.8230	40.3	A	0.1727	0.4	NC	0.2346
PA0246_at	35	P	0.0196	0.2	NC	0.5000	23.6	A	0.2108	0.1	NC	0.5000
PA0355_pfpI_at	47.9	P	0.0320	0.8	NC	0.3284	91.4	P	0.0232	3.6	I	0.0004
PA0417_at	26.8	A	0.1107	-0.1	NC	0.5000	61.8	P	0.0273	1.7	MI	0.0050
PA0424_mexR_at	247.7	P	0.0007	1.7	I	0.0000	450.3	P	0.0066	1.5	I	0.0000
PA0425_mexA_at	1241.4	P	0.0007	2.3	I	0.0000	711.7	P	0.0007	1.7	I	0.0000
PA0426_mexB_at	890.1	P	0.0007	2.1	I	0.0000	536.2	P	0.0007	1.6	I	0.0000
PA0427_oprM_at	1186.2	P	0.0007	2.1	I	0.0000	655.3	P	0.0007	1.8	I	0.0000
PA0445_s_at	59.7	P	0.0009	0.2	NC	0.5000	107.9	P	0.0232	0.7	I	0.0021
PA0526_at	24.8	P	0.0139	0.8	NC	0.5000	60.2	P	0.0096	1.7	NC	0.1641
PA0633_at	62.2	P	0.0007	-0.2	NC	0.9672	51.7	P	0.0044	0.9	NC	0.2505
PA0726_at	72.9	P	0.0066	0.6	I	0.0015	66.1	P	0.0116	3.7	MI	0.0050
PA0868_at	42.2	P	0.0066	-0.1	NC	0.5000	84.1	P	0.0080	3.4	I	0.0010
PA1230_at	17.7	A	0.0865	0.1	NC	0.4344	54.9	P	0.0096	2.7	I	0.0032
PA1281_cobV_at	51.5	P	0.0009	0	NC	0.5000	43.5	A	0.0665	1.2	NC	0.5000
PA1393_cysC_at	16.4	M	0.0503	0.2	NC	0.5000	53.4	A	0.0865	3.5	I	0.0007
PA1705_pcrG_i_at	28.2	P	0.0042	-0.1	NC	0.7367	129.2	P	0.0042	0.9	NC	0.2529
PA1780_nirD_at	19.8	P	0.0232	0	NC	0.5000	37.5	P	0.0165	3	MI	0.0053
PA1982_exaA_at	56.3	M	0.0579	-0.1	NC	0.9929	9.1	A	0.7466	0.1	NC	0.8598
PA2166_at	78	P	0.0023	1.1	I	0.0000	160.3	P	0.0007	1.9	I	0.0000
PA2394_at	21.3	P	0.0139	0.4	NC	0.4445	17.5	A	0.3248	1.6	NC	0.0912
PA2397_pvdE_at	30.4	P	0.0054	0.7	NC	0.3284	49.8	P	0.0080	0.2	NC	0.4445
PA2398_fpvA_at	34.4	P	0.0116	0.7	NC	0.0954	51.1	P	0.0232	0.2	NC	0.5000
PA2669_at	12	M	0.0503	-0.1	NC	0.5000	20.6	A	0.1727	2.2	NC	0.0088
PA2906_at	30.1	P	0.0232	-0.1	NC	0.9002	50.8	P	0.0196	1.8	NC	0.3284
PA2941_at	53.6	P	0.0196	0.1	NC	0.5000	61.5	M	0.0579	1.2	NC	0.5000
PA3136_at	77.2	P	0.0054	1.6	I	0.0000	101	P	0.0023	1	I	0.0003
PA3137_at	57.9	P	0.0044	1.6	I	0.0000	85.4	M	0.0579	1.7	I	0.0001
PA3278_at	22.6	P	0.0015	0	NC	0.5000	47	P	0.0054	1.9	I	0.0009
PA3369_at	537.4	P	0.0007	2.4	I	0.0000	2973.7	P	0.0007	4	I	0.0000
PA3370_at	122.4	P	0.0019	2	I	0.0000	448.7	P	0.0012	3.8	I	0.0000
PA3371_at	96.1	P	0.0029	1.3	I	0.0000	307.5	P	0.0054	3.1	I	0.0000
PA3547_algL_at	25.9	M	0.0503	0.3	NC	0.2118	49.5	P	0.0165	4.8	I	0.0037
PA3676_at	170.9	P	0.0116	1.7	I	0.0000	212.4	A	0.0665	1.3	I	0.0018
PA3677_at	94.1	P	0.0029	1.7	I	0.0000	158.9	P	0.0434	2.1	I	0.0005
PA3678_at	106.9	P	0.0007	1.2	I	0.0000	131.2	P	0.0023	1.2	I	0.0009
PA3679_at	52.6	P	0.0007	0	NC	0.5000	93.3	P	0.0007	1.7	I	0.0040
PA3719_at	429.6	P	0.0029	3.1	I	0.0000	508.2	P	0.0096	3.3	I	0.0000
PA3720_at	1205.8	P	0.0009	3.9	I	0.0000	1493.8	P	0.0029	4	I	0.0001
PA3721_at	413.8	P	0.0007	3.9	I	0.0000	214.6	P	0.0007	2.7	I	0.0000
PA3954_at	30	P	0.0012	0.7	NC	0.0534	56.3	P	0.0232	2.7	NC	0.1402
PA4033_at	30.3	P	0.0054	0.2	NC	0.3376	85.4	P	0.0065	1.7	I	0.0004
PA4037_at	28.7	P	0.0036	0.4	NC	0.4344	13.4	A	0.5139	1.3	NC	0.5953
PA4178_at	16.5	A	0.0760	0	NC	0.5000	48.2	M	0.0579	1.5	NC	0.0591

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	WTA 13 hours						WTB 13 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA4217_at	39.7	P	0.0066	0.2	NC	0.5000	23.9	A	0.0865	0.3	NC	0.5000
PA4344_at	25.8	P	0.0196	-0.2	NC	0.5000	60.5	P	0.0320	2.1	NC	0.2753
PA4738_at	306.8	P	0.0009	2	I	0.0000	2691.2	P	0.0007	4.3	I	0.0000
PA4739_at	741.8	P	0.0007	2.5	I	0.0000	2634.9	P	0.0007	4.9	I	0.0000
PA4816_at	33.7	P	0.0196	0.2	NC	0.5455	79.6	P	0.0273	1.4	NC	0.5000
PA4981_at	30.4	P	0.0054	0.6	NC	0.2586	57.6	P	0.0116	1.1	I	0.0005
PA5157_at	124.4	P	0.0023	2	I	0.0000	160.6	P	0.0273	4.2	I	0.0004
PA5158_at	194.8	P	0.0007	2	I	0.0000	264	P	0.0009	1.4	I	0.0000
PA5159_at	237.9	P	0.0009	2.9	I	0.0000	235.9	P	0.0029	2.5	I	0.0000
PA5160_at	177	P	0.0009	1.9	I	0.0000	274.1	P	0.0007	1.7	I	0.0002
PA5216_at	19.8	M	0.0503	0	NC	0.5000	87.7	P	0.0036	3.9	I	0.0000
PA5326_at	19.7	M	0.0503	0.1	NC	0.3469	60.9	P	0.0434	1.5	I	0.0029
PA5390_at	17.1	A	0.1727	0.3	NC	0.5000	58.4	P	0.0320	3.3	I	0.0002
PA5407_at	38.6	P	0.0007	0.3	NC	0.5000	62.6	P	0.0139	0.6	NC	0.5000
PA5469_at	13.8	A	0.1912	-0.3	NC	0.5000	4.7	A	0.7892	0.9	NC	0.0562
PA5481_at	196.4	P	0.0015	2.5	I	0.0000	470.8	P	0.0012	3.9	I	0.0000
PA5482_at	523.4	P	0.0007	2.6	I	0.0000	586.9	P	0.0009	4.7	I	0.0000

Time 26 hours

	WTA 26 hours						WTB 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0204_at	53.7	P	0.0054	1.2	NC	0.0101	40.3	A	0.1727	0.4	NC	0.2346
PA0246_at	48.8	P	0.0080	1	I	0.0002	23.6	A	0.2108	0.1	NC	0.5000
PA0355_pfpI_at	53.5	P	0.0080	1.2	NC	0.0066	91.4	P	0.0232	3.6	I	0.0004
PA0417_at	54.8	P	0.0320	0.6	I	0.0018	61.8	P	0.0273	1.7	MI	0.0050
PA0424_mexR_at	229.8	P	0.0015	1.7	I	0.0000	450.3	P	0.0066	1.5	I	0.0000
PA0425_mexA_at	1071.4	P	0.0007	2.1	I	0.0000	711.7	P	0.0007	1.7	I	0.0000
PA0426_mexB_at	775.3	P	0.0007	2.1	I	0.0000	536.2	P	0.0007	1.6	I	0.0000
PA0427_oprM_at	1271.4	P	0.0007	2.2	I	0.0000	655.3	P	0.0007	1.8	I	0.0000
PA0445_s_at	134.9	P	0.0009	0.9	I	0.0000	107.9	P	0.0232	0.7	I	0.0021
PA0526_at	46.5	P	0.0009	1.6	I	0.0000	60.2	P	0.0096	1.7	NC	0.1641
PA0633_at	67.8	P	0.0007	0.1	NC	0.5000	51.7	P	0.0044	0.9	NC	0.2505
PA0726_at	103.4	P	0.0012	1	I	0.0000	66.1	P	0.0116	3.7	MI	0.0050
PA0868_at	47	P	0.0116	0.1	NC	0.0793	84.1	P	0.0080	3.4	I	0.0010
PA1230_at	56	P	0.0066	1.1	I	0.0001	54.9	P	0.0096	2.7	I	0.0032
PA1281_cobV_at	47.4	P	0.0015	0.2	NC	0.2753	43.5	A	0.0665	1.2	NC	0.5000
PA1393_cysC_at	24.9	P	0.0196	0.7	NC	0.0217	53.4	A	0.0865	3.5	I	0.0007
PA1705_pcrG_i_at	78.6	P	0.0002	1.1	I	0.0017	129.2	P	0.0042	0.9	NC	0.2529
PA1780_nirD_at	57.7	P	0.0015	0.9	I	0.0001	37.5	P	0.0165	3	MI	0.0053
PA1982_exaA_at	45.1	A	0.0665	-0.4	NC	0.9568	9.1	A	0.7466	0.1	NC	0.8598
PA2166_at	67.4	P	0.0015	0.9	I	0.0000	160.3	P	0.0007	1.9	I	0.0000
PA2394_at	40.3	P	0.0196	1.1	I	0.0002	17.5	A	0.3248	1.6	NC	0.0912
PA2397_pvdE_at	36	P	0.0015	1	I	0.0023	49.8	P	0.0080	0.2	NC	0.4445
PA2398_fpvA_at	35.2	P	0.0023	1.1	I	0.0000	51.1	P	0.0232	0.2	NC	0.5000
PA2669_at	32.4	P	0.0054	1.2	I	0.0034	20.6	A	0.1727	2.2	NC	0.0088

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	WTA 26 hours						WTB 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA2906_at	59.5	P	0.0015	0.3	NC	0.0347	50.8	P	0.0196	1.8	NC	0.3284
PA2941_at	58.9	P	0.0066	0.3	NC	0.0534	61.5	M	0.0579	1.2	NC	0.5000
PA3136_at	71.9	P	0.0019	1.7	I	0.0000	101	P	0.0023	1	I	0.0003
PA3137_at	49.5	P	0.0023	1.4	I	0.0000	85.4	M	0.0579	1.7	I	0.0001
PA3278_at	40.4	P	0.0012	0.8	I	0.0002	47	P	0.0054	1.9	I	0.0009
PA3369_at	534.5	P	0.0007	2.2	I	0.0000	2973.7	P	0.0007	4	I	0.0000
PA3370_at	137.4	P	0.0015	2.1	I	0.0000	448.7	P	0.0012	3.8	I	0.0000
PA3371_at	128.5	P	0.0023	1.7	I	0.0000	307.5	P	0.0054	3.1	I	0.0000
PA3547_algL_at	33.7	P	0.0019	0.8	I	0.0000	49.5	P	0.0165	4.8	I	0.0037
PA3676_at	166.2	P	0.0116	1.8	I	0.0001	212.4	A	0.0665	1.3	I	0.0018
PA3677_at	111.1	P	0.0066	2.2	I	0.0000	158.9	P	0.0434	2.1	I	0.0005
PA3678_at	99.4	P	0.0007	1.2	I	0.0000	131.2	P	0.0023	1.2	I	0.0009
PA3679_at	76.6	P	0.0007	0.5	I	0.0025	93.3	P	0.0007	1.7	I	0.0040
PA3719_at	438.2	P	0.0044	3.2	I	0.0000	508.2	P	0.0096	3.3	I	0.0000
PA3720_at	1058.9	P	0.0009	3.8	I	0.0000	1493.8	P	0.0029	4	I	0.0001
PA3721_at	379.9	P	0.0007	3.5	I	0.0000	214.6	P	0.0007	2.7	I	0.0000
PA3954_at	48.5	P	0.0007	1.4	I	0.0001	56.3	P	0.0232	2.7	NC	0.1402
PA4033_at	37.1	P	0.0012	0.6	I	0.0000	85.4	P	0.0065	1.7	I	0.0004
PA4037_at	38	P	0.0012	1	I	0.0004	13.4	A	0.5139	1.3	NC	0.5953
PA4178_at	26.7	P	0.0374	0.4	NC	0.0793	48.2	M	0.0579	1.5	NC	0.0591
PA4217_at	48.2	P	0.0054	0.7	I	0.0004	23.9	A	0.0865	0.3	NC	0.5000
PA4344_at	45.1	P	0.0015	0.6	NC	0.0410	60.5	P	0.0320	2.1	NC	0.2753
PA4738_at	296.9	P	0.0007	2	I	0.0000	2691.2	P	0.0007	4.3	I	0.0000
PA4739_at	637.8	P	0.0007	2.3	I	0.0000	2634.9	P	0.0007	4.9	I	0.0000
PA4816_at	40.1	P	0.0007	0.2	NC	0.1346	79.6	P	0.0273	1.4	NC	0.5000
PA4981_at	59.9	P	0.0012	1.3	I	0.0003	57.6	P	0.0116	1.1	I	0.0005
PA5157_at	123.9	P	0.0015	2	I	0.0000	160.6	P	0.0273	4.2	I	0.0004
PA5158_at	185.1	P	0.0007	2	I	0.0000	264	P	0.0009	1.4	I	0.0000
PA5159_at	225.1	P	0.0009	2.9	I	0.0000	235.9	P	0.0029	2.5	I	0.0000
PA5160_at	188.1	P	0.0009	2.2	I	0.0000	274.1	P	0.0007	1.7	I	0.0002
PA5216_at	50.7	P	0.0196	0.5	MI	0.0058	87.7	P	0.0036	3.9	I	0.0000
PA5326_at	42.3	P	0.0036	1.3	I	0.0000	60.9	P	0.0434	1.5	I	0.0029
PA5390_at	40.4	P	0.0066	1.2	I	0.0001	58.4	P	0.0320	3.3	I	0.0002
PA5407_at	57.9	P	0.0007	0.4	I	0.0007	62.6	P	0.0139	0.6	NC	0.5000
PA5469_at	32.4	P	0.0116	1.1	I	0.0001	4.7	A	0.7892	0.9	NC	0.0562
PA5481_at	252.9	P	0.0007	2.3	I	0.0000	470.8	P	0.0012	3.9	I	0.0000
PA5482_at	545.4	P	0.0007	2.7	I	0.0000	586.9	P	0.0009	4.7	I	0.0000

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

RpoS⁻ mutant time 0, 6.5 hours

	RpoS⁻ 0 hours						RpoS⁻ 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0204_at	62.1	P	0.0019				117.9	P	0.0007	0.7	I	0.0001
PA0246_at	61.1	P	0.0066				89.2	P	0.0012	0.5	I	0.0000
PA0355_pfpI_at	59.2	P	0.0009				96.6	P	0.0007	1	I	0.0000
PA0417_at	70.9	P	0.0080				85.9	P	0.0023	0.7	I	0.0000
PA0424_mexR_at	110.1	P	0.0066				168	P	0.0007	0.7	I	0.0000
PA0425_mexA_at	268.8	P	0.0007				257.9	P	0.0007	0	NC	0.5000
PA0426_mexB_at	242.8	P	0.0007				192.6	P	0.0007	-0.2	NC	0.5000
PA0427_oprM_at	324.2	P	0.0007				223.4	P	0.0009	-0.4	MD	0.9943
PA0445_s_at	185	P	0.0007				285.9	P	0.0007	0.8	I	0.0000
PA0526_at	52.4	P	0.0012				108.3	P	0.0007	0.9	I	0.0000
PA0633_at	119.5	P	0.0007				178.3	P	0.0007	0.5	I	0.0001
PA0726_at	120	P	0.0012				217.1	P	0.0007	0.8	I	0.0000
PA0868_at	54.4	P	0.0023				105.7	P	0.0007	0.8	I	0.0000
PA1230_at	81	P	0.0023				146.9	P	0.0007	1.1	I	0.0000
PA1281_cobV_at	69.9	P	0.0012				107.1	P	0.0007	0.6	I	0.0000
PA1393_cysC_at	52.7	P	0.0015				97.5	P	0.0007	1.2	I	0.0000
PA1705_pcrG_i_at	92.7	P	0.0002				173.4	P	0.0002	0.8	I	0.0003
PA1780_nirD_at	55.8	P	0.0007				91.8	P	0.0007	0.8	I	0.0000
PA1982_exaA_at	74.1	P	0.0165				121.1	P	0.0012	0.5	I	0.0003
PA2166_at	68.6	P	0.0023				112.4	P	0.0009	0.8	I	0.0000
PA2394_at	68.4	P	0.0023				125.2	P	0.0012	0.8	I	0.0003
PA2397_pvdE_at	44.3	P	0.0009				97.2	P	0.0007	1.1	I	0.0000
PA2398_fpvA_at	114.6	P	0.0007				123.9	P	0.0007	0.1	NC	0.5000
PA2669_at	43.7	P	0.0015				106.7	P	0.0007	1.1	I	0.0000
PA2906_at	75.9	P	0.0009				117.6	P	0.0007	0.3	NC	0.0066
PA2941_at	64.3	P	0.0015				87.8	P	0.0007	0.4	I	0.0009
PA3136_at	48.4	P	0.0007				113.9	P	0.0007	1.1	I	0.0000
PA3137_at	36.5	P	0.0066				77.8	P	0.0007	0.6	I	0.0000
PA3278_at	49.7	P	0.0007				104.9	P	0.0007	0.9	I	0.0000
PA3369_at	69.8	P	0.0009				119.7	P	0.0007	0.7	I	0.0000
PA3370_at	54.7	P	0.0096				73.2	P	0.0012	0.8	I	0.0000
PA3371_at	83.3	P	0.0023				114.4	P	0.0009	0.5	I	0.0001
PA3547_algL_at	54.3	P	0.0029				105.2	P	0.0007	1	I	0.0000
PA3676_at	117.5	P	0.0116				150.7	P	0.0096	0.4	I	0.0020
PA3677_at	62.3	P	0.0066				99.4	P	0.0012	0.4	I	0.0013
PA3678_at	54.2	P	0.0007				98.2	P	0.0007	0.7	I	0.0000
PA3679_at	74.3	P	0.0007				111.1	P	0.0007	0.6	I	0.0000
PA3719_at	73.9	P	0.0273				125.6	P	0.0023	1	I	0.0000
PA3720_at	103.5	P	0.0009				189.9	P	0.0007	0.9	I	0.0000
PA3721_at	34.7	P	0.0015				82	P	0.0007	1.2	I	0.0000
PA3954_at	59.5	P	0.0007				140.8	P	0.0007	1.1	I	0.0000
PA4033_at	51.5	P	0.0009				116.5	P	0.0007	1.2	I	0.0000
PA4037_at	57.1	P	0.0007				118.4	P	0.0007	0.9	I	0.0000
PA4178_at	30.1	P	0.0029				83	P	0.0009	1.1	I	0.0009

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	RpoS- 0 hours						RpoS- 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	Sig.[c]	P/A	p-value	SLR[d]	I/D	p-value	Sig.[c]	P/A	p-value	SLR[d]	I/D	p-value
PA4217_at	74.7	P	0.0009				104.9	P	0.0007	0.5	I	0.0000
PA4344_at	82.7	P	0.0015				121.4	P	0.0007	0.8	NC	0.0076
PA4738_at	69.9	P	0.0054				167.4	P	0.0009	0.9	I	0.0000
PA4739_at	66.7	P	0.0007				113	P	0.0007	0.6	I	0.0000
PA4816_at	49.8	P	0.0007				67.1	P	0.0007	0.4	NC	0.0832
PA4981_at	95.8	P	0.0012				132.9	P	0.0007	0.7	I	0.0010
PA5157_at	53.1	P	0.0054				87.3	P	0.0007	0.9	I	0.0000
PA5158_at	69.6	P	0.0007				107.5	P	0.0007	0.4	I	0.0005
PA5159_at	44.1	P	0.0019				117.8	P	0.0009	0.9	I	0.0000
PA5160_at	75.1	P	0.0015				100.8	P	0.0007	0.5	I	0.0000
PA5216_at	59.9	P	0.0096				101.8	P	0.0029	0.6	I	0.0003
PA5326_at	38.9	P	0.0015				95	P	0.0007	1.3	I	0.0000
PA5390_at	31.1	P	0.0015				75.2	P	0.0007	0.9	I	0.0000
PA5407_at	77.4	P	0.0007				143.6	P	0.0007	0.8	I	0.0000
PA5469_at	47.2	P	0.0015				96.5	P	0.0007	1.1	I	0.0000
PA5481_at	54.8	P	0.0066				133.1	P	0.0019	1.2	I	0.0000
PA5482_at	70.3	P	0.0012				132.3	P	0.0007	1	I	0.0000

RpoS- mutant time 13, 26 hours

	RpoS- 13 hours						RpoS- 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	Sig.[c]	P/A	p-value	SLR[d]	I/D	p-value	Sig.[c]	P/A	p-value	SLR[d]	I/D	p-value
PA0204_at	34.2	P	0.0232	-0.9	D	1.0000	34.9	P	0.0139	-1	D	1.0000
PA0246_at	27.7	P	0.0116	-0.6	NC	0.9612	21.6	P	0.0116	-1	D	0.9999
PA0355_pfpI_at	35.9	P	0.0036	-0.8	D	0.9985	27.7	P	0.0096	-1	D	1.0000
PA0417_at	39.5	A	0.0760	-0.6	D	0.9999	32.6	A	0.0980	-1	D	1.0000
PA0424_mexR_at	226.8	P	0.0012	1.3	I	0.0000	229	P	0.0015	1.6	I	0.0000
PA0425_mexA_at	1965.2	P	0.0007	2.9	I	0.0000	1606.2	P	0.0007	2.7	I	0.0000
PA0426_mexB_at	1431.8	P	0.0007	2.7	I	0.0000	1174.5	P	0.0007	2.4	I	0.0000
PA0427_oprM_at	2109.8	P	0.0007	2.6	I	0.0000	1675.5	P	0.0007	2.4	I	0.0000
PA0445_s_at	79.1	P	0.0015	-1.1	D	1.0000	56.7	P	0.0015	-1.3	D	1.0000
PA0526_at	20.1	P	0.0019	-1.5	NC	0.9892	16.3	P	0.0066	-1.7	D	0.9999
PA0633_at	77.5	P	0.0007	-0.5	D	1.0000	68.9	P	0.0009	-0.8	D	1.0000
PA0726_at	47.1	P	0.0165	-1.3	D	1.0000	32.9	P	0.0054	-1.8	D	1.0000
PA0868_at	33.7	P	0.0096	-0.3	NC	0.9169	60.8	P	0.0066	-0.1	NC	0.9346
PA1230_at	22.1	M	0.0579	-1.4	D	1.0000	18.2	M	0.0579	-1.3	D	1.0000
PA1281_cobV_at	38	P	0.0012	-0.5	D	0.9989	30.8	P	0.0019	-0.9	D	0.9999
PA1393_cysC_at	20.7	P	0.0066	-1.2	D	1.0000	18.4	P	0.0165	-1.2	D	0.9999
PA1705_pcrG_i_at	51.6	P	0.0020	-0.7	D	0.9998	29	P	0.0007	-1.5	D	1.0000
PA1780_nirD_at	28.2	P	0.0080	-0.7	D	0.9995	22.9	P	0.0080	-0.8	D	1.0000
PA1982_exaA_at	85.8	P	0.0196	0.3	NC	0.0622	24.8	M	0.0503	-1.1	D	1.0000
PA2166_at	24.9	P	0.0165	-0.9	D	0.9993	21.8	P	0.0066	-1.4	D	0.9977
PA2394_at	32.4	P	0.0232	-0.9	D	1.0000	24.8	P	0.0232	-1.3	D	1.0000
PA2397_pvdE_at	23.6	P	0.0009	-0.7	D	0.9998	23.3	P	0.0015	-0.9	D	1.0000
PA2398_fpvA_at	164.4	P	0.0007	0.5	I	0.0001	93.1	P	0.0007	-0.2	D	0.9983
PA2669_at	22.8	P	0.0066	-0.7	D	0.9997	12.2	P	0.0066	-1.8	D	1.0000

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

	RpoS⁻ 13 hours						RpoS⁻ 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA2906_at	47.5	P	0.0080	-0.6	D	1.0000	36.8	P	0.0015	-0.8	D	1.0000
PA2941_at	53.9	P	0.0232	-0.3	D	0.9999	39.4	P	0.0139	-0.7	D	0.9999
PA3136_at	90.4	P	0.0015	0.8	NC	0.0192	112.3	P	0.0007	1.1	I	0.0009
PA3137_at	63.4	P	0.0044	0.7	I	0.0025	59.9	P	0.0019	0.5	I	0.0002
PA3278_at	19.9	P	0.0066	-1.9	D	1.0000	19.2	P	0.0019	-1.8	D	1.0000
PA3369_at	50.3	P	0.0023	-0.5	D	1.0000	58.5	P	0.0015	-0.3	MD	0.9947
PA3370_at	29.4	A	0.0760	-0.7	D	0.9999	27.3	A	0.1244	-0.9	D	1.0000
PA3371_at	43.4	P	0.0165	-0.9	D	1.0000	39.2	M	0.0503	-1.1	D	1.0000
PA3547_algL_at	31.6	P	0.0374	-0.8	D	1.0000	22.9	M	0.0503	-1.1	D	1.0000
PA3676_at	228.7	P	0.0116	1	I	0.0001	210.3	P	0.0116	0.9	I	0.0001
PA3677_at	108.5	P	0.0023	1.2	I	0.0000	141.6	P	0.0036	1	I	0.0004
PA3678_at	84.5	P	0.0007	0.6	I	0.0006	99	P	0.0007	0.7	I	0.0000
PA3679_at	64.4	P	0.0007	-0.3	D	0.9963	53.7	P	0.0012	-0.4	D	1.0000
PA3719_at	865.8	P	0.0019	3.7	I	0.0000	847.1	P	0.0015	3.9	I	0.0000
PA3720_at	2048.6	P	0.0009	4	I	0.0000	2076.1	P	0.0009	4	I	0.0000
PA3721_at	285.5	P	0.0007	3.2	I	0.0000	267.3	P	0.0007	3	I	0.0000
PA3954_at	32.4	P	0.0015	-0.7	D	0.9998	22.4	P	0.0029	-1.2	D	1.0000
PA4033_at	29.3	P	0.0012	-0.7	D	1.0000	39	P	0.0015	-0.4	D	1.0000
PA4037_at	36.3	P	0.0096	-1	D	1.0000	23	M	0.0503	-1.5	D	1.0000
PA4178_at	30.2	P	0.0096	-0.5	NC	0.9755	24.1	P	0.0066	-0.1	NC	0.9906
PA4217_at	35.3	P	0.0023	-0.8	D	1.0000	35.1	P	0.0009	-0.9	D	1.0000
PA4344_at	38.5	P	0.0044	-0.6	NC	0.9860	33.4	P	0.0139	-0.9	D	0.9989
PA4738_at	47	M	0.0579	-0.5	D	0.9999	39.2	P	0.0320	-0.6	D	0.9999
PA4739_at	58.3	P	0.0009	-0.3	MD	0.9947	62.4	P	0.0007	-0.1	NC	0.5354
PA4816_at	36.7	P	0.0054	-0.5	D	0.9995	49	P	0.0044	-0.3	D	0.9968
PA4981_at	34.9	P	0.0080	-1	D	1.0000	33.3	P	0.0116	-1.1	D	1.0000
PA5157_at	208.8	P	0.0009	1.8	I	0.0000	170.2	P	0.0019	1.4	I	0.0000
PA5158_at	317.9	P	0.0007	2	I	0.0000	245.2	P	0.0007	1.5	I	0.0000
PA5159_at	348.7	P	0.0007	2.8	I	0.0000	211.9	P	0.0007	2.2	I	0.0000
PA5160_at	229.8	P	0.0007	1.7	I	0.0000	155.6	P	0.0007	1.2	I	0.0000
PA5216_at	38.7	P	0.0434	-0.7	D	0.9998	31.6	A	0.0665	-0.9	D	1.0000
PA5326_at	24.5	M	0.0503	-0.9	D	0.9966	13.9	A	0.0865	-1.4	D	0.9995
PA5390_at	25.9	P	0.0273	-0.3	D	0.9998	23.3	P	0.0116	-0.4	D	1.0000
PA5407_at	34.6	P	0.0007	-0.7	D	0.9999	23.2	P	0.0007	-1	D	1.0000
PA5469_at	23.4	M	0.0503	-1.3	D	0.9999	16.6	P	0.0434	-1.5	D	1.0000
PA5481_at	16.9	A	0.1912	-1.9	D	1.0000	16.2	A	0.2762	-1.6	D	0.9999
PA5482_at	30.1	P	0.0196	-0.9	D	1.0000	22.5	P	0.0096	-1.3	D	1.0000

[a]WTA=June 22, 2004; WTB=July 8, 2004; RpoS⁻=Sept 5, 2005

[b]Detection call: P, present; A, absent. [b]Change call: I, increase; MI, moderate increase; NC, no change; MD, moderate decrease; D, decrease

[c]Signal intensity. [d]SLR, signal log ratio, $\log_2$(signal *x* hour/signal 0 hour)

Affymetrix ID	Descriptions
PA0204_at	PA0204 /DEF=probable permease of ABC transporter /FUNCTION=Membrane proteins; Transport of small molecules
PA0246_at	PA0246 /DEF=probable MFS transporter /FUNCTION=Membrane proteins; Transport of small molecules
PA0355_pfpI_at	PA0355 /GENE=pfpI /DEF=protease PfpI /FUNCTION=Translation, post-translational modification, degradation
PA0417_at	PA0417 /DEF=probable chemotaxis protein /FUNCTION=Chemotaxis
PA0424_mexR_at	PA0424 /GENE=mexR /DEF=multidrug resistance operon repressor MexR /FUNCTION=Transcriptional regulators
PA0425_mexA_at	PA0425 /GENE=mexA /DEF=RND multidrug efflux membrane fusion protein MexA precursor /FUNCTION=Transport of small molecules; Antibiotic resistance and susceptibility
PA0426_mexB_at	PA0426 /GENE=mexB /DEF=RND multidrug efflux transporter MexB /FUNCTION=Membrane proteins; Transport of small molecules; Antibiotic resistance and susceptibility
PA0427_oprM_at	PA0427 /GENE=oprM /DEF=outer membrane protein OprM precursor /FUNCTION=Membrane proteins; Transport of small molecules; Antibiotic resistance and susceptibility
PA0445_s_at	PA0445 /DEF=probable transposase /FUNCTION=Related to phage, transposon, or plasmid
PA0526_at	PA0526 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA0633_at	PA0633 /DEF=hypothetical protein /FUNCTION=Related to phage, transposon, or plasmid
PA0726_at	PA0726 /DEF=hypothetical protein of bacteriophage Pf1 /FUNCTION=Hypothetical, unclassified, unknown; Related to phage, transposon, or plasmid
PA0868_at	PA0868 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1230_at	PA1230 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA1281_cobV_at	PA1281 /GENE=cobV /DEF=cobalamin (5 -phosphate) synthase /FUNCTION=Biosynthesis of cofactors, prosthetic groups and carriers
PA1393_cysC_at	PA1393 /GENE=cysC /DEF=adenosine 5 -phosphosulfate (APS) kinase /FUNCTION=Central intermediary metabolism; Nucleotide Biosynthesis and metabolism; Amino acid biosynthesis and metabolism
PA1705_pcrG_i_at	PA1705 /GENE=pcrG /DEF=regulator in type III secretion /FUNCTION=Protein secretion/export apparatus
PA1780_nirD_at	PA1780 /GENE=nirD /DEF=assimilatory nitrite reductase small subunit /FUNCTION=Central intermediary metabolism
PA1982_exaA_at	PA1982 /GENE=exaA /DEF=quinoprotein alcohol dehydrogenase /FUNCTION=Carbon compound catabolism
PA2166_at	PA2166 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2394_at	PA2394 /DEF=probable aminotransferase /FUNCTION=Putative enzymes
PA2397_pvdE_at	PA2397 /GENE=pvdE /DEF=pyoverdine biosynthesis protein PvdE /FUNCTION=Adaptation, protection; Membrane proteins; Transport of small molecules
PA2398_fpvA_at	PA2398 /GENE=fpvA /DEF=ferripyoverdine receptor /FUNCTION=Transport of small molecules
PA2669_at	PA2669 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2906_at	PA2906 /DEF=probable oxidoreductase /FUNCTION=Putative enzymes
PA2941_at	PA2941 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3136_at	PA3136 /DEF=probable secretion protein /FUNCTION=Transport of small molecules
PA3137_at	PA3137 /DEF=probable MFS transporter /FUNCTION=Membrane proteins; Transport of small molecules
PA3278_at	PA3278 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA3369_at	PA3369 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA3370_at	PA3370 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA3371_at	PA3371 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3547_algL_at	PA3547 /GENE=algL /DEF=poly(beta-d-mannuronate) lyase precursor AlgL /FUNCTION=Adaptation, protection; Cell wall / LPS / capsule; Secreted Factors (toxins, enzymes, alginate)

Affymetrix GCOS data for genes increasing in response to PCP (cont.)

Affymetrix ID	Descriptions
PA3676_at	PA3676 /DEF=probable RND efflux transporter /FUNCTION=Membrane proteins; Transport of small molecules
PA3677_at	PA3677 /DEF=probable RND efflux membrane fusion protein precursor /FUNCTION=Transport of small molecules
PA3678_at	PA3678 /DEF=probable transcriptional regulator /FUNCTION=Transcriptional regulators
PA3679_at	PA3679 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3719_at	PA3719 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3720_at	PA3720 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3721_at	PA3721 /DEF=probable transcriptional regulator /FUNCTION=Transcriptional regulators
PA3954_at	PA3954 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4033_at	PA4033 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4037_at	PA4037 /DEF=probable ATP-binding component of ABC transporter /FUNCTION=Transport of small molecules
PA4178_at	PA4178 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4217_at	PA4217 /DEF=probable FAD-dependent monooxygenase /FUNCTION=Putative enzymes
PA4344_at	PA4344 /DEF=probable hydrolase /FUNCTION=Putative enzymes
PA4738_at	PA4738 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4739_at	PA4739 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4816_at	PA4816 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4981_at	PA4981 /DEF=probable amino acid permease /FUNCTION=Membrane proteins; Transport of small molecules
PA5157_at	PA5157 /DEF=probable transcriptional regulator /FUNCTION=Transcriptional regulators
PA5158_at	PA5158 /DEF=probable outer membrane protein /FUNCTION=Transport of small molecules
PA5159_at	PA5159 /DEF=multidrug resistance protein /FUNCTION=Transport of small molecules
PA5160_at	PA5160 /DEF=drug efflux transporter /FUNCTION=Adaptation, protection; Membrane proteins; Transport of small molecules
PA5216_at	PA5216 /DEF=probable permease of ABC iron transporter /FUNCTION=Membrane proteins; Transport of small molecules
PA5326_at	PA5326 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA5390_at	PA5390 /DEF=probable peptidic bond hydrolase /FUNCTION=Putative enzymes
PA5407_at	PA5407 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA5469_at	PA5469 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA5481_at	PA5481 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA5482_at	PA5482 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins

Affymetrix GCOS data for genes decreasing in response to PCP

Time 0 hours

	WTA 0 hours						WTB 0 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0224_at	334.2	P	0.0007				248.6	P	0.0012			
PA0262_at	54.1	P	0.0066				42.6	A	0.3001			
PA0263_hcpC_s_at	985.6	P	0.0007				2388.8	P	0.0007			
PA0284_at	575.9	P	0.0007				641.7	P	0.0007			
PA0579_rpsU_at	448.6	P	0.0007				502	P	0.0007			
PA0762_algU_at	652.5	P	0.0007				654.9	P	0.0015			
PA0848_at	124.4	P	0.0015				149.9	M	0.0579			
PA0962_at	806.9	P	0.0007				871	P	0.0007			
PA1178_oprH_at	840.4	P	0.0024				832.3	P	0.0030			
PA1193_at	76.9	P	0.0196				93.2	A	0.1727			
PA1198_at	572	P	0.0009				469.2	P	0.0012			
PA1493_cysP_at	411.1	P	0.0007				451.1	P	0.0007			
PA1544_anr_at	242	P	0.0007				155.7	P	0.0023			
PA1588_sucC_at	1553.2	P	0.0007				1211.3	P	0.0012			
PA1656_at	118.8	P	0.0009				142.3	P	0.0116			
PA1657_at	469.3	P	0.0007				559.8	P	0.0007			
PA1658_at	261.6	P	0.0007				376	P	0.0007			
PA1660_at	53.3	P	0.0007				70.6	P	0.0165			
PA1664_at	127	P	0.0007				182.1	P	0.0015			
PA2562_at	408.2	P	0.0007				567.4	P	0.0054			
PA2658_at	204.5	P	0.0015				166.7	P	0.0273			
PA2760_at	756.7	P	0.0007				905.5	P	0.0007			
PA2808_i_at	915.9	P	0.0010				600.7	P	0.0010			
PA2850_ohr_at	198.1	P	0.0007				144.8	P	0.0007			
PA2951_etfA_at	611.5	P	0.0009				370.1	P	0.0009			
PA3159_wbpA_at	816.4	P	0.0007				1047.5	P	0.0007			
PA3162_rpsA_at	1220.3	P	0.0007				1207.9	P	0.0007			
PA3266_capB_at	247	P	0.0007				332.1	P	0.0007			
PA3287_at	180.9	P	0.0009				194.3	P	0.0007			
PA3328_at	277.4	P	0.0007				208.2	P	0.0012			
PA3450_at	237.3	P	0.0015				386.7	P	0.0080			
PA3552_at	141.8	P	0.0023				106.5	P	0.0165			
PA3690_at	1906.2	P	0.0007				1657.2	P	0.0009			
PA3814_iscS_at	1001.5	P	0.0007				952.9	P	0.0009			
PA3815_at	530.6	P	0.0007				262.7	P	0.0009			
PA3819_at	459.6	P	0.0009				331.3	P	0.0036			
PA3904_i_at	529.4	P	0.0005				668.4	P	0.0005			
PA3905_at	76.8	P	0.0012				48.1	P	0.0374			
PA4053_ribE_at	457.7	P	0.0007				302.6	P	0.0012			
PA4386_groES_at	1690.2	P	0.0007				1262	P	0.0007			
PA4443_cysD_at	547.4	P	0.0007				383.8	P	0.0007			

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	WTA 0 hours						WTB 0 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA4563_rpsT_at	507.9	P	0.0007				577.8	P	0.0007			
PA4568_rplU_at	1549.7	P	0.0007				2214.5	P	0.0007			
PA4748_tpiA_at	195	P	0.0007				187.4	P	0.0007			
PA4847_accB_at	434.9	P	0.0007				423.1	P	0.0029			
PA5013_ilvE_at	770.6	P	0.0007				520.1	P	0.0012			
PA5182_at	463.9	P	0.0007				443.8	P	0.0007			
PA5212_i_at	363.5	P	0.0024				270.3	P	0.0030			
PA5253_algP_at	2061.2	P	0.0007				4107.5	P	0.0007			
PA5312_at	753.9	P	0.0007				443.1	P	0.0007			
PA5369_at	179	P	0.0007				333.2	P	0.0012			
PA5570_rpmH_at	294	P	0.0007				251.4	P	0.0007			

Time 6.5 hours

	WTA 6.5 hours						WTB 6.5 hours					
PA0224_at	190.9	P	0.0007	-0.8	D	1.0000	117.9	P	0.0019	-1	D	0.9986
PA0262_at	80.5	P	0.0012	0.6	I	0.0000	48.8	A	0.1727	0.4	NC	0.1641
PA0263_hcpC_s_at	528.6	P	0.0007	-1	D	1.0000	1858.4	P	0.0007	-0.4	D	0.9997
PA0284_at	178.1	P	0.0007	-1.8	D	1.0000	219.5	P	0.0009	-1.5	D	1.0000
PA0579_rpsU_at	159.2	P	0.0007	-1.6	D	1.0000	345.9	P	0.0007	-0.6	D	0.9999
PA0762_algU_at	208.6	P	0.0009	-1.7	D	1.0000	331.3	P	0.0044	-0.8	D	1.0000
PA0848_at	88.8	P	0.0023	-0.7	NC	0.9851	35.3	A	0.3248	-2	D	0.9999
PA0962_at	298.1	P	0.0007	-1.5	D	1.0000	650.7	P	0.0007	-0.6	D	0.9971
PA1178_oprH_at	221.2	P	0.0048	-2.1	D	1.0000	300.8	P	0.0060	-2	D	1.0000
PA1193_at	105.4	P	0.0232	0.4	I	0.0027	116.8	A	0.0665	0.2	NC	0.0192
PA1198_at	246	P	0.0012	-1.3	D	1.0000	292.9	P	0.0015	-0.8	D	0.9968
PA1493_cysP_at	173.6	P	0.0007	-1.1	D	1.0000	226.6	P	0.0007	-0.8	D	1.0000
PA1544_anr_at	120.7	P	0.0012	-1	D	1.0000	119.5	P	0.0096	-0.4	NC	0.5000
PA1588_sucC_at	515.3	P	0.0007	-1.5	D	1.0000	894.7	P	0.0019	-0.5	D	1.0000
PA1656_at	111.5	P	0.0012	-0.3	NC	0.6437	96.5	P	0.0139	-0.4	NC	0.9755
PA1657_at	220.7	P	0.0007	-1.3	D	1.0000	393.1	P	0.0007	-0.5	D	0.9999
PA1658_at	101.3	P	0.0009	-1.4	D	1.0000	232.4	P	0.0012	-0.5	D	1.0000
PA1660_at	54.7	P	0.0007	0	NC	0.1641	76.7	P	0.0138	-0.1	NC	0.5000
PA1664_at	139.2	P	0.0007	0.1	NC	0.0388	115.7	P	0.0012	-0.4	NC	0.8910
PA2562_at	130.9	P	0.0007	-1.6	D	1.0000	321.8	P	0.0080	-0.7	D	1.0000
PA2658_at	73.7	P	0.0054	-1.2	D	1.0000	111.6	P	0.0273	-0.4	NC	0.9207
PA2760_at	271.6	P	0.0009	-1.5	D	1.0000	664.5	P	0.0007	-0.5	D	0.9999
PA2808_i_at	369.1	P	0.0010	-1.4	D	0.9999	475.3	P	0.0010	-0.8	D	0.9996
PA2850_ohr_at	72.7	P	0.0007	-1.2	D	0.9999	43.7	P	0.0096	-1.4	D	1.0000
PA2951_etfA_at	226.7	P	0.0009	-1.6	D	1.0000	280.6	P	0.0019	-0.5	D	0.9994
PA3159_wbpA_at	235.1	P	0.0007	-1.7	D	1.0000	708.1	P	0.0007	-0.5	MD	0.9947
PA3162_rpsA_at	373.7	P	0.0007	-1.8	D	1.0000	795.2	P	0.0007	-0.6	D	1.0000
PA3266_capB_at	110.7	P	0.0007	-1.8	D	1.0000	194.3	P	0.0007	-0.7	D	0.9999
PA3287_at	94.4	P	0.0007	-0.9	D	1.0000	80.5	P	0.0096	-1.2	D	0.9997
PA3328_at	132.5	P	0.0007	-1.1	D	1.0000	191	P	0.0012	-0.1	NC	0.5000
PA3450_at	133.7	P	0.0019	-1.1	D	0.9989	271	P	0.0096	-1.1	D	0.9979
PA3552_at	118.2	P	0.0023	-0.2	NC	0.5000	98.1	P	0.0320	-0.4	D	0.9980

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	WTA 6.5 hours						WTB 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA3819_at	170.7	P	0.0029	-1.8	D	1.0000	199.5	P	0.0196	-1	D	1.0000
PA3690_at	508.6	P	0.0007	-1.8	D	1.0000	1227	P	0.0012	-0.4	D	0.9998
PA3814_iscS_at	304.5	P	0.0007	-1.6	D	1.0000	590.3	P	0.0044	-0.7	D	0.9998
PA3815_at	137.1	P	0.0015	-1.5	D	1.0000	155.1	P	0.0080	-0.6	D	0.9995
PA3904_i_at	200.4	P	0.0015	-1.4	D	1.0000	386.2	P	0.0015	-0.7	D	0.9999
PA3905_at	79.8	P	0.0066	-0.5	D	0.9973	19.9	A	0.5139	-1.4	D	0.9966
PA4053_ribE_at	212.2	P	0.0007	-1.1	D	1.0000	201.5	P	0.0036	-0.8	D	0.9997
PA4386_groES_at	550.8	P	0.0007	-1.6	D	1.0000	1021	P	0.0007	-0.4	D	0.9996
PA4443_cysD_at	194.3	P	0.0007	-1.6	D	1.0000	213.8	P	0.0007	-0.6	D	1.0000
PA4563_rpsT_at	219.5	P	0.0007	-1.3	D	1.0000	438.1	P	0.0007	-0.7	D	0.9998
PA4568_rplU_at	423.4	P	0.0007	-1.9	D	1.0000	1486.9	P	0.0007	-0.7	D	0.9996
PA4748_tpiA_at	79.9	P	0.0012	-1.4	D	1.0000	124.6	P	0.0012	-0.6	D	0.9989
PA4847_accB_at	188.9	P	0.0007	-1.3	D	1.0000	306.2	P	0.0096	-0.6	D	0.9979
PA5013_ilvE_at	304.4	P	0.0007	-1.3	D	1.0000	383	P	0.0015	-0.6	D	0.9998
PA5182_at	194.6	P	0.0007	-1.3	D	1.0000	270.9	P	0.0015	-0.7	D	1.0000
PA5212_i_at	183.5	P	0.0024	-0.8	D	1.0000	149	P	0.0093	-0.8	D	1.0000
PA5253_algP_at	697.3	P	0.0007	-1.6	D	1.0000	2675	P	0.0007	-0.6	D	1.0000
PA5312_at	316.7	P	0.0007	-1.4	D	1.0000	275.7	P	0.0007	-0.5	D	0.9999
PA5369_at	135.4	P	0.0007	-0.7	D	0.9995	213.1	P	0.0054	-0.8	NC	0.9841
PA5570_rpmH_at	98	P	0.0007	-1.6	D	1.0000	162.6	P	0.0023	-0.7	D	1.0000

Time 13 hours

	WTA 13 hours						WTB 13 hours					
PA0224_at	366.1	P	0.0007	-0.2	NC	0.9819	94.3	P	0.0012	-1.3	D	0.9999
PA0262_at	37.7	P	0.0116	-0.4	D	0.9991	50.6	A	0.1554	0.3	NC	0.5000
PA0263_hcpC_s_at	686.9	P	0.0007	-0.5	D	1.0000	544.9	P	0.0009	-2.3	D	1.0000
PA0284_at	282.6	P	0.0007	-1.1	D	1.0000	200.4	P	0.0019	-1.3	D	1.0000
PA0579_rpsU_at	460.2	P	0.0007	-0.1	NC	0.5000	463.3	P	0.0007	0.2	NC	0.5000
PA0762_algU_at	354.9	P	0.0007	-0.9	D	1.0000	471.3	P	0.0012	0	NC	0.5000
PA0848_at	38.9	P	0.0232	-1.5	D	1.0000	46.7	A	0.1107	-1.2	D	0.9993
PA0962_at	641.3	P	0.0007	-0.3	D	0.9993	609.9	P	0.0007	-0.5	D	1.0000
PA1178_oprH_at	160.3	P	0.0038	-2	D	1.0000	155.9	P	0.0048	-2.3	D	1.0000
PA1193_at	85.9	P	0.0320	0.1	NC	0.5855	99.5	A	0.1244	-0.1	NC	0.2425
PA1198_at	599.7	P	0.0007	0	NC	0.5000	433	P	0.0012	-0.1	NC	0.5000
PA1493_cysP_at	380.1	P	0.0007	-0.1	NC	0.5656	310	P	0.0007	-0.1	NC	0.9708
PA1544_anr_at	215	P	0.0007	-0.2	NC	0.9929	56	A	0.0760	-1.1	D	0.9989
PA1588_sucC_at	1946.2	P	0.0007	0.3	NC	0.2425	574.9	P	0.0036	-0.9	D	1.0000
PA1656_at	81.8	P	0.0036	-0.5	D	0.9997	53.5	A	0.0865	-1.4	D	1.0000
PA1657_at	334.5	P	0.0007	-0.5	D	1.0000	163.9	P	0.0009	-1.8	D	1.0000
PA1658_at	169.8	P	0.0007	-0.3	D	1.0000	73.9	P	0.0232	-2.3	D	1.0000
PA1660_at	38.9	P	0.0009	-0.4	NC	0.9841	45.1	A	0.2315	-1	D	0.9993
PA1664_at	84.2	P	0.0007	-0.6	D	1.0000	56.1	P	0.0139	-1.5	D	0.9999
PA2562_at	304.5	P	0.0009	-0.5	D	1.0000	477.1	P	0.0054	0	NC	0.5000
PA2658_at	107.5	P	0.0139	-0.8	D	1.0000	51.4	A	0.0980	-1.5	D	0.9996
PA2760_at	808.8	P	0.0007	0.1	NC	0.5000	440.3	P	0.0007	-0.7	D	1.0000
PA2808_i_at	950.7	P	0.0010	0	NC	0.5000	630.9	P	0.0010	0	NC	0.5000

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	WTA 13 hours						WTB 13 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA2850_ohr_at	65.3	P	0.0015	-1.3	D	1.0000	81	P	0.0434	-1.3	D	1.0000
PA2951_etfA_at	858.4	P	0.0007	0.3	NC	0.0871	282.8	P	0.0023	-0.1	NC	0.9346
PA3159_wbpA_at	759.3	P	0.0007	-0.1	NC	0.9279	560.5	P	0.0007	-0.9	D	1.0000
PA3162_rpsA_at	1302.1	P	0.0007	0.1	NC	0.5000	703.4	P	0.0007	-0.6	D	0.9980
PA3266_capB_at	223.5	P	0.0007	-0.1	NC	0.5000	234.2	P	0.0007	-0.3	NC	0.9938
PA3287_at	64.1	P	0.0044	-1.4	D	1.0000	55.8	A	0.1107	-1.6	D	1.0000
PA3328_at	218.1	P	0.0007	-0.3	D	0.9960	79.9	P	0.0139	-1.6	D	1.0000
PA3450_at	155.9	P	0.0080	-0.8	D	1.0000	227.9	P	0.0066	-1	D	0.9998
PA3552_at	94.2	P	0.0029	-0.5	D	0.9977	51.9	P	0.0374	-1.5	D	0.9973
PA3690_at	1639.3	P	0.0007	-0.1	NC	0.9129	1074.7	P	0.0029	-0.6	D	0.9986
PA3814_iscS_at	1034	P	0.0007	0.1	NC	0.5000	848.5	P	0.0019	-0.3	NC	0.6716
PA3815_at	334.7	P	0.0012	-0.7	D	1.0000	159.4	P	0.0080	-0.5	D	0.9971
PA3819_at	255.3	P	0.0023	-0.9	D	1.0000	385.3	P	0.0023	0.3	NC	0.0534
PA3904_i_at	504.7	P	0.0005	0	NC	0.5000	1008.7	P	0.0015	0.4	NC	0.0069
PA3905_at	64	P	0.0019	0	NC	0.7247	46.5	A	0.1394	-0.3	NC	0.9409
PA4053_ribE_at	380	P	0.0007	-0.1	NC	0.9313	293.4	P	0.0029	0	NC	0.6808
PA4386_groES_at	1785.6	P	0.0007	0	NC	0.5000	1300.9	P	0.0012	-0.3	D	0.9993
PA4443_cysD_at	489	P	0.0007	-0.1	NC	0.8708	479.7	P	0.0007	0.1	NC	0.5000
PA4563_rpsT_at	550	P	0.0007	-0.1	NC	0.5000	1054.5	P	0.0007	0.6	I	0.0040
PA4568_rplU_at	1444.3	P	0.0007	0	NC	0.5000	1667.5	P	0.0007	-0.4	NC	0.9493
PA4748_tpiA_at	160.8	P	0.0007	-0.5	D	0.9999	184.4	P	0.0012	-0.2	NC	0.8230
PA4847_accB_at	374	P	0.0007	-0.1	NC	0.6808	442.5	P	0.0096	0.1	NC	0.4344
PA5013_ilvE_at	698.5	P	0.0007	0	NC	0.5000	296.4	P	0.0009	-0.5	D	0.9993
PA5182_at	232.2	P	0.0009	-1	D	1.0000	355.4	P	0.0007	-0.1	NC	0.9851
PA5212_i_at	180.7	P	0.0024	-0.9	D	1.0000	672.9	P	0.0019	1.1	I	0.0000
PA5253_algP_at	2396.1	P	0.0007	0.3	NC	0.1090	2966.3	P	0.0007	-0.1	NC	0.5000
PA5312_at	859.8	P	0.0007	0.1	NC	0.5000	455.3	P	0.0009	-0.2	D	0.9985
PA5369_at	127.1	P	0.0009	-0.4	D	0.9999	103.8	P	0.0036	-1.4	D	0.9983
PA5570_rpmH_at	275.9	P	0.0007	0	NC	0.5000	250.1	P	0.0007	0.1	NC	0.5000

Time 26 hours

	WTA 26 hours						WTB 26 hours					
PA0224_at	160.2	P	0.0007	-1.1	D	1.0000	94.3	P	0.0012	-1.3	D	0.9999
PA0262_at	57.6	P	0.0029	0	NC	0.5000	50.6	A	0.1554	0.3	NC	0.5000
PA0263_hcpC_s_at	441	P	0.0007	-1.2	D	1.0000	544.9	P	0.0009	-2.3	D	1.0000
PA0284_at	229.5	P	0.0007	-1.4	D	1.0000	200.4	P	0.0019	-1.3	D	1.0000
PA0579_rpsU_at	355.2	P	0.0009	-0.4	D	1.0000	463.3	P	0.0007	0.2	NC	0.5000
PA0762_algU_at	305.8	P	0.0007	-1.1	D	1.0000	471.3	P	0.0012	0	NC	0.5000
PA0848_at	45.8	P	0.0139	-1.4	D	0.9999	46.7	A	0.1107	-1.2	D	0.9993
PA0962_at	554	P	0.0007	-0.4	D	1.0000	609.9	P	0.0007	-0.5	D	1.0000
PA1178_oprH_at	227.3	P	0.0030	-2.1	D	1.0000	155.9	P	0.0048	-2.3	D	1.0000
PA1193_at	98.6	P	0.0273	0.3	NC	0.4145	99.5	A	0.1244	-0.1	NC	0.2425
PA1198_at	443.1	P	0.0009	-0.3	D	1.0000	433	P	0.0012	-0.1	NC	0.5000
PA1493_cysP_at	324.4	P	0.0007	-0.3	D	0.9998	310	P	0.0007	-0.1	NC	0.9708
PA1544_anr_at	148.5	P	0.0007	-0.8	D	1.0000	56	A	0.0760	-1.1	D	0.9989
PA1588_sucC_at	1836.7	P	0.0007	0.1	NC	0.5000	574.9	P	0.0036	-0.9	D	1.0000

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	WTA 26 hours						WTB 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA1656_at	73.1	P	0.0036	-1	D	0.9999	53.5	A	0.0865	-1.4	D	1.0000
PA1657_at	138.9	P	0.0009	-1.7	D	1.0000	163.9	P	0.0009	-1.8	D	1.0000
PA1658_at	82.1	P	0.0009	-1.4	D	1.0000	73.9	P	0.0232	-2.3	D	1.0000
PA1660_at	26.5	P	0.0007	-0.9	D	0.9998	45.1	A	0.2315	-1	D	0.9993
PA1664_at	64.6	P	0.0007	-1.3	D	1.0000	56.1	P	0.0139	-1.5	D	0.9999
PA2562_at	228.9	P	0.0015	-0.9	D	1.0000	477.1	P	0.0054	0	NC	0.5000
PA2658_at	86.1	P	0.0116	-1	D	1.0000	51.4	A	0.0980	-1.5	D	0.9996
PA2760_at	754.2	P	0.0007	0.1	NC	0.5000	440.3	P	0.0007	-0.7	D	1.0000
PA2808_i_at	388.5	P	0.0010	-1	D	0.9999	630.9	P	0.0010	0	NC	0.5000
PA2850_ohr_at	74.1	P	0.0012	-1	D	1.0000	81	P	0.0434	-1.3	D	1.0000
PA2951_etfA_at	795.6	P	0.0007	0.1	NC	0.5000	282.8	P	0.0023	-0.1	NC	0.9346
PA3159_wbpA_at	661.2	P	0.0007	-0.4	D	0.9997	560.5	P	0.0007	-0.9	D	1.0000
PA3162_rpsA_at	1202.1	P	0.0007	-0.2	NC	0.5656	703.4	P	0.0007	-0.6	D	0.9980
PA3266_capB_at	164.1	P	0.0007	-0.5	D	0.9991	234.2	P	0.0007	-0.3	NC	0.9938
PA3287_at	60.9	P	0.0023	-1.6	D	1.0000	55.8	A	0.1107	-1.6	D	1.0000
PA3328_at	197.3	P	0.0009	-0.5	D	1.0000	79.9	P	0.0139	-1.6	D	1.0000
PA3450_at	143.7	P	0.0066	-0.8	D	1.0000	227.9	P	0.0066	-1	D	0.9998
PA3552_at	114.1	P	0.0044	-0.4	D	0.9985	51.9	P	0.0374	-1.5	D	0.9973
PA3690_at	915	P	0.0007	-1	D	1.0000	1074.7	P	0.0029	-0.6	D	0.9986
PA3814_iscS_at	937.9	P	0.0007	-0.1	NC	0.5000	848.5	P	0.0019	-0.3	NC	0.6716
PA3815_at	232.7	P	0.0015	-1	D	1.0000	159.4	P	0.0080	-0.5	D	0.9971
PA3819_at	213.8	P	0.0029	-1.3	D	1.0000	385.3	P	0.0023	0.3	NC	0.0534
PA3904_i_at	339.5	P	0.0015	-0.6	D	0.9999	1008.7	P	0.0015	0.4	NC	0.0069
PA3905_at	65.2	P	0.0036	-0.1	NC	0.9519	46.5	A	0.1394	-0.3	NC	0.9409
PA4053_ribE_at	332.3	P	0.0007	-0.1	NC	0.9378	293.4	P	0.0029	0	NC	0.6808
PA4386_groES_at	1286.9	P	0.0007	-0.5	D	1.0000	1300.9	P	0.0012	-0.3	D	0.9993
PA4443_cysD_at	311.3	P	0.0007	-0.7	D	1.0000	479.7	P	0.0007	0.1	NC	0.5000
PA4563_rpsT_at	492.9	P	0.0007	-0.2	NC	0.7247	1054.5	P	0.0007	0.6	I	0.0040
PA4568_rplU_at	1238.5	P	0.0007	-0.3	D	0.9994	1667.5	P	0.0007	-0.4	NC	0.9493
PA4748_tpiA_at	132	P	0.0007	-0.7	D	1.0000	184.4	P	0.0012	-0.2	NC	0.8230
PA4847_accB_at	361.2	P	0.0007	-0.2	NC	0.9544	442.5	P	0.0096	0.1	NC	0.4344
PA5013_ilvE_at	548.1	P	0.0007	-0.2	NC	0.9877	296.4	P	0.0009	-0.5	D	0.9993
PA5182_at	177.2	P	0.0007	-1.3	D	1.0000	355.4	P	0.0007	-0.1	NC	0.9851
PA5212_i_at	147.4	P	0.0030	-1.2	D	1.0000	672.9	P	0.0019	1.1	I	0.0000
PA5253_algP_at	1946.8	P	0.0007	-0.1	NC	0.5000	2966.3	P	0.0007	-0.1	NC	0.5000
PA5312_at	540.4	P	0.0007	-0.6	D	1.0000	455.3	P	0.0009	-0.2	D	0.9985
PA5369_at	116.8	P	0.0007	-0.6	D	0.9999	103.8	P	0.0036	-1.4	D	0.9983
PA5570_rpmH_at	227	P	0.0007	-0.4	D	0.9980	250.1	P	0.0007	0.1	NC	0.5000

RpoS- time 0, 6.5 hours

	RpoS$^-$ 0 hours				RpoS$^-$ 6.5 hours					
PA0224_at	365.1	P	0.0007		151.1	P	0.0007	-1.1	D	1.0000
PA0262_at	73.8	P	0.0012		102.3	P	0.0009	0.4	I	0.0000
PA0263_hcpC_s_at	1673.2	P	0.0007		400.7	P	0.0007	-2.1	D	1.0000
PA0284_at	603.7	P	0.0007		175.9	P	0.0007	-1.8	D	1.0000
PA0579_rpsU_at	191.4	P	0.0007		102.4	P	0.0007	-0.8	D	0.9993

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	RpoS⁻ 0 hours						RpoS⁻ 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0762_algU_at	348.9	P	0.0007				157.8	P	0.0007	-1.3	D	1.0000
PA0848_at	119.2	P	0.0007				104.8	P	0.0007	-0.3	NC	0.6531
PA0962_at	890.8	P	0.0007				183	P	0.0007	-2.3	D	1.0000
PA1178_oprH_at	1268.5	P	0.0014				215.8	P	0.0030	-2.6	D	1.0000
PA1193_at	98.8	P	0.0139				120.1	P	0.0116	0.2	I	0.0027
PA1198_at	349.7	P	0.0007				136	P	0.0007	-1.1	D	1.0000
PA1493_cysP_at	451.5	P	0.0007				166.8	P	0.0007	-1.5	D	1.0000
PA1544_anr_at	109.8	P	0.0007				111.4	P	0.0009	-0.3	NC	0.8957
PA1588_sucC_at	1207.5	P	0.0007				203.6	P	0.0007	-2.4	D	1.0000
PA1656_at	157.7	P	0.0012				110.4	P	0.0009	-0.5	D	0.9979
PA1657_at	763.8	P	0.0007				174.1	P	0.0007	-2.2	D	1.0000
PA1658_at	418.6	P	0.0007				108.9	P	0.0007	-1.9	D	1.0000
PA1660_at	65.8	P	0.0007				68.2	P	0.0007	-0.3	NC	0.5000
PA1664_at	276	P	0.0007				152.9	P	0.0007	-0.8	D	1.0000
PA2562_at	258	P	0.0012				95.2	P	0.0007	-1	D	1.0000
PA2658_at	106.9	P	0.0080				70.4	P	0.0019	-0.5	NC	0.9346
PA2760_at	815.3	P	0.0007				182.6	P	0.0009	-2.3	D	1.0000
PA2808_i_at	1937	P	0.0010				291.6	P	0.0010	-2.7	D	0.9999
PA2850_ohr_at	257.5	P	0.0007				91	P	0.0007	-1.7	D	1.0000
PA2951_etfA_at	584.4	P	0.0007				129.4	P	0.0012	-2.2	D	1.0000
PA3159_wbpA_at	291.8	P	0.0007				92	P	0.0007	-1.5	D	1.0000
PA3162_rpsA_at	627.9	P	0.0007				179.6	P	0.0009	-2	D	1.0000
PA3266_capB_at	98.6	P	0.0009				66.2	P	0.0007	-1.1	D	0.9968
PA3287_at	161.4	P	0.0007				100.9	P	0.0007	-0.7	D	1.0000
PA3328_at	456	P	0.0007				109.6	P	0.0007	-2	D	1.0000
PA3450_at	236.6	P	0.0012				124.7	P	0.0007	-0.9	D	0.9997
PA3552_at	140.5	P	0.0019				123.3	P	0.0012	-0.1	NC	0.5000
PA3690_at	1317.4	P	0.0007				154.6	P	0.0007	-3	D	1.0000
PA3814_iscS_at	1020.2	P	0.0007				151.3	P	0.0007	-2.2	D	1.0000
PA3815_at	381.5	P	0.0007				135.5	P	0.0007	-1.5	D	1.0000
PA3819_at	601.4	P	0.0009				119.6	P	0.0019	-2.3	D	1.0000
PA3904_i_at	335.8	P	0.0005				158.4	P	0.0015	-1.2	D	1.0000
PA3905_at	74.4	P	0.0066				52	P	0.0019	-0.5	NC	0.5000
PA4053_ribE_at	308.4	P	0.0007				134.5	P	0.0007	-1.2	D	1.0000
PA4386_groES_at	1287	P	0.0007				247.2	P	0.0007	-2.3	D	1.0000
PA4443_cysD_at	293	P	0.0007				160.6	P	0.0007	-0.9	D	0.9992
PA4563_rpsT_at	177.7	P	0.0007				161.9	P	0.0007	-0.3	NC	0.7247
PA4568_rplU_at	885.5	P	0.0007				239.9	P	0.0007	-1.9	D	1.0000
PA4748_tpiA_at	63.6	P	0.0007				59.6	P	0.0007	-0.1	NC	0.5000
PA4847_accB_at	272.6	P	0.0007				192.8	P	0.0007	-1.1	D	1.0000
PA5013_ilvE_at	631.5	P	0.0007				195.8	P	0.0007	-1.6	D	1.0000
PA5182_at	360.6	P	0.0007				175.9	P	0.0007	-1	D	1.0000
PA5212_i_at	440.7	P	0.0024				166.3	P	0.0024	-1.4	D	1.0000
PA5253_algP_at	1797.2	P	0.0007				319.5	P	0.0007	-2.4	D	1.0000
PA5312_at	614.3	P	0.0007				186.9	P	0.0007	-1.7	D	1.0000
PA5369_at	207.7	P	0.0007				170.3	P	0.0007	-0.4	NC	0.9046

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	RpoS⁻ 0 hours						RpoS⁻ 6.5 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA5570_rpmH_at	140.5	P	0.0007				70.1	P	0.0007	-1	D	1.0000

RpoS- mutant time 13, 26 hours

	RpoS- 13 hours						RpoS- 26 hours					
PA0224_at	210.4	P	0.0007	-0.7	D	1.0000	138.1	P	0.0007	-1.4	D	1.0000
PA0262_at	59.3	P	0.0019	-0.4	NC	0.9841	49.3	P	0.0080	-0.6	D	1.0000
PA0263_hcpC_s_at	1691.2	P	0.0007	0	NC	0.5000	1510.6	P	0.0007	-0.1	D	0.9957
PA0284_at	361	P	0.0007	-0.7	D	1.0000	552.7	P	0.0007	-0.2	D	1.0000
PA0579_rpsU_at	254.1	P	0.0007	0.5	I	0.0000	412.4	P	0.0007	1.3	I	0.0000
PA0762_algU_at	316.7	P	0.0007	-0.2	D	0.9971	380.1	P	0.0007	-0.1	NC	0.6342
PA0848_at	29.9	P	0.0232	-2	D	0.9999	26.3	M	0.0503	-1.9	D	0.9999
PA0962_at	747.6	P	0.0007	-0.2	D	0.9968	941.1	P	0.0007	0	NC	0.5000
PA1178_oprH_at	240.4	P	0.0024	-2.4	D	1.0000	292.3	P	0.0030	-2.1	D	1.0000
PA1193_at	86.7	P	0.0273	-0.1	NC	0.9002	107.3	P	0.0165	0.2	NC	0.5000
PA1198_at	440.5	P	0.0007	0.4	I	0.0023	766.4	P	0.0007	1.2	I	0.0000
PA1493_cysP_at	621.2	P	0.0007	0.3	NC	0.0562	689.6	P	0.0007	0.6	I	0.0000
PA1544_anr_at	143.2	P	0.0009	0.3	NC	0.5000	146.7	P	0.0007	0.5	I	0.0015
PA1588_sucC_at	2512.8	P	0.0007	1	I	0.0000	1799.9	P	0.0007	0.6	I	0.0000
PA1656_at	147.9	P	0.0029	0	NC	0.5000	99.2	P	0.0029	-0.5	D	0.9997
PA1657_at	681.1	P	0.0007	-0.2	NC	0.9633	605.8	P	0.0007	-0.3	D	0.9973
PA1658_at	479.6	P	0.0007	0.3	NC	0.2046	299.7	P	0.0009	-0.3	D	1.0000
PA1660_at	67.8	P	0.0007	0.1	NC	0.5000	40.7	P	0.0007	-0.6	D	0.9999
PA1664_at	178.9	P	0.0007	-0.7	D	1.0000	185.7	P	0.0007	-0.7	D	1.0000
PA2562_at	164.8	P	0.0023	-0.8	D	1.0000	154.7	P	0.0023	-0.7	D	1.0000
PA2658_at	101.6	P	0.0054	-0.2	NC	0.7162	86.7	P	0.0054	-0.2	NC	0.8862
PA2760_at	1423	P	0.0007	0.6	I	0.0000	1288.5	P	0.0007	0.6	I	0.0000
PA2808_i_at	923.9	P	0.0010	-0.8	D	0.9999	1693.7	P	0.0010	-0.2	NC	0.9861
PA2850_ohr_at	40.1	P	0.0036	-2.2	D	1.0000	51.3	P	0.0009	-2.3	D	1.0000
PA2951_etfA_at	1185.7	P	0.0007	1	I	0.0000	972.2	P	0.0007	0.7	I	0.0000
PA3159_wbpA_at	476.6	P	0.0007	0.7	I	0.0000	481	P	0.0007	0.8	I	0.0000
PA3162_rpsA_at	1191.6	P	0.0007	0.9	I	0.0000	1169.2	P	0.0007	0.9	I	0.0000
PA3266_capB_at	134.7	P	0.0007	0.8	I	0.0000	115	P	0.0007	0.8	I	0.0000
PA3287_at	29.6	P	0.0054	-2.7	D	1.0000	31.6	P	0.0029	-2.8	D	1.0000
PA3328_at	446.1	P	0.0007	-0.1	NC	0.7331	317.9	P	0.0007	-0.4	D	1.0000
PA3450_at	166.9	P	0.0080	-0.6	D	1.0000	187.6	P	0.0036	-0.3	D	0.9996
PA3552_at	77.6	P	0.0066	-0.9	D	1.0000	56.1	P	0.0273	-1.2	D	1.0000
PA3690_at	1610.7	P	0.0007	0.3	NC	0.0140	921.3	P	0.0007	-0.4	D	1.0000
PA3814_iscS_at	1329.8	P	0.0007	0.5	I	0.0001	1502.4	P	0.0007	0.6	I	0.0000
PA3815_at	369.2	P	0.0007	0	NC	0.5656	485.5	P	0.0007	0.2	NC	0.5000
PA3819_at	240.1	P	0.0019	-1.3	D	1.0000	343.3	P	0.0009	-0.7	D	1.0000
PA3904_i_at	433.8	P	0.0005	0.5	I	0.0009	536	P	0.0005	0.7	I	0.0001
PA3905_at	71.8	P	0.0023	0.3	NC	0.0076	84.4	P	0.0015	0.5	NC	0.0108
PA4053_ribE_at	403.2	P	0.0007	0.7	I	0.0001	461.9	P	0.0007	0.8	I	0.0000
PA4386_groES_at	1804	P	0.0007	0.4	I	0.0000	2159.3	P	0.0007	0.7	I	0.0000
PA4443_cysD_at	444.3	P	0.0007	0.7	I	0.0000	515.2	P	0.0007	0.9	I	0.0000
PA4563_rpsT_at	304.6	P	0.0007	0.8	I	0.0000	471.2	P	0.0007	1.3	I	0.0000

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

	RpoS- 13 hours						RpoS- 26 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA4568_rplU_at	1603	P	0.0007	0.8	I	0.0000	1938.9	P	0.0007	1	I	0.0000
PA4748_tpiA_at	122.3	P	0.0007	1.1	I	0.0000	133.4	P	0.0007	1.1	I	0.0000
PA4847_accB_at	501.8	P	0.0007	0.5	I	0.0007	473.1	P	0.0007	0.8	I	0.0000
PA5013_ilvE_at	936	P	0.0007	0.6	I	0.0000	1028.2	P	0.0007	0.7	I	0.0000
PA5182_at	145.9	P	0.0007	-1.2	D	1.0000	185.3	P	0.0009	-0.9	D	1.0000
PA5212_i_at	143.9	P	0.0038	-1.6	D	1.0000	147.8	P	0.0024	-1.7	D	1.0000
PA5253_algP_at	2401.9	P	0.0007	0.4	I	0.0012	2376.9	P	0.0007	0.4	I	0.0000
PA5312_at	886.9	P	0.0007	0.6	I	0.0000	790.8	P	0.0007	0.2	NC	0.0721
PA5369_at	179.2	P	0.0007	-0.1	NC	0.8026	141.6	P	0.0007	-0.6	D	0.9977
PA5570_rpmH_at	244.3	P	0.0007	0.9	I	0.0000	328.1	P	0.0007	1.3	I	0.0000

Affymetrix ID	**Descriptions**
PA0224_at	PA0224 /DEF=probable aldolase /FUNCTION=Putative enzymes
PA0262_at	PA0262 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA0263_hcpC_s_at	PA0263 /GENE=hcpC /DEF=secreted protein Hcp /FUNCTION=Secreted Factors (toxins, enzymes, alginate)
PA0284_at	PA0284 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA0579_rpsU_at	PA0579 /GENE=rpsU /DEF=30S ribosomal protein S21 /FUNCTION=Hypothetical, unclassified, unknown
PA0762_algU_at	PA0762 /GENE=algU /DEF=sigma factor AlgU /FUNCTION=Transcriptional regulators
PA0848_at	PA0848 /DEF=probable alkyl hydroperoxide reductase /FUNCTION=Adaptation, protection; Putative enzymes
PA0962_at	PA0962 /DEF=probable dna-binding stress protein /FUNCTION=Adaptation, protection
PA1178_oprH_at	PA1178 /GENE=oprH /DEF=outer membrane protein H1 precursor /FUNCTION=Adaptation, protection; Membrane proteins
PA1193_at	PA1193 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1198_at	PA1198 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1493_cysP_at	PA1493 /GENE=cysP /DEF=sulfate-binding protein of ABC transporter /FUNCTION=Transport of small molecules
PA1544_anr_at	PA1544 /GENE=anr /DEF=transcriptional regulator Anr /FUNCTION=Transcriptional regulators
PA1588_sucC_at	PA1588 /GENE=sucC /DEF=succinyl-CoA synthetase beta chain /FUNCTION=Energy metabolism
PA1656_at	PA1656 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1657_at	PA1657 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1658_at	PA1658 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1660_at	PA1660 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA1664_at	PA1664 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2562_at	PA2562 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2658_at	PA2658 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2760_at	PA2760 /DEF=probable outer membrane protein /FUNCTION=Transport of small molecules
PA2808_i_at	PA2808 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA2850_ohr_at	PA2850 /GENE=ohr /DEF=organic hydroperoxide resistance protein /FUNCTION=Adaptation, protection
PA2951_etfA_at	PA2951 /GENE=etfA /DEF=electron transfer flavoprotein alpha-subunit /FUNCTION=Energy metabolism

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

Affymetrix ID	Descriptions
PA3159_wbpA_at	PA3159 /GENE=wbpA /DEF=probable UDP-glucose/GDP-mannose dehydrogenase WbpA /FUNCTION=Cell wall / LPS / capsule; Putative enzymes
PA3162_rpsA_at	PA3162 /GENE=rpsA /DEF=30S ribosomal protein S1 /FUNCTION=Translation, post-translational modification, degradation
PA3266_capB_at	PA3266 /GENE=capB /DEF=cold acclimation protein B /FUNCTION=Adaptation, protection; Transcriptional regulators
PA3287_at	PA3287 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3328_at	PA3328 /DEF=probable FAD-dependent monooxygenase /FUNCTION=Putative enzymes
PA3450_at	PA3450 /DEF=probable antioxidant protein /FUNCTION=Adaptation, protection
PA3552_at	PA3552 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3690_at	PA3690 /DEF=probable metal-transporting P-type ATPase /FUNCTION=Membrane proteins; Transport of small molecules
PA3814_iscS_at	PA3814 /GENE=iscS /DEF=L-cysteine desulfurase (pyridoxal phosphate-dependent) /FUNCTION=Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA3815_at	PA3815 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3819_at	PA3819 /DEF=conserved hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins
PA3904_i_at	PA3904 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA3905_at	PA3905 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA4053_ribE_at	PA4053 /GENE=ribE /DEF=6,7-dimethyl-8-ribityllumazine synthase /FUNCTION=Biosynthesis of cofactors, prosthetic groups and carriers
PA4386_groES_at	PA4386 /GENE=groES /DEF=GroES protein /FUNCTION=Chaperones & heat shock proteins
PA4443_cysD_at	PA4443 /GENE=cysD /DEF=ATP sulfurylase small subunit /FUNCTION=Amino acid biosynthesis and metabolism; Central intermediary metabolism
PA4563_rpsT_at	PA4563 /GENE=rpsT /DEF=30S ribosomal protein S20 /FUNCTION=Translation, post-translational modification, degradation; Central intermediary metabolism
PA4568_rplU_at	PA4568 /GENE=rplU /DEF=50S ribosomal protein L21 /FUNCTION=Translation, post-translational modification, degradation
PA4748_tpiA_at	PA4748 /GENE=tpiA /DEF=triosephosphate isomerase /FUNCTION=Central intermediary metabolism; Energy metabolism
PA4847_accB_at	PA4847 /GENE=accB /DEF=biotin carboxyl carrier protein (BCCP) /FUNCTION=Fatty acid and phospholipid metabolism
PA5013_ilvE_at	PA5013 /GENE=ilvE /DEF=branched-chain amino acid transferase /FUNCTION=Amino acid biosynthesis and metabolism
PA5182_at	PA5182 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown; Membrane proteins

Affymetrix GCOS data for genes decreasing in response to PCP (cont.)

Affymetrix ID	Descriptions
PA5212_i_at	PA5212 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA5253_algP_at	PA5253 /GENE=algP /DEF=alginate regulatory protein AlgP /FUNCTION=Transcriptional regulators
PA5312_at	PA5312 /DEF=probable aldehyde dehydrogenase /FUNCTION=Putative enzymes
PA5369_at	PA5369 /DEF=hypothetical protein /FUNCTION=Hypothetical, unclassified, unknown
PA5570_rpmH_at	PA5570 /GENE=rpmH /DEF=50S ribosomal protein L34 /FUNCTION=Central intermediary metabolism; Translation, post-translational modification, degradation

Affymetrix GCOS data for control genes used in RT-PCR and rpoS

	WTA time 0 hours						WTB time 0 hours					
	Detection			Change			Detection			Change		
Affymetrix ID	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**	**Sig.[c]**	**P/A**	**p-value**	**SLR[d]**	**I/D**	**p-value**
PA0393_proC_at	90.5	P	0.0116				45.6	A	0.3766			
PA0761_nadB_at	123.6	P	0.0007				180	P	0.0012			
PA3622_rpoS_at	2378.1	P	0.0007				1519.9	P	0.0007			
	WTA time 6.5 hours						**WTB time 6.5 hours**					
PA0393_proC_at	88.4	P	0.0096	0	NC	0.2838	70.6	A	0.3001	0.4	NC	0.5000
PA0761_nadB_at	97.6	P	0.0007	-0.2	NC	0.5000	186.1	P	0.0007	0.2	NC	0.2925
PA3622_rpoS_at	920.5	P	0.0007	-1.4	D	1.0000	1836.9	P	0.0007	0.3	I	0.0000
	WTA 13 hours						**WTB 13 hours**					
PA0393_proC_at	96.8	P	0.0165	0.1	NC	0.5000	57.3	A	0.2108	0	NC	0.5000
PA0761_nadB_at	119.4	P	0.0007	0.1	NC	0.5000	140.6	P	0.0044	-0.2	NC	0.8230
PA3622_rpoS_at	2584.8	P	0.0007	0.3	NC	0.3013	955.8	P	0.0009	-0.9	D	0.9999
	WTA 26 hours						**WTB 26 hours**					
PA0393_proC_at	94.2	P	0.0116	0.1	NC	0.5000	57.3	A	0.2108	0	NC	0.5000
PA0761_nadB_at	140.7	P	0.0007	0.1	NC	0.5000	140.6	P	0.0044	-0.2	NC	0.8230
PA3622_rpoS_at	2395.7	P	0.0007	0	NC	0.5000	955.8	P	0.0009	-0.9	D	0.9999
	RpoS- time 0						**RpoS- 6.5 hours**					
PA0393_proC_at	147.2	P	0.0029				90.5	P	0.0036	-0.6	NC	0.9918
PA0761_nadB_at	117.8	P	0.0007				92.5	P	0.0007	-0.5	NC	0.9755
PA3622_rpoS_at	489.4	P	0.0007				200.3	P	0.0007	-1.7	D	1.0000
	RpoS- 13 hours						**RpoS- 26 hours**					
PA0393_proC_at	172	P	0.0023	0.3	NC	0.5000	175.9	P	0.0029	0.3	NC	0.1459
PA0761_nadB_at	137.3	P	0.0007	0.1	NC	0.5000	142.6	P	0.0007	0.2	NC	0.3949
PA3622_rpoS_at	639.5	P	0.0009	0.4	NC	0.0231	612.6	P	0.0007	0.5	I	0.0000

Affymetrix ID	**Descriptions**
PA0393_proC_at	PA0393 /GENE=proC /DEF=pyrroline-5-carboxylate reductase /FUNCTION=Amino acid biosynthesis and metabolism
PA0761_nadB_at	PA0761 /GENE=nadB /DEF=L-aspartate oxidase /FUNCTION=Amino acid biosynthesis and metabolism; Biosynthesis of cofactors, prosthetic groups and carriers
PA3622_rpoS_at	PA3622 /GENE=rpoS /DEF=sigma factor RpoS /FUNCTION=Transcriptional regulators
PA4268_rpsL_at	PA4268 /GENE=rpsL /DEF=30S ribosomal protein S12 /FUNCTION=Translation, post-translational modification, degradation

Figure 3.3

Data found in Excel file: *mexB_PCP_RT_final*.from RT-PCR data run May 2006

RT-PCR data determining *mexB* expression

sample	*mexB* SQ[a]	*nadB* SQ	*mexB/ nadB*	fold change[b]	*proC* SQ	*mexB/ proC*	fold change
RA1[c]	0.015	0.0021	7.109	1	0.032	0.4688	1
RA2	0.116	0.0014	82.857	11.655	0.0151	7.6821	16.389
RA3	0.106	0.0011	95.495	13.433	0.0207	5.1208	10.924
RA4	0.137	0.0012	119.13	16.758	0.0153	8.9542	19.102
RB1	0.010	0.0008	11.799	1	0.0125	0.7608	1
RB2	0.097	0.0014	67.431	5.7149	0.0269	3.6097	4.7446
RB3	0.095	0.0006	146.69	12.432	0.0122	7.8033	10.257
RB4	0.093	0.0008	111.28	9.4317	0.011	8.4273	11.077
WA1	0.013	0.0009	15.537	1	0.019	0.7	1
WA2	0.090	0.0009	95.117	6.1218	0.0114	7.8596	11.228
WA3	0.093	0.0008	111.03	7.1461	0.0176	5.2614	7.5162
WA4	0.129	0.0011	119.44	7.6876	0.0241	5.3527	7.6467
WB1	0.019	0.0017	11.149	1	0.0648	0.2994	1
WB2	0.033	0.0011	30	2.6907	0.0277	1.1805	3.9431
WB3	0.026	0.0007	37.997	3.408	0.0079	3.2741	10.936
WB4	0.021	0.0006	33.754	3.0274	0.0071	3.0141	10.068

[a]SQ=average starting quantity (ng) of RNA in triplicate RT-PCR reactions. RA, RB and WA, WB represent duplicate experiments of RpoS- mutant and WT, respectively.
[b]Fold change in comparison with initial timepoint (RA1, RB1, WA1, WB1). 1=0 hour, 2=6.5 hours, 3=13 hours, 4=26 hours.
[c]RA=Sept 5, 2005; RB=June 19, 2005; WA=Sept 15, 2005; WB=Nov 10, 2005

Average fold change[a] of the four data points using both *nadB* and *proC*

RpoS				WT			
hours post-PCP	fold change	S.D.	p-value	hours post-PCP	fold change	S.D.	p-value
-2.165	1	0		-0.875	1	0	
6.25	9.6258	5.4459	0.0506	6.275	5.9959	3.7652	0.0767
13	11.762	1.4387	0.0006	13.085	7.2516	3.0785	0.0269
26.085	14.092	4.5833	0.0106	25.915	7.1073	2.946	0.0255

[a]Example of calculation of average: $((SQ\textit{mexB}/SQ\textit{nadB})_{RA1} + (SQ\textit{mexB}/SQ\textit{nadB})_{RB1} + (SQ\textit{mexB}/SQ\textit{proC})_{RA1} + (SQ\textit{mexB}/SQ\textit{proC})_{RB1})/4$

Other comparisons of RT-PCR data

	comparison	t-test[a] p-value
WT		
	6.5 hours to 13 hours	0.6077
	13 hours to 26 hours	0.6698
	26 hours to 6.5 hours	0.617
RpoS$^-$		
	6.5 hours to 13 hours	0.493
	13 hours to 26 hours	0.3932
	26 hours to 6.5 hours	0.011
WT to RpoS$^-$ expression change		
	6.5 hours	0.3149
	13 hours	0.0378
	26 hours	0.0427

[a]Student's t-tests were done for comparison of timepoints within each strain. Unpaired t-tests were done for comparisons of WT to RpoS$^-$ mutant. α=0.05

Raw values for mexB expression

sample	ID	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
RA1	Jmuller1	25.98	-1.707	1.97E-02	1.50E-02	4.07E-03	26.42	3.77E-01
	Jmuller1	26.61	-1.893	1.28E-02				
	Jmuller1	26.66	-1.906	1.24E-02				
RA2	Jmuller2	23.77	-1.052	8.87E-02	1.16E-01	2.50E-02	23.4	3.34E-01
	Jmuller2	23.31	-0.915	1.21E-01				
	Jmuller2	23.12	-0.861	1.38E-01				
RA3	Jmuller3	23.5	-0.972	1.07E-01	1.06E-01	1.04E-02	23.51	1.45E-01
	Jmuller3	23.37	-0.935	1.16E-01				
	Jmuller3	23.66	-1.02	9.55E-02				
RA4	Jmuller4	22.83	-0.774	1.68E-01	1.37E-01	3.48E-02	23.17	3.97E-01
	Jmuller4	23.07	-0.844	1.43E-01				
	Jmuller4	23.6	-1.003	9.93E-02				
RB1	Jmuller5	27.11	-2.039	9.14E-03	9.51E-03	5.58E-04	27.05	8.49E-02
	Jmuller5	26.95	-1.994	1.02E-02				
	Jmuller5	27.09	-2.035	9.23E-03				
RB2	Jmuller6	23.49	-0.971	1.07E-01	9.71E-02	1.01E-02	23.64	1.53E-01
	Jmuller6	23.8	-1.061	8.69E-02				
	Jmuller6	23.63	-1.011	9.76E-02				
RB3	Jmuller7	23.67	-1.023	9.47E-02	9.52E-02	5.17E-03	23.67	7.96E-02
	Jmuller7	23.58	-0.997	1.01E-01				
	Jmuller7	23.74	-1.044	9.03E-02				
RB4	Jmuller8	23.71	-1.035	9.23E-02	9.27E-02	2.31E-03	23.7	3.65E-02
	Jmuller8	23.74	-1.043	9.06E-02				
	Jmuller8	23.66	-1.021	9.52E-02				
WA1	Jmuller9	26.56	-1.877	1.33E-02	1.33E-02	1.59E-03	26.57	1.77E-01
	Jmuller9	26.75	-1.933	1.17E-02				
	Jmuller9	26.39	-1.828	1.49E-02				
WA2	Jmuller10	23.65	-1.018	9.59E-02	8.96E-02	5.91E-03	23.76	9.64E-02
	Jmuller10	23.85	-1.075	8.41E-02				
	Jmuller10	23.77	-1.052	8.88E-02				
WA3	Jmuller11	23.42	-0.948	1.13E-01	9.26E-02	1.95E-02	23.73	3.11E-01
	Jmuller11	23.72	-1.039	9.15E-02				
	Jmuller11	24.04	-1.133	7.37E-02				
WA4	Jmuller12	23.37	-0.935	1.16E-01	1.29E-01	4.22E-02	23.26	4.63E-01
	Jmuller12	23.66	-1.02	9.54E-02				
	Jmuller12	22.76	-0.753	1.77E-01				
WB1	Jmuller13	25.96	-1.699	2.00E-02	1.94E-02	5.28E-03	26.04	4.22E-01
	Jmuller13	26.5	-1.859	1.38E-02				
	Jmuller13	25.67	-1.614	2.43E-02				
WB2	Jmuller14	25.13	-1.454	3.52E-02	3.27E-02	2.16E-03	25.24	9.55E-02
	Jmuller14	25.26	-1.494	3.20E-02				
	Jmuller14	25.31	-1.508	3.10E-02				
WB3	Jmuller15	26.41	-1.832	1.47E-02	2.58E-02	1.36E-02	25.71	7.61E-01
	Jmuller15	24.9	-1.386	4.11E-02				
	Jmuller15	25.84	-1.663	2.17E-02				

Raw values for *mexB* expression cont.

sample	ID	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
	Jmuller16	25.76	-1.64	2.29E-02	2.14E-02	2.09E-03	25.86	1.47E-01
	Jmuller16	26.03	-1.721	1.90E-02				
	Jmuller16	25.8	-1.653	2.22E-02				

Raw values for *nadB* expression

sample	Identifier	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
RA1	Jmuller1	27.48	-2.635	2.32E-03	2.11E-03	1.87E-04	27.62	1.27E-01
	Jmuller1	27.72	-2.709	1.96E-03				
	Jmuller1	27.65	-2.688	2.05E-03				
RA2	Jmuller2	28.28	-2.873	1.34E-03	1.40E-03	7.47E-05	28.21	7.71E-02
	Jmuller2	28.24	-2.863	1.37E-03				
	Jmuller2	28.13	-2.829	1.48E-03				
RA3	Jmuller3	28.07	-2.811	1.54E-03	1.11E-03	3.95E-04	28.61	5.10E-01
	Jmuller3	28.68	-2.995	1.01E-03				
	Jmuller3	29.08	-3.112	7.73E-04				
RA4	Jmuller4	28.19	-2.847	1.42E-03	1.15E-03	2.50E-04	28.52	3.12E-01
	Jmuller4	28.57	-2.962	1.09E-03				
	Jmuller4	28.81	-3.031	9.31E-04				
RB1	Jmuller5	28.64	-2.981	1.04E-03	8.06E-04	2.13E-04	29.05	3.73E-01
	Jmuller5	29.14	-3.132	7.38E-04				
	Jmuller5	29.37	-3.198	6.35E-04				
RB2	Jmuller6	27.96	-2.781	1.66E-03	1.44E-03	2.63E-04	28.19	2.80E-01
	Jmuller6	28.5	-2.94	1.15E-03				
	Jmuller6	28.09	-2.819	1.52E-03				
RB3	Jmuller7	29.6	-3.268	5.40E-04	6.49E-04	1.03E-04	29.35	2.37E-01
	Jmuller7	29.3	-3.178	6.63E-04				
	Jmuller7	29.13	-3.129	7.44E-04				
RB4	Jmuller8	28.59	-2.968	1.08E-03	8.33E-04	2.14E-04	29	3.56E-01
	Jmuller8	29.14	-3.131	7.39E-04				
	Jmuller8	29.26	-3.166	6.82E-04				
WA1	Jmuller9	28.66	-2.987	1.03E-03	8.56E-04	2.46E-04	28.97	4.65E-01
	Jmuller9	29.51	-3.24	5.75E-04				
	Jmuller9	28.76	-3.017	9.62E-04				
WA2	Jmuller10	28.93	-3.068	8.55E-04	9.42E-04	7.69E-05	28.79	1.22E-01
	Jmuller10	28.7	-3	1.00E-03				
	Jmuller10	28.74	-3.013	9.71E-04				
WA3	Jmuller11	29.54	-3.249	5.64E-04	8.34E-04	2.95E-04	29.03	5.20E-01
	Jmuller11	28.5	-2.94	1.15E-03				
	Jmuller11	29.05	-3.104	7.88E-04				
WA4	Jmuller12	28.54	-2.953	1.11E-03	1.08E-03	9.64E-05	28.59	1.33E-01
	Jmuller12	28.74	-3.012	9.73E-04				
	Jmuller12	28.49	-2.937	1.16E-03				

Raw values for *nadB* expression cont.

WB1	Jmuller13	28.1	-2.822	1.51E-03	1.74E-03	4.62E-04	27.92	3.66E-01
	Jmuller13	28.16	-2.84	1.45E-03				
	Jmuller13	27.5	-2.643	2.28E-03				
WB2	Jmuller14	28.55	-2.955	1.11E-03	1.09E-03	1.49E-04	28.58	2.03E-01
	Jmuller14	28.8	-3.03	9.33E-04				
	Jmuller14	28.4	-2.911	1.23E-03				
WB3	Jmuller15	29.65	-3.281	5.24E-04	6.79E-04	1.64E-04	29.3	3.54E-01
	Jmuller15	28.94	-3.07	8.51E-04				
	Jmuller15	29.31	-3.18	6.61E-04				
WB4	Jmuller16	29.32	-3.185	6.53E-04	6.34E-04	1.13E-04	29.38	2.70E-01
	Jmuller16	29.68	-3.291	5.12E-04				
	Jmuller16	29.15	-3.133	7.37E-04				

Raw values for *proC* expression

sample	Identifier	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
RA1	Jmuller1	29.04	-1.461	3.46E-02	3.20E-02	2.55E-03	29.15	1.15E-01
	Jmuller1	29.15	-1.496	3.19E-02				
	Jmuller1	29.27	-1.53	2.95E-02				
RA2	Jmuller2	30.61	-1.934	1.16E-02	1.51E-02	3.30E-03	30.25	3.27E-01
	Jmuller2	30.19	-1.807	1.56E-02				
	Jmuller2	29.96	-1.74	1.82E-02				
RA3	Jmuller3	30.03	-1.759	1.74E-02	2.07E-02	4.28E-03	29.79	2.88E-01
	Jmuller3	29.89	-1.717	1.92E-02				
	Jmuller3	29.47	-1.592	2.56E-02				
RA4	Jmuller4	30.36	-1.86	1.38E-02	1.53E-02	2.97E-03	30.23	2.68E-01
	Jmuller4	30.4	-1.873	1.34E-02				
	Jmuller4	29.92	-1.727	1.87E-02				
RB1	Jmuller5	30.67	-1.953	1.11E-02	1.25E-02	2.50E-03	30.52	2.74E-01
	Jmuller5	30.2	-1.812	1.54E-02				
	Jmuller5	30.68	-1.955	1.11E-02				
RB2	Jmuller6	29.38	-1.565	2.72E-02	2.69E-02	3.97E-03	29.41	2.17E-01
	Jmuller6	29.21	-1.514	3.06E-02				
	Jmuller6	29.64	-1.644	2.27E-02				
RB3	Jmuller7	30.68	-1.955	1.11E-02	1.22E-02	1.94E-03	30.56	2.22E-01
	Jmuller7	30.69	-1.959	1.10E-02				
	Jmuller7	30.3	-1.841	1.44E-02				
RB4	Jmuller8	30.55	-1.918	1.21E-02	1.10E-02	1.13E-03	30.69	1.49E-01
	Jmuller8	30.85	-2.007	9.84E-03				
	Jmuller8	30.67	-1.953	1.11E-02				
WA1	Jmuller9	29.89	-1.717	1.92E-02	1.90E-02	2.12E-03	29.91	1.63E-01
	Jmuller9	30.08	-1.774	1.68E-02				
	Jmuller9	29.75	-1.677	2.11E-02				
WA2	Jmuller10	30.95	-2.037	9.18E-03	1.14E-02	2.53E-03	30.66	3.15E-01
	Jmuller10	30.7	-1.963	1.09E-02				
	Jmuller10	30.32	-1.849	1.42E-02				
WA3	Jmuller11	30.02	-1.758	1.75E-02	1.76E-02	2.18E-03	30.02	1.79E-01
	Jmuller11	29.84	-1.702	1.99E-02				

Raw values for *proC* expression cont.

sample	Identifier	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
	Jmuller11	30.19	-1.81	1.55E-02				
WA4	Jmuller12	30.01	-1.753	1.76E-02	2.41E-02	8.31E-03	29.61	4.76E-01
	Jmuller12	29.75	-1.676	2.11E-02				
	Jmuller12	29.09	-1.476	3.34E-02				
WB1	Jmuller13	28.52	-1.305	4.96E-02	6.48E-02	1.43E-02	28.16	3.32E-01
	Jmuller13	28.08	-1.174	6.70E-02				
	Jmuller13	27.86	-1.108	7.80E-02				
WB2	Jmuller14	29.65	-1.646	2.26E-02	2.77E-02	4.60E-03	29.37	2.50E-01
	Jmuller14	29.3	-1.539	2.89E-02				
	Jmuller14	29.17	-1.501	3.16E-02				
WB3	Jmuller15	31.86	-2.312	4.87E-03	7.88E-03	3.59E-03	31.27	6.46E-01
	Jmuller15	30.58	-1.926	1.19E-02				
	Jmuller15	31.36	-2.16	6.93E-03				
WB4	Jmuller16	31.43	-2.183	6.56E-03	7.10E-03	2.94E-03	31.4	6.01E-01
	Jmuller16	31.99	-2.35	4.46E-03				
	Jmuller16	30.79	-1.989	1.03E-02				

Figure 3.4

Growth of WT PAO1 (HS = PAO1) and MexAB-OprM mutant (200 = PAO200) with and without PCP. Data is found in Excel file: *PAO200 on PCP*.

Experiment 1 (Data in Figure 3.4), March 31, 2006

		% transmittance (600 nm)				Absorbance = log(1/(trans/100))			
time	hours	HS	HS + PCP	200	200 + PCP	HS	HS + PCP	200	200 + PCP
10:50	0.00	99.8	100	100	99.5	0.001	0.000	0.000	0.002
11:00	0.17	95	94.5	96.8	96	0.022	0.025	0.014	0.018
12:30	1.67	94	93.5	95	96.2	0.027	0.029	0.022	0.017
2:00	3.17	86	91	89.8	96.5	0.066	0.041	0.047	0.015
4:30	5.67	37	62.5	59	93	0.432	0.204	0.229	0.032
5:30	6.67	24.5	41	40.5	91.5	0.611	0.387	0.393	0.039
6:40	7.83	18	28.5	27	88.5	0.745	0.545	0.569	0.053
8:00	9.17	16.5	17	20	85.5	0.783	0.770	0.699	0.068
10:10	11.33	17	18	21.5	68	0.770	0.745	0.668	0.167
12:50	14.00				38				0.420

Experiment 2, March 24, 2006.

		% transmittance (600 nm)				Absorbance = log(1/(trans/100))			
time	hours	HS	HS + PCP	200	200 + PCP	HS	HS + PCP	200	200 + PCP
5:45	0.00	99.5	99	98.8	99.2	0.002	0.004	0.005	0.003
6:00	0.25	96.5	94.5	96	96.5	0.015	0.025	0.018	0.015
7:10	1.42	95	94.5	97	97	0.022	0.025	0.013	0.013
9:00	3.25	89	90	92.5	94	0.051	0.046	0.034	0.027
10:40	4.92	64	74.5	86		0.194	0.128	0.066	
12:30	6.75	45	70.5	69	90	0.347	0.152	0.161	0.046
2:05	8.33	30.5	64	47	76.5	0.516	0.194	0.328	
3:50	10.08	20.5	39	30	84	0.688	0.409	0.523	0.076
5:10	11.42	17.5	24.5	21.5	73	0.757	0.611	0.668	0.137
6:15	12.50	18	17	21	59	0.745	0.770	0.678	0.229
7:30	13.75	18	17	21.5	42.5	0.745	0.770	0.668	0.372
9:50	16.08				38.5				0.415
12:00	18.25				45.6				0.341

Experiment 3, May 4, 2006.

		% transmittance (600 nm)				Absorbance = log(1/(trans/100))			
time	hours	HS	HS + PCP	200	200 + PCP	HS	HS + PCP	200	200 + PCP
10:33	0.05	98.9	93	95	94.2	0.005	0.032	0.022	0.026
12:40	2.17	93	91.2	96	93.5	0.032	0.040	0.018	0.029
14:37	4.12	82	82.8	92.8	93	0.086	0.082	0.032	0.032
16:35	6.08	67.5	77.5	88.2	94.2	0.171	0.111	0.055	0.026
18:34	8.07	52	73.8	73.2	93.2	0.284	0.132	0.135	0.031
20:30	10.00	36.8	66.2	50.2	93	0.434	0.179	0.299	0.032
10:50	24.33	29	37.2	39.8	77.8	0.538	0.429	0.400	0.109

Appendix B

Data for Chapter 4

Table 4.1 MICs in the presence and absence of PCP for WT and MexAB-OprM mutant

	Wild type (PAO1)					
	March 8, 2006		March 9, 2006		Feb 10, 2006	
Antibiotic	LB[a]	LB +PCP	LB	LB +PCP	LB	LB +PCP
Tetracycline	8.0	32	8	32	8	32
Chloramphenicol	16-8	64	16	64	16	64
Naladixic acid	32.0	256	32	256	32	512-256
Ciprofloxacin			0.05-0.1	0.2	0.05	0.2
Carbenicllin	32.0	128	32	128	32	128
Gentamycin	2.0	2	4	2	4	4

	MexAB-OprM mutant (PAO200)			
	March 9, 2006		Feb 10, 2006	
Antibiotic	LB	LB +PCP	LB	LB +PCP
Tetracycline	0.5	0.5	0.25	0.25
Chloramphenicol	0.5	0.5	0.5-1	0.5
Naladixic acid	2	4	4	4
Ciprofloxacin	0.0625	0.0625	0.0625	0.0625
Carbenicllin	0.5	0.5	0.5	0.5
Gentamycin	1	0.5-1	1	1

[a]LB=Luria-Burtani media only, LB+PCP=Luria-Burtani media + 30 mg/L PCP. Data is in notebook #06 and in Excel file: *chem_antibioticMICs*.

Table 4.2. Generational exposure to PCP and antibiotic resistance: MIC values (μg/mL) for all antibiotics and timepoints. Data are in Excel file: *gen_PCP_expanalysis*

Tetracycline

MIC[a]

	C1[c]			C2[c]				C3[c]			C4[c]		
generation[b]	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	8	8	8				0	8	8	8			
26	16	16	16	16	16	16	26	8	8	8	8	8	8
52	16	8	8	16	16	8	52	8	8	8	8	8	8
78	16	16	16	8	8	8	84.5	16	16	16	8	8	8
123.5	16	16	16	8	8	8	117	8	8	8	8	8	8
149.5	8	8	8	8	8	8	149.5	8	8	8	8	8	8
175.5	16	16	16	16	16	16	182	16	16	16	8	8	8
221	8	8	8	8	8	8	227.5	16	16	16	8	8	8
240.5	8	8	8	8	8	8							
273	8	8	8	16	8	8	266.5	16	16	16	≤4	≤4	≤4
305.5	16	8	8	8	8	16	305.5	8	8	8	≤4	≤4	≤4
370.5	8	8	8	8	8	≤4	331.5	8	8	8	≤4	≤4	≤4
429	8	8	8	8	8	8	396.5	8	8	8	8	8	≤4
617.5	16	16	16	16	16	16	565.5	8	≤4	8	8	8	16
669.5	8	8	16	16	32	16	630.5	8	8	8	8	8	8
702	16	8	16	8	8	8	663	8	8	8	32	≤4	≤4
728	8	8	8	16	8	8	689	8	8	8	8	8	8
747.5	16	16	16	8	8	8	715	16	16	16	16	8	16

MIC-PCP[a]

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	16	16	16				0	16	16	16			
26	32	32	64	32	32	32	26	64	32	64	32	64	32
52	32	32	32	32	64	32	52	16	16	16	16	16	16
78	32	32	32	32	32	32	84.5	32	32	32	32	32	32
123.5	32	32	32	32	32	32	117	16	16	32	32	32	32
149.5	32	32	32	32	32	32	149.5	32	32	32	16	16	16
175.5	32	32	32	32	32	32	182	32	32	32	32	32	32
221	32	32	32	32	32	32	227.5	32	32	32	32	32	32
240.5	16	16	16	16	16	16							
273	32	32	32	16	16	16	266.5	32	32	32	32	32	32
305.5	32	32	32	16	16	16	305.5	16	16	16	16	16	16
370.5	32	32	32	16	16	16	331.5	32	32	32	16	16	16
429	32	32	32	32	32	32	396.5	32	32	32	16	16	16
617.5	32	32	32	32	32	32	565.5	32	32	32	32	32	32
669.5	32	32	32	32	32	32	630.5	32	32	32	32	32	32
702	32	32	32	32	32	32	663	32	32	32	32	32	32
728	32	32	32	32	32	32	689	32	32	32	32	32	32
747.5	64	64	64	64	64	64	715	64	64	64	64	64	64

Generational experiment MIC values (μg/mL) (cont.), Tetracycline

MIC

	E1[c]			E2[c]				E3[c]			E4[c]		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	16	16	16	16	16	16	26	16	32	32	16	16	16
52	32	32	16	32	16	16	52	8	8	8	8	8	8
78	8	8	8	8	8	8	84.5	8	8	8	8	8	8
123.5	8	8	8	8	8	8	117	8	8	8	8	8	16
149.5	8	8	8	8	8	8	149.5	8	8	8	8	8	8
175.5	32	32	32	8	8	8	182	16	16	16	8	8	8
221	8	8	8	8	8	8	227.5	8	8	8	8	8	8
240.5	8	8	8	8	8	8							
273	8	8	8	8	8	8	266.5	8	8	8	8	8	8
305.5	8	8	8	8	8	8	305.5	≤4	≤4	≤4	≤4	≤4	≤4
370.5	8	8	8	8	8	8	331.5	≤4	≤4	8	≤4	≤4	≤4
429	8	8	8	8	8	8	396.5	8	8	8	8	8	8
617.5	16	16	16	32	64	32	565.5	8	16	8	8	8	8
669.5	8	8	8	8	8	8	630.5	8	16	8	≤4	≤4	≤4
702	≤4	≤4	≤4	≤4	≤4	≤4	663	≤4	≤4	≤4	≤4	≤4	≤4
728	8	8	8	8	8	8	689	8	8	8	8	8	16
747.5	8	8	16	8	8	8	715	16	16	16	16	8	8

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	32	32	32	32	32	32	26	128	64	64	32	64	32
52	32	64	64	32	32	32	52	32	32	32	32	32	32
78	32	32	32	32	32	32	84.5	32	32	32	32	32	32
123.5	32	32	32	32	32	32	117	32	32	32	16	16	16
149.5	16	16	16	32	32	32	149.5	16	16	16	16	16	16
175.5	32	32	32	16	16	16	182	32	32	32	32	32	32
221	32	32	32	32	32	32	227.5	32	32	32	32	16	16
240.5	32	32	32	16	16	16							
273	16	16	16	32	32	32	266.5	32	32	32	32	32	32
305.5	32	32	32	16	16	16	305.5	16	16	16	16	16	16
370.5	16	16	16	16	16	16	331.5	16	16	16	16	16	16
429	32	32	32	32	32	32	396.5	16	16	16	16	16	16
617.5	32	32	32	32	32	32	565.5	64	32	32	32	32	32
669.5	32	32	32	32	32	32	630.5	32	32	32	32	32	32
702	32	32	32	32	32	32	663	32	32	32	16	16	16
728	32	32	32	32	32	32	689	64	64	32	64	32	32
747.5	32	32	32	32	32	32	715	32	32	32	64	64	64

Chloramphenicol

MIC

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	≤16	≤16	≤16				0	≤16	≤16	32			
26	32	≤16	≤16	32	≤16	32	26	≤16	≤16	≤16	≤16	≤16	32
52	32	≤16	≤16	≤16	≤16	≤16	52	≤8	≤8	≤8	≤8	≤8	16
78	≤16	≤16	≤16	≤16	≤16	≤16	84.5	16	16	16	≤8	≤8	≤8
123.5	16	16	16	16	16	16	117	16	16	16	16	16	≤8
149.5	16	16	16	16	16	16	149.5	16	16	16	≤8	≤8	≤8
175.5	16	16	32	16	16	16	182	16	16	16	≤8	≤8	≤8
221	≤8	≤8	≤8	16	≤8	≤8	227.5	16	16	16	≤8	≤8	≤8
240.5	16	16	16	≤8	16	16							
273	32	16	16	16	≤8	≤8	266.5	16	16	16	≤8	≤8	≤8
305.5	≤8	16	≤8	16	≤8	≤8	305.5	≤8	16	16	≤8	≤8	≤8
370.5	≤8	≤8	≤8	≤8	16	≤8	331.5	16	≤8	≤8	≤8	≤8	≤8
429	≤8	≤8	≤8	16	16	16	396.5	16	16	16	≤8	≤8	≤8
617.5	16	≤8	16	16	16	32	565.5	32	32	32	32	32	32
702	16	≤8	16	≤8	≤8	≤8	663	≤8	≤8	≤8	≤8	≤8	≤8
728	≤8	16	≤8	≤8	≤8	≤8	689	16	16	16	≤8	≤8	≤8
747.5	≤8	≤8	16	≤8	≤8	≤8	715	≤8	≤8	≤8	≤8	16	≤8

MIC-PCP

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	64	64	64				0	64	64	64			
26	64	64	64	64	64	64	26	64	64	64	64	64	64
52	64	128	64	64	128	64	52	16	16	16	16	16	16
78	64	32	32	64	64	64	84.5	64	64	64	32	32	32
123.5	64	64	64	64	64	64	117	64	64	64	32	32	32
149.5	32	32	32	32	32	32	149.5	64	32	32	32	32	32
175.5	64	64	64	64	64	64	182	32	32	32	64	64	64
221	32	32	32	32	32	32	227.5	64	64	64	64	64	64
240.5	32	32	32	32	32	32							
273	64	64	64	32	32	32	266.5	64	64	64	64	64	64
305.5	64	64	64	64	64	64	305.5	32	32	32	32	32	32
370.5	64	64	64	64	64	64	331.5	32	32	32	32	32	32
429	32	32	32	64	64	64	396.5	64	64	64	64	64	64
617.5	64	64	64	64	64	64	565.5	128	128	128	64	64	64
669.5	32	32	32	128	128	128	630.5	128	128	128	64		64
702	32	32	32	32	32	32	663	32	32	32	64	64	64
728	32	32	32	64	64	64	689	64	32	32	32	32	32
747.5	32	32	32	32	32	32	715	32	32	32	32	32	32

Generational experiment MIC values (μg/mL) (cont.), Chloramphenicol

MIC

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	32	32	32	32	32	32	26	≤16	≤16	≤16	≤16	≤16	≤16
52	32	32	64	32	64	64	52	≤8	≤8	≤8	≤8	≤8	≤8
78	≤16	≤16	≤16	≤16	≤16	≤16	84.5	16	16	16	16	16	16
123.5	16	16	16	16	16	16	117	16	16	16	16	16	16
149.5	16	16	16	16	16	16	149.5	16	16	16	16	16	16
175.5	16	16	16	16	16	16	182	16	16	16	16	16	16
221	≤8	≤8	≤8	16	16	16	227.5	16	16	16	16	16	16
240.5	16	16	16	16	16	16							
273	32	32	16	16	16	16	266.5	16	16	16	16	16	16
305.5	16	16	16	16	16	16	305.5	≤8	16	16	≤8	≤8	≤8
370.5	≤8	≤8	≤8	16	≤8	16	331.5	≤8	16	≤8	≤8	≤8	≤8
429	≤8	16	16	≤8	16	16	396.5	16	32	16	16	16	16
617.5	16	16	16	64	64	64	565.5	32	32	32	32	32	32
669.5	32	32	32	32	32	32	630.5	16	16	16	32	16	16
702	16	16	16	16	16	16	663	≤8	≤8	≤8	≤8	≤8	≤8
728	16	≤8	≤8	16	16	16	689	32	16	16	16	16	16
747.5	≤8	≤8	≤8	≤8	≤8	≤8	715	≤8	16	≤8	≤8	≤8	≤8

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	128	64	64	64	128	64	26	64	64	64	128	128	64
52	*256	256	256	64	128	128	52	16	16	16	16	16	16
78	32	64	32	32	32	32	84.5	64	64	64	64	64	64
123.5	64	64	64	64	64	64	117	64	64	64	32	32	32
149.5	32	32	32	32	32	32	149.5	32	32	32	32	32	32
175.5	64	64	64	32	32	32	182	64	64	64	32	32	32
221	32	32	32	32	32	32	227.5	64	128	64	64	64	64
240.5	64	64	64	32	32	32							
273	32	32	32	64	64	64	266.5	64	64	64	64	64	64
305.5	64	64	64	64	64	64	305.5	32	32	32	32	32	32
370.5	64	64	64	32	32	32	331.5	32	32	32	32	32	32
429	64	64	128	32	32	32	396.5	32	32	32	16	16	≤8
617.5	64	64	64	64	64	64	565.5	128	128	128	64	64	64
669.5	256	256	128	64	64	64	630.5	64	64	64	64	64	64
702	32	32	32	64	64	64	663	32	32	32	32	32	32
728	32	64	32	64	64	64	689	64	64	64	64	64	64
747.5	32	32	32	32	32	32	715	32	32	32	32	32	32

Naladixic acid

MIC

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	≤32	≤32	≤32				0	64	≤32	≤32			
26	128	128	64	64	64	≤32	26	64	64	≤32	≤32	≤32	≤32
52	≤32	≤32	≤32	≤32	64	≤32	52	≤16	≤16	≤16	≤16	≤16	≤16
78	64	64	≤32	64	≤32	64	84.5	32	32	32	≤16	≤16	≤16
123.5	32	32	32	32	32	≤16	117	32	32	32	32	32	≤16
149.5	32	32	32	32	32	32	149.5	64	64	64	32	32	32
175.5	64	64	64	64	64	64	182	64	64	32	32	32	32
221	32	32	32	128	128	128	227.5	32	32	32	32	32	32
240.5	32	32	32	32	32	≤16							
273	32	32	32	32	64	64	266.5	32	32	32	≤16	≤16	≤16
305.5	64	32	32	≤16	≤16	≤16	305.5	32	32	32	32	32	32
370.5	32	32	32	32	32	32	331.5	32	32	32	32	32	32
429	64	64	64	64	64	64	396.5	64	64	64	32	32	32
617.5	64	64	32	32	32	32	565.5	64	64	64	64	64	32
669.5	64	64	64	128	64	64	630.5	64	64	64	64	32	32
702	128	64	64	32	32	32	663	64	128	64	32	32	32
728	64	64	64	32	32	32	689	64	64	64	64	64	64
747.5	64	64	64	32	32	32	715	64	64	64	32	32	32

MIC-PCP

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	256	256	128				0	512	256	256			
26	256	512	256	256	256	256	26	256	512	256	512	256	256
52	256	256	256	256	256	256	52	64	64	64	64	64	64
78	256	256	128	128	128	128	84.5	128	128	128	128	128	128
123.5	128	128	128	128	128	128	117	128	128	128	128	128	128
149.5	128	128	128	128	128	128	149.5	256	256	256	256	256	256
175.5	256	256	256	512	512	512	182	256	128	128	256	256	128
221	512	256	256	256	256	256	227.5	256	256	256	256	256	256
240.5	128	128	128	256	256	256							
273	256	256	256	256	256	256	266.5	128	128	128	128	128	128
305.5	128	128	128	128	128	128	305.5	256	256	256	256	256	256
370.5	128	128	128	128	128	128	331.5	128	128	128	128	128	128
429	256	256	256	512	256	256	396.5	256	256	256	256	256	256
617.5	256	256	256	256	256	256	565.5	256	256	256	256	256	256
669.5	256	256	256	512	256	256	630.5	256	256	256	256	256	256
702	256	256	256	256	256	256	663	256	256	256	256	256	256
728	256	256	256	256	256	256	689	256	512	256	512	256	256
747.5	256	256	256	256	256	256	715	256	256	256	256	256	256

Generational experiment MIC values (μg/mL) (cont.), Naladixic acid

MIC

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	64	≤32	≤32	≤32	≤32	≤32	26	64	64	≤32	≤32	≤32	≤32
52	128	64	128	64	≤32	64	52	≤16	≤16	≤16	≤16	≤16	≤16
78	≤32	≤32	≤32	≤32	≤32	≤32	84.5	32	32	≤16	32	≤16	≤16
123.5	32	32	≤16	32	32	32	117	32	32	≤16	32	32	≤16
149.5	32	32	32	32	32	32	149.5	32	32	32	64	32	32
175.5	64	64	64	32	32	32	182	32	32	32	32	32	32
221	64	32	32	32	32	32	227.5	32	32	32	64	32	32
240.5	32	32	32	32	32	32							
273	32	32	32	32	32	32	266.5	32	32	≤16	32	32	32
305.5	32	32	32	32	≤16	≤16	305.5	32	32	32	32	32	32
370.5	32	32	32	32	32	32	331.5	32	32	32	32	32	32
429	64	64	64	64	64	64	396.5	64	64	64	64	64	64
617.5	32	64	64	64	64	64	565.5	64	64	32	32	32	32
669.5	128	128	128	128	64	64	630.5	32	32	32	64	32	64
702	128	64	64	32	32	32	663	32	32	32	32	32	32
728	64	64	64	128	64	64	689	64	64	64	64	64	64
747.5	64	64	64	64	64	64	715	128	64	64	64	64	64

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	512	512	256	256	256	128	26	256	256	256	256	256	256
52	512	256	256	256	256	256	52	64	64	64	64	64	64
78	128	128	128	128	128	128	84.5	128	128	128	128	128	128
123.5	128	128	128	128	128	128	117	128	128	128	128	128	128
149.5	128	128	128	128	128	128	149.5	256	256	256	256	256	256
175.5	256	256	256	256	256	256	182	128	128	128	256	256	256
221	256	256	128	256	256	256	227.5	256	256	256	256	256	256
240.5	128	128	128	128	128	128							
273	256	256	256	256	256	256	266.5	128	128	128	128	128	128
305.5	128	128	128	128	128	128	305.5	128	128	128	128	128	128
370.5	128	128	128	128	128	128	331.5	128	128	128	128	128	128
429	256	256	256	256	256	256	396.5	256	256	256	256	256	256
617.5	256	256	256	256	256	256	565.5	256	256	256	256	256	256
669.5	256	256	256	512	512	512	630.5	256	256	256	256	256	256
702	256	256	256	256	256	256	663	256	256	256	256	256	256
728	256	256	256	512	512	512	689	256	512	256	256	256	256
747.5	256	256	256	256	256	256	715	256	256	256	512	256	256

Ciprofloxacin

MIC

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	0.1	0.1	0.1				0	0.1	0.2	0.1			
26	0.1	0.2	0.1	0.1	0.1	0.1	26	0.1	0.1	0.1	0.1	0.2	0.2
52	0.1	0.1	0.1	0.2	0.1	0.1	52	0.05	0.05	0.05	0.05	0.05	0.05
78	0.1	0.2	0.2	0.05	0.1	0.1	84.5	0.1	0.1	0.1	0.05	0.1	0.05
123.5	0.1	0.1	0.1	0.0.5	0.05	0.05	117	0.05	0.05	0.05	0.05	0.05	0.05
149.5	0.05	0.05	0.05	0.05	0.1	0.05	149.5	0.1	0.1	0.1	0.1	0.1	0.05
175.5	0.1	0.1	0.1	0.1	0.1	0.1	182	0.1	0.1	0.1	0.05	0.05	0.05
221	0.05	0.05	0.05	0.05	0.05	0.05	227.5	0.1	0.05	0.05	0.1	0.05	0.05
240.5	0.05	0.05	0.1	0.05	0.05	0.05							
273	0.05	0.05	0.05	0.1	0.05	0.1	266.5	0.05	0.05	0.05	0.05	0.05	0.05
305.5	0.05	0.05	0.05	0.1	0.05	0.05	305.5	0.1	0.1	0.1	0.05	0.05	0.05
370.5	0.1	0.1	0.1	0.05	0.05	0.05	331.5	0.05	0.05	0.05	0.05	0.05	0.05
429	0.1	0.1	0.1	0.05	0.05	0.05	396.5	0.05	0.05	0.05	0.05	0.05	0.05
617.5	0.05	0.05	0.05	0.05	0.05	0.05	565.5	0.05	0.05	0.05	0.05	0.05	0.05
669.5	0.1	0.05	0.05	0.05	0.05	0.05	630.5	0.05	0.05	0.05	0.05	0.05	0.05
702	0.05	0.05	0.05	0.05	0.05	0.05	663	0.05	0.05	0.05	0.05	0.05	0.05
728	0.05	0.05	0.05	0.05	0.05	0.05	689	0.05	0.05	0.05	0.05	0.05	0.05
747.5	0.1	0.05	0.05	0.05	0.05	0.05	715	0.1	0.05	0.05	0.05	0.05	0.05

MIC-PCP

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	0.2	0.2	0.2				0	0.2	0.2	0.2			
26	0.2	0.2	0.2	0.2	0.2	0.2	26	0.4	0.4	0.2	0.2	0.2	0.2
52	0.2	0.2	0.2	0.8	0.8	0.4	52	0.2	0.2	0.2	0.2	0.2	0.2
78	0.2	0.2	0.2	0.2	0.2	0.2	84.5	0.2	0.2	0.2	0.2	0.2	0.1
123.5	0.2	0.2	0.2	0.2	0.2	0.2	117	0.2	0.2	0.2	0.2	0.2	0.2
149.5	0.2	0.2	0.2	0.2	0.2	0.2	149.5	0.2	0.2	0.2	0.2	0.2	0.2
175.5	0.2	0.2	0.2	0.2	0.2	0.2	182	0.2	0.2	0.2	0.2	0.2	0.2
221	0.2	0.2	0.2	0.2	0.2	0.2	227.5	0.2	0.2	0.2	0.2	0.2	0.2
240.5	0.1	0.1	0.1	0.2	0.2	0.2							
273	0.2	0.2	0.2	0.2	0.2	0.2	266.5	0.2	0.2	0.2	0.2	0.2	0.2
305.5	0.2	0.2	0.2	0.2	0.2	0.2	305.5	0.2	0.2	0.2	0.2	0.2	0.2
370.5	0.2	0.2	0.2	0.2	0.2	0.2	331.5	0.2	0.2	0.2	0.2	0.2	0.2
429	0.2	0.2	0.2	0.4	0.2	0.2	396.5	0.2	0.2	0.2	0.4	0.2	0.2
617.5	0.2	0.2	0.2	0.4	0.2	0.2	565.5	0.2	0.2	0.2	0.2	0.2	0.2
669.5	0.2	0.2	0.2	0.2	0.2	0.2	630.5	0.2	0.2	0.2	0.4	0.2	0.2
702	0.2	0.2	0.2	0.2	0.2	0.2	663	0.2	0.2	0.2	0.2	0.2	0.2
728	0.2	0.2	0.2	0.2	0.2	0.2	689	0.2	0.2	0.2	0.2	0.2	0.2
747.5	0.2	0.2	0.2	0.2	0.2	0.2	715	0.2	0.2	0.2	0.2	0.2	0.2

Generational experiment MIC values (μg/mL) (cont.), Ciprofloxacin

MIC

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	0.1	0.1	0.2	0.1	0.1	0.1	26	0.1	0.2	0.1	0.1	0.1	0.1
52	0.1	0.1	0.1	0.1	0.2	0.2	52	0.1	0.1	0.1	0.1	0.1	0.1
78	0.05	0.05	0.05	0.05	0.1	0.1	84.5	0.05	0.05	0.05	0.1	0.05	0.05
123.5	0.05	0.05	0.05	0.05	0.05	0.05	117	0.05	0.05	0.05	0.05	0.05	0.05
149.5	0.05	0.05	0.05	0.05	0.05	0.05	149.5	0.1	0.1	0.1	0.1	0.1	0.1
175.5	0.05	0.05	0.1	0.1	0.1	0.1	182	0.1	0.1	0.1	0.1	0.05	0.05
221	0.05	0.05	0.05	0.05	0.05	0.05	227.5	0.05	0.05	0.05	0.05	0.05	0.05
240.5	0.05	0.05	0.05	≤0.025	≤0.025	≤0.025							
273	0.05	0.05	0.05	0.05	0.05	0.05	266.5	0.05	0.05	0.05	0.05	0.05	0.05
305.5	0.05	0.05	0.05	0.05	0.05	0.05	305.5	0.05	0.05	0.05	0.1	0.05	0.05
370.5	0.05	0.05	0.05	0.05	0.05	0.05	331.5	0.05	0.05	0.05	0.05	0.05	0.05
429	0.05	0.1	0.05	0.05	0.05	0.05	396.5	0.05	0.05	0.05	0.05	0.05	0.05
617.5	0.05	0.05	0.05	≤0.025	≤0.025	≤0.025	565.5	0.05	0.1	0.05	0.05	0.05	0.05
669.5	0.05	0.05	0.05	0.05	0.1	0.05	630.5	0.05	0.05	0.05	0.05	0.05	0.1
702	0.05	0.05	0.1	0.05	0.05	0.05	663	0.05	0.05	0.05	0.05	0.05	0.5
728	0.05	0.05	0.05	0.05	0.05	0.05	689	0.05	0.05	0.05	0.05	0.05	0.05
747.5	0.05	0.05	0.05	0.05	0.05	0.05	715	0.05	0.05	0.05	0.05	0.05	0.05

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	0.2	0.4	0.2	0.4	0.2	0.2	26	0.2	0.2	0.4	0.4	0.2	0.4
52	0.2	0.2	0.2	0.2	0.2	0.2	52	0.2	0.2	0.2	0.2	0.2	0.2
78	0.4	0.2	0.2	0.2	0.2	0.2	84.5	0.2	0.2	0.2	0.2	0.2	0.2
123.5	0.2	0.2	0.1	0.2	0.2	0.2	117	0.2	0.2	0.2	0.2	0.2	0.2
149.5	0.1	0.1	0.1	0.2	0.2	0.2	149.5	0.2	0.2	0.2	0.2	0.2	0.2
175.5	0.2	0.2	0.2	0.2	0.2	0.2	182	0.2	0.2	0.2	0.2	0.2	0.2
221	0.2	0.2	0.2	0.2	0.2	0.2	227.5	0.2	0.2	0.2	0.2	0.2	0.2
240.5	0.2	0.2	0.2	0.1	0.1	0.1							
273	0.2	0.2	0.2	0.2	0.2	0.2	266.5	0.2	0.2	0.2	0.2	0.2	0.2
305.5	0.2	0.2	0.2	0.2	0.2	0.2	305.5	0.2	0.2	0.2	0.2	0.2	0.2
370.5	≤0.025	≤0.025	≤0.025	0.2	0.2	0.2	331.5	0.2	0.2	0.2	0.2	0.2	0.2
429	0.2	0.2	0.2	0.2	0.2	0.2	396.5	0.2	0.2	0.2	0.2	0.2	0.2
617.5	0.4	0.2	0.2	0.2	0.2	0.2	565.5	0.2	0.2	0.2	0.2	0.2	0.2
669.5	0.2	0.2	0.2	0.2	0.2	0.2	630.5	0.2	0.2	0.2	0.2	0.2	0.2
702	0.2	0.2	0.2	0.2	0.2	0.2	663	0.2	0.2	0.2	0.2	0.2	0.2
728	0.2	0.2	0.2	0.2	0.2	0.2	689	0.2	0.2	0.2	0.2	0.2	0.2
747.5	0.2	0.2	0.2	0.2	0.2	0.2	715	0.2	0.2	0.2	0.2	0.2	0.2

Generational experiment MIC values (μg/mL) (cont.)

Carbenicillin

MIC

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	64	64	64				0	64	32	32			
26	32	64	32	32	32	32	26	32	32	32	64	128	64
52	32	32	64	64	64	128	52	16	32	32	16	32	16
78	32	32	32	32	32	32	84.5	32	32	32	32	32	32
123.5	32	32	32	32	32	64	117	32	32	32	16	32	32
149.5	32	32	32	32	32	32	149.5	32	32	32	16	16	32
175.5	32	32	32	32	32	32	182	16	16	16	16	16	16
221	32	32	64	32	32	32	227.5	32	32	32	32	32	32
240.5	32	32	32	16	32	32							
273	32	16	32	16	16	32	266.5	32	32	32	16	32	32
305.5	32	32	32	32	32	64	305.5	32	32	32	32	32	32
370.5	32	64	32	32	64	32	331.5	32	64	64	16	32	32
429	32	64	32	32	32	32	396.5	32	32	32	32	32	32
617.5	32	32	64	32	64	32	565.5	64	32	32	32	64	64
669.5	32	32	32	64	64	128	630.5	32	32	32	32	32	32
702	16	16	32	32	32	32	663	32	32	32	32	32	32
728	32	64	32	32	32	32	689	16	32	16	32	32	32
747.5	32	64	32	32	32	32	715	64	32	32	32	32	64

MIC-PCP

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	128	128	128				0	128	128	128			
26	128	128	128	128	128	128	26	128	256	256	128	256	128
52	128	128	128	128	128	128	52	64	128	128	64	128	128
78	128	128	256	128	128	128	84.5	128	128	128	128	128	128
123.5	128	128	128	128	128	128	117	128	128	128	128	128	128
149.5	128	128	128	128	128	128	149.5	64	64	64	128	128	128
175.5	64	64	64	128	128	128	182	64	64	64	64	64	128
221	128	256	128	128	128	128	227.5	128	128	128	128	128	128
240.5	128	128	128	128	128	128							
273	128	128	128	128	128	128	266.5	128	128	128	128	128	128
305.5	128	128	128	128	128	128	305.5	128	128	128	128	256	128
370.5	128	128	128	128	128	128	331.5	128	128	128	128	128	128
429	128	128	128	128	256	128	396.5	128	256	128	128	128	128
617.5	128	128	128	128	128	128	565.5	128	256	256	256	256	256
669.5	128	256	256	128	256	128	630.5	128	128	128	256	256	256
702	128	128	128	128	128	128	663	128	256	128	128	128	128
728	128	128	128	128	128	128	689	128	256	128	256	128	256
747.5	128	128	128	128	128	128	715	128	256	128	128	256	128

Generational experiment MIC values (μg/mL) (cont.), Carbenicillin

MIC

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	32	32	32	32	64	64	26	64	32	64	32	32	64
52	32	64	64	32	32	32	52	32	32	32	32	32	32
78	32	32	32	32	64	32	84.5	32	32	32	32	32	32
123.5	32	32	32	32	32	32	117	32	32	32	32	32	32
149.5	32	32	32	32	32	64	149.5	16	16	32	≤4	≤4	≤4
175.5	32	32	32	32	32	32	182	16	16	16	16	16	32
221	16	32	32	16	32	32	227.5	16	16	32	16	16	32
240.5	32	32	32	16	32	32							
273	16	32	32	16	32	16	266.5	16	32	32	16	32	32
305.5	32	32	32	32	32	32	305.5	32	32	32	16	32	32
370.5	32	32	32	32	32	32	331.5	16	16	32	32	64	32
429	32	64	32	32	32	32	396.5	32	64	64	32	32	32
617.5	32	32	64	32	64	32	565.5	32	64	64	64	128	64
669.5	64	64	32	32	64	64	630.5	32	64	32	32	64	64
702	32	32	32	32	32	64	663	32	32	32	32	32	32
728	32	64	64	64	128	128	689	32	32	32	32	64	32
747.5	32	64	32	64	128	64	715	32	64	64	64	64	64

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	128	128	128	128	128	128	26	128	256	128	128	128	128
52	128	128	128	128	128	128	52	128	128	128	128	128	128
78	128	256	128	128	128	128	84.5	128	128	128	128	128	128
123.5	128	128	128	128	128	128	117	128	128	128	128	128	128
149.5	128	128	128	128	128	128	149.5	64	128	128	64	64	64
175.5	64	128	128	128	128	128	182	64	64	64	64	64	64
221	64	128	128	64	64	64	227.5	128	256	128	128	128	128
240.5	128	128	128	128	128	128							
273	128	128	128	128	128	128	266.5	128	128	128	128	128	128
305.5	128	128	128	128	128	128	305.5	128	128	128	128	128	128
370.5	128	128	128	128	128	128	331.5	128	128	128	128	128	128
429	128	128	128	128	128	128	396.5	128	256	256	128	128	128
617.5	256	256	256	128	256	128	565.5	256	256	256	256	256	256
669.5	256	256	256	256	256	256	630.5	256	256	256	256	256	256
702	256	256	256	256	256	256	663	256	256	256	128	256	256
728	256	256	256	256	256	256	689	128	256	256	256	256	256
747.5	256	256	256	256	256	256	715	256	256	256	128	256	256

Generational experiment MIC values (μg/mL) (cont.)

Gentamicin

MIC

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	4	4	4				0	4	8	4			
26	4	4	4	4	4	4	26	4	4	8	4	8	4
52	4	4	8	4	8	8	52	4	4	4	4	4	4
78	4	2	4	4	4	4	84.5	2	4	4	4	4	4
123.5	4	4	4	4	4	4	117	4	4	4	4	4	4
149.5	4	4	4	4	4	4	149.5	2	2	2	2	2	2
175.5	2	2	2	1	1	1	182	2	2	2	2	2	2
221	2	2	2	2	2	2	227.5	4	4	4	4	4	4
240.5	2	2	4	2	4	4							
273	2	4	2	2	2	2	266.5	2	2	2	2	2	2
305.5	2	2	2	2	2	2	305.5	2	2	2	2	2	2
370.5	4	8	4	2	2	2	331.5	2	2	4	2	2	2
429	2	2	2	2	2	4	396.5	1	1	1	2	2	2
617.5	2	2	2	2	2	2	565.5	2	2	2	2	2	2
669.5	2	2	4	2	2	2	630.5	2	2	2	2	2	2
702	2	2	2	2	2	2	663	2	2	2	2	2	2
728	2	1	1	2	2	2	689	1	1	1	2	2	2
747.5	2	2	2	2	2	2	715	2	2	2	2	2	2

MIC-PCP

	C1			C2				C3			C4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0	4	4	4				0	2	2	2			
26	8	4	4	4	4	4	26	2	2	2	2	4	4
52	4	4	2	4	4	8	52	2	2	2	2	2	2
78	4	2	2	2	2	2	84.5	2	2	2	2	2	2
123.5	2	2	2	2	2	2	117	2	2	2	2	2	2
149.5	4	4	4	2	2	2	149.5	1	1	1	1	1	1
175.5	1	1	1	1	1	≤0.5	182	1	1	1	1	1	1
221	2	2	2	1	1	1	227.5	2	2	2	2	2	2
240.5	2	2	2	2	2	2							
273	1	1	1	1	1	1	266.5	1	1	1	2	1	1
305.5	2	2	2	2	1	1	305.5	1	2	2	2	1	2
370.5	2	4	2	2	2	2	331.5	1	1	1	1	1	1
429	1	1	1	2	2	2	396.5	1	1	1	2	2	1
617.5	2	2	1	2	2	1	565.5	1	1	2	4	4	2
669.5	1	1	2	2	2	2	630.5	1	1	2	2	4	2
702	1	1	1	1	1	1	663	1	1	1	1	1	1
728	1	1	1	1	1	1	689	2	1	1	1	1	1
747.5	1	2	1	2	1	1	715	2	1	1	1	1	1

Generational experiment MIC values (μg/mL) (cont.), Gentamicin

MIC

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	4	4	4	4	4	4	26	16	16	16	4	8	4
52	8	4	8	4	4	4	52	4	4	4	4	4	4
78	2	4	4	4	4	4	84.5	4	4	4	4	4	4
123.5	4	4	4	4	4	4	117	4	4	4	4	4	4
149.5	2	2	2	4	4	4	149.5	2	2	2	2	2	2
175.5	2	2	2	2	2	2	182	2	2	2	2	2	2
221	2	2	2	2	2	2	227.5	4	4	4	4	4	4
240.5	2	2	4	2	4	4							
273	2	2	2	2	2	2	266.5	2	2	2	2	2	2
305.5	2	2	2	2	2	2	305.5	2	2	2	2	2	2
370.5	2	2	2	2	2	2	331.5	2	2	2	2	2	2
429	2	2	2	2	2	2	396.5	2	2	2	2	2	2
617.5	2	2	2	1	1	1	565.5	2	2	2	2	2	2
669.5	4	2	2	2	2	2	630.5	2	2	2	2	2	4
702	2	2	2	2	2	2	663	2	2	2	2	2	2
728	2	2	2	2	2	2	689	4	2	2	2	2	2
747.5	2	2	2	2	2	2	715	2	2	2	2	2	2

MIC-PCP

	E1			E2				E3			E4		
generation	1	2	3	1	2	3	generation	1	2	3	1	2	3
0							0						
26	4	4	4	4	4	8	26	4	2	2	2	2	2
52	2	2	4	4	8	8	52	2	2	2	2	2	2
78	2	2	2	2	2	2	84.5	2	2	2	2	2	2
123.5	2	2	2	2	2	2	117	2	2	4	2	2	2
149.5	2	2	2	2	2	2	149.5	1	1	1	1	1	1
175.5	1	1	1	2	2	2	182	1	1	1	2	1	1
221	1	1	1	4	4	4	227.5	4	4	4	2	2	2
240.5	2	2	2	4	4	4							
273	1	1	1	2	1	2	266.5	2	1	1	2	1	2
305.5	1	1	1	2	2	2	305.5	1	1	1	2	2	2
370.5	2	2	2	1	1	1	331.5	2	1	1	2	1	1
429	2	1	1	1	2	1	396.5	1	1	1	2	1	1
617.5	2	2	2	1	1	1	565.5	4	2	2	4	2	2
669.5	4	4	2	2	2	2	630.5	1	2	2	2	2	2
702	2	1	2	1	1	1	663	1	1	1	2	2	1
728	1	2	2	1	1	1	689	2	1	1	1	1	1
747.5	2	2	1	1	1	1	715	1	1	1	2	1	2

[a]MIC=with LB only, MIC-PCP=with LB + 30 mg/L PCP.
[b]Generation times shown are actual time. For Table 4.2 the average of the two experiments was taken.
[c]C, control; E, experimental with PCP addition to medium. C1-C4 are the replicate flasks, and 1-3 are the replicate MICs at each timepoint.

Wilcoxon rank sum analysis[a] comparing experimental to control MICs
Data summary in Word file: *joy stats v01*.

Generation time	TET	CAM	NAL	CIP	CAR	GEN
591	0.8744	0.4292	0.7389	0.4533	0.6084	0.4533
682	0.06675	0.6084	0.6084	NA	0.4533	NA
731	0.2471	NA	0.1814	NA	0.0603	NA

[sa] Summary of p-values for relevant timepoints.

Figure 4.1 and 4.2 mexB **induction, RT-PCR results**
Data are in Excel file: *JFM_mexBindRT_final*, notebook #7, June 2006

		sample	*mexB*	*proC*	mexB/ proC	average fold change	S.D.[b]	p-value[c]
control[a]	early log	1AC	5.93E-04	5.86E-05	10.12	10.02	2.40	
		2AC	7.79E-04	1.03E-04	7.56			
		3AC	1.41E-03	1.14E-04	12.37			
	late log	1BC	8.05E-04	7.04E-05	11.43	10.67	1.26	
		2BC	1.12E-03	9.86E-05	11.36			
		3BC	5.21E-04	5.65E-05	9.22			
PCP	early log	4AP	3.06E-03	6.56E-05	46.65	47.74	0.97	0.000
		5AP	3.06E-03	6.31E-05	48.49			
		6AP	2.88E-03	5.99E-05	48.08			
	late log	4BP	1.11E-03	4.01E-05	27.68	28.74	4.56	0.003
		5BP	8.71E-04	2.58E-05	33.74			
		6BP	1.02E-03	4.11E-05	24.81			
PCP removed	early log	4CP	1.53E-05	7.15E-05	1.87	2.09	0.62	0.005
		5CP	3.00E-05	1.01E-04	2.78			
		6CP	8.79E-05	8.73E-05	1.60			
	late log	4DP	1.66E-05	8.27E-05	2.68	2.87	1.58	0.003
		5DP	1.82E-05	4.99E-05	4.53			
		6DP	2.35E-05	9.19E-05	1.38			
PCP added	early log	4EP	2.18E-03	7.57E-05	115.19	91.22	21.33	0.021
		5EP	1.48E-03	4.10E-05	84.15			
		6EP	3.15E-04	3.35E-05	74.33			

Figure 4.1 and 4.2 *mexB* induction, RT-PCR results, cont.

		sample	*mexB*	*proC*	mexB/ proC	average fold change	S.D.[b]	p-value[c]
PCP	late log	4FP	6.22E-04	5.05E-05	51.09	31.01	17.39	0.113
added		5FP	4.13E-04	8.47E-05	20.90			
		6FP	5.50E-04	6.15E-05	21.06			
2,4-DNP	early log	7AD	5.23E-04	3.74E-05	13.98	14.76	0.68	0.0302
		8AD	4.68E-04	3.07E-05	15.24			
		9AD	7.23E-04	4.80E-05	15.06			
	late log	7BD	2.46E-04	8.36E-05	2.94	4.27	1.87	0.0181
		8BD	1.05E-04	N/A				
		9BD	5.70E-04	1.02E-04	5.59			
Roundup	early log	10AR	9.10E-04	8.34E-05	10.91	10.30	0.63	0.8546
		11AR	6.26E-04	6.48E-05	9.66			
		12AR	9.67E-04	9.37E-05	10.32			
	late log	10BR	7.28E-04	1.15E-04	6.33	6.24	0.98	0.0086
		11BR	2.77E-04	5.30E-05	5.23			
		12BR	3.89E-04	5.42E-05	7.18			
Benzoate	early log	13AB	0.000545	4.88E-05	11.17	16.42	8.90	0.8546
		14AB	0.000985	3.69E-05	26.70			
		15AB	0.000402	3.53E-05	11.39			
	late log	13BB	0.000175	2.55E-05	6.86	5.23	1.70	0.0086
		14BB	0.00025	4.67E-05	5.34			
		15BB	0.00018	5.17E-05	3.47			

[a]Sample names correspond to the chemical addition: 1-3 are controls, AC=early log control, BC=late log control; 4-5 are PCP ammended flasks, AP=early log PCP, BP=late log PCP, CP=early log washed cells, etc.
[b]S.D. is standard deviation of fold change.
[c]p-value of t-test to determine difference from control expresssion, α=0.05.

Figure 4.2. Growth rates of PAO1 and PAO200 in minimal medium exposed to different chemical stressors. Data are in Excel file: *duplicate_chemscreen*, notebooks #6 and #7.

	Wild type (PAO1)				MexAB-OprM mutant (PAO200)			
2,4 DNP (mM)	A	B	Avg	AvgDev	A	B	Avg	AvgDev
5	0.031	0.038	0.034	0.004	0.016	0.022	0.019	0.003
2.5	0.036	0.049	0.043	0.007	0.022	0.029	0.026	0.004
1	0.038	0.067	0.052	0.014	0.043	0.044	0.043	0.001
0.5	0.055	0.062	0.058	0.003	0.042	0.078	0.060	0.018
0.25	0.053	0.071	0.062	0.009	0.044	0.074	0.059	0.015
Catechol (mM)								
5	0.015	0.037	0.026	0.011	0.000	0.000	0.000	0.000
4	0.024	0.033	0.028	0.004	0.000	0.022	0.011	0.011
3	0.023	0.039	0.031	0.008	0.012	0.033	0.022	0.010
2	0.035	0.047	0.041	0.006	0.011	0.053	0.032	0.021
1	0.051	0.059	0.055	0.004	0.029	0.075	0.052	0.023
Benzoate (mM)								
50	0.011	0.003	0.007	0.004	0.003	0.000	0.001	0.002
25	0.033	0.039	0.036	0.003	0.014	0.023	0.018	0.004
10	0.036	0.061	0.048	0.013	0.030	0.060	0.045	0.015
5	0.044	0.064	0.054	0.010	0.030	0.076	0.053	0.023
1	0.046	0.073	0.059	0.013	0.038	0.083	0.060	0.022
Roundup (μL/mL)								
10	0.118	0.151	0.134	0.016	0.100	0.083	0.091	0.008
1	0.107	0.110	0.109	0.002	0.089	0.084	0.086	0.003
0.1	0.091	0.099	0.095	0.004	0.084	0.076	0.080	0.004
Controls								
control	0.056	0.070	0.063	0.007	0.060	0.068	0.064	0.004
PCP (120 μM)	0.0159	0.025	0.020	0.005	0.012	0.008	0.010	0.002

Table 4.4. All antibiotics tested for resistance with disk diffusion assay in PAO1. Zone of inhibition (mm). Data are in file: *diskdiffchems*, notebook #7.

antibiotic	na[b] avg	PCP avg	PCP p-value	2,4 DNP avg	2,4 DNP p-value	benzoate avg	benzoate p-value	Roundup® avg	Roundup® p-value
Amikacin	21.3	23.9	0.0035	23.5	0.0761	20.3	0.1294	23.0	0.0486
Carbenicillin	21.3	16.3	0.0033	19.9	0.4620	21.3	1.0000	20.8	0.6540
Chloramphenicol	13.0	6.0	0.0004	6.0	0.0004	6.0	0.0004	6.0	0.0004
Ciprofloxacin	32.0	27.3	0.0108	28.5	0.0383	29.1	0.0431	30.3	0.2514
Gentamicin	17.1	19.0	0.0006	19.8	0.0376	16.3	0.1640	19.3	0.0011
Imipenem	24.8	24.0	0.5706	25.8	0.6073	11.4	0.0003	23.8	0.6073
Piperacillin	27.6	24.3	0.0023	27.5	0.9240	27.0	0.5498	26.0	0.0600
Tetracycline	12.4	6.0	0.0004	7.8	0.0004	6.0	0.0004	6.0	0.0004
Tobramycin	21.3	22.5	0.0764	22.9	0.0972	21.0	0.6376	21.0	0.7506

[a]The results are the average of duplicate tests in two trials (n=4), and the p-value is the result of a Student's t-test comparing the chemical with the control.
[b]na=no chemical addition.

Raw data from disk diffusion assay (mm zone of inhibition)

no chemical addition

antibiotic	code	mm				avg
Amikacin	AN 30	21	22	21.0	21.0	21.3
Carbenicillin	CB 100	23	22	20.0	20.0	21.3
Chloramphenicol	C 30	12	13	14.0	13.0	13.0
Ciprofloxacin	CIP 5	34	33	30.0	31.0	32.0
Erythromycin	E 15	6	6	6.0	6.0	6.0
Gentamicin	GM 10	17	17	17.0	17.5	17.1
Imipenem	IPM 10	27	25	23.0	24.0	24.8
Piperacillin	PIP 100	28	28	27.5	27.0	27.6
Tetracycline	Te 30	13	13	11.5	12.0	12.4
Tobramycin	NN 10	22	22	20.0	21.0	21.3

120 μM PCP

antibiotic	code	mm				avg
Amikacin	AN 30	25	24	23.5	23.0	23.9
Carbenicillin	CB 100	17	18	15.0	15.0	16.3
Chloramphenicol	C 30	6	6	6.0	6.0	6.0
Ciprofloxacin	CIP 5	27	27	27.0	28.0	27.3
Erythromycin	E 15	6	6	6.0	6.0	6.0
Gentamicin	GM 10	19	19	19.0	19.0	19.0
Imipenem	IPM 10	25	26	23.0	22.0	24.0
Piperacillin	PIP 100	25	25	23.0	24.0	24.3
Tetracycline	Te 30	6	6	6.0	6.0	6.0
Tobramycin	NN 10	22	23	22.0	23.0	22.5

Raw data from disk diffusion assay (mm zone of inhibition), cont.

5 mM 2,4 DNP

antibiotic	code	mm				avg
Amikacin	AN 30	25	25	22.0	22.0	23.5
Carbenicillin	CB 100	22	23	17.5	17.0	19.9
Chloramphenicol	C 30	6	6	6.0	6.0	6.0
Ciprofloxacin	CIP 5	31	29	27.0	27.0	28.5
Erythromycin	E 15	0	0	0.0	0.0	0.0
Gentamicin	GM 10	21	21	18.0	19.0	19.8
Imipenem	IPM 10	29	28	23.0	23.0	25.8
Piperacillin	PIP 100	25	26	29.0	30.0	27.5
Tetracycline	Te 30	7.5	7.5	8.0	8.0	7.8
Tobramycin	NN 10	24	24	22.0	21.5	22.9

50 mM Benzoate

antibiotic	code	mm				avg
Amikacin	AN 30	21	21	20.0	19.0	20.3
Carbenicillin	CB 100	22	23	20.0	20.0	21.3
Chloramphenicol	C 30	6	6	6.0	6.0	6.0
Ciprofloxacin	CIP 5	30	30	28.0	28.5	29.1
Erythromycin	E 15	0	0	0.0	0.0	0.0
Gentamicin	GM 10	17	17	15.0	16.0	16.3
Imipenem	IPM 10	11	11	11.5	12.0	11.4
Piperacillin	PIP 100	29	28	25.0	26.0	27.0
Tetracycline	Te 30	6	6	6.0	6.0	6.0
Tobramycin	NN 10	21	21	21.0	21.0	21.0

10 µL/mL Roundup

antibiotic	code	mm				avg
Amikacin	AN 30	24	24	22.0	22.0	23.0
Carbenicillin	CB 100	22	22	19.0	20.0	20.8
Chloramphenicol	C 30	6	6	6.0	6.0	6.0
Ciprofloxacin	CIP 5	32	32	29.0	28.0	30.3
Erythromycin	E 15	0	0	0.0	0.0	0.0
Gentamicin	GM 10	19	19	19.0	20.0	19.3
Imipenem	IPM 10	27	26	21.0	21.0	23.8
Piperacillin	PIP 100	27	27	25.0	25.0	26.0
Tetracycline	Te 30	6	6	6.0	6.0	6.0
Tobramycin	NN 10	22	22	20.0	20.0	21.0

Results of disk diffusion assay with PAO200 (data not shown in Chapter 4). Data are in file: *diskdiffchems*, notebook #7.

antibiotic	code	na		PCP		benzoate		2,4-DNP		Roundup®	
Amikacin	AN 30	27	27	27	27	25	25	29	31	30	30
Carbenicillin	CB 100	34	34	39	37	32	32	31	34	38	39
Chloramphenicol	C 30	32	32	32	32	31	30	34	35	31	30
Ciprofloxacin	CIP 5	42	41	40	42	39	38	44	44	40	39
Erythromycin	E 15	17	16	16	16	16	17	20	20	15	14
Gentamicin	GM 10	22	23	27	25	26	25	31	31	26	25
Imipenem	IPM 10	25	25	33	33	21	19	29	30	27	27
Piperacillin	PIP 100	33	34	35	37	34	35	36	35	37	37
Tetracycline	Te 30	27	27	26	26	25	26	26	26	29	29
Tobramycin	NN 10	25	25	27	26	23	23	29	28	28	28

Table 4.5 MICs (μg/mL) in the presence of different organic contaminants.

Data are in Excel file: *chem_antibioticMICs*, notebooks #6, #7.

PAO1

	June 1, 2006				June 17, 2006			
Inducing chemical	none	benzoate	2,4 DNP	Round-up®	none	benzoate	2,4 DNP	Round-up®
Antibiotic								
Carbenicllin	32	32	32	32	32	64	32	32
Tetracycline	8-4	8-16	16	8	16	32	16	8
Chloramphenicol	8	16	8	16	8	16	16	16
Naladixic acid	32	64	256	64	64	128	128	64
Ciprofloxacin	0.05	0.1	0.05	0.05	0.05	0.1	0.1	0.05
Gentamycin	2	2-4	0.25	2	2	2	0.25	2

PAO200

	June 1, 2006				June 15, 2006			
Inducing chemical	none	benzoate	2,4 DNP	Round up®	none	benzoate	2,4 DNP	Round up®
Antibiotic								
Carbenicllin	0.5	0.5	0.5	.25-.125	0.5	0.5	0.25-0.5	<0.125
Tetracycline	0.5	0.5	1	.25	0.25	0.25	0.5	.0.125
Chloramphenicol	0.5	0.5	0.5	.25	0.25	0.5-0.25	0.5	,0.125
Naladixic acid	2	16	8	8-2	2	16	8	4
Ciprofloxacin	0.005	0.005	0.005	0.005-0.01	0.01	0.005	0.005-0.01	0.005
Gentamycin	2-1	1	0.0625	0.5-1	1	1	0.0625	0.5

Jocelyn Fraga Muller
Curriculum Vitae

EDUCATION

Ph.D. **Civil Engineering**, expected July 2006
Virginia Tech. Advisors: Dr. Nancy Love and Dr. Ann Stevens
Future Professoriate Graduate Certificate
Dissertation: The Role of Multidrug Efflux Pumps in the Stress Response of *Pseudomonas aeruginosa* to Organic Contamination
GPA: 3.96/4.0

M.S. **Environmental Science and Engineering,** 2000
The University of North Carolina at Chapel Hill. Advisor: Dr. Frederic Pfaender
Thesis title: Examination of the bacterial diversity of a PAH contaminated soil.
GPA: High/High, Pass, Low (professional grading system)

B.S. **Environmental Sciences,** 1997
The University of Massachusetts at Amherst. Research advisor: Dr. Derek Lovley
Undergraduate thesis title: The effects of humic substances and plant phenolics on microbial iron reduction.
The University of Oviedo, Asturias, Spain, spring semester 1996.
GPA: 3.77/4.0

RESEARCH INTERESTS

- Microbial function and structure of contaminated environments.
- The effects of emerging and established anthropogenic contaminants on antibiotic resistance and disease transmission.
- Public health microbiology with a focus on achieving sustainable safe drinking water in developing countries.
- Understanding how biofilm growth of bacteria affects resistance to antibiotics and disinfection.
- Plant-microbial interactions and the effects on bio/phytoremediation.
- Discovery of novel microbial biochemical pathways using molecular techniques.

RESEARCH EXPERIENCE

Virginia Tech, Blacksburg, VA (August 2001 – present)
Graduate Research Assistant, Dept. of Civil and Environmental Engineering

- Investigating the role of RpoS under nutrient limiting growth conditions in the response of *Pseudomonas aeruginosa* to pentachlorophenol stress.
- Examining the whole-genome response of *Pseudomonas aeruginosa* to pentacholorophenol using Affymetrix microarray GeneChips, and correlating gene expression changes induced by the stressor to changes measurable at the whole cell level.

Virginia Tech, Blacksburg, VA (September 2000 – August 2001)
Laboratory Technician Senior, Dept. of Plant Pathology, Physiology and Weed Science

- Performed research assisting two different research programs.
- Supervised undergraduate researchers.

- Ensured lab safety in compliance with OSHA regulations; maintained all lab equipment, supplies, and plant and bacterial cultures.

The University of North Carolina, Chapel Hill, NC (May 1999 – December 2000)
Research Assistant, Dept. of Environmental Science and Engineering

- Worked with molecular methods to determine the community changes in PAH-contaminated soil microcosms under different enrichment conditions.
- Developed a method to gently remove contaminants allowing purification of PCR-ready DNA from PAH-contaminated soil.

University of Massachusetts, Amherst, MA (June 1997 – June 1998)
Departmental Assistant, Dept. of Microbiology

- Investigated the role of humic substances in electron shuttling using pure cultures of iron-reducing bacteria.
- Used 16S rDNA profiling methods to determine community structure of contaminated groundwater sediments.

University of Massachusetts, Amherst, MA (June 1996 – May 1997)
Senior Honors Research and Thesis, Environmental Sciences Program

- Used pure plant phenolics and humic substances and leaf-extracted compounds to investigate the use of reduced phenolics as electron donors for microbial iron reduction.

TEACHING EXPERIENCE

Virginia Tech, Blacksburg, VA (August – December 2002)
Instructor, Environmental Engineering Microbiology (CEE 5194)

- Taught one-half of a graduate level course, Environmental Engineering Microbiology (CEE 5194), with Dr. Nancy G. Love. Responsibilities included: preparing and giving a half-semester of class lectures; preparing and grading class assignments, exams, and projects.

Virginia Tech, Blacksburg, VA (May 2002 – March 2003)
Mentor for High School Research Study

- Worked with a colleague to mentor two Blacksburg High School senior students and assist with their research project on denitrification using natural organic matter as a sole carbon source.

The University of North Carolina, Chapel Hill, NC (August 1998 – May 1999)
Teaching Assistant, Dept. of Environmental Sciences and Engineering

- For both Ecology of Microorganisms (EVNR 134) and Environmental Microbiology (ENVR 135), prepared weekly laboratory exercises for students, assisted with individual student mini-research projects, and occasionally led lecture portion of labs.

FELLOWSHIPS AND AWARDS

Virginia Tech, Blacksburg, VA

Marion Via Fellowship, Virginia Tech Civil and Environmental Engineering Department;
3 years 100% funding, $32,500/year including tuition, fees, health insurance
GAANN Fellowship, U.S. Department of Education
3 years 50% funding, $12,000/year including travel and research expenses
P.E.O. Scholar, P.E.O. International
1 year, $8,000 for research

AAUW Selected Professions Scholar, American Association of University Women
1 year, $20,000 stipend
Chi Epsilon, Civil Engineering Honor Society
Virginia Tech Citizen Scholar
Paul E. Torgersen Graduate Research Excellence Award, 1st place poster
March 28, 2006

The University of North Carolina, Chapel Hill, NC

Alpha Epsilon Lambda, Graduate Honor Society

University of Massachusetts, Amherst, MA

University of Massachusetts Chancellor's Talent Award
4 years, $5,000/year
William F. Field Alumni Scholar
1 year, $750
Commonwealth Scholar, Departmental Honors
Recognition of Senior Honors Thesis, $500
Golden Key National Honor Society

PUBLICATIONS

- **Fraga Muller, J. F.,** J. Craig, A. M. Stevens, N. G. Love. 2006. Transcriptome analysis of the response of *Pseudomonas aeruginosa* to Pentachlorophenol. In preparation.
- **Fraga Muller, J. F**., A. M. Stevens, N. G. Love. 2006. Pentachlorophenol causes increased antibiotic resistance in the opportunistic pathogen, *Pseudomonas aeruginosa*. In preparation.
- Rooney-Varga, J. N., R. T. Anderson, **J. L. Fraga**, D. Ringelberg, and D. R. Lovley. 1999. Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. *Appl. Environ. Microbiol.* 65: 3056-3063.
- Lovley, D. R., **J. L. Fraga**, J. D. Coates, and E. L. Blunt-Harris. 1999. Humics as an electron donor for anaerobic respiration. *Enivr. Microbiol.* 1:89-98.
- Lovley, D. R., **J. L. Fraga**, E. L. Blunt-Harris, L. A. Hayes, E. J. P. Phillips, J. D. Coates. 1998. Humic substances as a mediator for microbially catalyzed metal reduction: *Acta Hydrochimica et Hydrobiologica*. 26: 152-157.

PRESENTATIONS

- **Fraga Muller, J. L.**, J. Craig, A. M. Stevens, and N. G. Love. Using Whole Genome Arrays to Investigate Functional Response to Contaminant Stress: the Response of *Pseudomonas aeruginosa* to Pentachlorophenol. July 2005. Oral Presentations, Association of Environmental Engineering and Science Professors Conference. Clarkson University, Potsdam, New York.
- **Fraga Muller, J. L.**, J. Craig, A. M. Stevens, and N. G. Love. The Stress Response of *Pseudomonas aeruginosa* to Pentachlorophenol. July 2005. Poster Presentation, American Society for Microbiology General Meeting. Atlanta, Georgia.
- **Fraga Muller, J. L.**, N. G. Love, and A. M. Stevens. Studying the Role of RpoS in Adaptation of *Pseudomonas putida* Biofilms to Chemical Perturbation. July 2002. Poster Presentation, Gordon Research Conference on Microbial Stress Responses. Newport, RI.
- **Fraga, J. L.**, and F. K. Pfaender. Bacterial Diversity of a PAH-Contaminated Soil. May 2000. Poster Presentation, American Society for Microbiology General Meeting. Los Angeles, CA.

INVITED SEMINARS

- **Fraga Muller, J. L.**, J. Craig, A. M. Stevens, and N. G. Love. February 10, 2006. *Pseudomonas aeruginosa* and pentachlorophenol: Increased antibiotic resistance revealed through whole-genome analysis of the stress response. Environmental and Water Resources Engineering Seminar, Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA.

PROFESSIONAL WORKSHOP PARTICIPATION

- **Successful Academic Careers: Mentoring, Funding, Winning CAREER Awards.** July 2005. Organizers: Pedro Alvarez, Susan Powers, and Andria Costello. Funded by National Science Foundation. Association of Environmental Engineering and Science Professors Biannual Meeting. Clarkson University, Postdam, NY.
- **Effective College Teaching for New Faculty and Graduate Students.** May 2002. Facilitators: Richard M. Felder and Rebecca Brent. Virginia Tech, Blacksburg, VA.

RESEARCH FUNDING AWARDS

Graduate Research Development Project, Graduate Student Assembly, Virginia Tech
- $300 Spring 2004
- $250 Spring 2005

COMMITTEE SERVICE AND ACTIVITIES

- Graduate School Ambassador, VA Tech, August 2005-present.
- Secretary of College of Engineering Graduate Student Committee, August 2002-May 2004.
- Active member of Student Action Committee, Environmental Engineering, VA Tech, August 2001-present; Co-Chair, August 2001-June 2003.
- Active member of Student Union Board for the UNC School of Public Health; Treasurer, 1998-1999; Co-Chair, 1999-2000
- Student Representative for the SPH Alumni Association, 1999-2000.
- Member of Class Gift Campaign Committee, SPH Class of 2000.
- Active member of the Student Association of Environmental Sciences, 1994-1997.

COMMUNITY SERVICE

- Life Teen Core Member (Catechist) and Life Teen Social Justice committee advisor, St. Mary's Church, Blacksburg, VA, August 2001-present.
- Paper recycling coordinator, CEE Department, July 2002-2003.

PROFESSIONAL MEMBERSHIPS

- American Society for Microbiology
- Association of Environmental Engineering and Science Professors