

## **Correlation of Specific Phenazine Derivatives with Gene Expression and Colony Morphology in *Pseudomonas fluorescens***

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The project that I am proposing to investigate this summer comes from some of the new research that I began during my pre-tenure sabbatical in the fall semester of 2011. Although I have had students supported by HHMI funds in the past, this is the first time that I have requested funding for research related to my new work on the physiological aspects of phenazine biosynthesis in the genera *Pseudomonas*. I am requesting funding to support one student researcher for 8-10 weeks.

### **Background Information**

The *Pseudomonads* are a widespread group of bacteria that inhabit both soil and water. Many of the members of this genera participate in beneficial interactions with plants, others are pathogens of various animals, plants and humans. Because of the widespread prevalence of these organisms in nature, and their varied interactions with other organisms in an ecosystem, the *Pseudomonad* genera represents an interesting group of organisms in which to ask comparative physiological questions. One of the most striking aspects of *Pseudomonad* biology is their extensive secretion of a group of molecules called phenazines.

Phenazines are small, pigmented, nitrogenous ring-based chemicals that are readily secreted by some bacteria in the *Pseudomonad* genera. Chemically different varieties of phenazine are made by differentially modifying the core phenazine molecule phenazine 1-carboxylate (PCA), and these chemical modifications lead to changes in the pigmentation of the molecule. It is the secretion of these phenazine molecules that leads to the striking blue coloration of *Pseudomonas aeruginosa*, the fluorescent yellow coloration of *Pseudomonas fluorescens*, and the bright orange pigmentation of *Pseudomonas chlororaphis*. While some *Pseudomonads* express several varieties of phenazine, others are limited to only one or two. Numerous studies have shown that phenazines have important physiological roles including redox modulation (Deitrich, 2008), cell signaling (Deitrich, 2006) and iron acquisition (Wang, 2011), although how specific types of phenazine molecules contribute to each of these processes is unknown. The experiments outlined below attempt to correlate individual types of phenazines, with specific biological properties.

### ***Specific Aim #1: Clone the phenazine modifying enzymes phzH, phzM, phzO, and phzS into expression constructs.***

Genetic studies in several well-characterized *Pseudomonads* have revealed important findings regarding the genes involved in phenazine biosynthesis. First, the genes for the core phenazine biosynthetic enzymes are located within a single operon. The resulting core phenazine molecule phenazine 1-carboxylate (PCA) is then “decorated” with additional functional groups by individual enzymatic reactions. The genes for these phenazine-modifying enzymes are located outside, and often distal to the core biosynthetic operon (Mavrodi, 2008), therefore these

orphan genes should easily be expressed in related *Pseudomonad* strains either from the native promoter, or via a constitutive promoter. PhzH, phzM, phzO and phzS are genes encoding different phenazine modifying enzymes. Based on available sequence information for these genes, they will be PCR amplified and cloned into suitable expression vectors.

**Specific Aim #2: Transform *Pseudomonas fluorescens* with each of the phenazine modifying genes cloned in Specific Aim #1.**

Several strains of *Pseudomonas fluorescens* express the core phenazine biosynthetic operon, but do not encode any of the phenazine modifying enzymes required to produce specific phenazine derivatives (Mavrodi, 2006). Transformation of these strains with the individual genes encoding the phenazine modifying enzymes should result in the production of the core phenazine molecule PCA, as well as the specific phenazine of interest. Observing a color change in the culture media due to the readily secreted phenazine molecules can assess successful production of the additional phenazine molecule. Successfully transformed *P. fluorescens* strains, can then be used in subsequent experiments to measure changes in specific physiological responses as a result of the additional phenazine molecules.

**Specific Aim #3: Correlate physiological changes with the expression of individual phenazine modifying enzymes.**

A. *Monitoring changes in gene expression using real-time RT-PCR.* In one study, twenty-two genes were shown to be upregulated by the presence of pyocyanin, a phenazine molecule produced by the organism *Pseudomonas aeruginosa* (Deitrich, 2006). Given the similarity in chemical structure, it is likely that other forms of phenazine might also produce changes in gene expression, however this has not been investigated directly. Following the expression of the individual phenazine modifying enzymes in the *P. fluorescens* strains produced in Specific Aim #2, changes in the top four previously identified differentially regulated genes will be monitored using real-time RT-PCR. Although it is possible that individual phenazines control the regulation of diverse sets of genes, this initial study will highlight any deviations from what has already been observed following expression of pyocyanin. Any specific engineered *P. fluorescens* strains that show significantly different levels of gene regulation, can be investigated more thoroughly using transcriptome-profiling experiments in the future.

B. *Monitoring changes in colony growth and morphology.* In the absence of phenazine, *Pseudomonas aeruginosa* produces large, highly wrinkled colonies when spotted on Congo-Red plates during exponential growth (Deitrich, 2008). It is hypothesized that presence of phenazines may directly impact the production and secretion of several exopolysaccharides important in the development of colony morphology. Following expression of individual phenazine modifying genes in *Pseudomonas fluorescens* (as indicated in specific aim #2), the same spotting techniques will be used to monitor colony size and morphology over the course of six days. Such studies could indicate that specific phenazine molecules play different roles in determining colony size and/or morphology.

### Significance and Feasibility

The research outlined in this proposal would be a significant contribution to the current understanding of the role of phenazines in bacterial physiology. The approach taken in the experiments proposed above represents a departure from the from previously published studies in that the effect of individual phenazine derivatives on bacterial physiology will be investigated in one single strain by simply adding specific phenazine modifying genes individually. Theoretically, the effect of these additional genes can be monitored individually and also in combination, thereby identifying physiological changes that might occur by synergistic, or antagonistic mechanisms.

The proposed experiments involve simple gene cloning and transformation, followed by relatively quick and easy physiological characterizations. The *Pseudomonas* strains necessary for amplification of the specific phenazine modifying genes, and the *Pseudomonas fluorescens* strain that will be used as the host strain in which these modifying genes are expressed, are readily available from an environmental collection of *Pseudomonads* in the laboratory of Dr. Christopher Taylor at the OARDC. It is my hope that my student and I can accomplish most, if not all of the proposed aims in a single summer.

### Literature Cited

Deitrich, L.E., Price-Whelan, A., Petersen, A., Whiteley, M., and Newman, D.K. (2006) The phenazine pyocyanin is a terminal signaling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Mol Microbiol* **61**: 1308-1321.

Deitrich, L.E., Teal, T.K., Price-Whelan, A., and Newman, D.K. (2008) Redox-active antibiotics control gene expression and community behavior in divergent bacteria. *Science* **321**: 1203-1206

Mavrodi, D.V., Blankenfeldt, W., and Thomashow, L.S. (2006). Phenazine compounds in fluorescent *Pseudomonas* Spp. biosynthesis and regulation. *Annu Rev Phytopathol* **44**: 417-45.

Wang, Y., Wilks, J.C., Danhorn, T., Ramos, I., Croal, L., and Newman, D.K., (2011). Phenazine-1-carboxylic acid promotes bacterial biofilm development via ferrous iron acquisition. *J Bacteriol* **193**: 3060-3617.