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UROP Final Report

Regulation of *Pseudomonas aeruginosa* Virulence Factors in Response to Products of Oropharyngeal Bacterial Mucin Fermentation

Background

Cystic fibrosis is a genetic disorder which results in dysfunction and disease of many mucus membranes, such as the lungs and sinuses[1]. The prolific dysfunction associated with CF is the accumulation of stagnant mucus in the lungs and inability to clear this mucus, which leads to acute and chronic lung disease[2]. Most often, lung disease associated with cystic fibrosis is perpetuated by bacterial infections: *Pseudomonas aeruginosa* is the major pathogen responsible for mortality and morbidity in individuals with CF. This bacterium is an opportunistic pathogen, harmless to those with fully functioning immune systems. However, in the CF lungs, *P. aeruginosa* can survive and proliferate.

Much research has been performed to investigate how *P. aeruginosa* uses so-called 'virulence factors' (enzymes, reactive-oxygen compounds, cyanide) to outcompete other microbes present or condition its environment. Our research group focuses on a more fundamental question: what is *P. aeruginosa* eating? Prior work by our lab has demonstrated that *P. aeruginosa* is likely getting its major source of carbon via the fermentation byproducts of other microbes present in the lungs[2]. These microbes are mucin-fermenting bacteria which breakdown the major component of mucus (mucin). These microbes are commonly found in the mouth, but have been found in the lungs[2,3]. The mucin-cross feeding hypothesis was supported when Flynn et al. observed *P. aeruginosa* could not grow to high abundance on pure mucin but could grow to robust population size on mucin fermentation byproducts from the oral microbes[2]. During these experiments, Flynn observed that *P. aeruginosa* cultures tended to appear more blue-green, illustrating the production of a blue compound called pyocyanin[4]. Pyocyanin has antimicrobial and host-damaging effects, so this response to mucin-degradation products—something which appeared to be feeding *P. aeruginosa*—was an interesting lead for a research project.

My UROP project focused on how *P. aeruginosa* is sensing and responding to mucin fermentation by the mucin-fermenting community. Many genes have been identified in *P. aeruginosa* that are responsible for the regulation and production of virulence factors, but we decided to focus on *pqsA* and *pqsR* initially. These genes are responsible for the production of PQS, a compound which globally regulates genes responsible for producing virulence factors such as pyocyanin[5]. We can quantify how these genes are being expressed in *P. aeruginosa* by cloning the promoter of a gene into a plasmid containing the *lux* operon[6]. When fully transcribed, the *lux* operon produces a luminescence signal. By using a specific promoter to control the *lux* operon, we can estimate the promoter activity of a gene by recording the luminescence signal in response to conditions such as growth in byproducts of mucin fermentation. Later in the project, I decided to also make *lasB* and *phzA1* promoter constructs. These genes (*lasB* and *phzA1*) directly code for elastase and the progenitor compound to pyocyanin, so I hypothesized using the promoters of these genes may more accurately capture how these factors are being regulated.

Results

The experimental pipeline for constructing the pMini CTX *lux* plasmids is illustrated in Figure 1. The majority of the work and time for this project was dedicated to designing and constructing the pMini CTX *lux* plasmids which contained the *lux* operon under the control of the chosen promoters (*pqsA, pqsR, lasB,* and *phzA1*). This was due to the appearance of an odd growth phenotype of one of our *E. coli* strains after transformation with pMini CTX *lux* plasmid. The phenotype also seemed to be associated with low plasmid yield, not yielding enough plasmid for transformation and screening the plasmid for successful ligation of the promoter. I decided to switch to a different strain (*E. coli* SM10) instead of our standard lab strain that had been used previously by a member of our lab for this plasmid work. I also tested other conditions such as different molar ratios for ligation to optimize all aspects of the experiment. However, all conditions were equally efficient when used with the new SM10 strain.

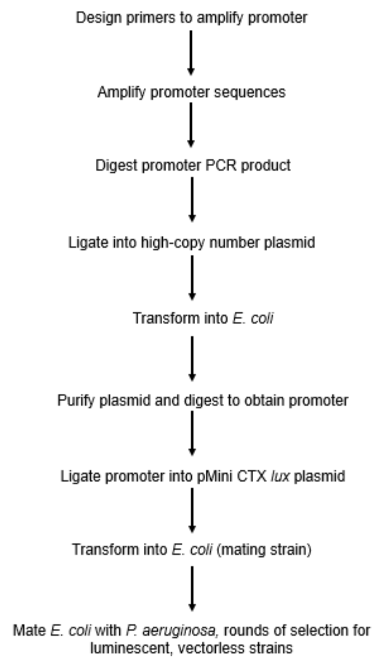

Figure 1. Experimental pipeline for constructing the pMini CTX *lux* plasmids.

Once the *P. aeruginosa* luminescent reporter strains were designed and archived (Figure 2), we then tested these strains against concentration gradients of mucin fermentation products, such as acetate and propionate[2]. Luminescence was recorded over a 24 hour period by recording the luminescence and the growth of each strain when grown in increasing concentrations of acetate, propionate, PQS (positive control), and no addition (negative control). These results imply the existence of a threshold concentration of acetate and propionate which activate virulence factor production (results not shown). Further work in the future using different concentrations of mucin fermentation products would answer further questions about how much acetate and propionate contribute to this effect and suggest the existence compounds which are inducing *P. aeruginosa* virulence factor production.

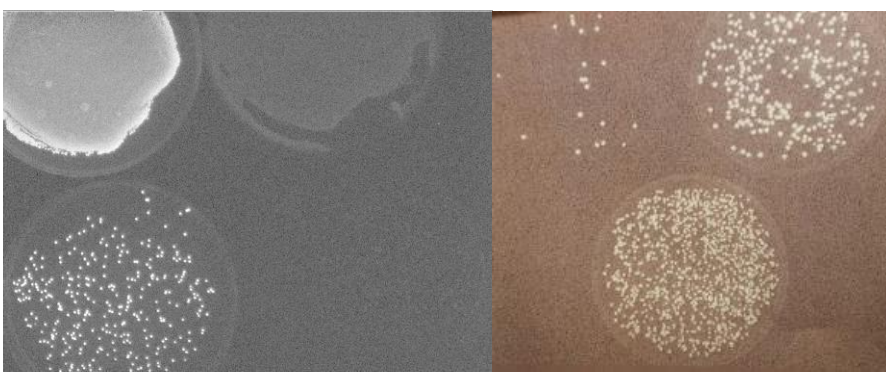

Figure 2. Picture of luminescent strains of *Pseudomonas aeruginosa* strain UCBPP-PA14 on LB nutrient agar plates.

Reflection on UROP Experience

This UROP experience was exciting, fruitful, and difficult. The experimental difficulties, although tough, were beneficial to me as they forced me to design more intelligent controls and think about all aspects of each procedure which could be optimized. Digging into the protocols and understanding them fully taught me more about the science behind my daily procedures and about how to set up experiments to minimize ambiguous results.

The results of this project, although not explicitly shown in this report, will be published in the coming year in an article and presented at a poster presentation at the American Society for Microbiology Microbe 2017 conference. I am very excited to represent the University of Minnesota UROP and disseminate my results. I appreciate all of the time, energy, and resources provided by Dr. Jeffrey Flynn and Dr. Ryan Hunter to help me complete this project. I thank UROP for the opportunity and funding to deepen my science education and research experience.

References

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