Virulence Factors of Pseudomonas aeruginosa: Investigating the Differential Expression of Pyocyanin Pigment in a Polymicrobial Setting

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1. Introduction

Bacteria almost always exist in polymicrobial communities where communication of quorum sensing between intra-species and inter-species is very important in regulating their virulence gene expression. The focus of our research is to understand the differential regulation of quorum sensing genes in the bacteria Pseudomonas aeruginosa. P. aeruginosa is an opportunistic bacterium that often results in serious infections in health-care settings especially in immunocompromised patients. One of the very important aspects of P. aeruginosa virulence is the production of the blue-green pigment pyocyanin and its association in the formation of biofilm. Our research is aimed to identify the role of pyocyanin on the formation of the biofilm by the bacteria. We are also interested in understanding at the genetic level how the quorum sensing circuit is modulated by P. aeruginosa in a polymicrobial community. We co-cultured the laboratory strain of P. aeruginosa with other various laboratory strains of gram positive and gram negative bacteria (Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis) that are frequently associated with nosocomial infections. Our preliminary results indicate that the expression of pyocyanin by the bacteria P. aeruginosa is highly dependent on the specific bacterial population that Pseudomonas encounters. We quantified the pyocyanin expression under various polymicrobial conditions using a colorimetric assay that we optimized. The future research efforts will be focused on generating the pyocyanin gene knockout strains with a goal to compare the relative gene expression differences between the wild type and mutant strains. Our findings would help understand how several other virulence factors of P. aeruginosa are co-regulated with pyocyanin. This could eventually provide valuable insight on the surveillance mechanisms of quorum sensing for P. aeruginosa in detection of its bacterial neighbors and response by producing antimicrobial factors. Our findings can also suggest novel therapeutic strategies for treating P. aeruginosa dominated polymicrobial infections.

2. Pyocyanin-A QS Phenomenon

One of the hallmark features of P. aeruginosa is its ability to produce the blue-green pigment pyocyanin. Pyocyanin belongs to the family of phenazines [5 methyl 1H]phenazines which are heterocyclic compounds that are produced naturally with side chains substituted at different points around their rings by different bacterial species. At neutral pH, pyocyanin exhibits a distinctive blue color and turns red in acidic pH conditions. It is a small and highly diffusible nitrogen-containing aromatic compound with a multitude of biological activities. Pyocyanin is involved in a variety of biological activities including quorum sensing, maintaining fitness of bacterial cells and biofilm formation. It is also known to exhibit antibacterial and antifungal properties. In addition, pyocyanin plays a major role in the survival of P. aeruginosa under oxygen-poor conditions.

In P. aeruginosa, pyocyanin production involves a stepwise process, beginning with the synthesis of the primary quorum sensing (QS) molecule N-acyl L-homoserine lactone (AHL) during the exponential growth phase followed by the secondary QS molecule Pseudomonas quinolone signal (PQS) during the late exponential phase. PQS directly controls the expression of phzG operon resulting in the production of phenazine-1-carboxylic acid (PCA) from its precursor chorismic acid. PCA is then modified to produce three metabolites during the early stationary phase of which pyocyanin is the major component and is regulated by the phzM gene. The two other types of phenazine products are phenazine-1-carboxamide (PCN) encoded by phzI and 1-hydroxyphenazine (1-OHPHZ, encoded by phzS).

3. Quantification of Pyocyanin Pigment Procedure

1. Actively growing bacterial suspension was used to inoculate the tryptic soy agar plates using a sterile spreader.
2. The plates were incubated at 37°C for more than 60 hours to maximize the yield of pyocyanin.
3. The agar was cut aseptically into 1-cm squares and placed in a sterile bottle.
4. Pyocyanin was extracted by adding chloroform & hydrochloric acid.
5. Absorbance at 520 nm is taken for the extracted pigment.
6. Concentration of Pyocyanin

4. Effect of Incubation Time on the Pyocyanin Pigment Production

Using the Tryptic Soy Agar, we cultivated the bacteria at 37°C for extended periods of time and harvested the cells every 12 hours to extract and measure the pyocyanin pigment. Approximately after 72 hours the pyocyanin production reaches maximum and stays the same after that. For all the subsequent experiments, P. aeruginosa was grown for 72 hours at 37°C to maximize the expression of pyocyanin.

5. Differential Regulation of Pyocyanin

P. aeruginosa was co-cultured with the two commonly found nosocomial gram positive bacteria Staphylococcus aureus (SA), Enterococcus faecalis (EF) and the two commonly found nosocomial gram negative bacteria Escherichia coli (EC), Klebsiella pneumonia (KP). (All the strains used were common wild type laboratory strains).

Procedure:
1. SA, EF, IC and KP (laboratory bacterial strains) each of the bacterial strains were grown individually on TSA plates by swabbing a freshly grown liquid culture with OD of 0.6 @ 600 nm.
2. A fresh culture of P. aeruginosa was suspended in a sterile Tryptic Soy broth tube to a reach a turbidity equivalent of a 0.6 OD @ 600nm.
3. 100 ul of the above P. aeruginosa was added at the center of each TSA plate having one of the following bacterial strains: EC, KP, EF and SA.
4. The plates were incubated at 37°C for 72 hours and pyocyanin pigment was measured.

6. Results: P. aeruginosa Differentially Regulates the Production of Pyocyanin

Pyocyanin is a highly versatile molecule recognized for its effect as a potent virulence factor of P. aeruginosa. Our results suggest that P. aeruginosa modulates the expression of pyocyanin in a mixed microbial population and we hypothesize that this regulation has an effect on the biofilm forming ability of P. aeruginosa. Biofilm formation is associated with increased resistance to antibiotic therapies and persistence of bacteria in nosocomial infections. Using versatile tools of Microbiology, Molecular Biology and Biochemistry we are working towards understanding the role of pyocyanin in the biofilm formation of P. aeruginosa in a polymicrobial setting. Eventually the goal is to identify novel drug targets that could serve as quorum quenchers or quorum-sensing inhibitors. This approach has a promising potential for the future in minimizing and delaying the problem of antibiotic resistance.

7. Experimental Strategy for Generating P. aeruginosa Gene Knockouts (Work under progress)


8. Conclusion

9. References & Acknowledgements

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