

Comparison of group behaviors in the wild-type versus mutant strains of the bacteria *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is an opportunistic bacterium that is frequently found in patients with cystic fibrosis, burn wounds, and various immuno-compromised conditions. The bacterium is known to produce various virulence factors through group communication behaviors known as quorum sensing. Here we report that autolysis or self-killing is a socially beneficial behavior of the bacteria and compare the wild-type and a mutant strain (deficient in one of the transcriptional activators of quorum sensing). Some of the group behaviors that are compared in the study are the ability of the bacteria to undergo programmed cell death or autolysis, the ability of the bacteria to produce the blue-green pigment pyocyanin which is toxic to many gram-positive bacteria and animal cells, and the ability of the bacteria to form biofilms.

Bacterial cell death is an interesting phenomenon because it plays a crucial role in many important processes. Bacterial cell death does not just occur in the late stationary phase when nutrient limitation can lead to starvation. But instead begins in the early stationary phase for some bacteria because of the phenomenon of autolysis. Our hypothesis is that there is a direct relationship between quorum sensing and programmed cell death of the bacteria and there are community benefits of the sacrificial suicide by autolysis of the bacteria *P.aeruginosa*. To address this, we are comparing the wild type and a mutant strain (RhIR mutant) of the bacteria in their abilities to undergo autolysis and biofilm formation (along with pyocyanin production). This study will provide a basic understanding of some of the fundamentally misunderstood concepts of bacterial behaviors. Group behaviors and community benefits are underestimated in bacteria and to this end, these studies provide an important insight into the global problem of antibiotic resistance.

RhIR Mutant

RhIR is a quorum sensing receptor and transcriptional regulator in *Pseudomonas aeruginosa* that activates the transcription of various virulence factors. Specifically, RhIR promotes the expression of genes coding for pyocyanin, biofilm formation, elastases, along with genes for many other virulence factors. Clinically, RhIR mutants almost never exist while bacteria containing mutations in other quorum-sensing genes naturally occur in patients. This implies that RhIR mutants are not as virulent and that this gene is essential to the pathogenicity of *P. aeruginosa*. The goal of our research is to use biofilm formation and pyocyanin production as biomarkers to compare the pathogenicity of wild type versus the RhIR mutant strains. Also, there is very limited information on the role of pyocyanin production on biofilm formation and we hypothesize that *P. aeruginosa* uses a novel mechanism known as “autolysis or sacrificial killing” which is bacterial apoptosis. Pyocyanin contributes to autolysis to form stronger biofilms. To test this hypothesis we looked at the autolysis ability of the wild type versus mutant strains along with the other biomarkers: biofilm formation and pyocyanin production.

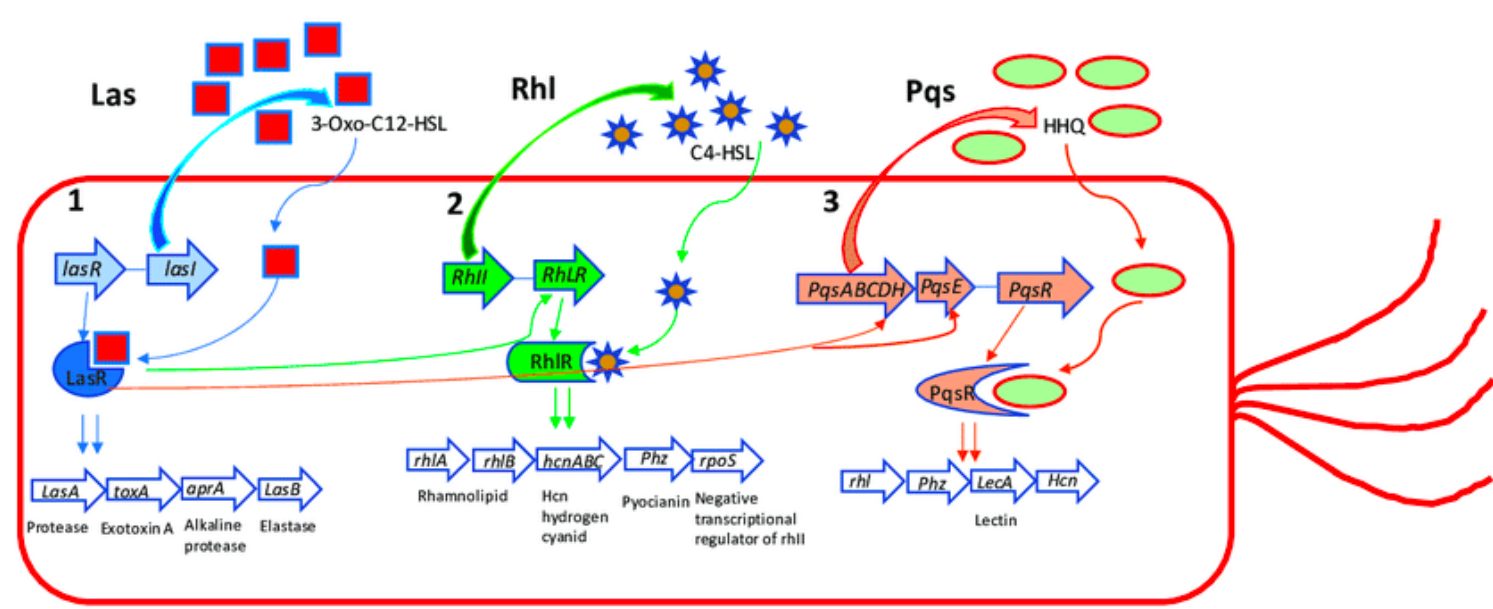
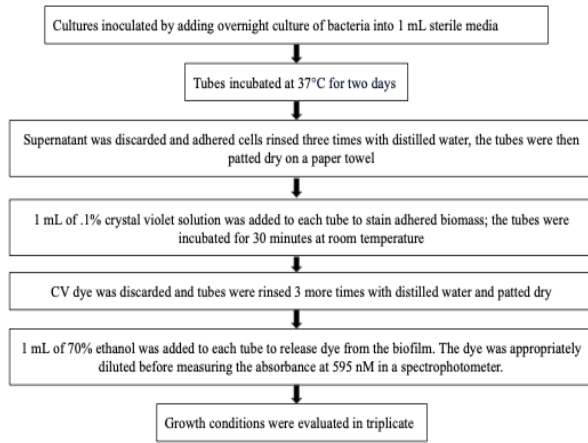


Figure 1. Major quorum sensing circuits in *P. aeruginosa*

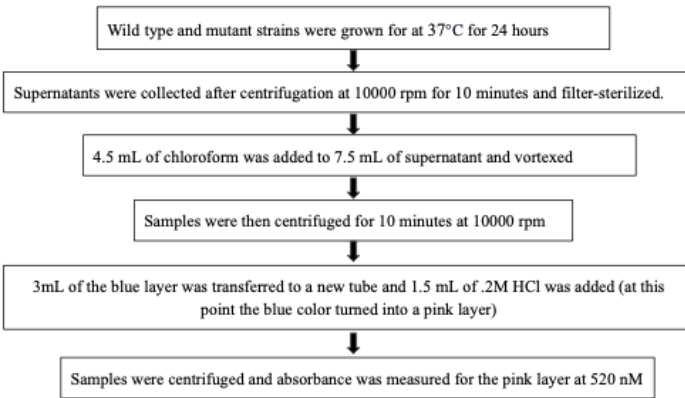
Guzzo, Francesca et al. “Plant Derived Natural Products against *Pseudomonas aeruginosa* and *Staphylococcus aureus*: Antibiofilm Activity and Molecular Mechanisms.” *Molecules* (Basel, Switzerland) vol. 25,21 5024. 29 Oct. 2020, doi:10.3390/molecules25215024

Methods

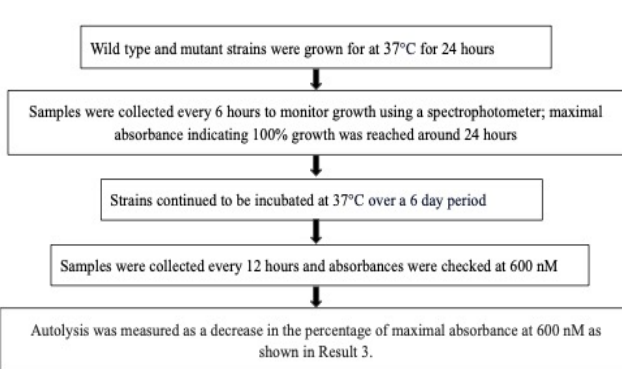
Biofilm Assay



Pyocyanin Assay

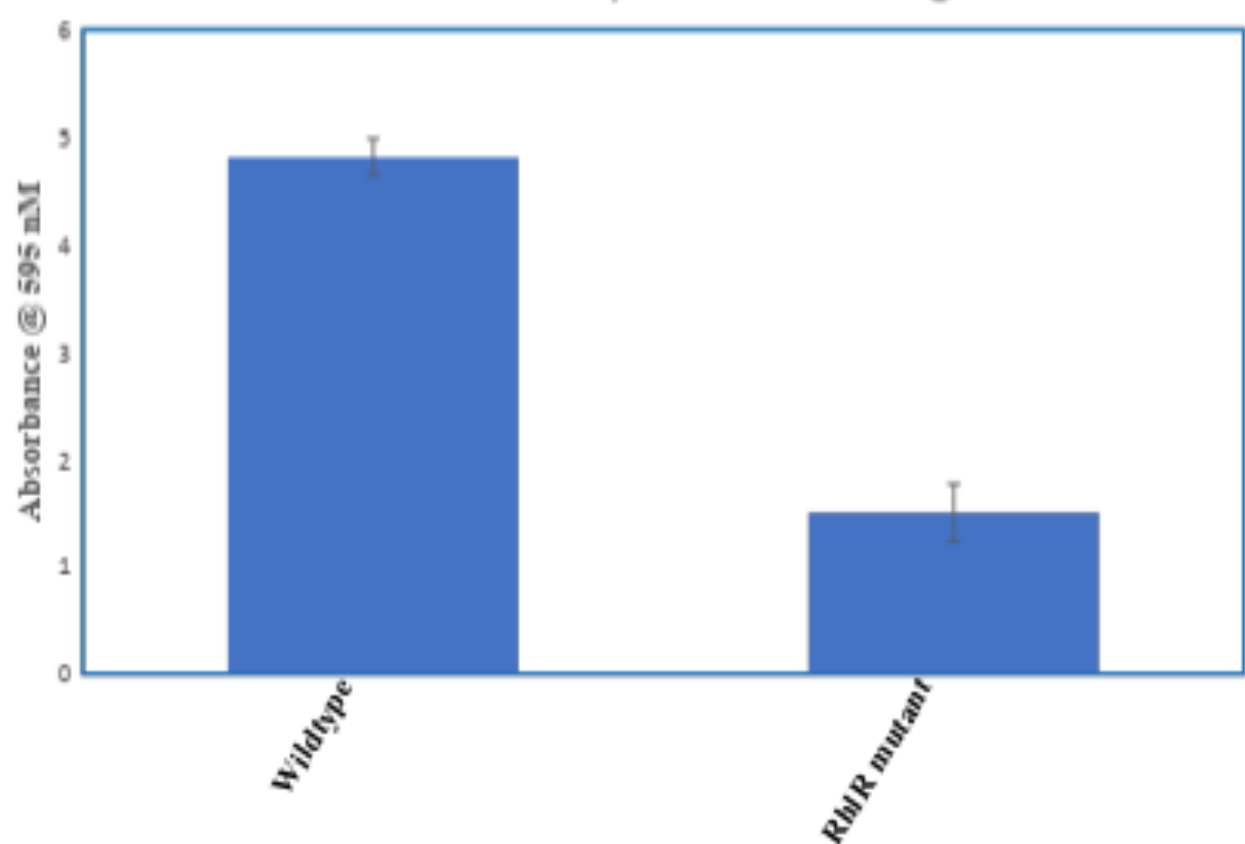


Autolysis Assay



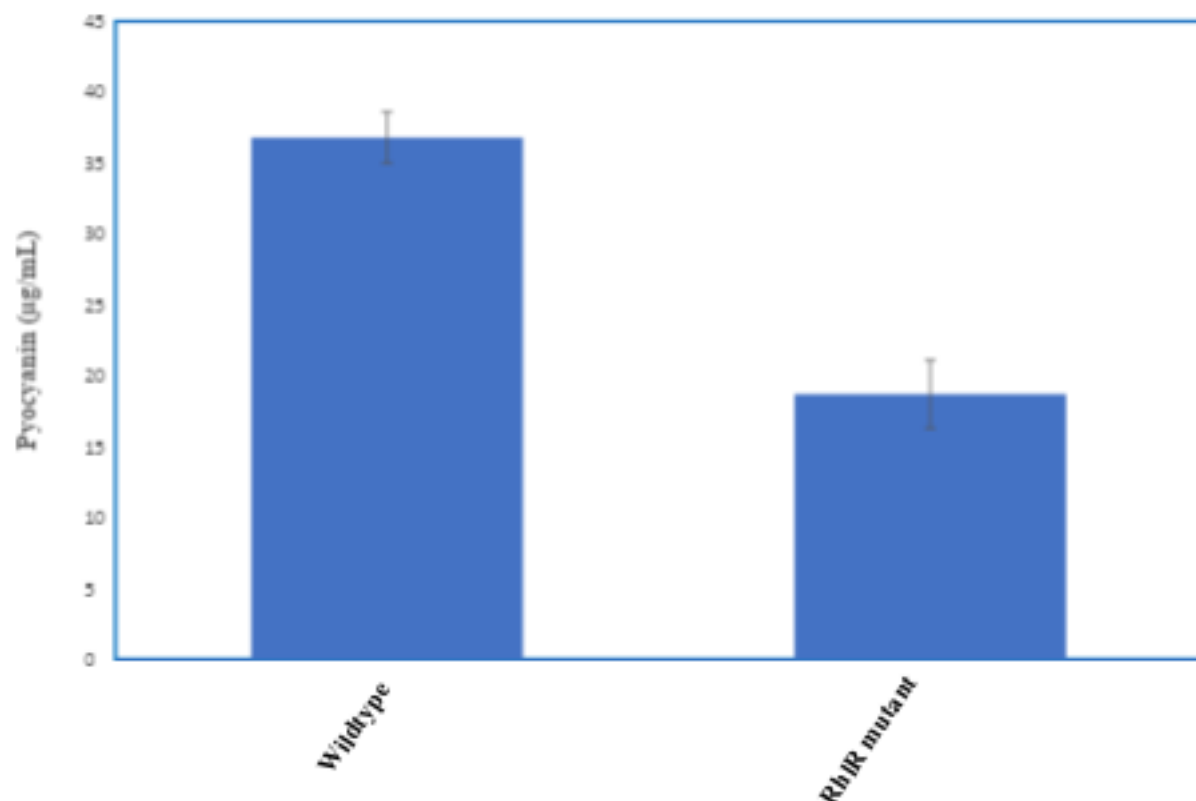
Results

Result 1: Comparison of biofilm formation between the wildtype versus RhIR mutant strains: measured as Crystal Violet absorbance @ 595 nM



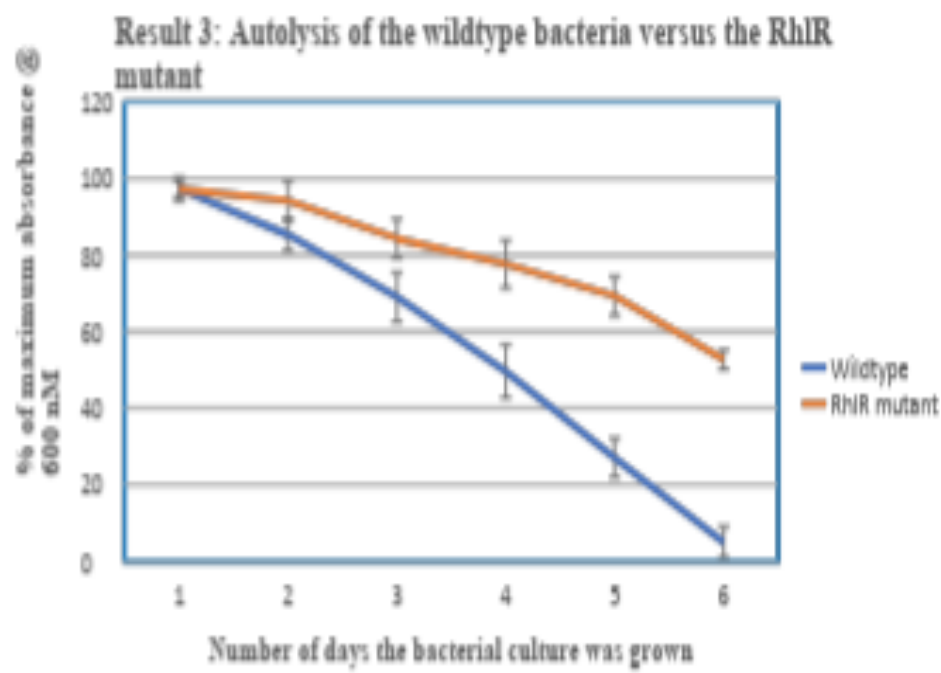
Result 1: As seen from the above graph, the biofilm formation was drastically reduced in the RhIR mutant strain compared to the wildtype strain. As RhIR is a QS transcription factor that controls the virulence of the bacteria and as biofilm formation is a critical step in the process, the mutant clearly fails to form stronger biofilm.

Result 2: Comparison of pyocyanin production in the wildtype strain versus the mutant RhIR strain



Result 2: As seen from the above graph, the pyocyanin production is attenuated in the RhIR mutant. As the pyocyanin operons *phzA-Z* are under the direct regulation of the quorum-sensing gene circuit of RhIR transcription factor, it can be deduced that RhIR mutant has a reduced virulence and thus becomes a very important drug target to combat the antibiotic resistance issue.

Results



Result 3: As seen from the above graph, the RhIR mutant strain shows a much-reduced autolysis than compared to the wild-type strain. This is a very interesting result as it supports our hypothesis that the production of pyocyanin formation triggers the autolysis of some of the *Pseudomona*'s own cells releasing various molecules like eDNA and eventually contributing to the formation of stronger biofilms that we see in the wildtype strain. The fact that the pyocyanin production is reduced in the mutant strain along with the autolysis and biofilm formation serves as a preliminary evidence to the relationship between pyocyanin production and biofilm formation.

Conclusion / Future Directions

- RhIR mutants have much attenuated virulence compared to the wild-type strain and various genes that are under the control of RhIR can be evaluated as potential drug targets
- Future experiments will include antibiotic testing on the wild type and the mutant strains; gene expression analysis of the wild type and the mutant strains to identify the differentially expressed genes in the QS circuit of the RhIR transcription factor

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