Eutrophication’s Impact on the Virulence of *Pseudomonas aeruginosa*

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Objective

Our project aims to evaluate the impacts of eutrophication and cyanobacterial blooms on the virulence of *Pseudomonas aeruginosa*. *P. aeruginosa* is a common Gram negative opportunistic pathogen found in nature. We tested five *P. aeruginosa* genes from the PAO1 strain under various states of eutrophic water conditions from different sites (Indian Brook Reservoir, Shelbourne Pond, and tap water). Transcriptional reporter constructs for five *P. aeruginosa* genes (tssA1, exsD, exoT, plcH, and lasB) were used to assess the impact of environmental perturbations on *P. aeruginosa* virulence. When published, our result will improve our understanding of human impacts on opportunistic pathogen using *P. aeruginosa* as a model. In addition, by understanding the behavior of *P. aeruginosa* in the aquatic environment, we can make inferences about the behaviors of other similar opportunistic pathogens.

Materials

• The strain being used was PAO1.
• Cultures of *P. aeruginosa* were grown overnight in MOPS [3-(N-morpholino) propane-sulfonic acid] medium and were incubated the optimal temperature of 37 °C, with glucose, pyruvate, and gentamycin added for nutrient sources and plasmid maintenance, respectively.

Methods

• We collected water samples at various states of eutrophic sites including Indian Brook Reservoir and Shelbourne Pond. The water samples were filtered through the Thermo Scientific Nalgene analytical test filter funnel (Figure B).
• Experiments were performed using reporter constructs for five genes. Initially, our goal was to verify induction of the transcriptional activators and genes under known conditions (Table 1). We did not run a positive control for the lasB, as it can be autoinduced in this strain upon reaching a moderate growth level.
• The Miller assay was conducted to measure the level of gene expression reported by the transcriptional reporters in Miller Units (Figure C).
• The expression of the transcriptional reporters was recorded according to the degree of the yellow color expressed, with the darker yellow color indicating higher enzymatic activity and vice versa (Figure D).
• DNA microarray test was conducted to further identify the specific relationship between genes under the various eutrophic freshwaters and tap water.

![Figure A. Example of *P. aeruginosa* gene.](image)

![Figure B. The Thermo Scientific Nalgene analytical test filter funnel.](image)

![Figure C. Mathematical formula for calculating one Miller Units.](image)

![Figure D. Example of the Miller assay with the degree of yellow color expressed.](image)

![Figure E1-E4.](image)

- The verification of the induction of transcriptional genes under their known inducing conditions.
- Significant statistical differences between inducing conditions was identified for tssA1.
- Gene expression differences among inducing conditions for exsD, exoT, and plcH were not statistically significant.

![Figure F1.](image)
- The differences among the means in lasB were not significant. The fold increases were static.

![Figure F2.](image)
- Gene expression differences in Negative vs. Tap and Indian Brook Reservoir water, Shelbourne Pond vs. Tap and Indian Brook Reservoir water were statistically significant; in Negative vs. Shelbourne Pond, Tap water vs. Indian Brook water were not statistically significant.

Conclusions

Our findings suggest that the *P. aeruginosa* genes responsible for the virulence via the general secretion apparatus and secretion systems had demonstrated non-significant level of gene expressions under various states of eutrophic water conditions compared to their known inducing conditions. We concluded that eutrophication does not increase the virulence of *P. aeruginosa*.

Acknowledgements

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Table 1. A list of the known conditions for the *P. aeruginosa* genes.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Inducer</th>
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<tbody>
<tr>
<td>plcH</td>
<td>Ciaolae</td>
</tr>
<tr>
<td>tssA1</td>
<td>Pig mucin</td>
</tr>
<tr>
<td>exsD</td>
<td>EGTA</td>
</tr>
<tr>
<td>exoT</td>
<td>EGTA</td>
</tr>
<tr>
<td>lasB</td>
<td>Noae</td>
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</tbody>
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Miller Units = \(1000 \times \frac{(A_{420} \times 1.75 \times \text{Abs})}{(\text{Abs} \times \text{OD}_{600})}\)

\(t\) = time of reaction in minutes

\(v\) = volume of culture used in reaction in mL

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Figure C. Mathematical formula for calculating one Miller Unit.

Table 2. A list of genes for nitrous oxide reductase in *P. aeruginosa* identified in the DNA microarray analysis. In tap water vs. freshwater, more genes expressions observed in tap water. Furthermore, some genes demonstrated copper ion resistance.

<table>
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<tr>
<th>Genes Number</th>
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<tbody>
<tr>
<td>P49312</td>
<td>Protein NsrF</td>
</tr>
<tr>
<td>P49324</td>
<td>Protein NsrL</td>
</tr>
<tr>
<td>P49325</td>
<td>Protein NsrD</td>
</tr>
<tr>
<td>P49317</td>
<td>Protein NsrC</td>
</tr>
<tr>
<td>P49318</td>
<td>Protein NrsH</td>
</tr>
<tr>
<td>P49319</td>
<td>Nitric oxide reductase, NsrS</td>
</tr>
<tr>
<td>P49335, pnsC</td>
<td>Nitric oxide reductase, pnsC</td>
</tr>
<tr>
<td>P49335, pnsB</td>
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</tr>
<tr>
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<tr>
<td>P49339, pnsD</td>
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