The Effects of C₆-HSL and Pyocyanin on *P. aureofaciens* Quorum Sensing and CFBE41o⁻ Inflammation Response

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**Abstract**
We investigated the role of the autoinducer C₆-HSL in the beginnings of bacterial Quorum Sensing (QS) and biofilm formation. QS is the density dependent response of bacterial colonies which triggers virulent behavior. C₆-HSL is the trigger molecule which prompts QS activity in bacteria such as *P. aeruginosa*, a type of bacteria which can lead to respiratory failure in Cystic Fibrosis patients. The bacterial QS response and biofilm formation was measured qualitatively using light microscopy following a modified staining protocol documented by Allison and Sutherland. We also examined the effects of C₆-HSL and other molecules produced by *P. aeruginosa* and their inflammatory effects on a Cystic Fibrosis (CF) patient cell line, which was determined through NF-kB translocation staining. The data is inconclusive in the case of C₆-HSL's effect on the CF cells, however it tentatively suggests that C₆-HSL may act as an inhibitor to biofilm growth in *P. aureofaciens*.

**Quorum Sensing Auto-induction in Gram-Negative Bacteria**
The diagram shows how QS acts in a density dependent manner as a result of the positive feedback loop.

**Biofilm Growth**
Bacteria were grown for four days directly on sterile coverslips in the supplemented medium described above at various concentrations of C₆-HSL. The coverslips were coated in fibronectin solution. Three tests were run. Below are fields of view from some of the plates from the third experiment. The blue images are the bacterial cells, while the orange outlines the biofilm growth.

**CF Inflammation Response**
The cells were plated at 20,000 cells per well on coverslips coated with fibronectin solution, and cultured for 24 hours before PYO and C₆-HSL exposure. Three trials were run. Below are images from the second trial. The nuclei of all cells were stained blue with Hoechst and the NF-kB was stained red with DyLight above the blue Hoechst signals.

**Cystic Fibrosis**
CF is a hereditary disease which causes complications in the respiratory and digestive systems. It is caused by a recessive mutation of the CFTR gene on chromosome 7. This leads to unusually thick mucus, which obstructs bronchi, bronchioles, and bile ducts. CF is considered the most common inherited lethal disease in those of European descent, with one in every 2000 Caucasians affected. Over 70% of CF patients die before their 30th birthday, usually due to infection associated complications. *P. aeruginosa* infections are the leading cause of death in CF patients, causing respiratory failure and death.

**Results**
The imaging and analysis performed on the *P. aureofaciens* cultures left purely qualitative results. The cultures grew well, however even with fibronectin coating, they did not attach very well to the coverslips. The results concerning C₆-HSL’s QS inhibitory effects on *P. aureofaciens* suggest that C₆-HSL inhibits biofilm growth as suspected. This trend was mirrored in each of our three trials, and while still inconclusive, this trend provides initial support to our hypothesis. The data set obtained is not large enough to show statistically significant trends.

**Conclusion and Future Aims**
-- If future experimentation does not support the idea of C₆-HSL as a QS and biofilm inhibitor, then colony density is not as important for biofilm formation as originally thought. If C₆-HSL is found to be a promoter of biofilm growth, it suggests that the biofilms are more dependent on time grown after QS begins than the conditions of that growth.
-- The time range we used, fixing cells after two days of experimental exposure was shown to be the community standard, but could be a bit excessive if pyocyanin does not cause the up-regulation of genes which catalyze an inflammation response. In future experimentation, we will attempt to examine NFkB translocation after shorter times, such as 30 minutes, in addition to continued testing at 2 days of exposure.