DETECTION OF PSEUDOMONAS AERUGINOSA CONCENTRATION VIA SYNTHETIC BIOSENSOR FOR QUORUM SENSING AUTOINDUCERS

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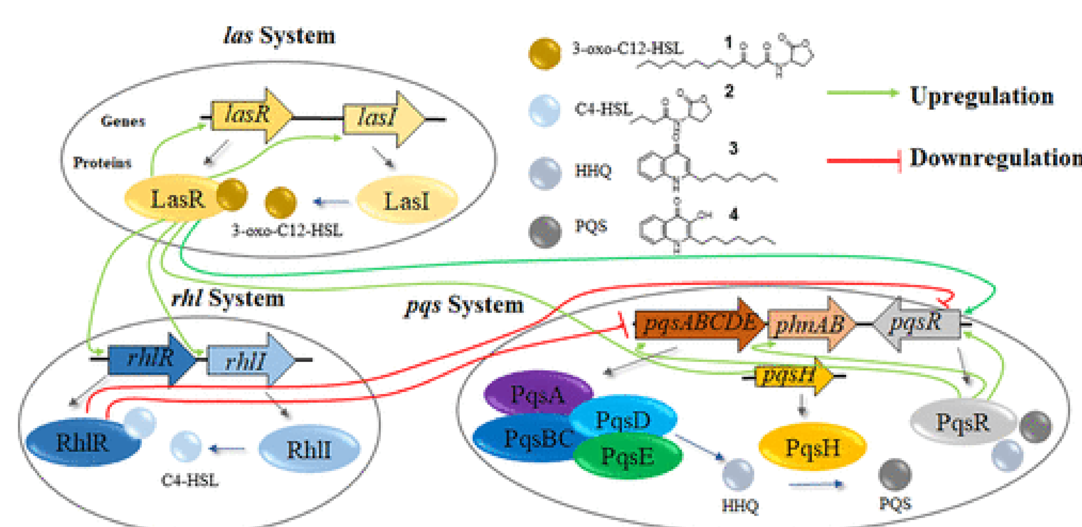
INTRODUCTION

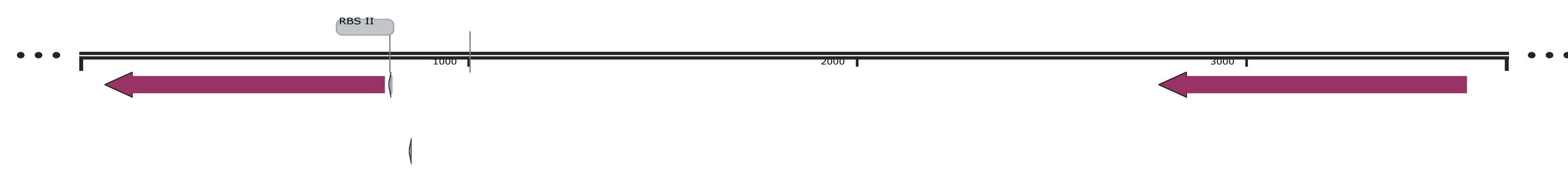
Pseudomonas aeruginosa is an opportunistic pathogen commonly found across various environments and hosts **(1, 2)**. *P. aeruginosa* prevalence (32,000 infections in hospitalized patients, 2,700 deaths in 2017 **(3)**) presents a severe threat with multiple drug resistance (MDR) **(1)**. Contributing to MDR, *P. aeruginosa* coordinates biofilm formation through quorum sensing (QS), in which diffusible molecules are secreted and sensed to detect local population density **(2,4)**. This work focuses on detecting *P. aeruginosa* QS signaling molecules to inform infection diagnosis and treatment. Specifically, we detect N-(3-Oxydodecanoyl)-L-homoserine lactone (3OC12HSL) from the *las* QS pathway, which coordinates virulence. A portion of the *P. aeruginosa* pathway can be seen in the figure below **(5)**.



3OC12HSL binds the protein LasR, forming a complex that activates Las QS-controlled promoters. We employ a synthetic plasmid that codes for the expression of LasR from a constitutive promoter and the expression of a colorimetric (blue) reporter, amilCP, from a QS-controlled promoter. The biosensor host is nonpathogenic *Escherichia coli*, offering a sensitive, specific, and low-cost detection method with visual readouts interpretable without additional equipment.

METHODS

Transformation



- 3uL of GA10-10 amilCP vector (seen above) was added to the DH5a *E. coli* cells and incubated on ice for 20 minutes.
- Cells were then heat shocked at 42°C for 30 seconds. The cells were then placed back on the ice for 2 minutes.
- 400uL of SOC broth was added, and the solution was transferred to a 14mL tube and then placed in a 37°C incubator for 1 hour.
- Cells were then plated on LB + kanamycin agar and grown overnight.
- For later testing, competent cells were frozen in a 30% glycerol stock solution.

Testing

- **Colorimetric Testing:**
  - Transformed DH5α+amilCP *E.coli* cells were then cultured in LB+kanamycin for 18 hours.
  - Conical tubes (n=12) were prepared as follows: 4mL with 10uM exogenous 3OC12HSL added to tube 1; 3mL with 1 mL serially diluted aliquots added to tubes 2-12.
  - Tubes were plated in 48-well plates, placed in a 37°C plate reader, and read at 588nm, 600nm, and 700nm for 24 hours.
  - In the presence of 3OC12HSL, DH5α+amilCP *E. coli* cells turn blue, providing easy visual readout without equipment.
- **Media Testing:**
  - A *P. aeruginosa* colony was inoculated in 3mL of PBS and vortexed.
  - Seven different culture types were prepared: 25-5% LB + 75-95% artificial wound fluid exudate (AWFE), 3mL each.
  - 300µL of *P. aeruginosa* in PBS was mixed into each solution.
  - 200µL of each solution was added to a 96-well plate in four replicates. The plate was read every 30 minutes at 600nm, 37°C 200RPM for 24 hours.
  - After 24 hours, a Crystal Violet assay was performed to measure biofilm formation at 590nm.

Sol-gel Preparation

- 5mL of 0.4M Sodium Silicate (SiNa) and 5mL of 6.5M of LUDOX were added to a 250mL beaker and mixed at 200 RPM for 30 seconds.
- 5mL of overnight DH5α+amilCP *E.coli* and 10mL of Phosphate buffer solution (PBS) were mixed for 1 minute in the beaker.
- Initial pH was measured (around 10.8), and 1M HCl was added to the solution until pH was between 7.0 and 7.3.
- 1mL aliquots were placed in a 24-well plate and allowed to gel.
- Gelation of solution occurs within 12 minutes of the addition of HCL.

RESULTS

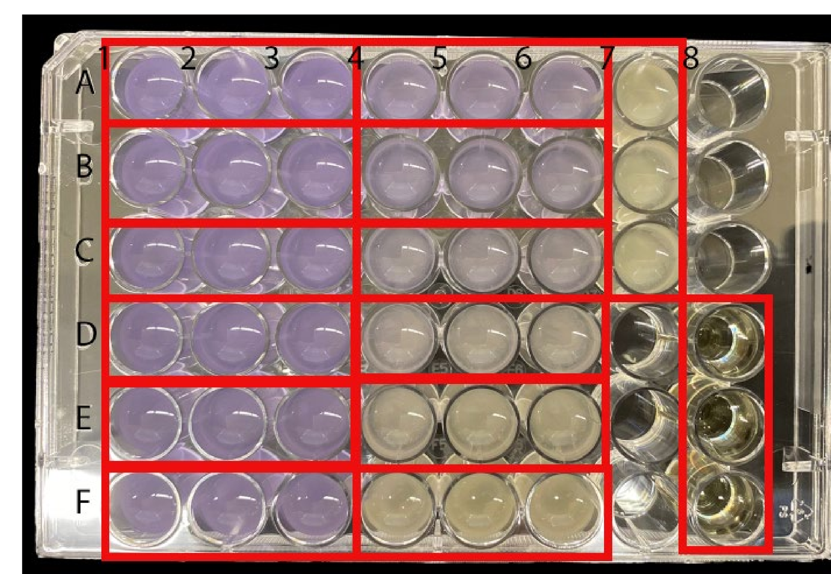


Figure 1: Detection of 3OC12HSL. Blocks indicate different concentrations of exogenous 3OC12HSL with dilutions starting at A1:A3 and ending at F4:F6. Bacterial control is seen at A7:C7 and media control at D8:F8.

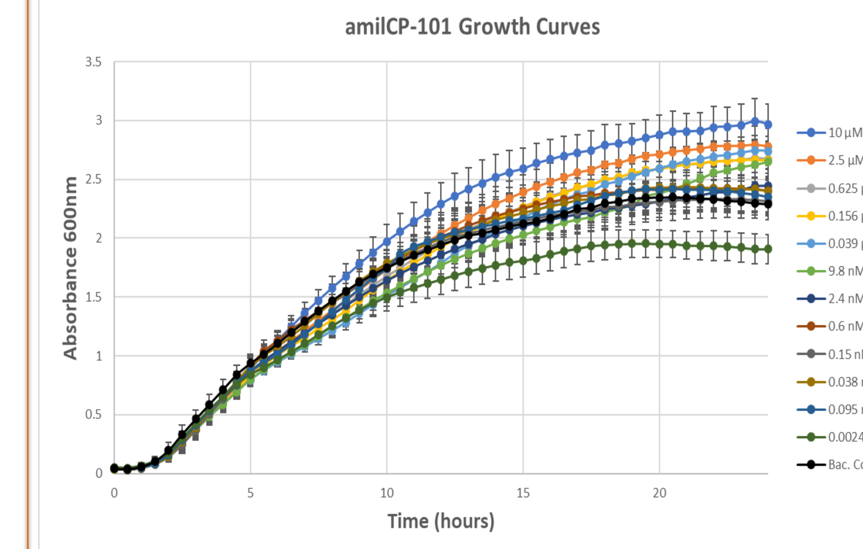


Figure 2a: Biosensor growth curves when exposed to varying concentrations of exogenous 3OC12HSL.

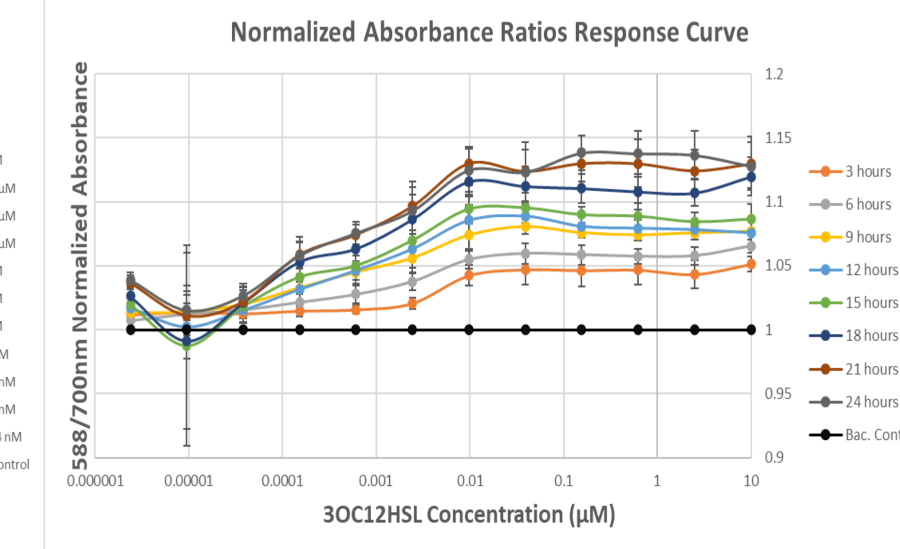


Figure 2b: Response curve showing the concentrations of exogenous 3OC12HSL normalized to bacterial control over time.

Figure 3: Encapsulation of amilCP biosensor. The reaction of SiNa and LUDOX with HCl allows for room-temperature encapsulation of bacteria.

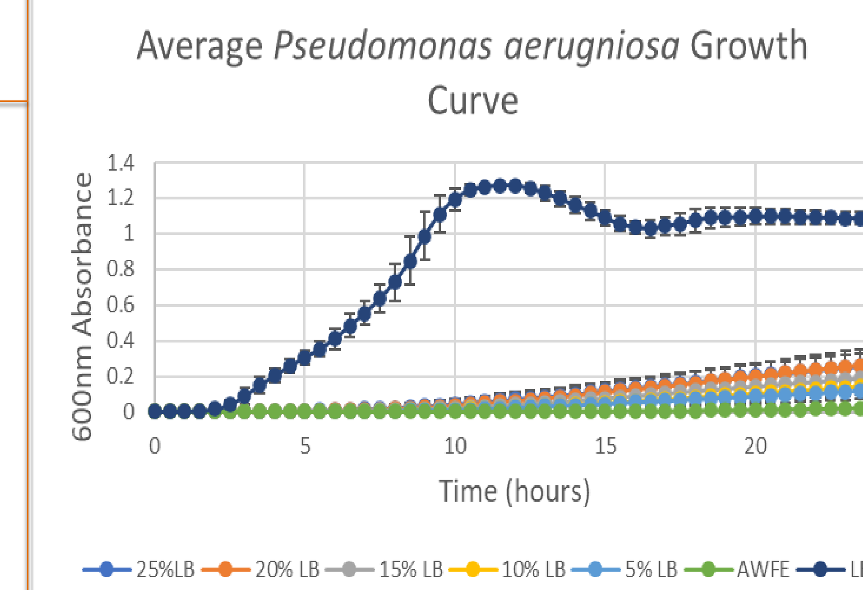


Figure 4a: *P. aeruginosa* growth curves based on varying LB concentrations.

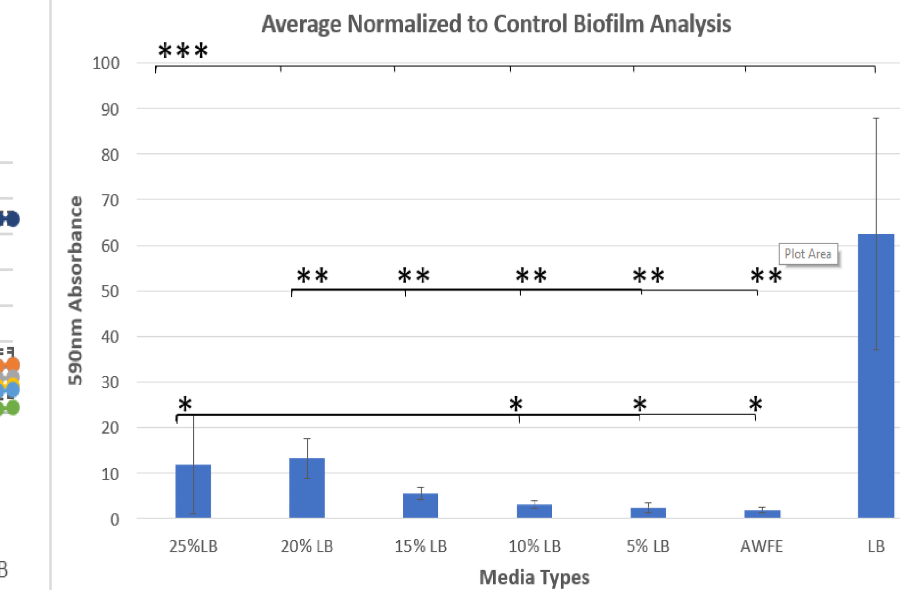


Figure 4b: Biofilm analysis of different media concentrations. P=0.05 for all statistical tests.

$$\#\ of\ molecules * \frac{1}{6.022 * 10^{23}} = 1.66 * 10^{-24} moles$$

$$(X) * 1.66 * 10^{-24} moles * \frac{1}{6 * 10^{16} L} = (X) * 2.77 * 10^{-9} M$$

$$0.000152588 \mu M * \frac{1 * 10^{-6} M}{1 \mu M} = 1.52588 * 10^{-10} M$$

$$(X) * 2.77 * 10^{-9} M = 1.52588 * 10^{-10} M$$

$$X = 0.055\ molecules/cell$$

Equation 1: Equation used to determine the number of 3OC12HSL molecules per biosensor cell at 0.15nM concentration.

DISCUSSION AND CONCLUSION

- DH5α+amilCP *E. coli* cells could detect exogenous 3OC12HSL in concentrations as low as 0.15nM.
- Growth curves and response curves can be seen in **Figures 2a** and **2b**.
  - 3OC12HSL may have some effect on biosensor growth.
  - We can see the correlation between **Figures 1 & 2.**
- Calculations in **Equation 1** indicate that our biosensor can detect as low as 0.055 molecules of 3OC12HSL/cell.
- DH5α amilCP *E. coli* could be encapsulated in sol-gel, as seen in **Figure 3**.
- *P. aeruginosa* growth and biofilm development depend on the LB concentration in liquid media (**Fig. 4a & Fig. 4b**).

FUTURE DIRECTION

- Usage of mass spectrometry may allow us to quantify the amount of 3OC12HSL molecules present in relation to bacterial density.
- Cell viability testing and biosensor validation within the sol-gel solution will allow us to determine if sol-gel is a valid method of detecting 3OC12HSL molecules.
- Testing the biosensor against polymicrobial communities will further validate its ability to detect 3OC12HSL molecules.

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