Quorum Sensing and Biofilm Production: Comparing the Efficiencies of AI-1 and AI-2

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Motivation

- Quorum Sensing (QS) is a paracrine signaling mechanism that allows colonies of bacteria to synchronize cooperative behaviors in response to fluctuations in population density.
- During QS, chemical signaling molecules known as autoinducers (AIs) are synthesized and released by bacteria, where they accumulate extracellularly in accordance with increasing population density.
- Upon reaching a threshold concentration, AI molecules bind to bacteria via specialized receptors; indicating that enough cells are present to perform cooperative behaviors. Consequently, the binding of AIs triggers alterations in gene expression throughout the entire population.

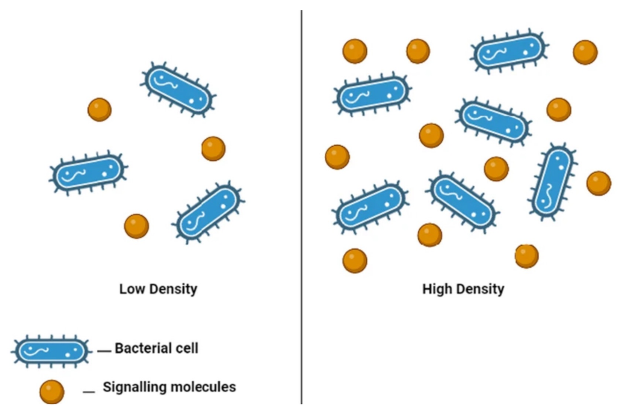

Fig. 1 Cell population density in relation to AI concentration (Boban, 2009).

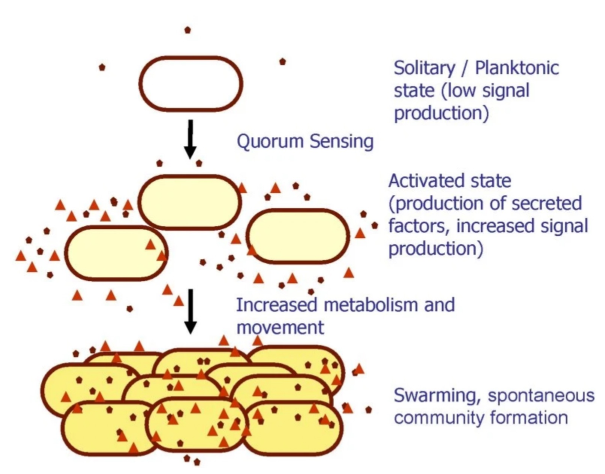

Fig. 2 Visualizing the role of QS in survival efficiency (Netotea, 2009).

Hypothesis

Our study design was driven by our desire to compare the functionality of AI-1 and AI-2. Due to the complexity of topics within the realm of Quorum Sensing, we decided to focus on biofilm formation as our singular application of study, with the quality and stability of biofilms serving as quantifiable variables. We hypothesized that biofilm formation would prove to be most efficient in AI-2 as opposed to AI-1, as AI-2 primarily relies on complex proteins to carry QS signals in and out of cells, reducing the prevalence of transcription errors.

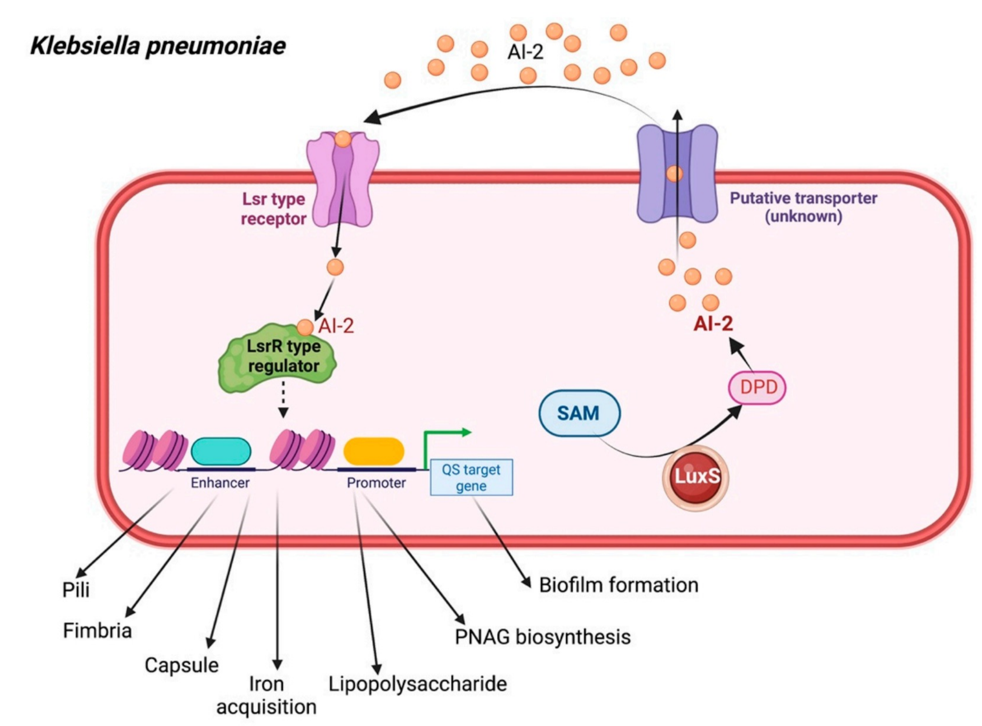

Fig. 4 AI-2 dependent QS circuit in *K. pneumoniae* (Santajit, 2022).

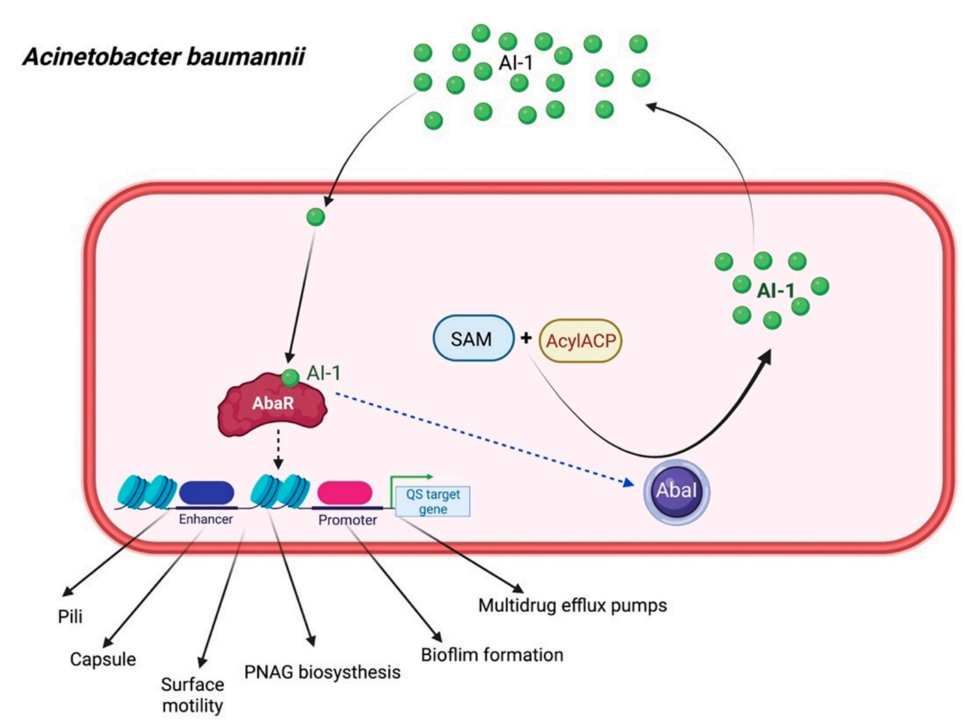

Fig. 5 AI-1 dependent QS circuit in *A. baumannii* (Santajit, 2022).

Background

- Communal gene expression and behavior coordinated by signal relay mechanisms and target genes, which are controlled by QS communication systems.
- While virtually all bacteria perform Quorum Sensing, three primary classifications have arisen to account for systematic differences between species: autoinducer-1 (AI-1), autoinducer-2 (AI-2), and autoinducer peptide (AI) pathways.
- While AI-1 and AI-2 depend on N-acyl homoserine lactone (AHL) molecules in cell-cell communication, AIP utilizes modified oligopeptides— AI-1 is most common in Gram-negative bacteria while Gram-positive bacteria often rely on AIP, but AI-2 has been recognized in both classes.
- In bacteria, QS and biofilm formation are closely interconnected social behaviors that allow both benign and pathogenic bacteria to efficiently colonize and survive within a host organism.
- As bacteria population grows, cells secrete and detect chemical signals to coordinate functioning and form a biofilm, then "attack" the host when large enough.
- Structural and functional differences between Gram-positive and Gram-negative bacteria in relation to QS raise the question of how the AI-1 and AI-2 pathways influence the efficiency of biofilm formation

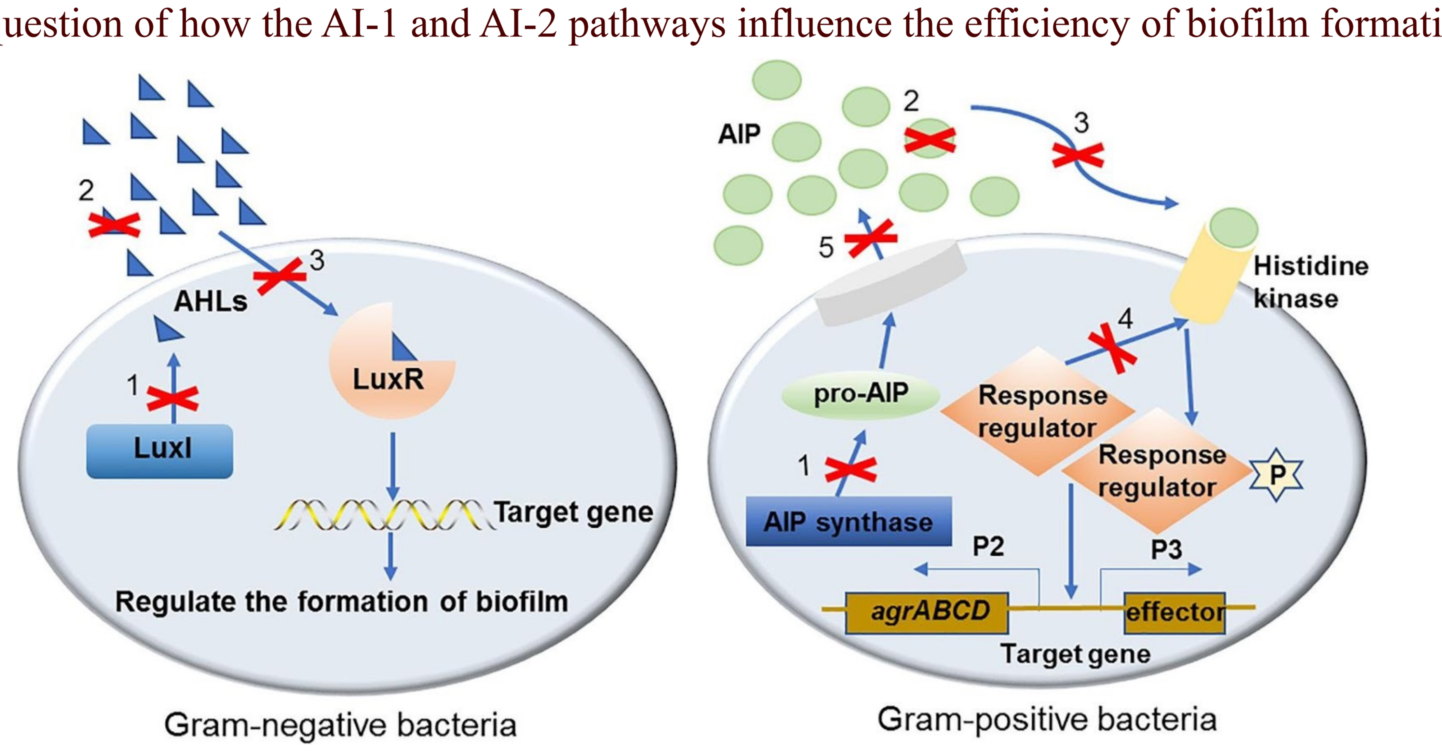

Fig. 3 Functionality of QS systems in biofilm formation for Gram-negative and Gram-positive species (Zhou, 2020).

Methods

Since little previous research has focused on comparing the efficiencies of AI-1 and AI-2 via biofilm formation, our project's design emphasizes the importance of collecting data from a multi-directional perspective:

- By analyzing the role of signaling molecules, such as acyl-homoserine lactones (AHLs) and peptides, we hope to gain further insight into understanding the basis of microbial communication in QS. Techniques such as LC-MS and HPLC allow for the precise identification and quantification of these molecules, offering insights into quorum sensing dynamics."
- Conducting genetic and transcriptomic analyses, both with and without genetic manipulation, assists in identifying target genes essential to proper QS functioning, while simultaneously unraveling individual contributions to overall efficiency.
- Additionally, live cell imaging techniques, such as fluorescence microscopy and bioluminescence assays, provide real-time visualization of quorum sensing dynamics within bacterial populations— offering a dynamic perspective on how colonies coordinate behavior to maximize survival efficiency under environmental stressors.
- Integration of computational modeling further aids in predicting QS outcomes and patterns under various scenarios, contributing to hypothesis formulation and experimental validation.
- Overall, a multifaceted approach combining molecular, and imaging techniques, complemented by computational tools, is crucial for a comprehensive understanding of bacterial quorum sensing, benefiting fields from microbiology to biotechnology and environmental science.

Discussion

While formulating experimental model and pinpointing a topic of study, our team kept these aspects in mind:

- What areas within bacterial Quorum Sensing research do you believe warrant further exploration or innovation, and why?
- How do you think the findings and insights gained from studying bacterial quorum sensing could be translated into practical solutions for issues in healthcare, agriculture, or environmental conservation?
- What ethical considerations should researchers keep in mind when conducting experiments involving genetic manipulation of bacterial quorum sensing components?

Future Directions

- While our research is ongoing and has not yet reached a finalized result, we have made significant progress towards our end goal of constructing and optimizing mathematical models to simulate Quorum Sensing dynamics within bacterial populations.
- Additionally, we are in the process of investigating and developing code to implement these models in Cello, enhancing our ability to engineer synthetic genetic circuits capable of Quorum Sensing.
- Moving forward, our primary focus will shift to refining these mathematical models, confirming their predictive accuracy through experimental validation, and exploring novel strategies to further quantify and compare Quorum Sensing efficiency among AI-1 and AI-2 with an emphasis on biofilm formation.
- By continuing to advance our understanding and manipulation of quorum sensing mechanisms, we aim to contribute to the development of innovative approaches for controlling bacterial behavior and combating infectious diseases.

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