# Eavesdropping on the Microbial World: The Search for Quorum Sensing Molecules

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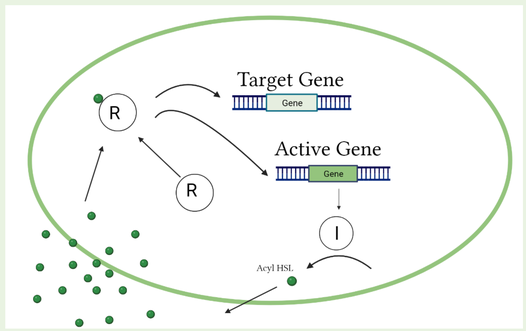
## Abstract:

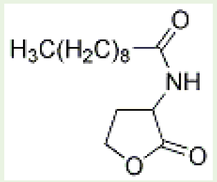
Nitrogen-fixing bacteria form symbiotic relations with legume plants and play a key role in crop health and productivity. Soybeans are an essential cash crop we must protect from drought. Our lab is exploring the development of drought-tolerant strains of *Bradyrhizobium japonicum.* Although strains of *B. japonicum* are already being produced, it is often outcompeted by non-drought-tolerant strains. It is believed that cell-cell communication plays a role in competitiveness. *B. japonicum* communicate with each other by what's known as quorum sensing. To improve competitiveness, we are dictating the produced strains to identify which molecules play a role in competition and developing strategies to manipulate the quorum sensing process.

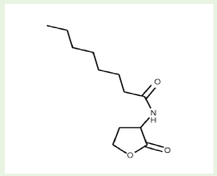
## What is Quorum Sensing?

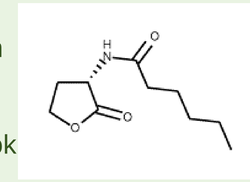
Quorum sensing is essentially the way that bacteria talk and communicate with one another. Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density.
Quorum-sensing bacteria produce chemical signaling molecules called autoinducers that increase in concentration as a function of cell density. Change in gene expression is caused by the presence of a minimal threshold concentration of an autoinducer.

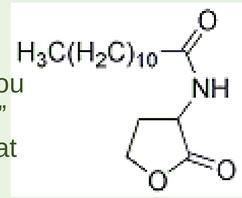
The diagram we created below shows how quorum sensing works: in this case, the bacterium uses quorum sensing to target a particular gene.





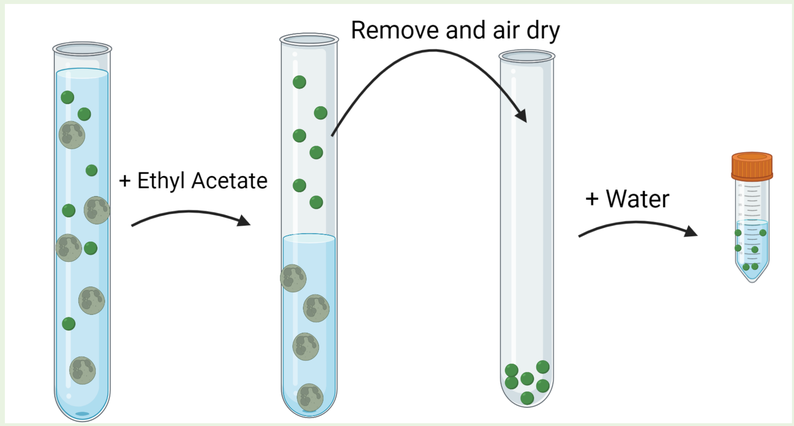



## Detect:

We use a common substrate known as “x-gal” to detect enzymatic activity of the target gene. If it yields a dark blue precipitate HSLs are present. Take a look at the test tubes to the right, the blue pigment indicates that all the strains produce HSLs, and we can begin purifying. The four molecule structures you see here are what we call our “standard.” We essentially compare our results to that of the standard (size, molecules, color, etc.) in hopes of a match.

## Purify:

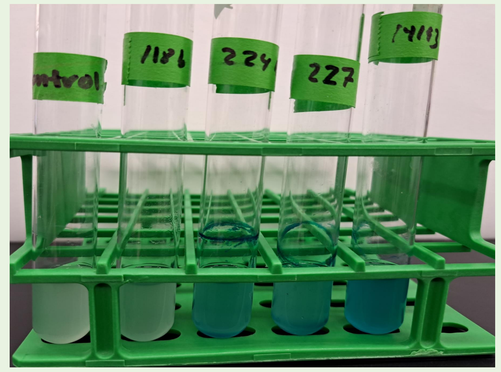
To purify our strains, we add about 1 mL of ethyl acetate to 1 mL of culture and then centrifuge the 2 mL tubes to separate the culture strains and ethyl acetate. We then pipette the ethyl acetate top layer out of the tube so only the culture solution is left. The tubes are then centrifuged with the lids open to evaporate the remaining liquid and leave only the dry HSLs at the bottom. A few microliters of distilled water is added through a pipette to "rinse" the side of the tube and collect all the HSLs, leaving a purified sample.




## Identify:

We characterize the molecules within the purified solution by performing a TLC separation. We marked four equally spaced lines on the plate and then added a drop of each strain on its respective line. In the process, the quorum sensing molecules move up through the TLC plate depending on weight. When the TLC separation is complete and the plate is dry, agar that contains the indicator strain and X-gal is poured over the plate. This is to identify the quorum sensing molecules in each strain It is then set to incubate for several days The spots containing quorum sensing molecules will appear as blue on the TLC plate.

1.



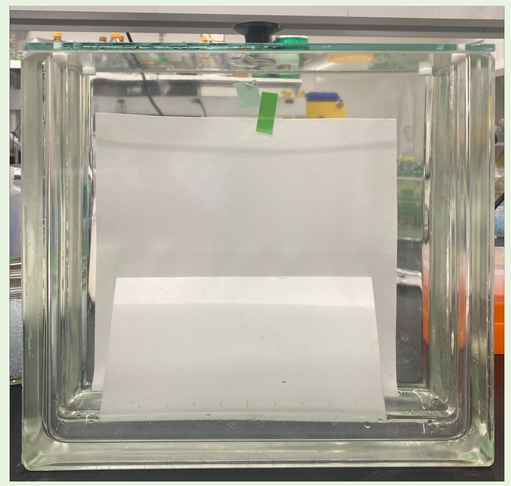

1. Picture of the test run with small bacterial cultures, each labeled with the strain.

2.

2. Results of the TLC with the blue pigment!

3. TLC Plate process that was used to separate molecules. This time, we used a glass plate and Ethyl Acetate.

3.



This project would not have been possible without the help of Dr. David J. Westenberg, Gabrielle Hightower, The MST Biological Sciences Department, and FYRE Program.