Microbial Matrix: Genetic and Chemical Aspects of Cell-Cell Communication

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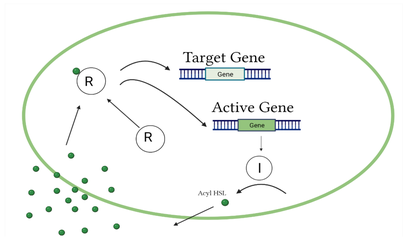
ABSTRACT

This research project focuses on looking at different quorum-sensing positive strains in *Bradyrhizobium japonicum*, a species of legume-root bacteria found in soybean plants across the Midwest. Soybean plants are a high-yield crop produced here in the Midwest, bringing in more than 8 billion dollars in monetary output for our state, making it Missouri's economic engine. Our objective is to use different chemical testing methods to distinguish genetic difference between negative strains. This can be done through polymerase chain reaction, gel electrophoresis, and gene editing. Our goal has been to PCR amplify a fine-tuned promoter, then clone it into the gene. In previous studies, drought has been shown to reduce nitrogen fixation in plants. When bacteria infect these plants, they leave behind nodules containing different chemical compounds, and so by studying these strains grown in Missouri and learning what we can about the existing bacteria, we could potentially create a drought-resistant bacteria strain and focus on the genetics and competition that go into each compound make-up.

BACKGROUND

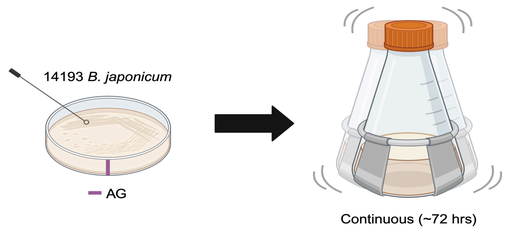
B. japonicum is a species of nitrogen-fixing bacteria, commonly found in soybean plant roots, that has drastically crop productivity. Bacterial cells of this species perform quorum sensing, a manner of communicating with one another via signal molecules in order to share information and adjust gene regulation accordingly. Previous research identified some strains able to produce multiple classic acyl-homoserine lactone (AHL) signal molecules, using *Agrobacterium tumefaciens* as a bacterial indicator. The chemical aspect of this project focuses on conducting a microbial time-growth analysis on AHL production via thin-layer chromatography spot test. It is to determine a possible trend between the quantity of AHLs produced by the *A. tumefaciens* bacterium versus different time points of its growth. On the other hand, we have also discovered that some strains do not synthesize a chemical signal, despite having the genetic capabilities to do so. With that said, the genetic aspect of the study aims to (1) determine if said strains produce a modified signal molecule and (2) test to see if different types of quorum sensing affects the competitiveness of different strains.



METHODOLOGY *(Chemical Aspect)*

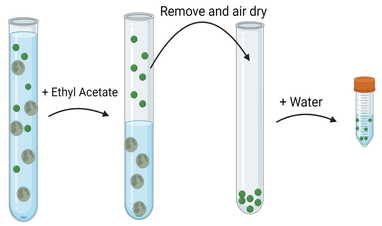
Bacterial Culture Preparation

The 14193 *B. japonicum* strain was grown on AG media. A culture of the strain was created in a shake flask and placed in a shaking incubator to promote continuous growth and prevent bacterial settlement.



AHL Extraction from Time-Growth Samples

A 5 mL sample of the culture was collected every 8 hours for 72 hours. 10 total time-growth samples (including an initial collection before being placed in the shaking incubator) were collected. 900 µL of ethyl acetate (ETAC) was added to 900 µL of each sample in separate 2 mL tubes. Said tubes were centrifuged afterwards. Once the mixtures settle, the top layer (containing the AHLs) of each sample was extracted into separate 1.5 mL tubes and dried.

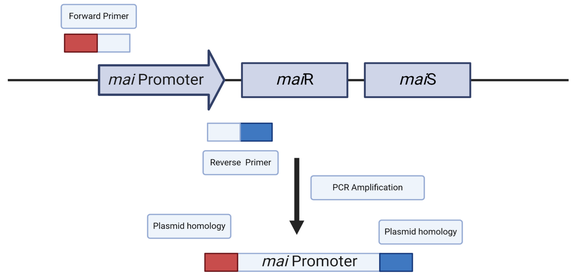


Thin-layer Chromatography Spot Test

Thin-Layer Chromatography (or TLC), is a commonly used method to separate molecules and compounds, based on polarity and size. In our case, we used TLC to separate the molecules found within the bacteria strain, and characterize each one. This way, we can use a substrate known as "x-gal" to yield a dark blue precipitate at the site of the enzymatic activity. We use the C8 AHL as a control, or a size comparison, and as shown on the white chromatography paper with blue spots, different size molecules were found and identified.

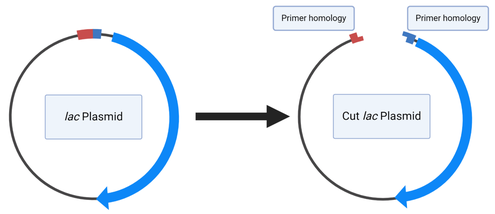
METHODOLOGY *(Genetic Aspect)*

Polymerase Chain Reaction



We've designed PCR primers for the promoter for the R gene which we predict will respond to the modified HSL.

Plasmid Preparation



The lux plasmid is an alternative to using the lac gene as an indicator but we will focus on the lac plasmid for this project.

Sub Cloning Genes into Plasmid Reactors

we cloned our promoter into our lac gene plasmid using homologous recombination – a technique called Gibson Assembly. If our hypothesis is correct, promoter will get turned on by the Mai the cells will turn blue in the presence of X-gal.

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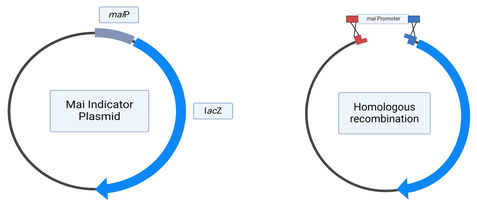


RESULTS/PROGRESS

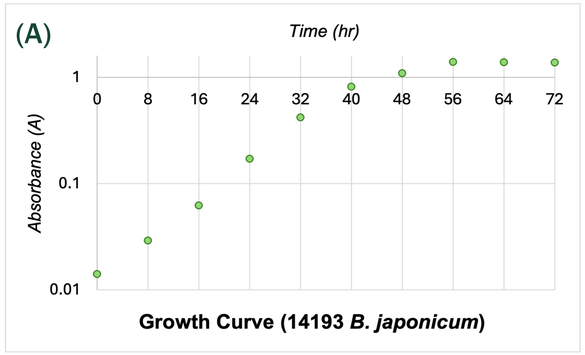
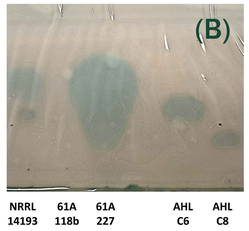
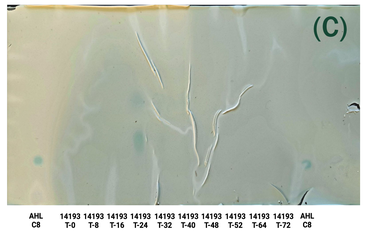
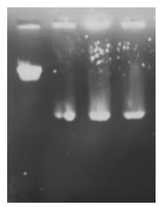
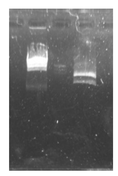


Figure B shows that the 14193 and 227 strains of *B. japonicum* are able to produce different AHLs. We grew the 14193 strain and showed that said strain starts producing AHLs at 24 hours of growth, as seen on Figure A and C.









For PCR we used 110 D as our template and obtained a positive PCR product of the expected size. Therefore, we can confirm that the primers used were correct. We now have a PCR product containing the R gene promoter to clone next to the lacZ indicator gene (Figures D&E).

Both plasmids were cut with restriction enzymes to facilitate cloning the PCR product into the plasmid. The DNA extraction was done through a kit, containing various buffers. By centrifuging and the suspended liquid and using fiberglass to filter out precipitate, we were left with just the raw DNA (Figure E).

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