

## Abstract

Quorum sensing is the process by which a variety of bacterial species communicates, coordinate their behavior, and express their genes based on cell density. There is an evolutionary benefit to quorum sensing. It allows bacterial populations to respond to different environments, especially as a response to biofouling and host-pathogen interaction. Also, it is essential to the production of biofilms. It has been shown that several terrestrial plants interfere with the quorum sensing signal to control bacterial colonization and biofilm formation. The aim of this project is to survey Louisiana's native plants for the production of quorum sensing inhibitors. Over the course of two semesters, we collected a variety of plants along the bayou at the University of Louisiana at Monroe and from the University's local greenhouse. During plant analysis, whole leaf, hole-punched leaf, and/or macerated leaf is placed on a LB agar plate, and overlaid with a culture inoculated with *Pseudomonas aerofaciens*. If culture pigmentation is reduced or nonexistent near sample, then quorum sensing inhibition is indicated. Likewise, inhibition of culture growth near sample may indicate possible antibiotic production. The two specimens named image 164 (unidentified at this time) and 221 (*Clemantis fpp*) resulted in potential quorum sensing inhibition and antibiotic production. In addition to the quorum sensing inhibition of *Ps. Aerofaciens*, image 164 (unidentified at this time) also proved to be an effective antimicrobial agent against *Bacillus subtilis*, *Serratia marcescens*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, and *Staptylococcus aureus*.

## Introduction

Diverse microorganisms can control specific processes in response to population density by Quorum sensing (QS). The knowledge of quorum sensing in bacteria can be of great benefit to the medical community as more and more bacteria are becoming resistant to many antibiotics. Bacterial resistance is normally associated with bacterial biofilms which are bacteria attached to surfaces and/or each other. Traditional antibiotics work to kill or inhibit growing bacteria while bacteria in a biofilm are more than one thousand times more resistant to the antibiotic because of their slow metabolism. The literature has shown that species interaction through quorum sensing signals help to increase biofilm diversity and density. One way to combat this new era of antibiotic resistant bacteria and the universal dilemma of biofouling is through quorum sensing inhibitors.

Bacterial cells communicate by sending chemical signals, called autoinducers, to one another to coordinate their actions. The cell signals are able to be deciphered by a diverse number of bacteria allowing bacterial mass to accumulate. This creates a biofilm, which is essential to the pathogenic bacterium's ability to invade and attack a host cell. Because quorum sensing is responsible for the bacterial accumulation into a biofilm, the medical community is searching for ways to inhibit quorum sensing by quorum sensing inhibitor (QSI). Several plants have been shown to inhibit biofilm formation and biofouling by secreting quorum sensing inhibitors. New studies are aimed at interfering with bacterial cell-to-cell signaling via the quorum-sensing (QS) pathway to inhibit bacterial virulence and/or the development of microbial biofilms. The goal of our research is to utilize native Louisiana plants to find quorum sensing inhibitors to reduce biofilm formation. Quorum sensing inhibitors (QSIs) hold great expectations in fighting bacterial infections.

## Results of Plant Testing

Plant	<i>Pseudomonas aerofaciens</i> Positive QSI	<i>Pseudomonas aerofaciens</i> Negative QSI	<i>Bacillus subtilis</i> QSI	<i>Serratia marcescens</i> QSI	<i>Bacillus cereus</i> QSI	<i>Micrococcus luteus</i> QSI	<i>Escherichia coli</i> QSI	<i>Staptylococcus aureus</i> QSI	<i>Agrobacterium tumefaciens</i> QSI
2:17	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2:19	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2:20	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2:21	Yes	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2:24	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
111	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
109	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
110	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
164	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
165	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
166	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
168	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
112	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
113	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
115	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A

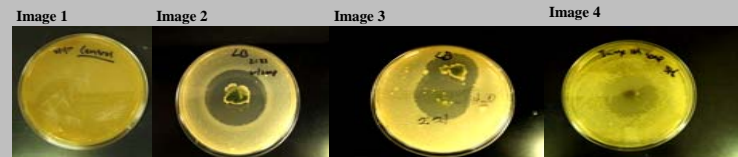


Image 1: Control plate of *Pseudomonas aerofaciens*

Image 2: macerated leaf from Plant 2:21 washed with soap and inoculated with *Pseudomonas aerofaciens*

Image 3: macerated leaf from Plant 2:21 washed with water and inoculated with *Pseudomonas aerofaciens*

Image 4: macerated leaf from Plant 164 washed with soap and inoculated with *Pseudomonas aerofaciens*

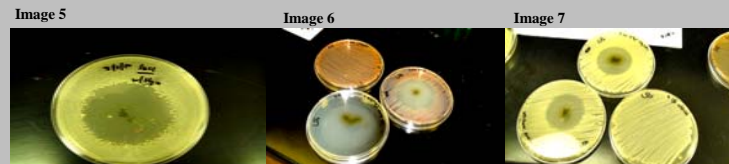


Image 5: Plant 164 macerated leaf washed with water and inoculated with *Pseudomonas aerofaciens*

Image 6: Control plate of *Serratia marcescens* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Serratia marcescens*

Image 7: Control plate of *Bacillus cereus* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Bacillus cereus*



Image 8: Control plate of *Agrobacterium tumefaciens* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Agrobacterium tumefaciens*

Image 9: Control plate of *Escherichia coli* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Escherichia coli*

Image 10: Control plate of *Staptylococcus aureus* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Staptylococcus aureus*

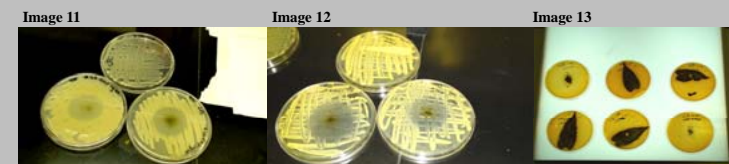


Image 11: Control plate of *Bacillus subtilis* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Bacillus subtilis*

Image 12: Control plate of *Micrococcus luteus* and macerated leaves of plant 164 washed with soap and water and placed on a LB agar plate of *Micrococcus luteus*

Image 13: Example of the entire screening assay carried out on a plant with *Pseudomonas aerofaciens* that showed no QSI or antimicrobial inhibition.

## Materials and Methods

### Plant Collection

Plant specimens were collected along the bayou at the University of Louisiana at Monroe and from the local greenhouse. Pictures were taken to identify the plants and they were placed in plastic bags in the refrigerator until use.

### Screening Assay

- Six (6) leaves from each plant were used for screening.
- Three (3) leaves were washed in deionized water and 3 leaves were washed in soapy water.
- We tested the leaves three different ways: using a whole leaf, a punctured leaf, and a leaf that had been macerated using a mortar and pestle.
- Each leaf treatment was placed on an individual LB agar plate.
- Soft agar overlay was melted and inoculated with 5 micro liters of an overnight culture of *Pseudomonas aerofaciens*.
- The overlay was then poured over every leaf and left to incubate overnight.
- The specimens were checked the next day for either loss of color or antimicrobial inhibition.

### Organisms Screened

The following organisms were test for quorum sensing inhibition and/or antimicrobial inhibition on LB agar: *Bacillus subtilis*, *Serratia marcescens*, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli*, *Staptylococcus aureus*, and *Agrobacterium tumefaciens*.

## Conclusion

As a whole, biofilm forming microorganisms have been able to survive millions of years. These organisms are involved in biofouling and infectious diseases that have cost millions of dollars to health care and industry. Current research focuses on finding compounds that may contain potential quorum sensing inhibitors (QSI). This study emphasizes the fact that there may be potential QSI compounds in our surrounding environment. With this simple protocol for screening plants, a large quantity of different specimens can simply be tested. Furthermore, specimens showing potential positive QSI can be investigated in more detail. The discovery of QSI compounds can aid in the control of microorganisms.

## Acknowledgments

We would like to take this opportunity to extend a thank you to all the people that helped make this research project a success and a special thank you to Dr. Dennis Bell for his plant identification expertise. This project was supported by the ULM Biology Department and the Howard Hughes Undergraduate Research Program.

## References

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