

Investigating the Potential of Endophytic Bacteria Found in the Seeds of *Sesbania Rostrata* for Promoting Plant Growth

Akshay Aribindi

School of Life Sciences
Hyderabad Central University Gowchibowli Hyderabad
*Corresponding author: aribindiakshay8@gmail.com

Abstract:

My investigation aimed to find out how the endophytic bacteria present in the seeds of a plant known as *Sesbania Rostrata* can benefit other plants by supplying them with essential nutrients. The goal of my investigation was to find such a bacteria that can benefit different types of plants in multiple ways by providing them with many of the essential nutrients that they need for survival. I conducted numerous biochemical tests on the bacteria that I isolated from the seeds of *Sesbania Rostrata* to find out all of the different biological nutrients they can supply plants with. These biochemical tests included the cellulase activity test, the zinc-solubilization test, the nitrogen fixation test, the phosphate-solubilization test, the phosphate-solubilization test, the protease activity test, the starch-hydrolysis test, the methyl red test, the Voges-Proskauer test, and the catalase test. In the end, I discovered that only one of my bacteria was positive for most of the tests and I learned that it would be the most beneficial. This bacteria was positive for all of the biochemical tests I conducted besides the Voges Proskauer test. I concluded that this bacteria would be the most beneficial because it can help plants grow by supplying them with the most amount of nutrients compared to the other bacteria that were found in the seeds of the plant that I used. As a result, the bacteria that I found in my experiment has potential to be used in agricultural applications including a biofertilizer as it offers a variety of benefits to plants.

Introduction:

Have you ever heard of seed endophytic bacteria that's found in the seeds of plants? They're essentially bacteria that live in the seeds of plants without causing disease. Seed endophytic bacteria live in the seeds of plants and they're believed to have a strong influence on the development of a plant. As a result, such bacteria can be used in biofertilizers to help plants develop or grow by receiving nutrients from their environment. In addition, my research focused on determining the most beneficial endophytic bacteria in the seed endophytes of *Sesbania Rostrata* by conducting a series of biochemical tests on all of the ones that were isolated from the seed endophytes. Adding on, I also characterized all of the bacterial species' visible morphological characteristics to gain a better understanding of what species they resembled or were. I found that the species of bacteria which I discovered that would be the most beneficial for plant growth was a species of bacteria from the genus, *Bacillus*. Its visible morphological characteristics are similar to *Bacillus* and it also produces similar biochemical results that a strain of *Bacillus* would produce. Furthermore, the purpose of my experiment was to find out which of the bacteria in the seed endophytes of *Sesbania Rostrata* would be the most beneficial for promoting plant growth so that it can potentially be used in a biofertilizer or a compost if applicable in the future.

Research Question: Which bacteria in the seed

endophytes of *Sesbania Rostrata* would be the most beneficial for promoting plant growth?

Literature Review:

Many articles about isolating bacteria from the seed endophytes of plants have already been made. However, there aren't many articles about experiments being done on the plant species, *Sesbania Rostrata*. But, one review article by Phumudzo Patrick Tshikhudo, Khayaletu Ntushelo, and Fhatuwani Nixwell Mudau (2023) explores the potential uses of endophytic bacteria from different species of plants in enhancing medicinal and herbal plant productivity while also simultaneously maintaining soil health. This research article discusses the different endophytic bacteria that have been isolated from many different species of plants that may prove to be useful in providing benefits for other plants and their surrounding environment. It also discusses how endophytic bacteria can also improve the nutrient availability of plants and how they provide protection against various biotic and abiotic stresses. In addition, this article identifies a genome of bacteria that's found in *Sesbania Rostrata* called *Azorhizobium caulinodans* and it cites a book about nitrogen-fixing bacteria in its references. However, it doesn't investigate the different strains of endophytic bacteria in that particular species of plant. The article includes the bacteria, *Azorhizobium caulinodans*, in it so that it can use it along with other types of bacteria that are beneficial for plants and their surrounding environment to describe their uses or sustainable applications. Since this is a review article, the authors of it weren't responsible for investigating any of the bacteria that they listed in their article.

Adding on, another research article describes how endophytic bacteria can be used in synergistic interactions and are prospects of promoting plant growth (Vandana, et al., 2021). This article discusses how different strains from many species of endophytic bacteria can be used to promote synergistic interactions, which essentially involve microbial cooperation that results in two or more microbial

populations supporting each other's growth and proliferation. Furthermore, these synergistic interactions are able to promote plant growth as they occur in different species of plants. One of the bacterial species that were isolated and used in this research experiment was a strain of *Azorhizobium caulinodans* from the plant, *Sesbania Rostrata*. The particular strain that was investigated in this study was *Azorhizobium caulinodans* ORS571. The researchers explored the different enzymes, including cellulase and pectinase, that this bacterium secreted to its host plants as well its nitrogen-fixing ability. In addition, this article delves into how the bacterial strain, *Azorhizobium caulinodans* ORS571, plays a role in synergistic interactions between different microbial species to promote plant growth. However, my research investigates for the most beneficial bacteria in the seed endophytes of *Sesbania Rostrata* to aid in promoting plant growth. The bacterial strain which I found to be the most effective as a result of its positive biochemical tests may be completely different from the species of bacteria that were used in the experiment with the other researchers. As a result, the bacteria I found has potential to be a completely different species of bacterium as it resembles the genus, *Bacillus*, according to the biochemical test results that I've received and the visible morphological analysis that I've conducted of it. My bacterium releases many different kinds of enzymes and it can be very useful for promoting plant growth. So, it's possible that my species of bacteria hasn't been discovered yet. Also, my experiment involves exploring the most beneficial bacteria in the seed endophytes of *Sesbania Rostrata*, which may have led me to discovering more than just a strain of *Azorhizobium caulinodans*. Plus, my bacteria may even have more benefits for plants compared to the strain of *Azorhizobium caulinodans* that those researchers investigated. This is because their study shows us how this bacterium doesn't release a lot of enzymes and that it only releases a few, including pectinase and cellulase. However, my bacteria uses around seven enzymes. So, my experiment is completely different from the one that these researchers conducted about

Investigating the potential of endophytic bacteria found in the seeds of *Sesbania rostrata* for promoting plant growth

synergistic interactions between different microbes.

Methods:

My research initially involved isolating all of the different species/strains of bacteria from the seed endophytes of *Sesbania Rostrata* and growing them on LB agar plates. This involved surface sterilizing and crushing around twenty-thirty seeds from the plant, *Sesbania Rostrata*. In addition, I conducted a serial dilution after crushing my seeds into a mixture. Then, I added each of the diluted mixtures to individual petri dishes and spread out all the mixtures in their petri dishes. I grew five different bacterial colonies throughout all of the petri dishes that I used for the serial dilution. Afterwards, I used the quadrant streaking method to streak all of the different colonies I isolated onto their individual petri dishes. I incubated the petri dishes for 48-72 hours afterwards. In the end, I obtained five petri dishes with all of the isolated bacterial species. I named these bacteria, "Sesbania 1," "Sesbania 2," "Sesbania 3," "Sesbania 4", and "Sesbania 5." Furthermore, I conducted biochemical tests on all of the bacterial species that I isolated. The biochemical tests that I conducted were the Voges Proskauer (VP) test, the methyl red test, the cellulase activity test (using carboxymethyl cellulose plates), the zinc-solubilization test (using zinc-solubilizing agar), the nitrogen fixation test (using Jensen's medium), the phosphate-solubilization test (using Pikovskaya's medium), the protease activity test (using a skim milk agar medium), the starch-hydrolysis test (using a starch agar medium), and

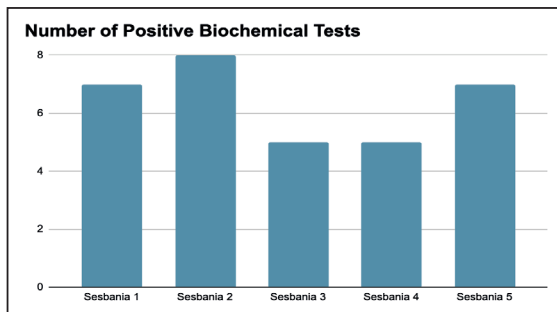
the catalase test (using hydrogen peroxide). I prepared each of the respective mediums for each of the different tests and conducted them. I made petri dishes with different kinds of mediums for each of the tests that required me to do so. Then, I streaked the different cultures of bacteria that I isolated onto each of them and incubated them. However, I had to prepare the right solutions for the Voges Proskauer and methyl red tests before I inoculated all of my species of bacteria into different test tubes with those solutions. Furthermore, I had to prepare more LB agar plates to inoculate my isolated bacteria into them before I conducted the catalase test. This is because it involved adding hydrogen peroxide to their original petri dish mediums. As a result, I made more mediums to ensure I preserved my original bacterial cultures. Adding on, I noted down all of the results that I obtained for all of the biochemical tests that I conducted for each of the bacteria that I isolated as I did each test. Afterwards, I compared all of the test results that I received and concluded which of the bacteria that I isolated would be the most beneficial for plant growth.

Results:

At the end of my experiment, I realized that only one of the species/strains of bacteria that I isolated was positive for most of the biochemical tests that I conducted. This bacterial colony was positive for all of the biochemical tests except for the Voges Proskauer test. Furthermore, you can view all of the bacterial cultures/colonies that I isolated from the seed endophytes of *Sesbania Rostrata* in the image below.



Bacterial Colonies/Cultures	Cellulase Activity Test	Zinc-Solubilization Test	Nitrogen Fixation Test	Phosphate-Solubilization Test	Protease Activity Test	Starch-Hydrolysis Test	Methyl Red Test	Voges Proskauer Test	Catalase Test
SB1 (Sesbania 1)	Single positive	Triple positive	Positive	Single positive	Single positive	Single positive	Negative	Negative	Positive
SB2 (Sesbania 2)	Double positive	Double positive	Positive	Single positive	Single positive	Single positive	Positive	Negative	Positive
SB3 (Sesbania 3)	Negative	Negative	Positive	Single positive	Single positive	Single positive	Negative	Negative	Positive
SB4 (Sesbania 4)	Single positive	Negative	Positive	Negative	Single positive	Single positive	Negative	Negative	Positive
SB5 (Sesbania 5)	Single positive	Negative	Positive	Single positive	Triple positive	Single positive	Negative	Positive	Positive



Adding on, as you can see in the table and chart above, “Sesbania 2” has the highest number of positive results compared to the other bacterial colonies for the biochemical tests that I’ve conducted. Adding on, “Sesbania 2” is the highlighted culture of bacteria that’s presented in the table. As a result, I can determine that this colony would be the most beneficial for promoting plant growth and effective in doing so. Furthermore, I analyzed the visible morphological characteristics of all of the colonies of bacteria

that I’ve isolated. A table with all of the data that I’ve collected from each of the cultures of bacteria that I’ve isolated is presented below. After conducting research about what the visible morphological characteristics and the biochemical test results of “Sesbania 2” attributed, I learned that they meant that this bacteria was similar to bacteria in the genus, *Bacillus*. As a result, it’s possible that this bacterial colony is a strain of the genus, *Bacillus*. Furthermore, “Sesbania 2” looks very similar to other *Bacillus* strains of bacteria. However, there are not many research articles that describe finding bacteria from the genus, *Bacillus*, in the seed endophytes of *Sesbania Rostrata*. But, it’s very common for many research studies to find *Azorhizobium caulinodans* in *Sesbania Rostrata*. As a result, it’s plausible that “Sesbania 2” may be a new strain of *Bacillus* or another species of bacteria and can possibly be more beneficial for plant growth compared to other types of bacteria.

Bacterial Culture/Colony	Shape	Elevation	Optical Density	Color
SB1 (Sesbania 1)	Circular	Raised	Transparent	Creamy White
SB2 (Sesbania 2)	Circular	Flat	Opaque	Creamy White
SB3 (Sesbania 3)	Circular	Raised	Opaque	Creamy White
SB4 (Sesbania 4)	Irregular	Flat	Transparent	Creamy White
SB1 (Sesbania 5)	Irregular	Raised	Opaque	Creamy White

Investigating the potential of endophytic bacteria found in the seeds of *Sesbania rostrata* for promoting plant growth

Discussion:

As a result, the most beneficial bacteria that I identified out of all of the ones that I isolated from the plant, *Sesbania Rostrata*, is completely different from the strain of *Azorhizobium caulinodans* that many researchers including the ones listed in this article have used in their experiment. As a result, it may be a completely new species of bacteria and most likely possesses many more benefits for plants than some strains of *Azorhizobium caulinodans*. This is because *Azorhizobium caulinodans* is mostly known for its nitrogen-fixing ability. In addition, the most beneficial bacteria that I found can be implemented into an agricultural application including a biofertilizer as it can have many beneficial effects for a wide variety of plants and promote their growth. In addition, particular strains of *Bacillus* haven't been found in *Sesbania Rostrata* in many past experiments. So, my findings may be relevant to the science community and can possibly be useful for agricultural applications in the future. However, my experiment and findings also have a few limitations. These include that my experiment hasn't verified the exact strain of the bacteria that I found as I haven't conducted a 16s rRNA sequencing procedure on the bacteria which I found to be the most beneficial. As a result, I can't determine exactly what species or strain my bacteria is even though its characteristics and test results resemble a strain of *Bacillus*. But, the bacteria I discovered resembles the genus, *Bacillus*, and it can have many benefits for plants according to all of the biochemical tests that I've conducted. Adding on, areas of future research include finding the exact species/strain of the most beneficial bacteria that I isolated from the seed endophytes of *Sesbania Rostrata*, conducting plant-growth promoting rhizobacteria tests on the most beneficial bacteria I isolated, and also finding out if this bacteria can be implemented into any agricultural applications including a biofertilizer.

Conclusion:

The main findings of my study were that "Sesbania 2" is the most beneficial bacteria out of all of the ones that I isolated from the seed endophytes of the plant, *Sesbania Rostrata*. In addition, according to the results that I received from the biochemical tests that I conducted on this specific bacteria, it's the most beneficial out of all of the other species/strains of bacteria that I isolated as it got a positive result for most of the tests. Furthermore, after conducting a visible morphological analysis of all the bacteria that I isolated and by using the results from my biochemical tests, I determined that "Sesbania 2" or the most beneficial bacteria that I isolated is similar to a strain from the genus, *Bacillus*. However, further testing needs to be done to find the exact species and strain of the bacteria I isolated. Adding on, I need to determine whether the bacteria that I isolated can be used in agricultural applications including biofertilizers in the future. In addition, my experiment supports my research question because it includes finding out what the most beneficial bacteria in the seed endophytes of *Sesbania Rostrata* is and I've done that by conducting biochemical tests on all of the bacteria that I isolated. Even though my experiment supports my research question, I've still got much research to complete to ensure that the bacteria that I've isolated can be utilized in an agricultural application.

Citations/References:

1. https://www.researchgate.net/publication/368396928_Sustainable_Applications_of_Endophytic_Bacteria_and_Their_PhysiologicalBiochemical_Roles_on_Medicinal_and_Herbal_Plants_Review
2. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7912845/#:~:text=Simple%20Summary,or%20indirectly%20promote%20plant%20growth.>