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ISOLATION OF *Rhizobium* sp. FROM *Mimosa pudica* GROWN IN NEUTRAL ALKALINE SOIL

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Abstract

Microbial diversity is considered as one of the most useful resources for bioprospecting. *Rhizobia* are of particular interest due to their symbiotic nitrogen fixing ability with legumes. In the present study, *Rhizobium* sp. was isolated from *Mimosa pudica* plant and characterized by Gram staining, Motility test, Colony morphology on YEMA medium and Biochemical tests. Multiple beneficial effects to the plants and 5 isolates which were grown on YEMA plates was studied in the present research. The isolates were screened for their abilities to fix Nitrogen and solubilize phosphates. The selected isolates were tested for IAA production and other Biochemical tests.

Key words: *Rhizobium* sp., *Mimosa pudica*, Nodulation, Characterization, YEMA medium, Phosphate solubilization, IAA production and Biochemical tests.

1. Introduction

Bacteria of genus *Rhizobium* sp. are fast growing Gram negative rods that are normally involved in nitrogen fixation for the atmosphere. *Rhizobia* are commonly found in the root nodules of legumes; they infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. We obtained our samples from the root nodules of a touch me not plant biologically called as *Mimosa pudica*. Yeast mannitol agar has been used for the isolation of *Rhizobium* sp. the YEMA medium contains yeast extract, which provides the bacteria with a nitrogen source, , a necessary element for natural *Rhizobium* sp. function. Our enrichment culture protocol also involves incubating the bacteria at room temperature at standard room pH 6 - 8 and regular lighting for atleast two days. The legume-*Rhizobium* interaction is the result of symbiotic relationship.

Specific recognition of the host legume was observed in *Rhizobium* sp. by Phillips (1991). Isolation typically involves handling each nodule individually while both sterilizing the nodule surface and disrupting the nodule to release bacteria. The identification of *Rhizobium* strains are based on antibiotic or phage resistance involve inoculating master plates of the nodule isolates, either of the total nodule suspension or of a single colony initially isolated from the nodule suspension and then plating individual isolates onto indicator plates.

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2. Materials and Methods

Preparation of bacteria samples from root nodules

Mimosa pudica plant inhabited with *Rhizobium* sp. was obtained from the garden in Chennai, Tamil Nadu, India. Brown nodules ~7 mm wide and a little bit of root were cut from the root system of the plant. The roots and nodules were rinsed with water. About 5 to 6, 1 cm long sections, each with one or two nodules, were cut from the *Mimosa* roots sections and immersed in 1 % chlorine solution in sterile petridishes for 15 minutes. Scalpels, and glass rods used to manipulate the nodules were contained in 70 % alcohol for sterilization. The lid was closed and the dish was swirled for about a minute. The solution was poured off, and nodules were immersed in distilled water. The largest root nodules were transferred to separate drops of water on a petridish and crushed. A large number of Yeast Extract Mannitol Agar (YEMA) plates were streaked with root nodule sample under sterile condition. The inoculated plates were inverted and left to grow at room temperature (20 - 25 °C) for two or three days.

Characterization of *Rhizobium* sp.

The *Rhizobium* strains were Gram stained and was incubated for 5 days on YEMA at different temperature, pH values and various NaCl concentration, Cell motility, Cell morphology was observed (Elsheikh and Wood, 1989). Oxidase activity was evaluated by touching a colony with a paper impregnated and observed the colour change. Catalase activity was determined by flooding a colony with 10 % (V/V) H_2O_2 and checking for the presence of bubbles. Other biochemical test was also performed. Antibiotic susceptibility tests were performed on YEMA using the antibiotics disc dispenser system with bio discs such as Ampicillin (10 µg and 25 µg), Chloramphenicol (30 µg and 50 µg), Erythromycin (10 µg and 25 µg) and Tetracycline (10 µg and 10 µg). The plates were incubated at 28 °C and evaluated after 4 days (Brockwell *et al.*, 1976). The strains were sensitive to most of the antibiotics tested, except for Erythromycin, which was similar to the profile of *Rhizobium mesoamericanum.* On the other hand, *Rhizobium grahami* has the only strain which was able to grow in the presence of Ampicillin discs. Enzymatic reactions were positive for Catalase, Urease, Hydrolysis of Esculin (Graham and Parker, 1964) and β-galactosidase and negative for Nitrate reduction, Tryptophan deaminase, Glucose fermentation and Hydrolysis of gelatin. There were several reports describing the characterization of *Rhizobia* based on morphological and biochemical features (Gachande and Khansole, 2011).

Nodulation

The nodulation capacity of the isolates was assessed in a Greenhouse condition. Seeds were surface sterilized by gently rinsing in 70 % ethanol for 2 minutes and $HgCl_2$ for 3 minutes. Three seeds of *Mimosa pudica* were sown in one plastic pot containing vermiculite-quartz sand mixture and moisturized with nitrogen free nutrient solution at regular intervals. Emerging seedlings were inoculated with a fresh suspension of bacterial strain. Uninoculated plants were included as control. The plants were harvested after 45 days of planting and observed for the presence and absence of nodules.

3. Result and Discussion

The interpretation shows there were 5 isolates of *Rhizobium* sp. collected from *Mimosa pudica*. The gummy colonies were found in all isolates of *Rhizobium* sp. after streaked on YEMA plates for 5 days at 37 °C with fast growing rate (Hussain *et al*., 2002). Gram negative was observed in Gram's reagent and also rod shape of bacteria cell with pink colour was observed under microscope. Dark black to purple granules were observed intracellularly with pink background when counterstained with Safranin. However, some pure *Rhizobium* isolates are unable to grow on Lactose. Casein utilization and peptonisation were resulted in Litmus Milk Test by *Rhizobium* isolates. Most of the biochemical tests were given the same results as reported for *Rhizobium* sp. in

literature of Shaluzad *et al.* (2008). Hence, the *Rhizobium* sp. was identified as *Rhizobium grahami* and *Rhizobium mesoamericanum* by their Morphology culture and Biochemical characterization (Barrett and Parker, 2006).

We were successful in proving our hypothesis that *Rhizobium* sp. can be isolated from root nodules of *Mimosa pudica* and grown on Yeast Extract Mannitol Agar (YEMA) plates. The enrichment method proved to be successful. We got the bacteria by crushing the nodules found on roots. We first prepared Liquid Nitrate Medium to be used as the medium to isolate *Rhizobium* sp. Since, we were not sure which species would be present and did not want to limit ourselves, we decided against using this medium. Finally, we decided to use Yeast Extract Mannitol Agar (YEMA) medium to grow *Rhizobium* sp. Because, this medium contains a source of Nitrogen (in Yeast extract) and Carbohydrate (Mannitol), it supports the growth of *Rhizobium* sp. The bacteria obtained from the root nodules were platted on YEMA plates, and streaked over and over again until a pure colony was seen growing on the plate. On the majority of the plates, white, mucilaginous, raised, semi-translucent colonies were seen. *Rhizobium* sp. is one of the few nitrogen-fixing bacterium that lives in close symbiotic association with legumes such as peas, beans, clovers and *Mimosa pudica*. The plant provides carbohydrate to the bacteria and, in return, the bacteria supplies fixed nitrogen compounds to the plant. *Rhizobia* sp. have a capacity to produce an enzyme called Nitrogenase that catalyzes the conversion of Nitrogen gas to Ammonia.

4. Conclusion

The isolation of *Rhizobium* sp. in the present research allows us to study the characterization of an organism and understand exactly how it fixes atmospheric nitrogen. This understanding can help in the field of agriculture to carry out the chemical free Organic farming. Fertilizers can be made using these bacteria in some way. It helps us understand symbiotic relationships between plants and bacteria. It is important to have a step by step protocol to isolate this bacterium so that bacterium can easily be obtained from the nodules. A systematic procedure is needed to make sure that the desired organism, *Rhizobium* sp. was isolated so that it can be grown on YEMA plates.

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