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Isolation, Identification & Characterization of *Rhizobium* sp. from *Arachis Hypogaea* L. (Groundnut)

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Abstract

Groundnut (*Arachis hypogaea* L.) is the important leguminous crop in tropical and subtropical area. Groundnut is cultivated in the rain – fed conditions. In Groundnut high protein content is present. Mostly in Maharashtra Dhule, Jalgaon, Nasik, Ahmednagar, Pune, Satara, Sangli, and Kolhapur in this districts Groundnut is cultivated. In the symbiotic relationship with the legume *Rhizobium* fixes the nitrogen (N). The experiment was conducted to characterize the *Rhizobium* bacteria in the Groundnut plant roots. In this experiment the colony characteristic and morphological characters were observed in the laboratory with the help of biochemical test.

Keywords: *Rhizobium*, Groundnut (*Arachis hypogaea*) and N2 Fixation

Introduction

Nitrogen is an essential nutrient for plant growth and development. Plants usually depend upon combined or fixed forms such as ammonia and nitrate because it is unavailable in its most prevalent form as atmospheric nitrogen. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers has led to worldwide ecological problems as well as affects the human health (Vitousek, 1997) [20] biological nitrogen fixation (BNF) is the cheapest and environment friendly procedure in which nitrogen fixing microorganisms, Interacting with leguminous plants, fix aerobic nitrogen into soil (Franche *et al.,* 2009) [7]. *Rhizobium* is the most well-known species of group of bacteria that acts as the primary symbiotic fixer of nitrogen. *Rhizobium* bacteria stimulate the growth leguminous plants they are able to fix atmospheric nitrogen into soil by interacting symbiotically with leguminous plants, Using the nitrogenize enzyme complex (Kiers *et al.,* 2003) [10] in addition *Rhizobium* Strains secrete growth hormone like Indole Acetic Acid (IAA), which shows positive influence on plant growth and also plays an important role in the formation and development root nodules (Nutman, 1977) [14]. *Rhizobia* are bacteria that spend most of their lives in soil, but they are better known for their work inside legume root nodules, where they convert atmospheric nitrogen to forms their plant hosts can use. When a legume host is abundant, *rhizobia* in nodules may outnumber those in soil. But hosts may not be available every month-or every year. *Rhizobia* in the nongrowing "persister" state can survive for over a year without food, especially if they start with abundant resources [6]. *Rhizobia* are free living, gram-negative, aerobic and facultative anaerobic, motile, chemo-heterotrophic bacteria. *Rhizobia* persist in soil as saprophytic heterotrophs when they are not infecting their hosts. Depending on the season, crop history, and management practice. The nucleus of the root cell guides the *Rhizobia* [7]. If legumes have ever been grown at a site, the higher value is more appropriate. If not, proper infection often requires inoculation. This is particularly true of those legumes in tropical forests. Nitrogen fixation rates vary enormously the air we breathe is 78% nitrogen by volume, yet many plants suffer N deficiency because this N is in a form unusable to plants. Some plants, however, have co-evolved with specific bacteria that can make this N available. When properly inoculated, legumes generally do not require N fertilizer because of this symbiotic relationship with beneficial bacteria. When a new legume is first introduced in a field, the soil may not contain appropriate *rhizobia*. Suitable organisms must be added for adequate nodulation and N fixation. Once the legume becomes established and is well modulated, inoculation in subsequent years is usually not required. Where a specific legume has been grown in a region for some time and the roots are extensively nodu-lated, most soils contain abundant *rhizobia* and even non-inoculated plants are well nodulated. Because of abundance of *rhizobia* in these soils, added *rhizobia* often produce only a small fraction of the nodules formed and yields are unresponsive [8].

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An association between two organisms or populations, which is stable in the absence of environmental change [9]. Restoring, maintaining and increasing soil fertility are major agricultural priorities in many parts of the developing world where soils are inherently poor in plant nutrients, and the demand for grain food and raw materials is increasing rapidly. Sustainable production of crops cannot be maintained by using chemical fertilizers alone. Nutrients need to be added from other sources such as organic manure and biofertilizer for providing greater stability in production and improving soil fertility (Mosharof *et al.,* 2012) [13].

Material and Methods

Rhizosphere Soil sample were collected in Sterilized polythene bag from Groundnut (*Arachis Hypogaea*) of Loni Devkar village in Taluka Indapur Dist. Pune. The samples brought in laboratory for isolation of *Rhizobium*. The serial dilution method was used for isolation of *Rhizobium*. The Yeast Extract Manitol Agar (YEMA) medium adapted. Inocuted culture plates were kept for Incubation for 24 Hours. After incubation slide preparation of isolated colonies for gram staining and observe under light Microscope. *Rhizobium* were identified by using Bergey's Mannual, on the basis of Morphological characters and with the help of biochemical test.

Colony Characteristics

Table 1: Colony Characteristics

Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
2 to 3mm Diameter	Circular	Greenish	Entire	Convex	Opaque	Smooth

After 24 hours Incubation, the colonies are observed colonies are 2 to 3 mm in diameter, circular in shape, Entire in margin. The colony having a surface that curves towards the outside (convex), opaque in opacity, smooth in consistency & gram negative bacteria because this colonies are pinkish in colour observed under the microscope

Observation Table in Biochemical Test

Table 2: Observation Table in Biochemical Test

S. No.	Test	Positive (+)/Negative (-)
1	Indole Production Test	-
2	Methyl Red	-
3	Voges Proskauer	+
4	Catalase	+
5	Oxidase	+
6	Urease	-
7	Sugar Fermentation	+
8	Starch Hydrolysis	-

Biochemical Characteristics

Different Biochemical tests were carried out which includes, Indole test, Methyled test, Voges Proskauer test, catalase test, Urease Test, Starch Hydrolysis, Oxidase Test and sugar fermentation test and Sugar Utilization Test.

Indole Test: A Loopful suspension of each isolate was inoculated into sterile 1% tryptone water medium and was incubated at 37°c for 24 hrs. After incubation, first xylene was added mixed well and then 1 ml Kovac's reagent was added. Positive test was indicated by development of pink coloured ring at the top of medium and Negative test indicated by Development of yellow coloured ring at top of medium.

Methyl Red Test: A loopful suspension of each isolate was incubated into sterile glucose phosphate broth medium and was incubated at 37°c for 24 hrs. After incubation, 5 drops of the methyl red indicator was added. Positive test was indicated by development of red colour in the medium and Negative test was indicated by development of yellow colour.

Voges-Proskauer Test: A loopful suspension of each isolate was inoculated into sterile glucose phosphate broth medium and was incubated at 37°c for 24 hrs. After incubation, 0.6 ml of a-naphthol and 0.2 ml of 40% of KOH were added. Positive test was indicated by development of red colour in the medium.

Catalase Test: The isolated colony of the isolate was picked up with sterile nichrome wireloop and dipped in 5 ml of H_2O, solution and observed for the evolution of gas bubbles as positive catalase test.

Urease Test: A loopful suspension of each isolate was streak inoculated on sterile Christensen's urea agar slant and incubated at 37°C for 24 hrs. The slant colour change to pink and indicated the urea hydrolysis property.

Sugar Fermentation: An inoculam from a pour culture is transfer aseptically to sterile tube of phenol red sucrose broth the inoculated to be is incubated at 370C for 24 hours. A positive test indicated by colour change from red to yellow indicating a PH change to acidic.

Starch Hydrolysis Test: Starch agar plate was prepared by using 1% starch in nutrient agar medium. A loopful suspension of test culture was spot inoculated at 37°c for 24 hrs. To test the amylase activity of isolates the plates were flooded with iodine solution. The clear zone of hydrolysis with purple background indicated the amylase activity.

Oxidase Test: A strip of filter paper was soaked in freshly prepared aqueous 1% oxidase reagent (N, N, N', N'-Tetramethyl para phenyl diamine dihydrochloride). The growth of the isolate was rubbed on the filter paper strip. The positive reaction was indicated by an intense deep purple appearance within 6-10 sec.

Results and Discussion

Isolates were obtained from groundnut nodules from Loni Devkar. After series of streaking pour culture of isolated strain obtained from soil nodules. The colonies of the isolates were greenish circular entire opaque and growth was observed after two days colonies circular greenish and convex. *Rhizobium* were isolated from root nodules of leguminous plants based on their gram staining, Physiological and Biochemical characteristics. Under an electronic microscope with magnification of 100X and observed for the presence of isolates. The isolate were gram-negative and found to be non-motile. *Rhizobium* were recovered and characterised by various biochemical tests like IMVIC, Catalase, Urease, Amylase test etc. They were also subjected various sugar fermentation test and were found lactose, glucose, sucrose confirming the bacterial species [15]. The chemical fertilizers negatively affects the environment and increase production costs, so eco-friendly and eco-effective and agro-Technologies to increase groundnut production are required, including microbial interventions through biofertilizers such as *rhizobium* inoculum. Even though several studies as documented above, have assessed to effect of *rhizobium* inoculation on the ability to enhance the growth and

productivity of groundnut [13]. The need of today's world is high output yield and enhanced production of the crop as well as fertility of soil to get in an eco-friendly manner. Fresh alternative should be explore for the use of bio inoculant for other high value crop such as vegetables, fruits and Flowers. The demand of agricultural productivity has increase dramatically as a result of civilization and Industrialization. Chemical fertilizers and pesticides increase agricultural yields, but they can degrade soil fertility and quality.
Rhizobium isolated from agricultural soil were used to prepare biofertilizers and when applied to the seeds, seed germination improved and also protected from plant diseases due to antifungal substances produced by them.

Conclusion

The bacteria isolated in this study are like growing *rhizobia* nodulate groundnut only. They promote groundnut growth better than commercial one. Biotic and Abiotic Stresses have ability to suppress the growth and adversely affects the productivity of leguminous plants symbiotic interaction between legume plants roots and microorganisms enhance the defence mechanism against these stresses. In the presence study, A bacterial isolate was characterised from the root noudules of Groundnut (*Arachis hypogaea*) and identified on the basis of morphological and biochemical characteristics. The isolated *Rhizobium* may promote growth and increase tolerance to different types of stresses and also play important part in sustainable agriculture system. It can be concluded that *Rhizobium* bacteria fix nitrogen in nodule which is essential in nitrogen deficient condition.

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