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Isolation, identification and screening of *Rhizobium* for plant growth promotion

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Abstract
The *Rhizobium* was isolated from the root nodules of groundnut plant using YEMA medium and identified. Then the *Rhizobium* was screened on the basis of pH resistance, temperature resistance, salinity resistance and antibiotic resistance. The plant growth promotion by *Rhizobium* was studied by Leonard jar experiment. The seeds of cowpea plants were treated with various concentration of *Rhizobium* culture at different time intervals. After the growth period, the plants were subjected for root length, shoot length, count of root nodules formed and chlorophyll content. The plants of T-4 (Treatment-4) showed better results than the other treatments.

Keywords: Isolation, screening of *Rhizobium,* plant growth promotion

1. Introduction
In legumes and few other plants, the bacteria live in small out growths on the roots called nodules. The plant roots supply essential minerals and newly synthesized substances to the bacteria (Burdass and Dariel, 2002) [2]. Within these nodules, the bacteria do nitrogen fixation and the plant absorbs the ammonia. The soluble form of nitrite and nitrate can be assimilated by plant's roots and utilized in synthesizing proteins and nucleic acids. This form of nitrogen can be converted to ammonia by plants, animals and microorganisms (Atlas, 1998) [1].
Rhizobium and *Bradyrhizobium* are described as soil bacteria that have ability to infect root hair of leguminous plant and it to induce nodule formation with subsequent fixation of nitrogen. Nitrogen fixing food and forage legumes tolerant of environmental stresses represent an important strategy to improve agricultural productivity. In this present study, the *Rhizobium* was isolated, identified and screened based on pH resistance, temperature resistance, salinity resistance and antibiotic (produced by some soil microbes) resistance. Then the screened *Rhizobium* was used to plant growth study.

2. Materials and methods
The *Rhizobium* was isolated from root nodules of groundnut plants using YEMA medium and identified based on morphology, motility, and various biochemical & physiological tests. After that, the *Rhizobium* was screened for various environmental stress such as pH (4.0, 5.0, 6.0, 7.0, 8.0 & 9.0), temperature (30˚C, 35˚C & 40˚C), osmotic pressure (salinity - 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% & 1%) and antibiotics (Tetracycline, Erythromycin, Ampicillin, Chloramphenicol & Kanamycin) **(**Gauri *et al.,* 2011) [3]. Then the screened *Rhizobial* culture was used for the plant growth study by Leonard jar experiment (Sunil A. Gosavi, 2013) [5]. The *Vigna unguiculata* plant was chosen for the plant growth study, because of its availability and nutritional content. The pre-germinated seed was treated with *Rhizobial* culture (T0-0, T1-5ml, T2-5+5ml, T3-5+5+5ml, T4-5+5+5+5ml) at different time intervals (T0-0th day, T1-1st day, T2 - 1st & 6th day, T3 - 1st, 6th & 11th day, T4 - 1st, 6^{th,} 11th &16th). Plant growth promotion was determined by length of shoot and root, count of root nodules formed and by the estimation of Chlorophyll content (Thimmaiah, S.K., 1999) [6]. After 21 days of plant growth, the results were observed.

3. Results and discussion

The growth of *Rhizobium* in the selective medium was observed in the YEMA media and selected for further studies.

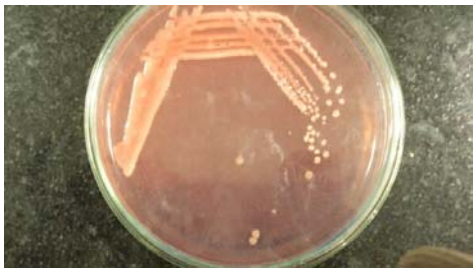

Fig 1: Growth of *Rhizobium* in YEMA medium

The isolated bacterial culture appeared as Gram negative rods and they are motile. It gives negative results to methyl red, voges-proskauer, citrate utilization, urease, Catalase, gelatin hydrolysis and ketolactose test. It gives positive result to starch hydrolysis and acid from glucose test. All this biochemical tests confirmed that the isolated bacterial culture is *Rhizobium.*

The *Rhizobium* shows the maximum growth in pH 7 at 35º C and it grows well in the medium containing 2%of Sodium chloride. The *Rhizobium* is resistant to Ampicillin (0.057g), Chloramphenicol (0.076g) and Tetracycline (0.052g) and sensitive to Erythromycin (0.133g) and Kanamycin (0.07g).

The result of enhanced plant growth using different concentration of *Rhizobium* culture added. The results represented the growth of the *Rhizobium* inoculated plants.

Table 2: Plant growth enhancement by *Rhizobium* bacteria in Leonard jar experiment

S. No	Sample	Shoot length	Root length	No. of Root nodules	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total Chlorophyll (mg/ml)
1	T_0	13	3	0	0.015	0.025	0.081
2	T_1	20	4.5	5	0.020	0.032	0.096
3	T_2	23	6	10	0.022	0.037	0.102
4	T_3	27	10.5	13	0.025	0.043	0.110
5	T_4	32	12	18	0.052	0.084	0.191

The plant treated with 20 ml (at 5 days of time interval) of *Rhizobium* culture (T_4) showed better result (25^{th} day). The shoot length of plant is 32 cm, the root length is 12 cm and the plant formed 18 nodules in its root. T_4 plants have higher chlorophyll content compare to control and other tested plant. Total chlorophyll value is (T_4 =0.191). The Treatment-4 showed the better plant growth than other treatment (T_1, T_2, T_3) and control.

4. Conclusion

The plant treated with higher concentration of *Rhizobial* culture (5+5+5+5 ml) at 5 days of time interval improved the plant growth than the other treatments (lower concentrations). The results indicates the leguminous plants needs the symbiotic association with the *Rhizobium* (nitrogen fixing microorganism) for its growth. So, the *Rhizobium* will be served as a biofertilizer to enhance the plant growth under various environmental stress conditions.

5. Reference

1. Atlas R, Bartha R. Microbial Ecology: Fundamentals and Applications. 4th Ed. Benjamin Cummings. Menlo Park, Canada, 1998, 694.
2. Burdass, Dariel. *Rhizobium,* Root nodules and Nitrogen fixation. Society for General Microbiology online, 2002. http://www.microbiologyonlinr.org.uk/forms/rhizobium.pdf
3. Gauri Ashok kumar singh, Rajendra Prasad Bhatt, Shailja Pant, Manjinder Kaur Bedi, Ashok Naglot. Characterization of *Rhizobium* isolated from root nodules of *Trifolium alexandrinum.* Journal of Agricultural Technology. 2011; 7(6):1705-1723.
4. Oblisami G. *In vitro* growth of five species of ectomycorrhizal fungi. Euro J. for Path. 1995; 1-7:204-210
5. Sunil Gosavi A. Studies on Legume-*Rhizobium* and Mycorrhizal interaction with special reference to *Phaseolus radiatusl* and *Phaseolus aconitifolius jacq.* Indian Streams Research Journal. 2013: III:8.
6. Thimmaiah SK. Standard methods of Biochemical analysis. Kalyani Publishers, Ludhiana. 1999, 534.