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Original Research Paper

Study of Root Nodulation Efficiency of Different *Rhizobium* Strains Found in Different Regions of Akola District and Developing Rhizobia Based Biofertilizer

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ABSTRACT

After introduction of chemical fertilizers in the last century, there is increased yield in agriculture in the beginning, but slowly they started displaying their ill-effects such as leaching out and polluting water basins, destroying microorganisms and friendly insects, increased salinity, reduced soil fertility, and making the crops more susceptible to the attack of diseases leading to damage of overall system, which is irreparable. Thus, to overcome this problem, it is necessary to find out alternatives to chemical fertilizers. Keeping this view in mind, the present work was undertaken to develop ‘microbial inoculants’ or ‘biofertilizers’. Biological nitrogen fixation offers an attractive and ecologically sound route for augmenting nutrient supply. Hence, biofertilizers are environmental and ecofriendly renewable resources. In present work, total 30 soil samples from different regions of Akola district were analysed for isolation of *Rhizobium*. Bioinoculant was prepared after isolation of maximum strains of *Rhizobium*. Its application was studied in pot experiments with soyabean seeds to visualize growth of soyabean plants along with its nodulation efficiency. After 2-3 months, it was observed that isolated strains have maximum nodulation efficiency as compared to control. Rhizobacterial inoculants were capable of forming root nodules in most leguminous plants. Thus, development of *Rhizobium* as biofertilizer contribute to increasing crop productivity through increased biological nitrogen fixation.

INTRODUCTION

Ever-increasing population of our country and rapidly decreasing land under cultivation have led to an increased use of chemical fertilizers to boost the crop yield and improve crop quality. But excessive use of chemical fertilizers results in environmental pollution. Hence, to combat this problem, it is necessary to develop an alternative method of supplying nutrients to plants. Also to overcome the adverse effect of chemical cultivation, it is suggested that efforts should be made to exploit all the available resources of nutrients under the theme of integrated nutrient management. Under this approach the best available option lies in the complementary use of biofertilizers. In recent years, use of biofertilizers has become a hope for most countries, as far as economical and environmental view points are concerned (Dubey 1999). The term ‘biofertilizers’ denotes all the nutrients inputs of biological origin for plant growth. It refers to microorganisms, which either fix atmospheric nitrogen or enhance the solubility and availability of soil nutrients. Biofertilizers include diverse category of bioinoculants such as nitrogen fixer, phosphate solubilizers and plant growth promoters.

Rhizobium is gram negative, aerobic rod shaped, non-spore forming and motile capable of forming root nodules in most leguminous plants fixing nitrogen for benefit of the host plants. The nitrogen fixing nodule bacterium (*Rhizobium leguminoserum*) was first isolated from legumes by Beijernick in 1888. Different species of *Rhizobium* can fix 50-200 kg of nitrogen per hectare/year in leguminous crops, therefore, they have been recommended as nitrogen biofertilizer in agriculture.

The use of *Rhizobium* increases the crop yield by 20-35% and improves soil fertility. However, it has been found that it works most efficiently in combination with chemical fertilizers. The use of biofertilizers is suitable for Indian conditions for improving the soil fertility and crop yields besides decreasing the input cost. Keeping this in mind, the present work was undertaken with objective to isolate *Rhizobium* strains from different soils, study their nodulation efficiency and to find out effective strains that can be developed as biofertilizers.

MATERIALS AND METHODS

All the analytical grade chemicals were procured from, Himedia, Mumbai. Soil for *Rhizobium* isolation was obtained from rhizosphere under leguminous plants from field areas of Akola district.

Chemical analysis of soil: Chemical analysis of soil for potassium, phosphorus and nitrogen was made following the methods of Admas & St John (1945) and Jackson (1967).

Isolation of *Rhizobium* species: Soil (1 g) from different regions was used as an inoculum in 100 mL of Yeast Extract Mannitol Agar for isolation of *Rhizobium* spp. Total 30 soil samples were analysed for isolation of *Rhizobium* spp. from which six Rhizobia strains were used for inoculant preparation (Table 1). Efficiency of *Rhizobium* strains were checked by conducting pot experiments. The isolated six strains of *Rhizobium* were proceeded for biofertilizer preparation. *Rhizobium* spp. were produced in bulk by inoculating Yeast Extract Mannitol Agar medium plate with *Rhizobium* and incubating at 26°C for 10 days. After incubation period, inoculum of bacterial suspension was prepared.

Selection and characterization of the isolates: *Rhizobium* utilizes Congo red dye very slowly and form white, circular and raised colonies. *Agrobacterium* displays colony characters like *Rhizobium* but colony colour is similar to Congo red, hence, white colour colonies are isolated and used for preparation of inoculum of bacterial suspension. Bacterial cells were stained with carbol fuchsin and visualized under compound microscope. The cells of those colonies having β-polyhydroxybutyrate granules were picked up for establishing *Rhizobium* inoculants, which utilise sugars, carbohydrates, produce hydrogen sulphide, and hydrolyse gelatin while showing positive reaction for citrate utilization, catalase, production of ammonia from peptone and urea. pH 7.0 and temperature 26±1°C were found to be stable for its growth. The isolated six strains of *Rhizobium* were proceeded for biofertilizer preparation. The steps involved in seed inoculation of *Rhizobium* culture are:

- 500 mL of sugar/jaggary solution was prepared and heated for 15 min.
- After heating 200 g of gum arabic was added into this solution and cooled at room temperature.
- Slurry was made by hand mixing it with inoculum and soyabean seeds were then added.
- The seeds were transferred on sheet of paper in shade.
- Finally a pot experiment was conducted to find out nodulation efficiency of six isolates.

Total 8 pots were taken each containing sterile soil except second one having unsterile soil, denoting as C2. Two pots of control (C1 & C2) were seeded without biofertilizers, remaining were seeded with prepared bioinoculants along with soyabean seeds. After 2-3 months effect of biofertilizers on soyabean plants and nodules per plant was seen. Morphology of isolated strains was studied under

Table 1: Chemical analysis of soils.

Soil Sample No.	Nitrogen concentration	Phosphorus concentration	Potassium concentration
23	450	50	280
30	463	53	265
25	545	62	270
15	470	45	255
20	495	48	249
05	501	50	278
Standard concentration	560 kg/ha	65 kg/ha	300 kg/ha

microscope along with colony characteristic. Biochemical sugars and enzyme studies of bacterial isolates were carried out by using specific media.

RESULTS AND DISCUSSION

The results of the study are given in Tables 1, 2, 3, 4 and 5. On the basis of colony characters, morphology and biochemical tests (Tables 3 and 4) six isolates were found to be the strains of *Rhizobium*, which were suitable to prepare inoculants as biofertilizers. Isolated strains showed maximum nodulation efficiency. As compared to control (C1), *Rhizobium* strain No. 2 and *Rhizobium* strain No. 6 have shown maximum nodulation efficiency, i.e., 180 nodules per plant and 250-260 nodules per plant respectively. Also, soyabean plants have grown more in height, with root length also increased having maximum root length of 9 cm reached in strain No. 6. *Rhizobium* strain No. 3 also shows higher nodulation. Each plant appeared large in shape and size along with increased number of pods per plant. On the basis of host specificity, the given

Table 2 : Isolation of *Rhizobium* strains from different soils.

Soil Sample No.	*Rhizobium* Isolates No.
23	R1
30	R2
25	R3
15	R4
20	R5
5	R6

Table 3: Characteristics of bacterial isolates.

Characters	1	2	3	4	5	6
A. Colony Characters						
Size	2-3 mm	2-4 mm	3 mm	2-4 mm	4 mm	2-4 mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Pink	Pink	Off-white	Pink	Off-white	Pink
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Opacity	Semi-translucent	Semi-translucent	Semi-translucent	Semi-translucent	Semi-translucent	Semi-translucent
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Margin	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
B. Morphology and Motility						
Shape	Rods	Rods	Short Rods	Rods	Rods	Rods
Gram Character	-ve	-ve	-ve	-ve	-ve	-ve
Motility	+ve	+ve	+ve	+ve	+ve	+ve

Table 4: Biochemical characteristics of bacterial isolates.

Sugars	Acid Production	Gas Production
A) Biochemical Sugars		
Glucose	+ve	+ve
Sucrose	+ve	+ve
Mannitol	+ve	-ve
Maltose	+ve	-ve
B)Enzyme Study		
Amylase	+ve	
Catalase	+ve	
Caesinase	+ve	
H_2S production	-ve	

isolates of *Rhizobium* species are of *Rhizobium japonicum*.

Soil samples Nos. 5, 15, 20, 23, 25 and 30 have comparatively higher concentrations of N, P, K (Table 1) and hence, further used for isolation of *Rhizobium*. N, P and K are most important elements of soil, which are available to the plants by biodegradation of organic matter by microorganisms. Due to continuous growing of plants year after year, soil erosion and reduction in concentration of N, P, K takes place. These directly affected the microbial flora of soil. Low concentration of N, P, K causes plants to become pale yellow with stunted growth.

As *Rhizobium* forms a good association with Leguminosae family, nodules on legume roots are responsible for fixing atmospheric nitrogen symbiotically and increase the crop yield by improving soil fertility. According to Regional Biofertilizer Development Centre, Nagpur, during field application of *Rhizobium* biofertilizers, 200 g of *Rhizobium* biofertilizers were suspended in 300-400 mL of jaggary solution/gum aerobic solution and mix thoroughly. This paste was mixed with 10 kg of seeds and dried in shade. These biofertilizers coated seeds were sown within 24 hours in the field.

Table 5: Effect of biofertilizers on nodules per soyabean plant.

Sr. No.	Pot experiment details	Soyabean ht. in cm	No.of nodules per plant	Root length in cm	No.of pods per plant
1	C1 (Sterile soil) (No fertilizer)	35 cm	80-90	3	12
2	C2 (Unsterile soil) (No fertilizer)	40 cm	100-120	4	15
3	*Rhizobium* strain No. 1 (R1)	65 cm	180	5	16
4	*Rhizobium* strain No. 2 (R2)	80 cm	200	7	18
5	*Rhizobium* strain No. 3 (R3)	60 cm	215	5	15
6	*Rhizobium* strain No. 4 (R4)	48 cm	225	6	18
7	*Rhizobium* strain No. 5 (R5)	55 cm	195	8	13
8	*Rhizobium* strain No. 6 (R6)	70 cm	250-260	9	24

The shape of the nodules is controlled by the plant and the nodules are vary considerably in size and shape. Nodulation can be impeded by low pH, metal toxicity (aluminium toxicity), nutrient deficiencies, salinity, water-logging and the presence of root parasites such as nematodes. Excessive moisture and water-logging prevent the development of root hair and site of nodulation and interfere with biological nitrogen fixation.

Thus, isolated *Rhizobium* strains can be developed as biofertilizers, which can prove to be a milestone in increasing crop yield of Leguminosae family. Biofertilizer technology can minimize production costs and at the same time avoid the environmental hazards by partially substituting for the chemical fertilizers.

CONCLUSIONS

Nowadays biofertilizers have gained more importance as they play an important role in improving economical status of farmers. They require less energy, not very costly and can be afforded by marginal farmers for getting more production. For getting increased food grain demands of increasing population, *Rhizobium* biofertilizers are safe, attractive and ecofriendly way of fertilization.

To popularize biofertilizer use in the community and also in country, much efforts are needed by not only by non-governmental and governmental Organizations, but also by farmers themselves. Training programmes should be conducted on a large scale, so that new information may be transmitted to farmers. For getting better results, biofertilizers are tested in the farmers' fields, so that farmers must be known of the potential of biofertilizers to increase the yield.

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