ISOLATION OF RHIZOBIA FROM SOYBEAN CULTIVATED IN LATUR AREA & STUDY OF ITS PHOSPHATE SOLUBILIZATION ACTIVITY

R N Jadhav

Dept. of Microbiology, R. S. Mahavidyalaya, Latur. (MS) INDIA
ramchandrajadhav30@hotmail.com

ABSTRACT

The present study deals with isolation of rhizobia from soybean crop cultivated in Latur area and study of its phosphate solubilization activity. Total 10 isolates of rhizobia were isolated from soybean crop and its phosphate solubilization activity was studied. YEMA & PVK media were used for this study. It was observed that only three isolates of rhizobia of soybean crop shows phosphate solubilization activity. The quantification of phosphorus solubilizing ability has been evaluated as PSE. It was also observed that PSE was increased by adding specific carbon and nitrogen sources in the media.

Key Words: Rhizobia, Phosphate solubilization, Soybean crop, Symbiotic nitrogen fixation.

INTRODUCTION

Soybean (Glycine *max* L.) is one of the important crops of the world. Production of soybean in India at the present time is restricted mainly to Madhya Pradesh, Uttar Pradesh, Maharashtra, Gujarat and Karnataka. Soybean posses a very high nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein. The oil being low in cholesterol has gained much attention from people suffering from heart problem. Rhizobia are soil bacteria that fix nitrogen after becoming established inside root nodule of legume. Rhizobia requires plant host, they cannot independently fix nitrogen. Soybean can be nodulated by different rhizobia belonging to three different genera (Albareda *et al.,* 2009). The soybean plant get nitrogen from bacteria while plant supplies carbohydrates, protein and sufficient oxygen so as not to interfere with fixation process. Healthy nodules are pink red in color inside & are actively fixing nitrogen. The significance of rhizobia forming root nodules and growth enhancement in soybean was widely studied by many workers in the recent past (Saeki *et al.,* 2006; Sharma, 2006).

The Rhizobia live freely in the soil and as soon as they come in contact with suitable host, starts process of infection. After infection of appropriate legume they can cause formation of nodules and participate in the nitrogen fixation. In general they are Gram negative, rod shaped but varieties of morphological shapes are observed when isolated from the root nodule. Rhizobia are unique in that they are the only nitrogen fixing bacteria living in a symbiotic relationship with legume. Phosphorus is most important plant nutrient. The organic phosphorus in soil is unavailable to plant. Plants cannot absorb organic phosphorus unless converted to inorganic forms. Microorganisms influence phosphorus availability to plant through the process of mineralization and immobilization The inorganic phosphorus which is unavailable to plants is solubilized by many microorganisms into solution Such bacteria are abundant on root surfaces of plants and account for the proliferation and metabolisms of numerous types of microorganisms. Bacteria, fungi and actinomycetes play an important role in solubilization of phosphates. Microbial involvement in the solubilization of inorganic phosphate was shown by Stalstrom (1903), who observed solubilization of tricalcium phosphate when it was inoculated with milk cultures and soil infusions. In the present study an attempt was made to isolate rhizobia from soybean crop cultivated in Latur area and study of its phosphate solubilization activity.

MATERIALS AND METHODS

Collection of samples:

20 Soybean plant samples were collected from different locations of Latur area such as Ekambi, Handolti, Janapur, Kasarshirshi, Kadepur, Kingaon,

Kumtha, Mangrule, Tandulja & Walandi. 10 pink, active nodules were randomly selected from it and were kept at low temperature until analysis.

Isolation and Identification of Isolates of Rhizobia:

Root nodules were washed first by water and then immersed in 0.1 % acidified HgCl2 for 5 minutes. The nodules were transferred in a beaker containing 10 ml of 95 % ethanol for 2-3 minutes. The nodules were again washed with sterile distilled water then crushed with sterile glass rod and suspension was made. The loopful suspension was streaked on YEM agar (M721 Hi-media) plate. The plates were incubated at 30ºC for 3-10 days. Isolates of rhizobia were identified by morphological, cultural & biochemical properties.

Study of Phosphate solubilization activity:

The 10 isolates of Rhizobia were spot inoculated on Pikovskaya's agar (M520 Hi-media) plates containing: Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, Ammonium sulphate-0.50 gm, Potassium chloride-0.20 gm, Magnesium sulphate - 0.10 gm, Manganese Sulphate – 0.0001 gm, Ferrous Sulphate- 0.0001 gm , Yeast extract- 0.50 gm & Agar-25gms in 01 liter of distilled water. The plates were incubated at 32 + or – 2.0 ºC for 5-10 days. After incubation phosphate solubilizing bacteria were detected by the formation of clear zone around its colony. The zone diameter around the colony was measured and PSE was calculated by using following formula.

$$\text{Phosphate Solubilization (Efficiency} = \frac{\text{Solubilization diameter}}{\text{Colony diameter}} \times 100$$

Gothwal *et al.*, 2006)

Effect of Carbon and Nitrogen source:

The phosphate solubilizzation was studied at different carbon sources such as glucose, galactose, starch, mannitol, maltose and different nitrogen sources such as ammonium sulphate, ammonium nitrate, sodium nitrate, potassium nitrate, urea. For study of effect of different carbon sources 1% glucose was replaced by galactose, starch, mannitol, maltose and for effect of different nitrogen source 0.05% ammonium sulphate was replaced by ammonium nitrate, sodium nitrate, potassium nitrate, urea in Pikovskaya's agar. Isolates of rhizobia were spot inoculated on it. The plates were incubated at 32 + or – 2.0 ºC for 5-10 days. After incubation phosphate solubilizing bacteria were detected by the formation of clear zone around its colony. The zone diameter around the colony was measured and PSE was calculated.

RESULTS AND DISCUSSION

20 different soybean plants along with intact root nodules were collected from different locations of Latur area. From this 10 isolates of rhizobia were isolated. Rhizobia were confirmed on the basis of morphological, cultural & biochemical characters (Table 01).

On YEM agar colonies of isolates of rhizobia were cream colored with mucoid transferent appearance. Isolates of rhizobia shows positive catalase, urease, oxidase & NR tests while negative MR, VP and Citrate & ketolactase test. Isolates of rhizobia produce blue coloration on BTB media. From this morphology along with cultural and biochemical tests suggested that these isolates were belonging to the genus *Bradyrhizobium.* Similar work of isolation of rhizobia by using YEM agar was done by Sadowasky *et al,* 1983 and Sharma *et al,* 2010. It was observed out of 10, only three isolates of rhizobia showed phosphate solubilization. The study of phosphate solubilization at different carbon & nitrogen source revealed that all isolates showed maximum phosphate solubilization in presence of glucose & ammonium sulphate. Maximum PSE was found in presence of ammonium sulphate followed by ammonium nitrate, potassium nitrate, sodium nitrate & urea. The time required for phosphate solubilization is high which indicates the solubilization of phosphate is a slow process (Alexander 1983). As the isolate of rhizobia of soybean RSB02 having dual potential of nitrogen fixation & phosphate solubilization, it can be exploited as good biofertilizer.

Table 1: Morphological & Biochemical characterization of Isolates of rhizobia of Soybean

Sr. no.	Test	Result
1	Gram nature	Gram negative
2	Motility	Motile
3	Spore	-
4	Catalase	+
5	Oxidase	+
6	Urease	+
7	MR	-
8	VP	-
9	3ketolactose production	-
10	Sucrose	-
11	Nitrate reduction	+
12	H2S	-
13	Gelitinase	-
14	Citrate	-
15	Color on BTB agar	Blue
16	2%NaCl tolerance	-
17	pH 9.0 tolerance	-

The isolates of rhizobia were then subjected for detection of phosphate solubilizing efficiency by using Pikovskaya's agar. (Table 2)

Table 2: Phosphate solubilization efficiency of isolates of Rhizobia:

Sr. no.	Bacterial Isolates	PSE
1	RSB01	--
2	RSB02	320
3	RSB03	--
4	RSB04	100
5	RSB05	--
6	RSB06	--
7	RSB07	---
8	RSB08	225
9	RSB09	--
10	RSB10	--

The efficient isolate of phosphate solubilizing rhizobia (RSB02) was then subjected for study of effect of different carbon (Table 03). & nitrogen sources (Table 04).

Table 3: PSE of Isolate of Rhizobia RSB02 at different 'C 'sources:

Sr. No.	Carbon source	PSE
1	Glucose	320
2	Galactose	250
3	Starch	100
4	Mannitol	80
5	Maltose	125

Table 4: PSE of Isolate of Rhizobia RSB02 at different 'N' sources:

Sr. No.	Nitrogen source	PSE
1	Ammonium sulphate	320
2	Ammonium nitrate	300
3	Sodium nitrate	150
4	Potassium nitrate	225
5	Urea	100

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