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Research Article

Phosphate Solubilisation Potential of Screened Nitrogen Fixing *Rhizobium leguminosarum* Strains Isolated from Nodules of Pea Plant

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ABSTRACT

The present study was undertaken to isolate Rhizobium leguminosarum strains from the nodules of Pea (Pisum sativum (L)) var AP-3 grown in the soil of Hoshiarpur. These isolated nitrogen fixing strains were also tested for their tendency to solubilize Tricalcium Phosphate (TCP) on Pikovskaya (PVK) agar, Modified Pikovskaya (MPVK) agar and National Botanical Research Institute (NBRIP agar). The halo size of R. leguminosarum SS-8 was reported to be biggest (13 mm) on NBRIP agar and the smallest (4 mm) in PVK agar. These strains were further inoculated in PVK and NBRIP broths, respectively to see their potentials to solubilize TCP. The strain R. leguminosarum SS-8 was observed to show maximum TCP solubilisation (261 µg/ml in NBRIP and 211 µg/ml in PVK broths) with maximum decrease in pH (4.14 in NBRIP and 4.20 in PVK broths).

Keywords: *Rhizobium, Nitrogen fixation, PVK, MPVK, NBRIP.*

INTRODUCTION

Biological Nitrogen fixation, a microbiological process which converts atmospheric Nitrogen into plant usable form, offers economically attractive and ecologically sound means of reducing external inputs and improving internal sources (Chatli et al., 2001). Of all the nitrogen fixing microorganisms, *Rhizobium* has the maximum ability to fix atmospheric Nitrogen. It is able to fix approximately 360 Kg of N/ha/year in association with different leguminous crops (Peoples et al., 1995; Sibbal et al., 2002). A few strains of *Rhizobia* besides fixing Nitrogen can also solubilize insoluble Phosphorous (P) and can prove to be highly efficient in fixing Nitrogen. Such kind of isolates can be used for the production of plant growth promoting formulations for improving output of different leguminous crops. These formulations can be used for various *in-vivo* studies along with a little dose of chemical fertilizers hence minimizing soil environmental pollution (Zhang et al., 2013).

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The present investigation was therefore carried out with following objectives:

- To collect soil samples from agricultural fields of Hoshiarpur and analyze them for texture, organic carbon, electrical conductivity (E.C.) and total nitrogen.
- To isolate *Rhizobia* from nodules of pea plant roots grown in fields and to screen these strains for P solubilizing capabilities on Pikovskaya (PVK), Modified Pikovskaya (MPVK) and National Botanical Research Institute (NBRIP) agar.
- These dual purpose solving *Rhizobia* were tested for their efficacy to solubilize TCP in PVK and NBRIP broths, respectively.

MATERIALS AND METHODS

The soil sample of Hoshiarpur fields was analyzed for various physio-chemical properties by standard AOAC methods (Fred et al., 1932). *R.leguminosarum* strains were isolated from healthy nodules of Pea (*Pisum sativum* (L.) *var* AP-3*)* grown in Hoshiarpur soil on Congo red Yeast Extract Mannitol Agar (YEMA). All cultures were purified and maintained on Yeast Extract Mannitol Agar (YEMA). These strains of *Rhizobia* were tested for their efficacy to solubilize TCP on PVK agar (Pikovskaya, 1948), MPVK agar (Gupta et al., 1994) and NBRIP agar (Nautiyal, 1999). The screened P solubilizing *Rhizobia* were used to study their capacities to dissolute TCP in PVK broth and NBRIP broth too (Watanable & Olsen, 1967).

RESULTS AND DISCUSSION

The soil sample analyzed was found to be Sandy loamy with E.C. (0.345 mmhos/cms), organic carbon (0.495%), organic matter (0.853%) and total Nitrogen content (0.035%) (Table 1). If the value of E.C. is less than 0.8 then the nature of soil is normal. But if its value falls between 0.8-1.6, then the soil is critical for salt sensitive crops. The nature of soil was found to be normal in Hoshiarpur.

Total 13 cultures of *R.leguminosarum* were isolated from nodules of pea plant. These strains appeared to be white, translucent, glistening and elevated with entire margins as compared with stained cultures of *Agrobacterium*. These were purified and further tested for their capabilities to solubilize TCP on PVK, MPVK and NBRIP agar. Out of 13 cultures of *Rhizobia* only two (*R.leguminosarum SS-8* and *R.leguminosarum SS-12*) were able to solubilize TCP. These strains were subcultured on PVK, MPVK and NBRIP agar media to measure their halo size. The clear zones around the colonies are formed due to TCP solubilisation. On MPVK agar, the halo zone was yellow in color due to acidification in media. The biggest halo zone was formed by *R.leguminosarum SS-8* on NBRIP agar followed by MPVK agar and PVK agar (Fig1, Table 2). It may be due to the basic Bromophenol blue dye which interacted with the gene producing *phosphatase* and hence inducing its potentials of P solubilisation. These screened strains were further inoculated in PVK broth and NBRIP broth and observed their TCP solubilisation potentials. *R.leguminosarum SS-8* dissoluted maximum TCP (261 µg/ml in NBRIP broth and 211 µg/ml in PVK broth). But *R.leguminosarum SS-12* solubilised the least TCP (250.7µg/ml in NBRIP broth and 187.2 µg/ml in PVK broth). The highest decrease in pH was observed in *R.leguminosarum SS-8* (4.14 in NBRIP broth and 4.20 in PVK broth). While the decrease in pH was minimum in *R.leguminosarum SS-12* (4.35 in NBRIP broth and 5.22 in PVK broth). The decrease in pH may be due to the production of organic acids, chelating agents or mineral acids production etc. (Table 3). Our results were in consonance with the results of some scientists (Kaur et al., 2019). These microbial cultures having dual properties can be further tested for Antibiotic resistance and hence used for pot and field experimental studies for commercial purposes too.

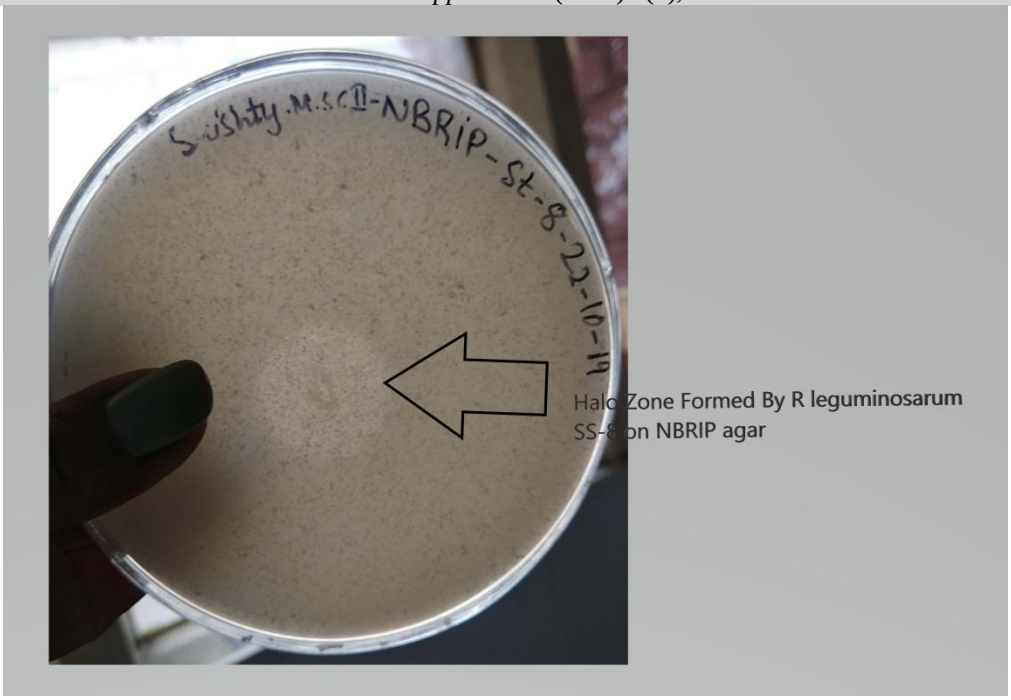

Fig. 1: Halo Zone formed by *R. leguminosarum* SS-8 on NBRIP agar

Table 1: Physico-chemical properties of soil of Hoshiarpur

Soil Sample	Nature Of Soil	E.C [Electrical Conductivity (mmhos/cm)]	Organic Carbon (O.C.) C%	Organic Matter (O.M. %)	Total Nitrogen (N %)
Dist. Hoshiarpur	Sandy Loamy	0.345	0.495	0.853	0.035

Table 2: Halo size (mm) of P solubilising *R.leguminosarum* strains on PVK, MPVK and NBRIP agar

Phosphate Solubilising *R.leguminosarum*	Treatment		
	Agar [Halo Size (mm)]		
	PVK agar	MPVK agar	NBRIP agar
R.leguminosarum SS-8	4	7	13
R.leguminosarum SS-12	2	5	12

Table 3 Tricalcium Phosphate (TCP) solubilisation (µgram/ml) and decrease in pH by P solubilising *R.leguminosarum* strains in PVK and NBRIP broth after three days of incubation

Phosphate Solubilising *R.leguminosarum*	PVK		NBRIP	
	TCP solubilized (µgram/ml)	pH	TCP solubilized (µgram/ml)	pH
R.leguminosarum SS-8	211	4.20	261	4.14
R.leguminosarum SS-12	187.2	5.22	250.7	4.35

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