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Studies on phosphate solubilization by *Rhizobium* sp. nodulating *Vigna unguiculata* (L.) Walp

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Six *Rhizobium* strains from *Vigna unguiculata* were examined for their tricalcium phosphate solubilizing activity on Pikovskaya's agar and broth. All the six isolates showed zone of phosphate dissolution on Pikovskaya's agar medium. These isolates showed varying phosphate solubilizing activity in Pikovskaya's broth. Glucose and sucrose were found to be the best carbon sources, while ammonium sulphate was the best nitrogen source. EDTA supported maximum phosphate solubilizing activity over controls. In these six isolates CPR5 and CPR2 are efficient phosphate solubilizers under various cultural conditions.

The root nodule bacteria of leguminous plants, which fix atmospheric nitrogen under symbiotic conditions, is now considered as an important member of PGPR under free living conditions in rhizosphere soil by their various activities including solubilization of insoluble phosphates. However, this aspect of phosphate solubilization by rhizobia has not received much attention. But it is essential for selection of efficient strains for legume crop improvement. Cowpea [*Vigna unguiculata* (L.) Walp] is an important food legume and an integral part of traditional cropping systems in the semi-arid regions of the tropics. Six *Rhizobium* strains were isolated from the fresh healthy root nodules of Cowpea plants grown in different soil samples and were designated as CPR1, CPR2, CPR3, CPR4, CPR5 and CPR6 (CPR stands for Cowpea *Rhizobium*). They were identified as *Rhizobium* sp. by morphological, cultural and biochemical characteristics. These strains were used to study for their phosphate solubilizing activity (PSA).

Six isolates of *Rhizobium* were screened for their PSA on Pikovskaya's tricalcium phosphate (TCP) agar plates. All the six isolates showed phosphate solubilization zone on TCP plates. After six days of incubation, the zone of phosphate solubilization on agar plates ranged from 12mm to 14mm. Maximum phosphate solubilization was observed with the isolate CPR5 followed by CPR2.

These isolates were used to study their phosphate solubilizing efficiency in broth cultures. The amount of phosphate solubilized was estimated by the method described by Subba Rao (1993). In these six *Rhizobium* isolates the isolate CPR5 showed maximum phosphate solubilization 56.0 µg/ml followed by CPR2 54.0 µg/ml. The isolate CPR6 is less efficient (18.2 µg/ml) than the other five isolates. CPR1 and CPR4 showed almost equal PSA (44.2 and 43.4 µg/ml).

Various factors like carbon sources, nitrogen sources and cell wall affecting agents are known to affect growth and PSA of rhizobia (Halder *et al*., 1991). The effect of carbon sources on phosphate solubilization was studied by replacing glucose in the medium by the equal amount of other carbon sources. These *Rhizobium* isolates utilized a variety of carbon compounds as energy source, but the amount of 'P' solubilization varied with different compounds. Among all the carbon sources tested, glucose supported maximum phosphate solubilization by these isolates. In these six *Rhizobium* isolates, the isolate CPR5 showed maximum PSA 56.0 mg/ml in glucose containing medium. Gaur (1990) reported that glucose was found to be the best carbon source for maximum phosphate solubilization. Almost all the isolates showed maximum PSA in glucose containing medium. PSA was high in glucose containing medium by the isolates CPR1, CPR4 and CPR5. The isolate CPR3 showed PSA in most of the carbon sources tested, but the efficiency of this isolate varied with the carbon source (20.0 mg/ml to 52.0 mg/ml). This isolate showed maximum PSA in sucrose containing medium followed by xylose. The isolate CPR6 showed maximum PSA (31.2 mg/ml) in lactose containing medium followed by sucrose and maltose. Statistical analysis (ANOVA) revealed that the variation between treatments and the difference between isolates were also insignificant.

Among all the nitrogen sources tested, maximum TCP solubilization (27.8 to 62.4 µg/ml) was recorded in presence of ammonium sulphate by all the six isolates. The isolate CPR3 showed PSA in almost all the nitrogen sources tested except potassium nitrate, sodium nitrate and glutamie acid. Statistical analysis showed that the variation between treatments and the difference between isolates were insignificant.

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Table 1 : Effect of carbon sources on phosphate solubilization by *Rhizobium* isolates from *V. unguiculata*

Carbon source (1%)	*Rhizobium* isolates					
	CPR1	CPR2	CPR3	CPR4	CPR5	CPR6
	Pr	Pr	Pr	Pr	Pr	Pr
*Control	12.0	16.0	24.2	22.0	20.6	16.0
Mannitol	25.0	24.6	36.8	23.4	50.0	22.2
Glucose	44.2	54.0	26.6	43.4	56.0	18.2
Maltose	22.6	--	--	--	--	24.3
Galactose	--	--	20.0	--	--	--
Lactose	--	--	--	--	36.6	31.2
Sucrose	--	--	52.0	--	--	28.6
Fructose	--	36.2	--	37.8	46.8	--
Inositol	--	--	32.0	--	--	--
Xylose	26.8	24.2	44.0	--	40.4	--
Rhamnose	--	32.4	22.2	--	--	--
Mannose	--	--	32.0	--	24.0	--
Arabinose	22.4	--	26.0	--	42.0	--
Sorbose	15.0	--	22.0	24.2	--	--

*without carbon source Pr = Phosphorus released

Table 2 : Effect of nitrogen sources on phosphate solubilization by *Rhizobium* isolates from *V. unguiculata*

Nitrogen source (1%)	*Rhizobium* isolates					
	CPR1	CPR2	CPR3	CPR4	CPR5	CPR6
	Pr	Pr	Pr	Pr	Pr	Pr
*Control	--	--	--	--	--	--
Potassium nitrate	24.2	--	--	--	--	--
Ammonium sulphate	62.4	27.8	38.4	45.0	60.2	44.0
Sodium nitrate	--	--	32.2	--	--	--
Sodium nitrite	--	--	--	36.2	--	--
L-aspargine	--	--	33.6	--	50.2	16.0
L-glycine	--	24.2	30.4	--	--	--
Glutamine	22.4	--	38.0	--	--	--
L-glutamic acid	--	16.4	--	--	--	--
Tyrosine	--	--	30.0	--	--	--
Alanine	--	--	36.0	--	--	--
Casamino acid	--	--	26.4	--	--	--
Cystein	--	--	22.4	--	--	--

*without nitrogen source Pr = Phosphorus released

Table 3: Effect of cell wall affecting agents on phosphate solubilization by *Rhizobium* isolates from *V. unguiculata*

Cell wall affecting agents	Concentration	*Rhizobium* isolates					
		CPR1	CPR2	CPR3	CPR4	CPR5	CPR6
		Pr	Pr	Pr	Pr	Pr	Pr
*Control		44.2	54.0	26.6	23.4	56.0	18.2
EDTA	0.1%	46.0	56.2	50.0	25.0	60.4	26.2
SDS	0.1%	28.0	30.0	18.0	36.0	26.0	20.8
Penicillin	50 IU	32.0	22.0	24.0	28.2	22.4	28.6
Lysozyme	50 IU	26.0	24.2	26.2	28.6	28.2	22.0

* without addition of cell wall affecting agents Pr = Phosphorus released

All the six isolates showed high PSA in EDTA (0.1%) containing medium (25.0 µg/ml to 60.4 µg/ml) over controls. The other cell wall affecting agents decreased PSA when compared to the controls. Statistical analysis revealed that the variation between treatments and the difference between isolates were also insignificant.

Phosphate solubilization is caused only by the production of organic acids (Halder *et al*., 1991). The final pH of the medium ranged from 2.4 to 6.9. The amount of TCP solubilized was low at pH 2.4 with glucose. This showed that low pH was ideal for phosphate solubilization.

This study suggests that the *Rhizobium* strains isolated from root nodules of *Vigna unguiculata* showed PSA under various cultural conditions may be exploited as efficient biofertilizers.

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REFERENCES

Gaur, A.C. (1990). *Phosphate solubilizing microorganisms as biofertilizers*, Omega Scientific Publishers, New Delhi, pp. 141-151.

Halder, A.K., Mishra, A.K. and Chakraborthy, P.K. (1991). Solubilization of inorganic phosphates by *Brodyrhizobium*. *Indian J. Expt. Biol.*, **29**: 28-31.

Subba Rao, N.S. (1993). Phosphate solubilizing Microorganisms. *In*: Biofertilizers in Agriculture and Forestry (Subba Rao, N.S. ed.), Oxford & IBH Publishing Co., 129-135.

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