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Effect of different carbon, nitrogen and cell wall affecting agents on phosphate solubilization by *Rhizobium* sp. nodulating *Macrotyloma uniflorum* (Lam.) Verdc.

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ABSTRACT

Thirty two *Rhizobium* strains from horsegram *Macrotyloma uniflorum* were examined for their tricalcium phosphate solubilizing activity on Pikovskaya's agar and broth. Only six strains showed zone of phosphate dissolution on Pikovskaya's agar medium. These strains showed varying phosphate solubilizing activities in Pikovskaya's broth. Various compounds of carbon, nitrogen and cell wall affecting agents were tested for their effect on tricalcium phosphate solubilization. Glucose and sucrose were found to be the best carbon source, while ammonium sulphate as best nitrogen source. EDTA supported maximum phosphate solubilizing activity over controls. In these six strains HGR 19 and HGR 22 were efficient phosphate solubilizers under various cultural conditions.

Key words : Phosphate solubilization, *Rhizobium* sp., *Macrotyloma uniflorum*, Horsegram.

INTRODUCTION

Phosphorus is one of the major plant nutrients required in optimum amount for proper plant growth. The role of micro-organisms is solubilizing insoluble phosphates in soil and making it available to plant (Kundu and Gaur, 1981). Soil microorganisms especially by bacteria (Garg *et al*., 1989 and Krishnaraj *et al*., 1999) play a very significant role in mobilizing P for the use of plants bringing about changes in pH of the soil microenvironment and producing chelating substances which lead to the solubilization of native as well as insoluble phosphates. Rhizobia share characteristics with plant growth promoting rhizobacteria (PGPR). They promote the growth of plants either directly through N_2 fixation, supply of nutrients, syntheis of phytohormones and solubilization of minerals.

Macrotyloma uniflorum (Lam.) Verdc. commonly known as horsegram is an important pulse crop of South India. It derives its importance for its adaptability to severe drought and environmental conditions. Very little is known about the *Rhizobium* sp. associated with root nodules of this host. Thirty two *Rhizobium* strains were isolated from the fresh healthy root nodules of *M. uniflorum* plants grown in thirty two soil samples collected from various parts in Andhra Pradesh. They were identified as *Rhizobium* sp. by morphological, cultural and biochemical characteristics. These strains were used to study for their phosphate solubilizing activity.

MATERIALS AND METHODS

All the thirty two isolates were screened for their phosphate solubilizing (PS) activity on Pikovskaya's tricalcium phosphate (TCP) agar plates. Six strains *viz*., HGR1, HGR18, HGR19, HGR20, HGR22 and HGR27 which showed phosphate solubilization zone on TCP plates were selected to study their phosphate solubilizing efficiency in broth cultures. For this, 50 ml of Pikovskaya's broth was inoculated with 1 ml of the inoculum of each isolate. They were incubated for 10 days on gyrorotary shaker at 28 ± 2°C. Culture broth was centrifuged at 10,000 g for 15 minutes. The pelleted bacterial cells were separated by filtration and the supernatant was used for the estimation of the amount of phosphate solubilized by the method described by Subba Rao (1993). Uninoculated flask from each set was kept as control.

The objective of this study was therefore to investigate the phosphate solubilizing activity of these strains and regulation of various factors like carbon sources nitrogen sources and the effect of cell wall affecting agents.

RESULTS AND DISCUSSION

Six strains showed zone of phosphate solubilization on TCP plates. After six days, the zone of phosphate solubilization on agar plates ranged from 10 mm to 12 mm. Maximum phosphate solubilization was observed with the strain HGR22 (50mg/ml) followed by HGR19. These results (Table 1) showed a lot of variation in P-solubilization efficiency. Maxwell and Bateman, 1967; Cerezine *et al*., 1988; Halder *et al*., 1991 reported that carbon and nitrogen compounds affect the microbial phosphate solubilization. These *Rhizobium* strains 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 was found best comparatively better. The strain HGR22 showed maximum PS activity 50.0 mg/ml in glucose

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

Carbon source (1%)	*Rhizobium* isolates					
	HGR1	HGR18	HGR19	HGR20	HGR22	HGR27
	Pr	Pr	Pr	Pr	Pr	Pr
*Control	12.2	6.0	24.2	11.0	20.4	6.0
Mannitol	25.0	14.6	36.6	23.4	50.0	10.2
Glucose	25.6	14.0	37.0	23.0	50.0	10.2
Maltose	12.6	--	--	--	--	14.3
Galactose	--	--	20.0	--	--	--
Lactose	13.2	--	--	--	36.6	51.2
Sucrose	--	--	50.0	--	--	38.6
Fructose	--	--	33.8	27.8	46.8	--
Inositol	--	--	30.0	--	--	--
Xylose	--	--	44.0	--	40.2	--
Rhamnose	--	--	22.2	--	--	--
Mannose	--	--	20.0	--	24.0	--
Arabinose	--	--	16.0	--	22.0	--
Sorbose	15.0	--	--	--	--	--

*Without carbon source Pr = Phosphorus released

The variation between treatments is insignificant (Fc=1.873; Ft=2.684) and the difference between strains is also insignificant (Fc=2.356; Ft=6.708). (Fc= F calculated value; Ft= F table value).

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

Nitrogen source (1%)	*Rhizobium* isolates					
	HGR1	HGR18	HGR19	HGR20	HGR22	HGR27
	Pr	Pr	Pr	Pr	Pr	Pr
*Control	--	--	10.2	--	--	--
KNO_3	--	--	34.2	--	--	--
$(NH_4)_2SO_4$	30.0	17.8	38.4	35.0	58.0	22.0
$NaNO_3$	--	--	32.2	--	--	--
$NaNO_2$	--	--	36.2	--	--	--
L-aspargine	--	--	33.6	--	50.2	6.0
L-glycine	--	14.2	20.4	--	--	--
Glutamine	26.2	--	38.0	--	--	--
L-glutamic acid	--	16.4	--	--	40.2	--
Tyrosine	--	--	30.0	--	--	--
Alanine	28.0	--	26.0	--	--	--
Casamino acid	--	--	36.4	--	30.4	--
Cystein	--	--	32.4	--	--	--

*Without nitrogen source Pr = Phosphorus released

The variation between treatments is insignificant (Fc=1.917; Ft=3.286) and the difference between strains is also insignificant (Fc=2.368; Ft=11.464).

containing medium. Glucose has been reported to be the best carbon source followed by sucrose and galactose by *Pseudomonas striata* (Gaur, 1990).

The strain HGR19 showed PS activity in almost all the carbon sources tested, but the efficiency of this strain varied with the carbon source (50.0 mg/ml to 16.0 mg/ml). This strain showed maximum PS activity in sucrose containing medium followed by xylose. The strain HGR20 showed maximum PS activity (27.8 mg/ml) in fructose containing medium. The strain HGR27 showed maximum PS activity in lactose containing medium. Statistical analysis (ANOVA) revealed that the variation between treatments and the difference between strains is also insignificant.

Among all the nitrogen sources (Table 2) maximum TCP solubilization of 58.0 μg/ml was recorded in HGR 22 and the presence of ammonium sulphate produced higher TCP solubilization in by all the six strains. The strain HGR19 showed PS activity in almost all the nitrogen sources tested. Statistical analysis (ANOVA) showed

Table 3: Effect of cell wall affecting agents on growth and indole acetic acid production by *Rhizobium* isolates from *M. uniflorum*

Cell wall affecting agents	Concentration	*Rhizobium* isolates					
		HGR1	HGR18	HGR19	HGR20	HGR22	HGR27
		Pr	Pr	Pr	Pr	Pr	Pr
*Control		25.0	14.6	36.6	23.4	50.0	10.2
EDTA	0.1%	36.0	24.2	50.0	35.0	60.2	30.4
SDS	0.1%	23.0	10.0	28.0	26.0	24.0	20.2
Penicillin	50 IU	20.0	12.0	24.0	20.3	22.2	18.0
Lysozyme	50 IU	16.0	14.2	20.3	18.4	20.0	10.0

*Without nitrogen source Pr = Phosphorus released

The variation between treatments is insignificant (Fc=2.866; Ft=11.918) and the difference between strains is also insignificant (Fc=2.710; Ft=7.560).

that the variation between treatments and the difference between strains is insignificant.

All the six strains showed high PS activity in EDTA (0.1%) containing medium (24.2 mg/ml to 60.2 mg/ml) over controls. (Table 3) The other cell wall affecting agents decreased PS activity over controls. Statistical analysis (ANOVA) revealed that the variation between treatments and the difference between strains is 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 2.9 to 6.9. The amount of TCP solubilized was low at pH 2.9 with glucose. This showed that low pH was ideal for solubilization.

The *Rhizobium* strains isolated from root nodules of *M. uniflorum* (horsegran) showed PS activity under various cultural conditions which may be exploited as efficient biofertilizers.

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