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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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