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Short Communication

In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria

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Crops absorb phosphorous in the form of soluble orthophosphate ion. The solubility of phosphate is inhibited by the presence of iron and aluminium in acidic soils and calcium in neutral and alkaline soils. This leads to fixation of phosphorous, making it not available to crop plants. The phosphate solubilizing bacteria (phosphobacteria) secretes some kinds of organic acids which act on insoluble phosphates and convert the same into soluble form, thus providing phosphorous to plant. An experiment was conducted to enumerate the population density of phosphobacteria in the rhizosphere soils of brinjal, chilly, cotton, green grain, groundnut, maize, paddy, ragi, sorghum and turmeric using Ketznelson and Bose medium following dilution plate technique. Efforts have been made to isolate phosphobacteria from these soils and isolated strains were inoculated in specific media containing specific substrates to produce growth regulating substances such as IAA and GA3 and phosphatase enzyme. The result showed that the population levels of phosphobacteria were higher in the rhizosphere soil of groundnut plant. Further, all the strains of phosphobacteria were able to produce phytohormones and phosphatase enzyme under in vitro conditions.

Key words: *In vitro*, phosphobacteria, growth regulators.

INTRODUCTION

Large scale use of chemical fertilizers causes pollution of soil. Among fertilizers constituents, phosphorous is one of the major nutrients for plants and it plays an important role in plant metabolism by supplying energy required for metabolic processes (Lal, 2002). However, plants cannot absorb insoluble forms of phosphorous and has to be converted into soluble forms by phosphatase enzyme such as acidic and alkaline phosphatase.

Several soil bacteria, particularly those belonging to phosphate solubilizing bacteria (phosphobacteria), posses the ability to solubilize insoluble inorganic phosphate and make it available to plants. The solubilization effect is generally due to the production of organic acids by these organisms. They are also known to produce amino acids, vitamins and growth promoting substances like indole acetic acid (IAA) and gibberellic acid (GA3) which help in better growth of plants.

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Phosphobacteria have been found to produce some organic acids such as monocarboxylic acid (acetic, formic), monocarboxylic hydroxy (lactic, glucenic, glycolic), monocarboxylic, ketoglucenic, decarboxylic (oxalic, succinic), dicarboxylic hydroxy (malic, maleic) and tricarboxylic hydroxy (citric) acids in order to solubilize inorganic phosphate compounds (Lal, 2002). The present study was undertaken to study in detail about the distribution and population density of phosphobacteria in rhizosphere soils of different crop plants and their production capacity of growth regulators and phosphatase enzyme under in vitro condition.

MATERIAL AND METHODS

Rhizosphere soil samples were collected from different field crops such as brinjal, chilly, cotton, green grain, groundnut, maize, paddy, ragi, sorghum and turmeric. Air dried soil samples were used to enumerate phosphobacteria using Ketznelsum and Bose medium (1959) following dilution plate technique. The phosphorous solubilization potential of selected strains of phosphobacteria was

Table 1. Population density of Phosphobacteria in rhizosphere soils of different field crops

Name of field Crops	Population density (x 10^5/g soil dry wt)	Designation of strain
Brinjal	12.34	BP07
Chilly	10.42	CP01
Cotton	13.41	CP02
Green gram	08.08	GP01
Ground nut	14.90	GP02
Maize	08.61	MP01
Paddy	10.08	PP04
Ragi	07.33	RP07
Sorghum	07.66	SP03
Turmeric	11.56	TP03

Table 2: *In vitro* production of growth promoting substances by Phosphobacterial strains

Name of strain	Growth promoting ubstances (ppm)	
	IAA	GA3
BP03	34.02	13.95
CP01	45.31	15.08
CP02	36.51	13.32
GP01	40.92	16.85
GP02	34.38	14.04
MP01	35.31	12.88
PP04	40.41	10.04
RP07	43.08	14.83
SP03	41.44	12.47
TP03	38.53	13.27

tested in vitro by estimating available phosphorous in the Pikovaskay's medium amended with tricalcium phosphate as a substrate.

Five days old cultures of phosphobacteria were transferred to Pikovaskay's broth containing L-tryptophan as a substrate for the production of IAA and GA3. Inoculated cultures were kept in a shaker for about five days under room temperature. Culture filtrates were centrifuged and subjected to IAA and GA3 analysis following the procedure of Tien et al. (1979).

For phosphate solubilization, phosphobacteria produce phosphatase enzyme. In an attempt to study the phosphatase activity in response to phosphorous enrichment, experiments were set up using known bacterial broth cultures in flasks with and without adding phosphorous source (-glycerophosphate used as a substrate). Culture filtrates were centrifuged and subjected to estimate phosphatase activity following the procedure of Tabatabai and Brimner (1969).

Table 3: *In vitro* phosphorous solubilizing capacity and phosphatase activity

Name of strain	Available P (ppm)	Phosphatase activity Micro moles/g/hr
BP07	29.41	17.55
CP01	30.44	21.31
CP02	28.08	16.23
GP01	24.88	14.74
GP02	44.08	36.87
MP01	35.56	24.57
PP04	29.41	19.08
RP07	40.69	30.34
SP03	42.38	32.38
TP03	34.47	22.76

RESULTS AND DISCUSSION

The result showed that there was a significant difference on the population density of phosphobacteria in different crop soils. The population level was higher in the rhizosphere soil of groundnut followed by cotton when compared to other crop plants (Table 1). This variation in the population of phosphobacteria might be attributed to many soil factors such as soil nutrients, pH, moisture contents, organic matter and some soil enzyme activities. The result thus throws light on the existence of microbial solubilizing of phosphorous in rhizosphere soils of different field crops. Baby et al. (2001) carried out an investigation on microbial dynamics in the rhizosphere of tea clones and reported that there was a significant difference on the population level of phosphobacteria in different clones of tea. Furthermore, they were reported that the population of nitrogen fixing Azospirillum and phosphate solubilizing bacteria were higher in young tea fields than older fields.

The result on the production of growth promoting substances indicated that all the strains of phosphobacteria were able to produce phytohormones such as IAA and GA3 under in vitro conditions (Table 2). The strain CP01 isolated from the rhizosphere soil of chilly plant produced higher of amount of IAA followed by RP07. In the case of GA3 production, strain GP01 isolated from green gram soil was the highest followed by CP01. Tien et al. (1995) reported that Azospirillum and phosphobacteria isolated from the soil of pearl millet produced IAA, GA3 and cytokinin-like substances which ultimately enhanced the plant metabolism. Bacteria isolated from rhizosphere soils are known to produce growth-regulating substances (Brown, 1972), and some of them are capable of dissolving phosphate (Barea et al., 1976). Some of the phosphobacteria are also able to produce vitamins towards the dissolution of bicalcium phosphate (Baya et al., 1981) . All the strains of phosphobacteria were able to solubilize inorganic phosphate. Phosphate solubilizing bacteria are capable of producing physiologically active auxins that may have pronounced effects on plant growth. The cultures release greater quantities of IAA in the presence of a physiological precursor, tryptophan, in a culture medium. Production of IAA varies greatly among different species and is also influenced by culture conditions, growth

stage and availability of substrate(s) (Brown, 1972; Vijila, 2000).

The result on the phosphatase enzyme activity showed that the strain GP02 which was isolated from groundnut rhizosphere soil had higher activity followed by the strain SP03 isolated from Sorghum soil (Table 3). However, there was a positive correlation between phosphate solubilizing capacity and phosphatase enzyme activity. This might be due to availability of higher amount of phosphorous in the medium (Barik and Purushothaman, 1998). There is increasing evidence that phosphobacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in releasing available phosphorous.

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REFERENCES

Baby UI, Tensingh, Baliah N, Ponmurugan P, Premkumar R (2001). Population dynamics of nitrogen fixing and phosphate solubilising bacteria in tea soil. UPASI Tea Res. Found. Newsletter, 10 (2) : 4.

Barea JM, Ocampo JA, Azcon R, Olivares J, Montoya E (1978). Effect of ecological factors on the establishment of Azotobacter in the rhizosphere. Ecological Bulletin (Stockholm) 26 : 325-330.

Barik SK, Purushothaman CS (1998). Phosphatase activity of two strain of bacteria on orthophosphate enrichment. Proc. Natl. Frontiers in Appl. Environ. Microbial . Dec.11-13, SES, CUSAT. Cochin. pp.165-170.

Baya, M.A., Boehhing, R.S. and Ramos-Cormenzana, A. 1981. Vitamin production in relation to phosphate solubilization by bacteria. Soil biol. Biochem. 13 : 527-531.

Brown, M.E. 1972. Plant growth substances produced by microorganism of soil and rhizosphere. J. Appl. Bacteriol. 35 : 443.

Ketznelson H, Bose B (1959). Metabolic activity and phosphate dissolving capacity of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Can. J. Microbiol. 5 : 79-82.

Lal L, (2002). Phosphatic biofertilizers. Agrotech, Publ. Academy, Udaipur, India, 224p.

Tabatabai MA, Bremner JM (1969). Use of P-nitrophenol phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301-307.

Tien TM, Gaskins MH, Hubbell DH (1979). Plant growth substances produced by Azospirillum brasilense and their effect on growth of pearl millet pennisetum americanum (L.) Appl. Environ.Microbiol., 37 :1016-1024.

Vijila K (2000). Estimation of IAA production in nitrogen fixing microorganisms. Practical manual-microbial interaction in soil. Tamil Nadu Agricultural University, Coimbatore. pp 38-39.