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PHOSPHORUS SOLUBILIZING PLANT GROWTH PROMOTING BACTERIA (PGPB) FROM MAIZE RHIZOSPHERE

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Abstract

In the present research about 40 isolates of PGPB from the bacterial group namely *Azospirillum brasilense, Bacillus megaterium, Pseudomonas fluorescens,* were isolated from the maize rhizosphere and *Gluconacetobacter diazotrophicus* was isolated roots of maize and all the isolates tested for phosphate solubilizing efficiency. Among the PGPB organisms *Azospirillum* failed to show efficiency in phosphate solubilization and remaining 30 isolates showed phosphate Solubilizing efficiency and among which G. *diazotrophicus* showed and recorded with maximum values for phosphate solubilization compared with other PGPB isolates. Further the efficiency of these isolates must be exploited under different environmental conditions namely in field.

Key words: Rhizosphere, PGPB, Maize and Phosphorus solubilizing

1. Introduction

PGPB organisms are known for many beneficial roles in the stimulation and enhanced growth and yield of agricultural and horticultural crops. These plant growth promoting bacterial organisms exerting benefits to the plants through 'N' fixation, 'P' solubilization, 'Z' solubilization, growth promoting hormones production and siderophore production. PGPB also protecting the root region namely rhizosphere by suppressing and eradicating pathogenic microbes. Hence, in the recent years soil microbiologist concentrating and focusing studies on the rhizobacteria and endophytic bacteria to find the growth promoting nature. These studies pave way for finding alternative for chemical inputs. In the recent years molecular studies on the isolation and enumeration studies reviewed the PGPR and likely to call as PGPB due to their presence everywhere in the soil as well as in plant parts as endophytes. The PGPB organisms able to promote crop yield with the reduction of chemical fertilizers virtually by efficient 'N' fixation, 'P' solubilization, production of growth promoting hormones and by biocontrol nature against pathogenic microbes.

Phosphorous (P) is the major essential macronutrients for plants and is supplied to soil in the form of phosphatic fertilizers. However, the phosphorous has applied to the soil as chemical fertilizer creates a condition in which phosphorus became unavailable to plants.

Rhizosphere bacteria can able to enhance the plant growth and plant productivity by different mechanisms. One of the most vital role is the dissolution of insoluble phosphorous in soil and converting phosphorous (P) available for plant intake.

Phosphorus is widely present in soil as organic and inorganic forms. The majority of total P in soils is present in organic forms (Speir and Ross, 1978) as phospholipids, nucleotides and

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inositol phosphate (Turner *et al*., 2002). Soil organic phosphorus (SOP) plays a major role in P nutrition of crops especially in high P-fixed calcareous soils (Tarafdar and Claasson, 1988). The SOP can contribute substantially to total phosphorus, ranging from 20 to 80% in most mineral soils and can supply a important portion of plant available P (Sharpley, 1985). It must be first converted into inorganic form after being mineralized and catalysed by different soil enzyme processes (Sarapatka, 2003). Soil phosphatases enzyme helps in hydrolysis of soil organic phosphorus which convert it into inorganic forms (HPO_4 - and H_2PO_4 -) before it can be utilized and taken up by plant roots from the soil solution (He *et al* ., 2004). This reaction is catalyzed by phosphatase enzymes present in soil, microorganisms, and plant roots and also in extracellular forms in soil. Phosphatase-catalyzed reactions are involved in the hydrolysis of both esters and anhydrides of H_3PO_4 (Tabatabai, 1994). Phosphatases are classified as acid phosphatases and alkaline phosphatases because their maximum activities can occur at low (pH 6.5) and high (pH 11) pH ranges, respectively. Acid phosphatases are produced by both microorganisms as well as higher plants but alkaline phosphatases are mainly produced by microorganisms (Tarafdar *et al*., 2001).

The release of organic substances by plant roots has an interesting ecological aspect, since it influences the nutrient availability in the rhizosphere and indirectly acts on the soil microorganisms that in turn influence plant growth (Graystone *et al*., 1998). Rhizosphere organism namely 'P' solubilizing organisms includes, *Bacillus megaterium* and *Pseudomonas fluorescens*. The present research aimed to isolate 'P' solubilizing PGPB organisms from the rhizosphere and plant parts of maize and to know the efficiency of PGPB organisms namely *Bacillus megaterium, Pseudomonas fluorescens* and *Gluconacetobacter diazotrophicus* on the dissolution of phosphorus.

2. Materials methods

Screening of phospahate solubilizing Bacteria for 'P' solubilization

The forty PGPB isolates (*Azospirillum brasilense, Bacillus megaterium, Pseudomonas fluorescens* and *Gluconacetobacter diazotrophicus*) were isolated from different location of Salem district. The efficiency of all the isolates on phosphate solubilization were determined by estimating the amount of soluble phosphorus released from tri-calcium phosphate (TCP) of the medium.

Screening of *Bacillus* isolates
Determination of soluble phosphorous

Take 50 ml of Pikovskaya's broth containing 100 mg of tri-calcium phosphate (TCP) were prepared and sterilized. The flasks were inoculated with *Bacillus* isolates and incubated at 30±1°C in controlled environmental conditions for 7 days incubation period in a shaker. After the incubation period, the culture media were centrifuged at 10000 rpm for 10 min and the clear supernatant was used for soluble P estimation by the method described by Olsen *et al*. (1954).

Screening of *Pseudomonas* isolates
Determination of soluble phosphorous

Take 50 ml of Pikovskaya's broth containing 100 mg of tri-calcium phosphate (TCP) were prepared and sterilized. The flasks were inoculated with *Pseudomonas* isolates and incubated at 30±1°C in controlled environmental conditions for 7 days incubation period in a shaker. After the incubation period, the culture media were centrifuged at 10000 rpm for 10 min and the clear supernatant was used for soluble P estimation by the method described by Olsen *et al*. (1954).

Screening of *Gluconacetobacter* isolates
Determination of soluble phosphorous

Take 50 ml of Pikovskaya's broth containing 100 mg of tri-calcium phosphate (TCP) were prepared and sterilized. The flasks were inoculated with *Gluconacetobacter* isolates and incubated at 30±1°C in controlled environmental

conditions for 7 days incubation period in a shaker. After the incubation period, the culture media were centrifuged at 10000 rpm for 10 min and the clear supernatant was used for soluble P estimation by the method described by Olsen *et al*. (1954).

Preparation of reagent A

A quantity of 12.0 g of ammonium molybdate was dissolved in 250 ml of distilled water. Separately 0.294 g of antimony potassium tartarate was dissolved in 100 ml of 0.5 ml Sulphuric acid. This solution was mixed thoroughly and the volume was made upto two litres with distilled water.

Preparation of reagent B

About 1.056 g of ascorbic acid was dissolved in 200 ml of reagent A.

Estimation of phosphorus

One ml of culture filtrate was pipette out into a 250 ml volumetric flask and diluted to 20.0 ml with sterile distilled water. Four ml of reagent B was added to the flask and the volume was made upto 25 ml with distilled water. The intensity of blue colour developed was read at 660 nm in UV –VIS spectrophotometer (M/s Elico) using appropriate reagent blanks. The standard graph prepared with known quantities of potassium dihydrogen orthophosphosphate was used for calculating the 'P' content of the sample. The quantity of 'P' solubilized was expressed as mg soluble of P 100 Mg^{-1} of TCP.

3. Results and Discussion

Phosphate solubilizing potentiality of PGPB isolates obtained from the rhizosphere of maize

The Phosphorus solubilization nature of PGPB organisms (*Bacillus, Pseudomonas* and *Gluconacetobacter*) is mainly due to the production of several organic acids by the organisms. Among the PGPB isolates *Gluconacetobacter* known to produce strong organic acids by utilizing sugars.

Screening of *Bacillus* isolates for phosphate solubilizing efficiency

The *Bacillus* isolates were screened for release of phosphorous from tri - calcium phosphate (TCP) in Pikovskaya's broth. *Bacillus* recorded appreciable values in 'P' solubilization compared with *Pseudomonas.* The results are presented in Table - 1.

Solubilization of tri-calcium phosphate by all the PGPB isolates were studied on the seventh day after inoculation and the values varied considerably. The isolate BMRS-6 recorded maximum solubilization of (27.20 mg of P released from 100 mg of tri – calcium phosphate) phosphorous followed by BMRT-7.

Screening of *Pseudomonas* isolates for phosphate solubilizing efficiency

The *Pseudomonas* isolates were screened for their phosphate solubilizing efficiency and the results are presented in Table 2. The amount of phosphate solubilization ranged from 4.80 to 23.22 mg of P released from 100 mg of tri – calcium phosphate. Among the ten different isolates *Pseudomonas,* the isolate PMRA-5 was showed maximum phosphate solubilizing capacity (23.22 mg of P released from 100 mg of tri – calcium phosphate) followed by Reference strain and PMRT-7.

Screening of *Gluconacetobacter diazotrophicus* isolates for phosphate solubilizing efficiency

All the ten *Gluconacetobacter diazotrophicus* isolates collected from maize root samples from ten different locations of Salem district, Tamilnadu. The *G. diazotrophicus* isolates showed appreciable amount of phosphates solubilizing capacity. All the isolates showed phosphate solubilizing capacity not less than 8.30 mg of P released from 100 mg of tri-calcium phosphate. The *G. diazotrophicus* isolate GdMRS - 6 showed maximum phosphate solubilizing capacity (30.70 mg of P released from 100 mg of tri – calcium phosphate). Among the ten isolates GdMRS - 6 recorded significant values when compared with other isolates except the isolate

GdMRT - 7, both are on par with one another (Table - 3).

Table - 1: Screening of *Bacillus* isolates for phosphate solubilizing efficiency

Name of the isolate	Phosphorous solubilized*
BMRV-1	14.40
BMRG-2	16.80
BMRT-3	6.30
BMRV-4	13.22
BMRA-5	20.00
BMRS-6	27.20
BMRT-7	22.60
BMRJ-8	8.99
BMRP-9	10.33
BMRP-10	9.48
Reference strain (MTCC 10127)	20.80
CD	14.40
S.E.	2.10

*mg of P released from 100 mg of tri – calcium phosphate

Table - 2: Screening of *Pseudomonas* isolates for phosphate solubilizing efficiency

Name of the isolate	Phosphorous solubilized*
PMRV-1	16.48
PMRG-2	12.60
PMRT-3	7.46
PMRV-4	4.80
PMRA-5	23.22
PMRS-6	14.40
PMRT-7	20.00
PMRJ-8	10.20
PMRP-9	9.39
PMRP-10	6.93
MTCC-9768	20.30
CD	2.52
S.Ed	1.20

*mg of P released from 100 mg of tri – calcium phosphate

Table – 3: Screening of *Gluconacetobacter* isolates for phosphate solubilizing efficiency

Name of the isolate	Phosphorous solubilized*
GdMRV-1	15.20
GdMRG-2	18.88
GdMRT-3	10.48
GdMRV-4	8.30
GdMRA-5	24.66
GdMRS-6	30.70
GdMRT-7	26.46
GdMRJ-8	9.99
GdMRP-9	12.33
GdMRP-10	21.36
PAL5 (Reference strain)	25.66
CD	4.80
S.E.	2.15

*mg of P released from 100 mg of tri – calcium phosphate.

In the present research, all the PGPB organisms from the rhizosphere soil samples of maize showed positive trend in the solubilization of 'P' in laboratory condition. Among the different isolates of PGPB, the isolates belongs to *G. diazotrophicus* showed and recorded maximum 'P' solubilizing efficiency compared with the isolates from *B. megaterium* and P. *fluorescens*. Hence it is a new future of *G. diazotrophicus* in 'P' solubilizing efficiency. In addition it's a wonder endophyte for 'N' fixation and growth promoting substances production.

Interestingly, the *G. diazotrophicus* showed superiority in 'P' solubilization when compared with *B. megaterium*. The present research outcomes are in accordance with the findings of (Muralikrishnan and Muthukaruppan, 1998; Vinoth and Prabudoss, 2015). Indian soils rich in P content where as the availability is minimum of phosphate. Hence, inoculation of solubilizing bacteria needed these PSB are common in the rhizosphere and secrete variety of organic acids and phosphatase enzyme which are converting the insoluble forms of P to soluble forms (Kim *et al*., 1998; Ponmurugan and Gopi, 2006).

The role of microorganisms in solubilizing insoluble phosphate in soil and making it available to plants is well known (Kundu and Gaur, 1984). Phosphate solubilizing microorganism includes several genera of bacteria *viz*., *Bacillus*, *Pseudomonas*, *Klebsiella* and *Serratia* (Nakas *et al*., 1987; Amardip *et al*., 2012 Sakthivel and Karthikeyan, 2012; Gandhimaniyan and Jayanthi 2013). Among the bacteria, most efficient phosphate solubilizing bacteria belonged to the genera *Bacillus* and *Pseudomonas* (Dave and Patel, 1999). The phosphate solubilizing bacterial isolates were screened based on the ability in medium (Banik and Dey, 1982; Dave and Patel 1999; Whitelaw *et al*., 1999). Further studies are needed to exploit the potentiality of these PGPB isolate on different environmental conditions.

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