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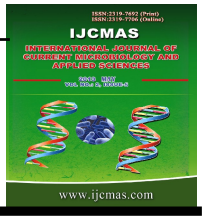

Original Research Article

Isolation, characterization and antagonistic effect of phosphate solubilizing microorganisms from *Vigna radiata* L. Rhizospheric soil

M.Kannahi* and N.Umaragini

P.G and Research Department of Microbiology and Division of Biotechnology
STET Women's college, Mannargudi, Tamil Nadu, India
**Corresponding author e-mail: kannahisri79@gmail.com*

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A B S T R A C T

Phosphorus is the second most important micronutrient required by the plants, next to nitrogen, is reported to be a critical factor of many crop production system. Microorganisms involved in the solubilization of insoluble phosphorus. From the rhizospheric soil of *Vigna radiate* L. phopsphate solubilizing bacteria like *P.fluorescens* and *B.subtilis* were isolated and fungi like *A.niger* and *F.oxysporum* were isolated. Then the PSM solubilizing activity was determined in National Botanical Reasearch Institute Phosphate Growth Medium and Pikovskayas agar medium. Then the phosphatase activity was determined by using spectrophotometer. Then finally antagonistic effect of PSM were also determined. These microorganisms mediate soil processes such as exudation of soluble compounds, storage and release of nutrients and water etc.

Introduction

Phosphorus, the second most important micronutrient required by the plants, next to nitrogen, is reported to be a critical factor of many crop production system, due to the fact that the limited availability in soluble forms in the soils (Xiao *et al*., 2011). Microbes present in the soil employ different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for plant to absorb. When phosphoric fertilizers are applied to the soil they often become insoluble (more than 70%) and are convert into complexes and in phosphate in the soil (Mittal *et al*., 2008 involved in the solubilization of insoluble phosphorus include bacteria, fungi, actinomycetes . Microorganisms and arbuscular mycorrhial (VAM) fungi (Khan *et al*., 2007; Wani *et al*., 2007; Xiao *et al*., 2009). Numerous rhizosphere microorganisms possessing phosphate solubilizing activity are reported (Mittal *et al*., 2008). Among the soil bacterial communities, ectorhizosphere strains from *Bacillus* and *Pseudomonas*(Wani *et al*., 2007). And endosymbiotic rhizobia have been described as effective phosphate solubilizers (Igual*et al*., 2001) strains from bacterial genera *Pseudomonas*,

Bacillus, Rhizobium, Enterobacter, Aspergillus and *Penicillium* from fungal genera (Wakelin *et al.,* 2004; Xieo *et al.,* 2012) are the most powerful phosphate solubilizers (Whtelaw *et al.,* 2012). The major microbiological means by which phosphate compounds are mobilized is the production of organic acids, accompanied by acidification of the medium. The type of organic acid produced and their amounts differ with different organisms. Among them glucuronic acid and 2-Ketogluconic acid seems to be the most frequent agent of mineral phosphate solubilisation (Song *et al.*,2008).Other organic acids such as acetic, citric, succinic, propionic, glycolic, oxalic, malonic, fumaric and sstartaric acid etc,have also been identified among phosphate solubilizers(Ahmed and Shahab,2001).

Tri and di –carboxylic acids are more effective as compared to mono basic and aromatic acids-Aliphatic acids are also found to more effective in phosphate solubilization compared to phenolic,citric and fumaric acids(Mahidi *et al.*,2007). Soil *Bacillus* and *Streptomyces sp* are able to mineralize very complex organic phosphates by production of extra cellular enzymes and phospholipases.

Several theories exist explaining the mechanisms of microbial phosphate solubilisation,namely: the sink theory, the organic acids theory and acidification by H+ excretion theory. In the sink theory,phosphate solubilizing organisms are able to remove and assimilate phosphate from the liquid medium and therefore stimulate indirect dissolution of Ca-p compounds by continuous removal of phosphate from broth (Halvorsson and Kornberg,1990).

The organic acid theory recognized as accepted by many researchers postulates that phosphate solubilising microorganisms produce organic acids leading to acidification of microbial cells and their surrounding and consequently the release of phosphate ions from the phosphate mineral by H^+ substitution for Ca^{2+} (Goldstein,1994).

The acidification by introduction of plants with Phosphate solubilizing microorganisms generally results in improved plant growth and yield, in particular under glass house conditions (Khan *et al.*, 2010). Enhancement of plant growth by improving biological nitrogen fixation is another beneficial effect of microorganisms with phosphate solubilizing potential (Ponmurugan and Gopi, 2006; Son *et al.*, 2006) have reported components, grain yield,nutrient availability and uptake in soybean were found to be enhanced by *pseudomonas sp. Pseudomonas* inoculation had favourable effect on salt tolerance of Zea mays L. under NaCl stress (Bano and Fatima, 2009).

Hence, the present study was under taken with the following objectives,Collection of sample from green gram rhizospheric soils at Lakshmangudi,Thiruvarur District, Tamilnadu,South India.Determination of phosphate solubilization and phosphatase activity. Antagonistic effect of phophate solubilizing microorganisms against *Fusarium oxysporum.*

Materials and Methods

Sample collection

Soil samples were collected from green gram rhizosphere soil at Lakshmangudi, Thiruvarur District, Tamilnadu, South India. The collected soil samples were

stored in polythene bags aseptically and maintained at the laboratory for further study

Isolation of phosphate solubilizing microorganisms

The serially diluted soil samples were placed on standard agar medium (pH 6.8 – 7.0) containing tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate. After 3 days of incubation at 30^{o}C, phosphate solubilizing bacteria developed clear zones around colonies. Colonies with clear zones were further purified by replating on the isolation of phosphate solubilizing fungi was done by serial dilution and plating method using soil extract agar medium supplemented with Tricalcium Phosphate.

Identification of phosphate solubilizing microorganisms

Bacterial identification

The morphological and biochemical tests were done by the methods described in experiments in Microbiology, Plant Pathology and Biotechnology (Aneja, 2005).

Phosphate Solubilization activity (Ngugen *et al.*, 1992)

All bacterial and fungal isolates were screened for inorganic phosphate solubilization. A loopfull of fresh bacterial and fungal cultures were streaked on to National Botanical Research Institute's Phosphate Growth Medium containing inorganic phosphate and plates were incubated at 28 ± 2°C for 3 days. After 3 days, the colonies showing the clear halo zone around them indicated solubilization of mineral phosphate. Phosphate solubilization activities were screened by measuring the clearing zone surrounding the developed bacterial colony via calculation of phosphate solubiliztion index (Nautiyal, 1999).

Phosphate solubilization Index = A/B×100.

A= total diameter (colony + halo zone).
B =diameter of colony.

Phosphatase activity (Tabatabai and Bremner, 1969)

3ml of aliquots, 1ml modified universal buffer (MUB) and 1ml of P-Nitrophenyl Phosphate were pipetted. The mixture was incubated at 37°C for 1hr. Phophatase activity was terminated by addition of 20ml 0.5N NaOH. The mixture transferred to a 50ml volumetric flask and the volume was made upto 50ml with distilled water. The absorbance was read with spectrophotometer at 410nm.

Antagonistic effect by Dual culture method (Skidmore and Dickinson, 1976)

Colony interaction between bacteria and fungi against test pathogens were studied invitro in dual culture method. The individual test organism *Fusarium* was grown separately on potato dextrose agar medium and the individual species of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Aspergillus niger* were grown separately on nutrient and potato dextrose agar medium respectively. Then the agar blocks (5 mm thickness) cut from the individual species of bacteria, fungi and test pathogen were inoculated just opposed to each other approximately 3cm apart, on PDA medium in petriplates. Three

replicates for each set were maintained and control was set in single.

The position of the colony margin on the back of the disc were recorded daily. Assessment were made for the fungi and bacteria achieved an equilibrium after which there was no further alteration in the growth. Since both of the organisms were naturally inhibited, the assessment was made for both organisms.

$$\% \text{ Inhibition in radial growth} = 100 \times \frac{r1 - r2}{r1}$$

Where,

r1 is the radial mycelia growth in control.
r2 is the radial mycelia growth in treatment.

Results and Discussion

In this present study, phosphate solubilizing organisms were isolated from green gram rhizosphere soil based on the screening technique. Phosphate solubilisation, Acid phosphatase activity and Antagonistic effects were also determined.

Phosphate solubilization activity

In this study, phosphate solubilizing bacteria and fungal strains were selected for phosphate solubilization. These strains were able to solubilize phosphate on National Botanical Research Institute Phosphate growth medium and pikovskayas agar medium(Perez *et al.*,1991).

In the concentration of phosphate released into the media varied from strain to strain which would be a consequence Phosphate precipitation of organic metabolites as reported earlier (Babenko *et al.*, 1984; Kan and Bhatnagar, 1977) An alternative explanation could be the difference in the rate of P release and uptake. When the concentration of P in the media and vice versa (Reyes *et al.,* 1999). . Compared with *Pseudomonas fluorescens, Bacillus subtilis and Fusarium oxysporum, Aspergillus niger* have maximum phosphate solubilization activity (45.8mm) (Table-1).

Phosphatase activity

Some of the strains produced high amount of phosphatase but released less amount of P from insoluble phosphate and vice versa. This explains clearly that through the amount of phosphatase released into the medium was higher. This finding indicates that there is a correlation between P release and phosphatase activity (Goldstein, 1994).

The acid phosphohydrolyases shows optimal catalytic activity at neutral and acid pH soil than the alkaline phosphatase. These phosphatase are classified into specific and non-specific and according to the substrate into specificity. The P released as a by product by the action of phosphatase providing the cells with essential nutrients (Ohtake and kuroda *et al.,* 1996).A strain of *Burkholderia cepacia* displayed a sigficant mineral p-solubilizing ability and a moderate phosphatase acitivity(Rodriquez *et al*., 1999). Compared with *Bacillus subtilis, Aspergillus niger* and *Fusarium oxyporum, Pseudomonas fluorescens* have maximum phosphatase activity(Table-1).

Antagonistic effect

The severity of tobacco black root rot was reduced when soil was added phytoroglucinol. Phloroglucinol metabolites are phenolic and are produced

Table.1 Phosphate solubilization and phosphatase activity of isolated microorganisms

Microorganisms	Phosphate solubilization (mm)	Phosphatase activity (IU/ml)
Pseudomonas fluorescens	34.6 ± 2.3	417.89± 4.2
Bacillus subtilis	25.0 ± 1.1	393.23± 3.8
Aspergillus niger	45.8 ± 2	123.12± 3.2
Fusarium oxysporum	36.2 ± 1.9	110.8 ± 2.7

Values are expressed as Mean ± Standard deviation

by bacteria with broad spectrum antibacterial, antifungal and phytotoxic properties (Maurphofer et al., 2002). Rice sheath blight caused by *Rhizoctonia solani*. It is one of the most destructive rice disease world wide (Ou, 1995). This fungal disease is secondary importance only to rice plant disease caused by *Pyricularia oryzae*. Management of sheath blight disease of green gram has been directed towards the integration of cultural practices with chemical control (Damicone *et al.,* 1993).

However chemical control using effective fungicides has various undesirable effects, such as phototoxic to gram plants and the requirements for critical timing of fungicide application may hinder its usage (Lee and Rush,1993) cause environmental pollution and decrease diversity of non target organisms. Compared with *Bacillus subtilis* and *Aspergillus niger,Pseudomonas fluorescens* shows higher antagonistc activity against *Fusarium sp* (Table-2).

Table.2 Antagonistic effect of phosphate solubilizing microorganisms against *Fusarium oxysporum*

Microorganisms	Zone of inhibition(mm)
Pseudomonas fluorescens	65.0 ± 2.7
Bacillus subtilis	50.0 ± 1.9
Aspergillus niger	55.0 ± 2

Values are expressed as Mean ± Standard deviation

In the present study, soil sample was collected from green gram rhizospheric soil. Among this study, two bacteria and two fungi were selected for phosphate solubilisation activity.

The phosphate solubilising organisms were identified based on cultural, morphological and biochemical characteristics. Hence, the isolated colonies were confirmed *as Pseudomonas fluorescence, Bacillus subtilis, Aspergillus niger* and *Fusarium oxysporum*. The maximum antagonistic activity was present in *Pseudomonas fluorescens.* Further research should be continued with the phosphate solubilising isolates may be used for plant growth and control the plant pathogens.

Approximately 70-90% of Phosphorus fertilizer applied to the soil is precipitated by Ca, Fe and Al metal cations making insoluble form which are not efficiently taken up plants. Inoculation of Phosphate solubilizing microorganisms in soil has been shown to improve solubilization of insoluble phosphate resulting in higher crop performances. All the isolated PSM were efficient phosphate solubilizer and

can be used as bioinoculants to increase the available phosphorus in the soil for *Vigna radiata* growth.

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