Phosphorus solubilizing microorganisms: mechanism and diversity

Zaffar Bashir, MY Zargar, FA Mohiddin, Shaheen Kousar, Mohit Husain and Farahnaz Rasool

Abstract
Phosphorus (P) is the second important nutrient after nitrogen. Plants acquire P from soil solution as phosphate anion. P is the least mobile element in plant and soil contrary to other macronutrients. An adequate supply of P is required for proper functioning and various metabolic activities like photosynthesis, nitrogen fixation, proteins and nucleic acid production etc. Majority of P is fixed in soil and therefore plant available P is scarcely available despite the abundance of both organic and inorganic forms in soil. only 0.1% of the total P is available to plants because of its fixation and poor solubility In soil. P fixes with other metallic elements in the soil such as Ca, Al, Fe to form calcium phosphate, aluminium phosphate and ferrous phosphate and thus becomes unavailable to plants. The use of chemical fertilizers has long term effect on environment in terms of soil fertility depletion, eutrophication; this attitude forced us to find a sustainable way for efficient P availability in agriculture to meet the overgrowing demand of food. The use of phosphorus solubilizing microorganisms (PSM) opens a new way for better crop productivity and greater yield performance without affecting soil health. Some of the microorganisms such as Pseudomonas sp, Bacillus sp, Aspergillus and Penicillium strains have been identified as P solubilizers. The present review focuses on the phosphate plant interaction, mechanism of phosphorus solubilization.

Keywords: Phosphorus Solubilizing microorganisms, Mechanism of phosphorus solubilization, pseudomonas, Bacillus, Aspergillus, Penicillium

Introduction
The word population is increasing day by day, so there is need for plenty of food crops to meet the requirement of growing population. Chemical fertilizers have negative impact on soil health. To overcome the above problems the efforts needed to focus the soil biological system. P is considered as one of the most essential macronutrient required for growth and development of plants (Saber et al., 2005)[99]. P is the master key element known to be involved in a plethora of functions in the metabolism of plant. P has several roles and is involved in various metabolic functions such as cell division and development, nitrogen fixation, crop quality, resistance to diseases (Sperberg, 1958, khan et al., 2014) [98, 99]. P is sequestered by adsorption to the surface of soil particles (Harris et al., 2006)[117] and became unavailable for plant consumption. When P is applied to the field it gets fixed. P is a structural component of many co-enzymes, Phosphoproteins, Phospholipids (Ozane et al., 1980) [116] and also forms apart of genetic memory i.e. “DNA” of all living organisms. P deficiency affects root architecture (Williamson et al., 2001)[114]. It is utilized by plants in inorganic form i.e. in orthophosphate (H₂PO₄⁻ and HPO₄²⁻) (Hinsinger, 2001) [112]. A number of microorganisms excreting organic acids like Oxalic acids, Malic acid, Gluconic acid, which solubilize P that chelate cationic partners of P ions and release P directly in to the solution (He et al., 2002). Microorganisms like Bacillus sp, Pseudomonas sp, Aspergillus, Penicillium etc assimilate soluble P and prevent it from adsorption or fixation (Khan and Goergesen, 2009) [110]. Microorganisms influences soil fertility through various processes like mineralization. Microbes increase P availability to plants through solubilization of inorganic form of P to available form (Kang et al., 2002., Chen et al.,2006)[20-23]. Microbes are used as alternate source for P deficiency which are eco-friendly and economic (Padmavathi and Usha, 2012) [72]. For many years PSM have isolated and identified which include Bacillus sp, Pseudomonas SP, Streptomycetes, Enterobacter Thibacillus, Erwinia, Serratia, Azotobacter, Enterobacter and Micrococcus, (Zhao and Lin,2001) [116], the other fungal strains functions in similar way include Penicillum, Aspergillus, Rhizopus and Fusarium (Zhu et al., 2011) [117]
Occurrence and Isolation of Psm
Microorganisms are ubiquitous and one gram of fertile soil contains $10^7$ to $10^{10}$ bacteria and their life may exceed 2000kg/ha (Hayat et al., 2010) [38-40]. They are present in various forms in soil like rods, Spherical and spiral etc. Spiral are very rare but mostly Bacilli are used in phosphate solubilization in soil (Boudoin et al., 2002) (11). The population of phosphate solubilizers in soil depend upon various factors of soil like organic matter, physical and chemical nature and phosphorus content of soil (Kim et al., 1988). PSBs are mostly found in Rhizosphere, in which complex reactions occur (Vazquez, 2000., Krishenvani., 2010) [107-110, 59, 60]. PSBs take part in biogeochemical cycles of P in the ecosystem (Saha and Biswash, 2009) [87, 88].

Isolation of PSB
Phosphate solubilizing bacteria are found in every ecological niche. There numbers are higher in the Rhizospheric soils, Phyllosphere, Rhizoplane and Phosphate rock deposits. First time PSB isolation was reported on Pikoviskayas agar medium, medium contains glucose 10.0 g, (NH4)2SO4 0.5 g, NaCl 0.3 g, KCl 0.3 g, MgSO4·7H2O 0.3 g, FeSO4·7H2O 0.3 g, MnSO4·4H2O 0.03 g, CaCO3 5.0 g and distilled water 1000 mL with the pH of 7.0. Pikoviska’s agar medium contains tricalcium phosphate as a source of insoluble P and uses selective pressure for isolation of PSB. In the presence of tricalcium phosphate media plates are opaque. Thus bacterial isolates solubilizing insoluble P display clear halos around their colonies (Zone of clearance) (Fig. 1). The visual observation and measurement of clear halo diameter allows semi-quantitative evaluation of PSB.

Fig 1: Zone of clearance

Protocol for isolation and development of effective inoculants of PSM based Biofertilizers:
Collection of soil sample
Serial dilution
Inoculation on medium (spread plate)
Clear zone around colony growth indicates PSM activity
Additional test in liquid media to assay P dissolution
Test isolates for abundant production of organic acids
Pure culture by reinoculation
Morphology and colony study
Biochemical tests and Molecular Characterization
Test a model plant
Green house trial/field trial then &Quality control and commercial Bio fertilizers

Phosphate Solubilizing Micro-Organisms
Naturally occurring Rhizospheric PSM dates back to 1903 (Khan et al., 2009) [47, 50, 53]. Various microorganisms play key role in solubilization of phosphorus which includes Bacteria, Fungi, and Actinomycetes. Bacteria are proved more effective in phosphorus solubilization than fungi. In addition to Pseudomonas and Bacillus, other bacteria reported as P-solubilizers these are Arthrobacter, Rhodococcus, Serratia, Chryseobacterium, etc. (Wani et al., 2005), Azotobacter (Kumar et al. 2001), Xanthomonas (De Freitas et al. 1997), Enterobacter and Klebsiella (Chung et al. 2005). PSB constitute 1 to 50% among all PSM in soil and fungi 0.1 to 0.5% (Chen et al., 2006) [20-23]. Recently phosphorus solubilizing bacteria have been used in soil for mineralization of pollutants i.e. bioremediation of polluted soil (Middlerop et al., 1990., Burd et al., 2000) [66, 17]. The insoluble forms of P such as Tricalcium phosphate, Iron phosphate and Aluminium phosphate etc. may be converted to soluble P by various P-solubilizing microorganisms inhabiting soil ecosystems (Gupta et al., 2007; Song et al.,2008; Khan et al.,2013; Sharma et al.,2013) [36, 97, 49, 92, 93]. Soil microorganisms in this regard have generally been found more effective in making P available to plants from both organic and inorganic sources by solubilizing (Toro 2007; Wani et al. 2007a) [105, 11] and mineralizing complex P compounds (Bishop et al., 1994; Ponmurugan and Gopi,2006) [12, 78], respectively. Several workers have documented their findings in order to better understand as to how the microbial populations cause the solubilization of insoluble P (Illmer and Schinner 1995; Khan et al.,2007, 2009; Buch et al., 2008) [42, 43, 52, 47, 50, 53, 14, 15] Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids secreted by microorganisms has been a well-recognized and widely accepted theory as a principal means of
P-solubilization, and various studies have identified and quantified organic acids and defined their role in the solubilization process (Malhi et al., 2004; Khan et al., 2010; Marra et al., 2012) [62, 63, 51, 64]. The Organic acids produced by many P-solubilizers, for example, bacterial cultures and fungi (Table 2.), in the natural environment or under in vitro conditions chelate mineral ions or decrease the pH to bring P into solution (Pradhan and Shukla, 2005) [79]. Consequently, the acidification of microbial cells and their surrounding leads to the release of P-ions from the P-mineral by H+ substitution for Ca2+ (Goldstein, 1994; Mullen, 2005; Trivedi and Sa, 2008) [32, 60]. Table 1 shows various solubilizers.

**Table 1:** PSB and PSF

<table>
<thead>
<tr>
<th>BACTERIA. Bacillus sp, Pseudomonas sp and fungi</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. mycoides, B. polymyxa</td>
<td>Gaind and Gaur, 1990, Kaul et al., 1999</td>
</tr>
<tr>
<td>B. licheniforms, B. amyloliquefaciens, B. atrophaeus</td>
<td>Vazquez, 2000</td>
</tr>
<tr>
<td>B. megaterium, B. phosphaticum</td>
<td>Sundara et al., 2002 [1]</td>
</tr>
<tr>
<td>B. cereus</td>
<td>Chandra et al., 2007</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>Chatti et al., 2008</td>
</tr>
<tr>
<td>Paenibacillus marcera</td>
<td>Krishnasewamy et al., 2009</td>
</tr>
<tr>
<td>P. syringae, P. aeruginosa</td>
<td>Bardiya and gaur, 1974</td>
</tr>
<tr>
<td>P. florescence</td>
<td>Nautiyal, 1999</td>
</tr>
<tr>
<td>P. striata</td>
<td>Peix et al., 2004</td>
</tr>
<tr>
<td>Pencillium expansum</td>
<td>Kucey et al., 1987, Cunningham et al., 1992</td>
</tr>
<tr>
<td>Pencillium sp</td>
<td>Fenice et al., 2000, Mittal et al., 2008.</td>
</tr>
<tr>
<td>Aspergillus niger, Aspergillus tubigensis</td>
<td>Richa et al., 2007.</td>
</tr>
<tr>
<td>Streptomyces acidiscabies</td>
<td>Dimpta et al., 2008.</td>
</tr>
<tr>
<td>Trichoderma sp</td>
<td>Altomere et al., 1999, Ahmed, 2010</td>
</tr>
<tr>
<td>Azotobacter sp</td>
<td>Santhi, 1998</td>
</tr>
</tbody>
</table>

**Mechanism of Phosphorus Solubilization**

PSB employ different mechanisms of P solubilization. The principle mechanism is the secretion of low molecular weight organic acids (Oxalic acids, Malic acid, Gluconic acid) (Sperberg, 1957., Goldstein, 1995., Buch et al., 2008) [99, 100, 14, 15]. The production of these organic acids results in the acidification of microbial cells and its surrounds (Rodriguez and fraga, 1999, Chen et al., 2006, Lin et al., 2006) [85, 20-23, 61]. Subsequent ionization of acid takes place and either proton produced becoming responsible for phosphorus release from mineral phosphate by proton substitution for Ca, Al, and Fe or carboxylic anions chelate cations and release phosphate anions. Organic acid production has also been well documented (Lin et al. 2006, Park et al., 2009, kumar and Rai., 2015) [61, 74, 75]. The genes are directly or indirectly involved in secretion of organic acids (Babu-khan et al., 1995, Buch et al., 2010) [8, 16].

**Some example of organic acids produced by Fungi and Bacteria**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Organic acid</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Aspergillus niger</td>
<td>Succinic</td>
<td>Vazquez et al., 2000</td>
</tr>
<tr>
<td>2. Pencillium regulosum</td>
<td>Citric, Gluconic</td>
<td>Rayes et al., 2001</td>
</tr>
<tr>
<td>3. Aspergillus flavus</td>
<td>Citric, Oxalic, Gluconic, Succinic</td>
<td>Malhia et al., 2004</td>
</tr>
<tr>
<td>4. Aspergillus candidus, Aspergillus flavus</td>
<td>Malic, Oxallic, Oxalic</td>
<td>Chin et al., 2006</td>
</tr>
<tr>
<td>5. Serratia marcescens (CC-BC14)</td>
<td>Lactic, Acetic, Tartaric, Fumuric, Citric</td>
<td>Chen et al., 2006</td>
</tr>
<tr>
<td>6. Chryseobacterium (CC-BC05)</td>
<td>Gluconic</td>
<td>Akinotokun et al., 2010</td>
</tr>
<tr>
<td>7. Trichoderma sp., A. terreus, A. wetti, Pencillium sp, Fusarium oxysporum</td>
<td>Oxalic, Citric</td>
<td>Arwiddson et al. 2010</td>
</tr>
<tr>
<td>8. Aspergillus niger, Pencillium sp,</td>
<td>Gluconic</td>
<td>Vyas and Glati, 2009</td>
</tr>
<tr>
<td>9. Pseudomonas Trivialis (BHB 769)</td>
<td>Formic, Malic</td>
<td>Jain et al., 2012</td>
</tr>
<tr>
<td>10. Aspergillus awamori S19</td>
<td>Citric, Fumuric, Oxalic</td>
<td>Shahid et al., 2012</td>
</tr>
<tr>
<td>11. Enterobacter sp., FS-11</td>
<td>Malic, gluconic</td>
<td>Mendes et al., 2013</td>
</tr>
<tr>
<td>12. Aspergillus niger FSI</td>
<td>Citric, Gluconic, Oxalic</td>
<td>Mendes et al., 2013</td>
</tr>
</tbody>
</table>

**Biodiversity Of Phosphorus Solubilizing Microorganisms**

Tricalcium phosphate, Rock phosphate and Organic phosphate solubilizing microorganisms have been isolated and characterized from diverse habitats (Goldstein, 1986., Illmer and Schinner, 1995., Kim et al., 1998, Perez et al., 2007., Aziz et al., 2012., Acevedo et al., 2014, Taktek et al., 2015) [33, 42, 43, 54-56, 7, 103]. PSB include Bacillus, pseudomonas, Serratia, Enterobacter, Micrococcus, Flavobacterium, Arthrobacter, Rhizobium. These were isolated from sea water and marine sediments (De Souza et al., 2000) [25], from rice and banana rhizosphere (Kumar et al., Naik et al., 2008) [69].
microorganisms (PSM) have been seen as best eco-friendly means for P nutrition of crop, that can be afforded by the farmers.

**Table 3:** Plant growth substances released by PSB

<table>
<thead>
<tr>
<th>PSB(PGPR)</th>
<th>Plant Growth Promoting Secretions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bacillus</td>
<td>IAA/HCN/Siderophore/lytic enzyme</td>
<td>Kumar et al., 2012</td>
</tr>
<tr>
<td>2. Azotobacter</td>
<td>IAA/Siderophores</td>
<td>Farazjzadah et al., 2012</td>
</tr>
<tr>
<td>3. Brevibacterium sp</td>
<td>IAA</td>
<td>Vivas et al., 2006</td>
</tr>
<tr>
<td>4. Xanthomonas sp</td>
<td>IAA</td>
<td>Sheng and xia, 2006</td>
</tr>
<tr>
<td>5. Pseudomonas putida</td>
<td>Siderophores</td>
<td>Tripathi et al., 2005</td>
</tr>
<tr>
<td>6. Pseudomonas fluorescence</td>
<td>Siderophore/IAA/P-solubilization</td>
<td>Gupta et al., 2005</td>
</tr>
<tr>
<td>7. Rhizobium sp</td>
<td>Gibberlic acid/zeatin/IAA</td>
<td>Boiero et al., 2007</td>
</tr>
<tr>
<td>8. rhizobium strain TAL 1145</td>
<td>ACC Deaminase</td>
<td>Tittaburth et al., 2008</td>
</tr>
<tr>
<td>9. Bradyrhizobium</td>
<td>Gibberlic acid/IAA</td>
<td>Afzal et al., 2010</td>
</tr>
<tr>
<td>10. Rhizobium leguminosarum</td>
<td>IAA</td>
<td>Stajkovic et al., 2011</td>
</tr>
</tbody>
</table>

**Table 4:** Plant growth promotion by PSM (Patil et al., 2002)

<table>
<thead>
<tr>
<th>Bio inoculant(PSM)</th>
<th>Crop Benefited</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. straita</td>
<td>Soybean in sandy alluvial soil</td>
</tr>
<tr>
<td>P. straita</td>
<td>Chick pea</td>
</tr>
<tr>
<td>Phosphobacterium</td>
<td>Sword bean variety SBS1</td>
</tr>
<tr>
<td>M. Biological</td>
<td>Banana</td>
</tr>
<tr>
<td>B. Firmus NCIM2636</td>
<td>Paddy in acid soils</td>
</tr>
</tbody>
</table>

**References**

2. Afzal A, Bano A, Fatima M. Higher soybean yield by inoculation with N-fixing and, 2010
7. Azziz G, Bajza N, Haghjou T, Taulé C, Valverde A, Iguat JM, Arias A. Abundance, diversity and prospecting of...
41. Hinsinger P. Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere. In: trace elements in the rhizosphere. Eds. GR
bacteria (PSB) in rhizosphere and non-rhizosphere
Krishnaveni MS. Studies on phosphate solubilizing


Park KH, Lee CY, Son HJ. Mechanism of insoluble phosphate solubilization by Pseudomonas fluorescens...


80. promoting characterization of indigenous Azotobacteria isolated from soils in Iran. Curr Microbiol. 64:397-403


83. Reyes I, Baziramakenga R, Bernier L, Antoun H. Solubilization of phosphate rocks and minerals by a wild-type strain and two UV induced mutants of Penicillium rugulosum. Soil Biol Biochem. 2001; 33:1741-1747


106. Tripathi M, Munot HP, Shouche Y, Meyer JM, Goel R. Isolation and functional characterization of siderophore producing lead and cadmium resistant...
International Journal of Chemical Studies


