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Phosphorus solubilizing microorganisms: mechanism and diversity

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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 ete. 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 Pencillium* 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, Pencillium*

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) [86]. 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, 48].P is sequestered by adsorption to the surface of soil particles (Harris *et al*., 2006) [37] 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, Phophoproteins, Phospholipids (Ozane *et al*., 1980) [71] and also forms apart of genetic memory ''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 ($H_2PO_4^{4-}$ and HPO_4^{2-}) (Hinsinger, 2001) [41]. A number of microorganisms excreting organic acids like Oxalic acids, M alic 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* ete assimilate soluble P and prevent it from adsorption or fixation (Khan and Goergesen, 2009) [47, 50, 53]. 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, Streptomyces, Enterobacter Thibacillus*, *Erwinia, Serratia, Azotobacter, Enterobacter* and *Micrococcus,* (Zhao and Lin,2001) [116], the other fungal strains functions in similar way include *Pencillium, Aspergillus, Rhizopus and Fusarium* (Zhu *et al*., 2011) [117]

Occurrence and Isolation of Psm

Microorganisms are ubiquitous and one gram of fertile soil contains 10^1 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 0.03 g, MnSO4·4H2O 0.03 g, CaCO3 5.0 g and distilled water 1000 mL with the pH of 7.0.Pikoviskaya'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.

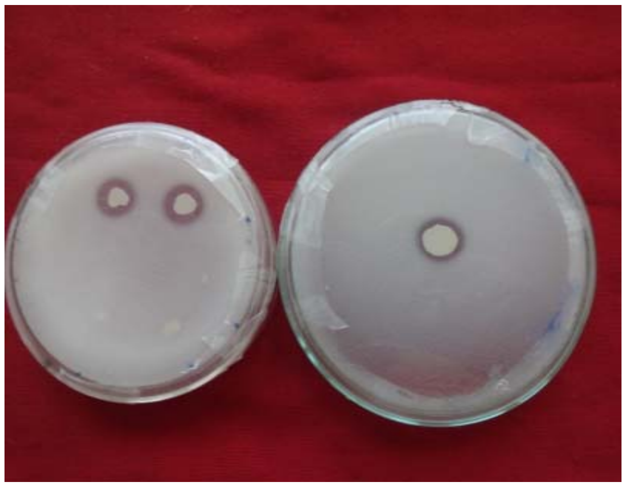

Fig 1: Zone of clearance

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 (Maliha *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, 68].Table 1.shows various solubilizers.

Table 1: PSB and PSF

BACTERIA. ***Bacillus sp, Pseudomonas sp and fungi***	**Reference**
B. mycoids, B. polymyxa	Gaind and Gaur, 1990.,Kaul *et al*., 1999
B. licheniforms, B. amyloliquefaciens, B. atrophaeus	Vazquez, 2000
B. megaterium, B. phosphaticum	Sundara *et al*., 2002 [2]
B. cerus	Chandra *et al*., 2007
B. subtilis	Chatli *et al*., 2008
Paenibacillus marceras	Krishnaewamy *et al*.,2009
P. syringae, P. aeruginosa	Bardiya and gaur,1974
P. flouresence	Nautiyal, 1999
P. striata	Peix *et al.,* 2004
Pencillium expansum	Kucey *et al*., 1987.,Cunningham *et al*., 1992
Pencillium sp	Fenice *et al*., 2000. Mittal *et al*., 2008.
Aspergillus niger, Aspergillus tubigensis	Richa *et al*., 2007.
Streptomyces acidiscabies	Dimpka *et al*., 2008.
Trichoderma sp	Altomere *et al*., 1999., Ahmed, 2010
Azotobacter sp	Santhi, 1998

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]. The carboxylic and hydroxyl groups of organic acids compete with cations (Ca, Al and Fe),make chelate complexes with metal ions and thus convert insoluble phosphorus in to soluble form.(Kpomblekou-a and tabatabai,1994) [57]. The organic acids can be measured by high performance liquid chromatography (Park *et al*.,2009, kumar and Rai,2015) [74, 75]. Iron chelating agents (Siderophores) and exopolysaccharides synthesized by PSB bring out fixed or locked P in to soluble form.(Yi *et al*.,2008.,Sharma *et al*., 2013) [115, 92, 93].Thus the organic acids, chelating agents and enzymes produced by PSB are responsible for solubilization of inorganic p. Table 2 shows organic acids produced by various bacteria and fungi.

Some example of organic acids produced by Fungi and Bacteria

Table 2

Organism	**Organic acid**	**Reference**
1. *Aspergillus niger*	1.Succinic	
2. *Pencillium regulosum*	2.Citric,Gluconic	Vazquez *et al*.,2000
3. *Aspergillus flavus*	3.Citric,Oxalic,Gluconic,Succinic	Rayes *et al*.,2001
4. *Aspergilluscandidus,Aspergillus flavus*	4.Malic,Gluconic, Oxalic	Maliha *et al*.,2004
5. *Serratia marcescens (CC-BC14)*	5.Citric, Lactic	Shin *et al*.,.2006
6. *Chryseobacterium (CC-BC05)*	6.Citric	Chen *et al*.,2006
7. *Trichoderma sp, A. terreus, A. wenti,*	7.Lactic,malic,acetic,Tartaric,Fumaric,Citric	Chen *et al*.,2006
Pencillium sp, Fusarium oxysporium	Gluconic	Akintokun *et al*.,
8. *Aspergillus niger, Pencillium sp,*	8.Oxalic,Citric	Arwidsson *et al*.,2010
9. *Pseudomonas Trivalis (BIHB 769)*	9.Gluconic,lactic,Succinic,Formic,Malic	Vyas and Glati,2009
10.Aspergillus awamori S19	10.Malic,Citric,Fumaric,Oxalic	Jain *et al*.,2012
11.*Enterobacter sp.FS-11*	11.Malic,gluconic	Shahid *et al*.,2012
12.*Aspergillus niger FSI,*	12.Citric,Gluconic,Oxalic	Mendes *et al*.,2013
Pencillium islandicum FS30		

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.,Parez *et al*., *2007*., Aziz *et al*.,2012., Acevedo *et al.,*2014.,Taktek *et al*.,2015) [33, 42, 43, 54-56, 7, 1 103]. 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]

Biodiversity of P Solubilizing Microorganisms

Groups	Examples
Nitrogen fixing microbes	
Symbiotic	*Rhizobium, Frankia, Anabaena Azollae*
Free living	*Azotobacter, Clostridium, Anabaena, Nostoc, klebsiella*
Associative	*Azospirillum*
P Solubilizing bacteria	*Bacillus, pseudomonas, Serratia, micrococcus,*
P Solubilizing fungi	*Aspergillus sp, Pencillium sp*
Actinomycetes	*Actinomyces, streptomyces*
Cyanobacteria	*Anabena sp, Nostoc sp, Scytonema sp*
VAM	*Glomus faciculatum*

Phosphate Solubilizers As Plant Growth Promoters

Phosphate solubilizing microorganisms exert direct or indirect effects on growth and development of plants. Direct effect include increased solubilization of nutrients and uptake of nutrients while indirect effect include suppressions of pathogens and secretion of iron chelating agents (Hayat *et al*., 2010) [38-40]. These solubilizers increase the plant growth and yield because:

1. They increase the nitrogen fixation (Kennedy *et al*., 2004) [46]
2. They prevent plants from pathogen attack (Gou *et al*., 2004. Saravana *et al*., 2008) [34]
3. They produce water soluble vitamins like niacin, thiamine, biotin and riboflavin for plant growth (Revillas *et al*., 2000., Sierra *et al*., 1999) [82, 96]
4. They produce different Hormones (Dey *et al*., 2004) [26]
5. They produce iron scavenging molecules (Pal *et al*., 2001) [73].

Table 3: Plant growth substances released by PSB

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

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

Bio inoculant(PSM)	Crop Benefited
P. straita	Soybean in sandy alluvial soil
P. straita	Chick pea
Phosphobacterium	Sword bean variety SBS1
B. Megaterium	Banana
B. Firlmus NCIM2636	Paddy in acid soils

Conclusion

P is essential macronutrient required for growth and development of plant. P is present in both organic and inorganic forms in soil but its availability is restricted as it occurs in insoluble form. Only 0.1% of the total P is available to plants because of its fixation and poor solubility in soil. Poor availability or deficiency of phosphorus (P) markedly reduces plant size. Phosphorus accounts about 0.2 - 0.8% of the plant dry weight. To satisfy crop nutritional requirements, P is usually added to soil as chemical P fertilizer, however synthesis of chemical P fertilizer is highly energy intensive processes, and is costly. Moreover, plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes and rapidly becomes fixed in soils. Microbiologists and soil scientists have found a ways and means of making phosphorus available to crops, an economically efficient substitute for fertilization of crops. Such environmental concerns have led to the search for sustainable way of P nutrition of crops. In this regards phosphate-solubilizing microorganisms (PSM) have been seen as best eco-friendly means for P nutrition of crop, that can be afforded by the farmers.

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