Endophytic Bacteria: Role in Phosphorous Solubilization

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Abstract

For complete growth and development of plants phosphorous (P) is the second key nutrient after nitrogen. Predominantly two major forms of phosphorous exist in soil: organic P and inorganic P, which are however mostly in insoluble forms. This unavailability of P is the result of fixation and precipitation, which causes P inadequacy and limits the growth of plants. To reassure the nutritional demand of crop, P is generally incorporated in soil in the form of chemical P fertilizer. However, the use of mineral P fertilizer has very long-term implications in the environment such as eutrophication, soil fertility depletion, and aggregation of harmful chemicals. So, it is important to generate alternative sustainable and economical method to fulfil the P requirements. In this regard, phosphate solubilizing microbes including P-solubilizing bacterial endophytes provided an unconventional and eco-friendly biotechnological solution to accomplish the phosphorous demands of crops. The bacterial endophytes are used as bio-inoculants and facilitate the growth of plants in many ways other than P-solubilization. This work emphasized on the plant colonizing ability of endophytic bacteria, their functional diversity and process involved in phosphorous solubilization or mineralization mechanism for their possible use to attain sustainable agriculture system.

Keywords

Soil P · Bacterial endophytes · P-solubilization/mineralization · Bio-inoculants · Sustainable agriculture

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5.1 Introduction

Phosphorous is the major vital macronutrient for the growth and development of plants. It plays a key role in numerous plant metabolic processes including energy transfer, biosynthesis of macromolecules, photosynthesis, and respiration (Fernández et al. 2007). In soil, the total P content is approximately around 0.05% (w/w); however, merely 0.1% of the total P is available to plants (Scheffer and Schachtschabel 1992; Otieno et al. 2015), as in acidic soil it is fixed as insoluble iron phosphates and in alkaline soil in the form of calcium phosphates. This insoluble form of phosphorous is not available and is not absorbed by plants and results in the elevated use of mineral phosphatic fertilizer to crops (Sharma et al. 2013) that cause environmental pollution like eutrophication. The increasing price of chemical P fertilizers, their adverse effect on environment and low efficiency of plant to use P from soil have highlighted the interest in the study of microbial solubilization of P in soil. The eco-friendly agriculture practices and sustainable evolution of food sector is anticipated to move towards enhancing the productivity without compromising the needs of forthcoming generations. Extensive productivity is expected to be achieved through developing new biotechnological methods and employing high crop yield strategies. In this view, the organisms with phosphate solubilizing ability, generally termed as P-solubilizing microbes, may offer feasible replacement to chemical phosphorous fertilizers. Among the various P-solubilizing microbes, bacterial endophytes are assessed as one of the principal group to escalate the bioavailability of soil insoluble P for plant biological growth and development (Zhu et al. 2011). Since the development of the rhizosphere concept in 1904 by Hiltner, many research studies have established that the rhizosphere soil environment is a hotspot of microbial activities, abundance and diversity because of the presence of root exudates and rhizodeposits (Hiltner 1904; Hartmann et al. 2008). The bacterial colonization in healthy plants has become an interest because of their capability for manipulation to enhance crop productivity (Turner et al. 1993). The group of microbes, either bacteria or fungi, that colonize within plant tissues symbiotically without harming the host plant are called as endophytes. The term endophytes was first introduced by De Bary (1866), which indicates the organisms that grow internally in plant tissues. Nowadays they are more appropriately explained, in respect to their various groups either bacterial or fungal associations, obligate or facultative with the host plant (Cabral et al. 1993; Hallmann et al. 1997; Rosenblueth and Martínez-Romero 2006). A large array of bacterial endophytes have been reported that are able to grow and survive on roots and in soil as well. The plant-associated bacteria that reside internally in plants are known as bacterial endophytes, which precisely regulate the host plant cells transmitting responses as a result of association (Hardoim et al. 2008) without any negative effect on host plant (Reinhold-Hurek and Hurek 2011). Bacterial endophytes can provide various beneficial aspects to host plants preferably plant growth promotion, defence from pathogens and under varied environmental situations endophytic bacteria are capable of communicating and interacting with the host plant more effectively in comparison to rhizosphere bacterial population (Ali et al. 2012;

Coutinho et al. 2015). In accordance to their life strategies, the endophytic bacteria possibly can be classified into three groups: obligate, facultative and passive endophytes. Obligate endophytes purely depend on host plant for their growth and viability and transmit to other plants through specific vectors. Whereas facultative endophytes complete their life cycle outside the host plants (Hardoim et al. 2008) and the third group (passive endophytes) colonizes the host plant tissue via several open injuries. The passive endophytes are less efficient, as for colonization it is necessary for the host to have cellular machinery (Verma et al. 2004; Rosenblueth and Martínez-Romero 2006). For the first time, endophytic bacteria was probably isolated by Mundt and Hinkle (1976) from plants, and till date, in 16 phyla over 200 bacterial genera are identified as endophytes. These genera of endophytic bacteria comprise both culturable and unculturable groups. The most extensively studied bacterial endophytes found abundantly across several phyla involving Proteobacteria, Firmicutes, Actinobacteria and Bacteriodetes (Hardoim et al. 2015; Bulgarelli et al. 2012; Wemheuer et al. 2017) and covers the members of *Pseudomonas, Enterobacter* (Taghavi et al. 2009, 2010), *Bacillus* (Deng et al. 2011), *Burkholderia* (Weilharter et al. 2011) and *Stenotrophomonas* (Ryan et al. 2009). The culturable methods for isolation of bacterial endophytes have been broadly reviewed (Hallmann et al. 1997; Reinhold-Hurek and Hurek 1998). Brígido et al. (2019) isolated, identified and characterized culturable endophytic bacteria inhabiting the roots of chickpea (*Cicer arietinum* L.) grown in different types of soils. They found that the most common endophytic bacteria were *Enterobacter* and *Pseudomonas*, which produced indole acetic acid (IAA) siderophores and facilitate dissolution of P. In another study, 55 isolates were isolated from sap, leaves and roots of maize crop that are able to solubilize tricalcium phosphate by producing organic acid (Abreu et al. 2017). In a similar study, 22 bacterial endophytes were isolated from rhizosphere and roots of wheat (*Triticum aestivum* L.) plants and these isolates solubilized P from tricalcium phosphate and liberated IAA (Emami et al. 2020). Endophytic bacteria play a crucial role in plant growth promotion by possessing favourable effect on host plant. These bacterial endophytes can stimulate plant growth in several ways such as increasing rate of germination of seeds, root and shoot biomass, chlorophyll content, and abiotic stress tolerance. (Wahla and Shukla 2017). They also enhance the growth of plants through nitrogen fixation, phytohormone production, and phosphorous solubilization (Iniguez et al. 2004). These bacteria play a significant role in the biocontrol of phytopathogens in the plant root zones by the production of antifungal/antibacterial compounds, siderophore production and elicitation of systemic acquired resistances (Rosenblueth and Martínez-Romero 2006). This chapter highlights the mechanism of phosphorous solubilization by endophytic bacteria as they are efficient in solubilizing the soil insoluble P and make it available to plants. This capability to transform insoluble P to available orthophosphate form is a very important aspect of plant growth promoting bacteria for enhancing yields (Rodríguez et al. 2006). Hence, it is crucial to have in-depth understanding of plant, soil and microbial phosphorous cycle to develop sustainable agriculture system.

5.2 Colonization of Bacterial Endophytes

Endophytic bacteria might have an interest over rhizosphere bacteria as it has characteristics feature of living inside the plants tissues and expresses opportunity to inevitably be in contact with cells of plant and consequently exert the direct favourable effect. Certainly rhizospheric bacteria may also have the ability to penetrate and colonize the plant root (Santoyo et al. 2016). This microhabitat has been extensively reported as one of the key source for colonization of endophytes (Hallmann et al. 1997). Actually, the diversity of endophytic bacteria can be accounted as a member of rhizospheric bacterial population (Marquez-Santacruz et al. 2010). The rhizospheric environment is very competitive for microbes to inhabit and acquire nutrients (Raaijmakers et al. 2002). Rhizospheric colonization has been linked to root exudation mechanism (Lugtenberg and Dekkers 1999). The endophytes utilize various mechanisms to enter inside the plant tissues, especially in roots. Primary and lateral cracks in the root and various tissue wounds are the most conventional routes of entry of bacterial endophytes into plant tissues (Sprent and De Faria 1989; Sørensen and Sessitsch 2007). Numerous nutrients providing plant metabolite for root-inhabiting bacteria like organic acids, amino acids and various other compounds are liberated in the rhizospheric region (Walker et al. 2003; Compant et al. 2010). Root wounds ooze plant metabolites and as a result they chemo-attract the bacteria (Hallmann et al. 1997). Root exudates and other nutrients captivate detrimental rhizobacteria and also beneficial bacteria, fungi and many other soil entities (Walker et al. 2003). Therefore, plant growth promoting bacteria (PGPB) or bacterial endophytes must be really very competitive to colonize successfully to the root zone. There are some other areas which allow endophytes to enter plant tissue such as stomata, young stems (Roos and Hattingh 1983), lenticels (Scott et al. 1996) as well as growing radicals (Gagnet et al. 1987). Rhizospheric region is studied as a hot spot for phosphate solubilizing bacteria (PSB) indicating that PSB rapidly grow in both rhizospheric and root endospheric region (Hui et al. 2011). The occurrence of high amount of PSB in rhizosphere is because of the availability of high intensity of nutrients, mainly root exudates, which support the growth and metabolism of bacteria (Sharma et al. 2007). In phosphorous-limited soils the population of phosphate solubilizers are high and they help in the solubilization of insoluble phosphorous in the vicinity of the roots in available form (Aranda et al. 2011; Zhou et al. 2011) by producing organic acids and enzymes (Otieno et al. 2015; Illmer and Schinner 1995).

5.3 Role of Bacterial Endophytes in Phosphorous Solubilization

The concentration of P in soil is very low around 1 ppm or less (Goldstein 1994). As it is well established that P in soil exists in two forms, the organic form of P is obtained from humus and other organic matter comprising dead decayed plant, animals and microbes, which accounts as a significant pool for nearly 20–80% of total soil P (Richardson 1994). A second main part is insoluble inorganic phosphate or mineral phosphorous. In soil they are represented as primary minerals such as dicalcium and tricalcium phosphate, hydroxyapatite, rock phosphate and oxyapatite (Goldstein 1986; Rodriguez and Fraga 1999). In majority of agricultural soils huge reserves of P are accumulated as a result of consistent application of chemical P fertilizers (Richardson 1994). However, large fraction of soluble chemical P fertilizer is quickly immobilized shortly after its implementation and as a result become inaccessible to plants (Dey 1988). The mineralization and solubilization of P are key processes to enhance its availability in the soil, and these activities can be efficiently conducted by endophytic bacteria. There are a vast majority of bacterial endophytic strains that efficiently serve as phosphate solubilizers like *Bacillus, Pseudomonas* and endosymbiotic *Rhizobia* (Igual et al. 2001). Many other bacteria have been isolated like *Klebsiella, Rhizobium, Erwinia, Micrococcus, Pseudomonas, Bacillus* and *Mesorhizobium*, which are associated with phosphate solubilization (Villegas and Fortin 2002). In soil the occurrence of P fixation and precipitation is generally dependent on soil type and pH. Thus, in acidic soil, P is fixed by free oxides and in alkaline soil it is fixed by calcium (Goldstein 1986, 1994; Jones et al. 1991).

5.4 Mechanism of Soil P-Solubilization

There are numerous mechanisms through which the solubilization of P takes place like lowering of pH, production of organic acid, secretion of extracellular enzyme like phosphatase, phytase. These processes is carried out by P-solubilizing microbes (Fig. 5.1) residing in various soil ecosystems (Rodriguez and Fraga 1999; Sharma et al. 2013; Khan et al. 2013). Both organic and mineral forms of complex phosphorous compound are solubilized and mineralized by soil endophytic bacteria making P available to plants (Wani et al. 2007a; Richardson and Simpson 2011).

The following processes are employed by endophytic bacteria for solubilization of phosphorous:

1. Organic P mineralization by release of extracellular enzymes.
2. Mineral P solubilization by production of organic acids.
3. By proton liberation.
4. By secretion of siderophores and exopolysaccharides.

5.4.1 Mechanism of Organic P-Solubilization

It is evident that soil includes a wide array of organic substances which possibly act as a prime source of P for growth and development of plant. In soil, organic P constitutes about 4–90% of total P (Khan et al. 2009). The organic form of P is mineralized to make it in available form i.e. first it must be hydrolyzed to inorganic P (Rodriguez and Fraga 1999). There are diverse microbes, especially the endophytic bacteria, which possess the potential to transform insoluble organic P into available

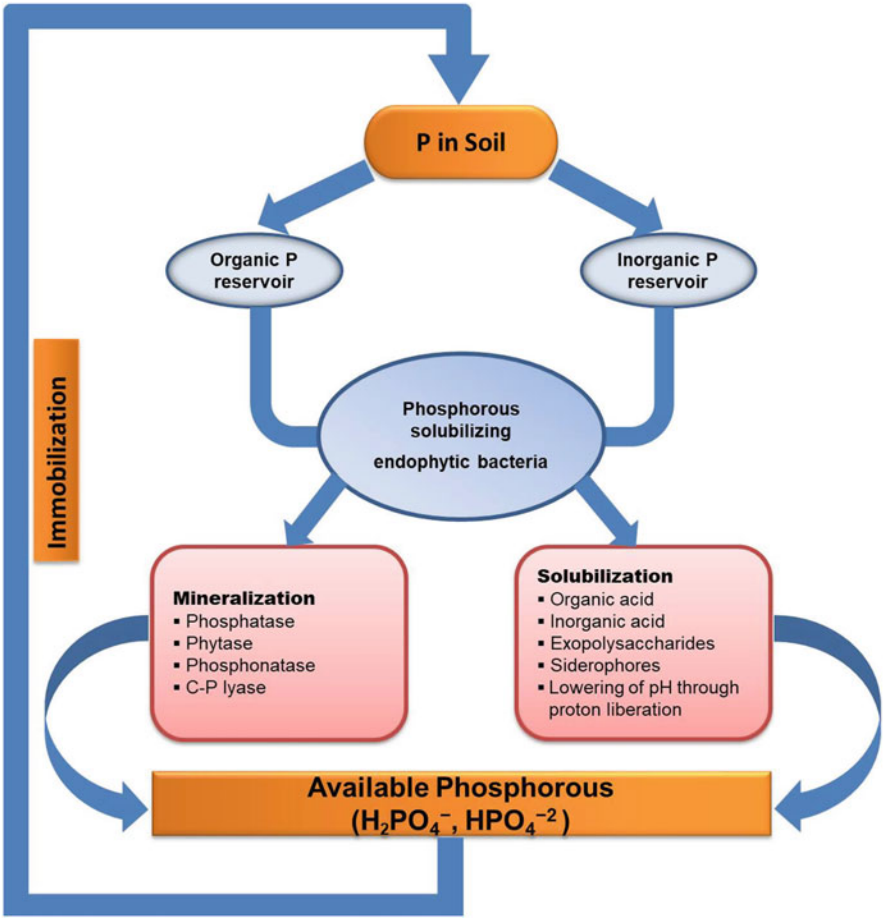

Fig. 5.1 Schematic presentation of soil P solubilization/mineralization by bacterial endophytes

form of P. This process of mineralization of P is carried out by extracellular enzymes, most importantly phosphatases (Tarafdar and Claassen 1988; Khan et al. 2014; Rodriguez and Fraga 1999), phytases (Nannipieri et al. 2011; Maogual et al. 2014) C–P lyase (Wahla and Shukla 2017), phosphonatases (Nannipieri et al. 2011).

5.4.1.1 Phosphatases

Phosphatases have been comprehensively explored in soil (Tabatabai 1994; Nannipieri et al. 2011). These enzymes catalyse the hydrolysis of both ester and anhydride bonds of phosphoric acid (Schmidt and Laskowski Sr 1961). Various kind of phosphatases are found in soil like phosphomonoesterases, phosphodiesterases, triphosphoric monoester hydrolases and enzymes functioning on phosphoryl-containing anhydrides and on P-N bonds (Nannipieri et al. 2011). Phosphomonoesterases comprising phytases are the studied enzymes for organic P mineralization by microbes (Jones and Oburger 2011). Further, based on the pH optima, phosphomonoesterases are categorized further into acid and alkaline phosphatases which catalyse the hydrolysis of monoester bonds of mononucleotides and sugar phosphates (Jorquera et al. 2008). Usually acid phosphatase prevails in the acidic soils and alkaline phosphatases are abundantly available in neutral to alkaline soil (Juma and Tabatabai 1977, 1998; Renella et al. 2006). Microorganisms capable of generating both acid and alkaline phosphatases (Nannipieri et al. 2011) and plant root produce only acid phosphatase (Hinsinger et al. 2018). The enzymes acid and alkaline phosphatases are exo-enzymes (liberated exterior to the cell), which are non-specific in nature and utilize organic form of P like a substrate and transform it into available inorganic P (Beech et al. 2001). There are a number of factors that regulate the phosphatase activities like availability of soil P and organic matter content (Štursová and Baldrian 2011). It is reported that the application of mineral P in soil inhibits the activity of phosphomonoesterases (Nannipieri et al. 2011). Phosphatases of microbial origin have elevated affinity for organic P in contrast to phosphatase originated from roots of plants (Chen et al. 2003; Walia et al. 2017). But the interaction between phosphorous solubilizing microbes in soil, phosphatase activity and mineralization of organic P is roughly understood till date (Chen et al. 2003).

5.4.1.2 Phytases

Phytases hydrolyses P from phytate degradation and is the key source of inositol. It is the stored form of P in seed and pollen and is dominant form of organic P in soil (Richardson 1994). All the six phosphate groups of inositol hexaphosphate are hydrolysed by phytase (Nannipieri et al. 2011). Soil microbes regulate the phytate mineralization in soil. In a study it is revealed that the vicinity of rhizospheric phosphate-solubilizing microbes render chance to plants to draw P straight from phytate (Richardson and Simpson 2011).

5.4.1.3 C-P Lyases and Phosphonatases

These are the enzymes that take part in the breakdown of C-P bond in organophosphonates (Rodríguez et al. 2006).

5.4.2 Inorganic P Solubilization

There are various bacterial species reported which solubilize insoluble inorganic phosphate compounds notably tricalcium phosphate, dicalcium phosphate, rock phosphate and hydroxyapatite (Rodriguez and Fraga 1999). Numerous bacteria have the ability to solubilize mineral phosphate compounds e.g. *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Micrococcus, Achromobacter, Flavobacterium, Agrobacterium* and *Erwinia*. Long et al. (2008) isolated 77 bacterial endophytes from *Solanum nigrum* grown in two different native habitats and reported that among the isolated strain; six were capable of solubilizing inorganic P. In another study from ginseng plant, Thamizhvendan et al. (2010) screened 18 endophytic isolates out of which nine isolates have P-solubilizing capability. The estimation of P solubilization potential of microbes has been achieved using serial dilution plate screening technique. Gerretsen (1948) suggested that microbes could solubilize unavailable form of phosphorous in soil and make it accessible to plants. From then, various methods and culture media have been proposed such as Pikovskaya (Pikovskaya 1948), Bromophenol blue dye method (Gupta et al. 1994), and National Botanical Research Institute P (NBRIP) medium (Nautiyal 1999). By using this method the P-solubilizing ability is detected by the formation of clear halo zone around the microbial colonies, in the culture media comprising mineral P mainly tricalcium phosphate as the only source of P. Although plate screening method is the most reliable technique for isolation and primary characterization of phosphate solubilizing microbes (Illmer and Schinner 1992). The sub-culturing of bacterial cultures is carried out to analyze the potential of P solubilization. When the potent PSB are chosen the P released by the PSB is quantitatively evaluated and the most potential phosphate solubilizers are further mass produced and evaluated under pot/field conditions with varying crops (Zaidi et al. 2009). Phosphate-solubilizing microbes solubilize mineral P by organic acid production (Table 5.1). Organic acids (OA) are the metabolic products released by microbes by the process of fermentation of organic carbon or oxidative respiration (Trolove et al. 2003). These OA originate in the periplasmic space of bacteria following direct oxidation pathway (Zhao et al. 2014; Alori et al. 2017). As a consequence of OA production the acidification of the microbial cell and its vicinity occurs (Goldstein 1994). Organic acid released in the vicinity of P-solubilizing microorganisms results in the decrease in pH to make P available in solution (Zaidi et al. 2009) and simultaneously results in the release of P ions out of mineral P by substituting H^+ for Ca^{2+} (Goldstein 1994).OA are capable of chelating cations like Al, Ca and Fe associated with P (Omar 1997; Sharma et al. 2013). There are numerous organic acids produced namely oxalic acid, 2-ketogluconic acid, succinic, gluconic, citric, lactic, malic, malonic, fumaric and tartaric acid (Ahmed and Shahab 2011). Among all these OA produced, gluconic acid is reported to be the predominant OA involved in solubilization of mineral P (Rodriguez and Fraga 1999). In several studies it is reported that gluconic acid (GA) is the main organic acid produced by PSBs such as *Burkholderia cepacia* (Rodriguez and Fraga 1999), *Pseudomonas* sp. (Illmer and Schinner 1992) and *Pseudomonas cepacia* (Goldstein 1994). Another important organic acid produced by PSB is 2-ketogluconic acid which is synthesized by *Rhizobium leguminosarum* (Halder et al. 1990), *Rhizobium meliloti* (Halder and Chakrabartty 1993), *Bacillus firmus* (Banik and Dey 1982), *Enterobacter intermedium* (Hoon et al. 2003) and *Bacillus subtilis* (Banik and Dey 1983). Since production of organic acids has been considered as the key process in phosphorous solubilization, some other mechanism have been also taken into consideration like the microbial production of chelating compounds (Sperberg 1958; Duff and Webley 1959) and production of some inorganic acids like HCl, nitric acid and carbonic acid for solubilizing P (Hopkins and Whiting 1916). However, in a study it is revealed that HCl has less ability to solubilize P from hydroxyapatite in comparison to that of organic acid at equal pH (Kim et al. 1997). *Nitromonas* and *Thiobacillus* species are also found to release P

Table 5.1 List of organic acid produced by P-solubilizing endophytic bacteria

P-Solubilizing Bacteria	Organic acids	References
Bacillus megatarium, Bacillus subtilis, Pseudomonas	Lactic acid, malic acid	Taha et al. (1969)
Arthrobacter sp.	Oxalic acid, malonic acid	Banik and Dey (1982)
Micrococcus sp.	Oxalic acid	Banik and Dey (1982)
Bacillus polymyxa, Bacillus licheniformis	Oxalic acid, citric acid	Gupta et al. (1994)
Pseudomonas cepacia	Gluconic acid, 2-Ketogluconic acid	Bar-Yosef et al. (1999)
Enterobacter intermedium	2-Ketogluconic acid	Hoon et al. (2003)
Bacillus megatarium	Propionic acid	Chen et al. (2006)
Serratia marscescens	Citric acid	Chen et al. (2006)
Pseudomonas fluorescens	Citric acid, malic acid, tartaric acid, gluconic acid	Fankem et al. (2006)
Enterobacter sp. FS-11	Gluconic acid, malic acid	Shahid et al. (2012)
Bacillus methylotrophicus CKAM	Gluconic acid, 2-Ketogluconic acid, formic acid	Mehta et al. (2014)
Burkholderia cepacia	Gluconic acid	Zhao et al. (2014)
Pseudomonas fluorescens	Gluconic acid	Otieno et al. (2015)
Burkholderia gladioli	Oxalic acid, acetic acid, butyric acid, lactic acid	Istina et al. (2015)
Bacillus sp.	Gluconic acid, acetic acid	de Abreu et al. (2017)

compounds by production of nitric acid as well as sulphuric acid (Sharma et al. 2013). It has however found that the effectiveness of these processes in the contribution of P solubilization appears to be insignificant (Rudolfs 1922).

5.4.2.1 Role of Proton Liberation P- Solubilization

Proton excretion from the cell is one of the main features of phosphate solubilization (Krishnaraj et al. 1998). Parks et al. (1990) suggested that the release of H^+ from NH_4^+ assimilation may be the other alternative process of P solubilization. In a study Illmer and Schinner (1995) reported that *Pseudomonas sp.* solubilized the P without producing organic acids as detected by HPLC analysis. They reported the release of protons accompanying NH_4^+ assimilation as one of the possible reason for P solubilization in absence of organic acid production. Different species of microbes possess different mechanism of proton release. The form of C i.e. glucose versus fructose had significant effect on proton release than the N (NH_4^+ versus NO_3^-)

supply (Park et al. 2009). In a study, the acidification of the cactus seedlings rhizosphere after inoculation with endophytic bacteria *Azospirillum brasiliense* in presence or absence of NH_4^+ and NO_3^-, the effect of inoculating this plant growth promoting bacteria was assumed to have effect on one or more metabolic processes of the plant which enhances efflux of proton and release of organic acid from roots which ultimately results in rhizosphere acidification (Carrillo et al. 2002).

5.4.2.2 Role of Siderophores in Mineral P- Solubilization

Siderophores are low molecular weight, iron-chelating compounds which form complexes with iron from mineral and make it soluble Fe^{3+} complexes under iron starvation condition by microbes and transport it to the cell. Siderophores production by P-solubilizing bacteria is a potent mechanism to ameliorate plant growth in iron limiting condition (Wani et al. 2007b; Ahmad et al. 2008). Several studies have revealed the excretion of siderophores from P-solubilizing microbes (Caballero-Mellado et al. 2007; Hamdali et al. 2008; Ahmad et al. 2008; Singh et al. 2008; Selvakumar et al. 2008; Jiang et al. 2008). As studies revealed that the mineral dissolution is dominant over ligand exchange via organic acid anions as a phosphate solubilizing process, it is obvious to consider the function of production of siderophores in increasing P-solubilization (Parker et al. 2005).

5.4.2.3 Role of Exopolysaccharides in Phosphate Solubilization

Exopolysaccharides (EPS) are polymeric material comprised of sugar residues secreted by microbes into their vicinity. EPS vary in their structure and composition. They can be homopolysaccharides or heteropolysaccharides. Moreover EPS may also include a wide variety of organic and inorganic substituents (Sutherland 2001). Yi et al. (2008) assessed the role of EPS in the solubilization of tricalcium phosphate by microorganisms. He studied for bacterial strain i.e. *Enterobacter* sp. EnHy-401, *Arthrobacter* sp. ArHy-505, *Azotobacter* sp. AzHy-510 and *Enterobacter* sp. EnHy-402 which have potential to solubilize TCP (tricalcium phosphate) to examine the possible role of EPS in P-solubilization. All these four strain have capacity to produce EPS and solubilize TCP, but despite that further studies are important to unravel the association between production of microbial EPS and P-solubilization.

5.4.3 Plant Growth Promoting Attributes of P-Solubilizing Endophytic bacteria

Phosphate solubilizing endophytic bacteria enhances the overall performances of plants by exhibiting multifunctional properties (Khan et al. 2013). They are not only potent P-solubilizers but also promote growth and development of plants by producing phytohormones like indole acetic acid (Wani et al. 2007b; Ahmad et al. 2008), siderophores (Wani et al. 2007c), cyanide and antibiotics (Wani et al. 2008). These bacteria also have capability to produce essential enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Madhaiyan et al. 2007) and can reduce the metal toxicity in stressed soils. In a study, endophytic bacteria was isolated from the cacti

Table 5.2 Plant growth promoting compounds produced by P-solubilizing bacteria

Endophytic bacteria	Plant growth promoting traits	References
Pseudomonas putida	HCN, Siderophore	Tripathi et al. (2005)
Pseudomonas fluorescens	IAA, Siderophore	Gupta et al. (2005)
Pseudomonas, Bacillus	Siderophore, IAA	Rajkumar et al. (2006)
Bacillus sp.	IAA, Siderophore	Wani et al. (2007c)
Burkholderia	ACC deaminase, IAA, Siderophore	Jiang et al. (2008)
Enterobacter sp.	ACC deaminase, IAA, Siderophore	Kumar et al. (2008)
Bacillus sp.	ACC deaminase, Siderophore	Farajzadeh et al. (2012)
Bacillus sp.	ACC deaminase, IAA, Siderophore	Kumar et al. (2012)
Bacillus methylotrophicus CKAM	Siderophore, IAA	Mehta et al. (2014)
Pseudomonas sp., *Paenibacillus*, *Bacillus* sp., *Enterobacter*	IAA	Emami et al. (2020)

rhizoplane growing on bare lava rocks which not only solubilize P but in addition also stimulated growth the wild cactus species (Puente et al. 2004a, 2004b, 2009). Some other physiological traits of PSB include the liberation of ecologically critical cyanide (Wani et al. 2007b). The plant growth promoting substances generated by these P-solubilizing bacteria are given in Table 5.2.

5.4.4 Genetics of Phosphate Solubilization

5.4.4.1 Genetics of Inorganic Phosphate Solubilization

The organic acid production is considered as a principal mechanism for mineral P solubilization (Rodriguez and Fraga 1999). In gram negative bacteria, glucose oxidation into gluconic acid is the principal mechanism for solubilization of mineral P i.e. MPS (Goldstein 1996). Biosynthesis of gluconic acid (GA) is catalysed by glucose dehydrogenase enzyme and pyrroloquinoline quinine (PQQ) as the cofactor by following direct oxidation pathway. PQQ is linked to the family of quinone and it performs as a cofactor for various bacterial dehydrogenases e.g. glucose and methanol dehydrogenase. A P-solubilizing gene was cloned from the *Erwinia herbicola*. When this gene was expressed in *E.coli* HB101 produces GA and is shown to solubilize hydroxyapatite (Goldstein and Liu 1987). The sequence analysis of this gene revealed its possible participation in the synthesis of enzyme PQQ synthase (Liu et al. 1992). This enzyme catalyses PQQ synthase, which is the essential cofactor in the formation of holoenzyme glucose dehydrogenase-PQQ, which helps in the synthesis of GA from glucose. PQQ synthesis genes from *Acinetobacter calcoaceticus* (Goosen et al. 1989) and *Klebsiella pneumoniae* (Meulenberg et al. 1992) have been cloned and 5 *pqq* genes were recognized and sequenced from *A. calcoaceticus* (Goosen et al. 1989). In a similar way another gene involved in PS and GA production, *gabY*, was cloned from *Pseudomonas cepacia* (Babu-Khan et al. 1995). This gene does not show visible homology along with the formerly cloned PQQ synthetase gene but showed similarity with histidine permeases membrane bound proteins. When *gabY* gene is present, the production of GA takes place only when *E.coli* expresses a functional glucose dehydrogenase (*gcd*) gene (Rodríguez et al. 2006). In *Pseudomonas cepacia* this *gabY* gene could possibly play an alternative role in expression/regulation of the direct oxidation pathway, hence behaving as a functional MPS gene in vivo. A fragment of DNA isolated from *Serratia marcescens* induces gluconic acid (GA) synthesis in *E.coli* but exhibited no homology to *pqq* or *gcd* genes (Krishnaraj and Goldstein 2001). Many other MPS genes isolated are not associated with *pqq* and *gcd* biosynthetic gene. A DNA fragment isolated from *Enterobacter agglomerans* exhibited MPS activity in *E. coli* JM109; however, the pH of the medium was not changed (Kim et al. 1997), which suggested that acid production is not a single method for bacterial P-solubilization (Illmer and Schinner 1995). The knowledge of molecular basis of P-solubilization trait is limited, and to bridge this knowledge gap the complete study of genetic basis of MPS is important.

5.4.4.2 Genetics of Organic P Mineralization

Since organic form of P can be mineralized to available form by group of enzymes: phosphatases, phytases, C-P lyases and phosphonatases (Rodríguez et al. 2006). Several genes involved in organic P mineralization have been isolated and characterized. The key mechanism involved in production of phosphatase is regulated by concentration of inorganic phosphorous i.e. Pi repressible phosphatases. As a part of phosphorous starvation mechanism, enhanced activity of phosphatases occurs as a result of phosphate deficiency. This process of regulation of phosphatase has been best received in *phoA* alkaline phosphatase gene isolated from *E.coli* (Rosenberg 1987). The genes regulated by inorganic phosphate (Pi) and activated by Pho B represent PHO regulon (Santos-Beneit 2015). Other bacteria like *Pseudomonas fluroscens* MF3 exhibit alkaline phosphatase activity in phosphorous-deficient condition (Gügi et al. 1991). Bacterial acid phosphatases are comprised of three gene families entitled as molecular class A, B and C (Thaller et al. 1995a). The *acpA* gene expressed acid phosphatase and shows optimal activity at pH 6 having broad range of substrate specificity (Reilly et al. 1996). Genes isolated from *Morganella morganii* encoding class A (Pho C) and class B (Nap A) acid phosphatase are very promising; moreover, they show broad substrate specificity at pH 6 and temperature 30 °C (Thaller et al. 1994; Thaller et al. 1995b). A gene has been isolated from rhizobacteria *Burkholderia cepacia* exhibiting phosphatase activity (Rodríguez et al. 2000). This gene was reported to code for protein present in outer membrane and increases the activity in P starving conditions and possibly participates in phosphorous transportation within cell from *Rhizobium meliloti.* Deng et al. (1998, 2001) cloned two non-specific periplasmic acid phosphatase gene *napD* and *napE*. A phosphatase gene (*napA*) from soil bacteria *Morganella morganii* was inserted into an endophytic bacterium *Burkholderia cepacia* IS-16 utilizing broad-host range pRK293 vector (Fraga et al. 2001). This recombinant strain shows improved phosphatase activity. Moreover, many other phosphatse encoding genes have been isolated from *E.coli* including *ushA* (Burns and Beacham 1986) *agp* (Pradel and Boquet 1988; Pradel et al. 1990) and *cpdB* (Beacham and Garrett 1980). The rhizospheric colonizing bacteria of the plant have significant impact on the host physiology (Antoun and Kloepper 2001). The comprehensive study of phosphate solubilization mechnism is still in infancy stage. Moreover, molecular level study to understand the mechanism involved in P solubilization mechanism by PSB is also ambiguous (Rodríguez et al. 2006). Although, various genes have been identified and cloned till date to characterize their role in inorganic and organic P solubilization (Sharma et al. 2013). Genetic manipulation of these genes is carried out by cloning and their expression in desired rhizobacterial strains to get improved phosphorous solubilizing capability for agricultural purpose as inoculants (Sharma et al. 2013). Several investigators have been worked on both phosphatase and MPS gene for their cloning and further characterization (Fraga et al. 2001; Krishnaraj and Goldstein 2001). Goldstein and Liu (1987) cloned a gene responsible for phosphate solubilization from *Erwinia herbicola*. The expression of *mps* genes in *E.coli* from *Renella aquatilis* showed enhanced gluconic acid production and solubilization of hydroxyapatite (Kim et al. 1998). Another rhizospheric bacteria *Pseudomonas* produces gluconic acid following oxidative glucose mechanism and overexpression of GDH gene, and PQQ biosynthesis enhances their P-solubilizing ability. In other study, expression of citrate synthase gene of bacteria in tobacco roots revealed increased exudation of organic acid and P availability. This unravels the possible role of organic acid synthesis gene in P assimilation. Genetic engineering or gene manipulation is a major conclusive method but there are some difficulties in gene insertion like dissimilarity of metabolic apparatus and regulating mechanism which should be addressed. In spite of problems and difficulties there is significant progress in acquiring genetically engineered microbes for improved agricultural purpose (Armarger 2002). However, further studies are required for better understanding of the different aspects of P-solubilizing bacteria for their better use in sustainable agriculture.

5.4.5 Bacterial Endophytes as Crop Bio-Inoculant

The conventional agricultural system is dependent on agrochemicals comprising phosphate fertilizers to achieve enhanced crop yields. These chemical fertilizers are not completely utilized by the crop plant and persist in the soil and disturb the rhizospheric microbial community (Ai et al. 2012). The extensive application of chemical fertilizers causes hazardous effect on environment sustainability. Therefore, due to high cost and hazardous impact of chemical fertilizer on environment, (López-Bellido et al. 2013) it become crucial to find cost-effective alternatives such as bio-inoculants which could be economical and environment friendly for sustainable agriculture (Adesemoye and Kloepper 2009). P-solubilizing microbes are considered as successful approach for providing proper nourishment (Martins et al. 2004) and used as soil inoculants to amplify growth and yield of crop plants (Otieno et al. 2015). Soil microbial communities play a significant role in solubilizing and mineralizing inorganic and organic form of phosphorous to make it accessible to plants (Adhya et al. 2015). P-solubilizing bacterial inoculants can be produced by the following steps:

1. Soil sample collection.
2. Serial dilution of soil samples.
3. Inoculation of serially diluted samples on desired media with sources of insoluble phosphorous.
4. Isolation, screening and selection of P-solubilizing bacteria producing clear halo zone around the colonies (halo zones indicate P solubilization).
5. Bioassay of phosphorous solubilizing ability of isolated bacterial strains.
6. Identification and characterization of PSB.
7. Plant-growth promoting activities were assessed.
8. Selection of appropriate carrier and development of bio-inoculants (microphos).
9. Field / pot trials of microphos.
10. Standardization.
11. Commercially prepared for agricultural implementation.

Hence, using P-solubilizing bacteria as bio-inoculants will reportedly enhance the uptake of P through plants (Chen et al. 2006). The microphos including P-solubilizing bacteria can be utilized in a distinct way i.e. as seed treatment, seedling root dip and soil application. Several studies revealed that P-solubilizing species of *Rhizobium, Bradyrhizobium* and *Azotobacter* in leguminous and non-leguminous plant enhance the P-content and growth of the plant.

5.4.6 Conclusions

Phosphorous is of paramount importance for plant nutrition after nitrogen. The adverse environmental effect, depleting rock phosphate and increasing price of chemical phosphate fertilizer have compelled to find sustainable method of agriculture to accomplish the increasing demand of food for the ever-increasing human population. Therefore, it is very important to make substitutes for chemical P fertilizers that are cost-effective. In this perspective, the research on P-solubilizing endophytic bacteria gained interest and used as economically efficient bio-inoculants or biofertilizers. Phosphorous solubilizing or mineralizing bacteria play a significant role in maintaining sustainable agriculture. However, limited knowledge and understanding of P-solubilizing bacteria has been achieved till date and require detailed study of interactions of microbes in the rhizosphere and their P- solubilizing potential from different fractions of soil. The phosphate solubilizing bacteria unfold new opportunities for extensive research to identify and characterize more phosphate-solubilizing endophytic bacteria with pronounced efficiency and as a result it can be used as biofertilizers in the field conditions. This can be achieved by extensive research on genetic engineering of specific P- solubilizing bacteria for strain improvement to get target results, and this technology should be transferred to farmers for better and eco-friendly agricultural practices.

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