Chapter 4
Endophytic Bacteria: Role in Phosphate Solubilization

Abhishek Walia, Shiwani Guleria, Anjali Chauhan and Preeti Mehta

Abstract The worldwide need to increase agricultural and horticultural production from a consistently diminishing and degraded land resource has set remarkable strain in light of agro biological systems. The current methodology is to keep up and enhance agricultural and horticultural productivity only by means of the utilization of chemical fertilizers and pesticides. Despite the fact that the utilization of chemical fertilizers is credited with almost fifty percent of increase in agricultural production yet they are closely associated with environmental contamination and health problems in human beings and animals. Microbial assorted qualities in the soil are viewed as critical for keeping up for the manageability of agriculture and horticulture systems. Nonetheless, the connections between microbial differences and environmental processes are not surely known. Rhizosphere soil strongly affects a range of procedures influencing crop yield. Rhizobacteria that are present inside plant roots, framing more close associations, are known as endophytes. These endophytes are likewise called intracellular plant growth-promoting rhizobacteria (PGPR) microorganisms dwelling inside plant cells, producing nodules and being present inside these specific structures. These incorporate an extensive variety of soil microorganisms framing less formal relationship than the rhizobia-legume advantageous interaction called symbiosis, endophytes may empower plant development, directly or indirectly and incorporate the rhizobia. In this review, we essentially concentrate on the plant development by Phosphate solubilization furthermore by different means. Phosphorus is normally lacking in most characteristic soils since it is settled as

A. Walia
Department of Microbiology, DAV University, Jalandhar, India

S. Guleria
Department of Biotechnology, Lovely Professional University, Jalandhar, India

A. Chauhan
Department of Soil Science and Water Management, Dr. YSPUH & F, Solan, Himachal Pradesh, India

P. Mehta (✉)
DBT-IOC Centre, R&D, Indian Oil Corporation Limited, Sec-13, Faridbad, Haryana 121007, India
e-mail: microbiology2preeti94@gmail.com

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insoluble iron and aluminum phosphates in acidic soils or calcium phosphates in soluble soils. Phosphate-solubilizing bacteria (PSB) as inoculants have the ability to convert insoluble forms of phosphorus to an usable form for high plant yields. This chapter mainly focuses on endophytic P-solubilizing bacteria, mechanism of P-solubilization, genetic diversity of P-solubilizers, and mass production of inoculants inoculant production and response of the crop to P-solubilizers bioinoculants.

Keywords Endophytes · Phosphate solubilization · PGPR · Bioinoculants Genetic diversity

4.1 Introduction

Microbial diversity in soil is viewed as critical for keeping up for the manageability of horticulture/agriculture creation frameworks. Notwithstanding, the connections between microbial diversity and ecosystem processes is not surely understood (Stark et al. 2007; Jha et al. 2014). Rhizosphere soil strongly influences the range of processes impacting crop yield. Numerous microorganisms are pulled in by supplements oozed from plant roots and this "rhizosphere impact" was initially depicted by Hiltner (1904). He observed higher number and activity of microorganisms in the region of plant roots. These microbes gain profit by the nutrient exudates by the plant roots, which ultimately advantageously impact the development of plants.

As of late, the interest in soil microorganisms has expanded, as they are a key component in supplement cycling and the support of soil fertility. Phosphorus is one of the essential macronutrient for plant growth and development In average soils, the P-content is about 0.05% (w/w) but only 0.1% of the total P is available to plants (Scheffer and Schachtschasel 1992; Otieno et al. 2015), since it is fixed as insoluble iron and aluminum phosphates in acidic soils or calcium phosphates in alkaline soils. These precipitated forms cannot be absorbed by plants, this leads to excessive and repeated application of P fertilizer to cropland (Sharma et al. 2013).

The capacity of a few soil microorganisms to change over insoluble types of phosphorus (P) to an accessible form is an imperative attribute in plant growth-promoting bacteria (PGPR) also known as P-solubilizing microorganisms (PSM). The utilization of PSM as inoculants enhances the P uptake by plants thus increasing plant yields (Ahemad and Khan 2010; Jain and Khichi 2014). Because of the negative ecological effects of compound composts and their expanding costs, the utilization of PSM is considered as a supplementary method for reducing the utilization of chemicals in agribusiness/cultivation (Welbaum et al. 2004; Hameeda et al. 2006; Mehta et al. 2013c; Walia et al. 2013a).

4.2 PSB and Their Hosts: Endophytic Region

For P-solubilizing PGPR to have an impact on plant development by means of an increment of the nutrient status of their host, there evident should be an intimate relationship between the phosphate-solubilizing bacteria (PSBs) and the host plant.

In any case, the level of closeness between the PSBs and the host plant can differ contingent upon where and how the PSBs colonizes the host plant. Connections between PSBs and their hosts can be ordered into two levels of complexity (1) Rhizospheric (2) Endophytic.

4.2.1 Endophytic Region

Rhizobacteria that build up and spends the entire piece of its life cycle inside plant roots, exhibit no outside contamination or negative impact on their host and forming more intimate associations, are endophytes or intracellular PGPR-(iPGPR). Perotti (1926) was the first to portray the event of nonpathogenic organisms in root tissues. Endophytic microorganisms have been considered to originate from the outside environment and enter the plant through stomata, lenticels, wounds, emergence of lateral roots and germinating radicals (Gaiero et al. 2013). Endophytic microbes can effectively or inactively colonize plants locally or systemically and both intercellularly and intracellularly. The endophytic niche provides protection from the environment for the colonizing bacteria that establish *in planta.* Subsequently, to be biologically effective, endophytes that infect plants from soil must be able to root colonizers. Despite the fact that, it is, for the most part, accepted that numerous bacterial endophyte groups are the result of a colonizing process started in the root zone (Compant et al. 2010), they might likewise begin from other sources than the rhizosphere, for example, the phyllosphere, the anthosphere, or the spermosphere. Lytic proteins created by root colonizing bacteria may likewise add to more effective penetration and colonization. The deliverable of endophytes like cellulolytic and pectinolytic catalysts are being considered for certain types of infection process, cell wall degrading chemicals, endogluconase and polygalacturonase causes infection of *Vitis vinifera* by *Burkholderia* sp. (Hallmann et al. 1997; Compant et al. 2005).

Endophytic microorganisms inhabiting vast assorted qualities of plants was looked into by Sturz et al. (2000) and Posada and Vega (2005). Rhizosphere is considered as a hot spot for P-solubilizing bacteria suggesting that these bacteria proliferates both in rhizosphere soil and root endosphere (Hui et al. 2011). But apart from that, population of endophytic bacteria is at the lower site as compare to rhizospheric bacteria or any other bacterial pathogens (Feng et al. 2013). Although, many researchers have confirmed the occurrence of least amount of endophytes in rhizosphere but Mehta et al. (2015) had given a strong evidence in their study with perpetually higher P-solubilizing bacteria in apple rhizosphere than those in roots endosphere (Table 4.1). The most acceptable reason for a higher population of rhizospheric bacteria could be due to high level of carbon fluxes creating the'rhizosphere impact' used to sustain bacterial growth (Reyes et al. 2006; Mittal and Johri 2007).

The population of PSB is always higher around the rhizosphere and around roots as compare non-rhizosphere. The high concentration of PSB around the roots

Table 4.1 Comparative data on P-solubilizing bacterial population in rhizosphere soil and roots of apple trees at different sites

Location	Percent P-solubilizers	
	Rhizosphere soil bacterial population	Root endophytic bacterial population
Chamba	79.2	38.8
Kinnaur	35.2	15.6
Shimla	29.2	18.7
Kullu	48.3	11.3
	T = 2.06	

occurs because of the presence of high levels of nutrients exuded from the roots of most plants that can support bacterial growth and metabolism (Glick 2003; Sharma et al. 2007). Higher the population of P-solubilizers is of direct significance to the plants as it helps in mobilization of insoluble P near the root, especially in P-deficient soils (Chatli et al. 2008; Gulati et al. 2008; Aranda et al. 2011).

In plant tissue, in general, endophytic P-solubilizing bacterial populations have been reported between 10^2 and 10^4 viable bacteria per gram (Sobral et al. 2004; Piromyou et al. 2010; Patel et al. 2012; Kumar et al. 2013a, b; Saini et al. 2015). Mehta et al. (2015) isolated one hundred and four and 85 of total 200 soil and root samples of apple trees harbored P-solubilizing bacteria. They observed that the proportion of rhizosphere soil and root endophytic P-solubilizing bacteria among culturable one varied greatly with respect to sampling sites, ranging from 0–79.2% to 0–60.6%, which was in agreement with previous study that showed large variation from 3 to 67 $\times$ 10^6 cfug^{-1} (Kundu et al. 2009). A large variation within and amongst different sites in population of P-solubilizing bacteria indicated their wide distribution within the crop and place of sampling. The poor population of P-solubilizing bacteria could be attributed to their meager natural population as a result of environmental factors along with physiochemical properties of the soil. Variation in the population of P-solubilizing bacterial status of samples within the sites is possible due to the collection of samples from a different point and an uneven population of competitive P-solubilizing bacteria.

Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species (Ryan et al. 2008; Mehta et al. 2015). The variation in endophytes occurrence might be a function of the different maturation stages specific to each plant, sampling time and environment condition, which contribute higher impact on different types and amounts of root exudates (Vendan et al. 2012). The presence of large population of bacteria isolated from all the sites unequivocally suggests the hypothesis that natural plant genotypic variants of a single species have a special choice for selection of specific microbiota consortia as a result of their unique root exudates profile (Micallef et al. 2009; Aranda et al. 2011).

4.2.2 Role of Phosphorus Solubilizing Microorganisms

The term microorganisms broadly encompass bacteria and fungi including other mini-creature only observed by microscope. Among the microorganisms, bacteria and fungi are more versatile to facilitate phosphate solubilization.

4.2.2.1 Phosphorus Solubilizing Bacteria and Fungi

PSM consist predominantly the bacteria and fungi among ectorhizospheric strains, *Pseudomonas*, *Bacillus,* and endosymbiotic rhizobia have been served as effective phosphate solubilizers (Igual et al. 2001). The vast majority of fungi are non-Phosphate solubilizers except for species of *Aspergillus* and *Penicillium* (Sagervanshi et al. 2012; Sahoo and Gupta 2014). Villegas and Fortin (2002) identified microorganism viz., *Rhizobium*, *Klebsiella*, *Mesorhizobium*, *Acinetobacter*, *Erwinia*, *Achrobacter*, *Enterobacter*, *Micrococcus*, *Pseudomonas* and *Bacillus* isolated from different soils as efficient P solubilizing strains. Majority of Gram-positive soil bacilli almost 95% belong to the genus *Bacillus* (Garbeva et al. 2003) and are capable to form endospores and for this reason survive beneath detrimental conditions; some species are diazotrophs along with *Bacillus subtilis* (Timmusk et al. 1999), while others have specific PGPR capacities (Kokalis-Burelle et al. 2002; Barriuso and Solano 2008). From rhizobial strains, two species of nodulating chickpea, *Mesorhizobium mediterraneum* and *Mesorhizobium ciceri*, are known for their high phosphate-solubilizing efficiency (Rivas et al. 2006). But, it is recognized that each aspect of nodule formation is limited due to the supply of P. legumes like alfalfa and clover displaying a positive effect in response to P supplementation (Gyaneshwar et al. 2002), however most of the supplemented P become unavailable when its reacts with soil components. The extracellular oxidation of glucose to gluconic acid via the quinoprotein glucose dehydrogenase results in efficient phosphate-solubilizing phenotype in Gram-negative microorganism (Otieno et al. 2012). Numerous soil microorganisms have the ability to solubilize this unavailable P through their metabolic activities exudating organic acids, which directly dissolve the rock phosphate, or chelating calcium ions that release P to the solution.

4.2.3 Microbes in Biogeochemical Cycle of P in Soil

Microorganisms are fundamental to the biogeochemical cycle of phosphorus and as such play crucial role in mediating the availability of phosphorus to flora (Richardson et al. 2011; Jain and Khichi 2014). Biogeochemical cycling of phosphorus is essential for various reasons. Every living cell requires phosphorus for nucleic acids, lipids, and a few polysaccharides. In soil, phosphorus exists in both

inorganic and organic forms. Inorganic phosphorous complexes without problems with cations (includes iron, aluminum, and calcium) in the environment as it is negatively charged. These compounds are relatively insoluble, and their separation is pH dependent, being accessible to plants and microorganisms between pH 6 and 7. Under such conditions, these organisms rapidly convert phosphate to its organic form in order that it becomes available to animals. A significant percentage of culturable bacterial and fungal communities were being accounted for inorganic P solubilizing activity (Barraquio et al. 2000; Chen et al. 2008; Ashrafuzzaman et al. 2009). The form of phosphorus found in biomass and materials such as humus and organic compounds is known as organic phosphorus. This organic phosphorus is recycled by microbial activity that involves transformation of simple orthophosphate (PO_4^-), with +5 valence state into more complex forms. These include the polyphosphate seen in metachromatic granules in addition to greater acquainted macromolecules.

4.2.4 P-Solubilizer as Biofertilizers

Microbial inoculants have provided a worth biological alternative to compensate agro chemicals and to sustain environment-friendly crop production (Dobbelaere et al. 2003; Musarrat and khan 2014). Phosphorus solubilizing microorganisms proved as an effectual approach for imparting balanced nutrition (Martins et al. 2004) and have recently attracted the attention of agriculturalists as soil inoculums to enhance the plant growth and yield (Fasim et al. 2002; Otieno et al. 2015).

The inorganic phosphates solubilization in soil by microorganisms and making them available to plants is the well-known mechanism (Bhattacharya and Jain 2000; Chen et al. 2006) and organisms responsible for this are referred as phosphate solubilizers. Population count of phosphate-solubilizing microorganisms is at the concentrated form in the rhizosphere, and they are metabolically more active than other sources (Vazquez et al. 2000). It is well known that both groups of microorganisms including phosphate-solubilizing bacteria and fungi are equally important to enhance plant growth by using solubilization mechanism and their acquisition to plant production via synthesis of plant growth-promoting substance and organic acid (Yadav et al. 2011).

The improvement of soil health in terms of fertility is one of the most common ways to increase agricultural production for which biological nitrogen fixation is considered to be the most important. After biological nitrogen fixation, phosphate solubilization is equally essential, as phosphorus (P) is significant key macronutrients for biological growth and development. Microorganisms provide a biological rescue system that enables to solubilize the insoluble inorganic P of soil and make it available to the plants. The ability of a few microorganisms to convert insoluble phosphorus (P) to an available form, like orthophosphate, is a critical trait in a PGPB for improving soil fertility and plant yields. Thus, the rhizospheric

phosphate-solubilizing microorganism can be a promising source for plant growth-promoting agent in agriculture (Rodriguez et al. 2006).

Using phosphate-solubilizing microorganism as inoculants will increase the P uptake through plants (Chen et al. 2006). The production of bioinoculants on a commercial scale and their acceptance by farming communities are closely linked as it is not easy. Furthermore, environmental variables including salinity, pH, moisture, temperature and climatic conditions of the soil largely affect the establishment and performance in field cum demonstrations trials of these PSM inoculants developed under laboratory conditions. Hence, there is a great need for proper development of suitable technology for the isolation of effective inoculants of PSM based biofertilizers for their adoption under farmer's fields. Current approach and developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi et al. 2009a).

4.3 Mechanism of P-Solubilization

Organic acid production by soil microorganisms is predominant mechanism of phosphate solubilization. Organic acids result in a decrease in pH of microbial cell and its surroundings (Halder et al. 1990; Khan et al. 2014a) (Fig. 4.1). In soil, phosphorus is present in the organic and inorganic form. Soil microorganisms release phosphorus by organic and inorganic P solubilization. Organic P solubilization is mineralization process (Richardson and Simpson 2011). Numerous mechanisms are opted by soil microorganisms in order to perform P solubilization such as lowering of pH, organic acid production, chelation and exchange reactions (Gerke 1992). Microorganisms secrete different types of organic acids during solubilization and lower the pH of rhizosphere and consequently dissociate the bond form of phosphates like $Ca_3(PO_4)$ (Tri Calcium Phosphate) in calcareous soil. Furthermore, these microorganisms also serve as a sink for P in the vicinity of labile C. Soil microorganisms immediately immobilize it even in low P soils. Ecological changes, for example, freezing–thawing or drying–rewetting, can bring about flush-events, a sudden increment in accessible P because of high extent of microbial cell lysis (Butterly et al. 2009).

The major processes employed by microorganisms for soil P solubilization summarized here:

(1) Secretion of mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions, CO, siderophores
(2) Biochemical P mineralization by release of extracellular enzymes and
(3) Biological P mineralization by liberation of P during substrate degradation

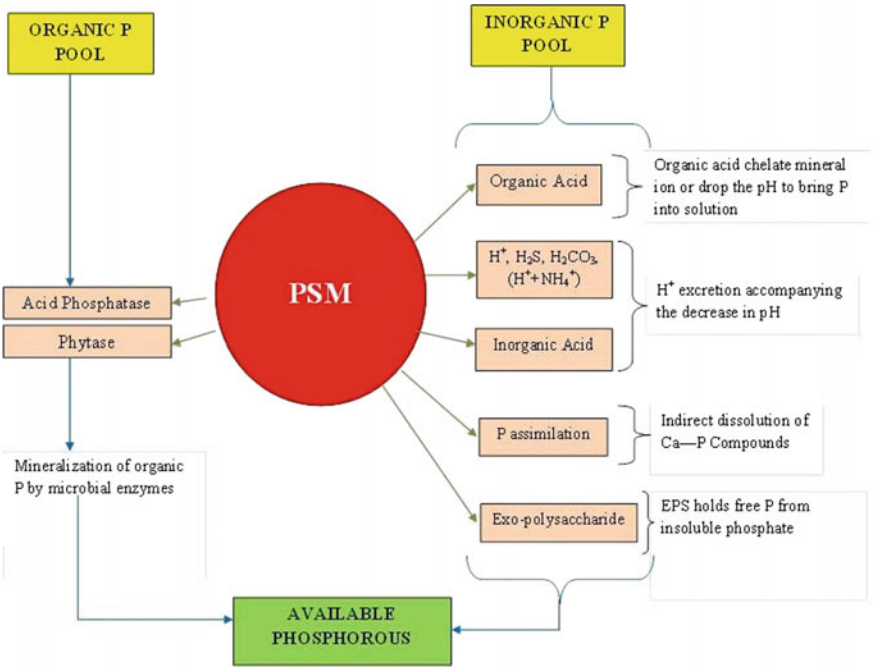

Fig. 4.1 Schematic representation of P solubilization/mineralization by various organic/inorganic substances produced by PSM

As described by Sims and Pierzynski (2005), the major processes of the soil P cycle that affect soil solution P concentrations are biologically mediated conversions of P between inorganic and organic forms, i.e., mineralization–immobilization; interactions between P in solution, and soil solid surfaces, i.e., sorption–desorption and dissolution–precipitation, i.e., mineral equilibria.

4.3.1 Different Microbial Mechanisms of P-Solubilization

Microorganisms are observed as proprietor of diverse mechanism to solubilize both organic and inorganic phosphate.

4.3.1.1 Organic P-Solubilization

Mineralization of organic phosphorus constituting 4–90% of the total soil P is referred as Organic P solubilization (Khan et al. 2009). Each organism can act in one or multiple ways to bring about the solubilization of insoluble P in soil. One of

which is use of enzyme, i.e., Non-specific acid phosphatases (NSAPs), phytases, C–P lyases, and Phosphonatases.

Non-specific acid phosphatases (NSAPs) have a capacity of dephosphorylate phosphoanhydride or phospho-ester bonds of organic matter. Among the different classes of phosphatase enzyme released by PSM, most studied and abundant class is Phosphomonoesterases (often called phosphatases) (Nannipieri et al. 2011). Depending upon the pH optima, phosphatases are further divided into acid and alkaline phosphomonoesterases (Jorquera et al. 2008). These enzymes (acid and alkaline phosphatases) are produced by plant roots as well as by PSM. Differentiation between phosphatases on the basis of their production source is very difficult (Richardson et al. 2009). However, plant roots can only produce large quantities of acid phosphatases. There are evidence proposing that phosphatases released from microbes have higher affinity for Po compounds as compared to phosphatases produced from plant roots (Chen et al. 2003), but still, there is not much understanding regarding the relationship between phosphatase activity of inoculated PSM and the subsequent mineralization of Po.

Phytases have a specific capacity of phytate degradation and cause P release. Phosphorous is stored in plant seeds and pollen in form of phytate. In the plant, it is primary inositol source. The key driver of regulation of phytate mineralization in soil is microorganisms. In spite of the fact that the capacity of plants to get P specifically from phytate is exceptionally restricted, but the vicinity of PSM inside of the rhizosphere provide an opportunity to plants to take up P directly from phytate (Richardson and Simpson 2011).

C–P lyases and phosphonatases are enzymes that act mainly in the breakdown of the C–P bond in organophosphonates (Rodriguez et al. 2006).

4.3.1.2 Inorganic P-Solubilization

Organic Acid Production

The major reason of inorganisc phosphorous solubilization is organic acid production by PSM. Primarily following organic acids are produced, i.e., acetic, citric, fumaric, glycolic, lactic, melonic, oxalic, propoionic, succinic acid, tartaric, etc. (Ahmad and Shahab 2011). Among all, the principal organic acid involved in inorganic P solubilization is gluconic acid. PSBs which produce abundant amount of gluconic acid are *Burkholderia cepacia*, *Erwinia herbicola, Pseudomonas* sp and *Pseudomonas cepacia* (Goldstein et al. 1994). However, sulphuric and nitric acids producing PBMs, i.e., *Thiobacillus* and *Nitrosomonas* species were also reported to solubilize phosphate compounds (Azam and Memon 1996).

HPLC (high-performance liquid chromatography) and enzymatic methods are mostly employed for the detection of organic acids produced by PSM (Whitelaw 2000). Mehta et al. (2013a) detected six different organic acids in culture filtrate of

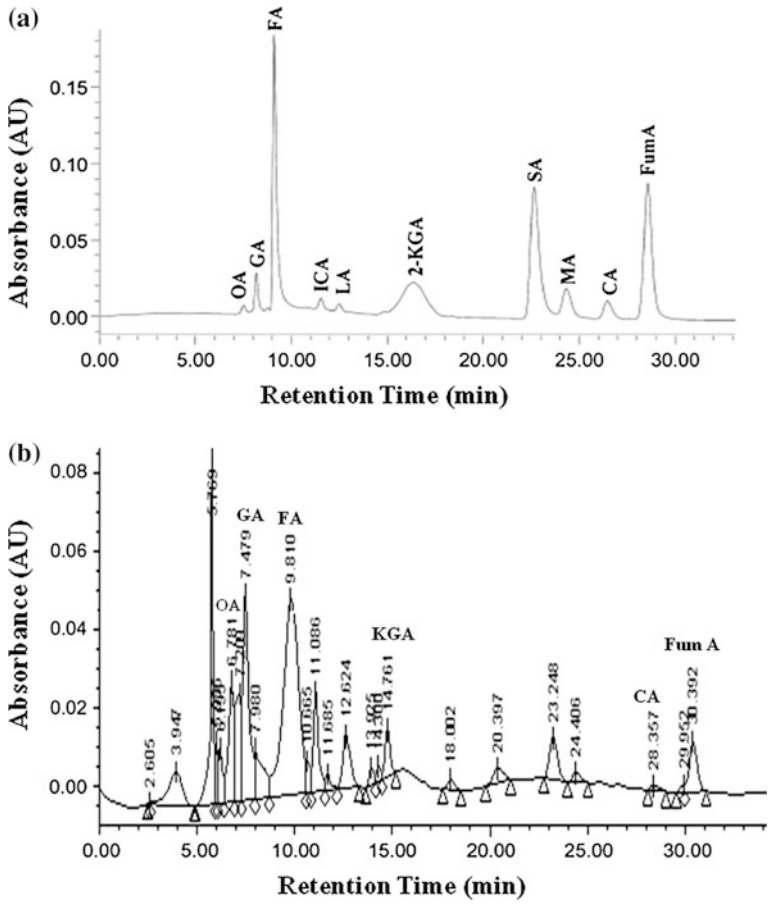

Fig. 4.2 HPLC chromatograms of authentic organic acids (**a**) and culture supernatant of *Bacillus subtilis* CB_8A grown for 3 days in PVK broth (**b**). Adopted from Mehta et al. (2013a). *OA* Oxalic acid; *GA* gluconic acid; *FA* formic acid; *ICA* isocitric acid; *LA* lactic acid; *2-KGA* 2-ketogluconic acid; *SA* succinic acid; *MA* maleic acid; *CA* citric acid; and *FumA* fumaric acid

Bacillus subtilis CB_8A by HPLC (Fig. 4.2). Six organic acids produced by *Bacillus subtilis* CB_8A are oxalic acid, gluconic acid, formic acid, 2-ketogluconic acid, citric acid, and fumaric acid. Out of these, major organic acids were gluconic acid (1.43%) and citric acid (0.67%) (Fig. 4.2). The reason of P solubilization by organic acid production may be: decrease in the pH; complex formation with metal ions of insoluble P (calcium phosphate, iron phosphate) and finally, P release; by competing with P for sites on the soil.

4.3.1.3 Important Facts of P-Solubilization by Organic Acid Production

i. Organic acids responsible for P-solubilization are the microbial metabolic product such as the product of fermentation of organic carbon sources (e.g., glucose) or oxidative respiration (Trolove et al. 2003).
ii. There is release of organic acids from the outer face of cytoplasmic membrane of P-solubilizing microorganisms which is the site of direct oxidation pathway. This organic acid release into the medium result in a decrease in pH (Zaidi et al. 2009b).
iii. PSM strains acidify the surrounding environment by synthesis and discharge of organic acid. Organic acids have the ability to chelate cations, i.e., Al, Ca, and Fe linked with P or they can result in exchange of acid anion with phosphate anion (Omar 1998).
iv. According to the abiotic study of Whitelaw et al. (1999), it was proved that HCl and gluconic acid can solubilize P. On the basis of above fact, solubilization of colloidal Al phosphate might be due to chelation of Al^{3+} by gluconic acid.
v. There is the presence of soluble inorganic phosphate i.e. H_2PO_4 at low pH. However, divalent and trivalent inorganic phosphate, i.e., HPO_4^{-2} and HPO_4^{-3} arise with the increase in soil pH.

However, acidification does not appear to be the main system of solubilization, as the capacity to decrease the pH at times did not associate with the ability to solubilize mineral P (Subba Rao 1982). The phosphate-solubilizing activity was ascribed both to reduction and to chelation processes.

4.3.1.4 Excretion of Proton

One of the major aspects responsible for P solubilization is pumping out of protons from cell (Krishnaraj et al. 1998). Some microorganisms release proton during NH^{4+} assimilation as the sole mechanism to promote P solubilization (Parks et al. 1990). Illmer and Schinner (1995) reported the absence of organic acids in culture solution by HPLC during P-solubilization by *Pseudomonas* sp. They also reported the probable reason of P-solubilization in culture solution, i.e., release of protons accompanying NH^{4+} assimilation or respiration. Participation of H^+ pump mechanism in P solubilization is also reported in *Penicillium rugulosum* (Reyes et al. 1999). Different mechanisms of proton release have been followed by different species. However, for P solubilization, only a few depends upon the presence of NH^{4+} ion (Carrillo et al. 2002).

4.3.1.5 Role of Siderophores and Exopolysaccharides in P-Solubilization

Siderophores are small, iron chelating molecules that bind with ferric ion and transport it to a cell. As, ligand exchange by organic acid anion is not a dominant P-solubilizing mechanism as compared to mineral dissolution. On the basis of this fact, the role of siderophores in enhancing P-solubilization is considered (Parker et al. 2005). There are various reports in the literature regarding the release of siderophores from PSM (Vassilev et al. 2006; Hamdali et al. 2008).

Microbial exopolysaccharides may play role in P-solubilization. Exopolysaccharides, secreted outside the cell by bacteria and fungi are mainly carbohydrate polymers. They are of different types, i.e., homo polysaccharides and heteropolysaccharides and may additionally contain a number of extraordinary organic and inorganic substituents. The role of microbial polysaccharides in P solubilization has been assessed by Yi et al. (2008). They reported significant production of EPS by highly efficient P-solubilizing bacteria, i.e., *Arthrobacter* sp. (ArHy-505), *Azotobacter* sp. (AzHy-510), *Enterobacter* sp. (EnHy-401), and *Enterobacter* sp. (EnHy-402).

4.3.1.6 Other Mechanisms

It has been suggested that processes such as sulphur oxidation, carbon monoxide, and nitrate production result in the formation of inorganic acids like sulphuric acid are a consequence of microbial phosphate solubilization (Swabyand Sperber 1958). The reaction between H_2S and ferric phosphate result in the formation of ferrous sulphate along with the simultaneous release of phosphate. So, production of H_2S can be one of the P-solubilization mechanisms.

4.3.1.7 Genetic Basis of Inorganic P-Solubilization

One of the major mechanism of P-solubilization is the production of organic acids, i.e., MPS. Therefore, understanding of the genetics behind MPS phenotype is necessary (Goldstein and Liu 1987). This assumption has been supported by cloning of PQQ gene responsible for gluconic acid production.

Pyrroloquinoline Quinone (PQQ) [(4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-] quinoline-2,7,9 tricarboxylic acid), aromatic, tricyclic ortho-quinone], belongs to the family of quinone cofactors. It serves as the redox cofactor for several bacterial dehydrogenases such as methanol dehydrogenase and glucose dehydrogenase (Fig. 4.3). PQQ-dependent glucose dehydrogenase (GDH) resides in the cytoplasmic membrane, can oxidize glucose to gluconate GDH, which needs PQQ for the holoenzyme. PQQ is derived from tyrosine and glutamic acid. It is characterized as a third class of redox cofactors following pyridine nucleotide and flavin-dependent cofactors (Houck et al. 1991).

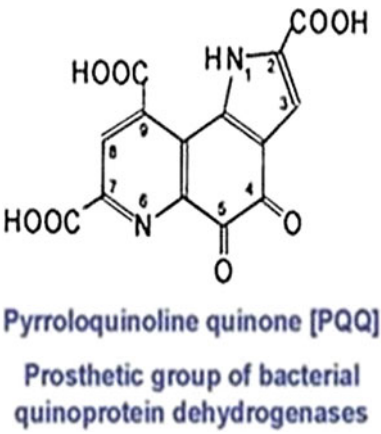

Fig. 4.3 Prosthetic group of bacterial quinoprotein dehydrogenases

Glucose dehydrogenase (GDH) Glucose dehydrogenase (GDH) is a quinoproteins which has the ability to oxidize glucose into gluconic acid. During catalytic reaction, GDH needs pyrroloquinoline quinone (PQQ) and metal ions such as Ca^{+2} or Mg^{+2} (in vitro). Membrane GDHs (m-GDHs) are monomeric proteins of 88 kDa with an N-terminal hydrophobic and large conserved PQQ-binding C-terminal domains. This C-terminal domain has catalytic activity (Yamada et al. 1994). However, N-terminal hydrophobic domain (residues 1–150) anchors the protein to the membrane. It consists of five trans-membrane segments which play a major role in anchoring the protein (Yamada et al. 1994). GDH plays a regulatory key in bioenergetic role in the bacteria. Uptake of exogenous compounds such as amino acids is due to trans-membrane proton motive force (PMF). Protons produced during oxidation participate directly in the generation of trans-membrane proton motive force (PMF). Therefore, this oxidative glucose pathway might be important for the survival of bacteria.

Very little is known regarding genetic or biochemical mechanisms involved in the synthesis of the GDH-PQQ halo enzyme. The possible inducers of halo enzyme are manitol, glucose, gluconate, and manitol (Van Schie et al. 1987). However, among several bacterial species, the difference in their constitutive and inducible phenotypes is observed (Goldstein 1994).

4.3.2 Genetic Diversity and Role of Genetic Engineering in P-Solubilization

4.3.2.1 Genetic Diversity of Phosphate-Solubilizing Microorganisms

Rhizosphere comprises of a huge microbial population of bacteria, fungi, protozoa, and algae. Bacteria are the most copious among them. The selection and

colonization of bacteria with in plant is based on their contribution to the fitness by releasing organic compounds through exudates (Lynch 1990), and therefore low diversity, selective environment is created (García et al. 2001). Since bacteria profusely colonize the rhizosphere and to the greater extent influence plants physiology, mainly taking into consideration of their competitiveness in root colonization (Antoun and Kloepper 2001).

The genotypic and phenotypic characteristic analysis of indigenous rhizobacteria can elucidate the mechanisms of interaction between them and plant roots. Studies on bacterial diversity are much more complex at taxonomic, functional and genetic levels in comparison to eukaryotes owing to the minute working scale and a large number of different bacterial species present in the environment.

Molecular basis behind phosphate solubilization by microorganisms is still limited and inconclusive (Rodriguez et al. 2006). Complete study of genes involved in P-solubilization and development of genetically engineered microbes is important not only for understanding their ecological role in the natural environment but also for their biotechnological application. As far as soil health is concerned exhaustive efforts are being made to explore indigenous soil microbial diversity with nutrient acquisition and mobilization potential with a special understanding of their distribution and behavior in soil habitats as well their influence on the quality of plant and soil health after introducing them as bioinoculants (Kumar et al. 2015).

A substantial number of phosphate-solubilizing culturable bacterial communities apart from genera *Pseudomonads* and *Bacilli*, there are some efficient P-solubilizing fungi that do not lose the P dissolving capacity even on repeated sub culturing under laboratory conditions as it occurs with most of the P-solubilizing bacteria (Kucey 1983). Generally, the release of organic acids by P-solubilizing fungi than bacteria consequently exhibit greater P-solubilizing activity. Among filamentous fungi that solubilize phosphate more efficiently belongs to genera *Aspergillus* and *Penicillium* (Reyes et al. 2002) although strains of *Trichoderma* and *Rhizoctonia solani* (Jacobs et al. 2002) have also been reported as good P solubilizers. Very few studies have been conducted in case of yeast to gauge their phosphate-solubilizing ability, these include *Yarrowiali polytica*, *Schizosaccharomyces pombe* and *Pichia fermentans* (Vassilev et al. 2001).

4.3.2.2 Genetic Engineering of PSM

High agricultural yield depends upon plant growth and nowadays it is achieved by employing high cost as well as environmentally hazardous phosphate fertilizers. To overcome this, an ecofriendly approach is to develop bacterial strains that can convert the form of phosphorus present in the soil to soluble forms which can be easily taken up by plants. Various attempts for developing such strains were made in past but failed due to incomplete knowledge of the phosphate-solubilizing genes, as well as the failure of the survival of bacterial strains under plant root environment. To deal with these challenges, this is desired to discover novel genes and pathways underlying solubilization of phosphorus sources which can be done by

the use of genome wise mutagenesis of phosphate-solubilizing bacteria. Validation of such novel genes and functions in *E. coli* is possible through advanced synthetic biological approaches which ultimately transfer novel phosphate-solubilizing capabilities associated with plant rhizosphere bacterium.

Several genes are isolated and characterized which are involved in mineral and organic phosphate solubilization. Cloning and expression of such genes in selected rhizobacterial strains through molecular biotechnology and genetic engineering have made a promising perception for obtaining recombinant strains with improved phosphate-solubilizing capability for agricultural purpose. Insertion of phosphate-solubilizing genes into microorganisms that lack P-solubilization trait may avoid the current need of using more than one strain of PGPR or consortia, when used as bioinoculants. The foremost success in cloning of a gene involved in mineral P solubilization in Gram-negative bacteria *Erwinia herbicola* was first time done by Goldstein and Liu (1987). The expression of this gene allowed the phosphate solubilization activity in *E. coli* HB101. *E. coli* can synthesize GDH, but not PQQ, thus it does not produce GA. This gene contributed in the synthesis of enzyme pyrrolo quinoline quinone (PQQ) synthase which was investigated through sequence analysis. For the synthesis of holoenzyme glucose dehydrogenase (GDH)-PQQ, PQQ is required which is a cofactor whose synthesis is directed by the enzyme pyrrolo quinoline quinone (PQQ) synthase. Formation of gluconic acid from glucose through direct oxidation pathway is catalyzed by glucose dehydrogenase (GDH)-PQQ. Sub cloning of the specific gene encoding mineral phosphate solubilization was done in a broad host range vector (pKT230). The recombinant plasmid expressed in *E. coli*, and further transferred to plant growth-promoting strains of *Burkholderia cepacia* and *Pseudomonas aeruginosa*, using tri-parental conjugation.

4.3.2.3 Mineral Phosphate-Solubilizing Genes for Strain Improvement

Genetic background, presence of number of copies of plasmids as well as metabolic interaction of recipient strains could highly influence the expression of an MPS gene in a different host. Thus, genetic transfer of any isolated gene involved in MPS to stimulate phosphate-mobilizing aptitude in PGPB strains, is an attention-grabbing approach.

Kim et al. (1998) reported the expression of MPS genes isolated from *Ranella aquatilis* which when cloned in *E. coli* boost a high-level production of gluconic acid (GA) and hydroxyapatite dissolution as compared to donor strain. It was suggested that different genetic regulation of the MPS genes might occur in both species. In another case study, an increase in exudation of organic acids as well as phosphate availability to plants was observed by the expression of bacterial citrate synthase gene when expressed in tobacco roots. More yield of leaf and fruit biomass was observed in citrate overproducing plants when grown under phosphate limiting conditions along with low P-fertilizer doze which depicted the putative role of organic acid synthesis genes in P uptake in plants.

Table 4.2 Microorganisms encoding phosphatase genes for P-solubilization

Microorganisms	Gene or plasmid	Features	References
Serratia marcesence	pKG3791	Produce gluconic acid and solubilizes P	Krishnaraj and Goldstein (2001)
Rahnella aquatilis	pKIM10	Solubilize P and produce gluconic acid in *E. coli* DH5α	Kim et al. (1998)
Enterobacter agglomerans	pKKY	Solubilize P in *E. coli* 109, does not lower pH	Kim et al. (1997)
Pseudomonas cepacia	Gab Y	Solubilize P and produce gluconic acid in *E. coli* JM 109	Babu-Khan et al. (1995)
Erwinia herbicola	*Mps*	Solubilize P and produce gluconic acid in *E. coli* HB 101, probably involve in synthesis of PQQ	Goldstein and Liu (1987)
Bacillus subtilis CB_8A	*Gdh*	Solubilise P and produce gluconic acid	Mehta et al. (2013c)

Most of the bacterial phosphatase-encoding genes were isolated by means of expression cloning systems entirely based on histochemical based screening of genomic libraries (Table 4.2). These procedures not only allow quick recognition of clones harboring, but also the expression of enzymatic activity.

Riccio et al. (1997) developed a selection system based upon indicator medium consisted of phosphatase substrate phenolphthalein diphosphate (PDP) and methyl green (MG) stain, resulted in green putative colonies with phosphatase positive phenotype (pho1) whereas, phosphatase negative (pho2) clones were grown as unstained colonies. This system offers an imperative approach for the isolation of several bacterial phosphatase-encoding genes from different species, such as *Providencia sturatii, Providencia rettgeri* and *Morganella morganii*.

Another important system for the expression of cloning of bacterial phosphatase-encoding genes (*phoC*) used by Pond et al. (1989) consists of Luria Agar amended with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) which was used for cloning of an acid phosphatase-encoding gene from *Zymomonas mobilis.* The transformant colonies were of dark blue which makes its easy direct selection on indicator plates.

Groisman et al. (1984) cloned the structural gene for the pH 2.5 acid phosphatase (*appA*) of *E. coli* for direct amplification of higher para-nitrophenyl-phosphate (pNPP) hydrolysis (phosphatase activity) responsible genes as a result acid phosphate colonies appeared yellow. Thaller et al. (1994) classified a non-specific phosphohydrolases into three different families: class A, class B, and class C phosphatases based on the cloning of phosphatase genes sequence analysis with other important parameters. Rossolini et al. (1998) studied the sequence level high homology in case of class A phosphatase genes from *M. morganii* and *P. stuartii*, which signifies that these genes are vertically derived from a common ancestor. A number of other phosphatase genes from *Escherichia coli* include: *ushA*, which encodes a 59-nucleotidase (Burns and Beacham 1986) *agp*, which encodes an acid

glucose-1-phosphatase (Pradel and Boquet 1988) and *cpdB*, encoding the 29–39 cyclic phosphodiesterase (Beacham and Garrett 1980).

Sharma et al. (2013) had suggested the application of genetically modified PSM as a potential candidate mover transgenic plants for improving plant performance: (1) with current technologies, a bacterium is much more easier to modify than complex higher organisms, (2) Multiple beneficial plant growth-promoting attributes can be introduced into a single organism, which could minimize the application of multi-strain bio-inoculant (3) Instead the engineering of crop by crop, a single, engineered inoculant can be used for several crops, especially when using a non-specific genus like *Azospirillum* (Rodriguez et al. 2006).

Gene recombination though an important conclusive approach but there are some barriers that needs be resolved first in order to achieve success, such as difference at the metabolic level and regulatory mechanisms between the donor and recipient strains. Despite many constraints and difficulties, significant and consistent progress are being done step by step in this field of molecular biology by genetically engineered microorganisms for sustainable and improved agriculture (Armarger 2002). On the whole, further advance studies on this aspect of PSM will provide key information in future for the better use of these PSM in diverse ecological conditions.

4.4 Phosphate-Solubilizing Bacteria as Plant Growth Promoters

P-solubilizers colonize plant roots and employ valuable effects on growth of plant and enhancement by a prevalent mechanism. To be an efficacious P-solubilizer, microorganisms need to set up itself in the rhizosphere at concentrations adequate to deliver the beneficial impacts. In this way, plant inoculation by P-solubilizer microorganism at a much higher rate than that regularly present in soil is important to exploit the property of phosphate solubilization for plant yield enhancement. There have been various reports on plant development and enhancement by microorganisms that can solubilize inorganic and/or natural P from soil after their inoculation in soil or plant seeds (Mehta et al. 2011; Kumar et al. 2015). The exact mechanism by which P-solubilizer stimulate plant growth is not clearly recognized, although several assumptions such as production of phytohormones, i.e., indoleacetic acid production, activation of P-solubilization, siderophore production, suppression of deleterious organisms, and promotion of the mineral nutrient uptake are usually accepted to be involved (Kumar et al. 2012; Walia et al. 2013b; Mehta et al. 2013a, b, c).

The P-solubilization capacity of the microorganisms is considered to be one of the most essential traits related with plant P-nutrition (Walia and Shirkot 2012). These PSMs render insoluble phosphate into available forms by the process of acidification, chelation, and exchange reaction (Pankaj and Sa 2008). This method

not only compensates the higher cost of industrial fertilizers but also mobilizes the fertilizers supplemented to the soil. In any case, at present, there is proof supporting the part of this component in plant development upgrade. For instance, a few soil microorganisms, including microbes, enhance the supply of P to plants as a result of their ability for inorganic or natural P solubilization (Lifshitz et al. 1987; Richardson 1994; Mehta et al. 2011). Considering that P accessibility is a restricting progress in plant sustenance, this confirmation proposes a basic assurance of phosphate-solubilizing microorganisms to plant nourishment and, consequently increase the performance of plant growth development. Mehta et al. (2013a, b) and Sharma et al. (2015) exhibited plant growth development of apple and tomato by a few microorganisms fit for mineral phosphate solubilization. There are so many strains indicating no indoleacetic acid production, however showing critical mineral phosphate solubilization and adequate movement of phosphatase has enhanced the yield of tomato, cauliflower, capsicum, apple, apricot, etc., among different cultivars, in field experiments.

Besides, a few illustrations of synchronous development and expansion in P uptake by plants as the consequence of phosphate-solubilizing microbial inoculations have been accounted for. Inoculation with two strains of P-solubilizers, i.e., *Rhizobium leguminosarum* has been showed to enhance root colonization and development advancement and to increase essentially the P application in tomato and apricot (Mehta et al. 2013c; Chauhan et al. 2014; Guleria et al. 2014a, b). Chabot et al. (1996) presumed that the P-solubilization impact of Rhizobia and other PSMs is by all accounts the most vital system of plant development advancement in reasonably rich and extremely fruitful soils. Then again, a strain of *Pseudomonas putida* too strengthened the development of roots and shoots and expanded 32P-named phosphate uptake in canola (Lifshitz et al. 1987). Inoculation of rice seeds with *Azospirillum lipoferum* strain 34H and tomato plants with *Bacillus subtilis* strain CKT1 expanded the phosphate particle content and brought about a huge change of root and shoot length and dry weights (Murty et al. 1988; Walia et al. 2013a). Concurrent expansions in P uptake and harvest yields have likewise been seen after inoculation with *Bacillus methylotrophicus* CKAM (Mehta et al. 2014), *Bacillus polymyxa* (Gaur and Ostwal 1972), *Bacillus subtilis* (Sharma et al. 2015), *Bacillus subtilis* CKT1 (Walia et al. 2013a) and *Bacillus circulans* (Mehta et al. 2013c), and others.

Another approach for the utilization of PSMs as microbial inoculants is the utilization of mixed or co-inoculation with different microbes. A few studies exhibit the useful impact of consolidated inoculation of P-solubilizing microbes and *Azotobacter* on yield, and in addition to nitrogen (N) and P accumulation in various crops (Kundu and Gaur 1984). Co-inoculation of *Pseudomonas striata* and *Bacillus polymyxa* strains demonstrating phosphate-solubilizing capacity, with a strain of *Azospirillum brasilense*, brought about a noteworthy change of grain and dry matter yields, with an increase in N and P uptake (Alagawadi and Gaur 1992). Likewise, phosphate-solubilizing *Agrobacterium radiobacte*r coinoculated with nitrogen fixer *Azospirillum lipoferum* showed enhanced grain yield as contrasted to single inoculations in pot and field tests (Belimov et al. 1995). These authors explained that

mixed inoculants gave more adjusted sustenance to the plants, and that the change in N and P uptake was the real mechanism involved. This proof focuses to the upside of the mixed inoculations of PGPR strains including PSMs.

Then again, it has been proposed that some PSMs act as mycorrhizal assistant microbes (Garbaye 1994). In such manner, a few studies have demonstrated that PSMs cooperate with vesicular arbuscular mycorrhizae (VAM) by discharging phosphate particles in the soil, which causes a synergistic connection that takes into consideration better use of ineffectively solvent P sources (Ray et al. 1981). It is likely that the phosphate solubilized by the microbes could be all the more effectively taken up by the plant through a mycorrhizae-intervened span in the middle of roots and encompassing soil that permits supplement translocation from soil to plants (Jeffries and Barea 1994). These authors concluded that the inoculated rhizobacteria could have released phosphate particles from insoluble rock phosphate and other P sources, and were then taken up by the outer VAM mycelium. Commercial biofertilizers affirming to experience phosphate solubilization utilizing mixed bacterial cultures have been produced. Extensive confirmation boosts the particular part of phosphate solubilization in the improvement of plant development by phosphate-solubilizing microorganisms. In any case, not all research center or field trials have offered positive results. For instance, an inoculant utilizing *Bacillus megaterium* var. phosphoricum, was used effectively in the previous Soviet Union and India, yet it did not demonstrate the same effectiveness in soils in the United States (Smith et al. 1962). Also, there are some deleterious species of bacteria present in the rhizosphere that have the potential to influence seed germination, plant growth, and crop yields significantly. These bacteria affect the plant growth through production of phytotoxins (Kumar et al. 2013a, b). Remarkably, in the study conducted by Walia et al. (2013a), a few isolates were found to significantly inhibit seed germination as demonstrated by a reduction in per cent of seed germination over uninoculated control, apparently by producing volatile metabolites. When studied, these deleterious bacterial isolates showed no HCN activity in vitro. Therefore, it is probable that some other gaseous metabolites produced by the bacteria under these conditions have repressed seed germination. This statement is supported by the increase in per cent seed germination by isolate N_{11} which otherwise produced HCN under in vitro conditions (Walia et al. 2013a, b; Alstrom and Burns 1989). Without a doubt, the productivity of the inoculation changes with the soil type, particular cultivar, and different parameters. The P substance of the soil is likely one of the critical elements in deciding the viability of the item.

4.4.1 Production of Phosphate-Solubilizing Microorganism Inoculants

Effective PSM cultures are mass-produced for supply to the agriculturists as microphos. The generation of microphos, i.e., a preparation containing

microorganisms with phosphate-solubilizing action, incorporates three stages: the main concerns choice and testing of phosphate-solubilizing strains; also, inoculant readiness, including determination and handling of the material carrier and mass culture of PSM; and thirdly, quality control methodology and dispersal. For microphos generation, peat, farmyard compost (FYM), soil and dairy animals waste cake powder have been recommended as suitable carriers (Kundu and Gaur 1981). For storage of cultures, these are packed in polybags for around three months at 30 ± 2 °C. In India, a microbial development termed Indian Agricultural Research Institute (IARI) microphos society (Gaur 1990; Khan et al. 2014b) was formed that contained two proficient phosphate-solubilizing microscopic organisms (*Pseudomonas striata* and *Bacillus polymyxa*) and three phosphate-solubilizing growths (*Aspergillus awamori*, *A. niger* and *Penicillium digitatum*).

4.4.2 Technology of Bioinoculants Production

Advancement of an effective inoculant includes a few basic components, for example, strain determination, choice of a carrier, mass duplication, detailing of the inoculant, and bundling and promoting. Stringent quality certification at different strides of generation guarantees the creation of reliably excellent inoculants. By and large, not long after the microbes are brought into the soil, the bacterial populace decays logically (Van Elsas et al. 1986; Bashan and Levanony 1988). This wonder might keep the development of an adequately vast microbial populace in the rhizosphere to acquire the expected plant reaction. The key snag is that the soil is a heterogeneous and flighty environment, even on the little scale (Van Elsas and Van Overbeek 1993). The inoculated microorganisms must contend with the frequently better adjusted local microflora. A noteworthy part of inoculant plan is to give a more suitable microenvironment to keep the fast decay of presented microorganisms in the soil. Although quite a bit of it is thought about the survival of microorganisms inside of the defensive environment of an inoculant transporter, little is thought about the burdens that microorganisms must persist upon exchange to the aggressive and regularly cruel soil environment (Rodriguez-Navarro et al. 1991; Heijnen et al. 1992). Inoculants must be intended to give a reliable wellspring of advantageous microorganisms that make due in the soil and get to be accessible to the plant. The assembling of bioinoculants requires four noteworthy steps (a) Selection of effective strain, (b) Mass culture, (c) Carrier materials and their handling and (d) Packaging, which are to be prepared after strictly ensure the quality of a production item.

4.4.2.1 Inoculant Formulation Technology for P-Solubilizers

Formulation is an urgent perspective for producing inoculants containing a compelling bacterial strain that can decide the achievement or disappointment of

organic workers. Formulation normally comprises of setting up the dynamic fixing, i.e., microorganism (s) in a suitable carrier together with added substances that guide in the adjustment and insurance of the microbial cells amid capacity and transport at the objective site. The formulation is difficult to protect after applying in the fields from destructive ecological components, and keep up or upgrade movement of the living beings in the field (Jones and Burges 1998). Another critical thought is the cost-viability of the plan.

To encourage the performance of high cell numbers and build survival of microorganisms in soil, diverse plans utilizing carrier materials have been utilized. The issue of value inoculant production relies on upon utilization of good carrier material in biofertilizer production unit. The carrier is the conveyance vehicle of live microorganisms from the production line to the field; nonetheless, no widespread carrier or plan is accessible for the arrival of microorganisms into the soil (Trevors et al. 1992). Carrier materials might act to improve survival of inocula by giving microorganisms a defensive domain keeping in mind the end goal to escape unfavorable conditions in the soil. The explanations behind a reduction in inoculum populace in the soil after some time incorporate inadequate supplements accessible for upkeep and replication, and imperfect ecological conditions, for example, pH, ionic quality, temperature and so forth (Van Elsas and Van Overbeek 1993). Predation by bacteriovorus microorganisms, for example, protozoa, and rivalry with indigenous microbes can likewise diminish inoculum application.

To be effective, a carrier material must upgrade survival of inocula amid capacity and after performance into the soil. The carrier must show two crucial properties, i.e., it must be backing the development of the objective produce and keep up a sought populace of inoculant over an adequate time period. To accomplish these objectives, a carrier should likewise show high water-holding limit and maintenance attributes, show compound and physical consistency and be nonlethal to inoculant strains and earth safe (Stephens and Rask 2000). Extra attributes for a decent inoculant should be as per the following:

1. The inoculants should be almost sterile or effectively cleaned, and as artificially and physically uniform as could be expected under the circumstances.
2. They must have steady quality, high water-holding limit (for wet transporters) and suitable for whatever number bacterial species and strains as could be allowed.
3. The inoculant must have an effectively movable pH, and be made of a sensibly valued crude material in satisfactory supply.
4. The inoculant must be nontoxic, biodegradable and nonpolluting, and have to minimize ecological dangers, for example, the dispersal of cells to the climate or to the ground water.
5. The inoculant must have an adequate time frame of realistic usability maybe a couple of years at room temperature.

Normally, no single carrier can have every one of these qualities, yet a decent one should have however many as could be expected under the circumstances.

4.4.2.2 Types of Carriers for P-Solubilizer Inoculants

The most useful carrier for inoculants are (i) Soils: peat, coal, soils, and inorganic soil (Smith 1995). (ii) Plant waste materials: fertilizers, barnyard compost, soybean and shelled nut oil, wheat grain, press-mud, spent mushroom manure. (iii) Inert materials: vermiculite, perlite, ground rock phosphate, calcium sulfate. These arrangements can later be fused into a strong carrier or utilized as they may be.

To produce an inoculant, the objective microorganism can be brought into a sterile carrier. From an absolutely microbiological perspective, the clean carrier has huge preferences yet from a commercial point of view, it is very costly to produce sterile carrier. In any case, sterile-originated inoculants have been effectively advertised even with their higher sticker price. But the less expensive non-sterile carriers, regardless of their potential burdens, have a much bigger market in the business sector (Olsen et al. 1994). The formulation is the key issue for inoculants containing a viable bacterial strain and can decide the achievement or failure of a biological agent.

Inoculants come in four essential dispersal frames. Powder form is utilized as a seed covering before planting. The little the molecule estimate, the better the inoculant will stick to the seeds. Standard sizes differ from 0.075 to 0.25 mm, and the measure of inoculant utilized is around 200–300 g/ha. These inoculants are the most well known both in developed and developing nations (Tang and Yang 1997). Slurries depend on powder-sort inoculants suspended in a fluid (typically water). The suspension is straightforwardly connected to the furrow or on the other hand, the seeds are plunged only preceding sowing. Granular form inoculants are connected straightforwardly to the furrow together with the seeds.

4.4.3 Applications of Endophytic P-Solubilizers in Agriculture and Response of Crops to Bioinoculants

High quality of planting material is a basic requirement for the achievement of any cultivation wander. To guarantee the nature of the planting materials, a successful production and assurance framework is of principal significance. Endophytic bacterial species can be conveyed stem or established cuttings of green plants. Such a conveyance system for endophytic microbes during ahead of schedule phase of its improvement would guarantee better establishing of the planting material. A few techniques for the conveyance of endophytic microorganisms are accounted for which incorporates seed treatment, bacterization of plant spread material, soil application and even foliar application. For vegetatively spread plant species, endophytic microorganisms can be specifically conveyed into the succulent plant framework before the planting in the soil (Panhwar et al. 2013). In these plants, shoots are amiable for bacterization by endophytic microorganisms. Endophytic

microbes from tomato, apricot, apple, seabuckthorn, and *Podophyllum hexandrum* (medicinal plant) illustrative of the overwhelmingly viewed genera *Bacillus, Pseudomonas*, *Enterobacter* and *Serratia* were tried for their abilities to enhance establishing of their host plant (Mehta et al. 2014; Kumar et al. 2015; Sharma et al. 2016). After endophytic inoculation and resulting development in soil, we saw that the root structures of inoculated apple cuttings were frequently denser with numerous fine attaches contrasted with those of the noninoculated control plants. Root arrangement was moderate for noninoculated plants. Interestingly, for cuttings that were permitted to establish in the vicinity of the chose endophytes, root development was started inside of 1 week, and shoot arrangement was more declared contrasted with that of the noninoculated plants.

The use of P-solubilizers is rapidly increasing in agriculture and horticulture and offers a finest way to replace chemical fertilizers and pesticides (Zaidi et al. 2014; Ahemad 2015). Earlier, Walia et al. (2013a) had isolated and characterized different P-solubilizers from the rhizosphere soils/roots of tomato having multiple plant growth-promoting traits (PGPTs). For the testing of effective P-solubilizers, a pot culture experiment was conducted where they reported a significant increase in shoot length, root length and dry matter production of shoot and root of tomato seedlings. Among seven P-solubilizers, strain CKT1 exhibited concomitant production of PGPTs, i.e., siderophore production, indoleacetic acid production, nitrogen fixation activity, and hydrogen cyanide production. Significant increase was observed in seed germination (36.08%), shoot length (5.22%), root length (21.12%), shoot dry weight (63.50%) and root dry weight (54.08%), nitrogen (18.75%), potassium (57.69%) and phosphorus (22.22%) as compared to uninoculated control. This study, therefore, suggests that the use of single strain inoculum of CKT1 with multiple PGPTs offers a new concept to address multiple modes of action.

In an another study by Mehta et al. (2013) endophytic P-solubilizing bacterial isolate *Bacillus circulans* CB7 isolated from apple rhizosphere soil of Himachal Pradesh, India exhibited PGPTs of auxin, nitrogenase activity, ACC deaminase activity, siderophore production, and antifungal activity against *Dematophora necatrix*. In vivostudies showed remarkable increase in seed germination (22.32%), shoot length (15.91%), root length (25.10%), shoot dry weight (52.92%) and root dry weight (31.4%). Also, the nutrient uptake by plants, i.e., nitrogen (18.75%), potassium (57.69%) and phosphorus (22.22%) was increased in shoot biomass. These results exhibited strongly that isolate CB7 has the favorable PGPR traits to be developed as a biofertilizer to boost soil fertility and enhance plant growth.

The synergistic effect of the combination of three PGPRs, *Bacillus licheniformis* CECT 5106, *Pseudomonas fluorescens* CECT 5398, and *Chryseobacterium balustinum* CECT 5399 with LS 213 on the growth promotion and biocontrol on tomato and pepper against *Fusarium* wilt and *Rhizoctonia* damping off was observed by Domenech et al. (2006). They concluded that when both rhizobacterium and strain LS213 were combined together to form an inoculum, the growth parameters were significantly higher than with individual rhizobacterium, in tomato and pepper, which revealed a synergistic and most effective effect on growth

promotion. Similarly, Pandey and Maheshwari (2006) studied the interaction for plant growth promoting comprising of two species i.e. *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3 which can produce IAA and solubilize inorganic phosphate. The consortium of two strains was tested on *Cajanus cajan* in sterile soil and their results revealed an increase in seedling length, yield and weight after inoculation with these species. A similar study was also conducted by Sharma et al. (2007) who isolated two phosphate-solubilizing strains namely *Pseudomonas fluorescens* and *Bacillus megaterium*. They coinoculated them into seeds of *Cicer arietinum* and observed that the consortium of two enhanced the seedling length, radical and plumule length.

Adesemoye et al. (2008) conducted a field study to test the effect of P-solubilizers microbial inoculants on corn plant growth, yield and nutrient uptake. The field results showed that inoculants promoted grain yields (kg/ha) 7717 for AMF (Arbuscular Mycorrhiza Fungi), 7260 for PGPR + AMF, 7313 for PGPR, 5725 for the control group and also enhanced nitrogen content per gram of grain tissues. Significantly higher amounts of N, P and K were taken up by microbes thus indicated the application of inoculants lead to a reduction in buildup of N, P, and K in agricultural soils which is measure of an integrated nutrient management system. Similarly, Yazdani et al. (2009) reported that use of PSM and PGPR in addition to conventional fertilizer applications (NPK) could improve root and shoot weight, and grain number per row and finally increased grain yield of *Zea mays* L. They concluded that application of PSM and PGPR together could reduce P application by 50% without any significant reduction of grain yield. PGPR can enhance plant growth by alleviating soil stresses experimentally observed by Mehta et al. (2013a). They hypothesized that the isolated strains of *Azospirillum* sp. and *Bacillus subtilis* CB_8A may alleviate the adverse effects of drought stress on wheat and apple growth.

4.5 Conclusion and Future Prospects

In intensive agricultural practices, the application of phosphatic fertilizer requires a greater input that cannot be afforded by the farmers furthermore due to impending impacts to the biological system. Keeping this in perspective, numerous researchers have occupied their examination in finding the shrouded treasure under the soil and thus, rhizosphere competent bacteria (RCB) or endophytic P-solubilizers came into light and gained interest as inoculants or economically efficient substitute for fertilization of crops by solubilization of phosphate from inadequately accessible sources in the soil. The characteristic state of plants is by all accounts in a nearby interaction with endophytes. In the endophyte–host communications, the base commitment of the plant to the endophyte is one of giving nutrition. Endophytic microorganisms are the rich wellspring of an extensive variety of bioactive mixes, bringing about the generation of each of the five classes plant development hormones (auxins, abscisins, ethylene, gibberellins, and kinetins). The accomplishment

of this microbiological approach, in any case, relies on upon identification, preparation and delivery of multifunctional endophytic phosphate solubilizers to farm practitioners. This would be amiable when a superior learning on endophyte environment and their molecular associations is achieved. Once recognized and physiologically portrayed, phosphate-solubilizing microorganisms are liable to give advantages to crops in sustainable agriculture. Further, keeping in mind the end goal to guarantee food security in developing nations, there is a dire requirement for the eco-friendly sustainable intensification of farming production systems. In this context, efficient indigenous or genetically modified region or crop specific endophytic PSM and advancements for their definitive exchange to the fields must be produced and delivered to farmers in a relatively brief time.

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