Chapter

Phosphate Solubilizing Rhizobacteria as Sustainable Management Strategy in Agrobiology

Aqsa Tariq and Ambreen Ahmed

Abstract

Phosphorous limits agricultural productivity due to its limited plant availability. Use of synthetic phosphate fertilizers disturbs soil fertility and ecosystem ecology as it contaminates environment. Plants have developed certain mechanisms to respond to P-scarcity, which involve release of specific chemical messengers through root exudates that attract rhizospheric phosphorbacteria to colonize plant root vicinity. Thus, use of phosphate-solubilizing bacteria/rhizobacteria (PSB/PSR) as biofertilizers is a safer approach toward sustainable agrobiology. These PSR are capable of solubilizing soil phosphate from insoluble to plant available form. Due to instability and slow movement of available phosphates in soils, they readily get incorporated with soil particles or chelates as metal complexes. In this scenario, PSR provide continuous chain of soluble phosphate to plants. PSR direct plant root system architecture toward available phosphate zones in soils. Moreover, there is an increased number of roots, root hair and lateral root, increase root absorbing surface area by increasing contact to soil particles. Hence, PSR-based root system morphology is a significant trait in measuring their agronomic efficiency. Moreover, PSB also possess phytostimulatory properties that significantly contribute to agricultural efficiency. Hence, the use of phosphate-solubilizing bacteria can improve crop productivity by increasing soil P-mobility and soil fertility.

Keywords: biofertilizers, phosphate solubilizing rhizobacteria, inorganic phosphorus, plant growth promotion

1. Introduction

Global food security greatly depends on soil fertility and agricultural sustainability. Most of the soils with high sorption capacity have finite phosphorous (P) resources which is far away from meeting the agricultural P demand, thereby, limiting agricultural fertility and productivity [1]. On the average, 0.05% (w/w) phosphorous is present in agricultural soils out of which only 0.1% is available to plants. Mostly inorganic phosphates (Pi) are present in higher concentrations but due to highly reactive nature of P-anions, it readily gets immobilized via complex formation with various mineral cations (Fe_3^+, Mg_2^+, Ca_2^+, and Al_3^+) [2]. Hence, application of animal

manure in traditional farming technique solves P-deficiency problems to some extend but this leads towards an unbalance ratio between various nutrients especially nitrogen and phosphorous in relation to relative crop demand and results in overfertilization [1]. Lower plant accessible P-concentrations and higher immobility in soils make it an essential mineral needed to be applied exogenously in the form of fertilizers. Therefore, conventional agricultural practices rely on high input of chemical fertilizers to boost crop productivity. Among various fertilizers, phosphate fertilizers are the major contributor to environmental contamination. Concentration of various metals in potassium and nitrogen fertilizers is significantly low as compared to phosphate fertilizers therefore, these are not regarded as serious threat to soil and environmental [3]. On the other hand, phosphate fertilizers contain traces of various metals including heavy metals i.e., cadmium (Cd), lead (Pb), arsenic (As), strontium (Sr), chromium (Cr), zinc (Zn) and radioactive metals such as thorium (Th), uranium (U), radium (Ra) etc. [4]. Consumption of such crops deteriorate our ecosystem by accumulating in agricultural soils and becoming part of food chain. Moreover, soil erosion facilitates the entry of P in waterbodies where it causes uncontrolled growth of algal blooms, deplete oxygen and cause risk to aquatic life. Even very low P- concentrations (10–20 μgPL^{-1}) can support luxurious growth of algal bloom. In addition, drinking highly eutrophicated water adversely affects human health [4].

Rock phosphate is the naturally occurring source of phosphate used for the manufacturing of various phosphate (P) fertilizers such as triple superphosphate (TSP), monoammonium phosphate (MAP), diammonium phosphate (DAP), and NPK mixtures [5]. Apatite, basic constituent of phosphate rock, is incorporated with various metals and radionuclides which later become distributed in environment by the application and formation of these fertilizers. Sometimes, besides commercially available P- fertilizers, its by-product phosphogypsum (PG) is also used to fertilize agricultural lands having potential environmental risk [6]. This uneven distribution of various metals in soil adversely affects its physiological properties which, in turn, affect nutrient availability to plants. This, in the longer run, reduces soil biodiversity and fertility by disrupting soil microbiota as these are very sensitive to environmental variations. The soil microorganisms play crucial role in regulating soil fertility as they are involved in nutrient cycling (particularly P- cycle) hence, maintaining plant health and crop productivity. Hence, keeping in view all the agrobiologically and environmental sustainability concerns, a greener and cleaner approach should be needed to compete this challenge. In this regards, utilization of phosphate solubilizing microbes (PSM) is the best possible solution. Phosphorous solubilization capacity of soil microbes have been extensively studied from the perspective of their utilization in agro-ecosystems and development of biological fertilizers. For this purpose, molecular prospects of bacterial transformation of organic phosphates through various mechanisms have received a great deal of attention. First ever report on plant growth improvement via. Inoculation using phosphate solubilizing microorganisms was published in 1948. Since then, after so many decades, there is no general agreement among the scientific communities on the benefits of these microbes in crop production, hence, their use is still limited. The current chapter summarizes the agricultural accountability and significance of phosphate solubilizing rhizobacteria (PSR) and the strategies acquired by these microscopic creatures to solubilize phosphate and the genetic aspects for better understanding of phosphate mineralizing mechanisms. This would lead scientific community to understand their nature that would be beneficial for the development of commercially available formulations used in agriculture.

2. Soil phosphorous dynamics and accessibility

Phosphorous is an important macronutrient constituting about 0.2–0.8% of plant dry weight [7]. Phosphorus is crucial in various plant metabolic processes including energy generation and transformation during developmental processes such as germination, flowering, root expansion, photosynthetic activities, nitrogen fixation, carbohydrate metabolism, enzymatic activities etc. In addition, it is integral part of various structural and functional macromolecules such as adenosine triphosphate, proteins, nucleic acids, lipoproteins etc. [8]. In soil, phosphorous is present in two basic chemical forms i.e., organic (P_o) and inorganic forms (Pi). Primary sources of inorganic phosphates include stable P minerals such as apatite ($Ca_5[PO_4]_3(OH,F,Cl)$), variscite ($AlPO_4\ 2H_2O$) and strengite ($FePO_4\ 2H_2O$). These minerals have P structural element and are very stable and considered as huge P- reservoirs existing naturally in soils. However, the phosphate liberation from these minerals is a gradual process, regulated particularly by soil pH [9]. Optimum pH for P availability to plants is 5.5–7. At high or low pHs', it forms chelates and become unavailable for plants [10, 11]. Under acidic conditions, adsorption of P on Fe and Al oxides and hydroxides (gibbsite and goethite) is increased. On the other hand, in alkaline conditions, Ca serves as primary P precipitated site. P can also readily bound with soil particles or adsorbed with cations to form complexes such as aluminum phosphate ($AlPO_4$), iron phosphate ($FePO_4$), and calcium phosphate ($Ca_3(PO_4)_2$) etc. These secondary sources of Pi are the major phosphorus sources for young plants [12, 13].

In addition, compounds originated mainly from soil organic matter (plant and animal residues and manure) are the source of organic phosphates. They include wide range of compounds varying in terms of their bioavailability and solubility. These compounds are categorized as various phosphate esters such as phospholipids, sugar phosphates, inositol phosphates, nucleic acids; phosphonatases such as C–P bonded compounds; and phosphoric acid anhydrides (adenosine di- and tri- phosphates) [14, 15]. Important organic source of P is soil microorganisms. Soil microbes have potential to inlock soil phosphorous by absorbing and incorporating in their cellular structures such as nucleic acids, coenzymes) or stored as polyphosphates which temporarily act as immobilized P-pool. This temporarily locked P can later be released into soil solutions through mineralization process [16]. Rhizobacteria accumulate polyphosphates or polymers of phosphoric acids under unfavorable conditions which serves as P-reserves within bacterial cells. These P-reserves are considered as high energy reserves providing anhydrides and can easily be used as energy source by releasing Pi [17]. Various enzymes are involved in consumption and degradation of accumulated polyphosphates. Poly P kinase catalyzes the synthesis of polyphosphates within microbes. Similarly, polypases (exopolypase (PPX)) and polyphosphate-specific kinases (polyP-fructokinase and polyP-glucokinase) are involved in phosphate utilization and degradation [9]. Bacterially mediated P-cycling process releases accumulated phosphorous back to the soil. However, the P- liberation from biomass is highly dependent on available soil carbon and phosphorous and composition of microbial communities [18, 19]. P_o constitute almost 30–65% of the soil out of which 3–14% become immobilized into soil microbial biomass [20]. Plant roots can efficiently uptake Orthophosphates ($H_2PO_4^-/HPO_4^{2-}$) but due to its weak stability and highly reactive nature, it loses its efficiency and becomes yield limiting factor in most of the agricultural soils [21, 22]. In addition to the microbial, plant and animal residues, a large quantity of xenobiotics (detergents, pesticides, antibiotics etc.) released in the environment also serves as source of organic P. These high molecular

weight organic compounds are resistant to chemical hydrolysis or biological degradation, thereby, the locked P within them is useless for plants unless converted to Pi or orthophosphates. However, some PSR studied have ability to degrade such complex compounds and release P from these sources [23].

Strong P- fixing capacity of soils and immobilization of soil P pool via precipitation, chelation or complex formation causes P scarcity in soils. Despite all these factors, P availability is generally a balanced process including desorption and adsorption mechanisms. Various rhizospheric phenomena particularly biological processes play critical role in soil P dynamics and its availability to plants. Both plants and rhizospheric biota contribute to bioavailability of P at root-soil interference by regulating specific signaling molecules such as release of H^+, chelation and ligand exchange etc. All these rhizospheric activities contribute to P-cycling process to improve P availability in agricultural soils [24].

3. Plant starvation responses

Plants uptake P- through roots by simple diffusion. The absorbed P- ions actively move across the plasmalemma against concentration gradient developed by existence of low orthophosphates. Plant response vary greatly from species to species in P-deficiency response. Generally, plants cannot respond and absorb soil P efficiently (plant P uptake rate: 10–12 to 10–15 m^2s^{-1}) due to its low mobility. This causes the formation of phosphate depleted areas adjacent to plant roots. Therefore, plants need subsidiary system that can help plants to receive optimum P requirement by developing nutrient pool around the plant roots [25]. Plants have developed generally various physiological, biochemical and morphological adaptations to respond P- scarcity and to endeavor P acquisition efficiency. These genetic modifications acquire by plants can be categorized as plant P- acquisition efficiency: capacity to absorb soluble P and P-utilization efficiency: capacity to utilize and assimilate the absorbed P. These include high expression of P transporters, carbon metabolism, secretion of various organic acids such as oxalate, citrate and malate), modification in root architecture, enhanced production of acid phosphatases and phytases. Modifications in root architecture is foremost response substantially studied in plants [26, 27]. A preferential allocations metabolic budget towards roots undoubtedly results in greater root hair formation and clustering of roots, providing greater surface area for P absorption but, on the other hand, it decreases root to shoot ratio resulting in reduced plant growth. However, greater root system allows plants for greater and easier nutrient acquisition [28]. Besides modifications in root architecture, root signaling is also significantly important parameter affecting P- acquisition efficiency. Under P-scarcity, release of organic acids by plant roots help to solubilize the nearby immobilized P-pool. Moreover, plants also release P- scavenging enzymes that also help in soil P- cycling mechanism. For instance, release of acid phosphatase catalyzes Pi hydrolysis process to release Pi from P_o residues [29]. In addition, plants enhance cellular P utilization efficiency by increasing activity of high affinity Pi/H^+ symporters (PHT1 gene family) associated with plasma membranes [30, 31]. Plants also regulate alternate metabolic pathways e.g., glycolysis pathways, tonoplast pyrophosphatase, and various respiratory electron transport pathways [32]. Despite of all these modifications in plants for improved P acquisition efficiency under P stress conditions, plants still are unable to full fil their P- demand, therefore, plants tend to establish symbiotic interactions with soil microbiota especially rhizobacteria to cope up with P- scarcity.

4. Phosphate solubilizing rhizobacteria (PSR): biological revolution

Rhizosphere is hotspot for various plant beneficial bacteria with potential to solubilize immobilized P sources (di- and tricalcium phosphates, hydroxyapatite, and rock phosphate). These rhizobacteria are known as phosphate solubilizing rhizobacteria (PSR). PSR are copious in nature. Various rhizobacteria belonging to genera *Paraburkholderia*, *Ralstonia*, *Burkholderia*, *Curtobacterium*, *Arthrobacter*, *Cronobacter*, *Massilia*, *Pseudomonas*, *Enterobacter*, *Bacillus*, *Serratia*, *Pantoea*, *Rhizobium*, *Klebsiella*, *Ochrobactrum*, *Staphylococcus*, *Arthrobacter*, *Acinetobacter* have phosphate solubilizing potential [33–40]. Visualizing the formation of clear halo zones around bacterial colonies on various phosphate media indicates their phosphate solubilizing ability. Quantitative analysis of P-solubilizing potential of PSR using rock phosphates (RP) and various Al-, Ca- and Fe- complexes revealed their efficiency to mobilize soil Phosphate for plant use. However, the extent to solubilize phosphorous is highly dependent on bacterial species. Agronomic efficiency of RP significantly increased using suitable PSR. This improvement is attributed to the positive effects of PSR on soil P-availability [20]. These microbes play significant role in P acquisition and nutrient management in soils and hence, serve as potential biofertilizers [41]. In addition, PSR exhibit diverse abilities and exert synergistic effect on plant growth and development besides solubilizing soil phosphate. They enhance plant growth by various plant growth promoting mechanisms including production of plant growth stimulating phytohormones such as auxins, gibberellins, cytokinins and various compounds such as siderophores, 1-aminocyclopropane-1-carboxylate (ACC) lytic enzymes, hydrogen cyanide (HCN), exopolysaccharides that lock up soil nutrients for plant availability and protect them from various unfavorable conditions [42]. Moreover, PSR also act as biocontrol agents protecting plants from pathogenic attacks by producing wide variety of antifungal compounds including certain phenolics and flavonoids [43]. The phosphate solubilization mechanisms are summarized in **Figure 1**.

4.1 Unearthing the mechanisms of P-solubilization: molecular insight

a. Inorganic phosphate solubilization

Principle mechanism of inorganic phosphate solubilization acquired by PSR is release of mineral dissolving compounds such as protons (H^+), siderophores, organic acids (OAs), carbon dioxide (CO_2) and hydroxyl ions (OH^-). Production of low molecular weight organic acids is common mechanism shared by PSR. Rhizobacteria produce these organic acids either during carbon metabolism through intercellular phosphorylation or through direct oxidation of glucose to gluconic acid and sometimes to 2-ketogluconic acid via quinoprotein glucose dehydrogenase (GDH), an enzyme involved in direct oxidation pathway in periplasmic space [44]. Pyrrolo quinoline quinine (PQQ) (product of pqq) acts as a cofactor which is essential for the activity of GDH. These organic acids lower down soil pH. Under alkaline conditions, soil P precipitates as Ca^{2+} phosphates and its solubility increase with decreasing soil pH. Increase in soil pH causes the formation of di- and tri- Pi (PO_4^{3-} and HPO_4^{2-}) [45]. The production of organic acids acidifies the surroundings and cellular environment by liberating H^+ in the vicinity of plants which regulates the accumulation of other cations that directs to P solubilization by substitution of H^+ for Ca^{2+}. For instance, assimilation of NH_4^+ along with H^+ causes P solubilization [46]. Moreover, there is no evidence of a correlation between pH and solubilized P [47]. The P- solubilizing efficiency of

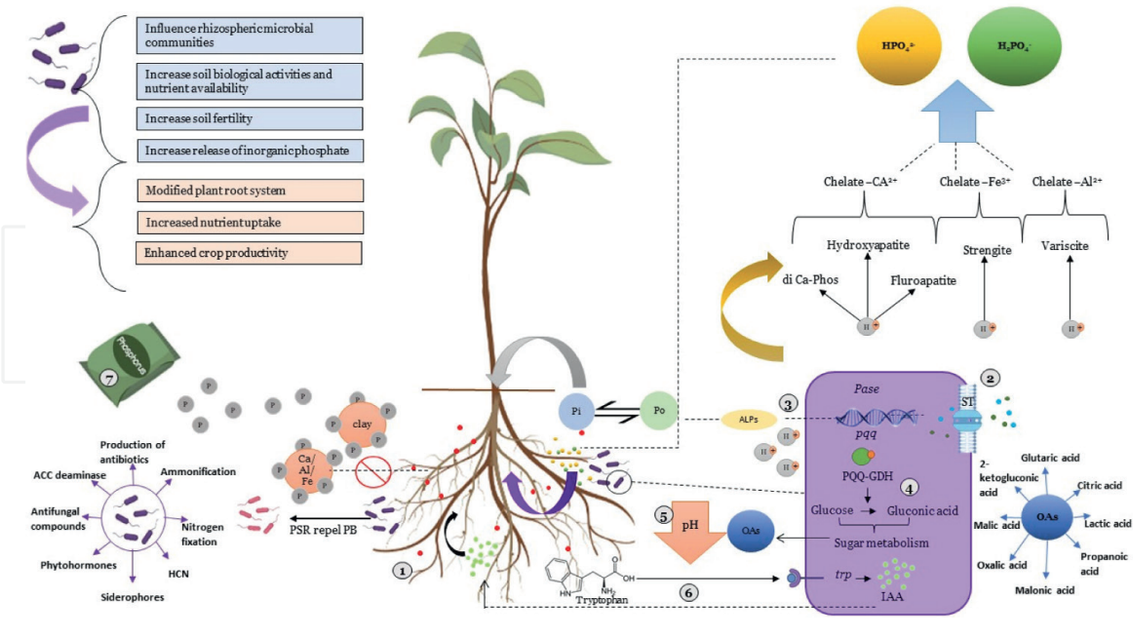

Figure 1.
Rhizospheric interactions between PSR and plants and their impact on plant growth. 1- Plant releases certain chemical messengers ( ) that attract beneficial PSR which in turn colonize plant roots and fight off pathogenic bacteria (PB). 2- Bacteria receive sugar chemical messengers (-glucose, -fructose) by sugar transporters (ST) which activate synthesis of phosphatases (Pase). 3- Synthesis of ALPs solubilize organic phosphate to inorganic phosphate that can be taken up by plants. Moreover, accumulation of H+ on bacterial surface also facilitates P-solubilization process by releasing Pi from various soil minerals. 4- Chemical messengers also activate pqq involved in sugar metabolism as a result of which it forms various organic acids (OAs). 5- Synthesized OAs lower soil pH which favors P-solubilization by PSR. 6- PSR also utilizes tryptophan released in root exudates to synthesize bacterial Indole acetic acid (IAA) that is released in the vicinity of plant roots and taken up by plants. 7- Pi present in phosphate fertilizers interacts with soil particles and form chelates before it is taken up by plants thus minimizing the advantage taken by the plants from the application of phosphate fertilizers.

PSR greatly depends on the strength and type of acid production. Variable nature of OAs leads them to respond differently. For example, di- and tri forms of carboxylic acids are more efficient as compared to monobasic or aromatic acids. In the same way. Aliphatic acids are more efficient as compared to fumaric, phenolic, or citric acids [48]. Moreover, the quantity of OAs produced is correlated to the concentration of soluble P. Hence, OAs production in P-deficit soils is greater as compared to the P- sufficient soils [49]. OAs produced by majority of PSR are glutaric, citric, propionic, lactic, glyoxalic, malonic, glicolic, 2-ketogluconic, oxalic acid, glyconic acid, acetic acid, malic acid, fumaric acid, succinic acid, tartaric acid, butyric acid, and adipic acids [50]. Among these OAs, gluconic and 2-ketogluconic acid are most commonly produced OAs. Gram-negative bacteria oxidize glucose to gluconic acids for mineral P- solubilization. Gluconic acids chelate the cations bounded with phosphate via OH^- or carboxyl (–COOH) groups making phosphate accessible to plants [51]. Pyrroloquinoline quinone-dependent periplasmic glucose dehydrogenase (PQQ-GDH), is responsible for the production of gluconic acid from glucose. PQQ-GDH is also responsible to produce gluconic acid. In most of the Gram-negative bacterial species, biosynthesis of PQQ is regulated by five genes comprising pqq operon (pqqA-BCDE) [52]. Until now, 11 pqq genes have discovered so far in various bacterial genera, however, pqqF and pqqG existing at proximal or distil end of operon are commonly found [53]. Various PSR genera exhibit this mechanism including *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Pantoea*, *Klebsiella*, *Rahnella*, *Serratia*, *Erwinia*, *Citrobacter*, *Burkholderia* and *Gluconobacter* [54, 55]. Another P-solubilizing mechanism acquired

by some PSR is release of H^+ microbes which release H^+ at their surfaces helping cation exchange via H^+ translocation or ATPase leading to the release of P from inorganic minerals (Ca-P) [9]. Production of chelating compounds and inorganic acid by some bacteria is also source of mineral P solubilization, however, effectiveness of these compounds is very less compared to other mechanisms of P-solubilization [43].

b. Organic phosphate mineralization

Mineralization of organophosphates highly depends on the environmental conditions. Alkaline conditions favor this process. Phosphate decomposition by PSR from organic substances is correlated with P- content of their biomass. This biological event plays an important role in solubilization of organic P and regulating P-cycling events in nature. These phosphate solubilizing bacteria secrete various enzymes responsible for organic P mineralization. Among these enzymes, phosphatases and phytases are important. Phosphatases (phosphohydrolases) belonging to the class phosphomonoesterases, dephosphorylate phosphoester or phosphoanhydride bonds present in organic compounds [56]. They can either be alkaline or acidic phosphomonoesterases (ALPs), however, acidic phosphatases are important and play significant role in decomposition having optimum catalytic activity. ALPs can mineralize up to 90% of organophosphatases, however, phytate is resistant to them [57]. The key ALPs encoding gene found in phosphorbacteria is *pho* (phoX, phoA, and phoD). Among these phoD is widely distributed among various PSR. However, phoD abundance has shown no correlation with the P-availability. phoD can mineralize phosphate even under low concentrations but causes immobilization of P in bacterial biomass under application of P fertilizers [58]. ALPs are categorized as specific acid phosphatases (SAP) and non-specific acid phosphatases (NSAP). Examples of SAP with different activites are: nucleotidases, hexose phosphatases, and phytases [59]. Several bacterial species have been known for their potential to produce phosphatases such as Pseudomonas sp., *Klebsiella aerogenes*, *Burkholderia cepacia*, *Enterobacter cloacae*, *Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Proteus mirabalis*, *Citrobacter freundi*, and *Serratia marcenscens* [9].

The enzyme phytase is responsible for releasing phosphorous locked in soil organic compounds such as seeds or pollens that were stored as phytate (inositol polyphosphate). Phytates are great source of phosphorous containing 60–80% of soil P. Phytates contain strong and stable ester bonds that can easily be hydrolyzed by PSR. They completely hydrolyzed phytates to lower molecular weight isomers of inositol polyphosphate and inorganic phosphates [60]. Several phosphorbacteria have been known for having their potential to produce phytases such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Pantoea*, and *Escherichia coli* [61, 62]. Four types of phytases identified so far from PSR are: β-propeller phytase (BPP; alkaline phytases), histidine acid phosphatase (HAP; acid phytases), protein tyrosine phytase (PTP; cysteine phytase) and purple acid phosphates (PAP; metalloenzyme) [63]. Acidic nature of these enzymes enhances their efficiency under various pH conditions. Some rhizospheric P- solubilizing *Bacillus* and *Streptomyces* also tend to produce phosphoesterases, phosphodiesterases and phospholipases to mineralize organophosphates [64].

4.2 Impact of exogenous P on phosphobacterial activities.

Soil phosphorous status directly influences plant metabolic activities, root exudates and carbon availability for rhizospheric microbes. Low soil P levels causes plants to activate P- stress responsive mechanisms involving various transcriptional

and physiological changes that indirectly affect its associated rhizobacterial communities [65, 66]. P- fertilizers are the yield limiting determinants of soil fertility which influence by disturbing soil nutrient equilibrium. The aggressive use of these fertilizers affects nutrient availability for biological processes and plant uptake [67]. Application of P- fertilizer significantly changes phosphorous turnover efficiency by recruiting rhizobacterial families and regulating bacterial genes involved in P cycling [68, 69]. P-fertilizers can shift soil microbial communities affecting soil biodiversity [70]. Environmental phosphate affects all the phenomena of inorganic P- solubilization, organic P mineralization, P-uptake and transport and plant responses. Phosphorbacteria respond differently to available phosphate conditions. Shifting of various phosphorbacteria in response to P fertilizer indicates their P- availability based selection criteria. Some bacteria such as Actinobacteria prefer high P areas whereas *Moraxellaceae* and *Pseudomonadaceae* prefer low phosphate soils. Similarly, bacterial genera *Bacillus*, *Clostrodium* and *Alicyclobacillus* have shown negative correlation with soil P-content [71]. Moreover, besides affecting rhizospheric bacterial taxonomy, soil nutrient also affects bacterial potential to solubilize immobilized phosphate. *Burkholderia* and *Collimonas* exhibit nutrient poor soils having efficiency for mineral decomposition to fulfill their nutritional demand [72]. *Burkholderia* is described as low phosphate responsive taxon. It is abundantly present in P deficient soils where it switches its interactions with plants i.e., commensal to opportunistic and utilize the stored inorganic shoot phosphate [73]. Nutrient acquisition ability of phosphate solubilizing bacteria makes them more competitive in nutrient poor soils [73].

Soil P-status leads to the upregulation of various P-solubilizing enzymes. Expression of gene (gcd) responsible for glucose dehydrogenase synthesis is suppressed under greater soil P levels through feedback mechanism. Moreover, plants growing under P-deficit conditions release certain signals through root exudates that influence P-solubilizing activity of PSR. The expression of pqq genes is increased by detecting root signals of plant growing under P-deficient conditions [74]. Moreover, the production of phosphatases is regulated by the availability of nitrogen and phosphorous. In the presence of sufficient nitrogen, their production is enhanced. On the other hand, phosphorous supply decreases their production [75]. This negative feedback creates strong correlation between exogenous P and phosphatases to increase P mineralization. Similarly, inorganic phosphate supply reduces the activity of phoD [76]. Under acidic conditions activity of acidic phosphatases and abundance of phoC are negatively correlated with P availability, whereas exogenous P- supply exceeds no significant effect on abundance and activity of alkaline phosphatases [77]. In some cases, long term P- fertilization causes bacterial dormancy leading to inactivation of bacterial P-solubilizing potential [74]. However, there are some controversies in bacterial response to available phosphorous. Sometimes PSR show no response to P-fertilization and the composition of soil bacterial communities remain uninterrupted [78]. Moreover, shift in bacterial communities in response to exogenous P supply is controlled by various biotic and abiotic factors such as nutrient level, drought, pH etc. hence, it is considered that rhizospheric microbial communities are initially determined by soil conditions, then scrutinized by root exudates and finally shaped by alterations in soil physiology [79].

5. Agronomic efficiency of phosphobacteria

Various plant traits have been extensively studied to develop an agronomic framework for the evaluation of PSR effects on crop yield parameters. These traits serve as

applicable indicators for evaluating the efficiency and potential of phosphorbacterial biofertilizers in agricultural fields (**Table 1**).

5.1 Plant-phosphobacterial interactions

Various plant physiological activities are involved in efficient use of soil phosphorous. Release of ions, organic acids and enzymes through root exudates favors plant, to recruit microbial communities especially PSR beneficial for their growth [88]. Soil microbes have affiliation with C-containing compounds, target plant root exudates and response chemotactically to plant chemical messengers. Several rhizobacteria especially phosphate solubilizing bacteria prefer to occupy the plant root zones. For instance, *Oceanobacillus*, *Massilia*, *Arthrobacter*, *Lactococcus* and *Bacillus* are recruited in the vicinity of wheat root zone to get benefit from organic acids released in the form of root exudated [89]. Similarly, some phosphorbacteria such as *Bacillus* sp. enhance root colonization in response to plant secreted organic acids. Plant root exudates activate root colonizing genes present in phophobacteria. This, in turn, significant in establishing plant-PSR interactions which is crucial in P-acquisition by plants [90, 91].

Phosphate solubilizing bacterial strains	**Expected mechanism**	**Experimental Plant**	**Agronomic efficiency**	**References**
Bacillus, *Pseudomonas*, *Massilia*, *Citrobacter*, *Arthrobacter* and *Acinetobacter*	Presence of P cycling related genes (gcd, bpp)	Chinese cabbage (*Brassica rapa*)	Increased plant fresh weight, dry weight, and plant height	[80]
Phosphate solubilizing bacteria (PSB)	Enhanced soil P content	Kasumbha (*Carthamus tinctorius*)	Increased number of leaves per plant, leaf area, number of seeds per capitulum, increased plant height, number of branches, number of capitulum per plant, seed oil and phenolic content	[81]
Bacillus siamensis, Rahnella aceris, Pantoea hericii, Bacillus paramycoides (Single and consortium)	Phosphate hydrolyzing enzymes (acid phosphatase and pyrophosphatase) and organic acids (glycolic acid)	Wheat (*Triticum aestivum*)	Modified root architecture (enhanced root hairs length, root length, root inorganic P content, plant biomass plant organic phosphate content, P translocation and soil phosphatases	[82]
Bacillus thuringiensis and *Pantoea ananatis*	Decreased soil Pb phytoavailability through dissolution of insoluble inorganic P and increase water-soluble phosphate concentrations	*Lactuca sativa* L.	Promoted plant growth and reduced shoot Pb concentrations	[22]

Phosphate solubilizing bacterial strains	Expected mechanism	Experimental Plant	Agronomic efficiency	References
Pseudomonas sp. (UC_1), *Klebsiella* sp. (UC_M), *Burkholderia* sp. (UC_J), *Chryseobacterium* sp. (UC_3)	Regulation of soil microbial communities	*Ulmus chenmoui*	Improved plant growth	[83]
Enterobacter sp. (J49) *Serratia* sp. (S119)	Interactions of P solubilizing activity and plant root exudation causes increased pectinase and cellulase activities	Soybean (*Glycine max*) and maize (*Zea mays*)	Improved plant growth	[84]
Bacillus mojavensis (B1), *Bacillus megaterium* (B2)	Enhanced soil P-solubilization	Sugarcane (*Saccharum officinarum*)	Increased yielding components such as increased stem height, internode, weight and diameter	[85]
Burkholderia sp. (N3)	Interactions with plant immune system by upregulating 129 genes and downregulating 33 genes involved in plant resistance against pathogen	Melon	Enhanced plant height, dry weight, leaf area, and uptake of nutrients of melon seedlings increased and suppression of bacterial fruit blotch in melon	[86]
Pseudomonas mallei, *Pseudomonas cepaceae* (Consortium)	Promotes soil biological activities, nutrient availability, enhanced productivity of calcareous soils	*Phaseolus vulgaris*	Increased fresh and dry weight of pods and seeds per plant, increases shoot fresh weight per plant, shoot dry weight per plant	[87]

Table 1.
Phosphate solubilizing rhizobacterial efficiency in agriculture.

5.2 PSR mediated regulation of phosphate related genes in plants

Phosphate solubilizing bacteria can either directly or indirectly trigger the expression genes responsible for Pi movement. These PSR regulate the expression of P transporters either by modulating the expression of plant metabolic genes (pheromone producing genes) or sometimes by increased P-supply in the vicinity of plant roots. Plants have two types of phosphate transporters (PHT) for the regulation of intracellular optimum phosphate concentrations. The high affinity transporter (PHT1) activates in roots whereas, low affinity transporter (PHT2) is responsible for Pi transfer in shoots, flowers, leaves etc. [92]. Phosphorbacteria regulate various phosphate related genes within plants in response to environmental conditions especially during low P supply. Plants growing in P deficit soils have shown upregulation of PHT1. The PSR *Pseudomonas putida* increased the expression of AT5G43350 gene responsible of the production of PHT1 in *Arabidopsis thaliana* [93]. Under combination of P and salt deficiency, PSR upregulated the expression of AT1G80050 gene responsible for the production of PHT2 in A.

thaliana. Contrary to this, the expression of gene (PHO2) responsible for Pi accumulation in shoots was down regulated. This phenomenon is referred to the fact that PHO2 is responsible for Pi signaling under low P supply [93]. Similarly, phosphate solubilizing *Bacillus* sp. enhanced P-acquisition in wheat plant by upregulating PHT1 transporter [94]. On the other hand, P solubilizing *Pseudomonas* sp., *Klebsiella* sp., *Stenotrophomonas* sp., *Serratia* sp. and *Enterobacter* sp. have been shown to down regulate the expression of Pi transporter in inoculated plants, however, plant growth is enhanced with improved P acquisition and biomass [95]. These changes in molecular patterns positively influence plant P acquisition that ultimately improved crop yield and productivity.

5.3 Effect of PSR on root system architecture

Root system is a paramount, fitness determining component of a plant. Phosphobacteria can modulate root system architecture through various mechanisms in favor of P acquisition. Modified root system stimulate enhanced root absorptive capacity for nutrients uptake [96]. Generally, under P scarcity, plants have adopted root modifications such as increased root biomass, greater number of roots, enhanced root length and surface area. This extensive, denser root system with larger surface area help plants in detecting localized higher phosphate content [97]. Moreover, spatial parameters in root architecture are important under P- stress. Sometimes for the P- acquisition, PSR affect plant roots to develop shallow root system by decreasing primary root growth and inducing laterals root formation. Thus, development of shallow and more proximate roots favor plants to acquire P from topsoil [98]. This phenomenon of detecting local phosphate concentration by modifying root system is termed as 'P-mining'. Under low P supply, inoculation of phosphate solubilizing *Bacillus megaterium* and *P. fluorescens* inhibited primary root formation and initiated lateral root and root hair formation in *A. thaliana* [99]. PSR also have positive influence on the development of root hair of inoculated plants. Plants with longer root hair are found to be more efficient in P- acquisition under P deficiency. Plants treated with phosphate solubilizing *Pseudomonas* sp. strongly influence root hair formation by increasing number of root hair and length of root hair [100]. Root functions related to phosphate foraging such as number of roots, root hair, lateral roots, frequency of root tips, branching intensity etc. have shown to be increased under the influence of PSR [101].

5.4 Mechanisms adopted by PSR for plant growth promotion

Phosphate solubilizing bacteria follow several other mechanisms influencing plant growth directly or indirectly such as production of phytohormones, quorum sensing signals, production of various enzymes etc. These mechanisms act synergistically, helping plant to better adopt the environmental conditions with improved growth yield. Some of these mechanisms affecting directly are described below.

5.4.1 Nitrogen fixation

Some phosphate solubilizing *Rhizobia* spp. with nitrogenase (nif) gene have potential to fix nitrogen. N is important macromolecule so PSR with potential N-fixing ability can significantly help plants to cope with its nitrogen demand having improved nitrogen acquisition [82]. Leguminous plants have developed symbiotic relation with nitrogen fixing rhizobacteria and modify plant roots by developing root nodules where these bacteria convert environmental nitrogen into

ammonia (plant available form of N) [102]. However, some non-nodule forming N-fixing phosphate solubilizing bacterial species such as Pseudomonas sp. also regulate legume-rhizobia symbiosis for improving the plant nitrogen levels. Increased ACC activity in Pseudomonas sp. trigger nodulation process in rhizobia [103, 104].

5.4.2 Siderophore production

These are iron chelating compounds secreted by some PSR bacteria to reduce inter- or intra-cellular iron that can be utilized by the associated plants. Due to iron scarcity, Phosphate solubilizing e.g., *Pseudomonas fluorescence,* can produce different kinds of siderophores i.e., pyoverdine pyochelin, and pseudobactin [105]. This phenomenon positively influences plant growth. For instance, Pseudomonas fluorescence produce pyoverdine which form complex with iron (pyoverdine-Fe) that can be easily taken up by plants. Iron acquisition is more important under stress conditions. Siderophores also help to alleviate the stress imposed on plants [106].

5.4.3 Phytohormone production

Phosphate solubilizing bacteria have potential of producing various plant hormones such as auxins, cytokinins, gibberellins, and ethylene. PSR release these hormones via interconnected series of signaling network and affect plant physiological activities [107]. Tryptophan present in plant root exudates acts as principle signaling molecule to produce bacterial Indole Acetic Acid (IAA). PSR generally detoxify tryptophan, or its analogs present in root exudates that cause IAA production [108]. Bacterial phytohormones can alter plant hormonal balance which is positively correlated with plant health. Many species of phosphate solubilizing *Bacillus* and *Pseudomonas* exhibit potential to produce auxin that triggers formation of lateral root and root hair in inoculated plants [109]. Moreover, auxin stimulates seed germination, enhance photosynthetic rate and produce other plant growth related metabolites [110, 111]. Similarly, gibberellins and cytokinins stimulate wide variety of plant processes such as seed germination, cell elongation etc. which play important role in plant growth and development. Various genera of phosphate solubilizing bacteria can produce phytohormones such as *Rhizobium*, *Pantoea*, *Azotobacter*, *Paenibacillus*, *Rhodospirillum*, *Bacillus*, *Pseudomonas*, *Microbacterium*, *Plantibacter*, *Sanguibacter*, *Buttiauxella*, *Microbacterium*, *Erwinia* [96, 112–114].

5.4.4 ACC-deaminase production

Sometimes bacterial IAA stimulate ACC synthase enabling the of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase using S-adenosyl methionine precursor which is also the intermediate of ethylene production in higher plats. ACC deaminases have potential to cleave ACC to ammonia and α-ketobutyrate that act as nutrient for plants. This enzyme is also responsible for the reduction of plant stress ethylene, thus alleviating stress effects imposed on plants. Plants inoculated with ACC producing PSR have shown increased shoot system [115]. Moreover, ACC deaminase producing PSR can also stimulate nodulation process. Various PSR have potential to produce ACC such as *Achromobacter*, *Azospirillum*, *Enterobacter*, *Acinetobacter*, *Serratia*, *Bacillus*, *Burkholderia*, *Pseudomonas* etc. [105].

5.4.5 Bacterial cyanide biosynthesis

Some phosphate solubilizing bacterial have hydrogen cyanide (HCN) production potential which is a volatile compound and protect plants from various biotic stresses including allelopathic effects. Moreover, they also protect other harmful rhizobcateria by colonizing plant roots. Most of the phosphate solubilizing *Pseudomonas*, *Bacillus*, *Serratia*, *Enterobacter*, *Pantoea* can produce HCN [116, 117].

5.4.6 Indirect methods of plant growth promotion

Various indirect mechanisms are also adopted by phosphate solubilizing rhizobacteria such as production of various antifungal compounds, antibiotics and lytic enzymes. Different antifungal compounds such as proteases, lipases, cellulases and chitinases degrade cell wall of pathogens. Different P solubilizing *Pseudomonas* and *Bacillus* species can produce antifungal compounds. These compounds can protect plant from various plant pathogens [118]. Hence, these phosphorbacteria can also act as biocontrol agents in agricultural fields. Moreover, some PSR can also release enzymes that act as antibiotics, protecting plants from other pathogenic bacteria. Thus, inducing plant systemic responses (ISR). *Bacillus* sp. can produce various compounds such as difficidin, bacillaene, rhizocticinsn chlorotetain, bacilysin, and mycobacillin. ISR positive plants can response stronger and faster to pathogenic attack due to their induced defense system [119, 120].

6. Future perspectives

Efficiency of phosphate solubilizing rhizobacteria as biofertilizer, biopesticides, phytostimulaors and bioremidiators have now become research priority owing to their importances as environmentally safe plant growth promoting agents. Various genera of rhizospheric bacteria are capable of solubilizing soil phosphate by either releasing organic acids or enzymes. But there is a need to investigate further indepth mechanisms for bacterial phosphate solubilization and their interactions with root exudates for the development of suitable biofertilizer. Also, study about the knowledge of impact of these biofertilizers on soil microbiota is necessary as the rhizobacteria are important candidate of P-cycling mechanism. Moreover, plant growth promotion by rhizobacteria is a complex network of mechanisms functioning synergistically, thereby particular interaction between phosphate solubilization and its influence on root morphology needs to be investigated. In addition, the interactions and coordination between various rhizobacterial traits and their impact on agronomic parameters should be considered as top priority research for sustainable agriculture economically. Hence, commercializing these biofertilizers can be a promising tool for agricultural sustainability.

Conflict of interest

Authors declare no conflict of interest.

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Author details

Aqsa Tariq and Ambreen Ahmed*
Institute of Botany, University of the Punjab, Lahore, Pakistan

*Address all correspondence to: ambreenahmed1@hotmail.com

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