Phosphobacteria as key actors to overcome phosphorus deficiency in plants

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Summary

Phosphorus (P), an essential macronutrient for all living organisms, is required in large amounts for the growth and development of plants. Although total soil P level is high, P bioavailability to plants is suboptimal in most soils due to high fixation rates into inorganic and organic insoluble complexes. Hence, plants are highly dependent on mechanisms that allow them to adapt to low-phosphate stress and/or achieve suitable levels of soluble P on the root surface. In this regard, the rhizospheric microbiota plays a key role in facilitating P nutrition and have long been recognized for their potential use as an environmental-friendly alternative to chemical P fertilization.

Herein, we outline the advances in the identification of phosphate solubilizing bacteria and phosphate mineralizing bacteria, collectively known as phosphobacteria, and their role in rendering P accessible to plants. We review and discuss research progress related to the introduction of phosphobacteria and/or P-mineralizing enzymes into soil, as well as plant transformation with bacterial genes that encode such enzymes, as strategies to

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improve plant growth and P-nutrition. We also provide an overview of studies about the impact of variations in soil P levels on the structure of soil and rhizospheric microbial communities and the potential consequences of such perturbations on plant growth. Finally, we discuss possible directions for future research to optimize the efficiency of biofertilization strategies based on the use of phosphobacteria.

Keywords

Phosphobacteria, Biofertilizers, Rhizospheric microbiota, Phytases, Phosphate solubilizing bacteria, Phosphate mineralizing bacteria, Phosphorus deficiency.

1 | Limited P availability, a usual restriction for plant growth

Phosphorus (P) represents around 0.2% of a plant's dry weight and is required in high amounts for growth and development. P is a component of nucleic acids, ATP and phospholipids, which in part explains its essential nature. In addition, inorganic P regulates several important enzyme reactions and metabolic pathways (Schachtman et al., 1998). Thus, low P availability represents a stressful condition for plants and imposes severe crop yield limitations (Bononi et al., 2020).

Even when the total amount of P in soils is usually high (between 400 and 1,200 mg kg^{-1} of soil), its bioavailability to plants is less than 1% (Rodríguez & Fraga, 1999; Zhu et al., 2018). Thus, P is a worldwide limiting nutrient for agricultural production. Inorganic P availability for plants is low due to P immobilization in P-insoluble complexes mainly with iron, aluminum and calcium, a phenomenon that strongly depends on soil pH. Besides, soils can contain organic P forms that cannot be easily assimilated by plants and also form complexes with the above-mentioned ions (Rodriguez & Fraga, 1999). In order to cope with P deficiency, large amounts of chemical fertilizers are applied. Commercial P fertilizers are obtained from mined phosphate rock, which can be used either directly or after chemical treatments that generate soluble phosphates, such as the widely used triple superphosphate (Mendoza et al., 2009).

Global reserves of rock phosphate are estimated in 300 billion tons (USGS, 2020), mostly located in oceanic seamounts (around 75%), the rest being located in continents. The increasing demand for P extraction could lead to a potential 'phosphate crisis' along with possible depletion of this element or an incontrollable increase in

commercial prices due to economic problems associated with P extraction (Zhu et al., 2018). In addition, the aforementioned processes of P immobilization also affect the efficiency of P fertilizer applied to the soil. Plants can absorb less than 20% of added P before it becomes unavailable due to immobilization or leaching, which implies wasting most of the applied fertilizer (Plaxton & Tran, 2011). Thus, excessive use of fertilizer leads to the eutrophication of fresh and seawater, posing an increasing environmental hazard. Previsions about the depletion of rock phosphate reserves range from total depletion by the year 2100 to P availability for 300–400 years (Sattari et al., 2012). In this regard, it is clear that legacy P could be of fundamental importance to sustain global crop yield production (Zhu et al., 2018). The term 'legacy P' refers to the soil P stock, represented by P accumulated in fertilized lands minus the possible P outflows, such as crop absorption, surface runoff and leaching, among others (Zhu et al., 2018). However, most of the legacy P is not available to plants. As a consequence, the ability of soil microorganisms to render legacy P available to plants, as well as to enhance the availability of P provided as added fertilizer, is of fundamental importance for agriculture.

2 | Phosphobacteria, a biological tool for increasing soil P availability

During their life cycle, plants interact with deleterious, neutral and beneficial microorganisms (Tilak et al., 2005). Beneficial microorganisms include a wide range of bacteria and fungi. Some of them establish sophisticated mutualistic symbioses involving the formation of specialized symbiotic structures on plant hosts. This is the case of rhizobial and actynorrhizal bacteria and mycorrhizal fungi. On its part, multiple free-living microorganisms that colonize the rhizosphere and/or endophytically invade

plant tissues exert a variety of beneficial effects on plant hosts. Collectively, microbes that exhibit the above-mentioned features are designated as plant growth promoting microorganisms (PGPM). These microorganisms can affect plant growth either directly or indirectly by several different mechanisms (Ali & Glick, 2019). In particular, phosphobacteria are an important subgroup of PGPM capable of providing plants with available P and promoting plant-growth (Jorquera et al., 2008) through the solubilization and/or mineralization of soil P (Widdig et al., 2019).

Research interest in phosphobacteria has been mainly focused on PSB because of their well-proven effect on plant growth and soil fertility (Liu et al., 2020). Secretion of low molecular weight organic acids is accepted to be the predominant mechanism involved in mineral P solubilization. These acids, through their carboxyl and hydroxyl groups, chelate the cations bound to P. In addition, the pH decrease derived from the release of organic acids (and in some cases inorganic acids or H^+ extrusion) leads to the transformation of PO_4^{3-} (unavailable to plants as a P source) into HPO_4^{2-} and $H_2PO_4^-$, which can be used by plants as a P source (Chen et al., 2006; Prabhu et al., 2019).

Organic acids reported to be produced by PSB include gluconic, 2-ketogluconic, oxalic, citric, acetic, malic, lactic, formic, succinic, propionic and tartaric (Vazquez et al., 2000; Chen et al., 2006; Marra et al., 2019). Among them, gluconic acid is the most frequently found to be produced by PSB (Castagno et al., 2011; Suleman et al., 2018). In bacteria, conversion of glucose to gluconic acid is facilitated by pyrroloquinoline quinone-dependent periplasmic glucose dehydrogenase (PQQ-GDH), which is a constitutive member of the direct oxidative pathway of glucose catabolism in several organisms (Goldstein et al., 2003). In some bacterial species, mostly Gram-negative, cofactor pyrroloquinoline quinone (PQQ) biosynthesis involves at least five genes organized in the *pqq* operon (*pqqABCDE*), all of which are indispensable for PQQ production (Choi

et al., 2008; Klinman & Bonnot, 2014; Shen et al., 2012). Up to 11 *pqq* genes have been identified; nevertheless, their exact number and position are variable among different bacterial taxa. Other commonly found genes include *pqqF* and *pqqG*, which can be located either proximal or distal to the *pqq* operon (Shen et al., 2012; Klinman & Bonnot, 2014). Even though the PQQ synthesis pathway has not been completely elucidated, the precursor peptide is known to be relatively conserved in size (Puehringer et al., 2008). The above-described mechanisms of gluconic acid production are shared by a range of bacterial genera, including *Pseudomonas*, *Pantoea, Enterobacter, Serratia, Erwinia, Rahnella, Citrobacter, Burkholderia, Klebsiella, Gluconobacter* and *Acinetobacter* (An & Moe, 2016; Castagno et al., 2011; Buch et al., 2008; Carreño-López et al., 2019).

As mentioned before, in addition to inorganic phosphates, a large fraction of total soil phosphorus is present in organic form (Balaban et al., 2017). Although organic P forms can account from 30–65% of total soil P, they are usually not accessible for plants (Richardson, 2001). Frequently, a high proportion of organic soil P is represented by phytate (*myo*-inositol phosphate) and phytate-metal complexes (Balaban et al., 2017). As opposed to plants, some soil phosphobacteria produce phosphohydrolases, known as phytases, a particular class of phosphatases able to mineralize organic P from phytate and related organic P sources, thus playing a central role in soil P-cycling (Singh et al., 2020). Phytase activity was first identified in rice grains more than 100 years ago and in the mycelium of *Aspergillus niger* (Richardson, 2001). During the last 20 years, phytases have attracted considerable attention because of their potential use in nutrition, environmental protection and other biotechnological applications. Phytases have several applications in food and feed industries, in the preparation of *myo*-inositol phosphates and as soil amendments for plant growth promotion (Jain et al., 2016). In

phosphobacteria, phytases are widespread and have been reported in a variety of genera (Azeem et al., 2015; Balaban et al., 2017). Phytases of soil and plant-associated phosphobacteria of the genera *Bacillus*, *Enterobacter* and *Pseudomonas* are extracellular, while those of *Pantoea* and *E. coli* are located in the periplasm (Gessler et al., 2018). Based on the catalytic mechanism of hydrolysis, four major groups of phytases are recognized: histidine acid phosphatases (HAPs; acid phytases), β-propeller phytases (BPP; alkaline phytases), purple acid phosphatases (PAP; metalloenzymes), and protein tyrosine phosphatases (PTP; cysteine phytase) (Singh et al., 2020). Members of each group exhibit different catalytic mechanisms that enable them to effectively utilize phytate at different pH values.

In addition to phytases, other microbial enzymes play important roles in organic P mineralization. Various phosphatases are produced by microorganisms in response to P starvation. In this regard, ester-phosphate bonds of many phosphoesters can be hydrolyzed by both acid and alkaline phosphomonoesterases (ALPs) (Nannipieri et al., 2011). ALPs are ubiquitous among microorganisms and can mineralize up to 90% of soil organic P, except for phytate (Wei et al., 2019). Microbial ALPs are encoded by three homologous genes (*phoA, phoD* and *phoX*), among which *phoD* is ubiquitously distributed in bacteria. This gene is highly abundant in various soil types, thus being considered a key ALP-encoding gene. Interestingly, *phoD* abundance was found to be negatively correlated with P availability. As a result, *phoD*-containing bacteria contribute to P mineralization under low P availability, but can lead to P immobilization in bacterial biomass in P-fertilized soils. Thus, in a similar way to phytases, ALPs are important drivers of P turnover in soil (Wei et al., 2019).

3 | Phosphobacteria-mediated amelioration of plant growth and P nutrition

The isolation and identification of PSB bacteria has been the object of a considerable number of studies (for reviews, see Alori et al. (2017) and Prabhu et al. (2019)). Many of these works involved the identification of bacteria able to solubilize inorganic P-compounds and promote plant growth *in vitro*. However, a much more limited number of studies reached the instance in which growth promoting effects of PSB are tested on plants cultivated in P-deficient soils. In the following paragraphs and Table 1 we summarize the main findings of studies providing evidences about the potential of selected strains of PSB for improving growth and yield of extensive crops under P-limiting conditions. As shown in some of the studies described below, PSB can be inoculated either alone or in combination with different types of P fertilizers.

A screening of PSB bacteria isolated from the rhizosphere of wheat (*Triticum aestivum*) plants grown on soils of Peshawar and southern Punjab region (Pakistan), identified two strains (*Pseudomonas* sp. MS16 and *Enterobacter* sp. MS32) that also exhibited multiple functions related to plant growth promotion (Suleman et al., 2018). Apart from showing *in vitro* wheat growth promoting effects, MS16 inoculation in soils with low P content increased grain yield by 38.5% and 17–18% in pot and field trials, respectively. Growth-promoting effects on different crops were also obtained by using a combination of rock phosphate and PSB such as *Pantoea cypripedii*, *Pseudomonas fluorescens*, *Pseudomonas plecoglossicida*, as well as *Serratia* strains not identified at the species level (Kaur & Reddy, 2015; Schoebitz et al., 2013). Thereafter, the use of an inexpensive yet weakly bioavailable fertilizer such as rock phosphate in combination with some of the above-mentioned bacteria could be a sustainable strategy to provide a continued and gradual supply of P during crop cultivation.

Under low P conditions, *Pseudomonas tolaasii* (strain IEXb) and *Pseudomonas koreensis* (strain SP28) were shown to increase maize (*Zea mays*) plant growth (plant height and shoot dry weight) and P content respectively, as compared to non-inoculated controls (Viruel et al., 2014). Further field tests demonstrated that IEXb inoculation enhanced seedling emergence (8%), shoot length (19%), grain yield (44%), 1000-grain weight (18%), plant biomass (32%) and P content (56%). In these trials, the efficiency of strain IEXb as a biofertilizer was higher in the absence than in the presence of P fertilizer (Viruel et al., 2014). In this way, these findings demonstrated the high potential of *P. tolaasii* IEXb as a PSB-based biofertilizer for cultivated maize (Viruel et al., 2014).

Peanut (*Arachis hypogaea* L.) is a widespread legume of high economic importance. Argentina is one of the most important peanut producers worldwide, and soils devoted to this crop have undergone a decrease in P levels as a consequence of intense use over years. Therefore, PSB represent an alternative to mitigate soil P deficiency in such soils. Eighteen strains belonging to the bacterial genera *Serratia*, *Enterobacter*, *Acinetobacter*, *Enterococcus* and *Bacillus*, originally isolated from stem, roots and root nodules of peanut plants were tested in soils with low P content under microcosm conditions. After plant inoculation, many of the strains were found to improve at least one of the various growth parameters analyzed, four of the strains increasing total aerial P and N content (Anzuay et al., 2015). Peanut is usually cultivated under rotation systems that include crops such as maize. On this basis, some of the bacteria analyzed in the above-mentioned study were evaluated under a simulated crop rotation system in soils with low P content. Inoculation with single or multiple PSB increased most of the plant growth parameters analyzed, such as aerial length, root length and dry-weight, as well as aerial dry-weight for both peanut and maize plants (Anzuay et al., 2017). In this

way, these two works identified interesting PSB candidates for biofertilization of peanut plants and also highlighted the potential of consortia based on multiple PSB as biofertilizers for this species.

In addition to P deficiency, cultivated peanut is also often exposed to soil salinity conditions. Salinity exerts deleterious effects on plants due to the decrease in soil water potential, the toxic effects of ion accumulation in plant tissues and oxidative stress derived from altered redox homeostasis (Munns & Tester, 2008). Soil salinity not only affects cultivated crops, but also the soil and rhizospheric microbiota, as well as the survival and performance of inoculated PGPM (de Souza Silva & Francisconi, 2012). In this regard, halotolerant PSB have been considered as potential biofertilizers to improve crop growth and yield in saline soils. A study by Jiang et al. (2019) identified four strains [*Bacillus megaterium* YM13, *Enterobacter* sp. (YM14), *Providencia rettgeri* TPM23 and *Ensifer adhaerens* TPMX5] highly tolerant to NaCl and capable to solubilize P at Na concentrations as high as 1.4 M. Moreover, the growth-promoting effects of these PSB were enhanced by the addition of tricalcium phosphate. This was the first report about the potential of *P. rettgeri* as an efficient biofertilizer for peanut plants cultivated under saline conditions.

On its part, plant species that naturally grow in hostile environments have been used as the source for isolating PGPM adapted to restrictive conditions. Strains of *Bacillus*, *Enterobacter* and *Acinetobacter* were isolated from the rhizosphere of desert plants (*Setaria viridis*, *Cenchrus ciliaris*, *Panicum antidotale*, *Amaranthus viridis* and *Dichanthium annulatum*) of the western region of Saudi Arabia (Daur et al., 2018). Among other plant growth promoting activities, all the strains exhibited P-solubilizing activity and improved different growth parameters and nutrient levels, including P content, when inoculated on plants of the forage legume *Medicago sativa* (alfalfa) on

low nutrient content soils. In particular, *Acinetobacter pittii* JD-14 caused a remarkable increase in fresh- (41%) and dry-weight (34%) of alfalfa plants, as compared to non-inoculated controls (Daur et al., 2018). However, *A. pittii* was found to be implicated in community and hospital acquired infections (Chusri et al., 2014), which raises doubt about the real potential of this species as a biofertilizer due to associated risks for human and environmental health.

Narrow leaf birdsfoot trefoil (*Lotus tenuis* L.), another forage legume, has been studied in relation to PSB-mediated plant growth promotion in soils with low nutrient content. Field experiments performed in soils of the Salado River Basin (Buenos Aires Province, Argentina) found *L. tenuis* inoculation with P-solubilizing *Pantoea eucalypti* M91 to increase shoot biomass and P concentration at early growth stages, as compared to non-inoculated controls (Castagno et al., 2014). Moreover, *Pantoea eucalypti* M91 inoculation increased the beneficial effects of chemical fertilization with triple superphosphate. Overall, results of this study demonstrated that *Pantoea eucalypti* M91 inoculation either alone or in combination with triple superphosphate, improves P use efficiency by *L. tenuis* plants in low nutrient content soils.

Crops cultivated in acid soils of tropical regions are usually exposed to limitations in P availability because of the fixation of soluble P forms to aluminum and iron-free oxides and hydroxides (Edwards, 1991). This is the case of common bean (*Phaseolus vulgaris*), a highly P-demanding food legume widely cultivated in Latin America and Africa. A collection of bacterial isolates obtained from yerba mate (*Ilex paraguaiensis*) plants growing on acid soils, was screened for PSB capable of improving growth of common bean plants under low P availability conditions. *Enterobacter aerogenes* was found to stimulate plant growth and also increase leaf P and N concentration (Collavino

et al., 2010). Hence, PSB native from acid soils can be used as efficient biofertilizers for plants grown under P deficiency caused by soil acidity.

Usual biofertilization practices not only involve PSB, but also nitrogen fixing bacteria. In particular, legumes establish a mutualistic relationship with diazotrophic bacteria collectively known as rhizobia, which induce the development of nitrogen fixing root nodules (Oldroyd et al., 2011). In this way, cultivated legumes are usually inoculated with specific rhizobia as an alternative to chemical nitrogen fertilization. The efficacy of biofertilization strategies based on legume co-inoculation with PSB and rhizobia has been evaluated, with variable results. Co-inoculation of soybean (*Glycine max*) with P-solubilizing *Pseudomonas putida* strains and its cognate rhizobia (*Bradyrhizobium japonicum*) improved growth and nodulation, as compared to plants inoculated only with *B. japonicum* (Rosas et al., 2006). However, no growth increase was observed after co-inoculation of the above-mentioned *P. putida* strains and their specific rhizobial partner (*Sinorhizobium meliloti*), as compared to plants inoculated only with *S. meliloti* (Rosas et al., 2006). The authors proposed that the differences in the ability of P-solubilizing *P. putida* to exert growth-promoting effects on both legumes could be due to the fact that the *S. meliloti* strain used in their experiments (3DOh13) is able to solubilize P, while *B. japonicum* TIIIB is not.

Similarly, chickpea (*Cicer arietinum*) inoculation with the rhizobial symbiont *Mesorhizobium mediterraneum* strain PECA21 increases the beneficial effects of the tricalcium phosphate on plant P content. In addition, dry matter, nitrogen, potassium, calcium and magnesium content were increased in plants inoculated with the rhizobial symbiont in combination with tricalcium phosphate, as compared with plants treated only with the P fertilizer. Therefore, this work suggested that selection of rhizobial strains for chickpea should not be based only on their nitrogen fixing efficiency, since

these bacteria can also promote plant growth by means of other mechanisms such as P solubilization.

It can be envisaged that further analysis of a wider range of rhizobial species and strains for their ability to solubilize P could expand their biofertilizing potential beyond their well-known role as nitrogen fixers (Peix et al., 2001). It is worth pointing out that *M. mediterraneum* was also able to exert growth-promoting effects on barley (*Hordeum vulgare*), a species cultivated in rotation systems with chickpea. This finding raises the possibility of using rhizobial strains for P solubilization and biofertilization of different plant species, including non-legumes, which are integrated into common rotation cycles of cultivation.

Along with natural inorganic P sources and exogenously added fertilizers, P can also be present in soils as part of the organic matter. Despite the relative abundance of organic P in some soils, the use of PMB for biofertilization purposes has been investigated in a lower extent than PSB, and the number of reports about field performance of PMB is also limited, as compared to PSB (Table 1). Inoculation of wheat seeds with the phytase-producing soil bacterium *Pseudomonas* sp. CCAR59 caused an increase in phytate-derived P availability and shoot dry-weight to similar levels of plants fertilized with soluble Na_2HPO_4 (Richardson et al., 2000).

A variety of phytate-hydrolyzing bacteria were isolated from the rhizosphere of white lupinus (*Lupinus albus*) plants, most of which belonged to the genus *Burkholderia*. A selected *Burkholderia* sp. strain (FpRpG4) caused a 3- and 6-fold increase in *Lotus japonicus* dry-weight and shoot length, respectively, with phytate as the sole P source (Unno et al., 2005). Phytase activity was also detected in *Pseudomonas* sp., *Enterobacter* sp. and *Pantoea* sp. strains obtained from the rhizosphere of pasture plants (Jorquera et al., 2008). Interestingly, this work demonstrated that the rhizosphere of

cultivated crops and pasture plants were differentially enriched in PSB and PMB, respectively.

In addition to the Gammaproteobacteria mentioned in preceding paragraphs, some Gram-positive bacteria have also shown the ability to mineralize P through the action of phytases. Phytate-producing *Bacillus amyloliquefaciens* FCB45, as well as culture filtrates of this strain, enhanced growth of maize seedlings cultivated under low phosphate conditions in the presence of phytate. Culture filtrates obtained from a *phyA*$^-$ mutant unable to produce phytase lost the ability to stimulate plant growth. These and other results of this work provided univocal evidence about the role of phytase in plant growth stimulation by *B. amyloliquefaciens* FCB45 (Idriss et al., 2002). A study by Liu et al. (2018) performed with maize plants grown in P-deficient soil found *Bacillus* sp. SD01N-014 inoculation to enhance plant growth and P availability. On its part, *Bacillus mucilaginosus* was found to improve growth of tobacco (*Nicotiana tabacum*) plants in pot and field experiments (Li et al., 2007).

Interestingly, some bacterial strains are able both to undertake P solubilization and mineralization. In this regard, PSB found to efficiently promote growth of Basmati rice plants in the field were also found to release P from phytate *in vitro* (Rasul et al., 2019). Although the contribution of phytate hydrolisis to the abovementioned growth-promoting effects was not analyzed by the authors, strains that combine P-solubilizing and mineralizing activities are interesting candidates for biofertilization purposes.

Alternatively to the use of PMB, direct addition of purified microbial phytases to soil has been proposed as a strategy to increase soil P use efficiency, but the ability of purified enzymes to enhance soil P availability has not been studied in depth. For example, phytase addition to low P content soils increased biomass production of maize plants (Findenegg & Nelemans, 1993). Recent analysis of biochemical and soil

adsorption properties of four phytases purified from *Escherichia coli* and *Aspergillus niger* suggested that purified phytases may be used as a complement of P fertilization (Caffaro et al., 2020). However, the use of purified phytases in the field is challenging, due to their potential degradation by soil proteases. In this regard, Menezes-Blackburn et al. (2014) demonstrated that P-mineralization from cattle manure by *E. coli* and *A. niger* phytases can be improved by enzyme immobilization in nanoclay complexes.

An additional approach to achieve P mineralization that goes beyond the use of phosphobacteria and purified microbial phytases is based on genetic transformation of plants with phytase genes of microbial origin. Studies in this field involved the transformation of a variety of plant species with fungal and bacterial phytases of the HAP type (Valeeva et al., 2018).

In some cases, improved plant growth and higher accumulation of inorganic P in plant tissues were obtained when transgenic plants were cultivated under laboratory conditions with phytate as the sole P source. Transgenic *Arabidopsis thaliana* and soybean plants expressing a phytase from *Aspergillus ficuum* exhibited a 3.5-fold increase in inorganic P content, as compared to wild-type plants (Li et al., 2009). Transgenic expression of an *Aspergillus niger* phytase gene allowed *A. thaliana* growth on a synthetic medium amended with phytate as the sole P source. Moreover, shoot dry-weight, shoot P concentration and shoot P content detected in transgenic plants cultivated in phytate containing medium were similar to those of plants grown in a medium with a soluble P source (Mudge et al., 2003). Similarly, transgenic expression of *A. niger* phytase improved the nutritional properties of wheat plants (Brinch-Pedersen et al., 2003).

As to BPPs, their high specificity for phytate potentially eliminates the risk of detrimental side effects towards other aspects of P metabolism in plant cells. Tobacco

and *A. thaliana* transformation with *B. subtilis* BPP led to increased plant growth on both species and increased P content in *A. thaliana* (Belgaroui et al., 2016; Lung et al., 2005; Yip et al., 2003). A recent study that compared the advantages of transforming *A. thaliana* with HAP and BPP concluded that, in principle, both approaches render similar results but their efficacy can be differentially affected by growth conditions (Valeeva et al., 2018). The above-mentioned studies suggest that the expression of bacterial phytases in transgenic plants could provide an important means for improving growth of cultivated crops under conditions of P deficiency.

4 | Rhizospheric microbial communities as affected by variations in soil P levels

Most soil microorganisms have developed a variety of evolutionary adaptations and physiological acclimation mechanisms to cope with variations in environmental and soil conditions (Schimel et al., 2007). Thus, abiotic stresses such as salinity, acidity and alkalinity, flooding and drought, extreme temperatures or nutrient deficiencies shape microbial communities according to each species genetic or physiological potential. In turn, changes affecting the activity of bulk-soil bacteria influence the interaction of these microorganisms with plants (Liu et al., 2019). The microbes living in the plant rhizosphere, rhizoplane and the root endosphere operate as the root microbiome and they actively affect plant development, nutrient acquisition and stress tolerances (Berendsen et al., 2012).

Much of the accumulated knowledge about abiotic stress effects on plant-microbe interactions has been gained through experiments performed under controlled conditions with a single strain, however there is still a need to understand the complexity of rhizosphere processes occurring under stress conditions. It should be kept

in mind that P deficiency could also be an indirect consequence of changes in soil physicochemical properties such as reduced water availability and pH shifts. These factors, in turn, are known to modify the microbial communities (Schimel et al., 2007; Naylor & Coleman-Derr, 2018), an issue that hampers the precise determination of P stress-specific effects on the structure and dynamics of the soil and rhizospheric microbiota.

Plants exposed to nutrient limitations change the quantity and composition of the rhizodeposits (such as sugars, organic acids, secondary metabolites, as well as complex polymers) released by roots. For example, N-deficiency reduces the release of amino acids in maize root exudates, P-deficiency stimulates the release of gamma-aminobutyric acid and carbohydrates, K-deficient plants release less sugars (particularly glycerol, ribitol, fructose and maltose), and Fe-deficiency increases the release of glutamate, glucose, ribitol and citrate (Carvalhais et al., 2011). Root exudates are often harnessed by bacteria, mainly as carbon sources, to rapidly proliferate in the rhizosphere and in this way, plants can modulate the assembly of their microbiomes by changing the composition of the released root exudates (Mommer et al., 2016; Pétriacq et al., 2017; Sasse et al., 2018). Differential metabolite exudation was also suggested to be the mechanism by which different *Arabidopsis* accessions could influence bacterial assemblages (Micallef et al., 2009).

Moreover, it has been shown that plants respond to P deficiency by releasing specific compounds at different growth stages (Pantigoso et al., 2020), this being a plausible mechanism by which they distinctively modulate their interaction with microbes during their life cycle (Marschner et al., 2006). *Arabidopsis* genotypes known to exhibit different local P starvation responses revealed a partial overlap between P and Fe

deficiency-induced changes in root exudate composition, with both deficiencies having a differential regulation of coumarin biosynthetic pathway genes (Ziegler et al., 2016). Specifically, the catecholic coumarins are hypothesized to have an antimicrobial effect on a dominant *Pseudomonas* strain, thereby altering the dynamics of the remaining community members (Voges et al., 2019). In pea (*Pisum sativum*), a higher abundance of Proteobacteria (*Rhizobium*, *Pseudomonas*, *Pantoea*, *Nitrobacter*, *Enterobacter*, and *Sphingomonas*) was found in the rhizosphere, whereas Firmicutes (*Massilia*, *Paenibacillus*, and *Planomicrobium*) were more abundant in bulk soil (Chaudhari et al., 2020). Besides phosphate solubilization, bacteria with well-described plant growth promoting activities (such as Indol acetic acid and siderophore production, and induced systemic resistance) were found to be enriched in soybean rhizosphere (Liu et al., 2019).

Despite the many works addressing the influence of the plant genotype on its associated microbiome (Liu et al., 2019; Gomes et al., 2018; Bulgarelli et al., 2015), Gomes et al. (2018) observed that soil P level has more impact on selecting maize root microbiomes under nutrient limiting conditions than the plant genotype or the plant compartment. However, there is no universal plant-microbiome response to P stress among different plant species, ranging from none or very subtle impact of P fertilization on the rhizospheric community (Sawyer et al., 2019; Pantigoso et al., 2020; Widdig et al., 2019) to large shifts in bacterial taxa composition and abundance, as well as microbial interactions (Gomes et al., 2018; Silva et al., 2017; Pantigoso et al., 2018; Leff et al., 2015; Gumiere et al., 2019). This is not surprising, given that each plant species uses different mechanisms to cope with nutritional deficits and therefore it seems reasonable that different P-transporting, P-solubilizing, and/or P-mineralizing microbes are selected to fit the plant requirements.

Disparate findings regarding the composition of PSB taxa are also found. On one hand, some studies showed that under low P conditions the bacterial community contains higher numbers of taxa involved in rendering insoluble P available for the roots (Pantigoso et al., 2018; Chaudhari et al., 2020). On the other hand, some reports demonstrated that PSB abundance is not affected by P addition (Tang et al., 2016; Fernández et al., 2015) or even decreases when P is combined with nitrogen addition, indicating that nitrogen availability may affect the functional traits of the PSB community (Widdig et al., 2019).

As previously described, different commercial products are widely used as P fertilizers and, as rock phosphate has less environmental impact than soluble phosphate fertilizers, the knowledge regarding the identity of rhizospheric taxa able to improve P release/acquisition by plants using rock phosphate fertilization could be central to a rational selection of phosphate solubilizers for sustainable agriculture. In maize, it was shown that P fertilization type induced changes in the composition of the rhizospheric microbiota; while Proteobacteria were found to be predominant in the microbial community in all treatments, different taxa related to this phylum responded differentially, suggesting some candidate groups (*Oxalobacteraceae*, *Burkholderiaceae*, *Bacillaceae*) of special relevance in rock phosphate solubilization (Silva et al., 2017). Similarly, different phosphate sources modified the structure of sugarcane bacterial communities, as well as the networks of bacterial and fungal interactions where the rock phosphate source led to a lowest level of competition and strongest system stability (Gumiere et al., 2019).

Under the assumption that important ecosystem functions are preserved even if the microbial community structure changes, recent studies have sought to compare the relative abundances of bacterial genes involved in P turnover processes such as

mineralization, solubilization and uptake of extracellular P sources. In a metagenomic approach comparing two sources of nitrogen fertilizers, no significant effect on the relative abundance of the analyzed genes was found, but a strong differential distribution of such genes amongst the families was observed (Grafe et al., 2018).

Interestingly, it was found that certain families (including *Verrucomicrobiaceae*, *Sphingomonadaceae*, *Anaerolinaceae*, *Planctomycetaceae*, *Chitinophagaceae*, *Acidobacteriaceae* and *Bradyrhizobiaceae*) harboured a 'universal' spectrum of functions, while others were specialized in single processes. In a related approach, (Liang et al., 2020) explored the presence of the gluconic acid gene (*gcd*) in 39 reconstructed bacterial genomes, among which novel phosphate-solubilizing microbial taxa were found. Besides, the relative abundance of these genomes was strongly correlated with bioavailable soil P, supporting their involvement in soil P cycling. Strikingly, many mobile genetic elements containing *gcd* were found in the 39 genomes, indicating that acquisition of new metabolic P cycling functions might occur through horizontal gene transfer (HGT).

Although HGT has been recognized as a major mechanism for gaining new activities in bacteria, very little attention has been given to its potential role in the acquisition of genes involved in P solubilization. On this basis, further research on this topic could provide valuable information about the mobility of P cycling functions within bacterial communities. Functional prediction for enriched rhizospheric bacterial taxa was performed for clover and maize under contrasting P availabilities, finding significant differences between the two plant species in terms of genes that are relevant for P metabolism and carbon cycling (Lang et al., 2019).

It should be noted that although the search for functions related to phosphate solubilization might be an interesting approach, the taxonomic identity of the bacteria

harboring such activities should not be disregarded, given the different potential of each microbial taxa to withstand the restrictions imposed by the soil physicochemical conditions.

In an effort to integrate arbuscular mycorrhizal symbionts into the general root microbiota responses, Bodenhausen et al. (2019) compared P-fertilizer induced changes in mycorrhizal (*Petunia* × *hybrida*) and non-mycorrhizal (*Arabidopsis*) plants. They observed that both plants contained different sets of bacterial communities under P-limiting conditions, sharing only four OTUs, among which *Dechloromonas* sp. was the most abundant.

In sugarcane, not only the phosphate source, but also arbuscular mycorrhizal fungi inoculation affected the structure of the soil microbial communities, explaining 41% of their variability. The authors suggested that hyphae of arbuscular mycorrhizal fungi recruit bacteria to support their own P acquisition (Gumiere et al., 2019).

The use of 'omic' strategies has thus far contributed to deepen our knowledge on microbial communities in their natural habitats, but culture-dependent approaches are still needed to test several hypotheses regarding molecular basis of plant-microbe interactions.

A midpoint between single strains and complex microbiomes, was achieved by enriching for a group of taxa (called synthetic communities, SynComs), as an alternative approach to develop experimental designs to provide insight into the selective processes occurring in plant-microbial community assembly.

The comparison of wild-type and mutant lines of *Arabidopsis* impaired in the phosphate starvation responses (PSRs) showed that the assembly of the root microbiome is mediated by the plant PSR genotype: PSR mutants developed different root bacterial microbiomes than wild-type plants even when grown under P-replete conditions. A

SynCom composed of 35 taxonomically diverse bacteria isolated from *Arabidopsis* was used to experimentally demonstrate that distinct bacteria are 'chosen' by mutant and wild-type plants under both low and high P concentrations. Moreover, the phosphate starvation response 1 (PHR1, a molecular switch controlling the phosphate stress responses) in *Arabidopsis* was also demonstrated to be involved in the suppression of plant immunity during P starvation (Castrillo et al., 2017).

More recently, a study using a SynCom, comprising 185 genome-sequenced endophytic bacterial isolates, was designed to resemble a natural bacterial community. From this study the genus *Burkholderia* emerged as a relevant player under P starvation conditions: when absent from the SynCom, plants accumulated higher P levels compared to plants exposed to the complete SynCom, this behavior being observed under P-limiting conditions but not in P-replete conditions. This behavior suggests that species from this genus might shift from a commensalistic to a pathogenic relationship, depending on the P status of the *Arabidopsis* plants (Finkel et al., 2019).

The fact that the presence/absence of a single strain has been able to modify plant P accumulation highlights the need to better understand the impact exerted by artificial introduction of bacteria used as biofertilizers, as well as their interactions with indigenous microorganisms. Gaining insight on these topics would contribute to improve the current efficacy of phosphobacteria inoculation and also understand its consequences on target ecosystems, thus preventing potential negative effects on them.

Although the impact of microbial inoculants on soil microbial communities has been examined (for a review see Trabelsi & Mhamdi (2013)), little attention has been given to the specific effect of phosphobacteria. Few studies, albeit indirectly, have addressed this issue. Recently, Nuzzo et al. (2020) analyzed the impact of commercial amendments containing PSB on growth of tomato plants (*Solanum lycopersicum*) and

communities of soil microorganisms. Overall, the work demonstrated that inoculation exerted no detectable effects on soil microbial community, excepting for very subtle changes on some bacterial taxa not related to the inoculated microbes. However, it is worth mentioning that an important finding of this work was that the actual microbial composition of the commercial formulations had little consistence with the composition provided in the product labels, being this a conceivable reason for the lack of efficacy in promoting growth of tomato plants. Thus, this work highlights the need to improve the validation requirements for commercial microbial inoculants.

As a final point, it should be kept in mind that correlating changes in the microbiota with the P-solubilizing or P-mineralizing activity of inoculated bacteria is not straightforward, because of the frequent presence of other traits in phosphobacteria that can affect the growth of indigenous organisms. In this regard, experiments comparing the effects of wild-type PSB or PSM and mutant strains unable to solubilize or mineralize P, combined with the use of SynComs, could provide valuable information about the specific effects of P-solubilization and P-mineralization activities on natural microbial communities.

5 | Conclusions and perspectives

Low soil P availability is a widespread constraint for agriculture, which demands high inputs of chemical P fertilizer that exert undesirable effects on the environment. As a consequence, microorganisms capable of increasing P availability to plants have attracted a great deal of attention, as an environmental-friendly alternative to increase plant yield.

Over years, research on PGPM provided solid evidences about the ability of

phosphobacteria to mitigate the deleterious effects of P deficiency on plants. A wide variety of bacteria capable of improving plant growth and nutrition under low P availability have been identified and tested at different levels, including field trials in some instances. Thus, it is not surprising that biofertilizers based on phosphobacteria are commercially available. Despite the significant advances achieved so far, several aspects of the use of phosphobacteria as biofertilizers could still be significantly improved.

One of such aspects is related to the use of PMB, which have been much less studied than PSB. In this regard, further studies about the ability of selected bacteria to efficiently mineralize P and render it available to plants under field conditions would be valuable, taking into account the relatively high levels of organic P that are normally present in many agricultural soil. Furthermore, suitable combinations of PMB and PSB specifically tailored for target soils on the basis of their relative contents of organic and inorganic forms could lead to a more efficient use of phosphobacteria as biofertilizers. Additionally, further identification of bacterial strains that exhibit both P solubilizing and mineralizing activities could provide a means to improve P acquisition from insoluble inorganic and organic sources by plants.

A frequent and obvious criterion for selecting phosphobacteria is their ability to solubilize or mineralize P either *in vitro* or *in vivo*. However, additional features not directly related with P mobilization are also important to increase the efficacy of phosphobacteria. In this regard, different studies have demonstrated that efficient phosphobacteria can be isolated from environments that closely resemble the conditions of the target ecosystems where they are inoculated. In addition to the relatively permanent constraints exhibited by particular ecosystems, such as soil acidity, alkalinity, salinity or low nutrient content, temporary stresses like drought also affect

survival of inoculated bacteria and indigenous microbial communities, as well as their interactions with plants. Therefore, bacterial ability to thrive in the rhizosphere or soils under low water availability or other temporary stress conditions could be a useful selection criterion to screen for phosphobacteria that are efficient as biofertilizers under field conditions. In this regard, biofertilizers based on genetically diverse phosphobacterial consortia can provide a means to ensure efficient P solubilization and/or mineralization under the varied range of conditions that bacteria need to cope with in target ecosystems.

An additional point to be considered in order to improve phosphobacteria-based biofertilizers is related to the need to include strict biosafety criteria for the selection of candidate bacteria. In fact, this issue not only affects the selection of phosphobacteria, but PGPM in general and is of particular importance considering the abundance of studies in which field tests are performed with bacterial strains that have not been characterized in depth at the taxonomical level.

In order to reduce environmental and health risks, a profound taxonomic characterization of bacterial strains is desirable prior to performing the evaluation of candidate strains under field conditions. In this regard, current accessibility to bacterial genome sequencing facilities at reasonable costs is a valuable tool not only for taxonomical identification and screening for the presence of interesting genes in bacteria, but also to detect non-desirable traits that could represent a risk for biofertilizer formulation.

Many studies based on massive sequencing of bacterial genes used as taxonomic markers have served to describe more or less accurately the consequences of P stress on the structure of bacterial communities. Current advances in the use of this kind of technologies are exerting a profound impact on the identification of rhizospheric core

microbiomes associated to particular plant species. It is thus expected that these approaches also serve in the future for specifically analyzing the effects of different environmental stresses on the abundance and diversity of phosphobacteria in the rhizosphere, thus identifying components of phosphobacterial cores with high potential for the development of efficient biofertilizers. In addition, the use of massive sequencing technologies would be of great help to understand the effect of inoculating phosphobacteria on the structure and functioning of rhizospheric bacterial communities, an issue that has not yet been thoroughly addressed. Such kind of studies would serve to analyze, for example, in which degree phosphobacteria exert their beneficial effects on plants in a direct way, or indirectly as a consequence of modifications in the structure of microbial communities. In this way, the combination of culture-independent massive analyses of microbial communities with traditional culture-dependent approaches is expected to exert a significant impact not only on our knowledge of ecological aspects of phosphobacteria, but also on the development of novel biofertilizers with increased efficiency under unfavorable conditions for crop cultivation.

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Conflict of interest

The authors have no conflict of interest to declare.

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Table 1. Improved growth and yield parameters of crop plants by phosphobacteria

Phosphobacteria	Bacterial type	Plant	Experimental conditions	Growth/yield parameters improved by phosphobacteria	References
Enterobacter sp. MS32, *Pseudomonas* sp. MS16	PSB	*Triticum aestivum*	Pot and field trial +DAP	Increased grain yield in pot and field trials (38.5% and 17-18%, respectively)	Suleman et al., (2018)
Pantoea cypripedii (PSB-3), *Pseudomonas plecoglossicida* (PSB-5)	PSB	*Triticum aestivum* *Zea mays*	Field trial + RP	Increased yield in maize (20%) and in wheat (16%)	Kaur & Reddy (2015)
Pseudomonas fluorescens, Serratia sp.	PSB	*Triticum aestivum*	Pot + RP	Increased P uptake (>50%)	Schoebitz et al., (2013)
Pseudomonas tolaasii IEXb	PSB	*Zea mays*	Pot and field trial + TSP	Enhanced seedling emergence (8%), shoot length (19%), grain yield (44%), 1000-grain weight (18%), plant biomass (32%) and P content (56%).	Viruel et al., (2014)
Acinetobacter L176*, Bacillus sp*. L55*, Enterococcus sp.* L191*, Pantoea sp.* J49*, Serratia sp*. J260, *Serratia sp.* S119.	PSB	*Arachis hypogaea* L.	Pot + TCP	Increased plant aerial length (up to 2.1-fold) and aerial DW (up to 4.4-fold). Increased total aerial P and N content	Anzuay et al., (2015)
		Arachis hypogaea L. *Zea mays*	Pot	Increased growth parameters and P content in soil and inoculated plants	Anzuay et al., (2017)
Bacillus megaterium YM13, *Ensifer adhaerens* TPMX5, *Enterobacter* sp. YM14, *Providencia rettgeri* TPM23.	PSB	*Arachis hypogaea* L.	Pot + NaCl and/or TCP	Increased root length (25–49%), stem length (19–48%) and number of leaves (12–37%). Growth-promoting effects enhanced by the addition of TCP	Jiang et al., (2019)

Acinetobacter pittii JD-14, *Bacillus* sp, *Enterobacter* sp.	PSB	*Medicago sativa*	Field trial	Improvement of different growth parameters and nutrient content, including P. FW and DW increased by *A. pittii* JD-14 (41 and 34% respectively)	Daur et al., (2018)
Pantoea eucalypti M91	PSB	*Lotus tenuis*	Field trial + PR/TSP	Increased dry matter yield (68%) and P concentration (15.4 %) at early growth stages	Castagno et al., (2014)
Bradyrhizobium japonicum + *Pseudomonas putida*	PSB	*Glycine max*	Pot + TCP	Improved growth and nodulation	Rosas et al., (2006)
Mesorhizobium mediterraneum PECA21	PSB	*Cicer arietinum* *Hordeum vulgare*	Pot + TCP	Increased dry-weight (56%), N and P content (1-fold) in barley. Increased dry-weight (18%), N and P content (1.25-fold) in chickpea. Increased K, Ca and Mg content in both species.	Peix et al., (2001)
Enterobacter aerogenes R4M-A	PSB	*Phaseolus vulgaris*	Pot + TCP	Increased plant dry biomass (1.7-fold) and leaf area. Increased P (2-fold) and N content in leaves.	Collavino et al., (2010)
Pseudomonas sp. CCAR59.	PMB	*Triticum aestivum* L.	Agar slants	Increased phytate-derived P availability. Shoot dry-weight similar to plants fertilized with soluble P.	(Richardson et al., (2000)
Burkholderia sp. FpRpG4	PMB	*Lotus japonicus*	Agarized medium + IHP	Increased shoot dry-weight (3-fold) and length (6-fold).	Unno et al., (2005)
Bacillus amyloliquefaciens FZB45	PMB	*Zea mays*	Agarized medium + IHP	Enhanced plant growth and chlorophyll content (6-fold).	Idriss et al., (2002)
Bacillus sp. SD01N-014	PMB	*Zea mays*	Pot	Enhanced plant growth and P availability	Liu et al., (2018)

Bacillus mucilaginosus D4B1 (WT), NKTS-3 (transgenic strain that produces highly active phytase)	PMB	*Nicotiana tabacum*	Pot and Field trial	Plant growth increased by both strains in pot (NKTS-3, 2.3-fold; WT,1.8-fold) and field trials (NKTS-3, 1.2-fold; WT, 1.1-fold)	Li et al., (2007)

PSB, phosphate-solubilizing bacteria; PMB, phosphate-mineralizing bacteria; DAP, diammonium phosphate; RP, rock phosphate; TSP, triple superphosphate; TCP, tricalcium phosphate; IHP, Inositol hexaphosphate.