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Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity

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Background: Increasing crop production to feed a growing population has driven the use of mineral fertilizers to ensure nutrients availability and fertility of agricultural soils. After nitrogen, phosphorus (P) is the second most important nutrient for plant growth and productivity. However, P availability in most agricultural soils is often limited because P strongly binds to soil particles and divalent cations forming insoluble P-complexes. Therefore, there is a constant need to sustainably improve soil P availability. This may include, among other strategies, the application of microbial resources specialized in P cycling, such as phosphate solubilizing bacteria (PSB). This P-mediating bacterial component can improve soil biological fertility and crop production, and should be integrated in well-established formulations to enhance availability and efficiency in use of P. This is of importance to P fertilization, including both organic and mineral P such as rock phosphate (RP) aiming to improve its agronomic efficiency within an integrated crop nutrition system where agronomic profitability of P and PSB can synergistically occur.

Aim of Review: The purpose of this review is to discuss critically the important contribution of PSB to crop P nutrition in concert with P fertilizers, with a specific focus on RP. We also highlight the need for PSB bioformulations being a sustainable approach to enhance P fertilizer use efficiency and crop production.

Key Scientific Concepts of Review: We first recognize the important contribution of PSB to sustain crop
production, which requires a rational approach for both screening and evaluation of PSB enabling an accurate assessment of the bacterial effects both alone and in intertwined interaction with plant roots. Furthermore, we propose new research ideas about the development of microbial bioformulations based on PSB with a particular focus on strains exhibiting synergetic effects with RP.

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**Introduction**

Human population is expected to reach 9 billion by 2050, an increase of 0.7% per year, accompanied by a 70% increase in food demand [1]. For many years, the aim for applying fertilizers is to supply nutrients to plants to sustainably secure adequate crop yield. Besides nitrogen (N) and potash (K), phosphorus (P) has been crucial to sustain crops yield for the production of both food and feed [2-4].

Phosphorus is an essential macronutrient directly involved in nucleic acids, cells division and growth of new tissues, which all regulate protein synthesis and energy transfer [5]. This nutrient is needed for diverse cellular processes like photosynthesis, carbohydrate metabolism, energy production, redox-homeostasis, and signaling [6]. Phosphorus plays a key role in root development, root traits anatomy modifications and root hair density with a significant contribution in increasing yield of crops [7]. Phosphorus can limit normal plant growth if not provided by the soil or by appropriate quantities of fertilizers. Consequently, P deficiency can cause significant reductions (up to 15%) of crop yield [8]. For this reason, P application remains one of the main agricultural practices to meet plant needs.

Obviously, application of water-soluble P fertilizers improves soil mineral fertility and increase P availability in soils, thereby plant P uptake will be enhanced leading to a higher plant productivity and yield [9]. Although P fertilizers are agriculturally vital to secure crop growth and productivity, their use efficiency by crops significantly may be very low due to P fixation to soil cations. For example, P fertilizers can rapidly react with soil divalent cations such as calcium (Ca), iron (Fe) and aluminum (Al) to form insoluble soil P mineral forms [10,11]. Therefore, improving use efficiency of P fertilizers in terms of nutrient uptake and crop yield remains highly important. Unlike water-soluble P fertilizers, RP directly applied in agricultural soils could be an efficient P form for crop production in high P retention soils [12]. The agronomic efficiency of RP has been extensively studied and reported over the past 50 years [13], and the positive effects of its direct application on soil properties and plants growth have been well reported to rely mainly on RP solubility [14-16]. However, the rate of RP dissolution needs to be improved in most agricultural soils, which is ultimately needed to meet plant P demand. To increase RP agronomic efficiency, use of agriculturally beneficial microorganisms involved in P-cycling is a promising biotechnological strategy that has gained worldwide interest in recent decades.

These microbes are commonly known as P solubilizers (PSM) belonging to the group of plant growth promoting microbes (PGPM) due to their phyto-stimulation capacities [17,18]. Among PGPM, plant growth promoting rhizobacteria (PGPR), exhibiting higher P solubilizing abilities, have been categorized as PSB exhibiting substantial benefits for plant growth and yield [19-21]. For example, *Pseudomonas* [22], *Azotobacter* [23], *Xanthomonas* [24], *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, and *Delftia* sp. [25,26] are known to exhibit higher P solubilization capacities along with multiple plant growth promoting activities.

In addition to their native P solubilizing capacity in soils, PSB can be combined to RP, as both are natural resources and their co-application has been demonstrated to improve RP agronomic efficiency [27-30]. Indeed, exploitation of microbial functional traits related to P solubilization, mainly in high P-retention agricultural soils, is paramount in order to propose microbial-based strategies enabling RP use efficiency, [31]. Many experimental studies provided evidence that synergies can occur when combining both PSB and RP likely leading to cost-effective P-based biofertilizers directly applicable in acidic or alkaline soils. For instance, dual application of RP and PSB (e.g., *Azotobacter*, *Azospirillum*, *Rhizobium* and *Klebsiella*) significantly improved plant P nutrition of both cereal and legume crops [32-37].

Indeed, various formulations containing PSB have been established to increase RP dissolution as well as achieving high yield of crops. The use of PSB becomes effective as it could continuously offer biological solutions in concert with mineral P fertilization as both are highly beneficial for plant growth. In this review, we focus on the importance of both RP and PSB in terms of agronomic profitability within an integrated P biofertilization approach. We also detail agronomic profitability of P (mineral and organic) and PSB co-application in amended soils and inoculated crops, establishing the connection between the influence of PSB co-application on agroeocystem production. Moreover, we discuss the importance of both a rational and functional screening approach of PSB, which is based on different screening levels to help construct efficient consortia. Additionally, we highlight PSB formulation to be a crucial step for bacterial survival and P solubilization activities within the root/rhizosphere interface. Specifically, our review discusses a real need to exploit PSB based on their ability to solubilize RP to advance research on a possible development of controlled-release P fertilizers as part of an environmentally sustainable approach alleviating low P availability issue while enhancing P use efficiency (PUE).

**Phosphorus is an essential nutrient for food production**

Phosphorus is considered as a macronutrient majorly involved in central and important molecules for living organisms including DNA, RNA, sugar, lipids, proteins, ATP, ADP and NADPH [38]. It is therefore an essential nutrient for plants development and growth given that P concentration reaches up to 0.5% of plant dry weight [5]. Adequate levels of P availability in soils, among other factors, significantly contributes to crop productivity assuming that P fertilizers are vital to meet plant P nutritional requirements that is partly responsible for sustaining crop production.

**Phosphorus in the soil–plant continuum**

Phosphorus in soil exists in different chemical forms, either organic or inorganic (Pi). Besides the readily available P fraction that P fertilizers can significantly provide, activities of both roots and associated microorganisms also contribute to improve P availability in the rhizosphere soil. Inorganic P forms include precipitated P containing minerals (Fig. 1) defined as minerals that contain P as a structural element [39] such as apatites, strengite and variscite that are very stable, and their solubility depends on soil pH [40]. Meanwhile the secondary forms are adsorbed or bound P (Fig. 1) such as P-sorbing minerals, mainly Al-, Ca- and
Fe- P whose dissolution depends on soil particles and soil pH [41,42]. Organic P forms consists of compounds varying in terms of bioavailability and solubility. For instance, most organic P forms usually exist as inositol P, anhydrides of phosphoric acid and phosphonates forms [43,44] originated from plant residues and animal manure [45-47]. Meanwhile, an important organic P proportion, between 3 and 14%, is bound in microbial biomass [48], which competes with plants for available P [49]. In the long term, this microbial biomass could represent a temporary immobilized pool of P, which can be mineralized and released in the soil solution as available P.

Despite the high capacity of P to bind strongly to soil particles [10], P availability for plant uptake is generally a balanced process of both adsorption and desorption phenomena. Indeed, rhizosphere biological processes play key roles in P dynamic and availability in agricultural soils. Both plants, via roots, and rhizosphere microbes significantly contribute to soil biological activities, thus driving P dynamic in the root-soil interface where P bioavailability highly dependent on organic and inorganic compounds such as mucilage, organic acids, phosphatases, and some specific signaling substances (proton release, chelation and ligand exchange) (Fig. 1). All considered to be key drivers of various rhizosphere processes [10] including P-cycling microorganisms that improve P availability in agricultural soils.

To facilitate P acquisition from soils, different modifications in root architectural traits are employed such as increasing root length and root hair density [50]. Root hairs are the most specialized for nutrient uptake [51]. In addition, formation of cluster roots is also considered among the major adaptations for a better P acquisition [52]. Crops may respond differently to soil P levels such as wheat, maize and rice exhibiting longer root hairs that improve PUE [53-55]. On the other hand, leguminous crops (such as Cicer arietinum and Vicia Faba) significantly change root physiology (such as exudates) than root morphology [56]. For example, exudation of organic acids such as oxalate and malate are involved in increasing P availability [57]. Another study on two contrasting soybean genotypes reported significant induction of oxalate, malate and citrate under P deficiency and/or aluminum toxicity [57]. Moreover, extracellular exudation of enzymes into the rhizosphere, either by roots or associated microorganisms, are additional mechanisms significantly contributing to improve P availability [58] with acid phosphatases being the most abundant P-hydrolyzing enzymes produced under low P conditions [59].

In connection with this research review, PSM can solubilize/mineralize unavailable P forms in soils through different mechanisms such as rhizosphere acidification and/or phosphatases excretion, resulting in enhanced plant P uptake [37]. For instance, PSM inoculation can modify root morphology and architecture through phytohormones production such as abscissic acid, cytokinin, indole-3-acetic acid and gibberellic acid [60,61]. Moreover, PSM could modulate the expression of auxin-responsive genes, hence playing a key role in the regulation of endogenous auxin level with positive consequences on P acquisition and plant physiological status [62-65]. In addition, positive effects on spatial rhizosphere/root heterogeneity can occur due to increased soil exploration leading to a more solubilization and root absorption of P, which can be achieved by inoculating roots with auxin-producing PSM isolates [66].

**Phosphorus is a key nutrient fertilizer for a sustainable crop production and food security**

Enhancing agricultural productivity to ensure food security is a matter of concern, which undoubtedly will require adequate amount of essential nutrients, including P. Commercial fertilizers are multiple and could be found as straight fertilizer when only a single nutrient is presented like single super P (SSP) or triple super P(TSP) or like urea or ammonium sulphate for nitrogenous straight
fertilizer, whereas di-ammonium P (DAP), monoammonium P (MAP), nitrophosphate (NP) and NPK, among other fertilizers, are considered mixed or complex fertilizers containing more than one essential nutrient [67]. For instance, application of urea P and monopotassium P increased soil P availability and leaf P content of Solanum tuberosum [68]. This is in line with recent findings by Otinga et al. [69] reporting that soil available P enhanced with the application of P fertilizers (TSP) with positive impacts on soil fertility. In addition, application of P fertilizers including mono-calcium P (MCP) and DAP improved nutrients (P and N) uptake of rice [70]. This fertilization could affect positively shoot and root P content generating higher plant biomass (80%) compared to unfertilized plants [71]. Similarly, under both greenhouse and the field conditions, growth, nutrient uptake, and grain yield of soybean increased under different P fertilization supplies including TSP and RP [72]. Furthermore, yield of sugarcane and Solanum tuberosum increased in response to different P fertilizers (e.g., DAP, SSP, TSP and RP) [68, 73, 74]. It is noted that application of P fertilizers improves soil fertility and increase P availability in soils, thereby plant P uptake will simultaneously increase leading to a better plant growth and yield.

Although P is an essential nutrient, however only a small fraction of it, estimated up to 25%, is taken up by plants [75]. Hence, application of P fertilizers must consider soil physicochemical properties (e.g., pH, redox potential) plausibly responsible for such a reduced utilization such as in acidic soils where P is mostly fixed by Al or Fe and in alkaline soils predominately by Ca [8]. Fertilizers such as TSP, DAP and MCP are water soluble P concentrated fertilizers, but their rapid reactivity with soil nutrients and clay particles significantly impact the fate of P in the soil as well as plant P uptake presumably will not synchronize with application of P fertilizers over time. Alternatively, RP may strongly be recommended to use as a low-reactive P fertilizer against fixation and adsorption phenomena, particularly in high-P retention soils. It was also demonstrated that RP remains a slowly dissolving P form enabling a gradual P release likely through acidification due to rhizosphere activities [15, 76, 77]. However, RP dissolution rate seems to be low given the higher plant P requirements throughout growth stages. To overcome RP low solubility, utilization of high P-dissolving crops (exhibiting robust and active rooting system) and PSM capable of solubilizing RP is highly recommended. Indeed, application of RP combined with PSM remains a highly interesting approach, which can offer opportunities to improve PUE.

Fertilizer best management practices improve PUE and meet sustainable agriculture goals

Agronomic practices, in terms of plant nutrition and soil fertility, through application of the right mineral fertilizers, while considering the right amount and composition, the right time, and the right place, ultimately improve use efficiency of nutrients [77] with positive consequences on yield in particular and on the demand for food and feed in general. A wiser “in field and in time” agricultural application of P-based fertilizers is of importance to make P nutrient use agronomically more efficient, environmentally beneficial, and economically vital. For this purpose, the 4Rs Nutrient Stewardship guidelines have been developed by the fertilizer industry as a process to guide fertilizer best management practices (FBMP) all around the world [78].

Bringing a single nutrient are usually used such as P containing fertilizers whose application is needed to raise P availability in soils [79]. For the right rate principle, it clearly links to soil nutrients status and plant requirements. An effective soil analysis (such as Olsen test [80], Bray test [81] and Kelowna and modified Kelowna tests [82]) should be available to determine the need for P fertilizer application and to estimate P rate needed. Application of P should be synchron-

ized with the crop’s nutrient requirements “Right Time”, especially at early growth stage of plant development, given that crops are often sensitive to P deficiency at the earliest growth stage [83]. Moreover, later P supply may be important for plant, which depends on the initial P status [84]. Optimization of P availability for crops can also be achieved with the right placement of P fertilizers “Right place” [85]. Indeed, application of P fertilizers nearby the root zone can help plants take up P efficiently and positively influence overall plant growth performance and yields. In this regards, banding of P fertilizer near to the root zone or it application with seeds have been reported among the best placement option more than P broadcast on the soil surface [86]. Placement of P fertilizers within the soil even at smaller placed P doses was reported to enhance rice development, P uptake, and yield under P deficiency [85]. Overall, fertilizers 4Rs practices have proven efficient to ensure a better agronomic efficiency of P fertilizers while considering the physical and chemical properties of soils and crop needs (Fig. 2).

In addition, ecological consideration should be taking as a starting point to create innovative fertilization strategies where both ecological and biological processes (e.g., nutrient-specific interactions in the rhizosphere and plants, soil, and plant microbiomes, etc.) and technologies can be co-exploited via concerted research and development efforts to achieve sustainable crop production goal. An example of integrated approach adopted to increase P fertilizers agronomic efficiency was proposed by Jayakumar et al. [87] suggesting a combination of both P mineral fertilizers (RP and TSP) and biological resources (PSB, egg shell and animal bone waste). This combination showed an increased (14%) efficiency of TSP when combined with bacterial inoculation. This exemplifies positive synergies between both mineral and microbial resources leading a better plant growth. Opportunities, therefore, exist to systematically deploy microbial resources as part of integrated crop fertilization systems [30].

Moreover, improvement of P use efficiency in agricultural ecosystem could be achieved through minimizing nutrient loss by developing smart fertilizers, which become a priority research among many agricultural research institutions. One of the most promising strategies is to develop controlled-release fertilizers (CRFs) (Fig. 2) deliberately made to release the active nutrient in a controlled manner while extending the duration of release and manipulating the rate of release to meet plants needs [88]. There are several CRFs marketed; for instance, application of coted polymer (MAP, DAP and SSP) increased PUE in rice as well as P availability in soil [89]. In a recent study, Pizzeghello et al. [90] reported an induced yields and P uptake of Hypericum moseriunm in response to polymer-coated MAP compared to MAP application. Nevertheless, there is still a limited number of studies on the development of controlled release P-fertilizers using low-grade RP. In this regard, Sarkar et al. [3] used different coating agents (polyvinyl alcohol and liquid paraffin) to produce a controlled release RP formulation and suggested this as a strategy to enhance P use efficiency. In other hands, being an integral component of soil biogeochemical processes, exploitation of free or encapsulated PSM in an environment friendly strategy can increase P availability in soil–plant systems using several mechanisms [91, 92]. Thus, the development of fertilizers based on the combination of RP nanocomposites and PSB along with best management practices could improve PUE and meet sustainable agriculture goals.

Phosphate bio-solubilization boosts rock phosphate agronomic efficiency

Increased agronomic efficiency of rock phosphate for a better PUE

Evidently, RP even in its natural form contributes to crop production mainly in specific soil conditions, but adequate technolo-
gies are needed to generate additional agronomic efficiency of RP. Across plant growth stages, it is essential to develop new strategies applied to the rhizosphere interface to dissolve maximum amount of available P from RP. One of the successful practices is the combination of RP with organic amendments such as manure [93-95]. Co-application of RP and vermicompost (cow dung, grasses, aquatic weeds and municipal solid wastes) was found to increase soil P availability more than single application of RP [96]. A study by Narayanan [97] demonstrated an increased RP use efficiency when combined with vermicompost or anaerobic digestate sludge considered to be excellent biofertilizers compared to mineral P fertilizers. Following the same approach, incorporation of different RP rates with organic manure, including cow dung and waste paper, increased soil soluble P by 39, 50 and 65% as compared to 2, 4 and 8% under a single RP application, respectively [98].

Another strategy consists of mixing RP with water soluble P fertilizers assumed to be both agronomically and economically effective. In this context, mixing RP with TSP was successful with the relative RP agronomic effectiveness (calculated relatively to a P conventional fertilizer) increased from 12.5 to 45% [99]. This improvement could be explained by the ability of TSP to provide a readily available P fraction for plants even at the earliest stages of their development with the assumption that RP use efficiency increases while plant roots develop and spatially exploit the rhizosphere soil [13]. Ndakidemi [99] also demonstrated the positive impacts of the combined use of RP and TSP on common bean whose seed yield significantly increased two fold (219%) compared to single RP fertilization. Similarly, co-application of RP and TSP (50/50%) was reported as effective as TSP (100%) in both field- and pot experiments-grown sorghum [100]. Besides, partial acidulation of RP can contribute to improve RP agronomic efficiency. The reaction of RP with acid waste (metallurgical acid residue and whether an acidic mine waste) was effective to produce more soluble P, thus improving plant P uptake and yield [101-103]. From the promising findings of these studies, it appears that practical formulations of mixed P fertilizers combining RP and other mineral P sources could be considered as a possible pathway enabling a higher RP agronomic efficiency, however more applied research are required to prove compatibility with both soil types and crops.

Furthermore, exploitation of PSM is among promising microbial technological applications used to increase RP agronomic efficiency. In this context, P-mediating microorganisms are integral components of the soil P dynamic as they strongly participate in the rhizosphere nutrient dynamic processes [104]. Many microorganisms, including fungi, bacteria, and yeast can solubilize different insoluble forms of P [92,105,106]. Xu et al. [107] showed that Pantoea ananatis and Bacillus thuringiensis could increase RP efficiency by producing organic acids such as gluconic, citric, and α-Ketoglutaric. Additionally, RP combined with PSB (Pantoea cypripedii and Pseudomonas plecoglossicida) increased soil P availability resulting in a higher crop yield of both maize and wheat [37]. Moreover, RP agronomic efficiency can be enhanced by adopting a mechanical–biological approach consisting of producing soluble P by growing Aspergillus niger using RP with particle sizes in the nanometric range. The mechanical treatment of RP (even for short periods of milling), combined with the biological cultivation process could be highly effective in increasing RP solubilization with gains ranged from 60 to 115% [108].

Phosphate solubilizing bacteria contribute to improve P use from rock phosphate

Evidently, PSB efficiency for PUE and crop production has been demonstrated through controlled and field studies, however RP that stands as a natural P source primarily used in the production of P fertilizers for the agriculture is still lacking technologies enabling a higher P solubilization as compared to well-developed technologies and processes that chemically transform RP into P mineral fertilizers. Over the last ten years, the effects of PSB on RP solubilization have been reported in more than 4640 research publications (Fig. 3) related to specific research fields, mainly sciences of the environment (494), soil (423), agronomy (308), plant (301), and microbial biotechnology (157). In these studies that have been trending up since 2000, attention has been paid
to the important role of microorganisms to enhance RP solubilization for a direct P application, and to develop appropriate technologies that enhance both RP solubilization and its agronomic efficiency. This also included identification of obstacles associated with the direct use of RP.

Phosphate solubilizing bacteria are capable to solubilize unavailable P form to soluble forms, thereby improving subsequent availability of P to plants [109-112]. Bacterial species belonging to several genera such *Pseudomonas* spp., *Agrobacterium* spp., and *Bacillus* spp., [113,114] are used as soil inoculants to increase P availability. In addition to P solubilization ability, other studies have reported *Rhizobium tropici* [115], *Azotobacter chroococcum* [116], *Enterobacter cloacae* [117] to be both solubilizing and mineralizing P bacteria. Park et al. [118] demonstrated that *Enterobacter* sp. can increase RP solubilization up to 17.5%, and this trait presumably increased soil P availability, showing that inoculation with PSB and amendment with RP could be a promising alternative option to use this potent source as P fertilizer and maintain higher nutrient availability in soils [118]. Similarly, Rezakhani et al. [119] reported a significant effect on RP solubilization indicated by increased soil P availability through inoculation with *Pseudomonas* sp. FA1. This PSB strain significantly promoted root and shoot biomass and uptake of P under RP fertilization. Moreover, PSB (MRS23) combined with RP resulted in a higher soil P availability (27%) compared to that solubilized (4%) from RP alone [37]. These results indicated that PSB increased the efficiency of RP by increasing its solubilization and providing more P into mineral P pool. In addition, inoculation of maize and wheat plants with PSB “*Pantoaea cyripedii* and *P. plecoglossicida*” under RP fertilization notably improved P content of shoots (37 and 186%), roots (76 and 91%) and yield (20 and 16%) of maize and wheat, respectively [33]. Similar effects were observed in wheat fertilized with RP and inoculated individually with five PSB *Pseudomonas plecoglossicida*, *P. reinekei*, *P. koreensis*, *P. japonica* and *P. frederiksbergensis* showing positive impacts on rhizosphere available P, shoot P content and root acid phosphatase activities [7].

In this regard, the efficiency of “RP-PSB” can also be improved by introducing a nutrient-rich organic component such as organic amendments including poultry manure and composts. Such a mixture have been reported to positively impact maize responses under RP and proposed to be a promising approach to enhance soil P availability and plant growth in intensive cropping systems [120]. Similarly, Alzoubi et al. [121] demonstrated that RP agronomic efficiency significantly increased in response to inoculation with PSB (*Bacillus megaterium*) and amendment with organic fertilizers (based on organic manure and olive residues). Moreover, growth and yield of legume plants (chickpea and lentil) significantly enhanced under application of RP enriched with organic amendments and PSB (*Bacillus thuringiensis* and *Bacillus* sp. Cp-h60). This improvement is attributed to the positive effect of PSB on P availability and particularly on nodulation that improved N nutrition of chickpea and lentil [36]. Likewise, combined use of RP, poultry manure and PSB (*Pseudomonas* spp, *Azospirillum* spp, and *Agrobacterium* spp strains) increased growth, P uptake and yield of chili equivalently to DAP application [28]. In this direction, it was demonstrated that PSB-RP utilization associated with application of biochar, in order to optimize microbial growth and reproduction, can be considered as a sustainable strategy to enhance RP solubilization and soil P availability [122,123].

A fine-tuned bacterial screening approach is needed for accurate selection of PSB

Generally, the adopted methodology for PSB screening (in vitro assays) is based mainly on soluble P quantification tests from either RP or other sparingly available P forms. Most likely, only PSB exhibiting high P solubilization rate are selected for additional traits, while the isolates with a low P solubilizing are excluded although they might be of importance in promoting plant growth rather than P solubilization and uptake of P. In a recent comparative study by Elhaissoufi et al. [7] using five contrasting PSB isolates, demonstrated that *Pseudomonas plecoglossicida* exhibiting the lowest P solubilization rate increased considerably shoot P content more than *Pseudomonas koreensis* and *Pseudomonas japonica* characterized for the high P solubilizing ability. Therefore, it is advisable to perform a thorough characterization that values the lowest PSB instead of making an exclusion decision, particularly both lowest and highest P solubilizing isolates might be of interest.
in constructing consortia exhibiting complementary traits. Indeed, an additional test in liquid and soil medium to assess P solubilization should simultaneously be performed. Liu et al. [124] showed that PSB (Bacillus megaterium, Bacillus Subtilis, Pseudomonas aeruginosa and Pseudomonas oryzihabitans) strains with the highest P solubilization values in liquid medium or agar plates exhibited a lowest P solubilization capacities once the PSB are inoculated in soil. In addition, empirical reports show that tricalcium P is inappropriate, as a universal selection, for most PSB isolated because P bio-solubilization is a very complex phenomenon affected by many factors, each of which cannot be evaluated and tested separately [125].

In this review, a rational screening approach is proposed based on both in vitro and in vivo characterization of PSB (Fig. 4). Several screening levels can be suggested with a starting level consisting of a fundamental characterization of main P solubilization traits in vitro conditions, particularly the qualitative (solubilization index) and quantitative traits (P available). Direct and indirect PGP traits (auxin production, N fixation, siderophore production etc.) are also needed in the first level of PSB characterization. At this first screening level, PSB either with or without halo of solubilization are selected, regardless of P solubilization capacities they exhibited. Halo production on solid agar medium should not be considered the sole test method for PSB screening, as it has been shown that bacterial strains exhibiting P solubilization in liquid medium did not produce solubilization halos when tested on agar plates [124]. The second level involves in-depth characterization of PSB, which involves in-soil inoculation experiments. In this approach, it is necessary to timely monitor the effect of PSB on P solubilization in unplanted soils. Due to the complex factors of soils, bacterial strains could solubilize insoluble P and release a soluble P form in liquid medium and into the soil without developing halo zones when tested on agar plates [124]. Thus, to avoid underestimating P solubilization bacterial capacity, P solubilization traits should be estimated in various media, concurrently. In addition to soil inoculation, the effect of PSB on plant growth at different stages of development (seedlings, vegetative and flowering) is proposed. The third level allows an efficient screening of PSB that could efficiently solubilize P along with increasing plant growth. The improvement of plant growth could be associated with multiple plant growth-promoting traits that PSB could exhibit rather than PSB solubilization traits [126,127]. In addition, it was demonstrated that PSB with low and medium P solubilization capacity induced a positive effect on root traits, wheat biomass and nutrient acquisition compared to PSB exhibiting high P solubilizing capacities [7]. The 4th level leads to a precise selection of PSB not only based on P solubilization under laboratory conditions, but also on the basis of monitoring P availability in unplanted soil experiments as well as the effect of PSB on plant development at different growth stages. Thus, the lowest and highest PSB isolates could be important candidates to select for consortia construction featuring multiple and different traits responsible for enhancing P availability and improving plant growth.

Physiological mechanisms implemented by PSB for P solubilization / mineralization

Phosphate solubilizing bacteria can be efficient in making P more available to plants from both organic and inorganic sources by solubilizing and mineralizing insoluble P compounds [128]. As mentioned in different studies in Table 1, the principal mechanism of P solubilization is the secretion of P-mineral dissolving compounds such as organic acids, protons, and siderophores [128-135]. Organic acids produced increased plant-available P into the rhizosphere by forming complexes with cations (Al- or Fr-P) or by block P absorption sites on soil particles [136]. These organic acids are the products of the microbial metabolism, mostly by oxidative respiration or fermentation of organic sources such as glucose [137]. For example, organic acids such as lactic, malic, acetic, oxalic, and gluconic are produced by different bacterial species such as Serratia sp., Bacillus sp., Enterobacter sp. and Azospirillum sp. [135,138,139]. In this regard, gluconic acid has been reported to be the most involved in P solubilization by chelating the cations bound to insoluble P [129]. In addition, many Gram-negative bacteria employ periplasmic glucose oxidation through pyrroloquinoline quinone-dependent glucose dehydrogenase.

Fig. 4. Illustration of bacterial screening steps proposed as an integrative approach for a qualitative and quantitative assessment and selection of phosphate-solubilizing bacteria based on four screening levels.
enzyme to produce gluconic acids that is encoded by gmd gene [140]. Pyrroloquinoline quinone acts as a redox cofactor in glucose dehydrogenase enzyme resulting in P solubilization [140]. Other mechanisms of P solubilizing bacteria include the release of protons with less or even no production of organic acids [141]. Moreover, an alternative mechanism to organic acid production is the production of inorganic acids (e.g., sulphuric, carbonic, and nitric acids) and chelating substances such as siderophores [22]. However, the efficiency of inorganic acids to solubilize P remains less important than organic acids [142].

In addition to P solubilization, PSB can be able to mineralize organic P. Different groups of P hydrolyzing enzymes are involved in the organic P mineralization processes. The first group produced by PSB has been characterized as phosphomonoesterases also referred to as phosphaetases (encoded by olpA) [143-145]. These enzymes can either be acid or alkaline phosphomonoesterases [146,147]. Alkaline phosphatase catalyzes the hydrolysis of P esters, namely Glucose-6 phosphate and ATP, releasing Pi [148]. Alkaline phosphatase optimal pH is above 7, most often between 9 and 10 while acid phosphatase has a pH optimum between 4 and 6 [149]. Another type of P hydrolyzing enzymes produced by PSB is phytase (encoded by appA) [150,151]. This enzyme is responsible for the mineralization of P from soil organic matter where P is stored in phytate, which hydrolyses bioavailable P. Previous findings have revealed that microbial phytases are the most suitable for application in commercial biotechnology enzyme production due to their catalytic properties [152]. Furthermore, phosphatase (encoded by phnX) and C-P lyase (encoded by phnJ), are able to release free P from recalcitrant organic P forms [153].

### Bioformulation of P-solubilizing bacteria to improve P use efficiency

**Design and market of PGPR bioformulations**

Formulation of microorganisms as biofertilizers is one of the environmentally friendly practices employed to improve performance of microorganisms used in crop production. According to a definition proposed by Malusa et al. [154], formulation contains one or more beneficial microbial strains prepared with an easy-to-use and economical carrier material. The bioformulation, as a process, is a crucial multistep consisting of providing a safe environment that protects microbial cells once they are introduced through a suitable carrier into the soil. The selection of an adequate carrier, and a design of correct delivery methods are paramount components to consider. Different microbial formulations have been developed, either liquid or solid, using various carrier materials. The carrier materials play a key role in the efficiency of the bacterial formulation. The main properties of a good carrier are: (i) non-toxicity to microbes, (ii) good exchange surface, (iii) ease of both sterilization and processing, (iv) available in high quantity, renewable and inexpensive, and (v) non-toxic to plant, human health and environment (Fig. 5). Data in Fig. 5 represents an innovative approach of bacterial formulation. A liquid formulation of microbial cells is prepared with water, oil or water-soluble polymer that improves stability and dispersion of microorganisms [155,156]. Both liquid and solid state-fermentation have also been developed for microbial formulation using various biomasses rich in nitrogen and carbon [157,158]. Solid bioformulations are often based on either inorganic or organic carriers, prepared in solid include granules, microgranules, wettable powders and dusts [159-162] and classified according to application mode and carrier design.

Solid and liquid formulations (Table 2), including encapsulations, are available in the market [163,164]. Granule formulations contain active ingredients, binder, and carrier materials. Most commonly carrier materials used are wheat meal [165], gluten [166], gelatin or acacia gum [167], semolina (durum), cottonseed flour and sugars [168] and sodium alginate [161]. For instance, commercial biofertilizers containing M. anisopliae var anisopliae strain F52 (MET52) [169] and Serratia entomophila [170] are considered to be effective granular biofertilizers. Moreover, wettable powders are of much interest because they are applied as a suspension in water and can be easily added to a liquid carrier just before its application. Commercial biofertilizers containing Trichoderma harrisonum, Pseudozyma flocculosa and Pseudomonas fluorescens AS506 are examples of wettable powders bioformulation [171,172]. Starch has been well studied with a dried beads or liquid core capsules [173,174]. It has successfully been used as a carrier in PGPR formulations [175]. The addition of mineral clay to alginate-based formulations was found to increase the physical properties of alginate polymer used as a carrier of Ruouitella planticola [176], improve cell survival, and serve as a protective micro-habitat accessible to bacteria due to its layer structure [177,178]. Dusts are also one of the oldest formulation types and contain a finely ground mixture of the active ingredients with particle size ranging from 50 to 100 μm [179]. For example, bioformulation of Beauveria bassiana conidia based on skimmed milk powder and glue was reported to achieve 100% of conidial germination and retained 78% conidial viability even after 12 months of storage at 30 °C [180]. Protein hydrolysates from animal and plant biomass were also used as a carrier for rhizospheric microbial formulations [181]. In this regard, Vejan et al. [182] reported that the bioformulation of Bacillus salmalaaya with chitosan-alginate-protein capsules, achieving an encapsulation index of 99.7.

### Table 1

Examples of organic acids produced by PSB and involved in P solubilization.

| References | PSB strains | Organic acids | Concentration | pH |
|------------|-------------|---------------|---------------|----|
| [128]      | *Pseudomonas fluorescens* | gluconic acid-formate acid - propanedioic acids | 11.1 mM | 4 |
| [129]      | *Pseudomonas prosrkeii* | 2,3-dimethylfumaric acid | 45 mg/l | 5-9 |
| [130]      | *Erwinia rhapontici, Bacillus subtilis,* | acetic acid-propionic acid- 2-keto-gluconic acid | 10 mM-4.7 mM–35 mM | 2.7- |
| [131]      | *Firmicutes SP, Proteobacteria sp,* | oxalic, lactic, citric, succinic, acetic and formic acids | 45.7 mg/g to 82.7 mg/g | 0.5 |
| [132]      | *Citrobacter, Pseudomonas, Staphylococcus,* | – | 1.4 | 3.81- |
| [133]      | *Bacillus strain* | acetic acid, citric acid – Oxalic acid | (56.7 μg/ml) | – |
| [134]      | *Enterobacter* | malic and lactic anions | (36.2 μg/ml) (5.93 μg/ml) | – |
| [135]      | *Serratia sp.* | malic acid- lactic acid- acetic acid | 80.48 ± 10.28 μg ml–64.03 ± 5.94 μg ml– | 6.5- |

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Establishment of liquid bioformulations has shown multiple advantages compared to solid bioformulations, including longer shelf life, high microbial viability, no contamination and high performance in field [183]. Liquid bioformulations consist in general of specific broth 10–40%, of dispersant 1–5%, ingredient for suspension 1–3%, surfactant at 3–8% and carrier liquid either oil or water, or a combination of both at 35–65% by weight [184]. Suspension concentrates, oil-miscible flowable concentrate, ultralow volume suspensions and oil dispersion are all types of liquid bioformulations [180]. Companies around the world are getting more and more interested in this new generation of biofertilizers. For instance, Japan and the United States companies produce and use different types of biofertilizers that contain *Rhizobium* sp. as inoculant on various crops including lentils, soybeans, corn, sorghum, sugar beets, wheat and canola [179,185].

Efficient "PGPR-RP" bioformulations are needed for direct application in agriculture

Biofertilizers have been widely applied in agriculture to help improve crop productivity and soil fertility. Biofertilizers are an essential component of sustainable agriculture and play a key role in supporting soil productivity while increasing the availability of various nutrients and inducing PGP traits (auxin, P solubilization,

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**Table 2** Examples of studies reporting beneficial effects of solid and liquid bio-formulations on various crops.

| Formulation types | References | Strains used | plants | Effects of formulation on plants |
|-------------------|------------|--------------|--------|---------------------------------|
| SOLIDE FORMULATION | [203]      | *Bacillus subtilis* | Lentil | Biocontrol activity against *Fusarium* promoting their growth and increased the dry weight of lentil plants. |
| [204] *Trichoderma parareesei*, *Pseudomonas fluorescens*, *Bacillus subtilis* | *Azotobacter chroococcum* | Tomato | Biocontrol activity against *Solania esculentum Mill* increase yield of tomato Increase maize growth parameters |
| [205] *Bacillus Subtilis* | *Pseudomonas corrugata* | – | Increase growth and yield crop in sodic/saline soil.sodic/saline soil. Enhanced plant biomass, increased the yield and accelerate the rhizosphere colonization |
| [206] *Enterobacter cloaceae* (PSB) | – | *Vicia faba* | *Biocontrol activity against Ralstonia solanacearum* |
| [207] *Bacillus megatherium* | *Pseudomonas fluorescens* | *Bacillus subtilis* *Trichoderma viride* | *tomato brinjal chill* | Increased root and shoot dry weights and lengths of wheat in field conditions |
| [209] *Bacillus endophyticus* *Bacillus sphaericus Enterobacter aerogenes Bacillus safensis Bacillus meigaterium(PSB)* | – | Wheat | *Biocontrol activity against Fusarium* Protect plants, enhance vegetative growth and contain pathogen populations |
| LIQUIDE FORMULATION | [210]      | *Pseudomonas fluorescens* | *Wheat* | Increase in plant biomass, nodule number and weight, and number of pods |
| [211] *Bacillus subtilis* | – | Variety of horticultural fruits trees ornamental crops | Pigropea | |
| [212] *Trichoderma spp.* | – | – | – | |
| [213] *Burkholderia sp.* | – | – | – | |
N fixation, etc.). For instance, bioformulated PSB strains (Pantoea sp. and Pseudomonas sp.) proved a great potential to enhance *Pisum sativum* growth [186]. Bioformulations, either individually or in consortium, containing *Bacillus licheniformis* and *Pseudomonas aeruginosa* significantly increased growth parameters and yield of *Brassica campestris* [187]. Indeed, bioformulated *Pseudomonas* sp. is a patented biofertilizer that increase plant growth with high market competitiveness [188]. This increase could be explained by directly providing plant with essential nutrients or indirectly by protecting plant against pathogens. Moreover, the application of biofertilizer based on PSB formulations showed an increase of 12.45, 78.11, and 34.4% in plant height, green fodder yield and grain yield of sorghum, respectively [189]. Furthermore, application of bioformulated *Rhizobium* enhanced nodulation by 42% along with increased germination and seed yield of *Lens culinaris* [190].

It is obvious that bacterial formulation is a green approach used to boost soil fertility and improve crop productivity. Over the past ten years numerous patents are invented for this purpose (Table 3).

Reddy and Janarthanam [191] invented microbial formulations that includes bacterial strains belonging to N fixing bacteria, PSB, other rhizobacteria, and biocontrol microbe isolates, and fungal strains (*Trichoderma viride*, *T. vixen*, *T. harzianum*, *T. harzianum* *LK*, *T. harzianum* *G*, and *T. longibrachiatum*). These polymicrobial formulations conferred for pea plant resistance against pathogens and increased nutrients (N, P, K) availability under reduced agrochemical applications [191]. Indeed, the invention of liquid bioformulation of the PSB *“Pseudomonas fluorescens* improved P, potash, boron and iron content of corn plants [192]. In addition, various capsule bioformulations have been invented to meet a specific plant need. For example, “Nitroset” is a consortium of symbiotic or non-symbiotic N2 fixing bacteria with a capacity to affect positively N fixation, “Phossol” is defined as a combination of PSB that increase P availability. “Potmob” include potash mobilizing microbe that effect potash mobilization, “Encounter”, “Encounter- 100”, “Rottucider” and “Nemuccider” are capsulated consortia of microbes acting as biopesticides and biofungicides [193]. In addition, to increase P availability and crop yield, bioformulation of PSB such as *Bacillus megathrixium* and *Pseudomonas putida* improve P uptake as well as fertilizers efficiency [194]. Indeed, Nadeem et al. [195] invented an efficient bioformulation with no synthetic process that allows P solubilization and mobilization from RP using either PSM (alone), or the combination of PSM and PGPM. Moreover, one or numerous PSM (such as *Penicillium spp* with one or more plant growth promoting traits (N fixation, P solubilization, seed germination, plant growth etc.) were used to formulate efficient biofertilizers containing RP, MAP, DAP, MCP, TSP and ammonium polyphosphate to increase soil P availability, plant P uptake and fertilizers use efficiency [196].

However, formulation of an effective biofertilizer requires a particular supporting carrier to protect the bacterial cells during storage and transport [197]. As mentioned earlier, there are different types of bioformulations used in agriculture, yet the application of this biofertilizers could be unprofitable because of a lack of adequate formulations and the low inoculant quality [198]. For the reason to achieve a successful bioformulation, numerous challenges should be tackled, among which, selection of a microbial strain exhibiting a better survival and colonization capacity, which in turn will ensure efficiency of bioformulations [199]. Under both natural and agricultural systems conditions, it is necessary to understand the bacterial community structure and functions in relation to environmental factors in order to avoid the multitrophic competition phenomena in the plant-soil-microbe continuum [200]. The second concern is to maintain the viability and functional properties of inoculant that could be enhanced using some additives such as of phytohormones (gibberellin acid and cytokinin), glycerol, a trihydroxyalcohol, poly-lactic acid and stirigalactones [201].

Free or encapsulate PSB are applied in soil to increase RP solubilization. However, up to date encapsulation techniques were not used to simultaneously formulate RP and PSM [202,203] as opposed to regular solid formulations. To our knowledge, only a few studies have developed materials integrating both RP particles dispersion and encapsulation of microorganisms in the same structure [106]. The production of a biofertilizer containing RP and PSM (individual or consortium) could be a potential approach to increase RP solubilization whose agronomic efficiency could be enhanced by PSM organic acids production. Formulation of PSM also requires integration of carrier materials, human health and environment risk, storage and transport. Selection of carrier materials suitable for liquid or solid formulations, while considering risks, costs and efficiency at each development step, is crucial for the development of eco-friendly biofertilizers (Fig. 5). Research efforts should also be oriented towards development of micro-environmental (using specific carrier materials) conditions to facilitate the growth and to harness functions of microbial bioformulations.

### Conclusions

Thanks to the high potential of beneficial PGPM in crop productivity and resource use efficiency, multiple PSB inoculants or bioformulations have been used in agriculture. On another hand, many bacterial inoculants show insufficient performance due to many constraints, which could directly link to the bacterial

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**Table 3**

Description of bio-formulation technology patents related to PSM application from 1991 to 2017.

| References | Patent title | Description of the invention | Date |
|------------|--------------|------------------------------|------|
| [214]      | Methods and compositions for increasing the amounts of phosphorus and/or micronutrients available for plant uptake from soil | The invention relates to a method and composition for increasing the amounts of phosphorus and micronutrients available for uptake by plants from the soil by introducing an inoculum of the fungus *Penicillium bilajii*. Formulation of liquefied biofertilizer comprise phosphate source and PSM, | 25/06/1991 |
| [215]      | Microbial solubilization of phosphate | Polymicrobial formulations comprise numerous bacterial and fungal strains to increase nutrients availability and plant growth | 12/09/2009 |
| [191]      | Polymicrobial Formulations For Enhancing Plant Productivity | 17/01/1992 |
| [216]      | Synergistic bacterial consortia for mobilizing soil phosphorus | Combination of synergistic bacteria strains (consortia) to transform organic phosphate to enhance soil P availability and other macronutrients and/or micronutrients to plants, and thereby enhancing their growth and yield | 06/11/2010 |
| [217]      | A kind of preparation of new biological organic fertilizer fermentation maturity agent | Biological organic fertilizers comprise *Bacillus spp* to improve soil moisture content, and drought-relief and protection of the harvest promote plant growth, and improving the yield and quality | 25/07/2017 |
formulation itself or indirectly to the environment, notably the low adaptive capacity in field. In general, most of knowledge reported in this review shows that bacterial solubilization could be unreproducible due to many experimental constraints, which reduce chances to provide efficacious bacterial formulations even though knowledge in this field has reached significant achievements in applied agricultural microbiology. In addition, recent findings pointed out inconsistencies in using bioformulations as a replacement to nutrient fertilizers such as P fertilizers, rather than exploring opportunities to a joint use of both resources. In this context, we outline three key research priorities for harnessing P bacterial solubilization in sustainable crop production:

- Implement new bacterial culture-dependent screening approaches that simulate both controlled and field conditions, which should offer an accurate evaluation of crop response to PSB in vitro and vivo conditions. This should consider variations, not only at the bacterial species level for P solubilization, but also to overall plant-PSB responses at both temporal and spatial levels likely enabling tight relationships between potentially efficient PSB and the surrounding rhizosphere environment.

- Construct microbial consortia uniting all desired characteristics, but mainly PUE, is an emerging research area that requires more attention on identifying synergistic microbial combinations that enhance both above- and below-ground crop performance. Although a microbial consortium is likely hard to engineer due to a dynamic state of species within the microbial mixture consisting of at least two different microorganisms, identification of the best isolates is inextricably dependent on a consortium-oriented isolation/construction approach enabling the use of compatible microbial strains with different modes of action. This should consider how much diversity is needed while ensuring complementarity in functions to generate major impacts on both P uptake and plant growth performance.

- Adopt multi-disciplinary approaches to design innovative microbial formulations in concert with rationalized use of agrochemicals, including P fertilizers with positive impacts on both crops and environment. This will require understanding the impact of fertilization on soil PSB abundance and function, which also reflects the need for fine-tuning fertilization levels, notably P and N.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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