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Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies

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ABSTRACT

Phosphorus is one of the main macronutrients for plant development. Despite its large deposits in soils, it is scarcely available for plants. Phosphate-solubilizing bacteria, belonging to the group of plant growth-promoting rhizobacteria (PGPR), are capable of mobilizing deposits of insoluble phosphates in the soil. The use of PGPR as inoculants provides an environmentally sustainable approach to increase crop production. The effectiveness of inoculants depends on their proper production, formulation and storage in order to ensure the application of the required number of viable microbial cells. In order to develop inexpensive technology, low-cost compounds for biomass production and protection should be used. After the biomass production process, the product should be formulated in a liquid or a solid form, taking into account required storage time, use of protectors/carriers, storage conditions (temperature, humidity, etc.), ease of application and maintenance of beneficial effects on crops. Careful determination of these optimal conditions would ensure a low-cost efficient inoculant that would promote the growth and yield of various crops.

1. Introduction

Microorganisms that inhabit the rhizospheric microenvironment are able to exert beneficial, neutral, variable, or deleterious effects on plant growth and development (Barea, 2015). Plant growth-promoting rhizobacteria (PGPR) are beneficial microorganisms such as nitrogen-fixing and phosphate-solubilizing bacteria (PSB) (Bharti and Barnawal, 2018). These bacteria present relevant properties involving essential nutrients for plants such as nitrogen (N) and phosphorus (P). Since PGPR are able to increase vegetable nutrient uptake by means of various widely studied mechanisms, they have become really interesting for the agricultural industry. Numerous formulations based on these microorganisms have been developed, with applications for different crops around the world (Saleem and Khan, 2017). However, the inconsistency in the results obtained, dependent on many factors such as climate, autochthonous microbiota, available nutrients and crop characteristics, makes optimization necessary for each particular system (Vassilev et al., 2015). Research including physiological and technological studies should be a priority in order to develop stable, functional and reliable inoculants as tools to support sustainable agriculture (Seema et al., 2018). In this review, interesting properties of PGPR, particularly of PSB, and their liquid and solid formulations are discussed. Moreover, a study of the formulation of a potential inoculant based on *Pseudomonas tolaasii* IEXb, a PSB isolated from the Puna region, Argentina, is also commented.

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; AMS, ammonium mineral salt medium; BNF, biological nitrogen fixation; CFU, colony forming units; IAA, indole acetic acid; NFb, nitrogen-free broth; PGPR, plant growth-promoting rhizobacteria; PSB, phosphate-solubilizing bacteria; SBM, Sporulation *Bacillus* Medium

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2. Phosphorus in the soil and its importance for plants

Phosphorus is one of the inorganic nutrients most required by plants. It is essential for plant growth and development since it is involved in many important functions such as energetic metabolism, structural functions, signal transduction functions and transfer of genetic features through successive generations. Thus, this element is essential for cell division and for the generation of new tissues (Dissanayaka et al., 2018).

Plants absorb P from orthophosphate anions ($H_2PO_4^-$ and HPO_4^{2-}) (Herrera-Estrella and López-Arredondo, 2016). In the soil, P is most often found in apatite, which is a group of phosphate minerals. Apatite wear causes the release of phosphate anions in low amounts (1% of total soil phosphorus) (Barea and Richardson, 2015). Phosphate anions participate in reactions that limit their availability to plants, forming compounds (e.g., several forms of tricalcium phosphate, iron phosphate or aluminum phosphate) in the form of salts in solution, crystalline salts or salts adsorbed by soil colloids. Moreover, phosphate ions can be directly adsorbed by soil colloids or form highly stable complexes with iron, aluminum or manganese hydroxides that are part of these colloids. These inorganic compounds have very low solubility (Liu et al., 2014). On the other hand, P can be found in organic forms including inositol phosphate, phosphomonoesters and phosphotriesters. These compounds can form insoluble complex molecules with some metals present in the soil. Based on the different aspects described, limited P bioavailability in the soil causes limited plant growth.

3. Chemical fertilization

Chemical fertilizers are applied to compensate for P deficiency in commercial crops, improving plant development and increasing yields. Plants are able to use P from soil solution, mainly from their root exudates, which contain organic acids and phosphatases (Novo et al., 2018). When phosphate fertilizers are introduced, several processes in rhizosphere, soil, and plants occur (Shen et al., 2011) (Fig. 1). In chemical fertilizers, P is included mainly as monocalcium phosphate [$Ca(H_2PO_4)_2$] or monopotassium phosphate (KH_2PO_4). A great part of soluble inorganic P applied as a fertilizer (more than 80%) immediately precipitates after its application by the formation of iron and aluminum phosphates in acid soils, monocalcium-bicalcium-tricalcium phosphates in alkaline soils, or adsorption on iron or aluminum oxides or clays. All these compounds are non-bioavailable complexes, whereby phosphorus is wasted (Urrutia et al., 2014; Wang et al., 2018). When phosphate fertilizers are applied in excessive amounts, they cause **P fixation**, characterized by the presence of enormous amounts of P in the form of phosphate minerals. Fixation mechanisms generally cause a slow release of P, generating great challenges to remediate these soils, with highly accumulated P and thus not available for crops (Roy, 2017).

The application of phosphate fertilizers may significantly affect the physicochemical properties of the soil (Li et al., 2017). However, with the necessary previous studies, it is possible to associate different types of fertilizers with soils that have different physicochemical characteristics (Gellings and Parmenter, 2016).

4. Phosphate-solubilizing bacteria

The rhizosphere is a critical zone where microorganisms, soil and plants interact. PGPR constitute a heterogeneous group that can be present in the rhizosphere as soil bacteria at the root surface. They are currently divided into three functional groups: plant growth-promoting bacteria, biocontrol agents, proposed by Bashan and Holguin (1997), and plant stress homeoregulating bacteria, proposed by Cassan et al. (2009). These functional PGPR groups can directly or indirectly facilitate plant growth under biotic or abiotic stress conditions (Singh et al., 2015). Bacteria of various genera such as *Bacillus*, *Pseudomonas, Mycobacterium, Azospirillum, Agrobacterium, Azotobacter, Rhizobium* and

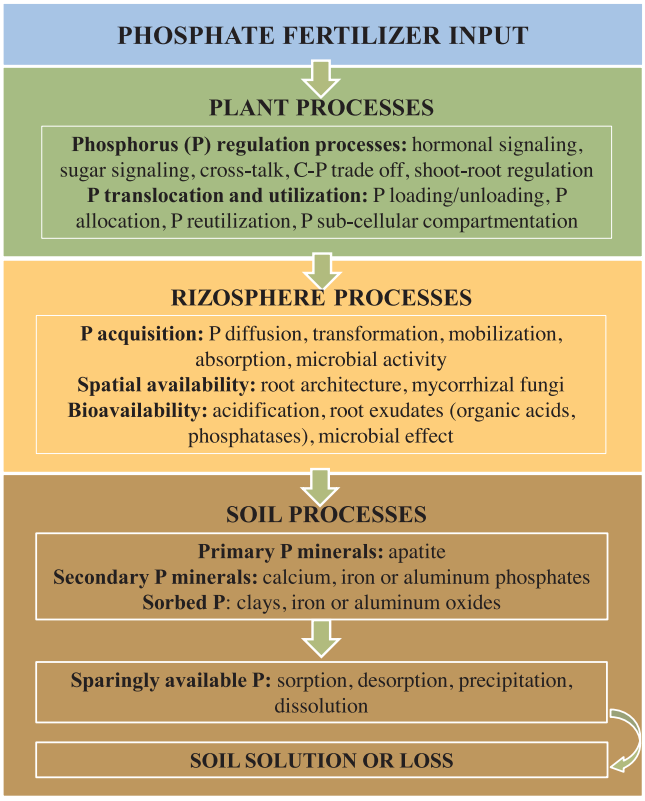

Fig. 1. Phosphorus dynamics in soil, rhizosphere and plants after the addition of chemical fertilizers (adapted from Shen et al., 2011).

Alcaligenes are included in the PGPR group (Pathak et al., 2017; Yadav et al., 2018).

PSB, which belong to the PGPR group, are ubiquitous and have different properties and population levels according to the physicochemical characteristics, organic matter content and P of the soil where they are found. In arid and semiarid zones such as the Puna region (Northwestern Argentina), there is a huge diversity of PGPR that can be biotechnologically exploited because of their high phenotypic plasticity (Viruel et al., 2011; Lamizadeh et al., 2016).

PSB can transform different P insoluble compounds into soluble forms available for plant uptake (Pathak et al., 2017). **Phosphate-solubilizing mechanisms** include solubilization of inorganic phosphates by the action of low molecular weight acids such as gluconic and citric acids, which are synthetized by soil bacteria. On the other hand, organic phosphate mineralization occurs through bacterial synthesis of phosphatases such as phytases and nucleases, which catalyze the hydrolysis of phosphoric esters, releasing the phosphate group (Novo et al., 2018). An important fact is that inorganic P solubilization and organic P mineralization are capabilities that can coexist in a same bacterial strain (Hanif et al., 2015).

5. Other mechanisms associated with plant growth-promoting effects

Phosphate solubilization is not the only mechanism through which PGPR can exert a beneficial effect on plants (Vejan et al., 2016). Other mechanisms include nitrogen fixation, production of siderophores, phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and biological control (Bharti and Barnawal, 2018) (Fig. 2).

5.1. Nitrogen fixation

N is the most important vital nutrient for plant growth and

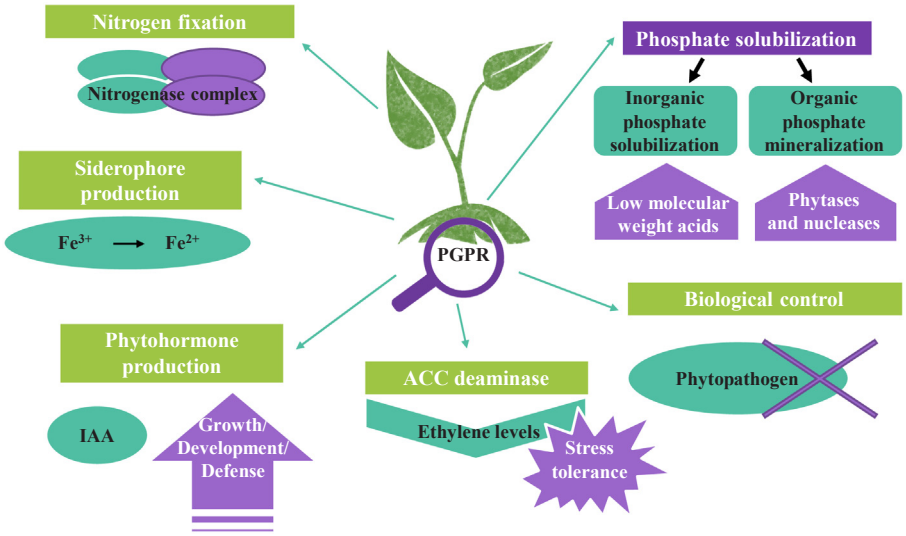

Fig. 2. Some of the most important beneficial effects that plant growth-promoting rhizobacteria (PGPR) exert on plants. IAA: indole acetic acid; ACC deaminase: 1-aminocyclopropane-1-carboxylate deaminase.

productivity. Although there is almost 78% of N_2 in the atmosphere, it is unavailable to plants. Atmospheric N_2 is transformed into forms available for plants by the biological nitrogen fixation (BNF) process, by which N_2 is transformed into NH_3 by N_2-fixing microorganisms (Singh et al., 2015). The N_2-fixation process is catalyzed by a complex enzyme known as nitrogenase complex (Choudhary and Varma, 2017). Dinitrogenase reductase provides electrons with high reducing power, while dinitrogenase uses these electrons to reduce N_2 to NH_3. Structurally, the N_2-fixing system has variations among different bacterial genera. Most BNF is catalyzed by the activity of molybdenum nitrogenase, which is found in all diazotrophs (Mus et al., 2018). The genes responsible for nitrogen fixation, called *nif* genes, are found in symbiotic and free living systems. The symbiotic activation of *nif*-genes in *Rhizobium* is dependent on low oxygen concentration, which is regulated by another set of genes called *fix*-genes, also commonly found in both symbiotic and free living nitrogen fixation systems (Wongdee et al., 2018). Several PGPR are able to colonize plant internal tissues and thus enhance their growth-promoting effect by providing a limiting oxygen environment required for activation of N_2-fixation and more efficient transfer of the fixed nitrogen to the host plants (Nyoki and Ndakidemi, 2018). BNF represents an alternative to chemical fertilizers due to its economic and environmental advantages.

5.2. Siderophore production

Iron (Fe) is a vital nutrient for almost all forms of life. In an aerobic atmosphere, where it is found mainly as Fe^{3+} ion, it forms hydroxides and oxyhydroxides that make it unavailable for microorganisms and plants, which require it in the Fe^{2+} form (Neilands, 2014; Pahari and Mishra, 2017).

Bacteria usually acquire Fe^{2+} by secretion of low molecular weight chelators known as **siderophores**. Fe^{3+} ion and a siderophore form a complex in the membrane in which Fe^{3+} is reduced to Fe^{2+}, which is released into the cell by the siderophore through an input mechanism that links the outer and inner membranes. During this reduction process, the siderophore can be destroyed or recycled (Kashyap et al., 2017).

Plants can assimilate Fe^{2+} from bacterial siderophores through different mechanisms such as directly uptaking Fe-siderophore complexes or by means of an exchange reaction using an appropriate ligand (Rasouli-Sadaghiani et al., 2014; Novo et al., 2018).

5.3. Phytohormone production

The auxin phytohormone (indole acetic acid, IAA), produced by rhizobacteria, is an effector molecule in plant-microorganism interactions as well as in pathogenesis and phytostimulation processes (Venturi and Keel, 2016).

IAA internal concentration in plants can be altered by the acquisition of IAA secreted by soil bacteria (Manasa et al., 2017). IAA is involved in growth and development aspects of plants as well as in defense mechanisms. IAA increases the root surface and length and provides greater access to soil nutrients by plants (Gowtham et al., 2017). IAA also weakens plant cell walls, resulting in an increase in the amount of radicular exudate, which in turn provides additional nutrients for rhizobacterial growth (Etesami et al., 2015).

5.4. ACC deaminase

Ethylene is an essential metabolite for plant normal growth and development (Van de Poel et al., 2015). Besides being a growth regulator, it is a hormone generated in stress situations resulting from salinity, drought or pathogenicity (Müller and Munné-Bosch, 2015). In these conditions, endogenous ethylene level increases significantly, with negative effects since it can act as a negative plant growth regulator, leading to shorter roots (epinasty), and premature senescence (Bharti and Barnawal, 2018). Thus, high ethylene concentrations can reduce crop yields. PGPR that have ACC deaminase are able to regulate ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) in α-ketobutyrate and NH_3 (Dar et al., 2018). In this way, PGPR facilitate plant growth and development since they are able to decrease ethylene levels. Thus, plant resistance to various stresses (e.g., presence of phytopathogenic bacteria, polyaromatic hydrocarbons, heavy metals, salinity and drought) is increased (Singh and Jha, 2016; Bharti and Barnawal, 2018).

5.5. Biological control

In general, competition for nutrients, exclusion of niches, systemic resistance induction and antagonistic metabolite production are the main modes of action through which PGPR exert biocontrol (Fukami et al., 2018). Through these mechanisms, beneficial bacteria can prevent the deleterious effect of phytopathogens on plant growth and/or development.

Antagonistic secondary metabolites produced by beneficial

microorganisms against phytopathogens are biodegradable molecules, in contrast with many agrochemicals. Biocontrol is used to control infectious diseases in both living plants and fruits during storage (Liu et al., 2018). It should be noted that some PGPR are also active against weeds and insects (Adnan et al., 2016).

Biological control is as complex process involving not only the biocontrol agent, the pathogen and the plant. It also includes the indigenous microbiota and macrobiota such as nematodes and protozoa, and the plant growth substrate such as soil, stonewool or vermiculite (Mahajan and Shirkot, 2014). The biological control agent should remain active against multiple plant diseases that often affect individual crops (Liu et al., 2017). However, broad-spectrum biocontrol activity is not easy to achieve, and more efficient and persistent biocontrol systems should be developed.

6. Phosphate-solubilizing bacteria as inoculants

Some PSB have been isolated and multiplied, allowing the development of various inoculants (Bashan et al., 2014). Inoculation with PSB belonging to genera such as *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, *Erwinia* and *Agrobacterium* has been associated with an increase in phosphate solubilization and crop yields (Rodríguez and Fraga, 1999; Ruzzi and Aroca, 2015).

The use of the inoculant either by itself or in combination with other products currently available on the market is a smart alternative, since it would lead to a reduction in the amount of chemical fertilizer required and to the use of the phosphate already present in the soil (Verma et al., 2015). Seed inoculation with PSB is an effective technique that can mitigate phosphorus deficiency (Qureshi et al., 2012). On the other hand, the positive effects of PSB can be more significant when co-inoculating PSB with bacteria having other physiological capabilities (e.g., N_2-fixation) or with mycorrhizal and non-mycorrhizal fungi (Muthukumar and Udaiyan, 2018).

Definitely, commercial crop inoculation with PSB would allow increasing yields, besides generating a more sustainable and eco-friendly agricultural practice because of the lower requirement for chemical fertilizers.

7. Inoculants: PGPR biomass production

Biomass production, formulation and shelf life determination are crucial steps during the development of bacterial inoculants, which should ensure the application of the required number of viable and active microbial cells (Bashan et al., 2014). The cost of biological fertilizers, which comprises mainly raw material costs, equipment, and staff, must be competitive in relation to the production cost of chemical fertilizers. In a general cost analysis, the choice of an appropriate culture medium for the development of high amounts of microbial biomass is an important issue (Xu et al., 2014; Liu et al., 2016).

In several studies, different culture media have been assayed or optimized for the growth of PGPR in submerged and solid-state fermentation processes (Table 1). Standard culture media such as ammonium mineral salt (AMS) broth, nitrogen-free broth (NFb) and nutrient broth are suitable for laboratory assays (Chanratana et al., 2017; Yaghoubi Khanghahi et al., 2018), but they are expensive for PGPR biomass production at larger scales (Trujillo-Roldán et al., 2013; Carrasco-Espinosa et al., 2015). Several new culture media were optimized based only on conventional ingredients (Vyas et al., 2014; Posada-Uribe et al., 2015; Camelo-Rusinque et al., 2017). However, the inclusion of some components such as peptones, yeast extract and tryptone continues to generate high inoculant production prices. In this sense, Vyas et al. (2014) and Posada-Uribe et al. (2015) argued that the decrease in nutrient concentrations or incubation time needed to reach maximum PGPR biomass can represent a reduction in production costs.

On the other hand, traditional culture medium components have been successfully combined with low-cost substrates for biomass production during inoculant development (Bashan et al., 2011; Singh et al., 2013; Peng et al., 2014; Zhang et al., 2018). In those studies, industrial wastes or by-products such as crude glycerol, corn flour, soybean meal, dairy sludge and maize bran residue were used as low-cost carbon or nitrogen sources for microbial growth (Table 1). Interestingly, glycerol and maize bran residue can be employed in both biomass production and formulation processes (glycerol as a protective agent of microbial cells and maize bran residue as a carrier) (Vassilev et al., 2017; Zhang et al., 2018). The use of culture media formulated with industrial wastes or by-products to produce bacteria of agronomic interest is a viable way to contribute to a sustainable agroindustry, handling low-cost substrates of high nutritional value.

As shown in Table 1, the most economical culture media recently proposed to produce inoculants were formulated only with waste or by-products (Xu et al., 2014; Zhang et al., 2014; Huang et al., 2015; Liu et al., 2016; Pastor-Bueis et al., 2017). For instance, different composts supplemented with novel additive nutrients were used in the solid-state fermentations of *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa* strains (Zhang et al., 2014; Huang et al., 2015; Liu et al., 2016).

Among the works reviewed, none has addressed the use of whey for PGPR growth. In a recent study carried out by our group, a culture medium composed of whey (1% w/v) and soybean meal (1% w/v) as low-cost substrates for *Pseudomonas tolaasii* IEXb biomass production was formulated (Table 1; unpublished results). *P. tolaasii* IEXb was previously isolated from Puna grass rhizosphere from Northwestern Argentina and selected for its beneficial characteristics as a bacterial candidate for inclusion in an inoculant (Viruel et al., 2011, 2014). This microorganism solubilizes tricalcium phosphate and hydroxyapatite, produces alkaline phosphatase, indole acetic acid and siderophore, and can increase maize growth and yield under both culture chamber and field conditions (Viruel et al., 2011, 2014). In plant trials, maize seeds were soaked in fresh *P. tolaasii* IEXb cultures conducted in a synthetic, liquid growth medium (Viruel et al., 2011, 2014). However, this procedure is not feasible under commercial conditions. Therefore, our subsequent studies were aimed at producing *P. tolaasii* IEXb biomass using low-cost culture media and at including it in suitable formulations, enabling its storage, transport and application with no drastic losses in viability or microbial contamination.

When scaling *P. tolaasii* IEXb biomass production in a 10 L fermenter, a satisfactory bacterial growth (around 10^9 CFU/mL at 8 h of culture) was obtained (Table 1; unpublished results). Therefore, alternative substrates used for *P. tolaasii* IEXb biomass production provided C, N and other nutrients necessary for bacterial growth. Whey and soybean meal are industrial by-products that can be used in both human food and animal feed; however, they must often be discarded, causing serious environmental pollution. Therefore, the use of these highly polluting by-products as low-cost culture media components for microbial biomass production could be an ecological solution for waste management. Soy flour is obtained from whole milled soy beans. It contains high protein and carbohydrate concentrations (35% and 34% w/w respectively), fat (19% w/w), minerals and a wide range of vitamins (Kang et al., 2017).

Whey is the main by-product of the cheese manufacturing process. About 9 liters of whey are produced per kilogram of cheese, its chemical composition depending on milk treatment (coagulation process, heating, centrifugation, homogenization, pasteurization, concentration, etc.), among other factors. Several main derived compounds are currently obtained from surplus whey such as condensed whey powders, whey protein concentrates/isolates/hydrolysates, whey permeate, individual whey proteins and lactose (Ryan and Walsh, 2016). Whey and some whey-derived compounds can be used as raw materials for microbial growth and biotechnological product formation (e.g., bioethanol, single-cell protein, bioplastics, antimicrobial peptides, enzymes, biogas and other organic compounds). Additional interesting information on the subject can be found in Cortés-Sánchez et al. (2015) and Ryan and Walsh (2016). In the case of whey powder, it is produced by

Table 1
Summary of studies on biomass production of plant growth-promoting bacteria (PGPR) for inoculant development.

Fermentation process	Microorganism	Assayed or optimized culture medium	Low-cost culture medium component	Other culture conditions assayed	Reference
Liquid fermentation	*Agrobacterium tumefaciens* and *Rhizobium* sp.	Nutrient broth at different concentrations of NaCl		Water content (at laboratory scale)	Yaghoubi Khanghahi et al., 2018
Liquid fermentation	*Azospirillum brasilense* Cd and Sp6, and *Azospirillum lipoferum* JA4,	Bashan-Trejo-Bashan (BTB)-1 and BTB-2 media form modified tryptone-yeast extract-glucose (TYG) medium (Bashan et al., 2002), in which glucose was substituted by Na-gluconate or glycerol	Industrial grade glycerol		Bashan et al., 2011
Liquid fermentation	*Azospirillum brasilense* strains (*Start* and *Calf*)	Modified enriched non-defined culture medium, based on the nitrogen-free broth (NFb), with a different C/N ratio (unspecified composition)		Volumetric mass transfer coefficient (K_La) during scaling-up in 10 and 1000 L bioreactors	Trujillo-Roldán et al., 2013
Liquid fermentation	*Azospirillum brasilense* strains (*Start* and *Calf*)	Modified NFb (DL-malic acid, K_2HPO_4, $MgSO_4$, NaCl, $CaCl_2$, $FeSO_4$, Na_2MoO_4, $MnSO_4$, KOH, NH_4Cl, H_3BO_4 and yeast extract)	DL-malic acid mixture (instead of L-malic acid stereoisomer)	Aeration rate in a 5 L pneumatic bioreactor	Carrasco-Espinosa et al., 2015
Liquid fermentation	*Azotobacter chroococcum* AC1	Optimized medium (yeast extract, sodium glutamate, glucose, K_2HPO_4, $MgSO_4$ and microelement solution)		pH, aeration rate, agitation in a bioreactor (working volume of 3.5 L)	Camelo-Rusinque et al., 2017
Liquid fermentation	*Bacillus siamensis*	Anaerobic digestate (from fruit and vegetable wastes) supplemented with sugar beet molasses	All culture medium components	Scaling-up in a 5 L pilot fermenter	Pastor-Bueis et al., 2017
Liquid fermentation	*Bacillus subtilis* EA-CB0575	Optimized Sporulation *Bacillus* Medium (SBM; glucose, yeast extract, special peptone, $MgSO_4$, KH_2PO_4, NaCl and stock salt solution)		pH, aeration rate, agitation during scaling-up in a 14 L bioreactor	Posada-Uribe et al., 2015
Liquid fermentation	*Methylobacterium oryzae* CBMB20	Ammonium mineral salt broth (AMS; Whittenbury et al., 1970) with sodium succinate as carbon source			Chanratana et al., 2017
Liquid fermentation	*Paenibacillus polymyxa*	Sweet potato starch wastewater	Whole culture medium	pH, temperature and agitation (at laboratory scale)	Xu et al., 2014
Liquid fermentation	*Pseudomonas putida* Rs-198	Corn flour, soybean meal, K_2HPO_4, $MnSO_4$ and NaCl	Corn flour and soybean meal		Peng et al., 2014
Liquid fermentation	*Pseudomonas tolaasii* IEXb	Whey and soybean meal	All culture medium components	Scaling-up in a 10 L pilot fermenter	Unpublished results
Liquid fermentation	*Pseudomonas trivialis* BIHB 745	Optimized trypticase soya broth (dextrose, NaCl, casein enzyme hydrolysate, soya peptone and KH_2PO_4)		Agitation during scaling-up in a 20 L bioreactor	Vyas et al., 2014
Liquid fermentation	*Rhizobium trifolii* (MTCC905 and MTCC906), and *Rhizobium meliloti* (MTCC100)	Dairy sludge supplemented with yeast extract and mannitol	Dairy sludge	pH (at laboratory scale)	Singh et al., 2013
Semi-solid fermentation	*Bacillus subtilis* Z1 and Z2	Maize bran residue (nutrient and carrier), peptone, NaCl and KH_2PO_4	Maize bran residue	pH, temperature, water content and flask filling capacity (at laboratory scale)	Zhang et al., 2018
Solid-state fermentation	*Bacillus amyloliquefaciens* SQR9	Chicken manure compost mixed with rapeseed meal, expanded feather meal, and dewatered blue algal sludge	All culture medium components		Huang et al., 2015
Solid-state fermentation	*Bacillus amyloliquefaciens* SQR9 and NJN-6, and *P. polymyxa* SQR21-*gfp*	Cattle manure compost containing algal sludge	All culture medium components		Zhang et al., 2014
Solid-state fermentation	*Bacillus amyloliquefaciens* SQR9 and NJN-6, and *Paenibacillus polymyxa* SQR21-*gfp*	Matured chicken or pig manure compost containing liquid amino acids from animal carcasses	All culture medium components		Liu et al., 2016

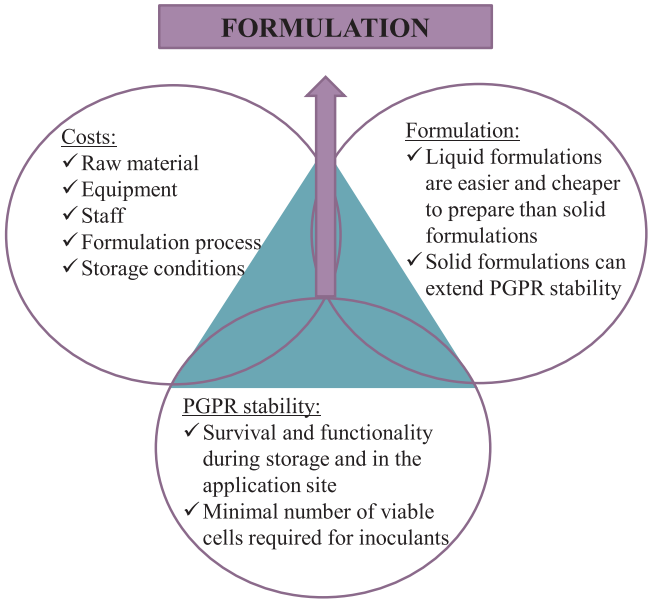

Fig. 3. Parameters to consider in the development of an inoculant formulation.

drying whey, which facilitates its handling, storage and transport. Concentrated whey powder contains (in % w/w): lactose, 50, proteins, 10, fat, 2, minerals, 5, and a broad range of vitamins, cofactors and mineral salts (Haug et al., 2007; Tunick, 2008). For biomass production, the use of whey powder instead of whey has relevant advantages such as high concentrations of lactose and other nutrients resulting in higher microbial growth levels (Lavari et al., 2014).

In addition to the selection of a suitable low-cost culture medium for optimal biomass production, scaling-up is a relevant issue in inoculant technology. Scaling-up is a process carried out in order to prevent environmental effects on microbial growth, and to introduce the required variations in equipment design when the bioreactor size changes (Trujillo-Roldán et al., 2013). As summarized in Table 1, as a first step, different culture conditions were assayed at laboratory scale in order to optimize PGPR growth parameters (Singh et al., 2013; Xu et al., 2014; Zhang et al., 2018). At larger scales, the most important factors in the culture such as incubation temperature and culture medium must remain constant (Garcia-Ochoa and Gomez, 2009; Ali et al., 2018). Other physicochemical factors such as agitation, oxygen availability and pH must be optimized during scaling-up to large bioreactors, as evidenced in several studies included in Table 1 (Trujillo-Roldán et al., 2013; Vyas et al., 2014; Carrasco-Espinosa et al., 2015; Posada-Uribe et al., 2015; Camelo-Rusinque et al., 2017). Scaling-up a biotechnological process to pilot scale enables the evaluation of the operational condition changes associated with industrial processes as well as the production of enough amounts of the product for market prospection or product registration (Schmidt, 2005; Trujillo-Roldán et al., 2013).

8. PGPR formulation development

An inoculant properly produced, formulated and applied can guarantee that the product will provide all the benefits that it is supposed to afford. Generally, many private companies offer commercial inoculants on the international market, where there is an increasing demand for a highly productive and effective product for a wide range of soils. However, the inoculants offered are often low quality ones. In developing and developed countries, some of the products used did not contain rhizospheric microorganisms or, if they did, these microorganisms showed contamination with other strains (Herrmann and Lesueur, 2013; Yadav and Chandra, 2014). This situation causes an inconsistency in the beneficial effect of inoculants in the field, thus affecting the market. Some inoculants fail to demonstrate their specific functions when their application is made, an outcome resulting from problems associated with their production and formulation (Vassilev et al., 2015; Biradar and Santhosh, 2018).

Inoculants should be developed as products with long-storage stability. The requirements with respect to their shelf life vary from 2 to 3 months at room temperature to1-2 years. Maximizing the initial amount of viable cells in inoculants is a strategy to make up for the fast deterioration rate (He et al., 2015; Martínez-Álvarez et al., 2016; Oliveira et al., 2017). Anyway, storage conditions should be optimized to support long-term cell survival (Joe et al., 2014; Berger et al., 2018; Berninger et al., 2018). There are studies that clearly show the relationship between number of bacteria applied on plants and crop yields (Bernabeu et al., 2018). Inoculant quality standards, which vary slightly between countries, establish that the amount of microorganisms must range from 10^7 to 10^9 colony forming units per gram or per milliliter of formulation (CFU/g or CFU/mL) (Malusá and Vassilev, 2014). Another approach to inoculum standards considers the number of viable cells per seed after the application as recommended by the producer. The minimum amounts of bacteria per seed are in the order of 10^3 for small-sized seeds, 10^4 for medium-sized seeds, and 10^5 for large-sized seeds (Bashan et al., 2014; Bharti et al., 2017).

The heterogeneity of soils is a huge obstacle for inoculants, because the bacteria introduced are unable to find an empty niche in them. The inoculated bacteria must compete against a better adapted indigenous microbiota and survive predation by soil microfauna, especially when they are inoculated in an unprotected form. Thus, inoculants should provide a more suitable microenvironment combined with physicochemical protection over a long period of time, which would prevent the fast decrease of the bacteria introduced (Berninger et al., 2016; Shahzad et al., 2017; Liffourrena and Lucchesi, 2018). Therefore, the purpose of inoculant formulations is to allow higher survival of PGPR both during storage and in the application site, in both suitable and available forms. Inoculants can be formulated as liquid or solid-based products, the latter being dry or wet formulations (Oliveira et al., 2017; Berninger et al., 2017; Berger et al., 2018). Fig. 3 summarizes some aspects to consider in order to develop appropriate liquid and solid formulations of PGPR.

8.1. Liquid formulations

Liquid inoculants are whole cultures or microbial suspensions combined with different compounds such as water, oil, or polymeric substances, which can increase adhesion, stability, and surfactant and dispersion capacity (Lee et al., 2016). The main advantages of liquid inoculants are their easier processing and lower costs compared to solid-based formulations (Kumaresan and Reetha, 2011). This is why liquid formulations constitute a significant percentage of the inoculant market (Lee et al., 2016).

Table 2 shows some recent studies on liquid formulations for potential use as inoculants for different crops. The presence in the culture medium of protective agents -or their addition after bacterial growth- can prolong cell survival during storage (Lee et al., 2016; Anith et al., 2016; Valetti et al., 2016; Pastor-Bueis et al., 2017; Bernabeu et al., 2018). However, although liquid inoculants can be packaged and stored for long periods of time, microorganisms are subject to abiotic stress, which can be caused by nutrient depletion, thermal shock or hypoxia, among other causes, resulting in a drastic decrease in viable and/or active cells (He et al., 2015; Berger et al., 2018; Bernabeu et al., 2018). The main challenge in this respect is to improve formulations to maintain the high quality of liquid inoculants (Lee et al., 2016).

Among protective substances, natural polymers (e.g., carrageenan, arabic gum, xanthan gum, gelatin, alginate, etc.), synthetic polymers (e.g., polyvinyl alcohol and polyvinylpyrrolidone), horticultural oil, glycerol, and mono- and disaccharides (e.g., glucose and lactose) are suitable for liquid formulation development (Table 2). In several liquid

Table 2
Plant growth-promoting rhizobacteria (PGPR): liquid formulations.

Assayed or selected protector, additive or carrier	Microorganism	Initial number of viable cells	Shelf life (viable cells)	Assayed crop	Reference
Arabic gum	*Bradyrhizobium* sp. J-81	10^{10} CFU/mL	6 months at room temperature (10^{8} CFU/mL)	Peanut	Valetti et al., 2016
Carrageenan	*Bacillus siamensis* SCFB3-1	10^{9} CFU/mL	N/A	Sweet pepper	Pastor-Bueis et al., 2017
Coconut water and polyvinylpyrrolidone or glycerol	*Pseudomonas fluorescens* AMB-8	10^{9} CFU/mL	6 months at room temperature (10^{7} CFU/mL)	Chilli and tomato	Anith et al., 2016
Corn flour, humic acid, urea, bentonite, alginate, K_2SO_4, KCl, $(NH_4)_2HPO_4$	*Pseudomonas putida* RS-198	10^{13} CFU/mL	6 months at room temperature (10^{8} CFU/mL)	Cotton	He et al., 2015
Glycerol	*Pseudomonas fluorescens* Pf1	10^{8} CFU/mL	N/A	Banana	Selvaraj et al., 2014
Glycerol or lactose	*Pseudomonas tolaasii* IEXb	10^{9} CFU/mL	6 months at 4 °C (10^{7} CFU/mL)	N/A	Unpublished results
Horticultural oil	*Rhodopseudomonas palustris* PS3	10^{9} CFU/mL	1 month at 4 °C (10^{8} CFU/mL)	Chinese cabbage	Lee et al., 2016
Isotonic phosphate buffer (in the presence or absence of KNO_3), or nutrient broth containing glycerol	*Pseudomonas putida* P13 and *Pantoea agglomerans* P5	10^{8} CFU/mL of *P. agglomerans* P5; 10^{7} CFU/mL of *P. putida* P13	3 months at room temperature (10^{6} CFU/mL)	N/A	Goljanian-Tabrizi et al., 2016
N/A (AviVital product carried out by ABiTEP GmbH, Berlin, Germany)	*Kosakonia radicincitans* DSM16656^{T}	Around 10^{11} CFU/g	6 months (around 10^{11} CFU/g at -20 °C; around 10^{8} CFU/g at 4 °C)	Maize	Berger et al., 2018
None	*Mesorhizobium ciceri* A13 and CR24 (on *Trichoderma viride* as a fungal matrix of biofilms)	10^{8} CFU/mL of *M. ciceri* A13 or CR24; 10^{7} CFU/mL *T. viride*	N/A	Chickpea	Das et al., 2017
Pero-dexin	Biofertile: *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus polymyxa*, *Enterobacter agglomerans* and *Pseudomonas putida*. Biocontrol: Three *B. polymyxa* strains, two *Bacillus macerans* strains, *B. circulans* and *E. agglomerans*.	10^{8} CFU/mL	N/A	Potato	Abbas et al., 2014
Polyvinyl alcohol, xanthan gum or gelatin	*Paraburkholderia tropica* MTo-293	10^{10} CFU/mL	12 months, unspecified storage temperature (10^{6} CFU/mL)	Wheat	Bernabeu et al., 2018
Stress-resistance biopolymers (exopolysaccharides and polyhydroxybutirate) produced by *Azospirillum brasilense* Ab-V5	*Azospirillum brasilense* Ab-V5	10^{9} CFU/mL	1 month at 20 °C (unspecified number of viable cells)	Maize	Oliveira et al., 2017

N/A: Not available.

Table 3
Plant growth-promoting rhizobacteria (PGPR): solid wet formulations.

Assayed or selected protector, additive or carrier	Microorganism/Immobilization process	Initial number of viable cells	Shelf life (viable cells)	Assayed crop	Reference
Alginate	*Methylobacterium oryzae* CBMB20 and *Methylobacterium suomiense* CBMB120 co-aggregated with *Azospirillum brasilense* CW903/Entrapment in alginate beads	10^9 CFU/g of *M. oryzae* CBMB20 or *M. suomiense* CBMB120	12 months at room temperature (around 10^8 CFU/g of *M. oryzae* CBMB20; 10^6 CFU/g of *M. suomiense* CBMB120)	Tomato	Joe et al., 2014
Alginate	*Pseudomonas plecoglossicida* R-67094 and *Rhizophagus irregularis* MUCL 41833 (a fungus)/Entrapment in alginate beads	Around 10^6 CFU/mL of *P. plecoglossicida* R-67094 and 5×10^2 spores of *R. irregularis* MUCL 41833	N/A	Tomato	Loján et al., 2017
Alginate, perlite, paraffin	*Pseudomonas putida* A (ATCC 12633)/Entrapment in alginate beads	10^8 CFU/g	5 months at 4 °C or room temperature (10^8 CFU/g)	Arabidopsis	Liffourrena and Lucchesi, 2018
Ammonium molybdate and peat	*Rhizobia meliloti*	10^9 CFU/g	N/A	Alfalfa	Zhou et al., 2017
Biochar	*Bacillus* sp. A30 and *Burkholderia* sp. L2	10^{10} CFU/mL (before desiccation)	8 months at 27 °C (10^7 CFU/g)	Tomato	Tripti et al., 2017
Biodegradable low-cost foam formed by cassava starch, sugarcane bagasse, glycerol, rock phosphate, crystal sugar, powdered skim milk, yeast extract, and phosphate buffered saline	*Azospirillum brasilense* Ab-V5/Biofilm formation inside the pores of the support	10^6 CFU/g	4 months at room temperature (around 10^6 CFU/g)	Maize	Marcelino et al., 2016
Biogas sludge and enriched soil	*Bacillus endophyticus*, *Bacillus sphaericus*, *Enterobacter aerogenes*, *Bacillus safensis*, *Bacillus megaterium* and *Virgibacillus* sp.	10^7 CFU/mL	N/A	Wheat	Mukhtar et al., 2017
Clay	*Azospirillum brasilense* and *Pantoea dispersa*/Attachment on clay pellets	10^9 CFU/g	N/A	Grey-leaved cistus	Schoebitz et al., 2014
Polyvinyl alcohol and glycerol	*Pantoea agglomerans* ISIB55 and *Burkholderia caribensis* ISIB40/Nanoimmobilization by electrospinning	Around 10^9 CFU/g	N/A	Soybean	De Gregorio et al., 2017
Stress-resistance biopolymers (exopolysaccharides and polyhydroxybutirate) produced by *Azospirillum brasilense* Ab-V5; peat	*Azospirillum brasilense* Ab-V5	10^9 CFU/mL	1 month at 20 °C (unspecified number of viable cells)	Maize	Oliveira et al., 2017

N/A: Not available.

Table 4
Plant growth-promoting rhizobacteria (PGPR): dry formulations.

Drying method	Assayed or selected protector, additive or carrier	Microorganism/Immobilization process	Initial number of viable cells	Shelf life (viable cells)	Assayed crop	Reference
Air drying	Peat	*S. mexicanum* ITTG R7^T, *R. calliandrae* LBP2-1^T and *R. etli* CFN42^T	10^9 CFU/mL	8 months at room temperature (10^9 CFU/mL)	Common bean	Ruíz-Valdiviezo et al., 2015
Air drying	Skimmed milk, zeolite and gelatin	*Paraburkholderia phytofirmans* PsJN/Entrapment in zeolite matrix	10^{6-7} CFU/mL	1 month at room temperature (10^{5-6} CFU/mL)	N/A	Berninger et al., 2017
Air drying	Talc, carboxymethyl cellulose and calcium carbonate	*Azospirillum brasilense* TNAU and *P. fluorescens* PF1	10^{10-11} CFU/mL (before desiccation)	N/A	Groundnut	Prasad and Babu, 2017
Desiccation	Sucrose, yeast extract, K_2HPO_4 and KH_2PO_4	*Azospirillum brasilense* Sp7, *Acinetobacter* sp. EMM02, *Sphingomonas* sp. OF178 and *P. putida* KT2440	10^9 CFU/mL (before desiccation)	N/A	Maize	Molina-Romero et al., 2017
Freeze-drying	Alginate, gum arabic and talc	*Bacillus amyloliquefaciens* FZB42/Entrapment in alginate beads	10^7 CFU/g	N/A	Maize	Berninger et al., 2016
Freeze-drying	Lactose	*Pseudomonas fluorescens* EPS62e	10^{11} CFU/g	12 months at 4 °C (around 10^{11} CFU/g)	Pear	Cabrefiga et al., 2014
Freeze-drying	Talc	*Streptomyces corchorusii* UCR3-16	Around 10^5 CFU/g	6 months at 4 °C (10^4 CFU/mL)	Rice	Tamreihao et al., 2016
Freeze-drying	Whey and sodium glutamate	*Pseudomonas tolaasii* IEXb	10^9 CFU/mL	6 months at 4 °C (around 10^9 CFU/mL), or room temperature (10^6 CFU/mL)	N/A	Unpublished results
Hot air drying	Talc, carboxymethyl cellulose, calcium carbonate and glucose	*Bacillus cereus* B25	10^9 CFU/g	12 months at room temperature (around 10^8 CFU/g)	Maize	Martínez-Álvarez et al., 2016
Shade drying	Talc, carboxymethyl cellulose and calcium carbonate	*Bacillus* sp. CaB5	10^7 CFU/g	45 days, unspecified storage temperature (10^7 CFU/g)	Cowpea and lady's finger	Basheer et al., 2018
Spray drying	Sodium alginate and maltodextrin	*Enterobacter* sp. 14/Microencapsulation by spray drying	N/A	N/A	N/A	Campos et al., 2014
Unspecified drying method	N/A (AviVital product carried out by ABiTEP GmbH, Berlin, Germany)	*Kosakonia radicincitans* DSM16656^T	Around 10^{11} CFU/g	6 months at -20 °C and 4 °C (around 10^{11} CFU/g)	Maize	Berger et al., 2018

N/A: Not available.

formulations, PGPR maintained at least the minimal number of viable cells required for several inoculants (around 10^7 CFU/mL or CFU/g) for six months of storage at room or refrigeration temperature (He et al., 2015; Anith et al., 2016; Valetti et al., 2016). Moreover, the beneficial effects of PGPR on different crops were evidenced after different storage times.

The various physiological mechanisms of action of protective agents include mainly nutrient provision, improvement of physical characteristics and osmoprotection of bacterial cells (Lee et al., 2016; Berninger et al., 2017). For instance, natural and synthetic polymers provide a protective microenvironment for bacterial cells, are able to limit heat transfer and possess high water activity (aw), all of which promote bacterial survival under different storage conditions (Mugnier and Jung, 1985). Horticultural oil is a safe and low-cost additive that can be catabolized by some bacteria such as *Rhodopseudomonas palustris* PS3 (Lee et al., 2016). Thus, it can act as an additional nutrient source to support bacterial growth during storage (Table 2). Other low-cost additives (pero-dexin, a by-product of the starch industry, and coconut water, an industrial waste) can also act as bacterial nutrients (Abbas et al., 2014; Anith et al., 2016). Moreover, their inclusion in PGPR formulations contributes to a significant decrease in inoculant production costs.

Glycerol is an economical polyol, well-known as a protective cell agent for its ability to protect bacterial cells from abiotic stress and participate in osmotic pressure balance and regulation of transmembrane traffic (Li et al., 2009). Moreover, glycerol is a carbon source for bacteria, has a high water-binding capacity, and can protect cells from the effect of desiccation by decreasing the drying rate. Its fluidity also promotes fast seed covering (Singleton et al., 2002). On the other hand, lactose is a disaccharide that acts as an effective protector because of its water-binding capacity, which decreases ice crystal formation when storing liquid formulations at low temperatures. Lactose is known to stabilize cell membranes and preserve the structure and function of proteins (Leslie et al., 1995). Moreover, the presence of eight hydroxyl groups in lactose molecules protects bacterial cells against free radicals produced during storage (Zárate et al., 2005).

Glycerol and lactose were used as protective agents in liquid formulations of *P. tolaasii* IEXb developed by our research group (Table 2; unpublished results). The procedure was as follows: cell suspensions of *P. tolaasii* IEXb, produced in a low-cost culture medium (Table 1), were combined with 20% glycerol or 10% lactose. In order to determine the shelf life of the formulated *P. tolaasii* IEXb cells, the different formulations prepared were stored at refrigeration temperature (4 °C) and at room temperature for six months. Liquid formulations of *P. tolaasii* IEXb were able to retain the minimal concentration of viable cells accepted for bioproducts containing PGPR (around 10^7 CFU/mL) but only during storage at refrigeration temperature. At this temperature, several processes are either prevented or delayed (e.g. microbial cell division and metabolic rate, nutrient depletion, toxic metabolite accumulation and moisture loss), favoring long-term storage of microorganisms (Mejri et al., 2013).

An interesting approach for the development of an improved inoculant technology is the use of biofilm-based biofertilizers (Das et al., 2017). Recently, a liquid formulation was developed including PGPR immobilized by biofilm formation on a fungal matrix (Table 2). Microbial immobilization in biofilms presents several advantages since it is a strategy based on a spontaneous microbial process that can be carried out through easy and inexpensive methods, and microbial cells in biofilms show increased survival with respect to planktonic (free) cells (Rabin et al., 2015). Moreover, bacterial cells in biofilms often exhibit higher plant growth promoting activity than their planktonic counterparts (Das et al., 2017).

8.2. Solid formulations

Solid formulations can be based on organic or inorganic carriers and prepared as granules or powders. They are classified on the basis of their particle size or their applications (Lee et al., 2016). In both granular and powder products, the organic or inorganic carrier is one of the most important component, and thus constitutes a crucial parameter in formulation processes. Tables 3 and 4 show several solid wet and dry formulations carried out with PGPR and different bacterial cell protectors, additives and carriers. Some studies evidenced that certain solid wet and dry formulations of PGPR showed appropriate stability during elaboration and storage of inoculants. However, in most of the works included in Tables 3 and 4, shelf life data of formulations were not available.

In solid wet formulations, no drying method was applied during formulation development; therefore, bacterial cells were exposed to a high water content during storage and/or application. As shown in Table 3, solid wet formulations of PGPR were based on alginate (Joe et al., 2014; Loján et al., 2017; Liffourrena and Lucchesi, 2018), clay (Schoebitz et al., 2014), peat (Oliveira et al., 2017; Zhou et al., 2017), biochar (Tripti et al., 2017) and biogas sludge combined with enriched soil (Mukhtar et al., 2017), among other materials. The selection criteria of carriers should include cost, availability, chemical stability, toxicity levels and farmer convenience with respect to management and flexibility (Malusá et al., 2012; Bashan et al., 2014).

In most solid wet formulations in Table 3, PGPR were immobilized using different methods such as adhesion/biofilm formation on solid supports or entrapment in alginate beads. As mentioned above, immobilization processes protect bacterial cells against various harsh environmental conditions (Rabin et al., 2015). Marcelino et al. (2016) successfully immobilized *Azospirillum brasilense* Ab-V5 using biofilm formation on an innovative biodegradable foam, which was formed by conventional compounds combined with several low-cost industrial by-products (e.g., cassava starch, sugarcane bagasse and glycerol). In the case of bacterial entrapment in alginate beads, the inclusion of perlite (a natural inorganic material with high chemical, physical and biological resistance) favored the mechanical stability of beads and the bacterial survival of *Pseudomonas putida* A (ATCC 12633) in the formulation (Liffourrena and Lucchesi, 2018). On the other hand, nanoimmobilization of PGPR by electrospinning is an alternative emerging method of bacterial immobilization (De Gregorio et al., 2017). Once in the soil, bacteria could be gradually released from macro- and microbeads or nanofiber matrixes (De Gregorio et al., 2017; Liffourrena and Lucchesi, 2018).

Dry formulations with low water content can extend microbial survival for longer periods and at higher temperatures than liquid formulations, thus reducing marketing and maintenance costs since refrigeration is not required (Melin et al., 2006). Dry formulations can be rehydrated to obtain cell suspensions to cover seeds, immerse roots, or be distributed on the soil (Malusá et al., 2012; Berninger et al., 2017). In contrast, liquid formulations can always be used directly, with no need for rehydration.

Dry inoculants can be produced using air drying, desiccation, freeze-drying (lyophilization) and spray drying, among other technologies (Table 4). Shade drying and air drying are low-cost drying methods (Ruíz-Valdiviezo et al., 2015; Berninger et al., 2017; Prasad and Babu, 2017; Basheer et al., 2018). In contrast, lyophilization and spray drying require specific equipment and are energy-consuming procedures; therefore, they are more expensive compared to other drying methods or to the development of liquid and solid wet formulations (Berninger et al., 2017). Freeze-drying is widely used to preserve the microbial viability of PGPR formulations (Cabrefiga et al., 2014; Berninger et al., 2016; Tamreihao et al., 2016). It is considered a soft dehydration method in which a cell-protector mixture is first carried out, forming a matrix to incorporate cells and protect them against hostile conditions (Wessman et al., 2013).

Several powder formulations have been produced using talc as a natural carrier (Table 4). Talc has relative hydrophobicity and reduced moisture absorption; moreover, it prevents the formation of hydrate

bridges, favoring long-term storage (Martínez-Álvarez et al., 2016). On the other hand, dry systems of PGPR immobilized by entrapment/microencapsulation in alginate or zeolite have been successfully developed (Campos et al., 2014; Berninger et al., 2016, 2017). In several granular and powder dry formulations, carrier materials were combined with adhesive/protective substances such as carboxymethyl cellulose (Martínez-Álvarez et al., 2016; Prasad and Babu, 2017; Basheer et al., 2018), gelatin (Berninger et al., 2017), arabic gum (Berninger et al., 2016), maltodextrin (Campos et al., 2014), disaccharides such as lactose and sucrose (Cabrefiga et al., 2014; Molina-Romero et al., 2017) and milk-derived compounds (Berninger et al., 2017). The use of protectors is a viable way to increase bacterial survival rates during drying and storage (Schoebitz et al., 2012). However, protector efficacy depends largely on the microbial species and strains involved, so each strain requires a specific optimization (Morgan et al., 2006).

A recent study performed by our group evidenced that a drying medium composed of 10% whey and 5% sodium glutamate effectively protected *P. tolaasii* IEXb cells during the lyophilization process, as no loss in viability was observed (Table 4; unpublished results). Lyophilization was mainly evaluated to compare the bacterial stability of liquid formulations with that of dry formulations (Tables 2 and 4). However, we do not advocate lyophilization as a large-scale drying method to produce a *P. tolaasii* IEXb inoculant since it is an expensive process. After six months of storage at refrigeration temperature, the mean value of viable cells recovered from *P. tolaasii* IEXb dry formulation was higher than the minimal number of viable cells accepted for several inoculants (Table 4; unpublished results).

Sodium glutamate, a dicarboxylic amino acid, can increase cell membrane fluidity and stabilize the headgroups, promoting membrane conservation and increasing bacterial resistance to the drying process (Martos et al., 2007). Proteins and lactose from whey could form a matrix embedding the bacterial cells and protecting them against several detrimental stresses during freezing and drying such as the formation of intracellular ice. Milk proteins allow the stabilization of protein structures by means of reactions between amino groups of bacterial cell proteins and the carrier (Sharma et al., 2014). Lactose also protects bacterial cells during storage at low temperatures (Urbański et al., 2017). In a similar way to glycerol and maize bran residue (Vassilev et al., 2017; Zhang et al., 2018), whey is a low-cost compound that can be used as a nutrient and a protective agent in biomass production and in formulation processes, respectively. Additional studies must be performed to evaluate innovative technologies (e.g., emerging strategies of immobilization/co-immobilization) for the development of cost-effective *P. tolaasii* IEXb-based formulations with improved efficiency and applicability in the commercial field.

9. Concluding remarks

PGPR, including PSB, exert beneficial effects on plant growth and yields through different mechanisms of action. Several PGPR have been isolated and included in formulations to be used as inoculants in agriculture, as a smart alternative to reduce the use of chemical fertilizers. The large-scale inoculant production using industrial by-products or wastes as components of culture media and formulations is a strategy to decrease the costs of beneficial agricultural technologies. PGPR cells are formulated in potential application forms, to provide a favorable microenvironment and to promote long-term microbial stability. Several liquid and solid-based formulations containing PGPR were able to promote the growth and yields of numerous crops. Liquid formulations are usually more easily prepared and applied than solid formulations, which is a relevant benefit, especially when substantial amounts are required. Moreover, the development of liquid formulations is less expensive compared to some drying processes. The selection criteria of the optimal strategy to develop the final bioproduct will depend on the balance between stability, efficacy, economic feasibility and ease of use, in addition to the generation of a more sustainable and eco-friendly agricultural practice.

Declaration of interest

The authors have no conflict of interests regarding the publication of this article.

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References

Abbas, M.T., Hamza, M.A., Youssef, H.H., Youssef, G.H., Fayez, M., Monib, M., Hegazi, N.A., 2014. Bio-preparates support the productivity of potato plants grown under desert farming conditions of north Sinai: five years of field trials. J. Adv. Res. 5, 41–48. https://doi.org/10.1016/j.jare.2012.11.004.

Adnan, M., Patel, M., Reddy, M.N., Khan, S., Alshammari, E., Abdelkareem, A.M., Hadi, S., 2016. Isolation and characterization of effective and efficient plant growth-promoting rhizobacteria from rice rhizosphere of diverse paddy fields of Indian soil. J. Agric. Biol. Sci. 9, 373–379.

Ali, S., Ali, M.F., Sameer, M., Rafique, Z., 2018. A review scale up fermentation procedure. Int. J. Sci. Res. Sci. Technol. 4, 1301–1307.

Anith, K.N., Vaishakhi, A.S., Viswanathan, A., Varkey, S., Aswini, S., 2016. Population dynamics and efficiency of coconut water based liquid formulation of *Pseudomonas fluorescens* AMB-8. J. Trop. Agric. 54, 184–189.

Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J. Soil Sci. Plant Nutr. 15, 261–282. https://doi.org/10.4067/S0718-95162015005000021.

Barea, J.M., Richardson, A.E., 2015. Phosphate mobilisation by soil microorganisms. In: Lugtenberg, B. (Ed.), Principles of Plant-Microbe Interactions. Springer International Publishing, Switzerland, pp. 225–234.

Bashan, Y., Holguin, G., 1997. *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can. J. Microbiol. 43, 103–121. https://doi.org/10.1139/m97-015.

Bashan, Y., Hernandez, J.P., Leyva, L.A., Bacilio, M., 2002. Alginate microbeads as inoculant carrier for plant growth-promoting bacteria. Biol. Fertil. Soils 35, 359–368. https://doi.org/10.1007/s00374-002-0481-5.

Bashan, Y., Trejo, A., de-Bashan, L.E., 2011. Development of two culture media for mass cultivation of *Azospirillum* spp. and for production of inoculants to enhance plant growth. Biol. Fertil. Soils 47, 963–969. https://doi.org/10.1007/s00374-011-0555-3.

Bashan, Y., de- Bashan, L.E., Prabhu, S.R., Hernandez, J.P., 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378, 1–33. https://doi.org/10.1007/s11104-013-1956-x.

Basheer, J., Ravi, A., Mathew, J., Krishnankutty, R.E., 2018. Assessment of plant-probiotic performance of novel endophytic *Bacillus* sp. in talc-based formulation. Probiotics Antimicrob. Proteins. https://doi.org/10.1007/s12602-018-9386-y.

Berger, B., Patz, S., Ruppel, S., Dietel, K., Faetke, S., Junge, H., Becker, M., 2018. Successful formulation and application of plant growth-promoting *Kosakonia radicincitans* in maize cultivation. Biomed Res. Int. 2018, e6439481. https://doi.org/10.1155/2018/6439481.

Bernabeu, P.R., García, S.S., López, A.C., Vio, S.A., Carrasco, N., Boiardi, J.L., Luna, M.F., 2018. Assessment of bacterial inoculant formulated with *Paraburkholderia tropica* to enhance wheat productivity. World J. Microbiol. Biotechnol. 34, 81. https://doi.org/10.1007/s11274-018-2461-4.

Berninger, T., Mitter, B., Preininger, C., 2016. The smaller, the better? The size effect of alginate beads carrying plant growth-promoting bacteria for seed coating. J. Microencapsul. 33, 127–136. https://doi.org/10.3109/02652048.2015.1134690.

Berninger, T., Mitter, B., Preininger, C., 2017. Zeolite-based, dry formulations for conservation and practical application of *Paraburkholderia phytofirmans* PsJN. J. Appl. Microbiol. 122, 974–986. https://doi.org/10.1111/jam.13360.

Berninger, T., González López, Ó., Bejarano, A., Preininger, C., Sessitsch, A., 2018. Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. Microb. Biotechnol. 11, 277–301. https://doi.org/10.1111/1751-7915.12880.

Bharti, N., Barnawal, D., 2018. Amelioration of salinity stress by PGPR: ACC deaminase and ROS scavenging enzymes activity. In: Singh, A.K., Kumar, A., Singh, P.K. (Eds.), PGPR Amelioration in Sustainable Agriculture: Food Security and Environmental Management. Woodhead Publishing, United Kingdom, pp. 85–106.

Bharti, N., Sharma, S.K., Saini, S., Verma, A., Nimonkar, Y., Prakash, O., 2017. Microbial plant probiotics: problems in application and formulation. In: Kumar, V., Kumar, M., Sharma, S., Prasad, R. (Eds.), Probiotics and Plant Health. Springer, Singapore, pp. 317–335.

Biradar, B.P., Santhosh, G.P., 2018. Cell protectants, adjuvants, surfactant and preservative and their role in increasing the shelf life of liquid inoculant formulations of *Pseudomonas fluorescens*. Int. J. Pure Appl. Biosci. 6, 116–122. https://doi.org/10.

18782/2320-7051.6821.
Cabrefiga, J., Francés, J., Montesinos, E., Bonaterra, A., 2014. Improvement of a dry formulation of *Pseudomonas fluorescens* EPS62e for fire blight disease biocontrol by combination of culture osmoadaptation with a freeze-drying lyoprotectant. J. Appl. Microbiol. 117, 1122–1131. https://doi.org/10.1111/jam.12582.
Camelo-Rusinque, M., Moreno-Galván, A., Romero-Perdomo, F., Bonilla-Buitrago, R., 2017. Desarrollo de un sistema de fermentación líquida y de enquistamiento para una bacteria fijadora de nitrógeno con potencial como biofertilizante. Rev. Argent. Microbiol. 49, 289–296. https://doi.org/10.1016/j.ram.2016.06.005.
Campos, D.C., Acevedo, F., Morales, E., Aravena, J., Amiard, V., Jorquera, M.A., Inostroza, N.G., Rubilar, M., 2014. Microencapsulation by spray drying of nitrogen-fixing bacteria associated with lupin nodules. World J. Microbiol. Biotechnol. 30, 2371–2378. https://doi.org/10.1007/s11274-014-1662-8.
Carrasco-Espinosa, K., García-Cabrera, R.I., Bedoya-López, A., Trujillo-Roldán, M.A., Valdez-Cruz, N.A., 2015. Positive effect of reduced aeration rate on growth and stereospecificity of DL-malic acid consumption by *Azospirillum brasilense*: improving the shelf life of a liquid inoculant formulation. J. Biotechnol. 195, 74–81. https://doi.org/10.1016/j.jbiotec.2014.12.020.
Cassan, F., Maiale, S., Masciarelli, O., Vidal, A., Luna, V., Ruiz, O., 2009. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. Eur. J. Soil Biol. 45, 12–19. https://doi.org/10.1016/j.ejsobi.2008.08.003.
Chanratana, M., Han, G.H., Choudhury, A.R., Sundaram, S., Halim, M.A., Krishnamoorthy, R., Kang, Y., Sa, T., 2017. Assessment of *Methylobacterium oryzae* CBMB20 aggregates for salt tolerance and plant growth promoting characteristics for bio-inoculant development. AMB Express 7, 208. https://doi.org/10.1186/s13568-017-0518-7.
Choudhary, D.K., Varma, A., 2017. Nitrogenase (a key enzyme): structure and function. In: Hansen, A., Choudhary, D., Agrawal, P., Varma, A. (Eds.), *Rhizobium* Biology and Biotechnology. Springer, Cham, pp. 293–307.
Cortés-Sánchez, A., De, J., Valle-González, E.R., Salazar-Flores, R.D., Ashutosh, S., 2015. Biotechnological alternatives for the utilization of dairy industry waste products. Adv. Biosci. Biotechnol. 6, 223–235. https://doi.org/10.4236/abb.2015.63022.
Dar, Z.M., Masood, A., Mughal, A.H., Asif, M., Malik, M.A., 2018. Review on drought tolerance in plants induced by plant growth promoting rhizobacteria. Int. J. Curr. Microbiol. Appl. Sci. 7, 412–422. https://doi.org/10.20546/ijcmas.2018.705.053.
Das, K., Rajawat, M.V.S., Saxena, A.K., Prasanna, R., 2017. Development of *Mesorhizobium ciceri*-based biofilms and analyses of their antifungal and plant growth promoting activity in chickpea challenged by Fusarium wilt. Indian J. Microbiol. 57, 48–59. https://doi.org/10.1007/s12088-016-0610-8.
De Gregorio, P.R., Michavila, G., Ricciardi Muller, L., de Souza Borges, C., Pomares, M.F., Saccol de Sá, E.L., Pereira, C., Vincent, P.A., 2017. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. PLoS One 12, e0176930. https://doi.org/10.1371/journal.pone.0176930.
Dissanayaka, D.M.S.B., Plaxton, W.C., Lambers, H., Siebers, M., Marambe, B., Wasaki, J., 2018. Molecular mechanisms underpinning phosphorus-use efficiency in rice. Plant Cell Environ. 41, 1483–1496. https://doi.org/10.1111/pce.13191.
Etesami, H., Alikhani, H.A., Hosseini, H.M., 2015. Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX 2, 72–78. https://doi.org/10.1016/j.mex.2015.02.008.
Fukami, J., Cerezini, P., Hungria, M., 2018. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express 8, 73. https://doi.org/10.1186/s13568-018-0608-1.
Garcia-Ochoa, F., Gomez, E., 2009. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol. Adv. 27, 153–176. https://doi.org/10.1016/j.biotechadv.2008.10.006.
Gellings, C.W., Parmenter, K.E., 2016. Energy efficiency in fertilizer production and use. In: Gellings, C.W. (Ed.), Efficient Use and Conservation of Energy. Eolss Publishers Co. Ltd, Oxford, pp. 123–136.
Goljanian-Tabrizi, S., Amiri, S., Nikaein, D., Motesharrei, Z., 2016. The comparison of five low cost liquid formulations to preserve two phosphate solubilizing bacteria from the genera *Pseudomonas* and *Pantoea*. Iran. J. Microbiol. 8, 377–382.
Gowtham, H.G., Duraivadivel, P., Hariprasad, P., Niranjana, S.R., 2017. A novel split-pot bioassay to screen indole acetic acid producing rhizobacteria for the improvement of plant growth in tomato [*Solanum lycopersicum* L.]. Sci. Hortic. 224, 351–357. https://doi.org/10.1016/j.scienta.2017.06.017.
Hanif, K., Hameed, S., Imran, A., Naqqash, T., Shahid, M., Van Elsas, J.D., 2015. Isolation and characterization of a β-propeller gene containing phosphobacterium *Bacillus subtilis* strain KPS-11 for growth promotion of potato (*Solanum tuberosum* L.). Front. Microbiol. 6, 583. https://doi.org/10.3389/fmicb.2015.00583.
Haug, A., Høstmark, A.T., Harstad, O.M., 2007. Bovine milk in human nutrition–a review. Lipids Health Dis. 6, 25. https://doi.org/10.1186/1476-511X-6-25.
He, Y.H., Peng, Y.J., Wu, Z.S., Han, Y., Dang, Y., 2015. Survivability of *Pseudomonas putida* RS-198 in liquid formulations and evaluation its growth-promoting abilities on cotton. J. Anim. Plant Sci. 3, 180–189.
Herrera-Estrella, L., López-Arredondo, D., 2016. Phosphorus: the underrated element for feeding the world. Trends Plant Sci. 21, 461–463. https://doi.org/10.1016/j.tplants.2016.04.010.
Herrmann, L., Lesueur, D., 2013. Challenges of formulation and quality of biofertilizers for successful inoculation. App. Microbiol. Biot. 97, 8859–8873. https://doi.org/10.1007/s00253-013-5228-8.
Huang, Y., Sun, L., Zhao, J., Huang, R., Li, R., Shen, Q., 2015. Utilization of different waste proteins to create a novel PGPR-containing bio-organic fertilizer. Sci. Rep. 5, 7766. https://doi.org/10.1038/srep07766.
Joe, M.M., Saravanan, V.S., Islam, M.R., Sa, T., 2014. Development of alginate-based aggregate inoculants of *Methylobacterium* sp. and *Azospirillum brasilense* tested under in vitro conditions to promote plant growth. J. Appl. Microbiol. 116, 408–423. https://doi.org/10.1111/jam.12384.
Kang, S.W., Rahman, M.S., Kim, A.N., Lee, K.Y., Park, C.Y., Kerr, W.L., Choi, S.G., 2017. Comparative study of the quality characteristics of defatted soy flour treated by supercritical carbon dioxide and organic solvent. J. Food Sci. Technol. 54, 2485–2493. https://doi.org/10.1007/s13197-017-2691-8.
Kashyap, A.S., Pandey, V.K., Manzar, N., Kannojia, P., Singh, U.B., Sharma, P.K., 2017. Role of plant growth-promoting rhizobacteria for improving crop productivity in sustainable agriculture. In: Singh, D., Singh, H., Prabha, R. (Eds.), Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore, pp. 673–693.
Kumaresan, G., Reetha, D., 2011. Survival of *Azospirillum brasilense* in liquid formulation amended with different chemical additives. J. Phytol. 3, 48–51.
Lamizadeh, E., Enayatizamir, N., Motamedi, H., 2016. Isolation and identification of plant growth-promoting rhizobacteria (PGPR) from the rhizosphere of sugarcane in saline and non-saline soil. Int. J. Curr. Microbiol. Appl. Sci. 5, 1072–1083. https://doi.org/10.20546/ijcmas.2016.510.113.
Lavari, L., Páez, R., Cuatrin, A., Reinheimer, J., Vinderola, G., 2014. Use of cheese whey for biomass production and spray drying of probiotic lactobacilli. J. Dairy Res. 81, 267–774. https://doi.org/10.1017/S0022029914000156.
Lee, S.K., Lur, H.S., Lo, K.J., Cheng, K.C., Chuang, C.C., Tang, S.J., Yang, Z.W., Liu, C.T., 2016. Evaluation of the effects of different liquid inoculant formulations on the survival and plant-growth-promoting efficiency of *Rhodopseudomonas palustris* strain PS3. Appl. Microbiol. Biotechnol. 100, 7977–7987. https://doi.org/10.1007/s00253-016-7582-9.
Leslie, S.B., Israeli, E., Lighthart, B., Crowe, J.H., Crowe, L.M., 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Appl. Environ. Microbiol. 61, 3592–3597.
Li, L., Ye, Y., Pan, L., Zhu, Y., Zheng, S., Lin, Y., 2009. The induction of trehalose and glycerol in *Saccharomyces cerevisiae* in response to various stresses. Biochem. Biophys. Res. Commun. 387, 778–783. https://doi.org/10.1016/j.bbrc.2009.07.113.
Li, R., Tao, R., Ling, N., Chu, G., 2017. Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: implications for soil biological quality. Soil Till. Res. 167, 30–38. https://doi.org/10.1016/j.still.2016.11.001.
Liffourrena, A.S., Lucchesi, G.I., 2018. Alginate-perlite encapsulated *Pseudomonas putida* A (ATCC 12633) cells: preparation, characterization and potential use as plant inoculants. J. Biotechnol. 278, 28–33. https://doi.org/10.1016/j.jbiotec.2018.04.019.
Liu, J., Yang, J., Liang, X., Zhao, Y., Cade-Menun, B.J., Hu, Y., 2014. Molecular speciation of phosphorus present in readily dispersible colloids from agricultural soils. Soil Sci. Soc. Am. J. 78, 47–53. https://doi.org/10.2136/sssaj2013.05.0159.
Liu, H., Chen, D., Zhang, R., Hang, X., Li, R., Shen, Q., 2016. Amino acids hydrolyzed from animal carcasses are a good additive for the production of bio-organic fertilizer. Front. Microbiol. 7, 1290. https://doi.org/10.3389/fmicb.2016.01290.
Liu, K., McInroy, J.A., Hu, C.H., Kloepper, J.W., 2017. Selection and assessment of plant growth-promoting rhizobacteria for biological control of multiple plant diseases. Phytopathology 107, 928–936. https://doi.org/10.1094/PHYTO-02-17-0051-R.
Liu, K., McInroy, J.A., Hu, C.H., Kloepper, J.W., 2018. Mixtures of plant-growth-promoting rhizobacteria enhance biological control of multiple plant diseases and plant-growth promotion in the presence of pathogens. Plant Dis. 102, 67–72. https://doi.org/10.1094/PDIS-04-17-0478-RE.
Loján, P., Demortier, M., Velivelli, S.L., Pfeiffer, S., Suárez, J.P., De Vos, P., Prestwich, B.D., Sessitsch, A., Declerck, S., 2017. Impact of plant growth-promoting rhizobacteria on root colonization potential and life cycle of *Rhizophagus irregularis* following co-entrapment into alginate beads. J. Appl. Microbiol. 122, 429–440. https://doi.org/10.1111/jam.13355.
Mahajan, R., Shirkot, C.K., 2014. PGPR: their role in plant growth and disease management. In: Gupta, S.K., Sharma, M. (Eds.), Approaches and Trends in Plant Disease Management. Scientific Publishers, Jodhpur, pp. 85–122.
Malusá, E., Vassilev, N., 2014. A contribution to set a legal framework for biofertilisers. Appl. Microbiol. Biotechnol. 98, 6599–6607. https://doi.org/10.1007/s00253-014-5828-y.
Malusá, E., Sas-Paszt, L., Ciesielska, J., 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. Sci. World J. 2012, 491206. https://doi.org/10.1100/2012/491206.
Manasa, K., Reddy, S., Triveni, S., 2017. Characterization of potential PGPR and antagonistic activities of *Rhizobium* isolates from different rhizosphere soils. J. Pharmacogn. Phytochem. 6, 51–54.
Marcelino, P.R.F., Milani, K.M.L., Mali, S., Dos Santos, O.J.A.P., de Oliveira, A.L.M., 2016. Formulations of polymeric biodegradable low-cost foam by melt extrusion to deliver plant growth-promoting bacteria in agricultural systems. Appl. Microbiol. Biotechnol. 100, 7323–7338. https://doi.org/10.1007/s00253-016-7566-9.
Martínez-Álvarez, J.C., Castro-Martínez, C., Sánchez-Peña, P., Gutiérrez-Dorado, R., Maldonado-Mendoza, I.E., 2016. Development of a powder formulation based on *Bacillus cereus* sensu lato strain B25 spores for biological control of *Fusarium verticillioides* in maize plants. World J. Microbiol. Biotechnol. 32, 75. https://doi.org/10.1007/s11274-015-2000-5.
Martos, G.I., Minahk, C.J., Font de Valdez, G., Morero, R., 2007. Effects of protective agents on membrane fluidity of freeze-dried *Lactobacillus delbrueckii* ssp. *bulgaricus*. Lett. Appl. Microbiol. 45, 282–288. https://doi.org/10.1111/j.1472-765X.2007.02188.x.
Mejri, D., Gamalero, E., Souissi, T., 2013. Formulation development of the deleterious rhizobacterium *Pseudomonas trivialis* X33d for biocontrol of brome (*Bromus diandrus*) in durum wheat. J. Appl. Microbiol. 114, 219–228. https://doi.org/10.1111/jam.12036.
Melin, P., Håkansson, S., Eberhard, T.H., Schnürer, J., 2006. Survival of the biocontrol

yeast *Pichia anomala* after long-term storage in liquid formulations at different temperatures, assessed by flow cytometry. J. Appl. Microbiol. 100, 264–271. https://doi.org/10.1111/j.1365-2672.2005.02778.x.
Molina-Romero, D., Baez, A., Quintero-Hernández, V., Castañeda-Lucio, M., Fuentes-Ramírez, L.E., del Rocío Bustillos-Cristales, M., Rodríguez-Andrade, O., Morales-García, Y.E., Munive, A., Muñoz-Rojas, J., 2017. Compatible bacterial mixture, tolerant to desiccation, improves maize plant growth. PLoS One 12, e0187913. https://doi.org/10.1371/journal.pone.0187913.
Morgan, C.A., Herman, N., White, P.A., Vesey, G., 2006. Preservation of micro-organisms by drying; a review. J. Microbiol. Methods 66, 183–193. https://doi.org/10.1016/j.mimet.2006.02.017.
Mugnier, J., Jung, G., 1985. Survival of bacteria and fungi in relation to water activity and the solvent properties of water in biopolymer. Appl. Environ. Microbiol. 50, 108–114.
Mukhtar, S., Shahid, I., Mehnaz, S., Malik, K.A., 2017. Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum* L.). Microbiol. Res. 205, 107–117. https://doi.org/10.1016/j.micres.2017.08.011.
Müller, M., Munné-Bosch, S., 2015. Ethylene response factors: a key regulatory hub in hormone and stress signaling. Plant Physiol. 169, 32–41. https://doi.org/10.1104/pp.15.00677.
Mus, F., Alleman, A.B., Pence, N., Seefeldt, L.C., Peters, J.W., 2018. Exploring the alternatives of biological nitrogen fixation. Metallomics 10, 523–538. https://doi.org/10.1039/C8MT00038G.
Muthukumar, T., Udaiyan, K., 2018. Coinoculation of bioinoculants improve *Acacia auriculiformis* seedling growth and quality in a tropical Alfisol soil. J. For. Res. 29, 663–673. https://doi.org/10.1007/s11676-017-0497-1.
Neilands, J.B., 2014. Microbial Iron Metabolism: a Comprehensive Treatise. Academic Press, New York and London.
Novo, L.A., Castro, P.M., Alvarenga, P., da Silva, E.F., 2018. Plant growth-promoting rhizobacteria-assisted phytoremediation of mine soils. In: Prasad, M.N.V., de Campos Favas, P.J., Maiti, S.K. (Eds.), Bio-Geotechnologies for Mine Site Rehabilitation. Elsevier Inc., Amsterdam, pp. 281–295.
Nyoki, D., Ndakidemi, P.A., 2018. Root length, nodulation and biological nitrogen fixation of *Rhizobium* inoculated soybean (*Glycine max* [L.] Merr.) grown under maize (*Zea mays* L.) intercropping systems and P and K fertilization. Adv. Biores. 9, 173–180. https://doi.org/10.15515/abr.0976-4585.9.1.173180.
Oliveira, A.L., Santos, O.J., Marcelino, P.R., Milani, K.M., Zuluaga, M.Y., Zucareli, C., Gonçalves, L.S., 2017. Maize inoculation with *Azospirillum brasilense* Ab-V5 cells enriched with exopolysaccharides and polyhydroxybutyrate results in high productivity under low N fertilizer input. Front. Microbiol. 8, 1873. https://doi.org/10.3389/fmicb.2017.01873.
Pahari, A., Mishra, B.B., 2017. Characterization of siderophore producing rhizobacteria and its effect on growth performance of different vegetables. Int. J. Curr. Microbiol. App. Sci. 6, 1398–1405. https://doi.org/10.20546/ijcmas.2017.605.152.
Pastor-Bueis, R., Mulas, R., Gómez, X., González-Andrés, F., 2017. Innovative liquid formulation of digestates for producing a biofertilizer based on *Bacillus siamensis*: field testing on sweet pepper. J. Plant Nutr. Soil Sci. 180, 748–758. https://doi.org/10.1002/jpln.201700200.
Pathak, D.V., Kumar, M., Rani, K., 2017. Biofertilizer application in horticultural crops. In: Panpatte, D.G., Jhala, Y.K., Vyas, R.V., Shelat, H.N. (Eds.), Microorganisms for Green Revolution. Springer, Singapore, pp. 215–227.
Peng, Y., He, Y., Wu, Z., Lu, J., Li, C., 2014. Screening and optimization of low-cost medium for *Pseudomonas putida* Rs-198 culture using RSM. Braz. J. Microbiol. 45, 1229–1237. https://doi.org/10.1590/S1517-83822014000400013.
Posada-Uribe, L.F., Romero-Tabarez, M., Villegas-Escobar, V., 2015. Effect of medium components and culture conditions in *Bacillus subtilis* EA-CB0575 spore production. Bioprocess Biosyst. Eng. 38, 1879–1888. https://doi.org/10.1007/s00449-015-1428-1.
Prasad, A.A., Babu, S., 2017. Compatibility of *Azospirillum brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogea* L.). Acad. Bras. Cienc. 89, 1027–1040. https://doi.org/10.1590/0001-3765201720160617.
Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F., Shakir, M.A., 2012. Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. J. Anim. Plant Sci. 22, 204–210.
Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E., Sintim, H.O., 2015. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med. Chem. 7, 493–512. https://doi.org/10.4155/fmc.15.6.
Rasouli-Sadaghiani, M., Malakouti, M.J., Khavazi, K., Miransari, M., 2014. Siderophore efficacy of fluorescent pseudomonades affecting labeled iron (^{59}Fe) uptake by wheat (*Triticum aestivum* L.) Genotypes differing in Fe efficiency. In: Miransari, M. (Ed.), Use of Microbes for the Alleviation of Soil Stresses. Springer, New York, pp. 121–132.
Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17, 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2.
Roy, E.D., 2017. Phosphorus recovery and recycling with ecological engineering: a review. Ecol. Eng. 98, 213–227. https://doi.org/10.1016/j.ecoleng.2016.10.076.
Ruíz-Valdiviezo, V.M., Canseco, L.M.C.V., Suárez, L.A.C., Gutiérrez-Miceli, F.A., Dendooven, L., Rincón-Rosales, R., 2015. Symbiotic potential and survival of native rhizobia kept on different carriers. Braz. J. Microbiol. 46, 735–742. https://doi.org/10.1590/S1517-838246320140541.
Ruzzi, M., Aroca, R., 2015. Plant growth-promoting rhizobacteria act as biostimulants in horticulture. Sci. Hortic. 196, 124–134. https://doi.org/10.1016/j.scienta.2015.08.042.
Ryan, M.P., Walsh, G., 2016. The biotechnological potential of whey. Rev. Environ. Sci. Biotechnol. 15, 479–498. https://doi.org/10.1007/s11157-016-9402-1.
Saleem, M., Khan, I., 2017. Bottlenecks in commercialisation and future prospects of PGPR. Appl. Soil Ecol. 121, 102–117. https://doi.org/10.1016/j.apsoil.2017.09.030.
Schmidt, F.R., 2005. Optimization and scale up of industrial fermentation processes. Appl. Microbiol. Biotechnol. 68, 425–435. https://doi.org/10.1007/s00253-005-0003-0.
Schoebitz, M., Simonin, H., Poncelet, D., 2012. Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. J. Microencapsul. 29, 532–538. https://doi.org/10.3109/02652048.2012.665090.
Schoebitz, M., Mengual, C., Roldán, A., 2014. Combined effects of clay immobilized *Azospirillum brasilense* and *Pantoea dispersa* and organic olive residue on plant performance and soil properties in the revegetation of a semiarid area. Sci. Total Environ. 466, 67–73. https://doi.org/10.1016/j.scitotenv.2013.07.012.
Seema, K., Mehta, K., Singh, N., 2018. Studies on the effect of plant growth promoting rhizobacteria (PGPR) on growth, physiological parameters, yield and fruit quality of strawberry cv. Chandler. J. Pharmacogn. Phytochem. 7, 383–387.
Selvaraj, S., Ganeshamoorthi, P., Anand, T., Raguchander, T., Seenivasan, N., Samiyappan, R., 2014. Evaluation of a liquid formulation of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *cubense* and *Helicotylenchus multicinctus* in banana plantation. Biocontrol 59, 345–355. https://doi.org/10.1007/s10526-014-9569-8.
Shahzad, S., Khan, M.Y., Zahir, Z.A., Asghar, H.N., Chaudhry, U.K., 2017. Comparative effectiveness of different carriers to improve the efficacy of bacterial consortium for enhancing wheat production under salt affected field conditions. Pak. J. Bot. 49, 1523–1530.
Sharma, R., Sanodiya, B.S., Thakur, G.S., Jaiswal, P., Sharma, A., Bisen, P.S., 2014. Standardization of lyophilization medium for *Streptococcus thermophilus* subjected to viability escalation on freeze drying. Microbiol. Res. 5, 5402. https://doi.org/10.4081/mr.2014.5402.
Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011. Phosphorus dynamics: from soil to plant. Plant Physiol. 156, 997–1005. https://doi.org/10.1104/pp.111.175232.
Singh, R.P., Jha, P.N., 2016. The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). PLoS One 11, e0155026. https://doi.org/10.1371/journal.pone.0155026.
Singh, A.K., Singh, G., Gautam, D., Bedi, M.K., 2013. Optimization of dairy sludge for growth of *Rhizobium* cells. Biomed Res. Int. 2013, e845264. https://doi.org/10.1155/2013/845264.
Singh, S.R., Biswas, D., Bahadur, L., 2015. PGPR for sustaining crop productivity under salt stress. In: Arora, S., Singh, Y.P., Singh, A.K. (Eds.), Advanced Techniques for Bioremediation and Management of Salt Affected Soils. ICAR-Central Soil Salinity Research Institute Regional Research Station, Lucknow, pp. 167–177.
Singleton, P., Keyser, H., Sande, E., 2002. Development and evaluation of liquid inoculants. Herridge, D. (Ed.), Inoculants and Nitrogen Fixation of Legumes in Vietnam. ACIAR Proceedings 52–66.
Tamreihao, K., Ningthoujam, D.S., Nimaichand, S., Singh, E.S., Reena, P., Singh, S.H., Nongthomba, U., 2016. Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiol. Res. 192, 260–270. https://doi.org/10.1016/j.micres.2016.08.005.
Tripti, K., Adarsh, U., Zeba, K., Vipin, A., 2017. Biochar and flyash inoculated with plant growth promoting rhizobacteria act as potential biofertilizer for luxuriant growth and yield of tomato plant. J. Environ. Manage. 190, 20–27. https://doi.org/10.1016/j.jenvman.2016.11.060.
Trujillo-Roldán, M.A., Valdez-Cruz, N.A., Gonzalez-Monterrubio, C.F., Acevedo-Sánchez, E.V., Martínez-Salinas, C., García-Cabrera, R.I., Gamboa-Suasnavart, R.A., Marín-Palacio, L.D., Villegas, J., Blancas-Cabrera, A., 2013. Scale-up from shake flasks to pilot-scale production of the plant growth-promoting bacterium *Azospirillum brasilense* for preparing a liquid inoculant formulation. Appl. Microbiol. Biotechnol. 97, 9665–9674.
Tunick, M.H., 2008. Whey protein production and utilization: a brief history. In: Onwulata, C., Huth, P. (Eds.), Whey Processing, Functionality and Health Benefits. Blackwell Publishing and the Institute of Food Technologists, Iowa, pp. 1–13.
Urbański, D., Jarosz, M., Stobińska, M., Sobecka, K., Mizielińska, M., Łukawska, B., Olchawa, E., 2017. Influence of whey on viability of *Lactobacillus gasseri* during freeze-drying process. World Sci. News. 83, 200–205.
Urrutia, O., Erro, J., Guardado, I., San Francisco, S., Mandado, M., Baigorri, R., Claude Yvin, J., Ma Garcia-Mina, J., 2014. Physico-chemical characterization of humic-metal-phosphate complexes and their potential application to the manufacture of new types of phosphate-based fertilizers. J. Plant Nutr. Soil Sci. 177, 128–136. https://doi.org/10.1002/jpln.201200651.
Valetti, L., Angelini, J.G., Taurian, T., Ibáñez, F.J., Muñoz, V.L., Anzuay, M.S., Ludueña, L.M., Fabra, A., 2016. Development and field evaluation of liquid inoculants with native *Bradyrhizobial* strains for peanut production. Afr. Crop Sci. J. 24, 1–13. https://doi.org/10.4314/acsj.v24i1.1.
Van de Poel, B., Smet, D., Van Der Straeten, D., 2015. Ethylene and hormonal cross talk in vegetative growth and development. Plant Physiol. 169, 61–72. https://doi.org/10.1104/pp.15.00724.
Vassilev, N., Vassileva, M., Lopez, A., Martos, V., Reyes, A., Maksimovic, I., Eichler-Löbermann, B., Malusà, E., 2015. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl. Microbiol. Biotechnol. 99, 4983–4996. https://doi.org/10.1007/s00253-015-6656-4.
Vassilev, N., Malusa, E., Requena, A.R., Martos, V., López, A., Maksimovic, I., Vassileva, M., 2017. Potential application of glycerol in the production of plant beneficial microorganisms. J. Ind. Microbiol. Biotechnol. 44, 735–743. https://doi.org/10.1007/s10295-016-1810-2.
Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Nasrulhaq Boyce, A., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. Molecules 21, 573. https://doi.org/10.3390/molecules21050573.

Venturi, V., Keel, C., 2016. Signaling in the rhizosphere. Trends Plant Sci. 21, 187–198. https://doi.org/10.1016/j.tplants.2016.01.005.

Verma, R.K., Verma, S.K., Pankaj, U., Gupta, A.K., Khan, K., Shankar, K., 2015. Improvement in the yield and quality of kalmegh (*Andrographis paniculata* Nees) under the sustainable production system. Nat. Prod. Res. 29, 297–300. https://doi.org/10.1080/14786419.2014.971791.

Viruel, E., Lucca, M.E., Siñeriz, F., 2011. Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. Arch. Microbiol. 193, 489–496. https://doi.org/10.1007/s00203-011-0692-y.

Viruel, E., Erazzú, L.E., Martínez Calsina, L., Ferrero, M.A., Lucca, M.E., Siñeriz, F., 2014. Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. J. Soil Sci. Plant Nutr. 14, 819–831. https://doi.org/10.4067/S0718-95162014005000065.

Vyas, P., Rahi, P., Chadha, B.S., Gulati, A., 2014. Statistical optimization of medium components for mass production of plant growth-promoting microbial inoculant *Pseudomonas trivialis* BIHB 745 (MTCC5336). Indian J. Microbiol. 54, 239–241. https://doi.org/10.1007/s12088-013-0425-9.

Wang, Z., Zhang, T.Q., Tan, C.S., Vadas, P., Qi, Z.M., Wellen, C., 2018. Modeling phosphorus losses from soils amended with cattle manures and chemical fertilizers. Sci. Total Environ. 639, 580–587. https://doi.org/10.1016/j.scitotenv.2018.05.141.

Wessman, P., Håkansson, S., Leifer, K., Rubino, S., 2013. Formulations for freeze-drying of bacteria and their influence on cell survival. J. Vis. Exp. 78, 4058. https://doi.org/10.3791/4058.

Whittenbury, R., Phillips, K.C., Wilkinson, J.F., 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. 61, 205–218. https://doi.org/10.1099/00221287-61-2-205.

Wongdee, J., Boonkerd, N., Teaumroong, N., Tittabutr, P., Giraud, E., 2018. Regulation of nitrogen fixation in *Bradyrhizobium* sp. strain DOA9 involves two distinct NifA regulatory proteins that are functionally redundant during symbiosis but not during free-living growth. Front. Microbiol. 9, 1644. https://doi.org/10.3389/fmicb.2018.01644.

Xu, S., Bai, Z., Jin, B., Xiao, R., Zhuang, G., 2014. Bioconversion of wastewater from sweet potato starch production to *Paenibacillus polymyxa* biofertilizer for tea plants. Sci. Rep. 4, e4131. https://doi.org/10.1038/srep04131.

Yadav, A.K., Chandra, K., 2014. Mass production and quality control of microbial inoculants. Proc. Indian Nat. Sci. Acad. 2, 483–489.

Yadav, S.K., Soni, R., Rajput, A.S., 2018. Role of microbes in organic farming for sustainable agro-ecosystem. In: Panpatte, D., Jhala, Y., Shelat, H., Vyas, R. (Eds.), Microorganisms for Green Revolution, Microorganisms for Sustainability. Springer, Singapore, pp. 241–252.

Yaghoubi Khanghahi, M., Ricciuti, P., Allegretta, I., Terzano, R., Crecchio, C., 2018. Solubilization of insoluble zinc compounds by zinc solubilizing bacteria (ZSB) and optimization of their growth conditions. Environ. Sci. Pollut. Res. Int. 25, 25862–25868. https://doi.org/10.1007/s11356-018-2638-2.

Zárate, G., Juárez Tomás, M.S., Nader-Macias, M.E., 2005. Effect of some pharmaceutical excipients on the survival of probiotic vaginal lactobacilli. Can. J. Microbiol. 51, 483–489. https://doi.org/10.1139/w05-031.

Zhang, M., Li, R., Cao, L., Shi, J., Liu, H., Huang, Y., Shen, Q., 2014. Algal sludge from Taihu Lake can be utilized to create novel PGPR-containing bio-organic fertilizers. J. Environ. Manage. 132, 230–236. https://doi.org/10.1016/j.jenvman.2013.10.031.

Zhang, H., Liu, Y., Wang, G., 2018. Integrated use of maize bran residue for one-step phosphate bio-fertilizer production. Appl. Biochem. Biotechnol. https://doi.org/10.1007/s12010-018-2874-4.

Zhou, J., Deng, B., Zhang, Y., Cobb, A.B., Zhang, Z., 2017. Molybdate in rhizobial seed-coat formulations improves the production and nodulation of alfalfa. PLoS One 12, e0170179. https://doi.org/10.1371/journal.pone.0170179.