

Perspectives for Biochar as a vehicle for inoculation of phosphate solubilizing bacteria: a review

Perspectivas do Biochar como veículo para inoculação de bactérias solubilizadoras de fosfato: uma revisão

Perspectivas del Biochar como vehículo para la inoculación de bacterias solubilizantes de fosfato: una revisión

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Abstract

Phosphorus (P) plays a vital role in many aspects of plant growth and development. The low amount of available P in agricultural soils reduces crop productivity and phosphate fertilizers are often applied. However, due to the high affinity of P for the soil constituents, the availability of this element becomes limited to plants. Thus, alternative, ecological, and low-cost techniques have been studied to improve P acquisition by crops. Microorganisms able to solubilize P, mainly phosphate-solubilizing bacteria (PSB) have stood out, since they offer an approach to overcome P scarcity by their introduction in agricultural systems via inoculants. In this paper, we showed the potential of P-solubilizing microorganisms and their mechanisms of action, the potential of different inoculation vehicles, also highlighting the biochar as a viable biological product for production of inoculants. The combined effects of these factors (PSB and biochar) add several benefits to the soil-plant system. Results from this review demonstrate that biochar amendments have great potential as a vehicle for inoculation of PSB. However, studies of biochar combined with PSB is still incipient. Future research should focus efforts on exploring highly efficient strains, optimizing conditions, and assessing several sources of waste for production of biochar and their efficiency in field experiments.

Keywords: Available phosphorus; Solubilizing microorganisms; Inoculants.

Resumo

O fósforo (P) desempenha um papel vital em muitos aspectos do crescimento e desenvolvimento das plantas. A baixa quantidade de P disponível em solos agrícolas reduz a produtividade da cultura e fertilizantes fosfatados são frequentemente aplicados. Porém, devido à alta afinidade do P pelos constituintes do solo, a disponibilidade desse elemento torna-se limitada às plantas. Assim, técnicas alternativas, ecológicas e de baixo custo tem sido estudadas para melhorar a aquisição de P pelas culturas. Microrganismos capazes de solubilizar P, principalmente bactérias solubilizadoras de fosfato (BSF), têm se destacado por oferecer uma abordagem para superar a escassez de P por meio de sua introdução em sistemas agrícolas via inoculantes. Neste trabalho, mostramos o potencial de microrganismos solubilizadores de P e seus mecanismos de ação, o potencial de diferentes veículos de inoculação, destacando também o biochar como um produto biológico viável para produção de inoculantes. Os efeitos combinados desses fatores (BSF e biochar) agregam vários benefícios ao sistema solo-planta. Os resultados desta revisão demonstram que os aditivos do biochar têm grande potencial como um veículo para inoculação de PSB. No entanto, estudos de biochar combinado com BSF ainda são incipientes. Pesquisas futuras devem concentrar esforços na exploração de cepas altamente eficientes, otimizando as condições e avaliando várias fontes de resíduos para a produção de biochar e sua eficiência em experimentos de campo.

Palavras-chave: Fósforo disponível; Microrganismos solubilizantes; Inoculantes.

Resumen

El fósforo (P) juega un papel vital en muchos aspectos del crecimiento y desarrollo de las plantas. La baja cantidad de P disponible en los suelos agrícolas reduce la productividad de los cultivos y a menudo se aplican fertilizantes fosfatados. Sin embargo, debido a la alta afinidad del P por los constituyentes del suelo, la disponibilidad de este elemento se limita a las plantas. Así, se han estudiado técnicas alternativas, ecológicas y de bajo costo para mejorar la adquisición de P por los cultivos. Se han destacado los microorganismos capaces de solubilizar P, principalmente bacterias solubilizadoras de fosfato (BSF), ya que ofrecen un enfoque para superar la escasez de P mediante su introducción en sistemas agrícolas a través de inoculantes. En este trabajo, mostramos el potencial de los microorganismos solubilizantes de P y sus mecanismos de acción, el potencial de diferentes vehículos de inoculación, destacando también el biocarbón como un producto biológico viable para la producción de inoculantes. Los efectos combinados de estos factores (BSF y biochar) agregan varios beneficios al sistema suelo-planta. Los resultados de esta revisión demuestran que las enmiendas de biocarbón tienen un gran potencial como vehículo para la inoculación de BSF. Sin embargo, los estudios de biocarbón combinado con PSB aún son incipientes. La investigación futura debe centrar los esfuerzos en explorar cepas altamente eficientes, optimizar las condiciones y evaluar varias fuentes de desechos para la producción de biocarbón y su eficiencia en experimentos de campo.

Palabras clave: Fósforo disponible; Microorganismos solubilizantes; Inoculantes.

1. Introduction

Phosphorus (P) constitutes molecules such as DNA, ATP, NADPH, and phospholipids of cell membranes, besides participating in processes such as photosynthesis, respiration, carbohydrate metabolism, N₂ fixation and protein activation (Vance et al., 2003). However, the interaction of P with soil constituents, its occurrence in organic forms and its slow rate of diffusion in the soil solution, turns P into a nutrient little available in the rhizosphere, reducing crop productivity in agricultural soils in different areas of the world (Zhu et al., 2018).

Located in the Neotropical zones, Brazilian soils have negligible amounts of P due to its source material and the interactions of this element with the soil, which direct the national agriculture towards the use of substantial amounts of phosphate fertilizers (Embrapa, 2017). Brazil imported about 80% of the raw material for NPK fertilizers manufacturing in 2019, when around 36.2 million tons are estimated to be sold. The use of fertilizers by Brazilian farmers has grown by around 450% in the last 30 years (Associação Nacional para Difusão de Adubos, 2020).

With slow mobility, most of the P is adsorbed to the soil colloids, which lessen the losses from percolation. Therefore, erosion is the main cause of P losses contained in soil organic matter and colloidal particles (Chintala et al. 2014). The risk of considerable P losses and the increase in eutrophication rates, due to high phosphate fertilizers accumulation in soils, has been observed in short, medium and long term studies (Oliveira Filho et al., 2020).

Organic P in soil organic matter is a relevant source for plant nutrition (Liang et al., 2017). The dynamics of organic P in the soil is associated with environmental conditions that influence the microbial activity, which immobilize or

release the orthophosphate ions (H_2PO_4), and with the physical-chemical and mineralogical properties of the soils (Santos et al., 2008). According to Gatiboni et al. (2007), the contribution of organic P to plant nutrition is 6% for soils that received phosphate fertilization and about 43% for native regions without anthropic influence.

In arable areas with seasonal or regular applications of phosphate fertilizers, pockets of P occur in the soil due to its adsorption on colloids or immobilization in microbial biomass (Nobile et al., 2020; Oliveira Filho et al., 2020). Such reservoirs are barely available to plants, representing a considerable financial investment, especially in a possible scenario of P scarcity in the market. Therefore, alternative sources of fertilizers are necessary to reduce dependence on mineral fertilizers (Medeiros et al., 2019).

The amount of mineral fertilizers applied to the soil is increasing every year in order to enhance crop productivity (Lee et al., 2019). Increasing the efficiency of fertilizers in agricultural production requires a multidisciplinary approach, which includes the optimization of fertilizer-soil-plant interaction. Brazil is facing a huge challenge for P management in cultivated soils, to attend the demand of increasing yields but preserving natural resources. However, the high Brazilian dependence upon P fertilizers may represent a unsustainable use of a finite resource (Withers et al., 2018).

In contrast to mineral fertilizers, which require revolve the soil for incorporation, the application of fixing or solubilizing microorganisms is less invasive to the environment, as they are inoculated into the soil with the seeds at sowing. This can reduce the risk of pollution of springs, effluents, and groundwater through eutrophication. The use of phosphate solubilizing microorganisms (PSM) is adaptable to more conservationist and organic production systems, given its biological nature.

A wide variety of organisms are involved in the natural cycle of P. Particularly, microorganisms play an important role, being responsible to turn insoluble P into soluble forms accessible to plants (Zhu et al., 2018). Phosphate solubilizing microorganisms have been the subject of study for decades (Kalayu, 2019). Several species of distinct taxonomic groups are reported as solubilizers (Actinomycetes, Cyanobacteria, Bacteria and Fungi) and among PSM, strains of bacteria have received considerable attention.

Thus, due to the high demand and low efficiency of phosphate fertilizers, beyond the importance of an efficient P management, is the search for new renewable and sustainable technologies for agriculture and the environment (Withers et al., 2018). There are several scientific articles about the selection of effective microbial strains for P solubilization in different agrosystems and soil types. However, there is still a deficit of papers that associate the effectiveness of low-cost inoculant formula that are accessible to produce. Thereupon, the objective of the present review is (i) to provide knowledge about studies of phosphate-solubilizing bacteria (PSB), (ii) to show what is the nature of an inoculant and what are the different materials commonly used to its production and (iii) to highlight advantages and disadvantages of biochar as a PSB inoculation vehicle.

2. Methodology

This study is a narrative review about the perspectives for biochar as a vehicle for inoculation of PSB. Narrative reviews are characterized by critical literature analyses, from a theoretical or contextual point of view (Grant & Booth, 2009). The selected literatures were extracted from the platforms: SciELO, ScienceDirect, Elsevier, PubMed, SpringerLink and Journal CAPES. Where the following keywords were applied, linked by Boolean operators (AND, OR): “available phosphorus; “solubilizing microorganisms”; “inoculants”; “black carbon” and “biochar”.

3. Development

3.1 History of bacterial inoculants

The microbial inoculant industry delivers more than 25 million doses annually to the Brazilian market (Franchini et al.,

2007), mostly focused on inoculating legumes and only 10% for growing grasses. These products are the main bio-based material produced and sold in the country, and the use of bacterial inoculants in agriculture is estimated to grow at a rate of 12.5% per year (Owen et al., 2015; Howieson & Dilworth, 2016).

The use of bacterial inoculants in agriculture has been traced for centuries. In remote times, farmers realized that the production of legumes could increase the yield of the subsequent harvest compared to areas without previous cultivation of legumes. At the end of the 19th century, the knowledge acquired from this practice was used to develop a recommended method for the inoculation of legumes in the United States of America (Smith, 1992). Later, the discovery of N₂ fixation by legumes and the isolation of rhizobia from the root nodules of these plants allowed the practice of bacterial inoculation (Cardoso & Estrada-Bonilla, 2019).

In 1895, Nobbe and Hiltner developed techniques to reproduce *Rhizobium* spp. in the laboratory and registered the first patent with these microorganisms, starting the production of these inoculants for large scale use (Hungria et al., 2005). Therefore, the commercialization of bacterial inoculants started in 1898 (Cardoso & Estrada-Bonilla, 2019), and the practice of *Rhizobium* inoculation became common since then.

At the end of the 1970s, Döbereiner and Day (1976) described the potential of the *Azospirillum* genus for promoting the development of non-leguminous plants. Bashan (1990) gathered data that demonstrated positive effects of *Azospirillum* inoculation for corn yield in the field, concluding that the responses were quite substantial due to biological nitrogen fixation. After observations that the promotion of plant growth caused by inoculation with *Azospirillum* sp. was mainly due to the promotion of root growth and not to nitrogen fixation (Okon & Labandera-Gonzalez, 1994), the experimental objectives and designs have been changed.

Döbereiner and team isolated the *Gluconacetobacter diazotrophicus* bacteria in sugarcane plants in the 1990s (Cavalcanti & Döbereiner, 1988). From there, it was possible to isolate and identify endophytic bacteria in samples of stems, leaves and roots, capable of colonizing sugarcane plants such as *G. diazotrophicus* (Reis et al., 1994), *Herbaspirillum seropedicae* and *H. rubrisubalbicans* (Olivares et al., 1996), *Azospirillum* spp. and new species of the *Burkholderia* genus (Perin et al., 2006).

Moreover, the co-inoculation of bacteria is already used in countries such as South Africa and Argentina, but studies are still incipient in Brazil. Co-inoculation provides greater root growth, in addition to greater nodulation potential and an efficient response in the interaction of diazotrophic bacteria, especially with the species *Azospirillum brasilense* and *Bradyrhizobium japonicum* (Zuffo et al., 2016). Other benefits of these bacteria are the ability to penetrate plant roots, the antagonism to phytopathogens, association with various grasses such as corn, production of phytohormones and tolerance to temperature variations (Araújo, 2008).

In addition to the mixtures of bacterial strains used in the formulation of inoculants, other groups were investigated alone or through co-inoculation, such as *Bacillus* (Szilagyi-Zecchin et al., 2015) and its association with fungi of the genus *Trichoderma* (Chagas et al., 2017) and bacteria belonging to *Pseudomonas* (Liffourrena et al., 2018; Manzoor et al., 2017), *Acetobacter* (Florentino et al., 2017) and various types of co-inoculation with *Azospirillum* and *rhizobia* (Galindo et al., 2018).

The first bacterial inoculants specific to solubilize P date back to 1950, when a product known as “Fosfobacterin”, derived from the mixture of kaolin and *Bacillus megatherium* var. *phosphaticum*, was released in the market in the former Soviet Union and Eastern Europe, increasing the mineralization of P in the soil by 20% (Kucey et al., 1989). However, these results were not confirmed by studies developed in the following years, in other countries such as the USA (Kucey et al., 1989). In several countries (Australia, Canada, Brazil, USA and Russia), since the 1950s, there are records of products based on bacteria such as *Acidithiobacillus* spp., *Bacillus* spp. and fungi such as *Penicillium bilaji* and *P. radicum*, aiming at the solubilization of P, however, in most cases the results are inconclusive and contradictory (Mendes et al., 2003).

Studies that explain the interactions between P and microorganisms have received a special attention, since phosphate solubilizing microorganisms are alternatives to improve the efficiency of P sources in the soil (Massenssini et al., 2015). Studies have been exploring the improved use of nutrients in the soil, by means of microorganisms able to solubilize P (Cisneros-Rojas et al., 2017) and potassium (K) (Bagyalakshmi et al., 2017). Dworzanski et al. (2006) registered the first patent using *Bacillus megaterium*, *B. cereus* and *B. pectobacterium* for dissolving P and K, fixing nitrogen and promoting plant growth, aiming to develop a multifunctional biofertilizer. The dissolution effects of P and K were proven in a greenhouse experiment; however, the product was not tested in the field (Dworzanski et al., 2006).

In 2016, the first inoculant that was successful for solubilization of P (BiomaPhos ®) with Brazilian technology was launched. The product is the result of the mixture of two bacterial strains (*Bacillus megaterium* and *B. subtilis*). The inoculation with these microorganisms can accelerate the release into the rhizosphere of non-available inorganic or organic P and enrich the soil biologically, thus increasing crops yields (Paiva et al., 2020). In corn cultivation in the cities of Santa Maria-RS and Palotina-PR, the mixture of BiomaPhos ® with phosphate fertilization using 50% of the recommended dose, resulted in gains of 28% on yield, compared to the isolate effects of treatments only with phosphate fertilizer or the isolate strains (Paiva et al., 2020). In addition, products containing *Bacillus* strains are more stable in the soil due to the ability of *Bacillus* to form endospores, which allows the bacteria to resist extreme abiotic conditions, such as changes in pH and temperatures and the presence of pesticides (Bahadir et al., 2018).

The Brazilian Ministry of Agriculture, Livestock and Supply (MAPA, 2011) provides a list of microorganisms authorized for the production of inoculants. Biological inoculants are evaluated in accordance with prerequisites established by MAPA, following the instructions established by regulation no. 13/2011. The MAPA estimates that approximately 150 strains of microorganisms have characteristics that fit the pre-established parameters and have been used in the development of inoculants for different cultures.

3.2 P-solubilizing bacteria

In 1948 Pikovskaya discovered the role played by bacteria in the biogeochemical cycle of P, by mineralizing organic P and solubilizing insoluble inorganic phosphate. This study paved the way for broader research on bacterial genera that have this ability (Zhu et al., 2018), the biochemical processes involved (Kumar, 2016; Ameen et al., 2019), geographic location (Zhu et al., 2018) and environmental conditions in which each strain presents better performance and is able to survive (Prabhu et al., 2019).

Among the species reported as P solubilizers, those classified in the genera *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas* and *Rhizobium* (Table 1) stand out for their representativeness in studies. Bacteria of the genus *Bacillus*, *Pseudomonas* and *Rhizobium* are among the most abundant in the rhizosphere (Cheng et al., 2017).

Table 1. Phosphate-solubilizing bacteria species.

Species	References
<i>Azospirillum brasilense</i>	Rodriguez et al., 2004; Moraes et al., 2019
<i>A. lipoferum</i>	Rodriguez et al., 2004
<i>Bacillus circulans</i>	Sharma et al., 2013; Satyaprakash et al., 2017
<i>B. megaterium</i>	Chen et al., 2006; Sharma et al., 2013; Satyaprakash et al., 2017; Hajjam et al., 2017
<i>B. polymyxa</i> ; <i>B. subtilis</i>	Khan et al., 2009; Sharma et al., 2013; Thakur et al., 2014; Hajjam et al., 2017
<i>B. pulvifaciens</i>	Arora & Gaur 1979
<i>B. coagulans</i> ; <i>B. fusiformis</i> ; <i>B. pumilus</i> ; <i>B. chitinolyticus</i>	Sharma et al., 2013
<i>B. sircalmous</i>	Khan et al., 2009; Kumar et al., 2018
<i>Burkholderia cenocepacia</i>	Liu et al., 2019; You et al., 2020;
<i>B. cepacia</i>	Pande et al., 2019
<i>B. multivorans</i>	Zeng et al., 2017
<i>B. stabilis</i>	Hong-Sin et al., 2018
<i>B. tropica</i>	Anillo et al., 2016; Bernabeu et al., 2016
<i>Enterobacter asburiae</i>	Kumar et al., 2016
<i>E. cloacae</i>	Borham et al., 2017
<i>E. ludwigii</i>	Lee et al., 2019
<i>Thiobacillus ferrooxidans</i>	Sharma et al., 2013
<i>Pseudomonas canescens</i>	Alam et al., 2002
<i>P. putida</i>	Alam et al., 2002; Ghaderi et al., 2008; Khan et al., 2009; Thakur et al., 2014
<i>P. calcis</i>	Sharma et al., 2013
<i>P. fluorescens</i>	Ghaderi et al., 2008; Khan et al., 2009; Sharma et al., 2013; Thakur et al., 2014; Hajjam et al., 2017
<i>P. striata</i>	Khan et al., 2009; Sharma et al., 2013; Thakur et al., 2014; Hajjam et al., 2017
<i>Pantoea agglomerans</i>	Son et al., 2006
<i>Rhizobium meliloti</i>	Sharma et al., 2013
<i>R. leguminosarum</i>	Afzal & Bano 2008; Walpola & Yoon 2012; Hajjam et al., 2017
<i>Mesorhizobium mediterraneum</i>	Peix et al., 2001

Source: Authors.

In addition of improving P availability to plants, the PSB are also capable of mobilizing heavy metals toxic to plants and humans (Mohamed & Almaroai, 2017; Teng et al., 2019), generating benefits to the biodiversity of the micro and macrofauna of the soil, thus enabling cultivation in previously contaminated soils. In these circumstances, Ahemad (2015) assesses the use of PSB as an economically viable alternative, causing less impact on the environment, without compromising the soil structure, nor its microfauna, compared to mineral inputs traditionally used.

Irfan et al. (2019), applying a mix of different strains of PSB and carriers of inoculum in saline soil in maize crop, observed a significant increase in plant growth and corn production, for all treatments in relation to control regardless the level of salinity. Shahzad et al. (2017) observed similar results with wheat, due to the production of ACC deaminase by PSB, which catalyze ACC synthase enzymes released by plants under stress conditions, reducing the effects of high salt concentration. According to Nadeem et al. (2013), this process also favors the growth of the introduced microbial community, since the degradation of ACC synthase produces α -ketobutyrate and as a by-product ammonia, which is absorbed by microorganisms promoting a positive symbiotic relationship.

Several species of distinct taxonomic groups are reported as P solubilizers (Actinomycetes, Cyanobacteria, Bacteria and Fungi) and among them the strains of bacteria have received considerable attention. With approximately 101 to 1010 individuals per gram of soil within soil microbial communities, bacterial populations can exceed 2,000 kg ha⁻¹ of the live weight in these habitats, while PSB can represent between 1 and 50% of the total communities of these microorganisms (Khan et al., 2009).

The physiological characteristics and biochemical properties of specimens of bacteria make these microorganisms colonizers of a wide variety of habitats. With a cosmopolitan distribution, species of PSB have been mainly isolated from rhizospheric soil samples. However, evidence demonstrates a symbiotic relationship with several groups of plants, also being able to occur as endophytes (Donato et al., 2019).

Vassilev et al. (2012) reported the occurrence of P solubilization by bacteria under adverse conditions. Abiotic stresses such as water scarcity, pH changes (high or low), salinity and temperatures, apparently, did not exert negative influences on the solubilization phenomenon. Prabhu et al. (2019) highlight these characteristics and classify tolerant and extremophile phosphate solubilizing bacteria as potential bioinoculants, especially in anthropized agricultural areas. The effectiveness of PSB species has been reported in several conditions, such as high levels of salinity (Nakbanpote et al., 2014) and pH variations (Prabhu et al., 2018).

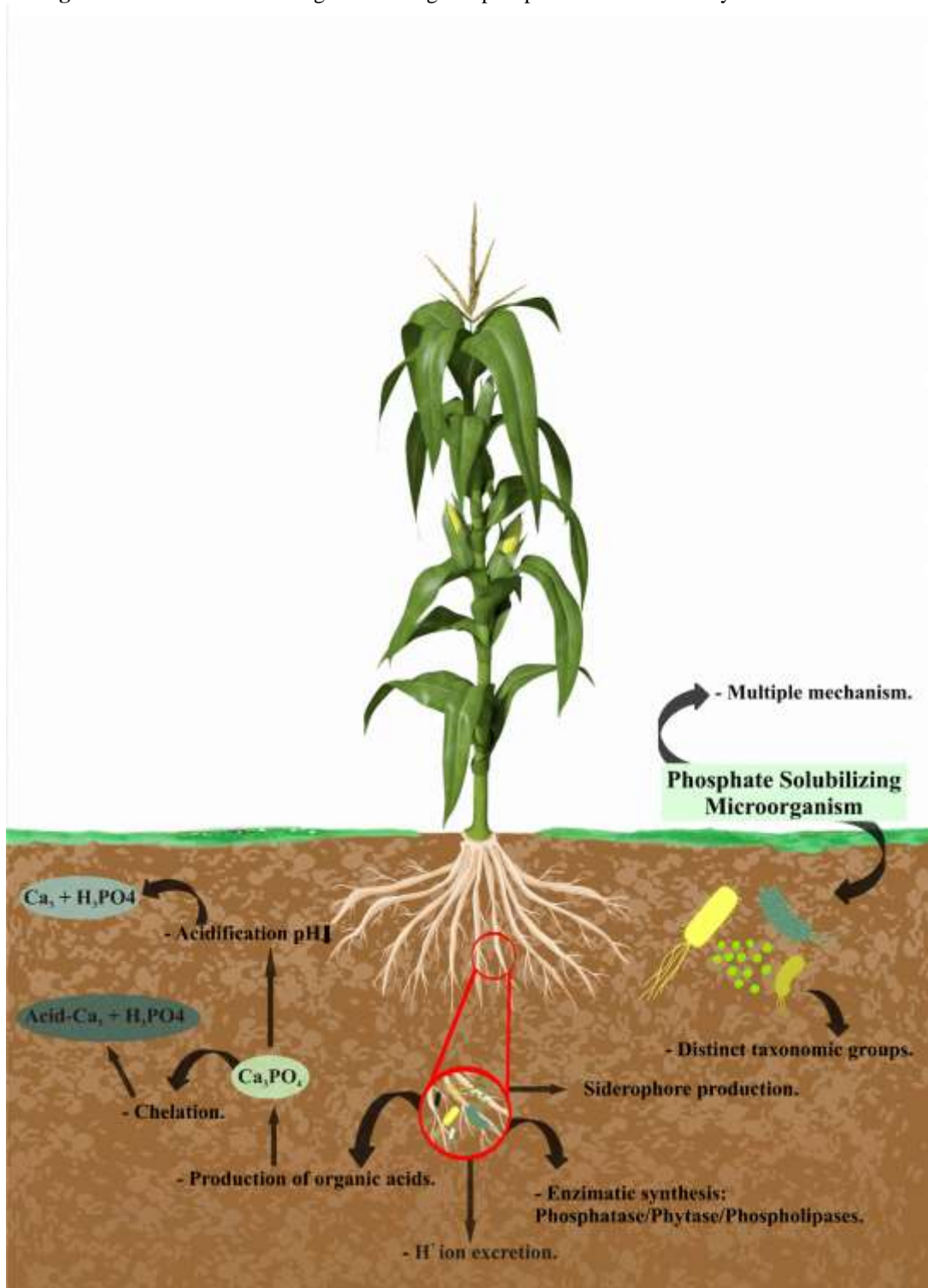
Benefits of the inoculation of PSB species have been reported in several crops, promoting the growth and development of eucalyptus (Foltran et al., 2019), cotton (Akhtar et al., 2010), beans (Belhardi et al., 2018), tomatoes (Anillo et al., 2016), and soybeans (Belhardi et al., 2018).

3.3 P solubilization mechanisms

Variations in the ability to solubilize P in soil, by PSB taxa, have been discussed in recent years (Gupta et al., 2012; Massenssini et al., 2015; Zhu et al., 2018). The increase in P availability has been documented, mainly, by species of the genera *Pseudomonas*, *Azotobacter*, *Burkholderia*, *Bacillus* and *Rhizobium* (Anillo et al., 2016; Hajjam & Cherkaoui, 2017; Belhardi et al., 2018). According to Zhu et al. (2018), the solubilization capacity can be attributed to factors such as species, lineage, soil properties (inherent and seasonal) and plant species present in the area. However, although the increase in productivity related to the promotion of P availability by the application of these microorganisms is widely recognized (Hajjam & Cherkaoui 2017; Belhardi et al., 2018), the mechanisms concerning this phenomenon are not entirely understood.

The microbial synthesis of different exudates, including organic acids, metabolites, and enzymes, has emerged as essential factors to P solubilization (Behera et al., 2014). Additionally, the reduction of soil pH, chelation and mineralization have been reported as important mechanisms for this phenomenon (Kalayu, 2019) (Figure 1).

Figure 1. Mechanisms of inorganic and organic phosphate solubilization by bacteria.



Source: Adapted from Prabhu et al. (2019).

In general, the action of solubilizing P by PSB would be closely related to four factors: i) synthesis of organic acids such as gluconic, isovaleric, oxalic, tartaric, succinic, citric, fumaric and butyric; ii) H⁺ proton extrusion mechanism, from the assimilation of NH₄⁺; iii) production of exopolysaccharides and iv) production of siderophores with high affinity for iron (Posso & De Prager, 2017).

Products of the bacterial metabolism, organic acids influence the natural dynamics of the P cycle. The synthesis of these acids has a strong positive correlation with the solubilization index and the decrease in soil pH (Satyaprakash et al., 2017). Studies conducted with bacteria of the genus *Acinetobacter*, have shown the solubilization of phosphates from gluconic, oxalic and butyric acids, providing better development of crops such as cabbage, cucumber, daisy, barley, wheat, and chickpeas (Kang et al., 2009; Ogut et al., 2010). According to Kalayu (2019), the efficiency of solubilization depends on the resistance and nature of the acids. Gluconic acid is reported as the main organic acid synthesized by PSB, such as *Acinetobacter* sp., *Pseudomonas* sp., *Enterobacter agglomerans* (*Erwinia herbicola*) and *Burkholderia cepacia* (Zhu et al., 2018).

Among the processes and mechanisms of P solubilization, chelating substances play an important role. The acids synthesized by PSB as 2-keto-gluconic acid, humic acid and fulvic acid convert insoluble forms of phosphate by cation chelation (Khan et al., 2009; Prabhu et al., 2019). These acids are strong chelators of tricalcium insoluble phosphates ($\text{Ca}_3(\text{PO}_4)_2$), aluminum phosphate (AlPO_4) and iron phosphate (FePO_4) (Khan et al., 2009).

The solubilization of organic phosphate in the soil occurs through the action of microbial enzymes (Jorquera et al., 2008). The production of extracellular enzymes such as phosphoesterases, phosphodiesterases, phytases and phospholipases by species of *Bacillus*, *Streptomyces* and *Pseudomonas*, for example, mineralize complex organic phosphates converting them into phosphate ions accessible for assimilation (Khan et al., 2009; Walpola & Yoon, 2012). Satyaprakash et al. (2017) also reported the production of siderophores, which hydrolyze organic P. However, Prabhu et al. (2019) warn that the relationship between siderophores synthesis and phosphate solubilization is not yet fully understood.

3.4 What a biological inoculant must have

Due to the expansion of agriculture and the high demand for fertilizers, alternatives that guarantee environmental security and that are economically viable are demanded by farmers. Among some of these alternatives, biofertilizers in the form of inoculants are characterized by being eco-sustainable, having low cost, however, they need specific formulation for use (Owen et al., 2015). Inoculants are inexpensive products, easy to purchase, have positive results regarding use in agriculture, providing great gains in production and savings in mineral fertilizers.

The success of microbial development after introduction of inoculants into the soil is linked to abiotic factors such as pH, temperature, humidity, and biotic factors such as competition for nutrients, substrates, predators and the presence of pathogens that compromise bacterial survival in the rhizosphere (Sivakumar et al., 2014; Reetha et al., 2014).

The microbial inoculant is a product that contains microorganisms favorable to plant growth. Classified as solids (powder, peat being a support for bacteria) and fluids (liquids, where bacteria already have their metabolic processes stabilized via cell protectors) (Prabhu et al., 2019).

According to the definition of the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA), in Decree 4.954 (2004), Art. 2, item V, inoculant is all the biological input with microorganisms capable of performing beneficial and necessary activities for the development of plants, produced in accordance with protocols stipulated by the Network of Laboratories for the Recommendation, Standardization and Diffusion of Technology of Microbial Inoculants of Agricultural Interest (RELARE). They are cheap products, offered in liquid, gel, peat and even new formulations. The inoculant in its liquid formula can be applied via seed and sowing furrow; the peat-based inoculant can only be applied via seed (Silva et al., 2009).

This material must have three essential characteristics: promote satisfactory bacterial development, keep the cells of microorganisms viable for a certain period, and ensure the gradual release of a bacterial population that will be beneficial to plants (Bashan et al., 2014; Cardoso & Estrada-Bonilla, 2019)

Biofertilizer is a liquid organic fertilizer, which can be produced in aerobic or anaerobic medium from a mixture of organic materials (manure, fruits, milk), minerals (macro and micronutrients) and water, from the fermentation of agricultural

residues or of animal waste. These biofertilizers can replace or complement chemical fertilization and can be used in soil or foliar applications (Sousa et al., 2013).

The term biofertilizer is also included in the Normative Instruction (IN) 64 of 2008 from the MAPA, which approves the technical regulation and the substances allowed for organic production systems. Biofertilizer is then defined as “product containing active components or biological agents, capable of acting directly or indirectly on all or part of the cultivated plants, improving the performance of production system and that is free of substances prohibited by the organic regulation”. Thus, this normative instruction incorporates beneficial microorganisms (fungi and bacteria) that can have a stimulating effect and could be considered biofertilizers in organic production.

3.5 Inoculant formulations

The inherent heterogeneity of any soil is the main obstacle to inoculation. The introduced bacteria can find niches in the plant rhizosphere colonized by other microorganisms. These introduced and unprotected bacteria must compete with the native microflora that is often better adapted and, in most cases, cannot withstand the predation of soil microfauna. In response, an important role for any inoculant formulation is to provide a more suitable microenvironment, combined with physical protection for an extended period (Bashan et al., 2016).

The potential substance must have stable physicochemical characteristics, which guarantee the integrity of its organoleptic properties, such as the conservation of its shape, composition, and molecular structure, as well as odor and texture, in order to not affect the number of viable cells, ensuring that after applying the product it will perform as expected (Arora & Mishra, 2016; Malusá et al., 2016).

Even with integrity, candidates for inoculum vehicles should not be a medium devoid of nutrients. It has to contain specific substances and chemical elements that can meet part of the energy demand that the microbial community needs to remain viable (Cortés-Patiño et al., 2015), as well as being able to present an adequate pH that promotes maintenance and propagation of microorganisms at the time of application. Inadequate formulations are the main barriers to the approval and commercialization of new inoculants (Stephens & Rask, 2000), as also the difficulty in adjusting formulas with physical, chemical, and biological characteristics capable of keeping the population of microorganisms viable over time (Silva et al., 2012).

Inoculation vehicles share the same fundamental principle, which is to ensure the survival of the microorganisms of interest, despite varying in physical condition (Husna et al., 2019; Bojkov et al., 2020). To this end, some criteria must be met before any substance or medium can be listed as a potential candidate, especially with regard to formulations that will be commercialized and must meet strict commercial and legislative standards.

Even though they are eligible for possible inoculating medium, raw materials must be significantly abundant, so their extraction and production costs turn them financially viable. Another key point that must be addressed is non-toxicity (Hale et al., 2015) and the use of renewable resources, which ensure continuous and sustainable exploration.

According to Bashan et al. (2014), there are five classes of inoculum-carrying materials: (1) peat, coal, biochar, clay and inorganic soil; (2) waste from plants of various industrial and agricultural origins; (3) inert materials, polymers and treated rock fragments; (4) freeze-dried microbial cultures, mixed with oil and dry bacteria; and (5) inoculating liquids, with the addition of chemicals that improve viscosity, stability, surface tension, function and dispersion.

Liquid formulations are suspensions that contain components that improve the viscosity and stability of the solution (Bashan et al., 2014). The main advantage of this formulation is its easy handling, being preferred by developed countries.

Polymeric formulations have been tested as an industrial substitute for commercial peat. They exhibit several advantageous characteristics including longer shelf life, viability and density of strains, ease of manufacture and better

performance in the field. Some examples of polymers are chitosan, alginate, agar, bean gum, among others (Bashan et al., 2014).

Peat is the most used worldwide as a rhizobium carrier, in powder or participating in the formulation of organic composts, except in Africa and Asia where this material is expensive. One of the problems of peat is its stability, due to its characteristics in terms of particle size, pH, humidity, shelf life (Deaker et al., 2004; 2011). Another problem is the finite source, since large world reserves of peat with characteristics conducive as vehicles of inoculation are not available. Alternative materials to peat were tested in the years 1980's and 1990's as charcoal, mud, coconut powder, sugar cane filter, but all obtained worse results when compared to peat (Singleton et al., 2002). Although other organic inputs have had positive results, the main obstacle is their availability in quantity to supply the demand of the market.

3.6 Viability of biochar as a component of microbial inoculants

Biochar is a fine-grained carbonaceous material with high organic carbon content and largely resistant to microbial decomposition. This material can be obtained through thermal decomposition (pyrolysis) of biomass, under limited oxygen conditions (Medeiros et al., 2020b) and temperature between 300 to 900 °C (Pandey et al., 2020). Several raw materials can be employed in biochar production, such as crop residues, bark like coffee (Lima et al., 2018), wood scraps (logs, bark, branches), grasses and other residues from agriculture, livestock and agro-industries (Medeiros et al., 2020a).

When applied to the soil, the biochar creates a carbon reserve, serving as a network that removes CO₂ from the atmosphere and stores it in the soil in the form of recalcitrant carbon (Lehmann & Joseph 2009), makes the soil suitable for carbon sequestration. Biochar has been widely stimulated and recognized as a C sequestering vehicle, and the United Nations meeting on climate change in Copenhagen describes in the draft negotiating text: “We must pay special attention to the role of soils in carbon sequestration, including the use of biochar and carbon sinks in arid lands”.

Furthermore, the application of biochar in agriculture brings several benefits as it helps to reduce nutrients leaching as well plant requirements of irrigation and fertilizers, due to the capacity of retaining nutrients and water (Lima et al., 2018; Razzaghi et al., 2020); acts as a soil conditioner (Aamer et al., 2020; Ye et al., 2020); increases the cation exchange capacity, as it presents negatively charged functional groups on its surface (Lara et al., 2013) and acts on soil aggregation (Amoah-Antwi et al., 2020). Biochar also increases carbon stability, adsorption and / or complexation of organic matter and toxic components and improves the microbial health of the soil (Li et al., 2017). The high specific surface area and the porosity of the biochar create an environment that promotes microbial growth, such as bacteria, ectomycorrhizal fungi, mycorrhizal fungi, and arbuscular mycorrhizal fungi (Palansooriya et al., 2019), enzymatic activities and nutrient cycling (Wang et al., 2016), and as an alternative for suppressing plant diseases (Debode et al., 2020).

As P is normally released at temperatures higher than 800 °C, it is most likely that pyrolysis is not sufficient to turn it available, therefore, P can be retained in the biochar in inorganic or organic form (Schmalenberger et al., 2016). Recent studies suggest that the interaction of biochar in the soil can also influence the availability of P, due to changes in pH, in enzymatic efficiency, in the formation of organo-mineral complexes, which can increase the solubility of P through changes induced in the soil microbial community (Du et al., 2019; Li et al., 2020; Shi et al., 2020). Given these advantages for the physical, chemical, and biological properties of the soil (Butnan et al., 2015), biochar emerges as a promising and ecologically favorable alternative for enhancing yields of agricultural crops (Butnan et al., 2015; Martins Filho, 2020).

Moreover, biochar can be an efficient inoculum component for some microorganisms (Medeiros et al., 2020a). Qian et al. (2020), when evaluating the biotransformation of P in the biochar on the behavior of *Pseudomonas putida*, proved that biochar can be a potential P fertilizer. However, it must be taken into account the type of raw material and temperature used for biochar production, which influences its performance. Medeiros et al. (2020a) evaluated biochars from coffee residues and found that it can be an inoculation vehicle for *Trichoderma aureoviridae*, a multifunctional microorganism in the soil. Yan et al. (2020)

reinforces the statement that the increase in the availability of P through biochar varies according to the residue used and the pyrolysis temperature, emphasizing that higher temperatures favor the supply of P.

The patent literature shows in document CN108083908 (A) that biochar mixed with maleic acid, ammonium nitrate and a mixed microbial agent composed of saccharomycetes, photosynthetic bacteria, *Bacillus subtilis*, rhizobia, *Trichoderma viride* and *Bacillus* is used as a microbial fertilizer, biofertilizer and / or soil modifier. Another product contains 95-98% biochar and 2-4% probiotic agent, consisting of *Paenibacillus polymyxa*, *Trichoderma* strains, rhizobia, nitrogen-fixing bacteria, phosphate solubilizers, potassium, cellulose decomposers, antibiotic, and photosynthetic generators (CN102660291 (A)). Another bean husk biochar was inoculated with *Trichoderma aureoviride* for the formulation of a biofertilizer (BR1020180163680). The biochar was also used to fix a mixture of microbial agents such as *Shewanella putrefaciens*, *Geobacter metallireducens*, *Trichoderma viride* and *Trichoderma harzianum* and a protective bacterium, *Bacillus subtilis* (CN109609417 (A)).

In this sense, biochar can be an economical and environmentally friendly alternative, as it serves as a tool for reusing waste generated by the agribusiness that generates 200 million tons of biomass from waste in Brazil (Martinez et al., 2019). In view of the extensive literature showing positive results from the use of biochar in agricultural soils, it was considered an ecological fertilizer for sustainable and modern agriculture (Chen et al., 2018), and as a member of microbial inoculants (Medeiros et al., 2020a, b).

4. Conclusion

As most Brazilian soils are deficient in phosphorus and the phosphate sources are non-renewable natural resources, the efficient use of P in agriculture is essential. The production of biofertilizers and bioinoculants are strategies for introducing microorganisms able to improve P availability to plants. In addition, the selection of strains of bacteria that are highly efficient in solubilizing P and adapting to different agrosystems are crucial to achieve the successful performance of P supply to plants. The use of biochar brings several benefits to the soil and plants, as well as to the environment, as a tool to recycle residues from the agribusiness. This review showed how biochar can be promising as component of a possible inoculant and for the formulation of new products. However, studies aiming the use of biochar combined with P-solubilizing bacteria is still incipient. Future research should focus efforts on exploring highly efficient strains, optimizing conditions, and assessing several sources of waste for production of biochar and their efficiency in field experiments.

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