Bacillus: a eco-friendly source for plant development and sanity

Bacillus: uma fonte eco-friendly para o desenvolvimento e sanidade de plantas

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Abstract

Bacillus are non-pathogenic gram-positive bacteria capable of producing resistant spores. They have several beneficial characteristics to crop production, such as increased plant growth, resistance to abiotic/biotic stresses, nutritional improvement, and potential for bioremediation of contaminated soils. The use of microorganisms in agriculture has increased due to the reduction in the use of agrochemicals, and sustainable production. Therefore, the present study was based on bibliographical research on the current state of the art of biotechnological prospecting and obtaining commercial products of *Bacillus* for the agricultural sector. The search was conducted through the Google Academic platform limiting the search to the years 2015 to 2020. Papers that explained in vitro or in vivo prospecting methodologies of *Bacillus* with promoter or protective activity of plants and those that described microbial inoculants with this genus were selected. Different species of *Bacillus* can modulate plant growth producing plant hormones. Molecular biology tools such as DNA sequencing and PCR are applied to the study of genes involved in plant growth modulation by *Bacillus* such as AcPho, ipdC and asbA. For the elaboration of agricultural bioproducts, vegetative structures or endospores are used, under an optimal concentration of 1 x 108 CFU/mL. In the face of species diversity, many have not yet been studied and are promising to become bioproducts. This fact is enhanced by the immeasurable applicability of *Bacillus*.

Keywords: Microbial inoculants; Modulation of plant growth; Plant protection.

Resumo

Bacillus são bactérias gram-positivas não patogênicas capazes de produzir esporos resistentes. Possuem várias características benéficas à produção agrícola, como promoção do crescimento das plantas, resistência a estresses abióticos/bióticos, melhora nutricional e potencial de bioremediação de solos contaminados. O uso de microrganismos na agricultura tem aumentado devido à redução do uso de agrotóxicos e à produção sustentável. Portanto, o presente estudo baseou-se em pesquisas bibliográficas sobre o estado atual da arte da prospecção biotecnológica e da obtenção de produtos comerciais do *Bacillus* para o setor agrícola. A pesquisa foi realizada através da plataforma Google Acadêmico, limitada a busca aos anos de 2015 a 2020. Foram selecionados artigos que explicavam metodologias de prospecção in vitro ou in vivo de *Bacillus* com atividade promotora ou protetora de plantas e aqueles que descreviam inoculantes com esse gênero. Diferentes espécies de *Bacillus* podem modular o crescimento das plantas produzindo hormônios vegetais. Ferramentas de biologia molecular, como sequenciamento de DNA e PCR, são aplicadas ao estudo de genes envolvidos na modulação do crescimento vegetal pelo *Bacillus*, como AcPho, ipdC e asbA. Para a elaboração de bioprodutos agrícolas, são utilizadas estruturas vegetativas ou endósporos, sob uma concentração ideal de 1 x 108 UFC/mL. Diante da diversidade de espécies, muitos ainda não foram estudados e prometem se tornar bioprodutos. Este fato é potencializado pela aplicabilidade imensurável do *Bacillus*.

Palavras-chave: Inoculantes microbianos; Modulação do crescimento vegetal; Proteção de plantas.

Resumen

Bacillus son bacterias grampositivas no patógenas capaces de producir esporas resistentes. Tienen varias características beneficiosas para la producción agrícola, como la promoción del crecimiento de las plantas, la resistencia al estrés abiótico/biótico, la mejora nutricional y el potencial de biorremediación de los suelos contaminados. El uso de microorganismos en la agricultura ha aumentado debido a la reducción del uso de pesticidas y la producción sostenible. Por lo tanto, el presente estudio se basó en la investigación bibliográfica sobre el estado actual del arte de la prospección biotecnológica y la obtención de productos de bacilo comercial para el sector agrícola. La búsqueda se realizó a través de la plataforma Google Academic, limitando la búsqueda a los años 2015 a 2020. Se seleccionaron artículos que explicaban metodologías de prospección de bacilos *in vitro* o *in vivo* con actividad promotora o protectora de plantas y aquellos que describían inoculantes con este género. Diferentes especies de *Bacillus* pueden modular el crecimiento de las plantas mediante la producción de hormonas vegetales. Las herramientas de biología molecular, como la secuenciación del ADN y la PCR, se aplican al estudio de genes implicados en la modulación del crecimiento de las plantas por *Bacillus*, como AcPho, ipdC y asbA. Para la elaboración de bioproductos agrícolas se utilizan estructuras vegetativas o esporas finales, bajo una concentración ideal de 1 x 108 UFC/mL. Dada la diversidad de especies, muchas aún no han sido estudiadas y prometen convertirse en bioproductos. Este hecho se ve reforzado por la incommensurable aplicabilidad de *Bacillus*.

Palabras clave: Inoculantes microbianos; Modulación del crecimiento de las plantas; Protección fitosanitaria.

1. Introduction

The application of beneficial microorganisms for agronomic use has been increasing, due to the food demand and to the need of environmental sustainability, booth favored by the lower use of agrochemicals and adoption of ecofriendly technologies (Gonçalves, et al., 2017; Mattei, et al., 2017). In consonance with this information, at 2020 was achieved a record of registers of biological products for agronomic use at Brazil (MAPA - Ministério Da Agricultura Pecuária E Abastecimento, 2020).

Bioprospection of microorganisms as plant growth promoters and to biological control is the aim of many studies that search organisms to support the production increase. The most frequently related bacteria include *Bacillus* sp., capable of the formation of resistance spores, commonly called endospores (Posada, et al., 2016).

Bacillus show a diversity of beneficial characteristics when applied to different crops, as increase of plants growth, resistance to biotic and abiotic stress, better nutrient uptake, and others, including the bioremediation potential to recover soils presenting xenophobic and recalcitrant substances as contaminants (Sessitsch, et al., 2018; Schulze, 2019).

The Genus *Bacillus* has a higher diversity of species and commercial strains indicated as insecticides, fungicides and nematicides (Adapar, 2020). Besides this, the literature confirms the induction of systemic resistance, increasing enzymes related to the oxidative stress and formation of reactive oxygen species (ROS), in plants treated with *Bacillus* (Garcia, et al., 2016).

The application of these microorganisms to crops production demand the formulation of bioproducts named Microbial Inoculants for Agriculture. These inoculants from microbial source, can have in its formulation the microorganism and subproducts, as enzymes, secondary metabolites or even nanoparticles, that can be applied by seed treatment or spraying (Dubey, et al., 2020).

Therefore, the present review was based on the actual status of biotechnological prospection and obtention of commercial products of *Bacillus* for agriculture use.

2. Methodology

The search was conducted using the Scholar Google platform, between the years of 2015 and 2020, using as descriptors: bioprospection, bacteria, agroindustry, plant growth promoting bacteria, vegetal sanity, microbial inoculants for agriculture. All these descriptors were used with *Bacillus* (Descriptor + *Bacillus*). Papers about bioprospection in vitro or in vivo of *Bacillus* sp. showing activity of plant growth promotion, plant protection, or describing microbial inoculants of

Bacillus were selected, totalizing 29 articles. Those articles were used to produce a narrative review.

3. Results and Discussion

3.1 The genus bacillus and the agricultural use

Bacillus are gram-positive nonpathogenic bacteria, used to produce extracellular enzymes, capable of production of resistance spores under to adverse environmental conditions, as extreme high temperatures (Alves, et al., 2018), and capable of capture exogen DNA, performing natural transformation. These genus show a widely use, related to the biodegradability, good ecological acceptance and low toxicity (Soares, et al., 2018).

The bacteria of Genus *Bacillus* are known for its potential to plagues and diseases control. The association *Bacillus*-plants can be observed by endophytic development of the bacteria or by biofilm formation covering plant tissue (Shameer, 2016; Dubey, et al., 2020; Soares, et al., 2020) (Figure 1).

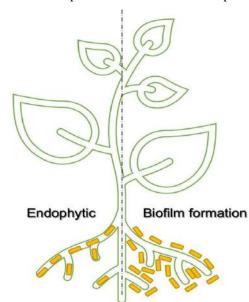


Figure 1 - Forms of development and colonization of plants by Bacillus.

Source: Adapted from Dubey, et al. (2020), Shameer (2016) and Soares, et al. (2020).

So, as the Figure 1 shows, benefic microorganisms can develop inside the plant tissue as endophytic microorganisms, or by formation of a biofilm, growing outside of plant tissues, consuming plant exudates. According Turubui, et al. (2018), among the plant tissues where endophytic microorganisms develop are roots, stalk and leaves of bryophytes and angiosperms, growing between or inside to plant cells, but without cause any damage.

Bacillus can play different rules at crop field, favoring the production increase, contributing to the defense system, mitigating plant stress, or even bioremediating herbicides (Sessitsch, et al., 2018; Schulze, 2019), consequently, this microorganism can act directly on plagues and disease control, or indirectly, favoring plant development and mitigating abiotic stress.

At selection of strains searching for the agriculture use, microorganisms with potential for inducting different crops show higher value. The Genus *Bacillus* is commonly related, probable because the plant-*Bacillus* interaction, due to the plant perception and response to this microorganism (Jochum et al., 2019). This genus was reported as friendly-associated with species as *Panicum antidotale*, *Tribulus terrestris*, *Zygophyllum simplex*,

Euphorbium officinarum, Lasiurus scindicus (Bokhari, et al., 2019), maize (Zea mays), soybean (Glycine max), cowpea (Vigna unguiculata) and rice (Oryza sativa) (Chagas, et al., 2017).

The isolation of *Bacillus* sp. from maize plants collected from experimental field at Juazeiro, Bahia, Brazil, named strain BS7, showed better results when compared to other strains from semiarid [BS24 (*Bacillus* sp.) and 6.2 (*Rhizobium* sp.)], presenting similarity to the commercial inoculant evaluated, applied to maize (Souza, et al., 2018), indicating potential to agronomic use. Thus, this perspective has shown potential of these microorganisms in the promotion of vegetal development and sanity, mitigating biotic and abiotic stress (Cruz, et al., 2018).

3.2 Modulation of plant growth by phytohormones production and selection of bacillus

At the plant rhizosphere, *Bacillus* can act on as plant growth promoter, favoring hormone production (Figure 2).

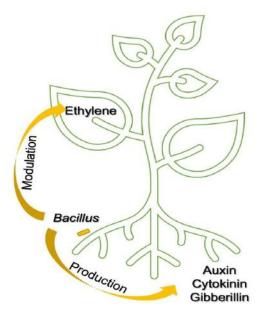


Figure 2 - Modulators of plant growth produced by Bacillus and modulated by the plant-Bacillus relation.

Source: Adapted from Garcia, et al. (2016), Lyngwi, et al. (2016) and Silva, et al. (2019).

The Figure 2, show Bacillus growing as a rhizosphere plant growth promoter bacteria, producing and providing to the plant auxin, cytokinin and gibberellin. Additionally, *Bacillus* promote ethylene modulation, by producing ACC deaminase, preventing ethylene production, which could be useful to mitigate abiotic stress promoted by water deficit. Those information were reported in littetarure. Concerning the *Bacillus* hormone production, cytokinins, gibereline (Silva, et al., 2019), and auxins, as indoleacetic acid (IAA), were reported. The ethylene production is also modulated by *Bacillus*. As an example, *Bacillus aryabhattai* can produce 1-amino ciclopropane-1-carboxilate (ACC) deaminase, preventing the ethylene production and regulating the ethylene-ACC balance (Garcia, et al., 2016; Lyngwi, et al., 2016).

Endophytic species as *Bacillus cereus*, described as plant growth promoters, enhanced plant top growth until 1,9 times (Maheshwari, et al., 2019). On this way, some authors are searching for the biological activities responsible for the promotion of plant growth and development (Table 1).

Table 1 - *Bacillus* species and respective biological activities of hormones production (H): auxin (AX), cytokinin (CK) e gibberellin (GA), modulation of ethylene (ET); antimicrobials production (Ant), better nutrition by solubilization of phosphate (SP) and zinc (SZn), production of siderophores (Sid), ammonia (NH3), organic acids (AO) and hydrogen cyanate (HCN).

Species	Н	Ant	SP	SZn	Sid	NH ₃	AO	HCN
B. aryabhattai			+					
B. badius	AX	+		+		+		
B. cereus	AX							
	ET	+	+		+	+		+
B. circulans	AX	+				+		
B. flexus	ET		+					
B. humi	AX							
	ET		+					
B. marisflavi	ET							
B. methylotrophicus	ET		+					
B. mojavensis		+				+		
B. mycoides	ET		+		+			
B. psychrosaccharolyticus	ET		+					
B. safensis	ET							
B. simplex	ET		+					
D	AX							
B. sonorensis	ET		+			+	+	
	ET	+						
B. subtilis	AX		+		+	+	+	+
B. tequilensis		+			+	+		
B. weihenstephanensis			+					
	AX							
Bacillus spp.	СК		+					
	GA							

Note: +: positive. Source: Adapted from Lyngwi, et al. (2016), Silva, et al. (2019), Maheshwari, et al. (2019) and Bokhari, et al. (2019).

Biochemical and molecular technic as Polimerase Chain Reaction (PCR) and DNA sequencing, can be used for strain selection of *Bacillus* with potential to plant growth promotion. Lyngwi, et al. (2016) using the 16S rRNA segment to sequencing this genus from soil samples, identified the biosynthesis of acid phosphatase, an enzyme responsible for phosphate solubilization, indolpiruvate descarboxilase and 1-amino ciclopropane-1-carboxilate desaminase, enzymes related to the ACC desamiase activity. Besides this, the authors also detected the presence of proteins associated to the siderophores biosynthesis. Those activities are important to the plant growth promotion.

Real time PCR (RT-PCR), can be a useful tool to bioprospection, allowing to identify the presence of several genes presented by the plant growth promotion microorganisms. As example, the AcPho genes, responsible for the acid phosphatase; ipdC, that codify the indolpuruvate descarboxilase synthesis; aacd, related to the 1- amino ciclopropane-1-carboxilate deaminase; asbA, associated to the siderophores biosynthesis. Those genes are related to the phosphate solubilization, auxin synthesis (indoleacetic acid), ethylene modulation, and siderophores productions, respectively (Lyngwi, et al., 2016).

Bokhari, et al. (2019) studying 58 strains of *Bacillus* by 16S rRNA region sequencing, pointed that the use of molecular biology techniques like DNA sequencing, allow separate species by groups of similarity, applying phylogenetic analysis. Those, are important due to the possibility to test an isolate from each similarity group, produced on specifics substrates, as liquids medium, essential to produce the commercial products, to investigate potential agents to apply to the plant growth promotion and development (Bokhari, et al., 2019). The knowledge of metabolic pathway responsible for the production and modulation offered by the microorganisms could be achieved by use of metagenomic techniques and genetic engineering, ensuring comprehension about the production and sponsoring optimization of the biosynthesis process (Nofiani, et al., 2018), supporting the large-scale manufacture of bioactive molecules and bioproducts of interest.

3.3 Plant nutrition supported through salt solubilization and siderophores production mediated by bacillus

Some mechanisms evolved on the nutrition and plant growth are mediated by Bacillus sp. (Figure 3).

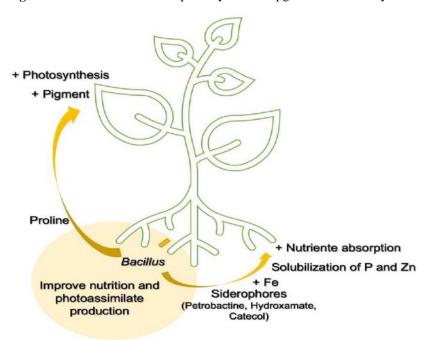


Figure 3 - Plant nutrition and photosynthesis upgrade mediated by *Bacillus*.

Source: Adapted from Bokhari, et al. (2019), Dubey, et al. (2020), Garcia, et al. (2016) and Lyngwi, et al. (2016).

The Figura 3 highlights the improvement of nutrition and photoassimilates productions by *Bacillus*, which is related to the proline production, promoting increase on pigments production and photosynthesis, besides the production of diverse siderophores, improving the Fe uptake, and the promotion of solubilization of P and Zn, improving the nutrient uptake. In accordance with this, Ikeda, et al. (2020) reported inorganic phosphate solubilization, siderophores production and biological nitrogen fixation are showed in *Bacillus*, corroborating its application as a microbial inoculant ecofriendly, reducing the chemical fertilizers requirement. Phosphate solubilization were reported by Garcia, et al. (2016) studying *B. safensis* and Lyngwi, et al. (2016) studying *B. thuringiensis*. Ikeda, et al. (2020) described the strain LMGB225 of *Bacillus* sp. as a phosphate solubilizer, increasing the levels of phosphate on maize leaf tissue.

Siderophores released by *B. methylotrophicus*, *B. thuringiensis* e *B. aryabhattai*, were studied. Those species exhibited capacity to produce petrobactin, a catecoic siderophore. Another report, carried out with *B. safensis*, detected hydroxamate siderophores. Regardless the type, the siderophores play a role on iron chelate production, increasing the plant uptake of iron (Garcia, et al., 2016; Lyngwi, et al., 2016).

Recently, the zinc solubilization by *B. cereus* was described (Bokhari, et al., 2019), as well as the intensification of proline production, increasing levels of photosynthetic pigments (Dubey, et al., 2020), resulting in a higher level of photo-assimilates and production.

3.4 antimicrobial compounds production, disease and plague control and systemic resistance induction in plants mediated by *bacillus*

The disease control through *Bacillus* can include district mechanisms, as the presence of antimicrobial metabolites, competition, and resistance induction against pathogens, are underlined. The siderophores production, as illustration, may induce the competition for iron uptake, and is one of the factors emphasized like an antagonism role played by *Bacillus* against a extensive range of plant pathogens, including a diversity of fungi and bacteria (Ikeda, et al., 2020). B. thuringiensis excrete some exotoxins (α , β , γ and δ , factor lice and factor rat), Vip3A, and enterotoxin which present distinct characteristics (thermostability, crystallin proteins, etc.) that can be useful to the biological control of plagues (Soares, et al., 2020).

Lytic enzymes produced in this group of bacteria might act on the cell wall of phytopathogens (Garcia, et al., 2016). Iturine and fengicine (peptides), and 14 volatile compounds produced by *B. subtilis* showed antimicrobial activity (Soares, et al., 2018). It is also those compounds responsible to produce a antimicrobial effect in vitro against Xanthomonas sp. and some phytopathogenic fungi as *Curvularia* sp., *Phytophthora* sp., *Rhizoctonia* sp., *Fusarium* sp., *Verticillium* sp. and *Sclerotinia* sp. (Gonçalvez, et al., 2017; Silva, et al., 2019; Gabardo, et al., 2020). On the other hand, *B. velezensis* show a wide-ranging potential as biopesticide and biofungicide, due to its potential to produce biofilms and lipopeptides (Dubey, et al., 2020).

Different strains of *Bacillus* promote the plant growth, specially under stress conditions, which is interconnected to the plant compounds production leading the bacteria to produce some factors improving and allowing its development under adverse conditions (Bokhari, et al., 2019). Jochum, et al. (2019) discovered a *Bacillus* sp. efficient to mitigate hydric stress in maize and wheat plants. Pranaw, et al. (2020) noticed that *B. altitudinis* (strain KP-14), capable of plant growth promotion on *Miscanthus giganteus*, notoriously expanded the biomass when plants were cultivated under saline stress or under soils presenting heavy metal contamination.

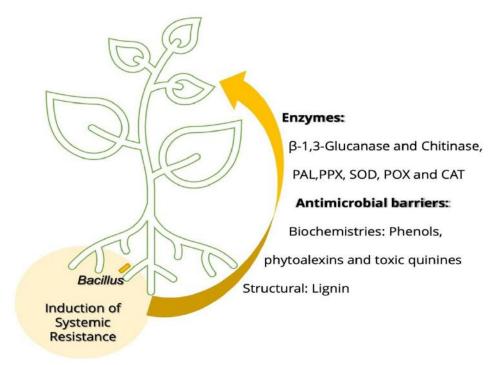
Promising results about *Bacillus* aptitude to control pathogens in vivo were reported by Garcia, et al. (2016), De Farias, et al. (2019) and Chamedjeu, et al. (2019). Garcia, et al. (2016) applying a suspension of *B. subtilis* (2,2 x 109 UFC/mL) on seeds and roots of rice to evaluate the plant growth and control of *Rhizoctonia solani*, noticed the seed germination promotion, and differences on height, tillering and grain production on treated and non-treated plants. The authors suggested that the results might be linked to the siderophores production (activity also showed by the strain), competition for the soil organic matter, and inhibition of the mycelial development of the pathogen.

De Farias, et al. (2019) studying the control of *Fusarium oxysporum* f. sp. vasinfectum wilt of cotton crop ('Mocó', 'Topázio' and 'BRS 286'), applying a suspension of 9 x 109 UFC/mL of *B. subtilis* as seed treatment, observed disease incidence between 39,5% to 59% for *B. subtilis*-treated plants, while 94,5% to 98,5% of disease incidence were noticed to non-treated plants, and 24% to 32% of disease incidence to commercial fungicide-treated plants.

Ralstonia solanacearum wilt on potato require the adoption of management, although many strategies do not present efficiency, owing to the host variability. *B. cereus* e *B. subtilis* applied to potato inhibited the disease, allowing the suggestion of its application as a management strategy (Chamedjeu, et al., 2019).

Despite of the phytopathogens control, this bacterias also presented potencial to systemic resistance intuction on plants. The induction could be mediated by different compounds, as effectors and molecular patterns associated to microorganisms, recongnized by receptors (Pascholati, et al., 2015) (Figure 4).

Figure 4 - Resistance mechanism role on the systemic resistance of plants mediated by *Bacillus*, favoring the resistance against abiotic and biotic stress.



Source: Adapted from Dubey, et al. (2020) and Garcia, et al. (2016).

The Figure 4 show *Bacillus* acting as a resistance inductor, promoting an upgrade to tissue structure, by improving the lignin content, and upgrading the biochemical resistance response by improving enzyme activity or content, of enzymes such as β -1,3-glucanase, chitinase, catalase (CAT), peroxidases (POX), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO). Those activities are shown by the species *B. subtilis* CSR-G-1, *B. pumilus* CSR-B-2, *B. marisflavi* CSR-G-4, *B. saffensis* CSR-G-5, *B. thuringiensis* CSRB-3 and *B. cereus* CSR-B-1. Those species might act as resistance inductors, displaying activity of enzimes as CAT, POX, PAL and PPO (Dubey, et al., 2020), favoring the production of phenol compounds, phytoalexins, lignin and toxic quinines. Moreover, β -1,3-glucanase and chitinase were related (Garcia, et al., 2016).

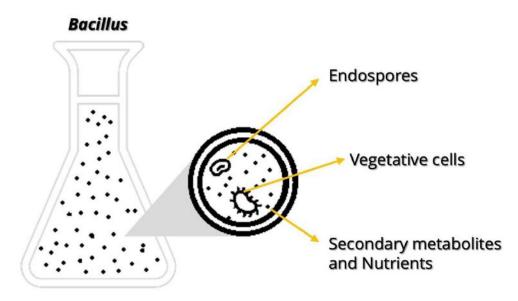
3.5 Bacillus: commercial products available for disease control in plants

In the face of the diversity of *Bacillus* species, many not yet studied and promising to become bioproducts, may act in future agricultural production (Bokhari, et al., 2019). This fact is enhanced not only by the wide applicability of bacteria use of this genus, but by the characteristics of degradability, ecological compatibility and low toxicity (Soares, et al., 2018).

The application of *Bacillus* for the development of commercial products is driven by being aerobic bacteria capable of producing endospores, which are cells resistant to high temperatures, desiccation, UV radiation, absence of nutrients, and exposure to chemical solvents. Endospores can remain dormant for years, germinating and resuming the vegetative state quickly, under favorable condition that involves availability of water and nutrients (Pereira & Martins, 2016). The elaboration of products using endospores favors resistance to the bio-controller in the field, in the formulation of stable products, and with longer shelf life (Liu, et al., 2015; Alves, et al., 2018).

For the elaboration of products consisting of *Bacillus*, the reproductive process of the bacteria is carried out by fermentation, and renewable raw materials can be used. Thus, the obtention of products presenting reduced cost, but with great added value, and environmentally correct, could be achieved (Soares, et al., 2018). Figure 5 shows the different components observed after fermentation processes of these products, including endospores, vegetative cells, secondary metabolites, and nutrients.

Figure 5 - Composition of *Bacillus* fermented, demonstrating different components that can be used in products for agricultural use.



Source: Adapted from Posada, et al. (2016).

The culture media in bioreactors is variable with species, strains, and objective. For example, the reproduction of *B. thuringiensis* may use higher concentrations of sugars and starch, while for *B. sphaericus* the culture medium presents higher demand for amino acids and proteins (Pereira & Martins, 2016).

Purification of these bioproducts, after the fermentation processes, could be carried out using salting-out and flocculation, which can be performed with or without the presence of ethanol, although, it is worth noting that ethanol impairs the enzymatic viability (Beretta & Júnior, 2017). Another alternative are the chromatography techniques that allow the identification of bioactive molecules (Wang et al., 2017).

Some of commercial *Bacillus* products, formulated using just one specie recorded in MAPA and released for use in the state of Paraná, Brazil, aiming at the control of pathogenic agents of plant disease, are presented at Table 2.

Table 2 – Commercial products (CP) based on a single species of Bacillus registered by the Ministério da Agricultura							
Pecuária e Abastecimento (MAPA) released, according to the Agricultural Defense Agency of Paraná, aiming at							
disease control.							

Active Ingredient	PC (Enterprise)	Strain (Concentration) ¹	Application ²	Target ³	
	Boneville [®] Chevelle [®] Veraneio [®] (Koppert [®])	UMAF6614 (1 x 10 ¹⁰ CFU/g)	ST	MI; MJ; PB	
B. amyloliquefaciens	Duravel [®] (BASF [®])	MBI600 (5,5 x 10 ¹⁰ EV/g)	Р	BC; BS; CP; PC; PU; RS; XC	
	Eco-Shot [®] (IHARA [®])	D-747 (5 x 10 ¹⁰ CFU/g)	Р	AS; BC; CG; CP; EP; PC; PF; SS	
	Nemacontrol [®] (Simbiose [®])	SIMBI BS10 CCT7600 (1 x 10 ¹⁰ CFU/mL)	ST	MJ; PB	
	No-Nema [®] (Biovalens [®])	BV03 (3 x 10 ⁹ CFU/mL))	ST; F; S; I	MI; MJ; PB; HG	
	Eficaz Nema [®] (Simbiose [®])	SIMBI BS10 CCT7600 (1 x 10 ¹⁰ CFU/mL)	ST	РВ	
B firmus	Votivo Prime [®] (BASF [®])	I-1582 (4,8x10 ⁹ CFU/mL)	ST	MJ; PB	
B. metylotrophicus	Onix OG [®] (Lallemand [®])	UFPEDA 20 (1 x 10 ⁹ CFU/mL	ST; F; P	MJ; PB	
B. pumilus To be continue	Sonata [®] (Bayer [®])	QST 2808 (1x10 ⁹ CFU/g)	Р	CL; AP; BC; PF; SM; UN; AS; CP	
Continuation table 2	Bio-imune [®] (Biovalens [®])	BV02 (3 x 10 ⁹ CFU/mL)	Р	CT; HV; XV; UN; AS; CG; CA; PS; PP; CL; SS	
	Biobac [®] (UPL [®])	Y1336 (1x10 ⁹ CFU/g)	S; P	HV; AP; BC; NP; RS; MI	
B. subtilis	BV09 (1x10 ⁸ CFU/mL)	I; E	MI; MJ; ME; MP; FO	Biobaci [®] (Biovalens [®])	
	Furatrop [®] (Biotrop [®])	CNPSo 2657 (1,9x10 ¹² CFU/L)	F	MJ	
	Rizos OG [®] (Lallemand [®])	UFPEDA 764 (3x10 ⁹ CFU/mL)	ST; P	MJ; PB	
	Serenade [®] (Bayer [®])	QST 713 (1x10 ⁹ CFU/g de ativo)	Р	AD; AP; BC; CA; CG; CP; FO; MF; PU; RS; SS; SM; PF; SSc; XCi; XVe	

¹Minimum guarantees; EV: viable spores; CFU: colony-forming units. ²ST: seed treatment; F: furrow; S: Seedlings; I: irrigation; P: pulverization; E: squirt. ³ AD: Alternaria dauci; AP: Alternaria porri; AS: Alternaria solani; BC: Botrytis cinerea; BS: B. squamosa; CA: Colletotrichum acutatum; CG: Colletotrichum gloeosporioides; CL: Colletotrichum lindemuthianum; CP: Cryptosporiose perennans; CT: Colletotrichum truncatum; EP: Erysiphe polygoni; FO: Fusarium oxysporum; HG: Heterodera glycines; HV: Hemileia vastatrix; ME: Meloidogyne exígua; MF: Mycosphaerella fijiensis; MI: M. incognita; MJ: M. javanica; MP: M. paranaensis; NP: Neofabraea perennans; PB: Pratylenchus brachyurus; PC: Phyllosticta citricarpa; PU: Pythium ultimun; PF: Podosphaera fuliginea; PP: Phakopsora pachyrhizi; PS: Pseudomonas syringae; RS: Rhizoctonia solani; SM: Sphaerotheca macularis; SS: Sclerotinia sclerotiorum; SSc: Streptomyces scabies; UN: Uncinula necator; XC: Xanthomonas campestris; XCi: Xanthomonas citri subsp. citri XV: Xanthomonas vasicola; XVe: Xanthomonas vasicola; NVe: Xanthomonas vasicola; NVe:

These bioproducts from *Bacillus* species must have their agricultural potential proved and after these, in Brazil, they are recorded in the Ministério da Agricultura Pecuária e Abastecimento (MAPA) as commercial products, presenting different recommendations and modes of application. Among these products, some are made up of unique species and strains, while others rely on the use of mixtures, containing different strains or species of *Bacillus*, or even with other genus of microorganisms (Adapar, 2020).

In this context, benefits as a guarantee of greater durability of the products, due to the combination of the various control agents is observed. It happens because there is a sum of the different methods of action of each one, which can increase and control effectiveness, giving more stable results, avoiding possible problems of loss of action (Bardin, et al., 2015). The bioproducts for agronomic use, generally uses *Bacillus* vegetative structures or endospores, applying concentrations between 1 x 107 UFC/mL and 1 x 108 UFC/mL, been those ones the lowest and the optima doses (Posada, et al., 2016).

However, not all species and isolates of *Bacillus* are safe to human health. *B. subtilis* and *B. megaterium* are considered safe, while *B. cereus* has restrictions in the United Kingdom for the preparation of biotechnological products (Beretta & Júnior, 2017; Posada, et al., 2016).

4. Final Considerations

Bacillus are among the bacteria commonly observed interacting with plants under different conditions. They may have an endophytic lifestyle or form biofilms externally to plant tissue. Bacteria of this genus can produce active molecules of biotechnological interest to the agricultural sector. Among the main mechanisms that classify these bacteria as promoters of plant growth, can be highlight the modulation/production of plant hormones, secondary metabolites with antimicrobial, nematicide or insecticide activity, solubilization of mineral salts, production of siderophores, induction of systemic plant resistance and mitigation of abiotic and biotic stresses.

The ability to form endospores of this group of bacteria becomes advantageous for the development of ecofriendly bioproducts that perform functions as pesticides and chemical fertilizers, however, without the adverse effects of them. Investigations involving metagenomic tools such as soil and plant microbiome studies could favor the knowledge of new *Bacillus* strains with potential for the development of a new microbial agricultural inoculant or a consortium of microbes beneficial to plants and ecologically sustainable. These independent studies of cultivation in comparison with those dependent on crops (isolation) are more advantageous since they would reduce the time of knowledge not only of the species of this genus of bacteria, but also of genes that may be involved in activities of agronomic interest.

Therefore, in order to optimize the obtention of new products, future research could use molecular bioprospection techniques and genetic engineering, to optimize the production of components of interest. The bioproducts obtained may consist of *Bacillus* and its metabolites, or each one of these components. The use of *Bacillus* endospores is attractive due to the high resistance to adverse conditions, which confers benefits in obtaining products with longer shelf life. Active metabolites can be attractive as long as they show some stability, been possible the obtention of products with potential shelf life and environmental resistance.

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References

Adapar - Agência de Defesa Agropecuária do Paraná. (2020). Agrotóxicos no Paraná: faça sua pesquisa. http://celepar07web.pr.gov.br/agrotoxicos/pesquisar.asp.

Alves, K. C. S., De Almeida, M. E. M., Glória, J. C., Dos Santos, F. A., Pereira, K. D., De Castro. D. P. & Mariúba, L. A. M. (2018). Bacillus subtilis: uma versátil ferramenta biotecnológica. Scentia Amazonia, 7 (2), 15–23.

Azevedo, J. L. (1998). Microrganismos endofíticos. In: Melo, I. S., & Azevedo, J. L. DE (Org.). Ecologia Microbiana. Jaguariúna: Embrapa-CNPMA. 117–137.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., & Nicot, P. C. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? *Frontiers in Plant Science*, 6, 1–14.

Beretta, D., & Júnior, A. E. (2017). Isolamento de biossurfactantes e hidrolases a partir do crescimento de Bacillus sp. 16-19.

Bokhari, A., Essack, M., Lafi, F.F., Andres-Barrao, C., Jalal, R., Alamoudi, S., Razali, R., Alzubaidy, H., Shah, K. H., Siddique, S., Bajic, V.B., Hirt, H., Saad, M. M. (2019). Bioprospecting desert plant *Bacillus* endophytic strains for their potential to enhance plant stress tolerance. *Scientific Reports*, 9, (1),1–13.

Chagas, L. F. B., Martins, A.L.L., De Carvalho Filho, M.R., Miller, L.O., De Oliveira, J.C., & Chagas Junior, A. F. (2017). *Bacillus subtilis* E *Trichoderma* sp. No incremento da biomassa em plantas de soja, feijão-caupi, milho e arroz. *Revista Agri-Environmental Sciences*, 3 (2), 10–18.

Chamedjeu, R., Masanga, J., Matiru, V., & Runo, S. (2019). Potential Use of Soil Bacteria Associated with Potato Rhizosphere as Bio-control Agents for Effective Management of Bacterial Wilt Disease Phenomics and Candidate genes for Production and Adaptive traits in Indigenous Poultry View project Genetic engineer. *Journal of Microbiology Research*, 9 (1)12–24.

Cruz, A. P., Calazans, G. M., Cordeiro, J., & Quintão, P. L. (2018). Avaliação da influência da salinidade na germinação, no desenvolvimento e diversidade de microrganismos endofíticos da leguminosa *Mucuna aterrima. Research, Society and Development*, 7 (2), e1272193.

Dubey, A., Malla, M. A., Kumar, A., Dayanandan, S., & Khan, M. L. (2020). Plants endophytes: unveiling hidden agenda for bioprospecting toward sustainable agriculture. *Critical Reviews in Biotechnology*, 1–22. https://doi.org/10.1080/07388551.2020.1808584.

Gabardo, G., Pria, M. D., Prestes, A. M. C., & Da Silva, H. L. (2020). Trichoderma asperellum E Bacillus subtilis Como Antagonistas No Crescimento De Fungos Fitopatogênicos in vitro. Brazilian Journal of Development, 6 (8), 55870–55885.

Garcia, T. V., Knaak, N., & Fiuza, L. M. (2016). Bactérias endofíticas como agentes de controle biológico na orizicultura. Arquivos do Instituto Biológico, 82, 1–9.

Gonçalves, O. S., Almeida, T. C., Vilela, C. S., & Machado, D. C. (2017). PROSPECTING OF Bacillus subtilis as biocontrol agents against biocontrole contra Fusarium sp. Revista Mirante, 10 (1), 132–142.

Ikeda, A. C., Savi, D. C., Hungria, M., Kava, V., Glienke, C., & Galli-Terasawa, L.V. (2020). Bioprospecting of elite plant growth-promoting bacteria for the maize crop. Acta Scientiarum - Agronomy, 42(e44364), 1–11.

Jochum, M. D., Mcwilliams, K. L., Borrego, E. J., Kolomiets, M. V., Niu, G., Pierson, E. A., & Jo, Y. K. (2019). Bioprospecting Plant Growth-Promoting Rhizobacteria That Mitigate Drought Stress in Grasses. *Frontiers in Microbiology*, 10, 1–9.

Liu, S. L., Wu, W. J., & Yung, P. T. (2015). Effect of sonic stimulation on Bacillus endospore germination. FEMS Microbiology Letters, 363 (1), 1-7.

Lyngwi, N. A., Nongkhlaw, M., Kalita, D., & Joshi, S. R. (2016). Bioprospecting of plant growth promoting bacilli and related genera prevalent in soils of pristine sacred groves: Biochemical and molecular approach. *PLoS ONE*, 11 (4), 1–13.

Maheshwari, R., Bhutani, N., Bhardwaj, A., & Suneja, P. (2019). Functional diversity of cultivable endophytes from *Cicer arietinum* and *Pisum sativum*: Bioprospecting their plant growth potential. **Bio***catalysis and Agricultural Biotechnology*, 20, 1–11.

Mapa - Ministério Da Agricultura Pecuária E Abastecimento. (2020). Mapa bate recorde de registros de defensivos agrícolas de controle biológico. https://www.gov.br/agricultura/pt-br/assuntos/noticias/mapa-bate-recorde-de-registros-de-defensivos-agricolas-de-controle-biologico.

Mattei, D., Henkemeier, N. P., Heling, A. L., Lorenzetti, E., Kuhn, O. J., & Stangarlin, J. R. (2017). Produtos fitossanitários biológicos disponíveis. In: ZAMBOM, M. A. et al. (Org.). Ética do cuidado, legislação e tecnologia na agropecuária. 1. ed. Marechal Candido Rondon: Centro de Ciências Agrárias/ Unioeste, 124–154.

Nofiani, R., De Mattos-Shipley, K., Lebe, K. E., Han, L. C., Iqbal, Z., Bailey, A. M., Willis, C. L., Simpson, T. J., & Cox, R. J. (2018). Strobilurin biosynthesis in Basidiomycete fungi. *Nature Communications*, 9 (1), 1–11.

Pascholati, S. F., Melo, T. A. De, & Durigan, J. (2015). Indução de resistência contra patógenos: definição e perspectivas de uso. Visão agrícola, 13, 110-112.

Pereira, E. L., & Martins, B. A. (2016). Processos Biotecnológicos Na Produção De Bioinseticidas. Revista da Universidade Vale do Rio Verde, 14, 714-734.

Posada, L. F., Ramírez, M., Ochoa-Gómez, N., Cuellar-Gaviria, T. Z., Argel-Roldan, L. E., Ramírez, C. A., & Villegas-Escobar, V. (2016). Bioprospecting of aerobic endospore-forming bacteria with biotechnological potential for growth promotion of banana plants. *Scientia Horticulturae*, 212 (81–90).

Sessitsch, A., Brader, G., Pfaffenbichler, N., Gusenbauer, D., & Mitter, B. (2018). The contribution of plant microbiota to economy growth. *Microbial Biotechnology*, 11 (5), 801–805.

Shameer, S. (2016). Haloalkaliphilic *Bacillus* species from solar salterns: an ideal prokaryote for bioprospecting studies. *Annals of Microbiology*, 66 (3), 1315–1327.

Silva, C. Dos S., Dos Santos, J. M. C., Da Silva, J. M., Tenório, F. A., Guedes-Celestino, E. L. F., De Cristo, C. C. N., Nascimento, M. S., Montaldo, Y. C., De Oliveira, J. U. L., & Dos Santos, T. M. C. (2019). Bioprospecting of endophytic bacteria (*Bacillus* spp.) from passionfruit (*Passiflora edulis* Sims f. *flavicarpa*) for plant growth promotion. *Australian Journal of Crop Science*, 13 (8), 1369–1374.

Soares, A. S., Costa, L. T. M., Da Silva, C. A., Do Santos, S. F., & De Souza Aguiar, R. W. (2020). Bioprospecting of *Bacillus thuringiensis* in the control of *Aedes aegypti* larvae. *Brazilian Journal of Biological Sciences*, 7 (16), 175–191.

Soares, C. C., Druzian, J. I., & Lobato, A. K. D. C. L. (2018). Estudo prospectivo de patentes relacionadas a utilização do *Bacillus subtilis* em bioprocessos. *Cadernos de Prospecção*, 11 (6), 295.

Souza, P. S. S., Nascimento, R. C., Da Silva, T. R., Nóbrega, R. S. A., & Fernandes Junior, P. I. (2018). Eficiência agronômica de bactérias promotoras de crescimento vegetal nativas do Semiárido na produtividade de milho ' BRS Gorutuba '. In: *Anais* da XIII Jornada de Iniciação Científica da Embrapa Semiárido. 2018, Petrolina: Embrapa Semiárido. 305–309.

Turibio, T. De O., Ferreira, E. M. S., Sousa, F. M. P., Silva, J. F. M., & Pimenta, R. S. (2018). Verificação da Produção de Substâncias Antimicrobianas por Fungos Endofíticos Associados à Soja (*Glycine max* (L.) Merrill) no Estado do Tocantins. *Revista Cereus*, 10 (3), 92–102.

Wang, W., Bai, R., Cai, X, Li, P., & Ma, L. (2017). Separation and determination of peptide metabolite of *Bacillus licheniformis* in a microbial fuel cell by high-speed capillary micellar electrokinetic chromatography. *Journal of Separation Science*, 40 (22), 4446–4452.