



Review Article

The genus *Bacillus* as biological control agent against pests and pathogens for sustainable agriculture

Fannie Isela Parra-Cota, Campo Experimental Norman E. Borlaug, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, CP. 85000, Ciudad Obregón, Sonora, México; **Isabeli Bruno**, Área de Agrometeorología, Ecofisiología y Tecnología de Alimentos, Instituto de Desarrollo Rural de Paraná (IAPAR-EMATER), Londrina 86047-902, Brasil; **Mónica García-Montelongo**, **Sebastián González-Villarreal**, Laboratorio Estatal de Salud Pública de Durango 34206, Nombre de Dios 1206, José Ángel Leal, Durango, México; **María Fernanda Villarreal-Delgado**, Sartorius de México, Libramiento Norte de Tepotzotlán, S/N Int. 5, CP 54605. Tepotzotlán, Estado de México; **Liliana Carolina Córdova-Albores**, Licenciatura en Agrobiotecnología. Centro Universitario del Sur. Universidad de Guadalajara. Enrique Arreola Silva No. 883. Ciudad Guzmán, Jalisco, México. C.P. 49000; **Alina Escalante-Beltrán**, **Sergio de los Santos-Villalobos***, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Col. Centro CP. 85000, Ciudad Obregón, Sonora, México.

***Corresponding Author:**

Sergio de los Santos-Villalobos
sergio.delossantos@itson.edu.mx

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ABSTRACT

Background/Objective. Bacteria of the *Bacillus* genus have been studied since their discovery in 1872, for their ability to synthesize metabolites of interest such as proteins used to control phytopathogens. The objective of this research was to analyze the most outstanding species of the *Bacillus* genus, as well as the main compounds produced by these species, and the perspectives on the use of this bacterial genus for pest and disease control.

Materials and Methods. An exhaustive search was carried out in scientific articles and books to gather the most relevant information regarding the *Bacillus* genus, focusing on its role as a biological control agent for pests and pathogens.

Results. The *Bacillus* genus includes more than 427 taxa, which can be classified into different groups. Among the biological control agents (BCA) are the *Bacillus cereus* group, which includes *B. cereus*, *B. anthracis*, and *B. thuringiensis*, and the *B. subtilis* group, which includes *B. subtilis*, *B. licheniformis*, and *B. pumilus*, mainly. *B. thuringiensis*, through cry genes, has molecular mechanisms to synthesize a crystalline inclusion during sporulation, which contains proteins known as endotoxins or Cry proteins. *B. subtilis* produces substances with a high potential for biological control, such as volatile organic compounds, as well as bioactive secondary metabolites.

Conclusion. The potential of the *Bacillus* genus to be used as biological control agents is evident. They are widely used for the development of different biopesticides that have advantages over other products. However, it is necessary to continue conducting research from the *in vitro* area in the laboratory to the field, to help guarantee their biosecurity and effectiveness.

Keywords: Beneficial bacteria, sustainable agriculture, biocontrol, action modes.

INTRODUCTION

The increase in the global population and the impact of climate change indicates that the food demand will double by 2050, which is currently satisfied by 80% by the agricultural sector (FAO, 2017). However, some of the factors that limit productivity and generate between 20 and 40% of losses in agricultural productivity are plant diseases caused by fungi, bacteria, viruses, and nematodes) (de los Santos-Villalobos *et al.*, 2021; García-Montelongo *et al.*, 2023). Due to the rising interest in controlling diseases of agricultural interest, the study and use of plant growth-promoting microorganisms (PGPM), as well as biological control agents (BCA) has grown in recent decades, as well as its use in the formulation of biopesticides incorporated in the Integrated Pest and Disease Management (IPDM). Conventionally, pests and diseases in plants are controlled with the use of chemical pesticides that contribute to the increase of environmental issues and consume a large volume of resources (de los Santos-Villalobos *et al.*, 2021; Montoya-Martínez *et al.*, 2024).

Microorganisms have a wide range of action mechanisms to protect plants and control pathogens. Some interact through competition for space and nutrients, parasitism, and antibiosis to interfere directly with the pathogen, whereas other interactions with plants can be produced by induced resistance (García-Montelongo *et al.*, 2023). Through the bioprospecting of selected strains as BCAs, it is possible to identify metabolites of interest; these should be identified, characterized, purified and their biological properties evaluated, while also monitoring their biocontrol activity (Montoya-Martínez *et al.*, 2024).

One of the most widely studied groups of endophytic and rhizosphere bacteria isolated are bacilli since they have been characterized as potential BCAs. The synthesis of enzymes with antibiotic activity (subtilin, subtilosin A, TasA, sublancin) is one of their antagonistic abilities, as well as the production of non-ribosomal peptides, which are synthesized by non-ribosomal peptide synthetases, such as bacilysin, bacilysin, chlorotetain, difficidin, mycobacillin and some rhizocytokine (Orozco-Mosqueda *et al.*, 2021).

Bacillus has currently been reported as the genus with the most strains used for the production of biopesticides, making up more than 85% of commercial bacterial products, due to their metabolic versatility which, via several biological mechanisms, helps it control pests and diseases (Villarreal-Delgado *et al.*, 2018).

This revision has the aim of critically analyzing the use of bacteria of the *Bacillus* genus, its main action mechanisms against pests and diseases in plants, as well as its implementation, free of biosecurity hazards, via the development of bioinoculants, since the scientific support of these products on their action mechanisms, ecological and biosecurity implications and their formulation are decisive for the development of sustainable agriculture.

THE *Bacillus* GENUS

The *Bacillus* genus was reported for the first time by Cohn (1872), who described it as bacteria that produce heat-resistant endospores (Villarreal-Delgado *et al.*, 2018). This bacterial genus, belonging to the Bacteria kingdom; Phylum Firmicutes; Class Bacilli; Order Bacillales, and Family Bacillaceae is characterized by its aerobic or anaerobic facultative growth, Gram-positive species, bacillary morphology, flagellar motility and variable size (0.5 to 10 μm), optimum growth at a neutral pH, and a variable optimal temperature range, although most species are mesophilic (they grow between 30 and 45 °C) (Figure 1). Its ability to produce endospores as a resistance mechanism is also noteworthy, and gives it the ability to prevail in ecosystems, even under adverse conditions (de los Santos-Villalobos *et al.*, 2018; San Miguel-González *et al.*, 2024).

Currently, this genus includes over 427 taxons with a publicly validated name (Parte *et al.*, 2020). Taxons, due to their genetic similarity, can be classified into different groups, the main BCAs include the group of *Bacillus subtilis*, composed of *B. subtilis*, *B. licheniformis*, and *B. pumilus*, and the *B. cereus* group, which includes *B. cereus*, *B. anthracis*, and *B. thuringiensis*, mainly; a wide variety of species of the *Bacillus* genus has displayed antagonistic activity against different phytopathogenic microorganisms that affect crops such as maize, rice, and others (Villarreal-Delgado *et al.*, 2018).

***Bacillus* AS BIOINSECTICIDES**

Biopesticides are a particular type of biological product, focused on the control of agricultural pests based on a living microorganism or a natural product (García De León and Mier, 2010). These may be classified into three types, according to the active substance: i) biochemical products, ii) semi-chemical products, and iii) microorganisms (Villarreal-Delgado *et al.*, 2018). The *Bacillus* genus has been

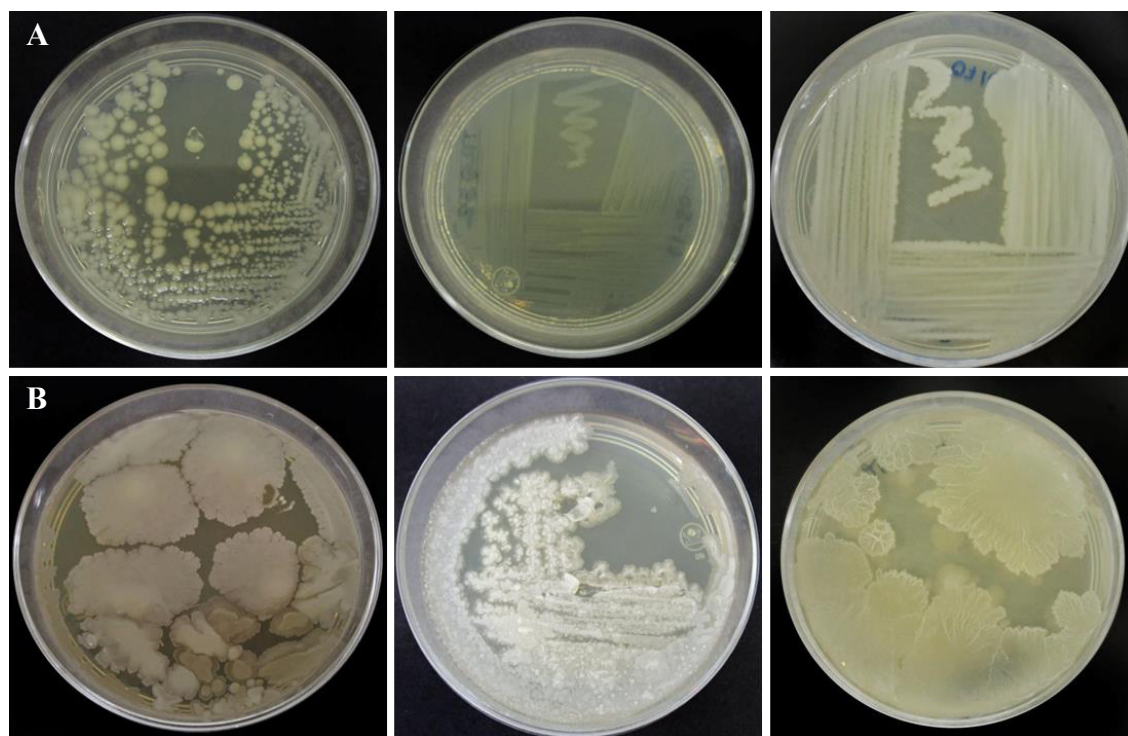


Figure 1. Macroscopic characteristics of *Bacillus cereus* (A) and *B. subtilis* (B), belonging to the Culture Collection of Native Soil and Endophytic Microorganisms, grown at 28 °C in nutrient agar (COLMENA) (www.itson.edu.mx/COLMENA) (de los Santos-Villalobos *et al.*, 2018).

widely studied due to its ability to produce spores, which facilitates its storage, encapsulation, and later application on the field, its wide metabolic diversity related to its prevalence in ecosystems and its ability to reduce the incidence of pests and diseases in crops (Santoyo *et al.*, 2019; Valenzuela-Ruíz *et al.*, 2020).

Bacillus thuringiensis (Bt)

Bacillus thuringiensis (Bt), through *cry* genes, has molecular mechanisms to produce toxins; it synthesizes a crystalline inclusion during sporulation, which contains proteins known as endotoxins or Cry proteins (Sarwar, 2015).

Crickmore *et al.* (2016) have created a special database for *Bt* toxins, which includes links to information on host insects and is currently updating (<https://www.bpprc.org/> consulted on May 30th, 2024), in which around 950 genes of toxins that codify different entomopathogenic proteins in *Bt* strains isolated from different parts of the world (Galvis and Moreno, 2018) have been identified and characterized. Most of these toxins, produced during the phase of sporulation, are

parasporal inclusions; these contain crystalline proteins known as delta-endotoxins, classified into two families: Cry and Cyt (Jouzani *et al.*, 2017).

The δ -endotoxins (Cry) are toxic to a wide range of insect pests, such as Lepidoptera, Coleoptera and Diptera (Maksimov *et al.*, 2020). *Bt* normally comprises a large family of several subspecies, each one categorized by different phylogenetic serotyped characteristics (such as *Bt* subsp. *kurstaki*, *Bt* subsp. *aizawai*, *Bt* subsp. *tenebrionis*, *Bt* subsp. *israelensis*, etc.); in addition, every subspecies of *Bt* includes multiple strains and serotypes (Bravo *et al.*, 2011).

In addition to the Cry proteins, other protein inclusions have been described, called Cyt proteins, which, unlike Cry proteins, have a much lower molecular mass (approximately 27 kDa) and also display cytolytic activity (Bravo *et al.*, 2007). Currently, over 300 *cry* genes have been reported from *Bt*, classifying into 73 Cry families and 3 Cyt families (Porcar and Juárez, 2004; Xu *et al.*, 2014). Both Cry and Cyt proteins are produced during the sporulation phases and are known for their specific toxic effects on a large variety of invertebrates, mainly Diptera, Lepidoptera, Coleoptera, and nematodes (Pérez-García *et al.*, 2011; Soberón *et al.*, 2007).

Cry proteins are highly specific regarding their form of action, and are present in sporangia in an inactive way. After the autolysis of the sporangium, the protein crystals are released, which become toxic after they are ingested by susceptible insects and partially digested in the midgut of the insect (Bravo *et al.*, 2011). The action mechanism begins once the Cry proteins are proteolytically processed through proteases found in the midgut of the host, separating a section of amino acids in the N-terminal region and in the C-terminal end (depending on the nature of the Cry protein), thus releasing active and toxic fragments that interact with the receptor proteins found in the intestinal cells of the larva (Figure 2). These fragments are recognized by the specific receptors in the membrane and inserted through the cadherin, giving succession to a series of signals for the formation of an oligomeric pre-porous structure, and consequently the lithic porous, through which an osmotic imbalance is produced, which finally destroys the intestinal epithelium and consequently, the death of the cell (Portela-Dussán *et al.*, 2013; Xu *et al.*, 2014).

It is important to mention that *Bt* is not responsible for the death of the insect *per se*, but once there is damage to the intestine of the insect, diverse bacteria of the intestine pass onto the hemolymph, and finally, the insect dies of septicemia. This action mechanism has been described in Lepidoptera, and other orders such as Coleoptera, nematodes, and Diptera, the mechanism has not been entirely elucidated (Vachon *et al.*, 2012). The key factors for this event are the presence of specific proteases, an alkaline environment, and the presence of specific receptors in the intestine of the insect, which explains that toxins are only effective in a small range of hosts and therefore often have a limited effect on non-target populations

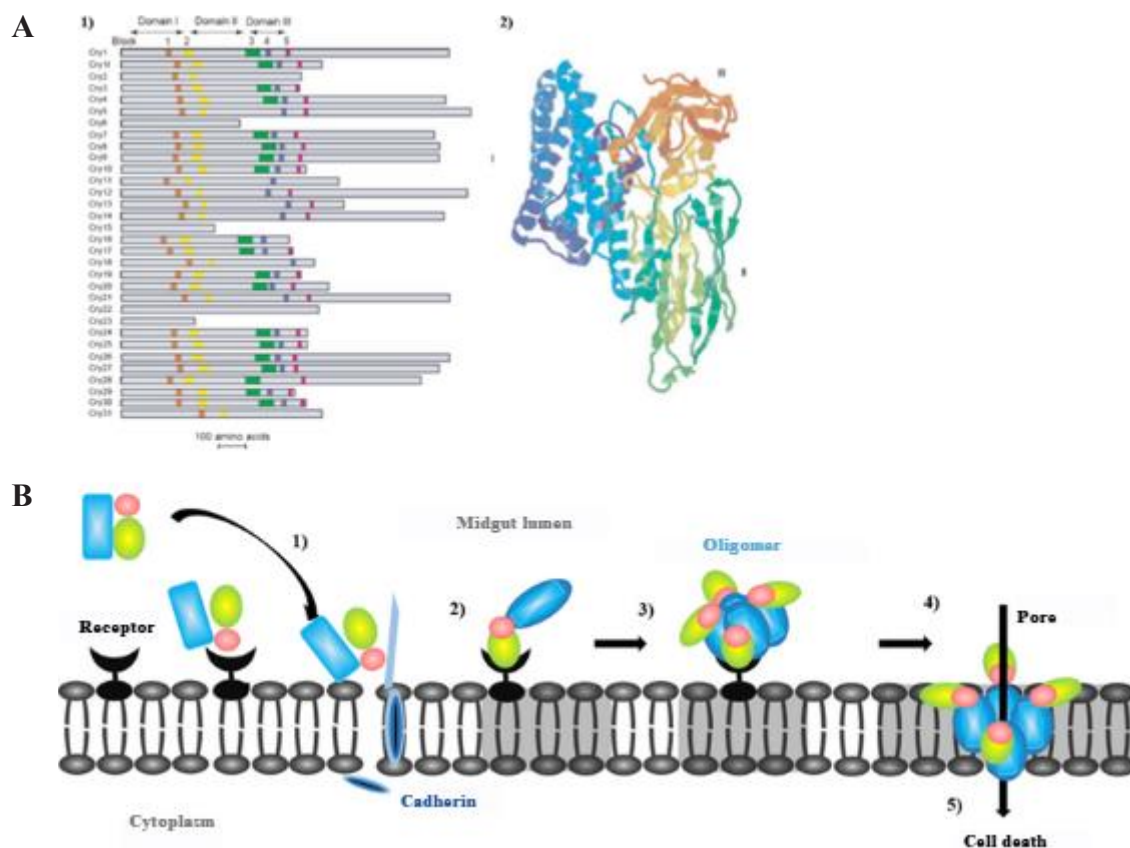


Figure 2. Cry proteins. 1-A) Primary structure, showing the organization of the domains of representative members from each Cry family, 2-A) Conserved tertiary structure, showing the positions of the three domains. B) Mechanism of action of Cry proteins in insects, initiating once the Cry proteins are proteolytically processed by proteases in the midgut of the host, separating an amino acid section in the N-terminal region and at the C-terminal end (depending on the nature of the Cry protein), thus releasing active and toxic fragments that interact with receptor proteins in the insect's intestinal cells. Modified from Villarreal-Delgado *et al.*, 2018.; de Maagd *et al.*, 2001; Xu *et al.*, 2014.

(Cawoy *et al.*, 2011). Additionally, the genes that codify the Cry/Cyt proteins are activated during the sporulation because they are controlled by a polymerase RNA that is also specifically synthesized while the spores are formed (Sanahuja *et al.*, 2011).

Despite the diversity of amino acid sequences, all Cry proteins share a similar general tertiary structure, as noticed in the six structures solved so far using X-ray crystallography X (Cry1Aa, Cry2Aa, Cry3Aa, Cry3Bb, Cry4Aa, and Cry4Ba); the C-terminal portion is involved in the formation of crystals, but it is not a part of the mature toxin, since a large part of it lies in the gut of the insect; the N-terminal

portion is the toxin itself and it comprises three domains: domain I is a set of seven α -helices, six of which are amphipathic and surrounds the seventh hydrophobic helix; this domain is responsible for the insertion of the membrane and the formation of lithic pores; domain II consists of three antiparallel β -sheets with exposed loop regions; domain III is a β -sandwich (Figure 2); domains I and II confer receptor-binding specificity, which helps define the host range (Boonserm *et al.*, 2006). On the other hand, the Cyt proteins bind directly to the lipid bilayer of intestinal cells and are composed of a single domain consisting of a β -sheet surrounded by two α -helices (Cohen *et al.*, 2008, 2011).

Diverse investigations have reported the potential of Cry proteins in controlling pests of agricultural importance. Niedmann and Meza-Basso (2006) displayed the potential of *Bt* for the control of the tomato leafminer (*Tuta absoluta*), highlighting that in tomato leaf trials added with Cry protein concentrates extracted from the *Bt* strains LM-11, LM-12, LM-14 and LM-33, a mortality of 20 of 60% of the *T. absoluta* larvae was achieved. Investigations have also been reported in which Cry protein extracts taken from the *Bt* strains LBIT-13, LBIT-44, LBIT-383, LBIT-418, and LBIT-428 proved to have a toxic effect on the fall armyworm (*Spodoptera frugiperda*) (Vázquez-Ramírez *et al.*, 2015).

The use of *Bt* is not limited to the control of agricultural pests, since its serovariety *B. thuringiensis* subsp. *israelensis* (*Bti*), discovered by Goldberg and Margalit (1977) in the Negev desert in Israel, has been used and sold for its high activity for the control of mosquitos and other Diptera of medical importance. *Bti* synthesizes three Cry proteins (Cry4A, Cry4B, and Cry11A) and one Cyt protein (Cyt1A); this combination is attributed to the high toxicity of *Bti*, as well as the lack of reports of resistance generation in susceptible insects (da Silva Carvalho *et al.*, 2018).

Vegetative insecticide proteins (Vip) are other interesting proteins derived from *Bt*, which are produced during the vegetative state of this bacteria. They include the binary toxin Vip1 and Vip2 with specificity against Coleoptera and Vip3 with a wide spectrum of activity against Lepidoptera. The insecticidal potential of Vip1/Vip2 has been proven on different pests such as the Coleoptera *Sitophilus zeamais*, known as the stored grain pest, and of Vip3 against the larvae of the Lepidoptera *Spodoptera frugiperda* and *Helicoverpa zea*, as well as against the cotton bollworm, *Helicoverpa armigera* (Mnif and Ghribi, 2015).

Other less studied *Bt* insecticidal toxins include secreted insecticidal proteins (Sip), which are proteins of approximately 41 kDa with reported activity against Coleoptera, and the β -exotoxins. These, as in Sip proteins, are secreted during the vegetative phase. Nevertheless, β -exotoxins are secondary metabolites secreted only by certain strains of *Bt*. Its toxic effect is not limited to invertebrates, and can also be toxic to mammals, therefore the use of *Bt* strains able to synthesize

β -exotoxins is restricted in several parts of the Americas and Europe (Chattopadhyay and Banerjee, 2018; Liu *et al.*, 2014; Palma *et al.*, 2014).

***Bacillus* AS BIOFUNGICIDES**

Biofungicides in general can be defined as microorganisms or natural compounds that can control pathogens that cause diseases in plants (Abbey *et al.*, 2019). There are multiple reports regarding the mode of action of various fungicidal agents:

Competence, in which the bio-fungicidal agent competes for nutritional factors (such as nitrogen or carbon) with fungi, limiting their growth.

Antibiosis, in which there are antagonistic biological interactions between organisms. **Parasitism**, a symbiosis in which one of the organisms subsists from the other, causing deterioration.

Induction of resistance, a mechanism in which the plant is induced to adopt biological measures that avoid the spreading of pathogens (García-Montelongo *et al.*, 2023; Köhl *et al.*, 2019).

Fungicides formulated with strains of the *Bacillus* genus offer several advantages over other microorganisms, as they form endospores and can tolerate extreme pH, temperature, and osmotic conditions. They can colonize the root surface, promote plant growth, and cause the lysis of fungal mycelium (El-Bendary *et al.*, 2016).

Bacillus subtilis

Bacillus subtilis is one of the most widely studied and used species for its production of substances with a high potential for biological control, such as volatile organic compounds, as well as bioactive secondary metabolites (Andrić *et al.*, 2020). Among these substances, lantibiotics stand out; they are low molecularweight peptides. These are antibiotics with a high level of antibacterial activity against Gram-positive bacteria, with subtilin being the most studied in the case of *B. subtilis*. This forms voltage-dependent pores in the cytoplasmic membranes of susceptible bacteria (Klein and Entian, 1994). Other lantibiotics synthesized by *B. subtilis* include plantazolicin, with a high selectivity against *B. anthracis* and ericin (Molohon *et al.*, 2016; Stein *et al.*, 2002). There are diverse antibiotics synthesized by *B. subtilis*, such as bacitracin, which acts mainly against Gram-positive bacteria by inhibiting the synthesis of peptidoglycan; bacilysin, which has activity against bacteria and fungi by inhibiting glucosamine synthesis and disrupting the formation of the microbial cell wall; and rhizoctin, an antibiotic

with activity mainly against fungi by inhibiting threonine synthase, affecting the synthesis of proteins in susceptible fungi (Borisova *et al.*, 2010; Molohon *et al.*, 2016; Rajavel *et al.*, 2009; Stein *et al.*, 2002).

The repertoire of proteins of interest of *B. subtilis* also includes fengycins and iturins, which have an inhibiting effect on fungal growth and antagonistic activity with the cell membranes of fungi (Caulier *et al.*, 2019). Other substances synthesized by *B. subtilis* with activity against plant pathogens are lipopeptides, low molecular-weight compounds with amphiphilic characteristics that protect plants by directly suppressing the growth of pathogens or inducing systemic resistance in host plants (Hashem *et al.*, 2019). It is worth noting that the substances synthesized by *B. subtilis* not only have activity on their own but also can induce an immune response in plants, making them less susceptible to infections (Andrić *et al.*, 2020).

The bacteria of the *Bacillus subtilis* species are widely studied for the biological control of phytopathogenic microorganisms, making their rational use easier; in this way, the United States Food and Drug Administration (USFDA) has granted this species the state “generally regarded as safe” (GRAS), thus being considered non-pathogenic (Cawoy *et al.*, 2011). *B. subtilis* is a ubiquitous bacterial species found in various habitats and occupies diverse ecological niches. It has no history of pathogenicity by contact with the environment, thus proving to be a potential agent of biological control of phytopathogenic fungi, mosquitos, and harmful nematodes (Mnif and Ghribi, 2015; Ongena and Jacques, 2008). The survival of *B. subtilis* in the rhizosphere and therefore its high efficiency as a biopesticide is due to its metabolic diversity (lytic enzymes and antimicrobial compounds) and its ability to produce spores (Cawoy *et al.*, 2011).

Diverse studies have reported the larvicide potential of metabolites (lipopeptides) derived from *B. subtilis*, which have been used in effective formulations for the control of *Drosophila melanogaster*, the Diptera *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti*, as well as the Lepidoptera *Prays oleae*, *Spodoptera littoralis* and *Ephestia kuehniella* (Mnif and Ghribi, 2015). Geetha and Manonmani (2008), reported that the pupal stages of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* [LC50: 2, 7.3 and 11.8 mg mL⁻¹, respectively] turned out to be more susceptible to the mosquitocidal lipopeptide that larval stages [LC50: 19, 23 y 34 mg mL⁻¹, respectivamente]; a noteworthy aspect of these insecticidal compounds is the report of its stability and that they maintain their larvicidal, pupicidal and adulticidal ability under conditions such as an extreme pH, high temperatures, solar radiation/UV, as well as the action of proteases (Geetha and Manonmani, 2010; Ghribi *et al.*, 2012).

On the other hand, other species of the *Bacillus* genus have been reported to have the ability for biological control. For example, *Bacillus cereus* is a spore-forming bacteria, widely distributed in the soil and used as a BCA. *B. cereus* is a

natural facultative pathogen of *A. aegypti*, the main vector for chikungunya and dengue since they can colonize the guts of the larvae of the insects. The insecticidal activity of *B. cereus* against *A. aegypti* larvae was approximately 90%, with the lethal concentration (LC50) being 2.5 ± 0.71 mg mL⁻¹ (Radhika *et al.*, 2011). In addition, new species of this genus with interesting metabolic abilities may lead to potential antagonistic bioproducts into phytopathogens. *B. cabrialesii* is a new species of *Bacillus*, characterized as an endophytic of the wheat crop. Although this strain has displayed biocontrol to *Bipolaris sorokiniana*, its strategies as a plant growth promoter (phosphorous solubilizer, producer of indoles, increase in chlorophyll) may make its use even more interesting, to reduce the use, not only of synthetic pesticides, but also of the fertilization doses (de los Santos-Villalobos *et al.*, 2018; Valenzuela-Aragón *et al.*, 2019; Villa-Rodríguez *et al.*, 2019).

COMMERCIAL USE OF *Bacillus*

Microbial pesticides offer advantages over other groups of pesticides, including those derived from fungi and plants and zooids, since the organisms used in the microbial insecticides are, in general terms, essentially not toxic or pathogenic for wildlife, humans, or other organisms not broadly related to the target pest. In addition, they do not damage the environment or water quality and they offer an eco-friendlier alternative than chemical pesticides (Chandler *et al.*, 2011; Sarwar, 2015). On the other hand, most microbial insecticides can be used alongside synthetic chemical insecticides because in most cases the microbial product is not deactivated or damaged by the effect of conventional insecticides (Mnif and Ghribi, 2015). Likewise, they may have a dual effect on the crops, since most BCAs have other mechanisms that improve the acquisition of nutrients and/or the development of the plant, as well as the soil properties, and also display the ability to stimulate the defense and other physiological processes of the plants, which may lead the treated crops to become more resistant to a variety of biotic and abiotic stress factors (Borriss, 2011; Sarwar, 2015). Additionally, biopesticides may be used when pests develop resistance to conventional pesticides.

An organism must fulfill several requirements before being released into the environment as a potential biopesticide, the most outstanding of which is that it must i) be highly specific and effective against the target pest, ii) display the potential to be processed successfully by a continuous production technology, iii) be available in formulations with a reasonable shelf life, iv) display stability and be harmless for humans, flora and non-target fauna (Menéndez and Paço, 2020). The *Bacillus* genus has been used as a model of study for agrobiotechnology for having different characteristics (Figure 3).

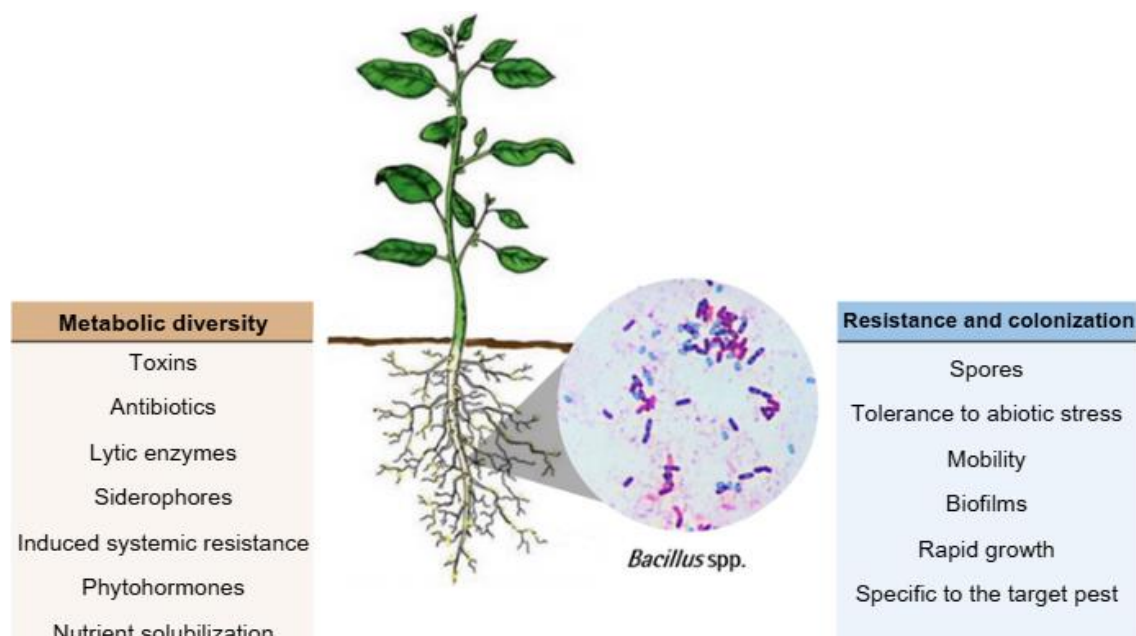


Figure 3. Characteristics of interest of *Bacillus* spp. for the formulation of bioinoculants.

For example, *Bacillus thuringiensis* (*Bt*) has been used as a biopesticide in agriculture, forestry, and the control of vectors of human diseases. Its advantages are the specific toxicity against target insects, the lack of polluting residues, and the safety of non-target organisms such as mammals, birds, amphibians, and reptiles (Sansinenea, 2012). Currently, products based on *B. thuringiensis* represent over 70% of the biopesticides sold globally (Figure 4) (Valenzuela Ruiz *et al.*, 2024). Most *Bt* formulations are obtained from different strains, including *B. thuringiensis* subsp. *kurstaki* (Btk) strain HD1 (Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa); *B. thuringiensis* subsp. *kurstaki* (Btk) strain HD73 (Cry1Ac); *B. thuringiensis* subsp.

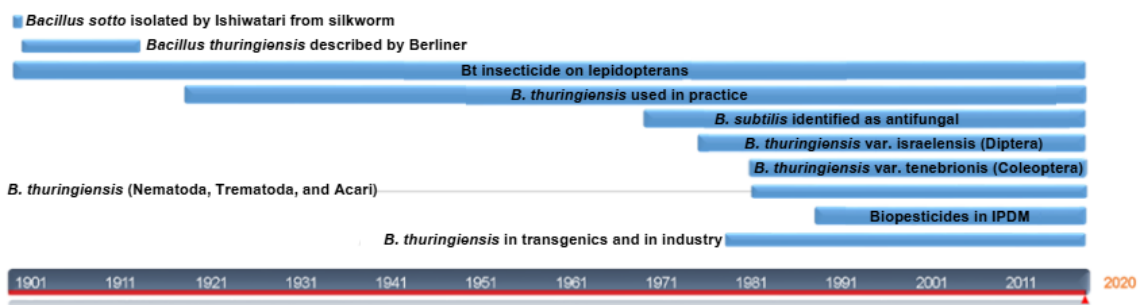


Figure 4. Evolution of the *Bacillus* genus as a biopesticide in the industry.

aizawai strain HD137 (Cry1Aa, Cry1B, Cry1Ca, and Cry1Da); *B. thuringiensis* subsp. *tenebrionis* (Cry3Aa) and *B. thuringiensis* subsp. *israelensis* (Cry4A, Cry4B, Cry11A, and Cyt1A) (Mnif y Ghribi, 2015). In Mexico, the use of *Bt*-based formulas represents an efficient alternative for the control of insects, presenting a percentage of use between 4 and 10% of the total insecticides used for the maize, cotton, and vegetable crops (Tamez Guerra *et al.*, 2001). Additionally, other species of the *Bacillus* genus, such as *B. subtilis*, *B. cereus*, and *B. licheniformis* have also shown their insecticidal potential to control agricultural crop pests and vectors of human diseases (Villarreal-Delgado *et al.*, 2018).

In recent years, the chemical pesticide production market has declined by 2% per annum, whereas the production of biopesticides displays an annual increase of 20% (Cheng *et al.*, 2020). Among the reasons behind the growing interest in biopesticides is the low probability of pathogenic organisms developing resistance, the reduction in the discovery of new insecticides, a greater public awareness regarding the risks of synthetic pesticides, an increase in studies on the specificity of biopesticides, advances in the production and technology of formulations and dissemination, as well as a better interaction with farmers and regulating authorities (Olson *et al.*, 2013; Olson, 2015).

It is important to highlight that the transition and integration of biopesticides in current agricultural practices must comply with certain requirements: a) effectiveness against the pest or disease, b) compatibility with other control methods, c) low or no environmental impact, d) long-lasting effect on the surroundings, e) profitability from the cost/benefit point of view, f) technical viability of its use, and g) acceptance by farmers and society in general. Therefore, its use is an opportunity to boost the development and modernization of current agricultural practices, contributing to food security through biosafety approaches (Villarreal-Delgado *et al.*, 2018).

CURRENT AND FUTURE CHALLENGES OF THE USE OF THE GENUS *Bacillus* IN AGRICULTURE

There are key issues to improve the efficiency of biopesticides and the consistency of protection, among which strain selection stands out, as the most commonly used method is currently in vitro screening, due to it being easy to implement. It presents the disadvantage that it generally does not take into account the environmental conditions in which it will be used, making it necessary to optimize the selection process by using more realistic methods that include the linking of field observations and verifiable parameters (Cawoy *et al.*, 2011).

Another aspect that must be taken into account to improve the efficiency of the biopesticides is the agro-system, that is, finding adequate combinations between biopesticides, chemical pesticides, plant fertilization, agricultural practices such as

different types of tilling, as well as integrating them into a MIPE that includes cultivation practices, plant resistance, chemical control and other BCAs (Cawoy *et al.*, 2011; Pérez-García *et al.*, 2011; Van Der Heijden *et al.*, 2008). The formulation and application methods are also key aspects worth considering that directly influence the efficiency of the biopesticides. For this, it is important to perform investigations on their application in specific environments, as well as to add compounds or other microorganisms to the formulations that are biocompatible and optimize the biological control activity (Cawoy *et al.*, 2011; Correa *et al.*, 2009). Likewise, it is necessary to study tolerance to heat, desiccation, exposure to ultraviolet radiation, as well as other types of stress that reduce the effectiveness of microbial insecticides. Consequently, special formulation, application, and storage procedures are needed for some microbial pesticides (Chandler *et al.*, 2011).

We can therefore specify that every BCA is a particular organism that carries out its action in a specific way, in which the studies of each selected microbial strain must be deepened to increase knowledge on how to enhance its biological control through effective formulation, considering ecological risk aspects and biosafety for the agro-system. It is therefore also necessary to delve deep into the correct identification of the BCA to discard the use of strains that may be pathogenic to humans. Particularly, within the group of *Bacillus cereus*, which includes species such as *B. cereus*, *B. thuringiensis*, *B. anthracis*, *B. mycooides*, *B. pseudomycooides*, *B. cytotoxicus* and *B. weihenstephanensis*, some strains have been identified as pathogenic to humans (Villarreal-Delgado *et al.*, 2018).

The virulence of these species has been mainly associated with the presence of two toxins: hemolysin BL (HBL) and the non-hemolytic enteric toxin (NHE), which form a protein complex (Kim *et al.*, 2015). In addition, other toxins have been found in pathogenic strains, including cytotoxin K (*cytK*), enterotoxin FM (*entFM*), enterotoxin S (*entS*), and enterotoxin T (*bceT*). Also involved in the pathogenicity of these strains are strains produced by other genes, such as hemolysin A (*hlyA*), hemolysin II and III (*hlyI*, *hlyII*), cereolysin A and B (*cerA*, *cerB*), and the pleiotropic transcription factor (*pclR*) (Ceuppens *et al.*, 2013).

The classification and differentiation of species within the *Bacillus cereus* group have been carried out using gene 16S rRNA and other characteristics such as i) virulence (*B. cereus*), ii) content of plasmids (*B. anthracis* and *B. thuringiensis*), iii) growth conditions (*B. cytotoxicus* and *B. weihenstephanensis*) and iv) morphological characteristics (*B. mycooides* and *B. pseudomycooides*). However, the differentiation between broadly related species, such as *B. cereus*, *B. anthracis*, and *B. thuringiensis*, is limited when virulence and plasmid content factors are used, due to the loss and transfer of these elements throughout their evolutionary history (Hoffmaster *et al.*, 2006; Liu *et al.*, 2015). Recent comparative studies of complete genomes using digital DNA-DNA Hybridization (dDDH) revealed a

wide distribution of *cry* genes and pXO plasmids in members of this group. These studies proved that there is a low correlation between the phylogenetic position and the presence or absence of these plasmids; they also showed that the multi-locus analysis (MLST) has a low resolution for the differentiation at a species level (Liu *et al.*, 2015).

To select and market the BCA of these species, it is necessary to identify and determine their virulence for human beings. The detection of the potential risk of these species can be carried out through taxonomy and β -hemolytic activity studies, and detection with defined virulence molecular markers, but the only accurate alternative for the classification and determination of its virulence is with the comparative genomic study using complete genomes. However, it is important to consider that these are costly tools, but as the genome sequences become more widely available, more in-depth studies can be conducted to ensure the biosafe strains in agriculture.

On the other hand, because the use of biopesticides has become highly relevant in the agricultural sector, which generally implies the application of large populations of the microorganism of interest to boost its establishment and colonization, studies on the environmental impact of the introduction of a BCA into agro-systems is necessary. This is because, under certain conditions, they may cause changes in the microbial communities with agro-ecologically unpredictable results, especially when BCAs are inoculated since their biological activity is not generally specific or selective for the phytopathogenic in question, which may lead to unpredictable changes in the microbial structure of these agro-systems (Trabelsi and Mhamdi, 2013). Therefore, it is important to monitor the impact of inoculating biological control agents on the structure and composition of microbial communities in agro-systems to ensure ecological balance, in addition to the desired insecticidal effect.

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