Review Article

Open Access CrossMark

Bacillus thuringiensis (Bt) is more than a special agent for biological control of pests

Abstract

Bacillus thuringiensis (*Bt*) is a bacterial genus known mainly for its capability to synthesize, in addition to spores, a parasporal body or crystal (δ—endotoxin), comprised of proteins which are toxic to pest *insects*, i.e., *Coleoptera*, *Diptera* and *Lepidoptera*, and other orders. As well as plant pathogenic nematode and many other applications as endophytic plant growth promoting bacteria, or cleaning enviornmental form some chemicals polluting agents, and even in human medicine for cancer prevention. The *Bt* coexists in a close relationship with insects to which eventually infects and poisons. It is likely that these unfavorable changes occurred during the evolutionary pathway in the interactions *Bacillus*–insect, which resulted in the present biochemical diversity between these species. The goal of this minireview is to show role of *Bt* in comparation with other genus *Bacillus* and explain why *Bt* is of interest for biological control of pests in conventional, protected and organic agriculture regarding other biotechnological applications to fully exploit the beneficial potential of *Bt*.

Keywords: pest biological control, endophytic plant promoting bacteria, bioremediation, cancer prevention

Volume 9 Issue 2 - 2022

Juan Manuel Sánchez–Yáñez,[1] José Luis Rico,[2] Gérard Ulíbarri[3,4]

[1]Microbiología Ambiental, Instituto de Investigación en Química y Biología, México
[2]Catálisis, Departamento de Ingeniería Química, México
[3]Department of Chemistry and Biochemistry, Laurentian University, Canada
[4]Translational Research Institute, México

Correspondence: Juan Manuel Sánchez–Yáñez, Microbiología Ambiental, Instituto de Investigación en Química y Biología, México, Email syanez@umich.mx

Received: February 08, 2022 | **Published:** March 23, 2022

Introduction

The genus and specie called *Bacillus thuringiensis (Bt)* is well known as an entomotoxic and/or entomopathogenic bacteria used against insect pests (IP) in agriculture and public health, today a key tool in Biological Control of the following orders: *Coleoptera, Diptera, Hymenoptera, Homoptera, Orthoptera* and *Phthiraptera*.[1–30]

Bt is applied to biological control of important human diseases which vectors are insects (VHD) belonging to the *Diptera* order with following genus involved in: malaria; *Anopheles*, dengue; *Aedes,* yellow fever; *Aedes* and *Haemogogus*, chikungunya; *Aedes* and zika; *Aedes.* The spores and crystals of *Bt israelensis* can intoxicate the young stage of larvae of a wide variety of *Diptera*, especially when solar radiation is minimal, *Bt* it is considered an ecological option to control these vector insects, although the lack of sufficient research on the persistence of *Bt israelensis* spores and crystals to the various aquatic conditions where oviposit mosquitoes limits a widespread application that ensures the disappearance of these vector insects for diseases of global importance.[1,3,4,18,28] Also, there is evidence that certain varieties of *Bt* can intoxicate and infect arthropods classified as part of the spiders, since some genera and species attack agricultural crops of commercial value, research in this regard is promising with the expectation that new varieties of *Bt* will be apply for the biological control of mites in substitution of chemical pesticides.[5,20,21]

One of the most interesting proprieties of *Bt* in human epidemiology its genetic capacity[29] for controlling snail of genera *Milax gagates* vector of the parasitic human and animal helminths such as *Angiostrongglus costaricensis*, *Angiostoma margaretae* and *Cryptosporidium parvum*. As well as the genus of snail *Biomphalaria,* vector of a trematode *Schistosoma mansoni* of importance in public health in the world.[6]

In addition, it has been reported that *Bt* crystals are also an alternative for the biological control of nematodes of veterinary importance such as the ruminant genera *Haemonchus, Teladorsagia, Nippostrongylus* and *Ancylostma* through a toxic action of these *Bt* crystals on the juvenile forms of these nematodes.[7,8] There is evidence that certain varieties of *Bt* synthesize crystals that are toxic to genera and species of phytopathogenic nematodes as the well–known *Meloidogyne hapla* responsible for millions of losses in agriculture, especially because chemical control causes serious problems for human health and environmental pollution.[8,19]

Beside this fact related to pest control. Another application of *Bt* for sustainable agriculture its ability to invade the root system and phylloplane[25] of various families of plants of commercial value as an endophyte, in which it can optimize the dose of mineral nitrogen fertilization, also is able to solubilize of phosphates to facilitate mineral absorption that allows plants to plants face soils with problems of elements necessary for agricultural production. While it can act in consortium with other genera and species of microorganisms that promote plant growth: actinomycetes, with mycorrhizal fungi and bacteria for different domestic crops.[9–12]

In terms of human health dealing with preventing several types of cancer there are reports that showed that parasporal crystals of *Bt* have anticancer activity, as well as the well–known MOLT–4, A549 associated to human lung cancer including HeLa (human uterus cervix cancer) cells, crystal from some strains *Bt* showed different toxicity like A1190 and A1462) were capable to destroy leukemic and normal T–cells, now those *Bt* proteins are called parasporin–2, 3, and 4 (PS2, PS3, PS4) to apply in treatment for preventing several types of cancer.[13–15]

Bt is regarding an ecological tool for recovering soil polluted by some pesticides, in that sense *Bt* is capable of synthesizing probiotics: organic substances and enzymes[21] that induce the mineralization of environmental toxins. This is the case of an organophosphate pesticide known as chlorpyrifos used in the control of domestic and agricultural insect pest. In the soil, chlorpyrifos at a relatively high concentration of 70 mg/Kg of soil can be effectively eliminated over a period of 80 days by the application of a *Bt* probiotic, analyzed by high pressure liquid chromatography, which showed that this probiotic induced the mineralization of chlorpyrifos, which allowed the reuse of the soil without causing negative environmental collateral effects.[16]

Bt exert their toxicity by crystal proteins (δ—endotoxins) or other toxins synthesized during its growth and sporulation.[14,17–21] Such proteins are very specific and safe to mammals' toxicity tests of *Bt* protein crystals for humans and animals support that the ingestion of these proteins does not allow them to solubilize, the Cry proteins rapidly degrade after being ingested. While both humans and animals lack specific recognition receptors for Cry proteins, necessary for them to be active. Consequently, no toxicity was detected when the dose for laboratory animals was oral. While there is no evidence of infection in rats, no skin damage was detected from the Cry proteins of Bt.[13,22–24]

Mode of action of *Bt* crystals on insect pests

In contrast, consumption of the crystal proteins by insects causes disturbances and eventually their death.[26,27,31–34] It is believed[35–39] that the first step of the *Bt* toxin is the recognition of target receptor or molecules located within or on the membrane surface of epithelial cells of the midgut of a susceptible insect. Although, the specific binding of Cry toxin proteins to brush border membrane vesicle (BBMV) of midgut has been reported,[11,28,39,40–44] less is known about the binding of Vip toxins. Recently, it has been suggested that the Vip3Aa toxin is bound specifically to a 48–kDa protein present at the BBMV prepared from the midgut epithelial cells of black cutworm (*Agrotis ipsilon)* larvae.[43,44]

Biopesticides based on *Bt* were first commercialized in France in 1930 to destroy insects in agricultural crops as well as urban plagues, i.e., *Coleoptera*, *Diptera* and *Lepidoptera.*[22,23,34,45–47] However, *Bt* also infects and kills beneficial insects such as: *Bómbix mori, Danaus plexippus.*[48] There exists a large variety of insects which are not considered IPs neither VHDs,[18,22,23,30,41,49] Biopesticides and anti–pest crops have been developed by genetically modifying the crops with *Bt* genes, resulting in thousands of *Bt* strains isolated and studied.[4,20,21,50–54] However, the evolution of resistance of pests to the insecticidal proteins from *Bt* produced by transgenic crops is drastically reducing the effectiveness of this method.[50,55–61] Indeed, in 2005 was reported that 1 out of 13 insect species were vulnerable to transgenic crops, whereas eight years later, 5 out of 13 had developed resistance to *Bt* modified crops.[44,62]

The sporulation of *Bt* could happen in soil, water or air,[63] however, it is generally accepted that it occurs inside the guts of insects[18,21,40,42,49,64] and inside other animals such as birds.[65] Although *Bt* is considered a typical bacterium, its sporulation in different environmental conditions may not occur, in contrast to *Bacillus cereus.*[30,66] In addition, a physiological difference between *Bt* and other members of the *Bacillus* species is the genetic stability to continuous sporulation. There are several reports in the literature which mention that *Bt* easily loses its sporulation capacity, under certain environmental conditions, providing strong evidence of a difference in the extrachromosomal DNA compared to other species.[67,68] Furthermore, the spores of the *Bt* are sensible to light and susceptible to some very specific pesticides, which is a drawback of the spore–crystal complex for foliar application.[69] However, *Bt* spores have been isolated from leaves of domestic and wild plants,[70] albeit susceptible to UV irradiation[71–77] It is still unclear whether these spores were transported by wind, insects or were merely isolated cases.[73] In another study, Ignoffo *et. al*. reported in 1978[78] that the viability was lost in less than 48h after aspersion of *Bt* spores on young leaves of corn and bean plants[79] which was attributed to solar irradiation.[78] It is believed that the *Bt* spores lack certain proteins that protect them against physical elements, this protection is a typical and basic characteristic of most spores of the *Bacillus* genus.[73,80] The lack of these proteins, partially explains the poor stability of the *Bt* spores to solar.[32,81] In contrast, the spores of *Bacillus cereus* reproduce and are resistant to solar irradiation, an advantage that is utilized to control pathogenic bacteria on leaves of commercial plants.[65,74]

Ecology of *Bacillus thuringiensis*

The spores of *Bt* are also sensitive to heavy metals: aluminium (Al), cupper (Cu), lead (Pb), etc, another aspect of the physiology of the *Bt* is its incapacity to sporulate under nutritional stress conditions[30,80] contrary to other members of the genus *Bacillus* that do not require mineral medium or glucose in order to induce sporulation.[73] Research using *Bt* showed that the genetically modified serotypes from Northern Mexico, as well as the Howard Raylord Deuchman strands from the U.S. Anti–doping Agency,[81] were incapable of growing in a mineral media, or in soil extracts used as growing media. In contrast, when *B. cereus* is introduced in the same media, it rapidly sporulates as most known saprobic *Bacillus* species do.[82]

It is generally accepted that *Paenibacillus* (before called: *Bacillus*) is a genus capable of atmospheric nitrogen fixation, which was expected since the species *Bacillus subtilis, B. lincheniformis,* and *B. cereus* grow and survive in soils with deficiency in.[30,80] These findings suggest that nutrients of *Bt* are obviously distinct to the rest of the *Bacillus* genus, which supports the hypothesis of its relationship and biochemical dependency on insects. As typical soil bacteria, *Bt* is incapable to synthetize vitamins or other compounds essential for its survival, in contrast to *B. cereus* or other species of this genus.[5,30,35,83,84]

Autecology of spore and crystals of *Bacillus thuringiensis*

Table 1 shows some of the diversity of *Bt*–based pesticides used to control pest insects in agricultural crops, stored grains, apiculture, forestry and urban areas.[37,82,85] Several North American[30] and European companies produce a variety of *Bt* formulations, geared to create different environmental, natural and artificial, applications. Some of these formulations are encapsulated in order to avoid the rapid denaturalization of the δ–endotoxin, such as MVP and M–One Plus commercialized by Mycogen. These products are efficient at *Lopelluis* controlling insects on infected leaves, as well as in aquatic environments.[86] The aforementioned unjustified the use of *Bt*–transgenic seeds in agriculture due to unknown risks associated with ecological factors and human health.[20,29,35,85,87,88] However, many reports have showed that crystal of proteins of *Bt* remnants from any application are binding to some physicochemical soil properties such as clay and humic acids by doing so these proteins has potential hazard to beneficial native animals like insects still not well known, in that sense research must be done in order to establish environmental rules to regulate any *Bt*–transgenic to prevent ecological disaster.[89–93]

Table 2 shows the biochemical and physiological differences of *Bt* compared to other typical microorganisms of the genus *Bacillus*. Unlike other species commonly found in soils, such as *Bacillus cereus*[83] and *Bacillus subtilis, Bt* is unable to synthesize growth factors, explaining its incapability of growing in mineral agar, or in the absence of combined organic nitrogen.[30,94,95] In addition to the normal tolerability of the spores of the *Bacillus* species to radiation, chemical agents, and thermal stability, the spores of the *Bacillus stearothermophilus* resist temperatures higher than 100 ºC, a genetic/biochemical property absent in the spores of *Bt*[37,82,84,96] or applied for bioremediation soil polluted by petroleum products mixing *Bt* with *Lysinbacillus sphaericus.*[97]

Table 3 presents selected features of some representative species of the *Bacillus* genus

Table 1 Some commercial biopesticides based in *Bacillus thuringiensis* species[20,24–26,28–30,32,35,37,38,46,51,81,82,85–93,96]

Commercial product	Company	*Bacillus thuringiensis* subspecies/varieties	Crop to protect
Bactospeine	Duphar	Kurstaki	Vegetables, fruits stored grains
Biobit	Novo labs		
Condor	Ecogen		
Cutlass	Ecogen		
Dipel	Abbot labs		
Javelin	Sandoz		
Larvo Bt	Knoll labs		
MVP		Encapsulated Kurstaki	
Certain	Sandoz	Aizawai	Honey producers
Skeetal	Novo labs	Israelensis	Toxic to mosquitoes
Teknar	Sandoz		
Vectorbac	Abbot labs		
M-One	Sandoz	San Diego	Stored grains, potatoes and leaves
Trident	Sandoz	Tenebrionis	Tree leaves
Tfoil	Ecogen	Tenebrionis	
M-One plus	Mycogen	Kurstaki San Diego encapsulated toxin	

Table 2 Biochemical and physiological differences of the *Bacillus thuringiensis* compared to other typical microorganisms of the genus *Bacillus*[30,35,37,64,71,75,77,82–84,94–97,99,105,108,112–115–117]

Characteristics	Response of *B. thuringiensis*	Response of representative *Bacillus* spp
Need of a growth factor	Positive	Negative
Induction to sporulation	No required	Stress required
Resistance of spores to UV	Negative	Positive
Growing in a mineral medium	Negative	Positive
Genetic stability	Negative	Positive

Table 3 Selected reproduction features for representative species of the *Bacillus* genus [21,32,35,46,48,66,67,71,75–77,80,82,88,98–105,112–117]

Species	Reproduction
Bacillus spp	Only in symbiosis with bees
Bacillus papillae and *B. larvae*	Obligate parasite in insects only
Bacillus anthracis.	Facultative parasite in animals and humans
Bacillus sphaericus and Bt	Facultative parasite in mosquitos (Diptera)
Bacillus cereus	Saprobe in soils and rhizosphere
Paenibacillus polymixa (before *Bacillus polymxya*)	Asymbiotic nitrogen bacteria in soils

Table 4 Hypothetic possible responses of *Bacillus thuringiensis* to abiotic and biotic natural environmental conditions[27,32,35,38,65,68,71,72,75–77,82,94,100–102,106–112]

Condition	Response	Consequence
Not germinating spores	lost viability	Not survival
	(due to nutritional requirements)	
Crystal protein	Mineralization	Inactivation (hours)
	(except when adhered to sand)	
Vegetative sporulating forms	No response to environmental stimulation	Dormancy of vegetative cells (but not for sporulating)

The interactions between insects and bacteria are well documented.[98] Natural and forced symbioses are often observed: mutualism in the case of *Bacillus sp* and bees[35,48,67,80,88,99–103] parasitism is observed with *Bacillus papillae*, *B.larvae, B.anthracis, B. sphaericus (*now *Lysinbacillus sphaericus),* and *B.cereus*.[35,46,66,104,105]

Table 4 presents a hypothetic response of *Bt* to the environment. It is assumed in this representation that the spores of *Bt* do not germinate under physicochemical stress conditions. Under this scenario, the *Bt* would only survive due to the interactions with insects[27,35,38,106] and plants as endophyte.[107] Furthermore, since the spores are unstable to the environment,[32,71,72,75,77,94,108–111] continuous sporulation of *Bt* would be only possible under favorable conditions: good oxygenation, simple carbon–source provision, suitable pH and absence of life–threatening physicochemical agents.[82,100,102]

Final comments

This short note shows that *Bacillus thuringiensis* behaves differently compared to other species of the *Bacillus* genus. *Bt* is considered as an entomotoxic and entomopathogenic bacteria, and its toxins are used to control insect pest, however, more research is needed to elucidate the effect of the remaining spores to continue the infection of other insects. It is also worth commenting that although *Bt* is genetically related to other species such as *Bacillus cereus, B. subtilis, B. licheniformis* and another *Bacillus,* their metabolism is strongly different. For instance, the *B. cereus, B. subtilis, B. licheniformis* species can grow in a medium with mineral nitrogen without B vitamins, or the *Paenibacillus polymyxa* species can even use atmospheric N_2 as a source of nitrogen, *Bt* can only grow in presence of organic nitrogen compounds and B vitamins. This last requirement for growth on *Bt* makes the interaction with the insect even stronger, ending the insect's offspring life from within as soon as *Bt* sporulation conditions are optimal. Finally, characterization of other toxins produced by *Bt* strains will further improved the application of these metabolites in other areas, however, the side effects resulted from the use of these toxins to the environment must necessarily be addressed.[112–116]

Acknowledgments

To project 2.7 of the CIC–UMSNH (2022), Morelia, Mexico, and to BIONUTRA, SA de CV, Maravatio, Mexico, for the support on this publication.

Author contributions: Conceptualization JMSY and GU.; data curation, JMSY, JL and GU; writing—original draft preparation, JMSY and GU.; writing—review and editing, JMSY and GU; Final version JMSY. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This manuscript received no external funding.

References

1. Ibrahim MA, Griko N, Junker M, et al. *Bacillus thuringiensis*. A Genomic and proteomic persperctive. *Bioeng Bugs.* 2010;*1*:31–50.
2. Palma L, Muñoz D, Berry C, et al. *Bacillus thuringiensis* Toxins: An Overview of Their Biocidal Activity. *Toxins.* 2014;*6*, 3296–3325.
3. Melo ALDA, Soccol VT, Soccol CR.. *Bacillus thuringiensis*:Mechanism of action, resistance, and new applications: A review *Crit Rev. Biotechnol.* 2016;*36*, 317–326.
4. Malovichko YV, Nizhnikov AA, Antonets KS. Repertoire of the *Bacillus thuringiensis* Virulence Factors Unrelated to Major Classes of Protein Toxins and Its Role in Specificity of Host–Pathogen Interactions. *Toxins. 2019;11*, 347.
5. Erban T, Nesvorna M, Erbanova M, et al. *Bacillus thuringiensis* var *tenebrionis* control of synanthropic mites (Acari: Acaridida) under laboratory conditions. *Exp Appl Acarol*. 2009;49 (4):339–346.
6. Abd El–Ghany A, Abd El–Ghany N. Molluscicidal activity of *Bacillus thuringiensis* strain against *Biomphalaria alexandrina* snails. *Beni–Suef University Journal of Basic and Applied Sciences.* 2017;6:391–393.
7. Du C, Cao S, Shi X, et al. Genetic and biochemical characterization of a gene operon for trans–aconitic acid, a novel nematicide from *Bacillus thuringiensis*. *J Biol Chem.* 2017;*292*, 3517–3530.
8. Vazquez–Pineda. Use of *Bacillus thuringiensis* products as alternative method of control against important veterinary parasitic nematodes. *Rev Mex Cienc Pecu*. 2012;3 (1):77–88.
9. Armada E, Azcón R, López– Castillo OM, et al. Autochthonous arbuscular mycorrhizal fungi and *Bacillus thuringiensis* from a degraded Mediterranean area can be used to improve physiological traits and performance of a plant of agronomic interest under drought conditions. *Plant Physiology and Biochemistry.* 90:64–74.
10. De la Fuente SNM, Casados VLE, Barboza CJE. Bacteriocins of *Bacillus thuringiensis* can expand the potential of this bacterium to other areas rather than limit its use only as microbial insecticide. *Canadian Journal of Microbiology.* 2013;59:515–522.
11. Fernandez–Chapa D, Ramirez–Villalobos J, Galan–Wong L. Toxic Potential of *Bacillus thuringiensis*: An Overview: in book: Protecting Rice Grains in the Post Genomic Era. 2019.
12. Jian Z, Wang F, Li Z, et al. 2014. Grain yield and nitrogen use efficiency responses to N application in *Bt* (Cry1Ab/Ac) transgenic two–line hybrid rice. *Field Crops Research*. 155:184–191.
13. Khorramnejad A, Talaei HR, Hosseininaveh V, et al. Characterization of new *Bacillus thuringiensis* strains from Iran, based on cytocidal and insecticidal activity, proteomic analysis and gene content. *Biocontrol*. 2018;63:807–818.
14. Ohba M, Mizuki E, Uemori A. Parasporin, a new anticancer protein group from *Bacillus thuringiensis*. *Anticancer Research.* 2009;29:427–433.
15. Periyasamy A, Kkani P, Chandrasekaran B, et al. Screening and characterization of a non–insecticidal *Bacillus thuringiensis* strain producing parasporal protein with selective toxicity against human colon cancer cell lines. *Annals of Microbiology.* 2016;1:1–12.
16. Aceves–Diez AE, Estrada–Castañeda KJ, Castañeda–Sandoval LM. Use of *Bacillus thuringiensis* supernatant from a fermentation process to improve bioremediation of chlorpyrifo in contaminated soils. *Journal of Enviromental Management*. 2015;157:213–219.
17. Shikov AE, Malovichko YV, Lobov AA, et al. The Distribution of Several Genomic Virulence Determinants Does Not Corroborate the Established Serotyping Classification of *Bacillus thuringiensis*. *Int J Mol Sci.* 2021;*22*, 2244.
18. Belousora ME, Malovicko YV, Shikov AE, et al. Dissecting the environmental consequences of *Bacillus trhuringiensis* application of natural ecosystems. *Toxin*. 2021;13:355.
19. Li XQ, Tan A, Voegtline M, et al. Expression of Cry5B protein from *Bacillus thuringiensis* in plant roots confers resistance to root–knot nematode. *Biol Control*. 2008;47 (1):97–102.
20. Vilas–Bôas GT, Peruca APS, Arantes OMN. Biology and taxonomy of *Bacillus cereus, Bacillus anthracis*, and *Bacillus thuringiensis*. *Can. Microbiol.* 2007;*53*, 673–687.
21. Martínez–Zavala SA, Barboza–Pérez UE, Hernández–Guzmán G, et al. Chitinases of *Bacillus thuringiensis*:Phylogeny, Modular Structure, and Applied Potentials. *Front Microbiol.* 2020;*10*:3032.
22. Bravo A, Likitvivatanavong S, Gill SS, et al. *Bacillus thuringiensis*: a story of successful bioinsecticide. *Insect Biochem Mol Biol.* 2011;41(7):423–431.
23. Raymond B, Johnston PR, Nielsen–LeRoux C, et al. *Bacillus thuringiensis*:nAn impotent pathogen? *Trends in Microbiology.* 2010;18(5):89–194.
24. Roh JY, Choi JY, Li MS, et al. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. International Journal of Microbiology *Biotechnology.* 2017;17:547.
25. Theoduloz C, Vega A, Salazar M, et al. Expression of a *Bacillus thuringiensis* δ–endotoxin *cry1Ab* gene in *Bacillus subtilis* and *Bacillus licheniformis* strains that naturally colonize the phylloplane of tomato plants (*Lycopersicon esculentum* Mill.). *J Appl Microbiol.* 94:1–7.
26. Adang MJ, Crickmore N, Jurat– Fuentes JL. Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. *Advances in Insect Physiology.* 2014;47:39–87.
27. Raymond B, Federici BA. In defence of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity—a response to EFSA. *FEMS Microbiol Ecol.* 2017;93:fix084.
28. Boyce R, Lenhart A, Kroeger A, et al. *Bacillus thuringiensis israelensis (Bti)* for the control of dengue vectors: systematic literature review. *Tropical Medicine and International Health*. 2013;18(5):564–577.

29. Arturo RR, Ibarra JE. Plasmid patterns of *Bacillus thuringiensis* type strains. *Appl Environ Microbiol*. 2008;74:125–129.

30. Brenner JD, Staley JT and Krieg NR. Bergey's Manual Determinative Systematic Bacteriology. In: Williaman W, Walkins W, editors. Baltimore. USA; 2006.

31. Li Y, Shu C, Shan Y, et al. Complete genome sequence of *Bacillus thuringiensis Bt185*, a potential soil insect biocontrol agent. *Journal of Integrative Agriculture*. 2017;16(3):749–751.

32. Broderick NA, Robinson CJ, McMahon MD, et al. Contribution of gut bacteria to *Bacillus thuringiensis*–induced mortality vary across a range of Lepidoptera. *BMC Biol*. 2009;7:11.

33. Fang S, Wang L, Guo W, et al. *Bacillus thuringiensis* bel protein enhances the toxicity of Cry1Ac protein to *Helicoverpa armigera* larvae by degrading insect intestinal mucin. *Appl Environ Microbiol.* 2009;*75*, 5237–5243.

34. Jouzani GS, Valijanian E, Sharafi R. *Bacillus thuringiensis*:A successful insecticide with new environmental features and tidings. *Applied Microbiology and Biotechnology.* 101:2691–2711.

35. Abdullah RR, Sukar NA, Ghanim NM. Improving the efficiency of *Bacillus thuringiensis* against insects of different feeding habits by plasmid transfer technique. *Life Sci J*. 2014;11:308–318.

36. Abulreesh HH, Osman GEH, Assaeedi ASA. Characterization of insecticidal genes of *Bacillus thuringiensis* strains isolated from arid environments Indian. *J Microbiol*. 2012;52(3):500–503.

37. Assaeedi ASA, Abulreesh HH, Osman GEH. The occurrence and insecticidal activity of *Bacillus thuringiensis* in the arid environments. *Aust J Crop Sci.* 2011;5(10);1185–1190.

38. Valicente FH, Lana UGP. Molecular Characterization of the *Bacillus thuringiensis* (Berliner) Strains 344 and 1644, Efficient Against Fall Armyworm *Spodoptera frugiperda* (J. E. Smith). *Revista Brasileira de Milho e Sorgo*. 2008;7(3):195–209.

39. Zhang L, Zhao G, Hu X, et al. Cry11Aa interacts with the ATP–binding protein from *Culex quinquefasciatus* to improve the toxicity. *Journal of Agricultural and Food Chemistry.* 2017;65:10884–10890.

40. Bizzarri MF, Bishop AH. The ecology of *Bacillus thuringiensis* on the Phylloplane: colonization from soil, plasmid transfer, and interaction with larvae of *Pieris brassicae*. *Microb Ecol*. 2008;56:133–139.

41. Lacey LA, Frutos R, Kaya HK, et al. Insect pathogens as biological control agents:Back to the future. *Journal of Invertebrate Pathology.* 2015;132:1–41.

42. Osman GEH. Detection, cloning and bioinformatics analysis of vip1/vip2 genes from local *Bacillus thuringiensis*. *Afri J Biotechnol*. 2012;11(54):11678–11685.

43. Osman GEH, Altaf WJ, Saleh IAS, et al. First report of detection of the putative receptor of *Bacillus thuringiensis* toxin Vip3Aa from black cutworm (*Agrotis ipsilon*), *Saudi Journal of Biological Sciences*. 2018;25:441–445.

44. Sajid M, Geng C, Li M, et al. Whole–genome analysis of *Bacillus thuringiensis* revealing partial genes as a source of novel *cry* toxins. *Appl Environ Microbiol*. 2018;84(14).

45. Land M, Miljand M. Biological control of mosquitoes using Bacillus thuringiensis israelensis:A pilot study of effects on target organisms, non–target organisms and humans. *Mistra Evi EM;PS4.* 2014.

46. Pardo–López L, Bravo A, Soberón M. *Bacillus thuringiensis* insecticidal three domain toxins mode of action, insect resistance and consequences for crop protection. *FEMS Microbiol Rev*. 2013;37:3–22.

47. Wirth MC, Federici BA, Walton WE. Cyt1A from *Bacillus thuringiensis* synergizes activity of *Bacillus sphaericus* against Aedes aegypti (Diptera:Culicidae). *Appl Environ Microbiol*. 2000;66:1093–1097.

48. Milius S. "Bt–Corn Pollen Can Kill Monarchs". *Science News.* 1999;155:324.

49. Badran A, Guzov V, Huai Q, et al. Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance. Nature. 2016;533:58–63.

50. Peng D, Lin J, Huang Q, et al. novel metalloproteinase virulence factor is involved in Bacillus thuringiensis pathogenesis in nematodes and insects. *Environ Microbiol.* 2016;18, 846–862.

51. Chougule NP, Bonning BC. Toxins for transgenic resistance to hemipteran pests. *Toxins.* 2012;4:405–429.

52. Panwar BS, Ram C, Narula RK, et al. Deconvolution approach for high–throughput gene mining from *Bacillus thuringiensis*. *Applied Microbiology and Biotechnology.* 2018;102:1467–1482.

53. Ye W, Zhu L, Liu Y, et al. Mining new crystal protein genes from *Bacillus thuringiensis* on the basis of mixed plasmid–enriched genome sequencing and a computational pipeline. *Applied Microbiology and Biotechnology.* 2012;78:4795–4801.

54. Carriere Y, Crowder YD, Tabashnik BE. Evolutionary ecology of insect adaptation to *Bt* crops. Evol Appl. 2010;3:561–573.

55. Chen M, Shelton A, Ye GY. Insect– resistant genetically modified rice in China: From research to commercialization. *Annual Review of Entomology.* 2011;56:81–101.

56. Jakka S, Shrestha R, Gassmann A. Broad–spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). *Scientific Reports*. 2016;6:1.

57. Li ZY, Sui H, Xu YB, et al. Effects of insect–resistant transgenic *Bt* rice with a fused Cry1AbþCry1Ac gene on population dynamics of the stem borers, *Chilo suppressalis* and *Sesamia inferens*, occurring in paddyfield. *Acta Ecologica Sinica*. 2012;32:1783–1789.

58. Sanchis V, Bourguet D. *Bacillus thuringiensis*:Applications in agriculture and insect resistance management. *A review Agronomy for Sustainable Development.* 2008;28:11–20.

59. Schnepf E, Crickmore N, Van Rie JD, et al. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol Mol Biol Rev*. 1998;62:775–806.

60. Soberón M, López–Díaz JA, Bravo A. *Bacillus thuringiensis*:A protein fold conserved in several pathogenic microorganisms. *Peptides*. 2013;2;41:87–93.

61. Tabashnik BE, Brévault T, Carrière Y. Insect resistance to *Bacillus thuringiensis* crops: lessons from the first billion acres. *Nature Biotechnology*. 2013;31(6):510–521.

62. Valadares G, Whittome B, Shore B, et al. Identification of *Bacillus thuringiensis* subsp. *kurstaki* strain HD–1 like bacteria from environmental and human samples after aerial spraying of Victoria British Columbia, Canada, with foray 48B. *Appl Environ Microbiol.* 2001;67(3):1035–1043.

63. Aronson AI, Beckman W, Dunn P. *Bacillus thuringiensis* and related insect pathogens. *Microbial Rev*. 1986;50(1):1–24.

64. Sthaly DP, Dingman DW, Bulla Jr, et al. Possible origin and function of the parasporal crystal in *Bacillus thuringiensis*. *Biochem Biophys Res Commun*. 1978;84 (3):581–588.

65. Burges HD, Hurst JA. Ecology of *Bacillus thuringiensis* in storage moths. *J Invertebr Pathol*. 1977;30(2):131–139.

66. Alekseev AN, Karabanova LN, Shevtsov VV.Initiators and inhibitors of *Bacillus thuringiensis* spore germination. *Mikrobiologiya*. 1982;51:780–783.

67. Chiang AS, Yen DF, Peng WK. Germination and proliferation of *Bacillus thuringiensis*, in the gut of rice month larva *Corcyca cephalica*. *J Invertebr Pathol.* 1986;48(1):96–99.

68. Benoit TG, Wilson CR, Bull DL, et al. Plasmid associated sensitivity of *Bacillus thuringiensis* to UV light. *Appl Environ Microbiol*. 1990;56:2282–2286.

69. Monnerat R, Soares CM, Capdeville G, et al. Translocation and insecticidal activity of *Bacillus thuringiensis* living inside of plants. *Microbial Biotecnol*. 2009;2 (4):512–520.

70. Akiba Y. Microbial Ecology of *Bacillus thuringiensis* VI Germination of *Bacillus thuringiensis* spores in the soil. Appl Ent Zool. 1986;21 (1):76–80.

71. Ferrerira LP, Suzuki TM, Hano NK, et al. Ecological aspects of *Bacillus thuringiensis* in on oxisol. *Scientia Agricola*. 2003;60 (1):19–22.

72. Germanine GR, Murrell WG. Effect of dipicolinic acid on the ultraviolet radiation resistence of *Bacillus cereus* spores. *Photochem Photobiol*. 1973;17(3):145–154.

73. Griego VM, Spence KD. Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. *Appl Environ Microbiol.* 1978;35(5):906–910.

74. Petras SF, Casida LE. Survival of *Bacillus thuringiensis* spores in soil. *Appl Environ Microbiol*. 1985;50 (6):1496–1501.

75. Pruett CJH, Burges HD, Wyborn CH. Effects of exposure to soil on potency and spore viability of *Bacillus thuringiensis. Journal of Invertebrate Pathology*. 1980;*35*(2):168–174.

76. Sekijima Y, Akiba Y, Ono K, et al. Microbial ecological studies on *Bacillus thuringiensis*. I. Dynamics of *Bacillus thuringiensis* in soil of mulberry field. *Japanese Journal of applied entomology and zoology*. 1977;21(1):35–40.

77. Ignoffo CM, García C. UV–Photorectivation of cell and spores of *Bacillus thuringiensis* effects of peroxidase of inactivation. *Environ Entomol*. 1978;7(2):270–272.

78. Sanchez–Yañez JM, Peña–Cabriales JJ. Persistence of *Bacillus thuringiensis* spores on soil and maize and bean leaves. *TERRA Latinoamericana*. 2000;18 (4):325–331.

79. Fonda MS, Salama HS, Selim M. Factors affecting the growth physiology of *Bacillus thuringiensis*. *Appl Microbiol Biotechnol*. 1985;22(1):50–52.

80. Van Frankenhuyzen K. Cross–order, cross–phylum activity of *Bacillus thuringiensis* pesticidal proteins. *Journal of Invertebrate Pathology*.2013;114:76–85.

81. Ejiofor AO, Johnson T. Physiological and molecular detection of crystalliferous *Bacillus thuringiensis* strains and from habitats in South Central United States. *J Ind Microbiol Biotechnol*. 2002;28:284–290.

82. Cherif A, Ettoumi B, Raddadi N, et al. Genomic diversity and relationship of *Bacillus thuringiensis* and *Bacillus cereus* by multi–REP–PCR fingerprinting. *Canadian Journal of Microbiology*. 2007;53:343–350.

83. Ferré J, Van Rie J. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annu Rev Entomol*. 2002;47:501–533.

84. Alper M, Güneş H, Tatlipinar A, e al. Distribution, occurrence of cry genes, and lepidopteran toxicity of native *Bacillus thuringiensis* isolated from fig tree environments in Aydın Province. *Turkish J Agric For.* 2014;38:898–907.

85. Canton PE, Reyes EZ, de Escudero IR, et al. Binding of *Bacillus thuringiensis subsp. israelensis* Cry4Ba to Cyt1Aa has an important role in synergism. *Peptides*. 2011;32:595–600.

86. Brar SK, Verma M, Tyagi RD, et al. Recent advances in downstream processing and formulation of *Bacillus thuringiensis* bases biopesticides. *Process Biochem*. 2006;41:323–342.

87. Regis L, da Silva SB, Melo–Santos MAV. The use of bacterial larvicides in mosquito and black fly control programs in Brazil. *Mem Inst Oswaldo Cruz*. 2000;95:207–210.

88. Lee L, Saxena D, Stotzky G. Activity of free and clay–bound insecticide proteins from *Bacillus thuringiensis sub, israelensis* against the mosquito *Culex pipiens*. *Appl Environ Microbial*. 2003;69:4111–4115.

89. Saxena D, Stotzky G. Fate and effects in soil of the insecticidal toxins from *Bacillus thuringiensis* in transgenic plants, in:Collection of Biosafety Reviews. International Centre for Genetic Engineering and Biotechnology, *Trieste*. 2003. p. 7–83.

90. Saxena D, Pushalkar S, Stotzky G. Fate and effects in soil of Cry proteins from *Bacillus thuringiensis* influence of physicochemical and biological characteristics of soil. *The Open Toxinology Journal*. 2010;3:133–153.

91. Stotzky G. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clay and humic acids. J *Environ Qual.* 2000;29:691–705.

92. Stotzky, G. Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*. In:DK. Letourneau, BE Burrows, editors. Genetically engineered organisms:assessing environmental and human health effects. CRC Press, Boca Raton, Florida, USA;2001. p. 187–222.

93. Shishir A, Roy A, Islam N, et al. Abundance and diversity of Bacillus thuringiensis in Bangladesh and their cry genes profile. *Front Environ Sci*. 2014;2:20.

94. Valicente FH, Mourão AHC. Use of by–products rich in carbon and nitrogen as a nutrient source to produce *Bacillus thuringiensis* (Berliner)–based biopesticide. *Neotropical Entomology*. 2008;37(6):702–708.

95. Giustolin TA, Vendramin JD, Alves SB, et al. Susceptibility of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae) reared on two species of *Lycopersicon* to *Bacillus thuringiensis* var. *kurstarki*. *J Appl Entomol*. 2001;125:551–556.

96. Fiuza LM, Polanczyk RA, *Crickmore N. Bacillus thuringiensis and Lysinibacillus sphaericus*:Characterization and Use in the Field of Biocontrol. Springer, Cham, Switzerland; 2017. ISBN:978–3–319–56678–8.

97. Dale C, Moran NA. Molecular interactions between bacterial symbionts and their hosts. *Cell.* 2006;126:453–465.

98. Agaisse H, Lerectus D. How does *Bacillus thuringiensis* produce so much insecticidal crystal protein? *Journal of Bacteriology*.1995;177 (21):6027–6032.

99. Crickmore N, Zeigler DR, Feitelson J, et al. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol. *Mol Biol Rev.* 1998;62(3):807–813.

100. Höfte H, Whiteley R. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol Rev*. 1989;53:242–255.

101. Iriarte J, Porcar M, Lecadet MM, et al. Isolation and characterization of *Bacillus thuringiensis* strains from aquatic environments in Spain. *Curr Microbiol*. 2000;40(6):402–408.

102. Li J, Carroll J, Ellar DJ. Crystal structures of insecticidal endotoxin from *Bacillus thuringiensis* at 2.5 Å resolution. *Nature*. 1991;353:815–821.

103. Da Silva RB, Valicente FH. Molecular characterization of *Bacillus thuringiensis* using rep–PCR. *Springer Plus* 2013;2:641.

104. De Maagd R, Bravo A, Crickmore N. How *Bacillus thuringiensis* has evolved specific toxin to colonize the insect world. *Rev Trend Genet*. 2001;17:193–199.

105. Armengol G, Escobar MC, Maldonado ME, et al. Diversity of Colombian strains of *Bacillus thuringiensis* with insecticidal activity against dipteran and lepidopteran insect. *J Appl Microbiol*. 2007;102:78–88.

106. Minu MA, Dipak V. *Bacillus thuringiensis* as endophyte of medicine plants:auxin producing biopesticide. *Int Res J Environment Sci.* 2014;3 (9) 27–31.

107. Bhalla R, Dalal M, Panguluri SK, et al. Isolation, characterization and expression of a novel vegetative insecticidal protein gene of Bacillus thuringiensis. *FEMS Microbiol Lett.* 2005;243:467–472.

108. Furlaneto L, Ostrensky SH, Nagy AOM. Survival and conjugal transfer between *Bacillus thuringiensis* strains in aquatic environment. *Brazilian Journal of Microbiology*. 2000;31:233–238.

109. Medrano–González MA, Luna–Olvera HA, Peña C J, et al. Survival of vegetative cells of *Bacillus thuringiensis* in the sphermosphere/rhizosphere of common bean. *TERRA Latinoamericana*. 2000;18 (4):333–337.

110. West AW, Burges HD and Wyborn CH. Survival of *Bacillus thuringiensis* and *Bacillus cereus* spore inocula in soil:effect of pH, moisture, nutrient availability and indigenous microorganisms. *Soil Biochem*. 1985;17:657–665.

111. Aronson A, Shai Y. Why *Bacillus thuringiensis* toxins are so effective? unique features of their mode of action. *FEMS Microbiol Lett*. 2001;195:1–8.

112. Arrieta G, Hernández A, Espinosa AM. Diversity of *Bacillus thuringiensis* strains isolated from coffee plantations infested with the coffee berry borer *Hypothenemus hampey*. *Biol Trop*. 2004;52(3):757–764.

113. Blackburn MB, Martin PA, Kuhar D, et al. Phylogenetic distribution of phenotypic traits in *Bacillus thuringiensis* determined by multilocus sequence analysis. *PLoS One.* 2013;8:e6606.

114. Martínez C, Caballero P. Contents of cry genes and insecticidal toxicity of *Bacillus thuringiensis* strains from terrestrial and aquatic habitats. *J Appl Microbiol*. 2002;92(4):745–752.

115. Guo R, Ruan H, Yang W, et al. Differential responses of leaf water–use efficiency and photosynthetic nitrogen–use efficiency to fertilization in Bt–introduced and conventional rice lines. *Photosynthetica*. 2011;49:507–514.

116. Cano, RJ, Boruck, MK, Schweitzer, MH, et al. *Bacillus* DNA in fossil bees: an ancient symbiosis. *Appl Environ Microbiol*. 1994;60(6):216.