J. Mycopathol. Res. ***57(4)*** *: 193-200, 2020;* (ISSN 0971-3719)
© Indian Mycological Society, Department of Botany,
University of Calcutta, Kolkata 700 019, India

REVIEW

BACILLUS THURINGIENSIS : importance and applications

NABANITA BARMAN[1], ANITA DEBNATH[1], JAHANUR HOSSAIN[2] SHIBU BARMAN[1], AND R. P. MUKHOPADHYAY[2]*

[1]Department of Botany, Cooch Behar Panchanan Barma University,Vivekananda Street, Cooch Behar 736101, West Bengal

[2] Department of Botany, Tufanganj Mahavidyalaya, Tufanganj 736160, Cooch Behar, West Bengal

Received : 11.11.2019 RMs Accepted : 11.12.2019 Published : 27.01.2020

Control of insect pest in agriculture and of insect vectors of important human diseases is mainly achieved by using chemical insecticide. However, use of these chemical insecticides has lead to several problems including environmental pollution and the human health problem such as cancer and several immune system disorders. Although microbial insecticides have been proposed as a substitute for chemicals, their use is limited since most microbes show a narrow spectrum of activity. Isolation and characterization of new insecticidal activities is the target of many pest control programme. *Bacillus thuringiensis* an endospore forming gram positive, rod shaped aerobic bacterium. *Bacillus thuringiensis* produces insecticidal proteins (cry and cytotoxin) during the spiculation phase and parasporalcrystals. These toxins are highly specific to their target insect are innocuous to humans vertebrates and plants are completely biodegradable. Therefore, *Bacillus thuringiensis* is a viable alternative for the control of insect pests in agriculture and disease vectors of importance in public health. Cry toxins have specific activities against insects species of the orders *Lepidoptera, Diptera,Coleoptera, Hymenoptera* and against nematodes. Moreover crystal proteins have not been Abel to provide protection against all the group of agronomical harmful pest . In view of this situation recently reported vegetative insecticidal proteins(VIP) a group of vegetative protein produced during the vegetative stage of bacterial growth from *BT* seems to provide possible solution.

Key words: *Bacillus thuringeiensis,* importance, applications

INTRODUCTION

*Bacillus thuringiensis*is an aerobic, spore forming, gram positive, (El-Menofy *et al.* 2014) rod shaped bacterium distributed widely in the natural environment from the Arctic to the Tropics. The entomopathogenic and insecticidal action ('Sotto' disease) of the bacterium was first noted by Ishiwata in Japan. The first *Bacillus thuringiensis* based microbial insecticide is commercial transgenic crop to express a *Bt* gene in 1996. *Bt* produce insecticidal proteins (cry and cytotoxin) during the spiculation phase and parasporal crystals.These crystals are predominantly comprised of one or more proteins, also called delta-endotoxins or cry proteins (El-Menofy, *et al.* 2014). These toxins are highly specific to their target insect and are innocuous to humans (Raymond *et.al.*2010; Bravo *et al.* 2011), vertebrates and plants are completely biodegradable. Another protein vegetative insecticidal protein produced during the vegetative stage of the bacterial growth which is non specific.

TAXONOMY AND DISCOVERY

Bacillus thuriengiensis (BT) is a member of the *Bacillus cereus* group and this group comprises of eleven closely related species : *B. anthracis, B. cereus, B. thuringienis, B. mycoides, B. pseudomycoides, B. weihenstepphanesis, B. cytotoxicus, B. toyonensis* (Jimenez *et al.* 2013) "B. gaemokensis" (Jung, *et al.* 2011), and *B. manliponensis* (Jung,*etal.* 2011), *and "B. bingma-yongensis"* (Liu, *et al.* 2014) (The names of the last three species are effectively but not yet validly published and thus are in quotation marks throughout this study). The former six species were identified during the 20th century, whereas the remaining five species were classified in recent

*Corresponding author : rampsdo783@gmail.com

years. Members of the *B. cereus* group have a significant impact on human health, agriculture and the food industry. The feature that disguishes *BT* from the other member of the *Bacillus cereus* is its entomopathogenic properties.

In Bt, three-domain Cry proteins display toxic activity against insect species of the following orders : Lepidoptera, Diptera, Coleoptera, Hemiptera (low to moderated toxicity for some aphids) and nematodes (Van Frankenhuyzen, 2009, 2013). To date, the Bt Toxin Nomenclature Committee (Ibrahim*, et al.,* 2010) has classified 73 different types (Cry 1 to Cry 73) of Cry proteins, including three-domain and ETX_MTX2 family proteins from Bt and Ls, with individual toxin showing well documented toxicity against lepidopterans, coleopterans, hemipterans, dipterans, nematodes (human and animal parasites, and free living; Rhabditida) some snails [Van Frankenhuyzen, 2009; Ben-Dov 2014; Chougule and Bonning, 2012; Van Frankenhuyzen, 2013; Ali *et al.,* 2010] and/or human-cancer cells of various origin. [Ohba, *et al.* 2009.

GENOME OF BT

Bt strain have the genomic size of 2.4 to 5.7million bp. Physical map have been constructed by two *Bacillus thuringiensis* strains. Comparison with bacterial chromosomes chromosomal map suggest that all of there chromosome have a similar organization in the half near the replication origin while displaying greater variability in the terminal half. Most *Bacillus thuringiensis* isolates have several extra chromosomal elements, some of them circular and other linear. It is long been recognized that the protein comprising the parasporalerystal are generally encoded by large plasmids.

TOXIN

According to WHO, 9 different toxins have been described in different strains. These toxins are Alfa- exotoxin (Phospholipase C), beta – exotoxin (thermostable) exotoxin), gamma – exotoxin (toxic to sawflies), delta – exotoxin (protein parasporal crystal), louse factor exotoxin (active only against lice), mouse factor exotoxin (active only against mouse and lepidopteran insects), water soluble toxin, VIP 3 A (*BT* vegetative insecticidal protein)and enterotoxin (produced by vegetative cells). Among those several toxins produced by *BT* strain, delta - endotoxin received much attention and has been exploited as a commercial production of bioinsecticides which more efficiency utilize for protection a variety of crops from various insects pests . *BT* crystals have been found to be of various forms(bipyramidal , cuboidal , flat rhomboid , spherical or a composite with two or more crystal types). Structurally the crystal toxins (delta –endotoxins) belongs to the two different types

- Cry family, with specific cytolytic activity as cry 1Aa1, cry1Ba1 , Cry2 Aa1 etc
- Cyt family, which is a nonspecific cytolytic and hemolytic as cytAa1, Cyt2 Aaetc (WHO, 1999).

The *BT* proteins can be divided into two groups the crystal forming proteins including in *BT* spores (cry and cyt toxins) and the protein produced in vegetative state (VIP toxins).

The cry toxins can be divided into several groups the largest of these is the group of 3 domains cry toxins).

DELTA–ENDOTOXIN PROTEIN PARASPORAL CRYSTALS

One of the most dramatic aspects of Bt sporulation is the formation of parasporal crystals. The insecticidal toxin (cry proteins) of Bt, often times reffered to as delta – endotoxins are some what specific to certain insects. The family of genes codeing for these toxins is the cry gene family. The term delta – endotoxin, relative to B. cry toxin is a misnomer. Based on its mode of action, a cry toxin is a ‘simple’ toxin, defined as a monomer or oligomer of a toxic simple protein. The parasporal crystals of Bt are oligomers composed of polypeptide protein subunits. The protoxin is the immediate toxic precursor of cry toxins ie upon activation of an insecticidal cry toxin is generated. Different parasporal crystals are made either of single or multiple crystal of Bt subsp. Kerstaki HD – 73 contains Cry 1 Ac protein, whereas the parasporal crystal of HD 1 sfrain, which belongs to the same subspecics is comprised of five, different cry toxins – cry 1 Aa, cry 1 Ab, cry 1 Ac, cry 2 Aa and cry 2 An.

A universal nomenclature and classification scheme for Cry proteins and their genes based on host range was also proposed. Accordingly, cry genes were classified into four major classes based upon their protein toxicity towards insects and primary reactivity with corresponding genes viz cry I (lepidopteran specific), cry II (lepidopteran and dipteran specific), cry III (coleopteran specific) and cry IV (dipteran specific).

3Aa (Fig. 1) cry 1 As cry 1 As, cry 2 Aa, cry 3 Bb, cry 4 Ba, cry 4 Aa and cry 8Ea 1 (Guo *et al*. 2009).

REGION IN *BACILLUS THURINGIENSIS* TOXIN REQUIRED FOR TOXICITY

All toxins contain three structural domains and share a high degree of topological similarity.

Fig. 1 : Three-dimensional structure of activated Cry3Aa toxin. Schematic diagram showing the three domains of the protein. (Image courtesy of D.J. Ellar, University of Cambridge, UnitedKingdom)

Table1 : Nomenclature and classification scheme for cry Genes based on host range.

Gene	Crystal Shape	Protein size (kDa)	Insect activity
Cry I [several subgroup: A (a), A (b), A (c), B, C, D, E, F, G]	Bipyramidal	139–138	*Lepidoptera* larvae
Cry II [subgroups A, B, C]	Cuboidal	69 — 71	*Lepidoptera*
Cry III [subgroups A,B, C]	Flat / irregular	73—134	*Cloeoptera*
Cry IV [subgroups A,B,C,D]	Bipyramidal	73—134	*Diptera*
Cry V IX	Various	35—129	Various

Later, another nomenclature, based on amino acid sequence similarity, was proposed. According to this classification cry genes are divided into 51 groups and the cry toxins are separated into six major classes according to their insects host specificities that includes:

Group 1—lepidopteran (cry 1, cry 9 and cry 15) ; *Group 2*— lepidopteran and clipteran (cry 2) ; *Group 3*— coleopteran (cry 3, cry 7 and cry 8) ; *Group 4* — dipteran (cry 4, cry 10, cry 11, cry 16, cry 17, cry 19 and cry 20); *Group 5* — lepidopteran and coleopteran (cry II) and *Group 6 — nematodes* (cry 6). The three – dimensional structures of a number of cry toxins have been published : Cry

Domain I is composed of a bundle of seven Alfa – helices connected by loops. The Alfa – helical has a central amphipathic alfa- helix that well conserved among all the toxins described. Various mutations in **Domain I** appear to cellular receptors. Whether these mutation affect overall conformation of the toxin molecule and the compromising toxicity is not known.

Domain II consists of three sets of anti parallel 1 beta – sheets each terminating with a loop. The beta sheets are packed around a central hydrophobic core forming a so called beta-prism structure.

Domain III is a sandwich of two anti parallel Beta-sheets that form a “jelly – roll” topology. Results of site – directed mutagenesis and truncation analysis provide strong evidence for the involvement of Domains II and III in receptor binding and insecticidal activity.

MECHANISM OF CRY TOXIN ACTION

There are several models reviewed in the literature that seek to explain how cry toxins exert their killing capacity (Fig. 2; 3A and B).

The first one: Whalon and Wingered. Before toxicity can occur, the protoxin must be proteolytically processed. This requires the high pH found in the midgut as well as digestive enzymes from the insect. Activation involves the removal of both the carboxy 1 terminal and the amino terminal ends of the protein. Once activated, the cry toxin diffuses from the lumen thought the periplasmic membrane into the endoperiplasmic space. The fully processed and active cry toxin now has access to the surface of the columnar epithelial cells. At the cell surface, a critical handshake occurs as the cry protein binels to its receptor. Aminopeptidases are involved in digestion and call adhesion molecules similar to

The second one: Binding of Cry 1 Ab toxin to the BT–R1 receptor induces a molecular signal that stimulates heterotrimeric G protein and adenyly 1 cyclase with anal accompanying dramatic increase production of cAMP. The cAMP activates protein kinase A, bringing about an array of cellular alterations, which includes cytoskeletal rearrangement and ion. Acceleration of this second messenger pathway alters the chemistry of the cell and brings about cell death. Furthermore killing mechanism involves promotion by the toxin of exocytotic translocation of BT – R1 from intracellular membrane vesicles to the cell membrane (Zhang *et al.* 2008). Movement of the receptor is mediated by toxin – induced singnal –

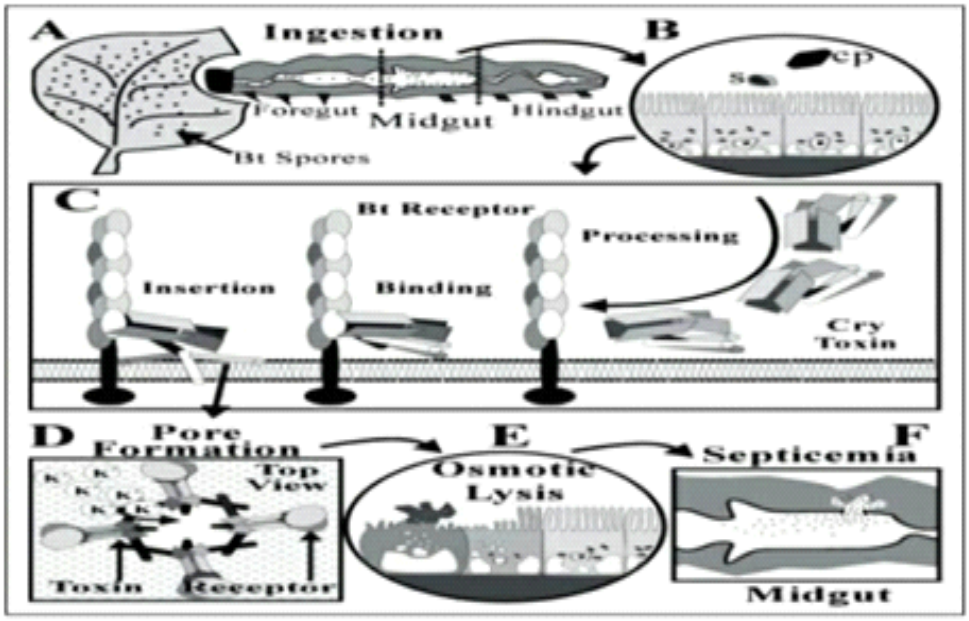


Fig. 2 : Mechanism of cry protein toxicity . A – Ingestion of spores or recombinant protein phytophagous larva B – In the midgut endotoxins are solubilized from *Bacillus thuringiensis* spores and inclusion of crystalline protein . C – Cry toxins are proteolytically processed to active toxins in the midgut , active toxins binds receptors on the surface of columnar epithelial cells. Bound toxin inserts into the cellular membrane . D– Cry toxins aggregate to form pores in the membrane .E – Pore formation leads to the osmotic lysis. F – Heavy damage to midgut membranes leads to starvation or septicemia [Bravo *et al.* 2011].

cadherins function as receptors for the cry proteins. Binding of the cry proteins is thought to occur at a membrane proximal region of these membrane bound proteins. Evidence from receptor binding studies has demonstrated that some cry proteins bind to more then one glycosylation is critical for some of the interactions. As a result, resistance to one cry toxin does not guarantee universel resistance to all cry proteins, although the presence of multiple resistancealleles in one individual has been shown to contribut to cross – resistance to multiple cry toxins (Gamal *et al.,* 2015).

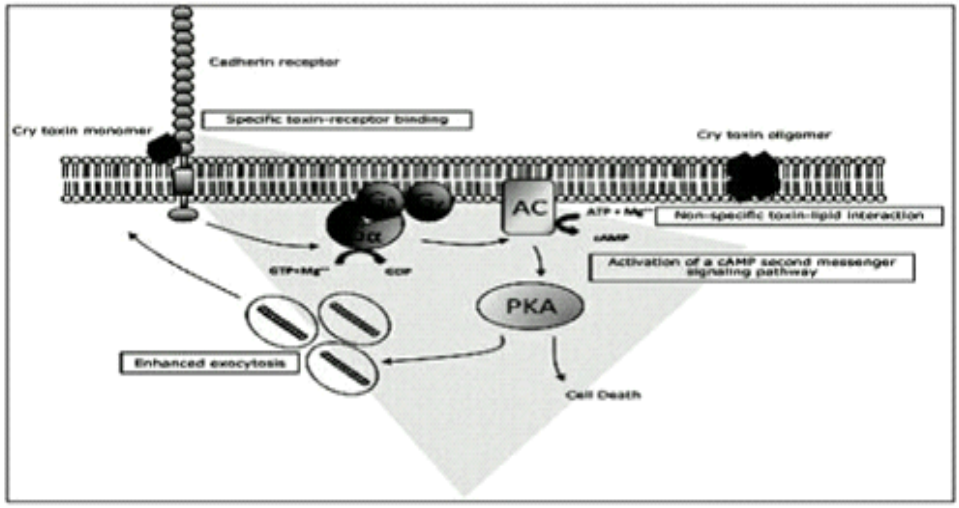
transduction and correlated directly to the execution of cell death (Ibrahim *et al.* 2010; Vachon *et al.* 2012).

VEGETATIVE INSECTICIDAL PROTEIN'S TOXINS

In addition to Delta – endotoxins, Bt produces a novel family of insecticidal proteins named vegetative insecticidal proteins (Vip) during its vegetative stage. Two classes of vip toxins are described. The first consists of a binary system composed of two proteins, Vip 1 and Vip 2, which

are 100 kDa in size, respectively. These proteins are highly toxic to certain coleopteran species.The second class consists of an 88.5 k da protein, VIP -3 which is active against a wide spectrum of lepidopteran insects. These two classes of protein do not display sequence of homology with cry proteins. To date there are approximately 82 kinds of vegetative insecticidal protein genes that have been identified and cloned . These genes can be classified into three groups, eight subgroups, 25 classes, and 82 subclasses according to the encoded amino acid sequences similarity (http:// www.llifesci.sussex.ac.uk/home/NeilCrimore/ Bt/ vip:html/). The Vip proteins, bearing no similarity to Delta-endotoxins have became an important new insecticidal proteins .

a mixture of spores and the insecticidal crystals. By 1995, 182 Bt-based products are registered by the U.S. Environmental Protection Agency (EPA), but in 1999 Bt formulations constitute less than two per cent of the total sales of all insecticides and represented around 80% of all biopesticides sold. The use of Bt has increased as insect pests have become resistant to chemical insecticides.Bt sprays are estimated to bring in U.S. $8 billion per annum. At one time, Bt sprays constitute $100 million in annual sales, but with the advent of transgenic plants engineered with the insecticidal cry gene, sales have decreased to $40 million. Half of current sales are used in Canadian forests to control the gypsy moth, spruce budworm, and other lepidopteran pests. The



A

Fig. 3: Proposed model for Cry toxin action. The univalent binding of Cry toxin monomer to BT-R initiates the progression of cell death by transmitting a death signal into the cell. A signal transduction pathway, involving G protein (Ga) adenylyl cyclase (AC) and protein kinase A (PKA), is activated. Activation of the signaling pathway mediates exocytosis of the BT-R receptor from intracellular vesicles to the cell membrane. The resulting enhanced display of BT-R on the cell surface facilitates recruitment of additional toxin molecules which, in turn, amplifies the original signal in a cascade-like fashion. The signaling kinase PKA modifies downstream molecules that promote the biochemical activities that destroy the cell. Toxin oligomers incorporated into the plasma membrane of living cells do not form lytic pores and are not toxic. (Ibrahim *et al.* 2010).

DEVELOPMENT OF BIOPESTISIDES

The Insecticidal properties of Bt were recognized many years before the bacterium was identified, with some accounts suggesting that Bt spores may have already been in use in ancient Egypt (Sanahuja *et al.* 2011). Insecticidal Bt products are first commercialized in France in the late 1930s.For over 60 years, Bt has been one of the most consistent and significant biopesticides for use on crops as an insecticidal spray, containing

OECD (Organization for Economic Cooperation and Development) predicts that the biopesticide may grow to 20% of the world's pesticide market by 2020.Bt sprays are used sporadically and typically over small areas. Sprayable Bt formulations have penetrated cotton, fruit and vegetable, aquatic, and other insecticide markets. New Bt formulations have consistently made gains in a limited number of fruit and specialty vegetable markets over the last number of years. Sprays are also chosen by organic farmers to meet guidelines

for using strictly non synthetic materials. An additional use of Bt is in the protection of stored commodities from pest infestation. In the early 1950s, Stein has began to experiment with Bt, produced Bt, and the agent Thuricide has been soon available. The name Thuricide has survived of industrial transformations and is a product of Valent Biosciences today. Standardization is based on spore count rather than potency, the products often contained a heat-tolerant exotoxin, and most are based on subsp*. thuringiensis* and are of low potency. The isolates of Kurstak and Damage are serotyped by de Barjac and Lemille and designated subsp. KurstakiHD-1. They became the basis for products that are competitive with chemical insecticides in performance and cost, and before long, all of the Bt companies that produced Bt are producing subsp. Kurstaki, it remains, by far, the greatest commercial success of microbial control, in the strictest sense of the word. Much of Bt's commercial success prior to the introduction of transgenic plants is in forestry. According to Lewis *et al.* its use against the spruce budworm and gypsy moth in North American forests accounts for 60% or more of world sales. However, other varieties, such as the Coleoptera-active Bt subsp.*tenebrionis*, discovered by Krieg *et al.* and the Diptera-active subsp*. israelensis* isolated by Goldberg and Margalit , have come to be used extensively for the control of larvae of pest and vector black flies and mosquitoes around the world, providing both medical and environmental benefit. An example of an outstanding success in cooperation between industry and a governmental organization to achieve those benefits is the Onchocerciasis, Control Programme of the World Health Organization(WHO), wherein Bt subsp. *Israelensis* applications comprise up to 50% of all insecticide applications. The relevant works on the screening and isolation of new Bt strains have been performed and finally resulted in the production of commercial products (Table 2).Among them, we isolated a Bt strain designated NT0423,belonging to Bt subsp. *aizawai,* an isolate from the soil of sericultural farms . The manufactured article, using Bt strain NT0423, (named "Tobaggi" and produced from Dongbu Hannong Chemicals) is one of the registered Bt biopesticides in Korea . This strain has at least five known crystal protein genes, cry1Aa, cry1Ab, cry1C,cry1D, and cry2A, and one new gene, cry1Af1 (Gen bank Accession No. U82003). It has dual toxicity against lepidopteran larvae-like *Plutella xylostella*, *Spodoptera exigua,* and *Hyphantria cunea,* and dipteran larvae-like *Culex pipiens* and *Musca domestica*. The developmental procedure for the Bt NT0423 product might be a typical example of Bt sequential research for Bt biopesticides (Table 2).

Table 2: Bt-based biopesticide active ingredients and product

Bt subspecies	Strain	Product name	Target insect
Kurstaki			
	-c	Foray	Lepidopteran
	-	Biobit	Lepidopteran
	BMP123	BMP123	Lepidopteran
	EG2348	Condor	Lepidopteran
	EG2371	Cutlass	Lepidopteran
	ABTS-351	Dipel	Lepidopteran
	EG7481	Crymax	Lepidopteran
	EG7826	Lepinox	Lepidopteran
	SA-11	Javelin	Lepidopteran
	SA-12	Thuricide	Lepidopteran
Aizawai			
	GC-91	Agree	AW
	NB200	Florba	AW
	ABTS	XenTari	AW
	NT0423	Tobagg	AW
	GB413	Solbicha	AW
Israelensis			
	-	Gnatro	Mushroom fly
	-	Bactiomas	Mosquito & BF
	AM-65-5	VectoBa	Mosquito & BF
	SA3A	Teknar	Mosquito & BF
	BMP144	BMP	
Tenebrioni			
	NB-176	Novodor	CPB, ELB

The information about Bt strains and product names is from EPA-registered ingredients and products list of biopesticides

Two products are produced by a Korean company.

USE OF *BACILLUS THURINGIENSIS*

Insecticidal action: Upon sporulation *B. thuringiensis* froms crystals of proteinaceous

insecticidal Delta – endotoxins (called crystal proteins or cry proteins) which are encoded by cry gene . In most of strains of *B. thuringiensis* the cry gene are located on a plasmid[cry is not a chromosomal gene in most strains. Cry toxins have species of the orders *Lepidoptera* (moths and butter flies); *Diptera* (flies and mosquitoes); *Coleoptera* (beetles) ; Hymenoptera (wasps, bees, ants and sawflies) and against nematodes. Thus, *Bacillus thuringiensis* serves as an important reservoir of cry toxins for production if biological insecticides and insect resistant biologically modified crops, When insect ingests toxin crystals, their alkaline digestive tracts denatured the insoluble crystals making them soluble and thus amenable to being cut with protease found in the insect guts, which liberate the toxin from the crystal. The cry toxin is then inserted into the insect gut cell membrane, paralyzing the digestive tract and forming a W. S. Cranshow (2013). The insect stop eating and starves to death; live Bt bacteria may also colonize the insect which can contribute to death. It has been shown that a small RNA called BT R1 can silence the cry toxin when outside the host by binding to the RBS side of the crystal 5 Bt toxin transcript and inhibiting its expression. The silencing results in increase ingestion by *C. elegans* and is relieved inside the host, resulting in the host death. [Peng *et al.* 2018].

In 1996 another type of insecticidal protein in *Bt* was discovered and is known is the vegetative insecticidal proteins. Vip proteins do not share sequence homology with cry proteins, in general do not compete for the same receptors and some kill different insect than do cry protein.

IMMUNE INHIBITOR A

In addition to the toxic factor, others protein are involved in invasion of the host and blocking the host immune defenses. Two protein materials are isolated that could block the immune factors of the hemolymphsaturated pupae and named the immune inhibitors as A& B. Immune A is heat sensitive and is able to block the lysis of coli by hemolymph taken from pupae of *Hyalophora ceropia* .The immune inhibitor A was purified.

Recent studies with *Bacillus thuringiensis* have discussed promising applications in the branches of other branches of science —

- Chitinase that degrades chitin , increase the efficiency of *Bacillus thuringiensis* insecticides , and there has been of increasing interest in the industry.
- Another promising field is the potential of *Bacillus thuringiensis* protein to act against cancer cells.
- Parasporin toxins of *Bacillus thuringiensis* that do not have an entomopathogenic effect , have a cytotoxic effect on the cells changed by some cancers.

Bt bacteria synthesize different insecticidal proteins named Cry, Vip and Cyt that are able to kill different insect orders, or nematodes .This proteins have been extensively used to control insects and is in practice in agriculture as sprays or expressed In genetically modified (GM) plants. Commercial production of the *Bt* as a spray began in France in 1938, and was introduced to the United States in 1958. Originally it was not widely used due to the availability of effective synthetic insecticide and its practical limitation; it rapidly washes away in rain and is degraded by sunlight.

REFERENCES

Ali, B.A., Salem, H.H. , Wang, X.M., Huang, T.H., Xie, Q.D. and Zhang, X.Y. 2010. Effect of Bacillus thuringiensis var. israelensis endotoxin on the intermediate snail host of Schistosomajaponicum. *Curr. Res. Bacteriol.* **3:** 37-41.

Ben-Dov E. 2014. *Bacillus thuringiensis* subsp. *Israelensis* and its Dipteran-Specific Toxins. *Toxins.* **6:** 1222-1243.

Bravo, A., L. ikitvivatanavong, S., Gill, S.S. and Soberon, M. 2011. *Bacillus thuringiensis* : A story of a successful bioinsecticide*. Insect Biochemistry and Molecular Biology,* **41:** 423-431.

Chougule, N.P. and Bonning, B.C.2012. Toxins for transgenic resistance to hemipteran pests. *Toxins.* **4:**405-429.

Cranshaw, W.S, 2013. Colorado State University extension office. *Bacillus Thuringiensis* Fact sheet.

El-Menofy, W.H. Osman, G.H. Assaeedi, A, and Salama, M.S. 2014. Construction of A Novel Recombinant Baculovirus Containing Cry1Ab Insecticidal Protein from *Bacillus thuringiensis. Biol. Online Proced. J.* **4 :** 7-16.

Gamal Osman, Sameer OrganjiAbdulrahan S Assaeedi, Dooak. Elhareed. 2015. *Bacillus thuringiensis* comprehensive review.

Guo, S., Ye, S., Liu, Y., Wei, L., Xue, J., Wu, H., Song, F., Zhang, J., Wu., X., Huang, D. and Rao, Z. 2009. Crystal structure of *Bacillus thuringiensis* Cry 8Ea1 : An insectidal toxin toxic to underground pests, the larvae of Holotrichiaparallela. *J. Struct. Biol.* **2:** 259-266.

Ibrahim, M.A., Griko, N., Junker, M., and Bulla, L.A. 2010, *Bacillus thuringiensis.* A genomics and proteomics perspective. *Bioeng. Bugs,* **1:** 31-50.

Jimenez, G. *et al.,* 2013. Description of *Bacillus toyonensis* sp. nov.., a novel species of the *Bacillus cereus* group and pairwise genome comparisons of the species of the group by means of ANI calculations. *Syst. Appl. Microbial.* **36:** 383-391.

Jung, M.Y. *et al.,* 2011. *Bacillus manliponensis* sp. nov., a new member of the *Bacillus cereus* group isolated from foreshore tidal flat sediment. *J.Microbial,* **49:** 1027-1032.

Liu, B. *et al.,* 2014. *Bacillus bingmayongesis* sp. nov., isolated from the pit soil of Emperor Qin's Terra-cotta warrious in China. *Antonie Van Leeuwenhoek* . **105:** 501-510.

Melo AL , Soccol VT, and Soccol CR, 2016 . a program de Pos Graduacoa em Engenharia de Bioprocessor Biotechnologia , Universidade Federal do Parana , Curitiba Parna , Brazil . *Crit Rev Biotechnol.* **36:**317-326.

Ohba, M., Mizuki, E. and Uemori, A. 2009. Parasporin, a new anticancer protein group from *Bacillus thuringiensis. Anticancer Res.* **29:** 427-433.

Okumura S., Ohba M., Mizuki E., Crickmore N., Cotć J.-C., Nagamatsu Y., Kitada S., Sakai H., Harata K. and Shin T., 2014. Parasporin Nomenclature. Available online:http:// parasporin.fitc.pref.fukuoka.jp/

Osman, G. Assaeedi, A., Osman, Y., El-Ghareeb, D., and Alreedy, R. 2013. Purification and characterization of *Bacillus thuringiensis* vegetative insecticidal toxin protein(s) *Lett. Appl. Microbiol.* **57:** 310-316.

Peng D , Luo X , Zhang N , Guo S , Zheng J ,Chen L, and Sun M. 2018. Small RNA mediated cry toxin silencing allows *Bacillus thuringiensis* to evade caenorhabditis elegant avoidance behavioral defense. *Nucleic acids Research* **46**: 159-173.

Raymond, B., Johnston, P.R., Nielsen-LeRoux, C., Lereclus, D. and Crickmore, N. 2010. *Bacillus thuringiensis :* An impotent pathogen ? *Trends in Microbiology,* **18:** 189-194.

Sanahuja, G., Banakar, R., Twyman, R.M., Capell, T. and Christou, P. 2011. *Bacillus thuringiensis :* a century of reasearch, development and commercial applications. *Plant Biotechnol J.* **9:** 283-300.

Soberon, M., Lopez-Diaz, J.A. and Bravo, A. 2013. Cyt toxins produced by *Bacillus thuringiensis :* A protein fold conserved in several pathogennic microorganisms. *Peptides.* **41:** 87-93.

Vachon, V., Laprade, R., and Schwartz, J.L. 2012. Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins : A critical review. *J. invertebr. Pathol.* **111 :** 1-12.

Van, Frankenhuyzen K. 2009. Insecticidal activity of *Bacillus thuringiensis* cristal proteins. *J. Invertebr. Pathol.* **10:** 1-16.

Van, Frankenhuyzen K. 2013. Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. *J. Invertebr. Pathol.* **114:** 76-85.

Yang Liu, Qiliang Lai, Markus Goker,Jan P.Meir-Kollthoff, Meng Wang,Yamin Sun, Lei Wang and Zongze Shao, 2015.Genomic insight into the taxonomic status of the *Bacillus cereus* group.

Yu X., Liu T., Sun Z., Guan P., Zhu J., Wang S., Li S., Deng Q., Wang L., Zheng A., *et al.,* 2012. Co-expression and synergism analysis of Vip3Aa29 and Cyt2Aa3 insecticidal proteins from *Bacillus thuringensis. Curr. Microbial.* **64:** 326-331.