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An Overview on the Crystal Toxins from *Bacillus thuringiensis*

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ABSTRACT

Strains of *Bacillus thuringiensis* (*Bt*) are known to produce crystalline proteins (*δ*-endotoxins) concomitantly with sporulation during their stationary phase of growth, which are demonstrated as lethal to lepidopeterous, coleopeterous and dipterous insects in addition to mites, nematodes, protozoa and flukes. Upon ingestion, the *δ*-nascent endotoxin is an inactive protoxin complex of (*Cry* alone or *Cry* and *Cyt* toxins together) high molecular mass, which is cleaved upon ingestion into the active component proteins at the high alkaline environments in the digestive tract of these agricultural pests. Conventionally, *Bt*-crystals are being produced employing submerged or liquid fermentation techniques in commercial media, but recently many workers have used solid-state fermentation strategy for the enhanced production of *Bt*-toxin at low cost. Apart from *δ*-endotoxin, some isolates of *Bt* produce another class of insecticidal small molecules called *β*-exotoxin (thuringiensin), which may be harmful to humans. Moreover, resistance to *Bt* developed in various target pest is yet another concern for *Bt*-industry. Following a brief introduction, this review addresses various toxins produced by various strains of *Bt*, *Bt* production media and media formulations with emphasis to solid-state fermentation, general structure of *Cry* toxin, its mode of action, target pests, bioassay, resistance to *Bt* toxins and resistance management. Briefly, this review would provide the readers an overview on the general aspects of *Bt* toxin, its general structure and mechanism of action.

Keywords: *Bacillus thuringiensis*; *δ*-Endotoxin; Resistance

1. Introduction

Biological pesticide is one of the most promising alternatives over conventional chemical pesticides, which offers less or no harm to the environments and biota. *Bacillus thuringiensis* (commonly known as *Bt*) is an insecticidal Gram-positive spore-forming bacterium producing crystalline proteins called delta-endotoxins (*δ*-endotoxin) during its stationary phase or senescence of its growth. *Bt* was originally discovered from diseased silkworm (*Bombyx mori*) by Shigetane Ishiwatari in 1902. But it was formally characterized by Ernst Berliner from diseased flour moth caterpillars (*Ephestia kuhniella*) in 1915 [1]. The first record of its application to control insects was in Hungary at the end of 1920, and in Yugoslavia at the beginning of 1930s, it was applied to control the European corn borer [2]. *Bt*, the leading biorational pesticide was initially characterized as an insect pathogen, and its insecticidal activity was ascribed largely or completely to the parasporal crystals. It is active against more than 150 species of insect pests. *Bt* is normally marketed (as a mixture of dried spores and toxin crystals) under various trade names worldwide for controlling many plant pests, mainly caterpillars belonging to Lepidoptera (represented by butterflies and moths), mosquito larvae and a few others including unconventional targets like mites. The share of *Bt* products in agrochemical (fungicide, herbicide and insecticide) market is about only 1%. The first commercial *Bt* product was produced in 1938 by Libec in France, but the product was used only for a very short time due to World War II, and then in the USA in the 1950s [3]. The toxicity of *Bt* culture lies in its ability to produce the crystalline protein, this observation led to the development of bioinsecticides based on *Bt* for the control of certain insect species among the orders Lepidoptera, Diptera, and Coleoptera [3-5]. Nowadays, *Bt* isolates are reported also active against certain nematodes, mites and protozoa [6]. It is already a useful alternative or supplement to synthetic chemical pesticide for applications in commercial agriculture, forest management, and mosquito control, and also a key source of genes for

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transgenic expression to transfer pest resistance in plants. Due to this economic interest, numerous approaches have been developed to enhance the production of *Bt* bioinsecticides. The insecticidal activity of *Bt* is known to depend not only on the activity of the bacterial culture itself, but also on abiotic factors, such as the medium composition and cultivation strategy.

2. *Bt* Toxins

Bt produces one or more types of parasporal crystalline proteins (called *δ*-endotoxins) concomitantly with sporulation. Cryastal (*Cry*) or cytolytic (*Cyt*) proteins singly or in their combination constitute the *δ*-endotoxins [7]. *Cry* proteins are parasporal crystalline inclusions produced by *Bt* that exhibit experimentally verifiable toxic effect to a target organism or have significant sequence similarity to a known *Cry* protein. *Cyt* proteins are also parasporal inclusions exhibiting hemolytic (cytolitic) activity with obvious sequence similarity to a known *Cyt* protein. These toxins are highly specific to their target insect, but innocuous to humans, vertebrates and plants, and are completely biodegradable [8]. These crystalline proteins are mainly encoded by extra-chromosomal genes located on the plasmids. The parasporal crystalline proteins produced during the stationary (senescence) phase of its growth cycle account for 20% - 30% of the dry weight of the cells of this phase [9]. Expression of most *Cry* genes (e.g., *cry*1Aa, *cry* 2A, *cry* 4A, *etc*.) are well regulated in the sporulation phase of growth. Studies have shown that several *Cry* proteins—when expressed in either *E. coli* or *B. subtilis*—expressed as 130 to 140 kDa protoxin complex molecules that retain their biological activity. More than 200 types of endotoxin gene have been cloned from various strains of *Bt*, and sequenced so far. The plasmid profiles of most *Bt* strains are rather complex, with molecular weight varying from 2 to 200 kb and the number of plasmids ranging from 1 to 10 in most strains [10]. The self-assembly of these 130 kDa proteins is spontaneous, mediated primarily by the C-terminus of the protein. Their cysteine-rich carboxyl terminus is highly conserved among lepidopteran-specific *Cry* proteins, which generates a number of disulfide bridges that allow good crystal packing and also protects the toxin from the attack of various proteases. Commercial insecticides derived from *Bt* have a long history of successful use in the biocontrol of insect pests [11,12]. Many studies examined the composition and methods of preparation of nutrient media for entomopathogenic bacteria [13,14]. Chromsomal insertion of *Cry* gene may enhance the production of *δ*-endotoxins in *Bt* strains [15]. Erythromycin resistance may affect the sporulation processes in *Bt* and *B. subtilis* [16,17]. Most *Bacillus* strains produce a mixture of structurally different insecticidal crystal proteins (*Cry* proteins), which are encoded by different *Cry* genes which target different insect orders (**Table 1**). Each of these proteins may contribute to the insecticidal spectrum of a strain that makes it selectively toxic to a wide variety of insects belonging to the Lepidoptera, Coleoptera, Diptera, Hymenoptera and Mallophaga, as well as to other invertebrates [11,18-20]. *Bt* strains are able to produce exoenzymes, such as proteases and *α*-amylases [21]. Apart from *δ*-endotoxin, some isolates of *Bt* produce another class of insecticidal small molecules called *β*-exotoxin, the common name for which is thuringiensis [22].

Table 1. Endotoxin producing Bacilli and target organisms.

***Bacillus* spp.**	**Target pest**	**Reference**
B. laterosporus & *Brevibacillus laterosporus*	*Musca domestica* and *Aedes aegypti*	[25]
B. sphaericus & *Bt israelensis*	*Culex quinquefasciatus*, *C. pipiens*, *C. tarsalis*	[26-28]
Bt	*Acyrthosiphon pisum*, *Aedes aegypti*, *Autographa californica* , *Cacyreus marshalli*, *Epinotia aporema*, *Lobesia botrana*, *Manduca sexta*, *Manduca sexta*, *Pectinopho gossypiella*, *Pieris brassicae*, *Plutella xylostella*, *Rhizoglypus robini* *Spodoptera exigua*, *Spodoptera frugiperda*	[29-43]
Bt berliner	*Lygus hesperus*	[44]
Bt BRL 43	First instar larvae of cotton leaf worm, cotton boll worm and black cut worm	[45]
Bt finitimus B-1166 VKPM	-	[46]
Bt galleriae	*Galleria mellonella* L.	[47]
Bt H14	*Leishmania. major*	[48]
Bt IPS 78/11	*Manduca sexta*	[49]
Bt israelensis IPS78/11	*Lucilia cuprina*, *L. sericata*, and *Calliphora stygia*	[50]
Bt sotto	Cabbage butterfly	[51]
Bt kurstaki	*Helicoverpa zea*; *Scrobipalpula absoluta*: *Malacosoma neustria* and *Lymantria dispar* larvae	[52,53]
Btk (serotype H3a, 3b, 3c) strain BNS3	*Prays oleae*	[54]
Btk HD1 & *Btk* HD73	*Manduca sexta Heliothis irescens*	[55]
Bt tolworthi	*Spodoptera frugiperda*, *Ostrinia nubilalis* and *Plutella xylostella*, *S.exigua*	[56,57]
Bt. tenebrionis	synanthropic mites	[58]

Beta-exotoxin and the other *Bacillus* toxins (*δ*-endotoxins) may contribute to the general insecticidal toxicity of the bacterium to lepidopteran, dipteran, and coleopteran insects. *Beta*-exotoxin is known to be toxic to humans and almost all other forms of life and, in fact, its presence is prohibited in *Bt* products [23]. Engineering of plants to contain and express only the genes for *δ*-endotoxins avoids the problem of assessing the risks posed by these other toxins that may be produced in microbial preparations [24].

3. General Structure of *Cry* Toxin

The major component of crystals toxic to lepidopteron larvae is a 130 kDa protein (protoxin), which upon cleavage in the insect yields the functional (insecticidal) proteins of lower molecular weight; very often the crystal formed is an assemblage of many proteins [59]. A *Bt* isolate (*Soil*-47) showed distinct bands of 32.1 and 34.6 kDa. The band corresponding to 32.1 kDa protein could arise from the type C*ry*1 and/or C*ry* 4 gene, while the other (34.6 kDa) protein is possibly encoded by the type C*yt* gene [60]. An unexpected finding was that a 20 kb heterologous DNA fragment was found intimately associated with the crystals from *Btk* HD73, The DNA is not susceptible to nuclease attack unless the protoxin is removed or proteolyzed to toxin. The active toxin is not associated with DNA; however, evidence was obtained which indicated that the DNA was involved in the generation of toxin from the crystal protein. [61].

Structure determination of *Bt* toxins remains one of the most important tools in understanding and improving the utility of these proteins. Crystal structure of *Cry* III A has been published first [62] and several others are now available. Xia *et al*. [63] predicted the first theoretical model of the three dimensional (3D) structure of a Cry (*Cry* 5Ba) toxin by homology modeling on the structure of the *Cry*1Aa toxin, which is specific to Lepidopteran insects. The three-domain structure of *Cry*IIIA consisted of the following: an *α*-helical barrel (domain I) which shows some resemblance to membrane-active or spore-forming domains of other toxins [64]; a triangular prism of "Greek key" beta sheets (domain II); and a *β*-sheet jelly-roll fold (domain III) [62]. Members of this 3-domain *Cry* family are used worldwide for insect control, and their mode of action has been characterized in some details [8].

4. Mode of Action

Mode of action of *δ*-endotoxin involves several events that must be completed several hours after the ingestion in order to lead to insect death. Following ingestion of the inactive protoxin, the crystals are solubilized by the alkaline conditions in the insect midgut and are subsequently proteolytically converted into a toxic core fragment [65]. This activated toxin binds to receptors located on the apical microvillus membranes of epithelial midgut cells. For *Cry*1A toxins, at least four different binding sites have been described in different lepidopteran insects: a cadherin-like protein (CADR), a glycosylphosphatidylinositol (GPI)-anchored aminopeptidase-N (APN), a GPI-anchored alkaline phosphatase (ALP) and a 270 kDa glycoconjugate [66]. *Cry* toxins interact with specific receptors located on the host cell surface and are activated by host proteases following receptor binding, which would result is in the formation of a pre-pore oligomeric structure that is insertion competent. In contrast, *Cyt* toxins directly interact with membrane lipids and insert into the membrane. Recent evidence suggests that *Cyt* synergizes or overcomes resistance (for instance, to mosquitocidal-*Cry* proteins) by functioning as a *Cry*-membrane bound receptor [8].

Once activated, the endotoxin binds to the gut epithelium and causes cell lysis by the formation of cation-selective channels, which leads to death. The activated region of the *δ*-endotoxin is composed of three distinct structural domains: an N-terminal helical bundle domain involved in membrane insertion and pore formation; a *beta*-sheet central domain involved in receptor binding; and a C-terminal beta-sandwich domain that interacts with the N-terminal domain to form a channel. After binding, toxin adopts a conformation suitable for allowing its insertion into the cell membrane. Subsequently, oligomerization occurs, and this oligomer forms a pore or ion channel induced by an increase in cationic permeability within the functional receptors contained on the brush borders membranes. This allows the free flux of ions and liquids, causing disruption of membrane transport and cell lysis leading to insect death [65,55]. The complete nature of this process is still elusive.

Differences in the extent of solubilization sometimes explain differences in the degree of toxicity among *Cry* proteins [67]. A reduction in solubility is speculated to be one potential mechanism for insect resistance [68]. *Cry*3A protein may be necessary for the solubilization of toxins in the midgut of insects [69]. Most recently, two models were proposed for the action of crystal proteins *i.e.*, the sequential binding model and signaling pathway model [70].

5. Target Pests

It is well documented that many insects are susceptible to the toxic activity of *Bt*; of them, lepidopterans have exceptionally been well studied, and many toxins have shown activity against them [66]. The order Lepidoptera encompasses majority of susceptible species belonging to agriculturally important families such as Cossidae, Ge-

lechiidae, Lymantriidae, Noctuidae, Pieridae, Pyralidae, Thaumetopoetidae, Tortricidae, and Yponomeutidae [71]. A novel crystal proteins exhibiting insecticidal activity against lepidopterans has been reported from *Bt* strains [72].

Dipterans are also important target pests, and many of them are highly susceptible to *Bt*. Discovery of novel strains of *Bt* containing parasporal crystal proteins having pesticidal properties against whiteflies, aphids, leaf hoopers, and possibly other sucking insects of agronomic importance extended the potential applications of this bacterium. However, the novel toxic activities found in these novel strains are not limited only to insects, as some of them produce crystals with activity against nematodes, protozoans, flukes, collembolans, mites and worms, among others [66].

6. Production Media and Media Formulations

Indeed, large quantities of spores with high insecticidal activity are required for practical applications. This means that while handling *Bt* as bioinsecticide, a high spore count is not sufficient to ensure toxicity, but it is necessary to reach high *δ*-endotoxin titers. One of the most underreported aspects of *Bt* is that of the production and formulation, although there are certain work existed in connection with *Bt* growth on several synthetic or complex media [73]. There are several formulations of media proposed by different authors. Our group explored the efficacy of various raw agricultural products as supplement to LB for enhancing the toxin production, and found potato flour as an efficient supplement to commercial Luria-Bertani (LB) medium [7]. To develop a cost-effective process for the production of *Bt*-based insecticide, it is imperative to cultivate the bacterial strain in a nutrient rich medium to obtain the highest yields of spore-crystal complexes. Conventionally, *Bt*-crystals are being produced employing submerged or liquid fermentation (SmF) techniques, but recently many workers use nutrient-rich waste water or sludge from various treatment plants as the medium for the production of *Bt*-toxin [74].

***Solid-state fermentation*:** Solid-state fermentation (SSF) has been developed in eastern countries over many centuries, and has enjoyed broad application in these regions to date [75]. The term SSF denotes cultivation of microorganisms on solid, moist substrates in the absence of a free aqueous phase (water). There are several advantages for SSF; for example, high productivities, extended stability of products and low production costs, which say much about such an intensive biotechnological application. With increasing progress and application of rational methods in engineering, SSF will reach higher levels regarding standardization and reproducibility in future. This can make SSF as the preferred technique in the special fields of application such as the productions of enzymes and secondary metabolites, especially foods and pharmaceuticals [76].

Different production media and media compositions can change either the relative toxicity against several target insects or the insecticidal potency of products obtained from the same *Bt* strains [77]. According to Farrera *et al.*, [78], media with different composition showed changes in crystal production, *i.e.* different amounts of *Cry* proteins produced per spore would vary. The ingredients in the media affect the rate and synthesis of the different *δ*-endotoxins and also the size of the crystals produced. Using barley as the carbon source, Amin [79] developed a cost-effectively protocol for the mass production of *Bt.*

Several media based on complex substrates such as corn steep liquor [80], peptones [81] blackstrap molasses and Great Northern White Bean concentrate [82], or LB supplemented with agricultural products [7] have been found efficient for *Bt* bioinsecticide production. Various investigators modified such commercial media by supplementing it with mineral nutrients or various salts, *i.e.*, enriched medium. Zouari *et al.* [73] showed that *Bt* subspecies *kurstaki* produced 1 g/L of *δ*-endotoxin in 4.5 g/L total dry biomass in a complex liquid medium, in which the sugar was replaced by gruel hydrolysate. A mixture of extracts from potato and Bengal gram or bird feather and de-oiled rice bran or wheat bran, chickpea husk and corncob was used to cultivate *Bt israelensis* and found that the mosquitocidal activity of the crude toxin was higher than that produced in the conventional medium [83]. Valicente *et al.* [84] used LB medium supplemented with various salts, and agricultural by-products like soybean flour (0.5%) and liquid swine manure (4%) to increase *Bt* biopesticide production by SmF, which resulted in 1.18 g/L dry cell mass. Zhuang *et al*. [85] also claimed that they have purified *δ*-endotoxin (up to 7.14 mg/g medium) by one step centrifugation from wastewater sludge-based medium, however they did not provide any physical evidence for the purified crystals. From these reports, it seems that maximum yield of *Bt* toxin could be attained is 3.6 g/L [84] in SmF or 7.14 g/Kg medium in SSF [85], where they did not provide the actual cost effect.

7. Bioassay

Well-designed studies under confined conditions required to understand the effect of *Bt* toxins on different organisms. It is considered that *Bt* toxins also to be toxic to lepidopeterous, coleopeterous and dipterous insects in addition to mites, nematodes, protozoa and flukes [18, 19,40]. These proteins are usually thought to act only on the actively feeding larvae of susceptible species by a

mechanism involving consumption and proteolytic processing of the protein followed by binding to, and the lysis of midgut epithelial cells. It was found that proteolytically activated insecticidal crystal proteins significantly reduced the lifespan of adult *Heliothis virescens* and *Spodoptera exigua* at concentrations of 500 µg/ml, but not 167 or 25 µg/ml at their assay conditions [86]. *Bt* crystal proteins showed *in vitro* cytotoxicity against human cancer cells and leukemic T cells [87]. Interestingly, Xu *et al.* [88] demonstrated that the *Bt* crystal proteins can protect plasmodium-infected mice from malaria. Moreover, non-conventional targets such as *Caenorhabditis elegans* (nematode) has been demonstrated for the first time [89].

Toxins of *Btk* strain HD1 have widely been used to control the forest pests such as gypsy moth, spruce bud worm, the pine procesionary moth, the European pine shoot moth and the nun moth [90]. Direct feeding of crude pellet containing *Bt*-toxin [91], pollen diet formulation [92] are the normal mode of applications being practiced in entomotoxicity assays. A different feeding strategy was successfully used for the bioassay of *A. guerreronis*, in which the dried solid fermented powder directly brushed on the infested coconut buttons [7,21]. Many authors used surfactants like BIT (1,2-benzisothiazolin-3-one), one of the inertingredients in Foray 48 B(a *Btk* formulation); the siloxane (organosilicone) Triton-X-100, Tween 20 and Latron CS-7 are some surfactants for *Btk* formulations [93].

The mortality rate of *Thaumetopoea solitaria* on the application of *Btk* toxin has been demonstrated by Er *et al*. [94]. Purified *Btk* toxin inhibited the growth of monarch larvae, but did not cause mortality [95]. The LC_{50} value of *Btk* was found to be 398.1 µg/ml against caterpillars of *Arctornis submarginata* [96]. Toxicity of several formulations of *Btk* to beet armyworm (*Spodoptera exigua*) was determined using neonate larvae in a diet incorporation bioassay.

Probit analysis (LC_{50}) has been used by many authors for ascertaining the efficacy of various *Bt* formulations. For instance, Yashodha and Kuppusamy [97] successfully used dipping method for testing the efficacy of *Btk* formulation in Tween 20 on Brinjal. Gobatto *et al*. [98] used various concentrations of spore suspension of *Bt* for estimating the probit value on mosquito and a moth. Payne *et al*. [40] employed artificial feeding assay for Two-spotted spider mite (*T. urticae*), a related mite to *E. orientalis* with different feeding regime. They fed the mite with 5 mg spray-dried powder of *Bt* broth (a mixture of pores, crystals, cellular debris) in 1 ml sucrose (10%) containing preservatives and surfactant.

Possible use of *Bt* preparation (Dipel 2X) as a substitute for chemical insecticides (Lannate and Hostathion) was evaluated against two major pests of potato crop, *Agrotis* sp. and *Spodoptera exigua.* The toxicity studies of *Bt* to four instars larvae of diamondback moth, *Plutella xylostella* (L.) suggested that *Bt* could be an important agent for the control of larval instars of *Plutella xylostella*. [42]. The *Bt* diet suppressed the growth of the four mite species such as *Acarus siro* L., *Tyrophagus putrescentiae* (Schrank), *Dermatophagoides farinae* Hughes, and *Lepidoglyphus destructor* (Schrank) *via* feeding tests [58].

8. Resistance to *Bt* Toxins

***Laboratory-selected strains*:** In the past, it was believed that insects would not develop resistance to *Bt* toxins, since *Bt* and insects have coevolved. Starting in the mid-1980s, however, a number of insect populations of several different species with different levels of resistance to *Bt* crystal proteins were obtained by laboratory selection experiments, using either laboratory-adapted insects or insects collected from wild populations [99,100]. Examples of laboratory-selected insects resistant to individual *Cry* toxins include the Indian mealmoth (*Plodia interpunctella*), the almond moth (*Cadra cautella*), the Colorado potato beetle (*Leptinotarsa decemlineata*), the cotton leafworm (*Spodoptera littoralis*), the beet armyworm (*S. exigua*), *etc*. [19]. Given the multiple steps in processing the crystal to an active toxin, it is not surprising that insect populations might develop various means of resisting intoxication. It is important, however, to keep in mind that selection in the laboratory may be very different from selection that occurs in the field. Insect populations maintained in the laboratory presumably have a considerably lower level of genetic diversity than field populations. Several laboratory experiments to select for *Bt* resistance in diamondback moths failed, although the diamondback moth is the only known insect reported so far to have developed resistance to *Bt* in the field [19]. It is possible that the genetic diversity of the starting populations was too narrow and thus did not include resistance alleles. In the laboratory, insect populations are genetically isolated; dilution of resistance by mating with susceptible insects—as observed in field populations—is excluded [19].

In addition, the natural environment may contain factors affecting the viability or fecundity of resistant insects, *i.e*., factors excluded from the controlled environment of the laboratory. Resistance mechanisms can be associated with certain fitness costs that can be deleterious under natural conditions [101]. Natural enemies, such as predators and parasites can influence the development of resistance to *Bt* by preferring either the intoxicated, susceptible or the healthy resistant insects. In the former case, one would expect an increase in resistance development, while in the latter, natural enemies can help to retard resistance development to *Bt*. Never-

theless, selection experiments in the laboratory are valuable because they reveal possible resistance mechanisms and make genetic studies of resistance possible.

***Field-selected strains*:** The first case of field-selected resistance to *Bt* was reported from Hawaii, where populations of diamondback moth showed different levels of susceptibility to a formulated *Bt* product (Dipel). Populations from heavily treated areas proved more resistant than those populations treated at lower levels, with the highest level of resistance at 30-fold [100].

The resistance trait is conferred largely by a single autosomal recessive locus [102]. This "Hawaii" resistance allele simultaneously confers cross-resistance to *Cry*1Aa, *Cry*1Ab, *Cry*1Ac, *Cry*1Fa, and *Cry*1Ja but not to *Cry*1Ba, *Cry*1Bb, *Cry*1Ca, *Cry*1Da, *Cry*1Ia, or *Cry*2Aa (369). At least one Cry1A-resistant diamondback moth strain has been shown to be very susceptible to *Cry*9C [31].

Resistance to *Btk* products and resulting failure in diamondback moth control has resulted in the extensive use of *Bt* subsp. *aizawai*-based insecticides in certain locations [19]. Insects in two colonies from Hawaii showed up to a 20-fold resistance to *Cry*1Ca, compared to several other colonies, including one obtained earlier from the same location, as well as moderately high resistance to *Cry*1Ab and *Btk*-based formulations [19].

A Malaysian strain simultaneously highly resistant to the *kurstaki* and the *aizawai* subspecies was apparently mutated in several loci [31]. A *Cry*1Ab resistance allele associated with reduced binding to brush border membrane vesicles receptors was partially responsible for resistance to both subspecies. Genetic determinants responsible for subspecies *kurstaki*-specific and subspecies *aizawai*-specific resistance segregated separately from each other and from the Cry1Ab resistance allele in genetic experiments [103].

After less than 2 decades of intensive use of *Btk* in crucifer agriculture, resistant insects have evolved in numerous geographically isolated regions of the world, and subspecies *aizawai* resistance is beginning to appear even more rapidly.

Defying the expectations of scientists monitoring transgenic crops such as corn and cotton that produce insecticidal proteins derived from *Bt*, target insect pests have developed little or no resistance to *Bt* crops thus far, according to US Department of Agriculture-funded scientists. These findings suggest that transgenic *Bt* crops could enjoy more extended, more profitable commercial life cycles and that the measures established to mitigate resistance before the crops were introduced are paying off [104].

Evolution of resistance in pests can reduce the effectiveness of insecticidal proteins from *Bt* produced by transgenic crops. Field outcomes support theoretical predictions that factors delaying resistance include recessive inheritance of resistance, low initial frequency of resistance alleles, abundant refuges of non-*Bt* host plants and two-toxin *Bt* crops deployed separately from one-toxin *Bt* crops. The results imply that proactive evaluation of the inheritance and initial frequency of resistance are useful for predicting the risk of resistance and improving strategies to sustain the effectiveness of *Bt* crops [105].

9. Resistance Management

Resistance management strategies try to prevent or diminish the selection of the rare individuals carrying resistance genes and hence to keep the frequency of resistance genes sufficiently low for insect control [106,107].

Proposed strategies include: the use of multiple toxins (stacking or pyramiding), crop rotation, high or ultrahigh dosages, and spatial or temporal refugia (toxin-free areas). Retrospective analysis of resistance development does support the use of refugia [99]. Experience with transgenic crops expressing cry genes grown under different agronomic conditions is essential to define the requirements of resistance management. In transgenic plants, selection pressure could be reduced by restricting the expression of the crystal protein genes to certain tissues of the crop (those most susceptible to pest damage) so that only certain parts of the plant are fully protected, the remainder providing a form of spatial refuge. It has been proposed that cotton lines in which *Cry* gene expression is limited to the young bolls may not suffer dramatic yield loss from *Heliothis* larvae feeding on other plant structures, since cotton plants can compensate for a high degree of pest damage [108].

Another management option is the rotation of plants or sprays of a particular *Bt* toxin with those having another toxin type that binds to a different receptor. A very attractive resistance management tactics is the combination of a high-dose strategy with the use of refugia [19].

10. Conclusion

Development of resistance to *Bt* toxin is one of the concerns of *Bt*-based agroindustry. It was expected that resistance would be developed in transgenic crops such as corn and, interestingly target insect pests have developed little or no resistance to these *Bt* crops. It suggests that transgenic *Bt* crops could enjoy more extended, more profitable commercial life cycles and the measures established to mitigate resistance before the crops were introduced are paying off. Nevertheless, making *Bt* toxin at low cost for the farmers, especially in the developing and underdeveloped countries remains one of the major challenges, wherein SSF offers great potentials. In fact, the ill effects of the exotoxin, thuringiensin from *Bt* on humans (and other animals too) are a growing concern.

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REFERENCES

[1] R. J. Milner, "History of *Bacillus thuringiensis*," *Agriculture Ecosystems and Environment*, Vol. 49, No. 1, 1994, pp. 9-13. doi:10.1016/0167-8809(94)90014-0

[2] J. C. Lord, "From Metchnikoff to Monsanto and beyond: The Path of Microbial Control," *Journal of Invertebrate Pathology*, Vol. 89, No. 1, 2005, pp. 19-29. doi:10.1016/j.jip.2005.04.006

[3] E. W. Nester, L. S. Thomashow, M. Metz and M. Gordon, "100 Years of *Bt*, a Critical Scientific Assessment," American Academy of Microbiology, 2002.

[4] L. A. Lacey, R. Frutos, H. K. Kaya and P. Vail, "Insect Pathogens as Biological Control Agents: Do They Have a Future?" *Biological Control*, Vol. 21, No. 3, 2001, pp. 230-248. doi:10.1006/bcon.2001.0938

[5] R. A. de Maagd, A. Bravo and N. Crickmore, "How *Bacillus thuringiensis* Has Evolved Specific Toxins to Colonize the Insect World," *TRENDS in Genetics*, Vol. 17, No. 4, 2001, pp. 193-199. doi:10.1016/S0168-9525(01)02237-5

[6] M. Marvier, C. Mc Creedy, J. Regetz and P. Karieva, "A Meta-Analysis of Effects of *Bt* Cotton and Maize on Nontarget Invertebrates," *Science*, Vol. 316, No. 5830, 2007, pp. 1475-1477. doi:10.1126/science.1139208

[7] R. B. Smitha, V. N. Jisha, S. Pradeep, M. K. Sarath Josh and Sailas Benjamin, "Potato Flour Mediated Solid State Fermentation for Enhanced Production of *Bt*-Toxin," *Journal of Bioscience and Bioengineering*.

[8] A. Bravo, S. S. Gill and M. Soberón, "Mode of Action of *Bacillus thuringiensis* Cry and Cyt Toxins and Their Potential for Insect Control," *Toxicon*, Vol. 49, No. 4, 2007, pp. 423-435. doi:10.1016/j.toxicon.2006.11.022

[9] H. Agaisse and D. Lereclus, "How does *Bacillus thuringiensis* Produce so Much Insecticidal Crystal Protein?" *Journal of Bacteriology*, Vol. 177, No. 21, 1995, pp. 6027-6032.

[10] D. G. Mc Dowell and N. H. Mann, "Characterization and Sequence Analysis of a Small Plasmid from *Bacillus thuringiensis var. kurstaki* Strain HD1-DIPEL," *Plasmid*, Vol. 25, No. 2, 1991, pp. 113-120. doi:10.1016/0147-619X(91)90022-O

[11] C. C. Beegle and T. Yamamoto, "History of *Bacillus thuringien*sis Berliner Research and Development," *Canadian Entomologist*, Vol. 124, No. 4, 1992, pp. 587-616. doi:10.4039/Ent124587-4

[12] A. Bravo, S. Likitvivatanavong, S. Gill and M. Soberón, "*Bacillus thuringiensis:* A Story of a Successful Bioinsecticide," *Insect Biochemistry and Molecular Biology*, Vol. 41, No. 7, 2011, pp. 423-443. doi:10.1016/j.ibmb.2011.02.006

[13] A. Sierpinska, "Toxicity of *Bacillus thuringiensis* to Some Defoliating Forest Lepidoptera," *Folia Forestalia Polonica. Seria A—Leśnictwo*, Vol. 39, 1997, pp. 79-92.

[14] H. Bti, "Production of *Bacillus thuringiensis* subsp. *Israelensis* from Agro-Based Product (corncob)," *Current Science*, Vol. 101, No. 8, 2011, p. 1009.

[15] S. Kalman, K. L. Kiehne, N. Cooper, M. S. Reynoso and T. Yamamoto, "Enhanced Production of Insecticidal Proteins in *Bacillus thuringiensis* Strains Carrying an Additional Crystal Protein Gene in Their Chromosomes," *Applied and Environmental Microbiology*, Vol. 61, No. 8, 1995, pp. 3063-3068.

[16] F. Fargette and N. Grelet, "*Bacillus thuringiensis Berliner* Mutants Resistant to Various Antibiotics and Showing Alterations of Sporulation," *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences. Série D*: *Sciences Naturelles*, Vol. 280, No. 4, 1975, p. 499.

[17] I. Mahler and H. O. Halvorson, "Two Erythromycin-Resistance Plasmids of Diverse Origin and Their Effect on Sporulation in *Bacillus subtilis*," *Journal of General Microbiology*, Vol. 120, No. 1, 1980, pp. 259-263.

[18] J. S. Feitelson, J. Payne and J. Kim, "*Bacillus thuringiensis*: Insects and beyond," *Nature Biotechnology*, Vol. 10, No. 3, 1992, pp. 271-275. doi:10.1038/nbt0392-271

[19] H. E. Schnepf, N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler and D. H. Dean, "*Bacillus thuringiensis* and Its Pesticidal Crystal Proteins," *Microbiolopgy and Molecular Biology Review*, Vol. 62, No. 3, 1998, pp. 775-806.

[20] R. A. de Maagd, A. Bravo, C. Berry, N. Crickmore and H. E. Schnepf, "Structure, Diversity, and Evolution of Protein Toxins from Spore-Forming Entomopathogenic Bacteria," *Annual Review of Genetics*, Vol. 37, No. 1, 2003, pp. 409-433. doi:10.1146/annurev.genet.37.110801.143042

[21] R. B. Smitha, "Dual Production of Endotoxin and Amylase from *Bacillus thuringiensis* Subsp *kurstaki* by Fermentation and Efficacy Studies of Endotoxin against Eriophyid Mite," Ph.D. Thesis, University of Calicut, India, 2010.

[22] S. Guo, M. Liu, D. Peng, S. Ji, P. Wang, Z. Yu and M. Sun, "New Strategy for Isolating Novel Nematicidal Crystal Protein Genes from *Bacillus thuringiensis* Strain YBT-1518," *Applied Environmental Microbiology*, Vol. 74, No. 22, 2008, pp. 6997-7001. doi:10.1128/AEM.01346-08

[23] D. L. Berlitz, A. O. D. Azambuja, A. Sebben, J. V. D. Oliveira and L. M. Fiuza, "Mortality of Oryzophagus Oryzae (Costa Lima, 1936) (Coleoptera: Curculionidae) and Spodoptera Frugiperda (JE Smith, 1797) (Lepidoptera: Noctuidae) Larvae Exposed to *Bacillus thuringiensis* and Extracts of *Melia azedarach*," *Brazilian Archives of Biology and Technology*, Vol. 55, No. 5, 2012, pp. 725-731. doi:10.1590/S1516-89132012000500012

[24] P. Toledo, T. Liedo, T. Williams and J. Ibarra, "Toxicity of *Bacillus thuringiensis*-Exotoxin to Three Species of Fruit Flies (Diptera: Tephritidae)," *Journal of Economic Entomology*, Vol. 92. No. 5, 1999, pp. 1052-1056.

[25] L. Ruiu, I. Floris, A. Satta and D. J. Ellar, "Toxicity of a *Brevibacillus laterosporus* Strain Lacking Parasporal Crystals against *Musca domestica* and *Aedes aegypti*," *Biological Control*, Vol. 43. No. 1, 2007, pp. 136-143. doi:10.1016/j.biocontrol.2007.07.002

[26] M. Boisvert and J. Boisvert, "Effects of *Bacillus thuringiensis* var. *israelensis* on Target and Nontarget Organisms: A Review of Laboratory and Field Experiments," *Biocontrol Science and Technology*, Vol. 10, No. 5, 2000, pp. 517-561. doi:10.1080/095831500750016361

[27] H. Park, D. K. Bideshi, M. C. Wirth, J. J. Johnson, W. E. Walton and B. A. Federici, "Recombinant Larvicidal Bacteria with Markedly Improved Efficacy against Culex Vectors of West Nile Virus," *American Journal of Tropical Medicine and Hygiene*, Vol. 72, No. 6, 2005, pp. 732-738.

[28] S. Cohen, O. Dym, S. Albeck, E. Ben-Dov, R. Cahan, M. Firer and A. Zaritsky, "High-Resolution Crystal Structure of Activated Cyt2Ba Monomer from *Bacillus thuringiensis* Subsp. *Israelensis*," *Journal of Molecular Biology*, Vol. 380, No. 5, 2008, pp. 820-827. doi:10.1016/j.jmb.2008.05.010

[29] N. Jiménez-Juárez, C. Muñoz-Garay, I. Gómez, G. Saab-Rincon, J. Y. Damian-Almazo, S. S. Gill, M. Sobero and A. Bravo, "*Bacillus thuringiensis* Cry1Ab Mutants Affecting Oligomer Formation Are Non-Toxic to *Manduca sexta* Larvae," *Journal of Biological Chemistry*, Vol. 282, No. 29, 2007, pp. 21222-21229. doi:10.1074/jbc.M701314200

[30] R. Peng, A. Xiong, X. Li, H. Fuan and Q. Yao, "A *δ*-Endotoxin Encoded in *Pseudomonas fluorescens* Displays a High Degree of Insecticidal Activity," *Applied Microbiology and Biotechnology* Vol. 63, No. 3, 2003, pp. 300-306. doi:10.1007/s00253-003-1343-2

[31] B. Lambert, W. Theunis, R. Agouda, K. Van Audenhove, C. Decock, S. Jansens, J. Seurinck and M. Peferoen, "Nucleotide Sequence of Gene Cry IIID Encoding a Novel Coleopteran-Active Crystal Protein from Strain BTI109P of *Bacillus thuringiensis* Subsp *kurstaki*," *Gene*, Vol. 110, No. 1, 1992, pp. 131-132. doi:10.1016/0378-1119(92)90457-Z

[32] M. A. F. Abdullah and D. H. Dean, "Enhancement of Cry19Aa Mosquitocidal Activity against *Aedes aegypti* by Mutations in the Putative Loop Regions of Domain II," *Applied and Environmental Microbiology*, Vol. 70, No. 6, 2004, pp. 3769-3771. doi:10.1128/AEM.70.6.3769-3771.2004

[33] N. Zouari, O. Achour and S. Jaoua, "Productionof Delta-Endotoxin by *Bacillus thuringiensis* Subsp *kurstaki* and Overcoming of Catabolite Repression by Using Highly Concentrated Gruel and Fish Meal Media in 2- and 20 dm^3 Fermenters," *Journal of Chemical Technology and Biotechnology*, Vol. 77, No. 8, 2002, pp. 877-882. doi:10.1002/jctb.650

[34] F. Rajamohan, O. Alzate, J. A. Cotrill, A. Curtiss and D. H. Dean, "Protein Engineering of *Bacillus thuringiensis* Delta-Endotoxin: Mutations at Domain II of CryIAb Enhance Receptor Affinity and Toxicity toward Gypsy Moth Larvae," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 93, No. 25, 1996, pp. 14338-14343. doi:10.1073/pnas.93.25.14338

[35] D. H. Sauka, J. Sánchez, A. Bravo and G. B. Benintende, "Toxicity of *Bacillus thuringiensis* Delta-Endotoxins against Bean Shoot Borer (*Epinotia aporema Wals.*) Larvae, a Major Soybean Pest in Argentina," *Journal of Invertebrate Pathology*, Vol. 94, No. 2, 2007, pp. 125-129. doi:10.1016/j.jip.2006.09.002

[36] S. Herrero, J. González-Cabrera, B. E. Tabashnik and J. Ferré, "Shared Binding Sites in Lepidoptera for *Bacillus thuringiensis* Cry1Ja and Cry1A Toxins," *Applied and Environmental Microbiology*, Vol. 67, No. 12, 2001, pp. 5729-5734. doi:10.1128/AEM.67.12.5729-5734.2001

[37] G. M. S. Lima, R. W. S. Aguiar, R. F. T. Corrêa, E. S. Martins, A. C. M. Gomes, T. Nagata, M. T. De-Souza, R. G. Monnerat and B. M. Ribeiro, "Cry2A Toxins from *Bacillus thuringiensis* Expressed in Insect Cells Are Toxic to Two Lepidopteran Insects," *World Journal of Microbiology and Biotechnology*, Vol. 24, No. 12, 2008, pp. 2941-2948. doi:10.1007/s11274-008-9836-x

[38] M. Porcar, A. M. Grenier, B. Federici and Y. Rahbe, "Effects of *Bacillus thuringiensis δ*-Endotoxins on the Pea Aphid (*Acyrthosiphon pisum*)," *Applied and Environmental Microbiology*, Vol. 75, No. 14, 2009, pp. 4897-4900. doi:10.1128/AEM.00686-09

[39] J. D. Tang, A. M. Shelton, J. V. Rie, S. D. Roeck, W. J. Moar, R. T. Roush and M. Peferoen, "Toxicity of *Bacillus thuringiensis* Spore and Crystal Protein to Resistant Diamondback Moth (*Plutella xylostella*)," *Applied and Environmental Microbiology*, Vol. 62, No. 2, 1996, pp. 564-569.

[40] J. Payne, K. E. Narva and J. Fu, "*Bacillus thuringiensis* Genes Encoding Nematode Active Toxins," US Patent No. 5831011, 1998.

[41] M. E. Carter, M. G. Villani, L. L. Allee and J. E. Losey Berlin, "Absence of Non-Target Effects of Two *Bacillus thuringiensis* Coleopteran Active *δ*-Endotoxins on the Bulb Mite, *Rhizoglypus robini* (Claparède) (Acari, Acaridae)," *Journal of Applied Entomology*, Vol. 128, No. 1, 2004, pp. 56-63. doi:10.1046/j.1439-0418.2003.00788.x

[42] S. Ranjbari, M. Safaralizadeh and H. S. Aramideh, "Insecticidal Effect of *Bacillus thuringiensis* Var *kurstaki* on the Various Instars Larvae of *Plutella xylostella* L. (Lep.: plutellidae) under Laboratory Condition," *Egyptian Academic Journal of Biological Sciences*, Vol. 3, No. 1, 2011, pp. 27-32.

[43] S. Naimov, M. Weemen-Hendriks, S. Dukiandjiev and R. A. Maagd, "*Bacillus thuringiensis* Delta-Endotoxin Cry1 Hybrid Proteins with Increased Activity against the Colorado Potato Beetle," *Applied and Environmental Microbiology*, Vol. 67, No. 11, 2001, pp. 5328-5330. doi:10.1128/AEM.67.11.5328-5330.2001

[44] E. Wellman-Desbiens and J. C Cote, "Development of a *Bacillus thuringiensis*-Based Assay on *Lygus Hesperus*," *Journal of Economic Entomology*, Vol. 98, No. 5, 2005, pp. 1469-1479. doi:10.1603/0022-0493-98.5.1469

[45] C. Chandi, G. S. Mandal, A. Basu, K. S. Ghosh, S. D. Gupta, M. K. Maiti and S. K. Sen, "Prediction-Based Protein Engineering of Domain I of Cry2A Entomocidal Toxin of *Bacillus thuringiensis* for the Enhancement of

Toxicity against Lepidopteran Insects," *Protein Engineering, Design & Selection*, Vol. 20, No. 12, 2007, pp. 599-606. doi:10.1093/protein/gzm058

[46] J. Wojciechowska, "Two Novel Delta-Endotoxin Gene Families *cry*26 and *cry*28 from *Bacillus thuringiensis* ssp. *Finitimus*," *FEBS Letters*, Vol. 453, No. 1-2, 2009, pp. 46-48. doi:10.1016/S0014-5793(99)00650-X

[47] I. M. Dubovskii, O. A. Olifirenko and V. V. Glupov, "Level and Activities of Antioxidants in Intestine of Larvae *Galleria mellonella* L. (Lepidoptera, Pyralidae) at Peroral Infestation by Bacteria *Bacillus thuringiensis* ssp. *Galleriae*," *Journal of Evolutionary Biochemistry and Physiology*, Vol. 41, No. 1, 2005, pp. 20-25. doi:10.1007/s10893-005-0030-6

[48] A. Hanan, H. A. A. El-Sadawy, J. M. Georgy, S. Shaaban, E. I. Hossary and H. A. Kassem, "Fusion of *Bacillus stearothermophilus* Leucine Aminopeptidase II with the Raw-Starch-Binding Domain of *Bacillus* sp. Strain TS-23 α-Amylase Generates a Chimeric Enzyme with Enhanced Thermostability and Catalytic Activity," *Journal of Indian Microbiology Technology*, Vol. 3, 2008, pp. 23-29.

[49] N. Tigue, J. Jacoby and D. J. Ellar, "The Triple Helix 4 Residue, Asn135, Is Involved in the Oligomerization of Cry1Ac1 and Cry1Ab5 *Bacillus thuringiensis* Toxins," *Applied and Environmental Microbiology*, Vol. 67, No. 12, 2001, pp. 5715-5720. doi:10.1128/AEM.67.12.5715-5720.2001

[50] C. N. Chilcott, P. J. Wigley, A. H. Broadwell, D. J. Park and D. J. Ellar, "Activities of *Bacillus thuringiensis* Insecticidal Crystal Proteins Cyt1Aa and Cyt2Aa against Three Species of Sheep Blowfly," *Applied Environmental Microbiology*, Vol. 64, No. 10, 1998, pp. 4060-4061.

[51] T. Iizuka, S. Ishikawa, S. Asano, H. Bando, Z. Zheng and N. Murai, "Insecticidal Activity of the CryIA(a) and Cry IB Delta-Endotoxin of *Bacillus thuringiensis* Was Retained after the Coding Region of the Gene Was Truncated and Expressed in *Escherichia coli*," *Journal of Sericulture Science*, Vol. 63, No. 4, 1994, pp. 303-309.

[52] M. Vasquez, C. Parra, E. Hubert, P. Espinoza, C. Theoduloz and L. Meza-Basso, "Specificity and Insecticidal Activity of Chilean Strains of *Bacillus thuringiensis*," *Journal of Invertebrate Pathology*, Vol. 66, No. 2, 1995, pp. 143-148. doi:10.1006/jipa.1995.1077

[53] H. Kati, S. Kazim, N. Remziye and Z. Demirbag, "A Highly Pathogenic Strain of *Bacillus thuringiensis* Serovar Kurstaki in Lepidopteran Pests," *The Journal of Microbiology*, Vol. 45, No. 6, 2007, pp. 553-557.

[54] S. Rouis, M. Chakroun, I. Saadaoui and S. Jaoua, "Proteolysis, Histopathological Effects, and Immunohistopathological Localization of Delta-Endotoxins of *Bacillus thuringiensis* subsp. *kurstaki* in the Midgut of Lepidopteran Olive Tree Pathogenic Insect *Prays oleae*," *Molecular Biotechnology*, Vol. 35, No. 2, 2007, pp. 141-148. doi:10.1007/BF02686109

[55] A. Aronson, C. Geng and L. Wu, "Aggregation of *Bacillus thuringiensis* Cry1A Toxins upon Binding to Target Insect Larval Midgut Vesicles," *Applied and Environmental Microbiology*, Vol. 65, No. 6, 1999, pp. 2503-2507.

[56] D. M. F. Capalbo, F. H. Valicente and I. M. Oliveira, "Solid-State Fermentation of *Bacillus thuringiensis tolworthi* to Control Fall Armyworm in Maize," *Electronic Journal of Biotechnology*, Vol. 4, No. 2, 2001, pp. 9-10.

[57] B. Lambert, L. Buysse, C. Decock, S. Jansens, C. Piens, B. Saey and M. Peferoen, "A *Bacillus thuringiensis* Insecticidal Crystal Protein with a High Activity against Members of the Family Noctuidae," *Applied and Environmental Microbiology*, Vol. 62, No. 1, 1996, pp. 80-86.

[58] T. Erban, M. Nesvorna, M. Erbanova and J. Hubert, "*Bacillus thuringiensis* Var. *tenebrionis* Control of Synanthropic Mites (Acari: Acaridida) under Laboratory Conditions," *Experimental and Applied Acarology*, Vol. 49, No. 4, 2009, pp. 339-346. doi:10.1007/s10493-009-9265-z

[59] N. Crickmore, D. R. Zeigler, J. Feitelson, E. Schnepf, J. van Rie, D. Lerecus, J. Baum and D. H. Dean, "Revision of the Nomenclature for the *Bacillus thuringiensis* Pesticidal Crystal Proteins," *Microbiology and Molecular Biology Reviews*, Vol. 62, No. 3, 1998, pp. 807-813.

[60] M. H. Hasan, A. Akter, M. Ilias, S. N. Khan and M. M. Hoq, "Growth, Sporulation and Toxin Production by *Bacillus thuringiensis* Isolates in Media Based on Mustard-Seed Meal," *Bangladesh Journal of Microbiology*, Vol. 27, No. 2, 2010, pp. 51-55.

[61] F. R. Clairmont, R. E. Milne, V. T. Pham, M. B. Carrière, and H. Kapla, "Role of DNA in the Activation of the Cry1A Insecticidal Crystal Protein from *Bacillus thuringiensis*," *The Journal of Biological Chemistry*, Vol. 273, No. 15, 1998, pp. 9292-9296. doi:10.1074/jbc.273.15.9292

[62] J. Li, J. Carroll and D. J. Ellar, "Crystal Structure of Insecticidal δ-Endotoxin from *Bacillus thuringiensis* at 2.5A Resolution," *Nature*, Vol. 353, 1991, pp. 815-821. doi:10.1038/353815a0

[63] L. Q. Xia, X. M. Zhao, X. Z. Ding, F. X. Wang and Y. J. Sun, "The Theoretical 3D Structure of *Bacillus thuringiensis* Cry5Ba," *Journal of Molecular Modeling*, Vol. 14, No. 9, 2008, pp. 843-848. doi:10.1007/s00894-008-0318-8

[64] D. M. Ojcius and I. D. E. Young, "Cytolytic Pore-Forming Proteins and Peptides: Is There a Common Structural Motif?" *Trends in Biochemical Sciences*, Vol. 16, 1991, pp. 225-229. doi:10.1016/0968-0004(91)90090-I

[65] H. Höfte and H. R. Whiteley, "Insecticidal Crystal Proteins of *Bacillus thuringiensis*," *Microbiological Reviews*, Vol. 53, No. 2, 1989, pp. 242-255.

[66] N. M. Rosas-García, "Biopesticide Production from *Bacillus thuringiensis*: An Environmentally Friendly Alternative," *Recent Patents in Biotechnology*, Vol. 3, No. 1, 2009, pp. 28-36. doi:10.2174/187220809787172632

[67] C. Du, P. A. Martin and K. W. Nickerson, "Comparison of Disulfide Contents and Solubility at Alkaline pH of Insecticidal and Non-Insecticidal *Bacillus thuringiensis* Protein Crystals," *Applied and Environmental Microbiology*, Vol. 60, No. 10, 1994, pp. 3847-3853.

[68] W. H. McGaughey and M. E. Whalon, "Managing Insect Resistance to *Bacillus thuringiensis* Toxins," *Science*, Vol. 258, No. 5087, 1992, pp. 1451-1455. doi:10.1126/science.258.5087.1451

[69] J. Carroll, D. Convents, J. Van Damme, A. Boets, J. Van Rie and D. J. Ellar, "Intramolecular Proteolytic Cleavage of *Bacillus thuringiensis* Cry3A *δ*-Endotoxin May Facilitate Its Coleopteran Toxicity," *Journal of Invertebrate Pathology*, Vol.70, No. 1, 1997, pp. 41-49. doi:10.1006/jipa.1997.4656

[70] V. Vachon, R. Laprade and J. L. Schwartz, "Current Models of the Mode of Action of *Bacillus thuringiensis* Insecticidal Crystal Proteins: A Critical Review," *Journal of Invertebrate Pathology*, Vol. 111, No. 1, 2012, pp. 1-12. doi:10.1016/j.jip.2012.05.001

[71] J. Iriarte and P. Caballero, "Biología y Ecología de *Bacillus thuringiensis*," *Phytoma. España*, 2001, pp. 15-44.

[72] J. A. Baum, T. Bogaert, W. Clinton, G. R. Heck, P. Feldmann, O. Ilagan and J. Roberts, "Control of Coleopteran Insect Pests through RNA Interference," *Nature Biotechnology*, Vol. 25, No. 11, 2007, pp. 1322-1326. doi:10.1038/nbt1359

[73] N. Zouari, A. Dhouib, R. Ellouz and S. Jaoua, "Nutritional Requirements of a *Bacillus thuringiensis* Subsp *Kurstaki* Strain and Use of Gruel Hydrolysate for the Formulation of a New Medium for Delta-Endotoxin Production," *Applied Biochemistry and Biotechnology*, Vol. 69, No. 1, 1998, pp. 41-52. doi:10.1007/BF02786020

[74] A. Yezza, R. D. Tyagi, J. R. Valéro and R. Y. Surampalli, "Bioconversion of Industrial Wastewater and Wastewater Sludge into *Bacillus thuringiensis* Based Biopesticides in Pilot Fermentor," *Bioresource Technology*, Vol. 97, No. 15, 2006, pp. 1850-1857. doi:10.1016/j.biortech.2005.08.023

[75] S. Benjamin, R. B. Smitha, V. N. Jisha, S. Pradeep, S. Sajith, S. Sreedevi, P. Priji, K. N. Unni and M. K. Sarath Josh, "A Monograph on Amylases from Bacillus Spp.," 2013.

[76] U. Holker and J. Lenz, "Solid-State Fermentation—Are There Any Biotechnological Advantages?" *Current Opinion in Microbiology*, Vol. 8, No. 3, 2005, pp. 301-306. doi:10.1016/j.mib.2005.04.006

[77] Z. Tokcaer, W. Bayraktar, U. Mehmetoglu, G. Ozcengiz and N. J. Alaeddinogly, "Response Surface Optimization of Antidipteran Delta-Endotoxin Production by *Bacillus thuringiensis* Subsp. *israelensis* HD 500," *Process Biochemistry*, Vol. 41, No. 2, 2006, pp. 350-355. doi:10.1016/j.procbio.2005.02.030

[78] R. R. Farrera, F. Perez-Guevara and M. Torre, "Carbon: Nitrogen Ratio Interacts with Initial Concentration of Total Solids on Insecticidal Crystal Protein and Spore Production in *Bacillus thuringiensis* HD-73," *Applied Microbiology and Biotechnology*, Vol. 49, No. 6, 1998, pp. 758-765. doi:10.1007/s002530051243

[79] G. Amin, S. Alotaibi, Y. A. Narmen and W. D. Saleh, "Bioinsecticide Production by the Bacterium *Bacillus thuringiensis*. 1. Pattern of Cell Growth, Toxin Production and By-Product Synthesis," *Archives of Agronomy and Soil Science*, Vol. 54, No. 4, 2008, pp. 387-394. doi:10.1080/03650340802158201

[80] I. Goldberg, B. Sneh, E. Battat and D. Klein, "Optimization of a Medium for a High Yield Production of Spore-Crystal Preparation of *Bacillus thuringiensis* Effective against the Egyptian Cotton Leaf Worm *Spodoptora littoralis* Boisd," *Biotechnology Letters*, Vol. 2, No. 29, 1980, pp. 419-426. doi:10.1007/BF00162348

[81] D. P. Sikdar, M. K. Majundar and S. K. Majundar, "Effect of Minerals on the Production of the Delta-Endotoxin by *Bacillus thuringiensis*," *Biotechnology Letters*, Vol. 13, No. 7, 1991, pp. 511-514. doi:10.1007/BF01049209

[82] O. N. Morries, V. Converse, P. Kanagaratnam and J. S. Davies, "Effect of Cultural Conditions on Spore-Crystal Yield and Toxicity of *Bacillus thuringiensis* Subsp *aizawai* HD133," *Journal of Invertebrate Pathology*, Vol. 67, No. 2, 1996, pp. 129-136. doi:10.1006/jipa.1996.0020

[83] H. Bti, "Production of *Bacillus thuringiensis* Subsp *israelensis* from Agro-Based Product," *Current Science*, Vol. 101, No. 8, 2011, pp. 1009-1010.

[84] F. H. Valicente, T. E. de Souza, M. I. S. Leite, F. L. Freire and C. M. Vieira, "Production of *Bacillus thuringiensis* Biopesticide Using Commercial Lab Medium and Agricultural By-Products as Nutrient Sources," *Revista Brasileira de Milho e Sorgo*, Vol. 9, No. 1, 2010, pp. 1-11.

[85] L. Zhuang, S. Zhou, Y. Wang, Z. Liu and R. Xu, "Cost-Effective Production of *Bt* Biopesticides by Solid-State Fermentation Using Wastewater Sludge: Effects of Heavy Metals," *Bioresource Technology*, Vol. 102, No. 7, 2011, pp. 4820-4826. doi:10.1016/j.biortech.2010.12.098

[86] M. Grove, W. Kimble and W. McCarthy, "Effects of Individual *Bacillus thuringiensis* Insecticidal Crystal Proteins on Adult *Heliothis virescens* (F) and *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae)," *Biocontrol*, Vol. 46, No. 3, 2001, pp. 321-335. doi:10.1023/A:1011424400297

[87] E. Mizuki, M. Ohba, T. Akao, S. Yamashita, H. Saitoh and Y. S. Park, "Unique Activity Associated with Non-Insecticidal *Bacillus thuringiensis* Parasporal Inclusions: *In Vitro* Cell-Killing Action on Human Cancer Cells," *Journal of Applied Microbiology*, Vol. 86, No. 3, 1999, pp. 477-486. doi:10.1046/j.1365-2672.1999.00692.x

[88] Z. Xu, B. Yao, M. Sun and Z. Yu, "Protection of Mice Infected with *Plasmodium berghei* by *Bacillus thuringiensis* Crystal Proteins," *Parasitology Research*, Vol. 92, No. 1, 2004, pp. 53-57. doi:10.1007/s00436-003-0990-7

[89] L. D. Marroquin, D. Elyassnia, J. S. Griffitts, J. S. Feitelson and R. V. Aroian, "*Bacillus thuringiensis* Toxin Susceptibility and Isolation of Resistance Mutants in the Nematode *Caenorhabditis elegans*," *Genetics*, Vol. 155, No. 4, 2000, pp. 1693-1699.

[90] G.-M. Li, X.-Y. Zhang and L.-Q. Wang, "The Use of *Bacillus thuringiensis* on Forest Integrated Pest Management," *Journal of Forestry Research*, Vol. 12, No. 1, 2001, pp. 51-54. doi:10.1007/BF02856801

[91] M. Fadel and M. Sabour, "Utilization of Dairy by Product in the Production of Bioinsecticide," *Journal of Biological Sciences*, Vol. 2, No. 2, 2002, pp. 116-120.

[92] S. Buchholz, P. Neumann, K. Merkel and R. R. Hepburn, "Evaluation of *Bacillus thuringiensis berliner* as an Alternative Control of Small Hive Beetles, *Aethina tumida Murray* (Coleoptera; Nitidulidae)," *Journal of Pest Sci-*

ence, Vol. 79, No. 4, 2006, pp. 251-254. doi:10.1007/s10340-006-0141-x

[93] N. Helassa, H. Quiquampoix, S. Noinville, W. Szponarski and S. Staunton, "Adsorption and Desorption of Monomeric *Bt* (*Bacillus thuringiensis*) Cry1Aa Toxin on Montmorillonite and Kaolinite," *Soil Biology and Biochemistry*, Vol. 41, No. 3, 2009, pp. 498-504. doi:10.1016/j.soilbio.2008.12.008

[94] M. K. Er, S. Karada and C. Mart, "Effectiveness of *Bacillus thuringiensis* Var. *kurstaki* on *Thaumetopoea solitaria* Frey. (Lepidoptera: Thaumetopoeidae) Larvae in Laboratory Conditions," *Turkish Journal of Agriculture and Forestry*, Vol. 31, No. 4, 2007, pp. 255-261.

[95] R. L. Hellmich, B. D. Siegfried, M. K. Sears, D. E. Stanley-Horn, M. J. Daniels, H. R. Mattila, T. Spencer, K. G. Bidne and L. C. Lewis, "Monarch Larvae Sensitivity to *Bacillus thuringiensis* Purified Proteins and Pollen," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 98, No. 21, 2001, pp. 11925-11930. doi:10.1073/pnas.211297698

[96] S. Khewa and A. Mukhopadhyay, "Biocontrol Potential of a Newly Isolated Bacterial Agent against *Arctornis submarginata* (Walker) (Lepidoptera: Lymantriidae) Occurring in Darjeeling Terai Region," *Journal of Biopesticides*, Vol. 3, 2010, pp. 114-116.

[97] P. Yashodha and C. Kuppusamy, "A Bioefficacy of *Bacillus thuringiensis* Var *kurstaki* against Eggplant Borer, *Leucinodes orbonalis* (Pyraustidae: Lepidoptera)," *International Journal of Integrative Biology*, Vol. 3, No. 3, 2008, pp. 209-213.

[98] V. Gobatto, S. G. Giani, M. Camassola, A. J. P. Dillon, A. Specht and N. M. Barros, "*Bacillus thuringiensis* Isolates Entomopathogenic for *Culex quinquefasciatus* (Diptera: Culicidae) and *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)," *Brazilian Journal of Biology*, Vol. 70, No. 4, 2010, pp. 1039-1046. doi:10.1590/S1519-69842010000500018

[99] J. Ferre, B. Escriche, Y. Bel and J. Van Rie, "Biochemistry and Genetics of Insect Resistance to *Bacillus thuringiensis* Insecticidal Crystal Proteins," *FEMS Microbiology Letters*, Vol. 132, 1995, pp. 1-7. doi:10.1016/0378-1097(95)00271-6

[100] B. E. Tabashnik, "Evolution of Resistance to *Bacillus thuringiensis*," *Annual Review of Entomology*, Vol. 39, No. 1, 1994, pp. 47-79. doi:10.1146/annurev.en.39.010194.000403

[101] A. Trisyono and M. E. Whalon, "Fitness Costs of Resistance to *Bacillus thuringiensis* in Colorado Potato Beetle (Coleoptera: Chrysomelidae)," *Journal of Economic Entomology*, Vol. 90, No. 2, 1997, pp. 267-271.

[102] B. E. Tabashnik, Y. B. Liu, N. Finson, L. Masson and D. G. Heckel, "One Gene in Diamondback Moth Confers Resistance to Four *Bacillus thuringiensis* Toxins," *Proceedings of the National Academy of Sciences*, Vol. 94, No. 5, 1997, pp. 1640-1644. doi:10.1073/pnas.94.5.1640

[103] D. J. Wright, M. Iqbal, F. Granero and J. Ferre, "A Change in a Single Midgut Receptor in the Diamondback Moth (*Plutella xylostella*) Is Only in Part Responsible for Field Resistance to *Bacillus thuringiensis* Subsp. *kurstaki* and *B. thuringiensis* Subsp. *aizawai*," *Applied and Environmental Microbiology*, Vol. 63, No. 5, 1997, pp. 1814-1819.

[104] J. L. Fox, "Resistance to *Bt* Toxin Surprisingly Absent from Pests," *Nature Biotechnology*, Vol. 21, No. 9, 2003, pp. 958-959. doi:10.1038/nbt0903-958b

[105] B. E. Tabashnik, T, Brévault and Y. Carrière, "Insect Resistance to *Bt* Crops: Lessons from the First Billion Acres," *Nature Biotechnology*, Vol. 31, 2013, pp. 510-521.

[106] D. N. Alstad and D. A. Andow, "Managing the Evolution of Insect Resistance to Transgenic Plants," *Science*, Vol. 268, No. 5219, 1995, pp. 1894-1896. doi:10.1126/science.268.5219.1894

[107] F. Gould, "Potential and Problems with High-Dose Strategies for Pesticidal Engineered Crops," *Biocontrol Science and Technology*, Vol. 4, No. 4, 1994, pp. 451-461. doi:10.1080/09583159409355357

[108] F. Gould, "Genetic Engineering, Integrated Pest Management and the Evolution of Pests," *Trends in Biotechnology*, Vol. 6, No. 4, 1988, pp. 15-18. doi:10.1016/0167-7799(88)90007-8

