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Diversity in *cry* genes of *Bacillus thuringiensis*

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INTRODUCTION

The *Bacillus thuringiensis* Berliner research began when the Japanese bacteriologist, Ishiwata (1901) isolated the bacillus from diseased *Bombyx mori* larvae. He named it “sottokin”, which means “sudden death bacteria” in Japanese. A decade later, Ernst Berliner (1915) isolated a similar organism from diseased granary populations of *Ephestia kuehniella* Zell (Mediterranean flour moth) larvae obtained from Thueringen, Germany, and named the bacterium *Bacillus thuringiensis* (Bt). As Ishiwata did not formally describe the organism he found, Berliner is credited with naming it. Application of Bt was first reported by Husz in 1928 who isolated a Bt strain from *Ephestia* and tested it on European corn borer, *Ostrinia nubilalis* (Kumar *et al.,* 1996). In 1960, stimulated by the growing concern over the use of chemical insecticides, a Bt strain was commercialized for the first time and marketed as “Thuricide”. Slowly, the first strain was replaced by a more potent strain (HD-1) isolated by Dulmage (1970).

In earlier days, it was believed that Bt was mostly active against lepidopterans, until Goldberg and Margalit (1977) isolated a strain *Bacillus thuringiensis israelensis* from a mosquito breeding pond in Negev desert, which proved highly toxic to mosquito and blackfly larvae. Later in 1983, Kreig and co-workers investigated a strain from dead mealworm pupae and designated as *Bacillus thuringiensis tenebrionis* which was found to be highly toxic to elm leaf beetle and Colorado potato beetle grubs. These findings ultimately led to more screening programs and now there are thousands of Bt isolates in various collections. The advancement

in genetic engineering and molecular biology in the early eighties led to the cloning of Bt crystal protein *(cry)* gene for the first time successfully (Wasano *et al.*, 2001). Initially several *cry* genes were expressed in plant colonizing microorganisms to target the stem and root dwelling insect pest. Today, the most efficient way to deliver *cry* genes seems to be the development of transgenic plants expressing them (Betz *et al.*, 2000; Kannaiyan, 2000; De Gosa *et al.*, 2001).

CLASSIFICATION OF Bt SUBSPECIES

Bt is a gram positive, spore forming bacteria that exists in a diverse locations, such as the soil, plant surfaces, insect cadavers and in grain storage dusts (de Maagd *et al.*, 1999). During the sporulation stage of its life cycle, Bt produces one or more proteinaceous crystal inclusions (Fig.1), possessing varying degrees of insecticidal activity (Bulla *et al.*, 1977). The presence of these parasporal crystals in the sporangia and their insecticidal activity has been the unique character that was available for differentiating Bt from its closely related species, *Bacillus cereus* (Claus and Berkeley, 1986 and Andrews *et al.*, 1987).

Helgason *et al.* (2000) observed a close similarity of the genomes of *Bacillus anthracis* strain to those of *Bacillus thuringiensis* and *Bacillus cereus* strains and suggested that they should be considered as belonging to one and the same species. These three species show differences in their phenotypes mostly due to plasmid borne genes.

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In early days, Bt strains were classified into sub species based mainly on morphological and biochemical characters (Lynch and Baumann, 1985 and de Barjac and Franchon, 1990). In recent times, scientists used different methods for classification such as phage-typing (Ackermann *et al.* 1995), esterase pattern of vegetative cells (Norris, 1964), crystal serology (Lynch and Baumann, 1985), plasmid pattern (Lereclus *et al.,* 1982), oligonucleotide probing (Prefontaine *et al.,* 1987), and H -Flagellar serotyping (de Barjac and Frachon, 1990).

Although several methods were tried for classification, serotyping using' H' flagellar antigen remains the most widely used, simplest and practical method to classify Bt strains (de Barjac and Frachon, 1990). In 1993, Bouroque and co workers reported that there might be variation in the biochemical characters, plasmid patterns, shape, stability and the insecticidal activity of the Bt isolates even if placed within the same serotypes. A list of 80 Bt serotypes based on H serotyping is given in Table 1 (Lecadet *et al.,* 1998).

Table 1. Serovars of *Bacillus thuringiensis* strains

S.No	Serovar	H.antigen	Toxicity*	S.No	Serovar	H.antigen	Toxicity*
1.	*thuringiensis*		L, D	41.	*Jagathesan*	28a, 28c	D
2.	*finitimus*	2		42.	*amagiensis*	29	
3.	*alesti*	3a,3c	L	43.	*medellin*	30	D
4.	*kurstaki*	3a,3b,3c		44.	*toguchini*	31	
5.	*sumiyoshiensis*	3a,3d		45.	*cameroun*	32	
6.	*fukuokaensis*	3a,3d,3e	D	46.	*leesis*	33	
7.	*sotto*	4a,4b	L	47.	*konkukian*	34	
8.	*kenyae*	4a,4c	L,D	48.	*seoulensis*	35	
9.	*galleriae*	5a,5b		49.	*malaysiensis*	36	D
10.	*canadensis*	5a,5c	L	50.	*andaluciensis*	37	
11.	*entomocidus*	6	L	51.	*oswaldocruzi*	38	
12.	*aizawai*	7	L,D	52.	*brasiliensis*	39	
13.	*morrisoni*	8a,8b	L,D,C	53.	*huazhongensis*	40	
14.	*ostriniae*	8a,8c	L	54.	*sooncheon*	41	
15.	*nigeriensis*	8b,8d		55.	*jinghongiensis*	42	
16.	*tolworthi*	9	L,D	56.	*guiyangiensis*	43	
17.	*darmstadiensis*	10a,10b	L,D	57.	*higo*	44	
18.	*londrina*	10a,10c		58.	*roskildiensis*	45	
19.	*toumanoff,*	1 la, 1lb		59.	*chanpaisis*	46	
20.	*kyushuensis*	1 la, 1lc	L,D	60.	*wratislaviensis*	47	
21.	*thompsoni*	12	L,D	61.	*balearica*	48	
22.	*pakistani*	13		62.	*muju*	49	
23.	*israelensis*	14	D	63.	*navarrensis*	50	
24.	*dakota*	15		64.	*xiaguangiensis*	51	
25.	*indiana*	16		65.	*kim*	52	
26.	*tohokuensis*	17		66.	*asturiensis*	53	
27.	*kumamotoensis*	18a, 18b	C	67.	*poloniensis*	54	
28.	*yosoo*	18a, 18c		68.	*palmanyolensis*	55	
29.	*tochigiensis*	19		69.	*rongseni*	56	
30.	*yunnanensis*	20a, 20b	L	70.	*pirenaica*	57	
31.	*pondicheriensis*	20a, 20c	L	71.	*argentinensis*	58	
32.	*colmeri*	21		72.	*iberica*	59	
33.	*shandongiensis*	22	L	73.	*pingluonsis*	60	
34.	*japonensis*	23	C	74.	*sylvestriensis*	61	
35.	*neoleonensis*	24a, 24b		75.	*zhaodongensis*	62	
36.	*novosibirsk*	24a, 24c		76.	*bolivia*	63	
37.	*coreanensis*	25		77.	*azorensis*	64	
38.	*silo*	26		78.	*pulsiensis*	65	
39.	*mexicanensis*	27	L	79.	*graciosensis*	66	
40.	*monterrey*	28a, 28b		80.	*vazensis*	67	

* Toxic to L: lepidopteran larvae; D: dipteran larvae; C: coleoplteran larvae
(Based on Lecadet *et al.,* 1998)

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INSECTICIDAL CRYSTAL PROTEINS OF Bt

Cry protein synthesis

The insecticidal crystal proteins are formed during sporulation stage of the bacterium's life cycle. About 20 -30 per cent of the dry weight of the matured sporangium is accounted for these proteins (Lilley *et al.,* 1980). Crystal formation can be observed microscopically during the later part of stage II (Somerville., 1971 and Mikkola *et al.,* 1982). In Bt H-14, electron microscopic studies revealed the presence of nascent inclusions even at stage I (Abdel-Hameed *et al.,* 1990). Once crystal formation is initiated, crystal protein is synthesised until the end of stage IV (Lecadet and Dedonder, 1971), although crystals may continue to enlarge until stage VI. Over 80 per cent of crystal proteins produced during sporulation is synthesised *de novo* from amino acids supplied by break down of proteins in the sporulation process (Monro, 1961). It is believed that all subunits of the crystals are synthesised simultaneously (Lecadet and Dedonder, 1971).

Structural features of Cry proteins

The crystal morphology in Bt is highly complex and it shows different forms like bipyramidal, cuboidal, spherical, squares and irregular (Chilcott and Wigley, 1994). These various forms of true crystals have been observed using phase contrast and electron microscopes (Huber and Luthy, 1981). Bipyramidal crystals synthesised in host cells are typically about 1.1 µm long and

0.511μm wide (Oeda *et al.,* 1989). Bipyramidal crystals show a greater frequency of toxicity than all the other types. Most of the lepidopteran active isolates contain such inclusions (Attamthom *et al.,* 1995); cuboidal crystals are active against lepidopteran and dipteran larvae or lepidopteran larvae alone (Yammato and Mclauzhtin, 1981); spherical and irregular crystals are mostly mosquitocidal, often active against certain coleopteran species (Krieg *et al.,* 1983). Irregular crystals also include those with very little or no identified toxicity (Zelanzy *et al.,* 1994). Based on Cry protein composition, the crystals have various forms. Most Cry1 type proteins form bipyramidal crystals, Cry2 type assume cuboidal form and the Cry3 proteins form flat rhomboidal crystals. The crystals formed by Bt *israelensis* are typically spherical. Many investigations carried out to study the chemical nature of the toxin showed that it is a glycoprotein. The toxicity of Cry proteins was affected by the glycosylation process (Bulla *et al.,* 1977). Protein content ranges from 88 to 95 per cent and carbohydrates value range from 0.5 to 12 per cent have been reported. Further analysis carried out showed, crystals of Bt were found to contain 0.36 per cent silicon by weight and significant concentratiorls of Ca, Fe and Mg as revealed by emission spectroscopy (Meenakshi *et a/.,* 1993 and Abdel -Hameed *et a/.,* 1991). The crystal toxin is insoluble in water or inorganic solvents, but soluble in alkaline solvents. Cry1 proteins are soluble at pH 9.5, while the Cry2 proteins are soluble at a pH of about 12. Similarly, Cry4A, Cry5B and Cyt toxins are soluble at pH 9.5, while the Cry40 toxin requires a pH of 12. The Cry3A toxin on the other hand, dissolves at pH's

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Navon, A. 2000. *Bacillus thuringiensis* insecticides in crop protection -reality and prospects. *Crop Protection,* 19: 669-676

below 4 and above 9.5. The crystals can also be dissolved at neutral pH in the presence of detergents and denaturing agents like urea, β-mercaptoethanol, OTT and SOS (Gill *et al.,* 1992). The presence of cysteine residues at the C-terminal part of protoxin is attributed to the relative insolubility and the requirement of denaturing and reducing agents for further solubilization (Hofte and Whiteley, 1989). Separation of crystals from the spores and cell debris involves isopycnic centrifugation in sucrose or caesium chloride gradients (Fast, 1972), zonal gradient centrifugation using NaBr (Ang and Nickerson, 1978). In addition, relatively large scale and rapid purification of crystals from a broad range of Bt strains is possible by linear and discontinuous gradients using Renongrafin-76 (66.7% N-methyl glucamine and 94% sodium salt of 3, 5 diacentamido 2, 4, 6, Tridobenzoic acid) (Sharpe *et a/.,* 1975).

Bt toxins usually termed protoxins are activated by the insect midgut proteases to yield the toxic fragment. Gill *et al.* (1992) reported that the larger protoxin of about 130 -140 kDa undergoes proteolysis yielding a toxic fragment of 60 -70 kDa derived from N-terminal half of the protoxin. Structurally the activated toxin can be divided into 3 structural regions.

I. N-terminal region, which is the toxic domain (amino acid sequence 1 -279) consisting of several conserved hydrophobic regions.

II. A conserved C-terminal region (amino acid sequence 461 -695) and

III. A variable region between these two region that contains most of the amino acid differences.

Hofte and Whiteley (1989) investigated sequences among a number of toxins and found five well conserved regions and designated as blocks from one to five. According to this it was predicted that the crystal toxin, consists of three domains namely

Domain I : Consists of a bundle of seven anti parallel α-helices in which helix five is enriched by the remaining helices.

Domain II : Consists of three antiparallel **β**-Sheets.

Domain III : It has two twisted antiparallel **β**-sheets.

This has been confirmed by X-ray crystallographic studies (Li *et al.* 1991; Grochulski *et al.,* 1995). The long hydrophobic and amphipathic helices of domain I suggest that this domain might be responsible for the formation of lytic pores in the intestinal epithelium of the target organism. The involvement of Domain II in receptor binding was supported by site directed mutagenesis and segment swapping experiments (Rajamohan *et al.,* 1996; Jurat-Fuentes and Adang, 2001). The **β**-sandwich structure of domain III could playa number of roles. The main function consisting of maintaining the structural integrity of the toxin molecule, mostly it shields the molecule from proteases during proteolysis within the gut of the target pest (Mackinnon and Miller, 1989 and Li *et al.,* 1991). The insecticidal bacterium

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Maqbool, S. B., T. Husnain, S. Riazuddin, L. Masson and P. Christou. 1998. Effective control of yellow stem borer and rice leaffolder in transgenic rice *indica* varieties Basmati 370 and M7 using the novel delta-endotoxin *cry2A Bacillus thuringiensis* gene. *Mol. Breed.,* 4: 1-7.

Bt synthesizes δ-endotoxin Cry proteins in two size classes, 135 and 70 kDa, and both form crystalline inclusions in cells after synthesis. Crystallization of 135-kDa proteins is due to intermolecular attraction of regions in the C-terminal half of the molecule, and the N-terminal half fails to crystallize when synthesized in vivo. In this crystallization process domain I of Cry3A plays a vital role (Park and Federici, 2001).

Mode of action of Cry protein

The Cry protein when ingested by the insect is first converted from the inactive form to active toxin protein by proteolysis in the alkaline mid gut region (Huber and Luthy, 1981; Lightwood *et al.,* 2000), which then diffuses through peritrophic membrane and binds to high affinity receptors present on the midgut epithelium. When the activated toxin binds to the specific receptors (Hoffmann *et al.,* 1988; Maclintosh *et al.,* 1991), the interaction of receptor-toxin becomes irreversible and generates pores on the membrane. The formation of pore eventually disturbs the ionic gradient, further leading to the swelling of microvilli and destruction of epithelial membrane, leading to the cell death.

CLASSIFICATION OF *cry* GENES

The crystal proteins of Bt have been studied for their insecticidal properties and their high natural levels of production. The increasingly rapid characterization of new crystal protein genes, triggered by an effort to discover proteins with new insecticidal properties have made the classification of cry genes

more difficult. Earlier scientists used arbitrary designations like icp (McClinden *et al.,* 1985), Bta (Sanchis *et al.,* 1989), P1 and P2 (Donovan *et al.,* 1988), type B and type C (Hofte *et al.,* 1988) and 4.5 Kb and 6.6 Kb (Kronstad and Whiteley, 1986). The first attempt to systematically classify Bt crystal genes were undertaken by Hofte and Whiteley (1989). The systematic designations followed by Hofte and Whiteley were as follows. The principal toxicity spectrum was denoted by Roman letters (from I to IV) grouped under different classes. Within the class major and minor amino acid differences were denoted by upper and lower case letter, respectively (eg:*cryIAa).* Since, Bt genes encoding cytolytic proteins were totally unrelated to *cry* genes, they were designated as *cyt* genes. In general the term *'cry'* (first given by Heid *et al.,* 1982) was followed to designate the genes encoding crystal proteins and Cry for toxins.

cry 1 Class

The *cryl* genes code for lepidopteran specific bipyramidal crystal proteins having molecular range of proteins having molecular range of 130 -140 kDa. Upon proteolytic cleavage they yield 60 -70 kDa core toxin fragment. The Cry1 proteins have related amino acid sequence; they display greater than 55 per cent identity (Hofte and Whiteley, 1989). The cry 1 gene class contains subclasses of genes *(cry1 A to* K). The carboxy terminal half of the all *cry* I encoded proteins are highly conserved, whereas the N terminal end is more variable. N-terminal variable region

Lereclus, D., *J.* Ribier, A. Klier, G. Menou and M. M. Lacadet. 1984. A transposon like structure related to the δ-endotoxin gene of *Bacillus thuringiensis. EMBO* J., 3: 2561-2567.

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Lenin, K. , M. Asia Mariam, and V. Udayasuriyan. 2001. Expression of a *cry2Aa* gene in an acrystalliferous *Bacillus thuringienis str*ain and toxicity of Cry2Aa against *Helicoverya al-nligera. World* J. *Microbiol. Biotechnol* 17 (3) 273-278.

represents the active fragment whereas the conserved C-terminal region is involved mostly in crystallization.

cry 2 Class

The *cry2* class of genes encode about 65 -71 kDa proteins which form cuboidal inclusions during sporulation. It has three subclasses *(cry2A, cry2B, cry2C).* The Cryll proteins share about 80 -90 per cent amino acid sequence homology, but are dissimilar to the other Cry proteins, except in the first, N-terminal conserved domain. The Cry2 protein are toxic to either lepidopteran or lepidopteran and dipteran larvae (Widner and Whiteley, 1989).

cry 3 Class

It encodes 73 kDa coleopteran specific proteins (CryIII) (Herrnstadt *et al.,* 1987). There are 5 sub classes (CryIII A to E) in this group of genes. Cry3 A and Cry3 B share about 67 per cent amino acid sequence identity and are homologous to the amino terminal half of the Cry1 protoxins. Cry3 D shows limited homology to other Cry3 toxins. The Cry3C encode proteins which form bipyramidal crystals unlike other Cry3 crystals which form rhomboidal proteins (McPherson *et al.,* 1988).

cry 4 Class

This class of genes is composed of a mixed group of dipteran -specific crystal protein genes. This class contains 4 sub classes namely *cry4A, cry4B, cry4C* and *cry4D* encoding polypeptides with predicted molecular mass of 135, 128, 74 and

72kDa respectively. The Cry4C and Cry4D are naturally occurring truncated versions. The protoxins are protelytically activated to a toxic fragment of undetermined molecular weight (Chilcott and Ellar, 1988; Chungjatupomchai *et a/.,* 1988).

Cyt genes

Cyt genes encode a 27 kDa polypeptide which shows no homology to other crystal protein genes. These genes are cytolytic or haemolytic for a variety of invertebrate and vertebrate cells (Thomas and Ellar, 1983).

Other *cry* genes

The *cry 5* class of genes encode a protein having molecular weight of about 80 kDa. They show toxicity towards coleopteran and lepidopteran larvae. The *cry 6* class on the other hand are reported to exhibit activity against nematodes (Feitelson *et al.,* 1992).

Apart from these well-characterized crystal proteins, there are several proteins which do not seem to be active against any of the .insects tested (Martin and Travers, 1989).

Crickmore classification of Bt genes

When using Hofte and Whiteley system of classification it had certain limitations like

Krieg, V. A., A. M. Huger, G. A. Longenbruch and W. Schnetter. 1983. *Bacillus thuringiensis* var. *tenebrionis:* a new pathotype effective againstlarvae of coleoptera. Z. *Angew. Entomol..* 96: 500.

Kronstad, J. W. and H. R. Whiteley. 1984. Inverted repeat sequences flank the *Bacillus thuringiensis* (crystal protein gene. J.*Bacteriol.,* 160: 95-105.

Kronstad, J. W. and H. R Whiteley. 1986. Three classes of homologous *Bacillus thuringiensis* crystal- protein genes. *Gene,* 43: A9-40.

Kumar, P. A., R. P. Sharma and V. S. Malik. 1996. The insecticidal proteins of *Bacillus thuringiensis. Advances in Applied Microbiology.* 42: 1-43.

Lambert, B., and M. Peferoen. 1992. Insecticidal promise of *Bacillus thuringiensis. Bioscience,* 42: 112-122

Lampe1, J.S., G.L. Canter, M.B. Dimock, J.L. Kecly, J.J. Anderson, BB. Vratani, J.S. Foulke, Jr. and J.T. Turner. 1994. Integrative cloning, expression and stability of the *crylAc* gene from *Bacillus thuringiensis* subsp. *kurstaki* in a recombinant strain of *Clavibactor xyli* subsp. *cynodontis. Appl. Environ. Microbiol.,* 60: 501-508.

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Kalman, S., K. L. Kichne, J. L. Libs and T. Yamamoto. 1993. Cloning of a novel *CrylC-type* gene from a strain of *Bacillus thuringiensis* subsp. *galleriae. Appl. Env. Microbiol.,* 59: 1131-1137.

Kalman, S., K. L. Kiehne, N. Cooper, M. S. Reynoso and T. Yamamoto. 1995. Enhanced production of insecticidal proteins in *Bacillus thuringiensis* strains carrying an additional crystal protein gene in the chromosomes. *Appl. Env. Microbiol.,* **61**: 3063-3068.

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i. Toxins showing homology have different insecticidal spectrum.

Eg: CryllA is toxic to both lepidopterans and dipterans, whereas CryIIB is toxic to lepidopteran larvae only. But they are grouped together.

ii. Since, it is mainly function based, bioassay of new toxin against target organism is a must.

Recently, in 1998 under the chairmanship of Neil Crickmore, a standing committee of the *Bacillus* Genetic Stock Centre (BGSC) was instituted in assigning names to new *cry* and *Cyt* genes. Based on the recommendations of the committee Crickmore *et al*. (1998) revised the nomenclature for the *cry* and *crt* genes. The Table 2 shows a list of holotype toxins. In this newly revised system, a broad definition was given to Cry Protein: a parasporal inclusion (crystal) protein from Bt that exhibits some experimentally verifiable toxic effect to a target organism, or any protein that has obvious sequence similarity to a known Cry protein. Similarly Cyt protein denotes a parasporal inclusion (Crystal) protein from Bt that exhibit haemolytic activity, or any protein that has obvious sequence similarity to a known Cyt protein.

Table 2. Known cry and cyt gene sequences with revised nomenclature assignments

Revised gene name	Original gene or protein Name	Accession No.	Coding region[a]	Refer-ence	Revised gene name	Original gene or protein name	Accession No.	2125-3990>	Refer-ence
Cry1Aa1	CryIA(a)	M11250	527-4054	92	Cry2Ab2	cryIIB	X55416	874-2775	17
Cry1Aa2	CryIA(a)	M10917	153->2955	98	Cry2Ac1	cryIIC	X57252	2125-3990	124
Cry1Aa3	CryIA(a)	D00348	73-3600	99	Cry3Aa1	cryIIIA	M22472	25-1956	39
Cry1Aa4	CryIA(a)	X13535	1-3528	62	Cry3Aa2	cryIIIA	J02978	241-2172	93
Cry1Aa5	CryIA(a)	D17518	81-3608	113	Cry3Aa3	cryIIIA	Y00420	566-2497	41
Cry1Aa6	CryIA(a)	U43605	1->1860	63	Cry3Aa4	cryIIIA	M30503	201-2132	65
Cry1Ab1	CryIA(b)	M13898	142-3606	119	Cry3Aa5	cryIIIA	M37207	569-2500	22
Cry1Ab2	CryIA(b)	M12661	155-3622	111	Cry3Aa6	cryIIIA	U10985	569-2500	1
Cry1Ab3	CryIA(b)	M15271	156-3620	31	Cry3Ba1	cryIIIB2	X17123	25->1977	101
Cry1Ab4	CryIA(b)	D00117	163-3627	50	Cry3Ba2	cryIIIB	A07234	342-2297	85
Cry1Ab5	CryIA(b)	X04698	141-3605	40	Cry3Bb1	cryIIIBb	M89794	202-2157	24
Cry1Ab6	CryIA(b)	M37263	73-3537	37	Cry3Bb2	cryIIIC(b)	U31633	144-2099	23
Cry1Ab7	CryIA(b)	X13233	1-3465	36	Cry3Ca1	cryIIID	X59797	232-2178	59
Cry1Ab8	CryIA(b)	M16463	157-3621	69	Cry4Aa1	cryIVA	Y00423	1-3540	121
Cry1Ab9	CryIA(b)	X54939	73-3537	13	Cry4Aa2	cryIVA	D00248	393-3935	95
Cry1Ab10	CryIA(b)	A29125	-[b]	28	Cry4Ba1	cryIVB	X07423	157-3564	16
Cry1Ac1	CryIA(c)	M11068	388-392	13	Cry4Ba2	cryIVB	X07082	151-3558	112
Cry1Ac2	CryIA(c)	M35524	239-3769	117	Cry4Ba3	cryIVB	M20242	526-3930	125
Cry1Ac3	CryIA(c)	X54159	339->2192	18	Cry4Ba4	cryIVB	D00247	461-3865	95
Cry1Ac4	CryIA(c)	M73249	1-3534	84	Cry5Aa1	cryVA (a)	L07025	1->4155	102
Cry1Ac5	CryIA(c)	M73248	1-3531	83	Cry5Ab1	cryVA (b)	L07026	1->3867	67
Cry1Ac6	CryIA(c)	U43606	1->1821	63	Cry5Ac1		I34543	1->3660	76
Cry1Ac7	CryIA(c)	U87793	976-4509	38	Cry5Ba1	PS86Q3	U19725	1->3735	76
Cry1Ac8	CryIA(c)	U87397	153-3686	71	Cry6Aa1	cryVIA	L07022	1->1425	68
Cry1Ac9	CryIA(c)	U89872	388-3921	33	Cry6Ba1	cryVIB	L07024	1->1185	67
Cry1Ac10		AJ002514	388-3921	107	Cry7Aa1	cryIIIC	M64478	184-3597	58
Cry1Ad1	CryIA(c)	M73250	1-3537	79	Cry7Ab1	cryIIIC (b)	U04367	1->3414	75
Cry1Ae1	CryIA(e)	M65252	81-3623	60	Cry7Ab1	cryIIIC (c)	U04368	1->3414	75
Cry1Af1	icp	U82003	172->2905	49	Cry7Ab2	cryIIIE	U04364	1->3471	29
Cry1Ba1	cryIB	X06711	1-3684	10	Cry8Aa1	cryIIIG	U04365	1->3507	66
Cry1Ba2		X95704	186-3869	105	Cry8Ba1	cryIIIF	U04366	1-3447	70
Cry1Bb1	ET5	L32020	67-3753	25	Cry8Ca1	cryIG	X58120	5807-9274	104
Cry1Bc1	cryIB(c)	Z46442	141-3839	6	Cry9Aa1	cryIG	X58534	385->3837	32
Cry1Bd1	cry E1	U70726		12	Cry9Aa2	cryX	X75019	26-3488	97
Cry1Ca1	cryIC	X07518	47-3613	45	Cry9Ba1	cryIII	Z37327	2096-5569	57

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Revised gene name	Original gene or protein Name	Accession No.	Coding region[a]	Refer-ence	Revised gene name	Original gene or protein name	Accession No.	2125-3990>	Refer-ence
Cry1Ca2	cryIC	X13620	241->2711	88	Cry9Ca1	N141	D85560	47-3553	4
Cry1Ca3	cryIC	M73251	1-3570	79	Cry9Da1		AF042733	<1->1937	122
Cry1Ca4	cryIC	A27642	234-3800	114	Cry9Ca2	cryIVC	M12662	941-2965	111
Cry1Ca5	cryIC	X96682	1->2268	106	Cry10Aa1	cryIVD	M31737	41-1969	21
Cry1Ca6	cryIC	X96683	1->2268	106	Cry11Aa1	cryIVD	M22860	<1-235	2
Cry1Ca7	cryIC	X96684	1->2268	106	Cry11Aa2	Jeg80	X86902	64-2238	19
Cry1Cb1	cryIC(b)	M97880	296-3823	48	Cry11Ba1	94 kDa	AF017416		72
Cry1Da1	cryID	X54160	264-3758	42	Cry11Bb1	cryVB	L07027	1->3771	67
Cry1Db1	prtB	Z22511	241-3720	56	Cry12Aa1	cryVC	L07023	1-2409	90
Cry1Ea1	cryIE	X53985	130-3642	115	Cry13Aa1	cryVD	U13955	1-3558	77
Cry1Ea2	cryIE	X56144	1-3513	7	Cry14Aa1	34kDa	M76442	1036-2055	11
Cry1Ea3	cryIE	M73252	1-3513	82	Cry15Aa1	cbm71	X94146	158-1996	5
Cry1Ea4		U94323	388-3900	47	Cry16Aa1	cbm72	X99478	12-1865	5
Cry1Eb1	cryIE(b)	M73252	1-3522	81	Cry17Aa1	cryBPI	X99049	743-2860	126
Cry1Fa1	cryIF	M63897	478-3999	14	Cry18Aa1	Jeg65	Y07603	719-2662	86
Cry1Fa2	cryIF	M73254	1-3525	80	Cry19Aa1		D88381		87
Cry1Fb1	prtD	Z22512	483-4004	56	Cry19Ba1	86kDa	U82518	60-2318	61
Cry1Ga1	prtA	Z22510	67-3564	56	Cry20Aa1		I32932	1-3501	74
Cry1Ga2	cryIM	Y09326	692-4210	96	Cry21Aa1		I34547	1-2169	76
Cry1Gb1	cryH2	U70725		12	Cry22Aa1				
Cry1Ha1	prtC	Z22513	530-4045	56					
Cry1Hb1		U35780	728-4195	53	cyt1Aa1	cytA	X03182	140-886	118
Cry1Ia1	cryV	X62821	355-2511	108	cyt1Aa2	cytA	X04338	509-1255	120
Cry1Ia2	cryV	M98544	1-2157	34	cyt1Aa3	cytA	Y00135	36-782	26
Cry1Ia3	cryV	L36338	279-2435	100	cyt1Aa4	cytA	M35968	67-813	30
Cry1Ia4	cryV	L49391	61-2217	54	cyt1Ab1	cytM	X98793	28-777	109
Cry1Ia5	cryV159	Y08920	524-2680	94	cyt1Ba1		U37196	1-795	78
Cry1Ib1	cryV465	U07642	237-2393	100	cyt2Aa1	cytB	Z14147	270-1046	51
Cry1Ja1	ET4	L32019	99-3519	25	cyt2Ba1	"cytB"	U52043	287-655	35
Cry1Jb1	ET1	U31527	177-3686	116	cyt2Bb1		U82519	416-1204	15
Cry1Ka1		U28801	451-4098	52					
Cry2Aa1	cryIIA	M31738	156-2054	20					
Cry2Aa2	cryIIA	M23723	1840-3738	123					
Cry2Aa3		D86064	2007-3911	89					
Cry2Ab1	cryIIIA	M23724	1-1899	123					

[a] The symbols < and > indicate that the coding region extends up-or downstream, respectively, from the known sequence data.
[b] Only the polypeptide sequence has been reported. (Crickmore et al., 1998 MMBR 807-813)

To organize the wealth of data produced by genome sequencing efforts, a new nomenclatural paradigm is emerging, exemplified by the internationally recognized cytochrome P 450 superfamily nomenclature system (White *et al.,* 1998). The revised nomenclature is based mainly on the above system both in conceptual basis and in nomenclature format.

Some of the important features of revised nomenclature are:

I. The *cry* genes whose products share < 45% amino acid sequence homology are characterized by different Arabic numbers, designated as primary ranks (eg. *cry* 1 , *cry2* etc.).

II. The *cry* genes of the same primary rank whose products show < 78% amino acid sequence homology are differentiated by secondary ranks by using uppercase letters (eg. *cry1A, cry1B* etc.,).

III. The *cry* genes having same primary and secondary ranks whose products share less than 95% amino acid sequence homology receive separate tertiary rank, designated by lowercase letters (eg. *cry1Aa, cry1Ab* etc.,).

IV. The *cry* genes whose products are different in amino acid sequence, but are more than 95% identical to each other are given separate quaternary ranks by another Arabic number (eg. *cry1Aa1, cry1Aa2* etc.,).

Under the revised system almost all *cry* genes retained the names they received under Hofte and Whiteley (1989) after a substitution

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Goldberg, L. J., and J. Marglit. 1977. A bacterial spore demonstrating rapid larvicidial activity against *Anopheles serengetii, Uranotenia unguiculata, Culex univittatus, Aedes aegypti and Culex pipiens. Mosquito News.,* 37:355-358.

of Arabic for Roman numerals. There are few notable exceptions *crylG* becomes *cry9A, crylllC* becomes *cry7Aa, crylllD* becomes *cry3C, crylVC* becomes *cry10A, crylVD* becomes *cry11A, crt* becomes *cyt1A* and *criB* becomes *cyt2A.* The known Cry and Cyt proteins now fall into 30 sets including Cyt1, Cyt2 and Cry1 through Cry28.

LOCALIZATION AND REGULATION OF *cry* GENES

The majority of *cry* genes are localized in the self replicating plasmids, having a size ranging from 140 to 150 MDa (Lereclus *et al.,* 1989; Whiteley and Schnepf, 1986). The role of plasmids were evident, after the cloning of the toxin structural genes were used as probes to determine their localization in most of the known strains (Lereclus *et al.,* 1989; Whiteley and Schnepf, 1986). Further investigation showed that in some strains (Bt var. *entomocidus,* 8t var. *aizawal) cry* genes are localized on the chromosome (Udayasuriyan *et al.,* 1996; Sekar *et al.,* 1987). The involvement of transposons as a part of *cry* genes composite structure was first demonstrated by a variety of approaches (Kronstad and Whiteley, 1984; Lereclus *et al.,* 1984). The *cry4A* gene, in the dipteran acitve Bt *israelensis* was flanked by 2 repeated seqeunces in opposite orientation (80urgouin *et al.,* 1988). In most of the *cry2* family of crystal protein genes, they are present as a part of operon. Widner and Whiteley (1989), Wu *et al.* (1991) reported that *cry2Aa* and *cry2Ac* are distal part of operon containing two more open reading frames.

The synthesis of crystal proteins in Bt is controlled by a variety of mechanisms occurring at the transcriptional, post transcriptional and post translational levels. The development of sporulation is controlled by the successive activation of sigma factors which binds to the core RNA polymerase to direct the transcription from sporulation specific promoters (Moran, 1993). The *cry*1*Aa* gene is a typical example of a sporulation dependent *cry* gene expressions. At post transcriptional level the stability of the *cry* mRNA is an important factor for high level of toxin production in Bt. The half life of *cry* mRNA is about 10 min, which is almost five fold greater than the half-life of an average bacterial mRNA (Glatron and Rapoport, 1972). One of the possible reasons for *cry* mRNA stability is due to the presence of a stem loop structure at 3' end which acts as a positive retroregulator by preventing 3' 5' exoribonuclease activity on *cry* mRNA.

CLONING OF *cry* GENES

The main objective of cloning *cry* gene using recombinant DNA methodologies is to improve Bt strains available for better production and to improve the toxicity. The first ever report on cloning of Bt crystal protein gene was reported in 1981. Schenpf and Whiteley (1981) cloned a crystal protein gene from Bt subsp. *kurstaki* and expressed in *E. coli.* The recombinant strains of *E. coli* synthesised a 130 kDa protein that showed positive reaction with antibody raised against a peptide of the same size from Bt crystals. In 1993, Kalman *et al.,* cloned a new *cry1C* gene

Donovan, W. P., C. C. Dankocisk, M. P. Gillbcrt, M. C. Gawron Burke, I. G. Groat and B. C. Carltill. 1988. Amino acid sequence and entomocidal activity of the P2 crystal protein. *J. Bioi. Chem.,* 263: 561-567.

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Feitelson, J. S., J. Payne and L. Kim. 1992. *Bacillus thuringiensis:* Insects and beyond. *BioTechnology,* 10: 271-275.

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de Maagd, R. A., D. Bosch and W. Stiekema. 1999. *Bacillus thuringiensis* toxin mediated insect resistance in plants. *Science,* 4: 09-13.

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(cry1Cb) from Bt *gallariae,* based on distinct electrophoretic mobility of PCR products amplified using specific primers. Chak *et al.* (1994) placed a *crylC* gene, along with the alpha-amylase promoter from B. *subtilis* in a B. *thuringiensis* -derived cloning vector, generating a pair of recombinant plasmids pSB 744 and pSB 745 and expressed in Bt subsp *kurstaki cry-B* and HD73. Shin *et al.* (1995) cloned two cryV type genes, *cryV1* and *cryV465,* from Bt *kurstaki* HD- 1 and Bt subsp *entomocidus* BP 465 respectively and determined their nucleotide sequence. The cloned CryV1 protein was toxic to *Plutella xylostella* Lin. and *Bombyx mori,* whereas the CryV465 protein showed toxicity towards *Plutella xylostella* only.

Delecluse *et al.* (1995) cloned a mosquitocidal gene, designated cry11 B, encoding a 81 kDa crystal protein in Bt subsp *jegathesan.* The sequence of Cry11 B protein showed high homology towards Cry11A toxin (CryIVD) from Bt subsp *israelensis.* A novel mosquitocidal protein gene, *cry20Aa* was cloned from Bt subsp *fukuokaensis* (H-3a; 3d: 3e). The gene product was naturally truncated and had a molecular weight of 86 kDa. The amino acid comparisons showed *cry20Aa* to be an entirely different protein (Lee and Gill, 1997). Two new crystal protein genes, *cry19A* and Orf2, were isolated from Bt subsp. *jegathesan* encoding a 74.7 kDa protein and 60 kDa protein respectively. The orf2 amino acid sequence is very much similar to carboxy terminus of Cry4 proteins (Rosso and Delecluse, 1997). Sasaki *et al* (1997) cloned a *cry2A* -type gene from Bt

serovar *sotto* SKWO1 -10.2-06 and designated as *cry2* (SKW). The cloned protein was toxic to *Bombyx marp.* The Cry11a was active on both *Plutella xylostella* and *Bombyx man.* A new Cry 11d protein was toxic to *Plute//a xyloste//a* as Cry11a but less active on *Bombyx man* (Choi *et al.,* 2000). The Cry2Aa of a new Bt strain (47-8) was effective against *Helicoverpa armigera,* cotton bollworm (Lenin et al, 2001).

EXPRESSION OF *cry* GENES IN RECOMBINANT BACTERIA

For many years conjugation (Gonzalez J.M. Jr and Carlton; 1992) and transduction (Lecadet *et al.,* 1992) have been used to transfer recombinant plasmids. At present a variety of *E.coli* -Bt shuttle vectors have been constructed to facilitate the introduction of cloned *cry* genes in Bt. Some of these plasmids employ replicons derived from other Gram positive bacteria (eg: PBC16, PC194) while others employ replicons isolated from native Bt plasmids (Chak *et al.,* 1994). In addition to these shuttle vectors, integrational vectors have been used to insert cloned *cry* genes into resident plasmids (Lereclus *et al.,* 1992; Adams *et al.,* 1994) or into the chromosome (Kalman *et al.,* 1995), by homologous recombination. In several cases, the cloned *cry* gene transfer into Bt host strain has resulted in improved spectrum for toxicity (Chak *et al,* 1994; Lereclus *et al,* 1992; Kalman *et al,* 1995).

Heterologous promoters may also be used to improve the expression of certain *cry* genes, including promoters for

Cheng, X., R. Sardana, H. Kaplan, and I. Altosaar. 1998. *Agrobacterium-transformation* rice plants expressing synthetic *cry/Arb)* and *cry/Arc)* gcnes arc highly toxic to striped stem borer and yellow stem borer. *Proc. Natl. Acad. Sci. USA.* 95 : 2767-2772.

Chilcott, C. N. and P. *J.* Wigley. 1994. Isolation and toxicity of *Bacillus thuringiensis* from soil and insect habitats in New Zealand. J.*Invertebr.. Pathol.,* 61: 244-247.

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Chak, K. F., M. Y. Tseng and T. Yammoto. 1994. Expression of the crystal protein gene under the control of the a-amylase promoter in the *Bacillus thuringiensis* strains. *Appl. Env. Microbiol.* 60: 2304-2310.

Chaufaux. *J., J.* Muller-Cohn, C. Buisson, V. Sanchis, D. Lereclus, and N. Pasteur. 1997. Inheritancc of resiStance to the *Bacillus thuringiensis* crylC toxin in *Spodoptera littoralis* (Lepidoptera: Noctuidae). J.*Econ. Entomol.,* 90 : 873-878.

B. subtilis a. -amylase gene (Chak *et al,* 1994), *cry3Aa* (Baum and Malvar, 1995) and *cry3Bb* (Agaisse and Lereclus, 1995). The unique feature of these *cry* genes, the *cry3Aa* and *cry3Bb* gene is that it is sporulation -independent and it may be induced or depressed during stationary phase, probably by transition -phase regulators. These sporulation -independent promoters may be useful in improving the production of sporulation -dependent Cry proteins. Heterologous recombination may be used, not only to integrate *cry* genes into a resident plasmid, or into the chromosome, but also to disrupt genes of interest.

In recent times., enhancing the stability of crystal proteins under field conditions has had a great attention. To combat this, scientists went for DNA manipulations and expressed the cloned *cry* genes in other microorganisms preferably plant associating microbes. The first report of such an expression was established, when Monsanto scientists successfully expressed a cry1Ab gene into a root colonizing bacterium *Pseudomonas fluorescens.* After that numerous attempts were made to introduce *cry* genes into a variety of organisms, for improved environmental delivery of Cry proteins (Table 3)

Table 3. Transgenic microbes as delivery agents for Cry proteins

S.No.	Cry proteins	Microbial host	Advantages	References
1.	*Cry1Ac*	*P.fluorescens*	Foliar resistance	Cell Cap[12] – product of mycogen crop since 1987
2.	*Cry3A*	*Rhizobium leguminosarum*	Control of root feeding insects of legumes	Skok *et al.* (1990)
3.	*Cry1Ac*	*P.cepacia*	Control of *Maduca sexta* & *Heliothis virescens* Tobacco	Stock *et al.* (1990)
4.	*Cry4B*	*Caulobacter crescentus*	Control of mosquito larvae in aquatic environment	Thanabalu *et al.* (1992
5.	*CryIVD*	*Agmenellum quadruplicatum PR-6 (Cyanobacterium)*	Control mosquito larvae	Murphy and Stevens Jr. (1992)
6.	*Cry1Aa*	*Bacillus megaterium*	Prolonged protection of cotton plants against *Helicoverpa armigera*	Bora *et al.* (1992)
7.	*Cry1Ac*	*Clavibacter xyli*	Control of internally feeding insects	Incide R product of crop genesis international, Lampel *et al.* (1994)
8.	*Cry2A*	*Bacillus cereus*	Control of *Heliothis virescens* on Tomoto	Moar *et al.* (1994)
9.	*Cry1Aa*	*Azospirillum lipoferum*	Control of hidden pest was aimed but transgene was not stable	Udayasuriyan *et al.* (1995)
10.	*Cry4A Cry11A*	*Anabaena sp.Stain PCC 7120*	control mosquito	Xiao Qiang *et al* (1997)
11.	*Cry1Ac*	*P.flurorescens*	Control sugarcane borer *Eldana saccharina*	Herrera *et al.* (1997)
12.	*Cry11A*	*B.sphaericus*	Control Mosquitol	Poncet *et al.* (1997)

Bentur, J. S., Andow, D. A, Cohen, M. B., Romena, A. M., Gould, F. 2000a. Frequency of alleles conferring resistance to a *Bacillus thuringiensis* toxin in a Philippine population of *Scilpophaga incertu/as* (Lepidoptera: Pyralidae). *J Econ Entomol 93(5):* 151 5-21

Bentur, J. S., Cohen, M. B., Gould, F. 2000b. Variation in performance on cry 1 Ab-transformed and nontransgenic rice varieties among populations of *Scirpophaga incerlu as* (Lepidoptera: Pyralidae) from Luzon Island, Philippines. *J Econ Entomol 93(6):* 1773-8

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Barton. K. A., H. R. Whiteley and N. Yang. 1987. *Bacillus thuringiensis* delta-endotoxin in transgenic *Nicotiana tobacum* provides resistance to lepidopteran insects. *Plant Physiol.,* 85: 1103-1109.

Baum. J. A. and T. Malvar. 1995. Regulation of insecticidal crystal protein production in *Bacillus thuringiensis. Mol. Microbiol.,* 18: 1-12.

INSECT RESISTANCE TO CRY PROTEINS

The increasing use of chemical insecticides led to the development of resistance and resurgence among insect populations. This led to the switching over of chemical insecticides to Bt-based products and bioformulations. In 1985, McGaughey reported that Indian meal moth populations from grain storage bins treated with Bt formulation showed high LC50 values relative to populations in untreated bins. Later resistance to Bt insecticides was reported from field population *Plutella xylostel/ a* (Tabashnik *et al.,* 1990). Recently de Maagd *et al.* (1999) showed that Bt toxins are not an exception for insects to develop resistance. Development of resistance to CrylllA and Cry1Ac proteins have been reported in Colorado potato beetle and in *Plutella xylostella* (Wierenga *et al.,* 1996 and Tang *et al.,* 1999). Globally the development of resistance to P. *xylostella* towards Bt subsp *kurstaki* has been reported from Florida (Shelton *et al.,* 1993), Japan (Hama *et al.,* 1992), The Philippines (Ferre *et al.,* 1991) and China. Recently laboratory studies reveal 76 folds increase in resistance towards Cry1Ac ä-endotoxin for *Helicoverpa armigera* (Hubner) by the end of the 10th generation (Kranthi *et al.,* 2000). Due to the escalation of resistance to Bt ä -endotoxins in recent times, scientists were forced to undertake studies for understanding the mechanism involved in resistance development in insects. A variety of studies conducted on different resistant populations shows that the primary reason of resistance

lies in the lowering of the affinity of the toxin to the brush border membrane (Hoffman *et al.*,1988 and Ferre *et al.*, 1991). In contrast, studies made by Gould *et al.* (1992) and T abashnik (1994) demonstrated cross resistance to Bt toxins. The reasons for this cross resistance can be attributed to proteolysis of protoxins or decreased solubilization of crystals in the midgut of the larvae. The inheritance of resistance to Bt toxins is also studied (Chaufux *et al.*, 1997, Hama *et al.*, 1992, Ramirez *et al.*, 1995 and Bentur *et al.*, 2000b).

SEARCH FOR NEW Bt PROTEINS

The insecticidal proteins of Bt are characterized by their non-toxicity to mammals. Bt is a major source for transfer of genes into plant genomes to impart insect resistance. A few kind of insecticidal crystal protein genes *(cry1Ab and cry* 1 *Ac)* of Bt are now widely used to develop insect resistant transgenic crops. Despite an earlier view that insects would not develop resistance to Bt toxins, now it is realized that insect resistance to Bt toxins can evolve under situations of continuous exposure and or selection pressure. This has caused a great concern for continuous use of transgenic plants with a single kind of Bt toxin. Due to differential binding specificity to the insect midgut membrane vesicle, a new Bt toxin may be effective in avoiding or at least delaying the development of insect resistance by alternate or combinatorial use.

integrate the use of a wide range of crop-protection agents, e.g., synthetic, naturally-occurring and behaviour modifying chemicals together with biorational, microbial and cultural methods.

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activities well beyond that associated with Bt until recently (Feitelson *et al.,* 1992). As long as it can be demonstrated that the toxicological activity of these novel isolates does not extend to non-target (beneficial) species, i.e., that they retain the environmental and safety advantages traditionally associated with Bt, then an extension in the spectrum of activity of selected Bt isolates can be exploited commercially. Various mutant forms of insecticidal proteins with improved biological activity will be created in the future by fusing diverse domains and *in vitro* mutagenesis of genes that codes for these biological agents (Rajamohan *et al.,* 1995). Bt transgenic plants hold great promise as an important new tool in integrated pest management programs. This technology allows the crop plant to deliver its own means of protection against insect attack. The expected result is a very specific and directed biological control method that is environmentally sound and that can be expected to reduce the need for manual and chemical inputs by the grower. Commercial bioinsecticide formulations are generally ineffective in controlling cotton bollworm and European corn borer in which topical applications of the powder do not reach the inside of the plant tissue where the insects bore and feed. Such transgenic crops provide farmers a means of controlling a serious insect pest that is not easily controlled by current chemical pesticides (Kumar *et al.,* 1996). However total reliance on anyone method (or tool) for pest control will inevitably lead to rapid build-up of resistance in the pest populations. Thus strategies are needed which

Commercial Bt-based bioinsecticides, are usually the formulations of spores and crystalline inclusions that are released upon lysis of Bt during its stationary phase of growth. The products are applied at 10-50 g or about 10^{20} molecules per acre. The molecular potency of Bt toxins is higher compared to that of other pesticides, 300 times higher than synthetic pyrethroids or 80,000 times higher than organophosphates (Feitelson *et al.,* 1992). Most of the Bt product is based on subsp. *Kurstaki* strain HD-1. It is effective on over 200 crops and against more than 55 lepidopteran species, whereas subsp. *tenebrionis* and/or *san diego* are effective against coleopteran insects, such as Colorada potato beetle (Table 5).

Bt products account for 90-95% of total biopesticide market. About 75% of Bt market is in North America and Far East (Lambert and Peferoen, 1992). Efforts are needed to expand the use of Bt in other parts of the world in order that the use of hazardous chemical pesticides is reduced. There is great need for higher potency Bt toxins to have effective control over less susceptible and/or hidden pests such as *H. armigera.* and S. *litura* (Hardy and Quinlon, 1986; Perlak *et al.,* 1990; Whitlock *et al.,* 1991). Loss due to a single polyhagous pest *(H. armigera)* alone is estimated at about Rs. 1000 crores per year in India (Singh, 1996). Variation of a single amino acid in the Bt toxin can remarkably influence the level of toxicity (Udayasuriyan *et al.,* 1994; Rajamohan *et al.,* 1995). The discovery rate of new Bt toxin is more because of its genome diversity. Therefore, it is reasonable to search for new Bt toxins which are more effective against insect

Table 5 Natural and genetically modified B.t products registered for agricultural use*

BT STRAIN	COMPANY	PRODUCT	TARGET INSECTS
(a)Natural			
kurstaki HD-1	Abbot, USA	Biobit, Dipel, Foray	Lepidoptera
kurstaki HD-1	Thermo Trilogy, USA	Javelin, Steward, Thuricide	Lepidoptera
kurstaki	Abbot	Bactospeine, Futura	Lepidoptera
kurstaki	Thermo Trilogy	Able, Costar	Lepidoptera
aizawai	Abbot	Florbac, Xentari	Lepidoptera
tenebrionis	Abbot	Novodar	Coleoptera
tenebrionis	Thermal Trilogy	Trident	Coleoptera
kurstaki	BioDalia, Israel	Bio-Ti	Lepidoptera
kurstaki	Rimi, Israel	Bitayon (granular feeding baits)	Btrachedra amydraula
galleriae	Tuticorin Alkali chemicals & fertilizers, India	Spicturin	Lepidoptera
YB-1520	Huazhong Agric. University, China	Mainfeng pesticide	Lepidoptera
CT-43	Huazhong Agric. University, China	Shuangdu	Lepidoptera Coleoptera Diptera
(b) Genetically	**modified**		
aizawai recipient	Thermo Trilogy	Agree, Design	Lepidoptera
kurstaki donor		(transconjugant)	Lepidoptera
kurstaki recipient	Ecogen, USA	Condor, Cutlass (transconjugant), CRYMAX, Leptino	Lepidoptera Lepidoptera
kurstaki	Ecogen	Leptinox (recombinant)	Lepidoptera
kurstaki recipient	Ecogen	Raven (recombinant)	Lepidoptera Coleptera
d-endotoxin	Mycogen, USA	MVP	Lepidoptera
encapsulated in		MATTCH	Lepidoptera
Pseudomonas		MTRACK	Coleptera
fluorescens		CellCap ®	

* Based on Navon (2000)

pests of Indian crops. Our studies on screening of indigenous Bt strains for toxicity (Fig. 2) and molecular characterization of new Bt strains revealed difference in nature and composition of Bt toxins, between native and standard Bt strains. Hence, cloning of toxin genes from new Bt strains and their characterization are essential to expand the use of Bt and for the management of resistance development in insects.

CONCLUSION

Due to the various constraints that has emerged with the use of chemical pesticides such as, development of resistance and resurgence in insects, residual toxicity, secondary outbreak of minor insect pests and biological magnification in the ecosystem, scientists are now looking for new avenues to control or manage insect pests. One of the earliest solutions that was answered to the above problems was the use of biological agents in pest control. Among the various options that are available as on date, use of soil bacterium, the Bt has a great potential for use as a biopesticide.

In environmental impact terms, the narrow spectrum of biopesticides such as Bt has been seen as an advantage. However, this has proved to be a double- edged sword in that, in commercial terms, a broader spectrum of activity is usually required (Cannon, 1993). In practice, these demands are not mutually exclusive, and the recent explosion of research into Bt biology has yielded novel isolates (and an increasing number of *cry* genes) with