Israel Journal of Entomology

Vol. XXIII(1989) pp. 157-173

THE INSECTICIDAL CRYSTAL PROTEIN GENES OF *BACILLUS THURINGIENSIS*

ROBERT M. FAUST AND JEANR. ADAMS
United States Department of Agriculture,
Agricultural Research Center, Beltsville, Maryland 20705, USA

ABSTRACT

In a number of instances the potential to manipulate the genes of *Bacillus thuringiensis* has become a reality. Within the past ten years much has been learned about the genetics and molecular biology of the organism and advances in gene manipulation and gene transfer technology have made possible the introduction and expression of foreign genes, including the *B. thuringiensis* entomocidal protein toxin, in transgenic organisms. The basic information about the toxin genes of *B. thuringiensis* has allowed investigators to combine the most desirable attributes and create arational biocide tailor-made for specific animal, human and plant protection applications with increased effective traits and an improved system for delivery to the target pest. The toxin genes have been cloned from more than 24 strains representing nine of the known subspecies, DNA and deduced amino acid sequences of the toxin from 13 strains are known, and possible regulatory mechanisms of gene expression have been described.

INSECTICIDAL CRYSTAL PROTEIN GENES HAVE BEEN CLONED FROM *BACILLUS THURINGIENSIS* ISOLATES TOXIC TO LEPIDOPTERAN, DIPTERAN, AND COLEOPTERAN INSECTS AND EXPRESSED IN OTHER BACTERIA

A number of the genes encoding the insecticidal crystal protein of *B. thuringiensis* have been cloned into *Escherichia coli* and in some instances into B. *subtilis, B. megaterium, Pseudomonas fluorescens, Clavibacter xyli* and *Agrobacterium tumefaciens.* These gene cloning experiments in addition to curing, mating and hybridization studies have confirmed that the insecticidal crystal protein genes of the various subspecies of *B. thuringiensis* are in almost all cases plasmid-borne. The studies on localization of plasmid encoded insecticidal crystal protein genes using curing, transconjugal mating, hybridization and gene cloning methodology are summarized in Tables 1 and 2. Table 1 also includes a list of the various subsp. of B. *thuringiensis.* The techniques used include a number of gene identification strategies and host-vector cloning systems. Verification of the cloned genes has been obtained by gene product analysis, e.g., reactivity with antibodies raised against B. *thuringiensis* crystal proteins and larvicidal activity. The cloned genes have been used as probes to localize the insecticidal crystal protein gene in the other subspecies in which there are extensive homologous DNA sequences. Gene identification has revealed that there are multiple heterogeneous copies of the protoxin gene in some of the subspecies.

The first insecticidal crystal protein gene to be cloned was isolated from the lepidopteran-killing strain, *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel, which is a variant of strain HD-1 and is the active ingredient of the commercial biocide Dipel (Schnepf and Whileley, 1981). Since then protein toxin genes have been cloned from more than 24 strains representing 9 of the known subspecies (see Table 2).

Generally, the cloning studies have revealed that the crystalline inclusions lethal to lepidoptera insects consists of a single protoxin polypeptide of about 130 kilodalton (Kdal) that is subsequently

TABLE 1
Localization of plasmid encoded insecticidal crystal protein genes in *Bacillus thuringiensis*

H-serotype subspecies		Reported plasmid (Mdal) location determined by: Curing/mating	Hybridization/cloning	References[2]
1	*thuringiensis*[1]	HD-2:(54),75; HD-225:110	Berliner 1715:42, 60, 150; chr[1]; HD-2:54, 57, chr; "F":8, 50; 3A:11; HD-290:50, 60, 150	1-17
2	*finitimus*	98	98	18
3a	*alesti*	105	120-150	2-4, 10, 17
3a,3b	*kurstaki*	HD-1:44, 105, 110-115; HD-73:50; HD-74:55; HD-263:44, 60, 110	HD-1:30, 43-44, 50, 60, 100, 120, 150, chr; HD-73:50, 54; HD-172:50, 150; HD-244:50, 150; HD-279: 44, 60, 150	1-4, 10-11, 17, 19-46
4a,4b	*sotto*	–	40	10, 17, 30, 47-48
4a,4b	*dendrolimus*		chr	8
4a,4c	*kenyae*	–	–	–
5a,5b	*galleriae*	130	150-160	2, 4, 10, 17, 27, 33
5a,5c	*canadensis*	–	–	–
6	*subtoxicus*	–	56,chr	8,30, 34, 49
6	*entomocidus*	105	chr	3, 50
7	*aizawai*	–	7-29: 45; IPL7:51; IC1:110	30, 33, 51-52, 71
8a,8b	*morrisoni*	–	PG14:74, 94, 145, 150	10, 17, 53-54
8a,8b	*tenebrionis/san diego*	–	90	55-57
8a,8c	*ostriniae*	68	–	3
8b,8d	*nigeriensis*	–	–	–
9	*tolworthi*	44,110	44-45, 50-70, 150	3, 10, 17
10	*darmstadiensis*	65,70	47, 50-60	3, 10, 58
11a,11b	*toumanoffi*	–	150	10, 17
11a,11c	*kyushuensis*	–	60, chr	54, 59
12	*thompsoni*	100	–	2, 4, 17
13	*pakistani*	–	–	–
14	*israelensis*	72-75	75	2-3, 28, 43, 60-70
15	*dakota*	–	–	–
16	*indiana*	–	–	–
17	*tohokuensis*	–	–	–
18	*kumamotoensis*	–	–	–
19	*tochigiensis*	–	–	–
20a,20b	*yunnanensis*	–	–	–
20a,20c	*pondicheriensis*	–	–	–
21	*colmeri*	100-110	–	3
22	*shandogiensis*	–	–	–
23	*japonensis*	–	–	–
–	*wuhanensis*	–	chr?	10, 17
–	*fowleri*	–	–	–

[1]In some cases the ICP genes have been detected in the chromosome (chr).

[2]References: 1. Aronson et al., 1986; 2. Carlton and Gonzalez, 1984; 3. Carlton and Gonzalez, 1985a; 4. Carlton and Gonzales, 1985b; 5. Gonzalez et al., 1981; 6. Hofte et al., 1986; 7. Honigman et al., 1986; 8. Klier et al., 1986; 9. Klier and Rapoport, 1984; 10. Kronstad et al., 1983; 11. Kronstad and Whiteley, 1986; 12. Lereclus et al., 1984; 13. Mahillon et al., 1985; 14. Mahillon et al., 1987; 15. Wabiko et al., 1986; 16. Whiteley et al., 1985; 17. Whiteley and Schnepf, 1986; 18. Debro et al., 1986; 19. Adang et al., 1985; 20. Adang et al., 1987a; 21. Adang et al., 1987b; 22. Anonymous, 1987; 23. Barton et al., 1987; 24. Fischkoff et al., 1987; 25. Geiser et al., 1986; 26. Gonzalez and Carlton, 1982; 27. Gonzalez et al., 1982; 28. Gonzalez and Carlton, 1984; 29. Held et al., 1982; 30. Klier et al., 1985; 31. Kondo et al., 1987; 32. Kronstad and Whiteley, 1984; 33. Lereclus et al., 1982; 34. Lereclus et al., 1985; 35. McLinden et al., 1985; 36. Obukowicz et al., 1986a; 37. Obukowicz et al., 1986b; 38. Obukowicz et al., 1987; 39. Schnepf and Whiteley, 1981; 40. Schnepf and Whiteley, 1985; 41. Schnepf et al., 1985; 42. Shivakumar et al., 1986; 43. Thorne et al., 1986; 44. Watrud et al., 1985; 45. Whiteley et al., 1984; 46. Wong et al., 1983; 47. Shibano et al., 1985; 48. Whiteley et al., 1981; 49. Padua et al., 1984; 50. Lereclus et al., 1983; 51. Haider et al., 1987; 52. Oeda et al., 1987; 53. Earp and Ellar, 1987; 54. Sekar, 1987; 55. Hofte et al., 1987; 56. McPherson et al., 1988; 57. Sekar et al., 1987; 58. Ohba and Aizawa, 1979; 59. Negamatsu et al., 1978; 60. Angsuthanasombat et al., 1987; 61. Bourgouin et al., 1986; 62. McLean and Whiteley, 1987; 63. Sekar and Carlton, 1985; 64. Waalwijk et al., 1985; 65. Ward and Ellar, 1983; 66. Ward et al., 1984; 67. Ward and Ellar, 1986; 68. Ward et al., 1986; 69. Ward and Ellar, 1987; 70. Ward and Ellar, 1988; 71. Lereclus et al., 1989.

degraded in the insect gut to about 60–70 Kdal (Wabiko et al., 1986). Kronstad and Whiteley (1986) have grouped the lepidopteran-killing toxin protein genes of *B. thuringiensis* subsp. *kurstaki* and subspecies *thuringiensis* into three classes designated the "4.5-, 5.3- and 6.6 kb class" based on differences in length of Hind III restriction fragments containing the 5' ends of the insecticidal crystal protein genes.

It is known that the expression of the genes in *B. thuringiensis* begins at about stage II of sporulation (Bulla et al., 1980; Ward and Ellar, 1986; Wong et al., 1983) and synthesis continues until late sporulation. In *B. subtilis* expression of the lepidopteran-killing (and dipteran-killing) cloned protein toxin gene has been observed during vegetative growth (Shivakumar et al., 1986; Ward et al., 1986) and in *E. coli* the lepidopteran-killing protein gene of *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel was expressed at all stages of growth (Wong et al., 1985). In several of the reports crystalline inclusions similar or identical in appearance to the inclusions produced by the parent strain were formed (e.g., in *E. coli*, *B. subtilis*, and *B. megaterium*) (Klier et al., 1982; Sekar and Carlton, 1985; Whiteley et al., 1987). The crystalline inclusion can account for 20 to 30% of the dry weight of sporulated cells in *B. thuringiensis*.

In cloning studies of the mosquitocidal crystal protein gene from *B. thuringiensis* subsp. *israelensis*, it appears that several different toxins are present and as shown in Table 2, several investigators have reported cloning and sequencing of a 26–28 Kdal protein gene (located on a 72–75 Mdal plasmid) that is responsible for a generalized hemolytic/cytolytic activity (Sekar, 1986; Tyrell et al., 1981; Visner et al., 1986). The gene for a 130 Kdal mosquitocidal crystal protein (also located on the 72–75 Mdal plasmid) has also been cloned and it has been shown that two non-identical copies of these genes are present in the same strain (Bourgouin et al., 1986; Ward and Ellar, 1988). One of the gene copies was sequenced and was found to have little homology with the genes coding for the lepidopteran-killing protein toxin. Thorne et al. (1986) and Donovan et al. (1988a) have found that in close proximity to one copy of the 130 Kdal protein gene is a gene coding for a putative 72 Kdal protein that has low toxicity for mosquito larvae. It is not known definitely whether the 72 Kdal protein is identical to the 68 Kdal protein found in solubilized crystal preparations. Recently, Ward and Ellar (1988) cloned two homologous genes of *B. thuringiensis* subsp. *israelensis* into *E. coli* and *B. subtilis* which code for two 130 Kdal mosquitocidal proteins, doublets that are often seen in protein extracts resolved on polyacrylamide gels.

There has been confusion as to which of the proteins found in solubilized crystal preparations are lethal to mosquito larvae and hemolytic/cytolytic, although the 26–28 Kdal protein seems to be responsible for the hemolytic activity. Davidson and Yamamoto (1984), Ward et al. (1984), and Armstrong et al. (1985) have reported that the 26–28 Kdal protein was responsible for the larvidical activity. Hurley et al. (1985) showed that a 65 Kdal protein was the mosquitocidal factor, and Wu and Chang (1985) reported that the toxic factor resides within the 130 Kdal protein, and also that toxicity involves the synergistic action of at least the two of the proteins (e.g. 130- and the 26–28 Kdal protein). A synergistic action has also been proposed by Ibarra and Federici (1986). The evidence, however, seems to indicate that the 130 Kdal protein is the major mosquitocidal material (Angsuthanasombat et al., 1987; Bourgoin et al., 1986; Sekar and Carlton, 1985; Ward and Ellar, 1988).

Angsuthanasombat et al. (1987), after cloning the 130 Kdal mosquitocidal protein gene, found that a 1.9 kb fragment of the 3.8 kb insert that coded for the 130 Kdal protein coded for a protein of 65 Kdal. It has also been demonstrated that the 26–28 Kdal protein has homology with the corresponding C-terminal portion of the *B. thuringiensis* subsp. *kurstaki* HD-1 protein toxin (Thorne et al., 1986).

A few other mosquitocidal strains of *B. thuringiensis* have also been investigated and include *B. thuringiensis* subspecies *colmeri*, *kyushuensis*, *darmstadiensis* 73–E–10–2 and 73–E–10–16, *morrisoni* PG–14 and *aizawai* IC1 (Haider et al., 1986; Ohba and Aizawai, 1979; Padua et al., 1980, 1984). Additionally, a mosquitocidal protein of 65 Kdal (P–2 protein) has been identified in the

TABLE 2
Insecticidal crystal protein genes cloned from *Bacillus thuringiensis* into other bacteria

Subspecies	Strain designation	Location[1]	Host	Sequence reported	Reference[4]
		Toxic to lepidoptera			
thuringiensis	(berliner 1715)	42 Mdal, chr[2]	*E. coli*, *B. subtilis*	Yes	1-7
	HD-2	53,57 Mdal	*E. coli*	No	8-9
	3A	11 Mdal	*E. coli*	No	10
kurstaki	HD-1;Dipel	30, 43, 47, 150 Mdal, chr, or N.R.[3]	*E. coli*	Yes	11-21,57
			B. subtilis		8,22-28
			P. fluorescens	–	
			A tumefaciens.		
		N.R.	*C. xyli* subsp. *cynodontis*	–	
	HD-73;KTo	50,54 Mdal or N.R.	*E. coli*, *A tumefaciens*	Yes	8,29-32
	HD-244	50 Mdal	*E. coli*	No	33
sotto	–	35-45 Mdal	*E. coli*	Yes	24,34-36
subtoxicus	–	56 Mdal	*E. coli*	No	24,32
entomocidus	–	–	*E. coli*	–	53
aizawai	HD-133	N.R.	E. coli	No	37
	IPL7	51 Mdal	*E. coli*	Yes	38
	IC1	110 Mdal	*E. coli*	No	39
	7-29	45 Mdal	*E. coli*	Yes	24,32,51,54,55
		Toxic to diptera (mosquitocidal protein)			
israelensis	HD567-61-9	72-75 Mdal	*B. subtilis*, *B. megaterium*	No	40
	4Q2-72	72-75 Mdal	*E. coli*	Partial	41
	IPS 78	72-75 Mdal	*E. coli*, *B. subtilis*	Yes	42-43
	ONR 60A	72-75 Mdal	*E. coli*, *B. subtilis*	Yes	19,52,56

TABLE 2 (Continued)

Subspecies	Strain designation	Location[1]	Host	Sequence reported	Reference[4]
		(26-28 Kdal protein)			
	IPS 78	72-75 Mdal	*E. coli*, *B. subtilis*	Yes	44-46
	4Q2-72	72-75 Mdal	*B. subtilis*	No	47
	IPS 84(1884)	N.R.	*E. coli*	Yes	48
	N.R.	N.R.	*E. coli*	No	49
	N.R.	N.R.	Blue-green alga	–	(anonymous)
		(26-28 Kdal protein)			
morrisoni	PG14	74 Mdal	*E. coli*	Yes	50
		(mosquitocidal protein)			
aizawai	IC1	110 Mdal	*E. coli*	No	39
		Toxic to coleoptera			
tenebrionis	–	90 Mdal	*E. coli*, *P. fluorescens*	Yes	40-42
san diego	–	N.R.	*E. coli*	Yes	1,40

[1]Masses indicated are for plasmids
[2]chr = chromosomal DNA.
[3]N.R. = not reported.
[4]References: 1. Hofte et al., 1986; 2. Klier et al., 1982; 3. Klier and Rapoport, 1984; 4. Lereclus et al., 1984; 5. Mahillon et al., 1985; 6. Mahillon et al., 1987; 7. Wabiko et al., 1986; 8. Kronstad and Whiteley, 1986; 9. Whiteley et al., 1985; 10. Honigman et al., 1986; 11. Adang et al., 1987b; 12. Geiser et al., 1986; 13. Held et al., 1982; 14. Kondo et al., 1987; 15. Schnepf and Whiteley, 1981; 16. Schnepf and Whiteley, 1985; 17. Schnepf et al., 1985; 18. Shivakumar et al., 1986; 19. Thorne et al., 1986; 20. Whiteley et al., 1984; 21. Wong et al., 1983; 22. Barton et al., 1987; 23. Fischoff et al., 1987; 24. Klier et al., 1985; 25. Obukowicz et al., 1986a; 26. Obukowicz et al., 1987; 27. Watrud et al., 1985; 28. Anonymous, 1987; 29. Adang et al., 1985; 30. Adang et al., 1987a; 31. Kronstad and Whiteley, 1984; 32. Lereclus et al., 1985; 33. McLinden et al., 1985; 34. Kronstad et al., 1983; 35. Shibano et al., 1985; 36. Whiteley et al., 1981; 37. Chak and Ellar, 1987; 38. Oeda et al., 1987; 39. Haider et al., 1987; 40. Sekar and Carlton, 1985; 41. Angsuthanasombat et al., 1987; 42. Ward and Ellar, 1987; 43. Ward and Elar, 1988; 44. Ward et al., 1984; 45. Ward and Ellar, 1986; 46. Ward et al., 1986; Bourgouin et al., 1986; 48. Waalwijk et al., 1985; 49. McLean and Whiteley, 1987; 50. Earp and Ellar, 1987; 51. Lecadet et al., 1988; 52. Sen et al., 1988; 53. Visser et al., 1988; 54. Sanchis et al., 1988; 55. Sanchis et al., 1989; 56. Donovan et al., 1988a; 57. Donovan et al., 1988b

crystal inclusions of *B. thuringiensis* subspecies *kurstaki*, *thuringiensis*, *kenyae*, and *tolworthi* (Hall et al., 1977; Yamamoto and McLaughlin, 1981; Yamamoto, 1983). In one report only the toxin proteins of *morrisoni* PG–14 have been demonstrated to exhibit immunological relatedness to *B. thuringiensis* subsp. *israelensis* (Ellar et al., 1985). Sekar (1987) using a cloned gene encoding the mosquitocidal 130 Kdal protein of *B. thuringiensis* subsp. *israelensis* as a probe, found that the crystal toxin genes of *B. thuringiensis* subsp. *kyushuensis* and *morrisoni* PG–14 shared homology to the crystal toxin gene of *B. thuringiensis* subsp. *israelensis*, while the mosquitocidal genes of *B. thuringiensis* subspecies *colmeri* and *kurstaki* did not exhibit any homology. Hybridization studies using the clones *B. thuringiensis* subsp. *israelensis* 26–28 Kdal crystal protein gene as a probe against

morrisoni PG–14 demonstrated that the coding region of the subspecies *morrisoni* PG–14 gene was highly homologous to the subspecies *israelensis* 26–28 Kdal toxin gene, and the gene product expressed in *E. coli* cross-reacted with anti-*israelensis* 26–28 Kdal toxin serum (Earp and Ellar, 1987).

Cloning and expression of the coleopteran-killing crystal protein genes (subspecies *tenebrionis/san diego*) have revealed that, unlike the 130 Kdal protein toxin genes of the lepidopteran-killing strains of *B. thuringiensis* subsp. *kurstaki*, the coleopteran-killing insecticidal crystals (which are "chicklet" or wafer shaped as opposed to bipyramidal-shaped) harbor an insecticidal 64–68 Kdal protein (Herrnstadt et al., 1987; Sekar et al., 1987). Only limited regional homology with the lepidopteran-killing and dipteran-killing toxins have been found (Sekar et al., 1987). Working with *B. thuringiensis* subsp. *san diego*, Herrnstadt et al. (1987) have found that there are short stretches of exact homology existing throughout the amino acid sequences of the lepidopteran, dipteran and coleopteran protoxins. Further, based on the deduced amino acid sequence, they calculated that the coleopteran-killing toxin is synthesized as a 73 Kdal precursor that is processed to about 65 Kdal prior to incorporation into the crystalline inclusion (Herrnstadt et al., 1986). It is also known that neither the C-terminal half of the 130 Kdal lepidopteran-killing protoxin nor the hemolytic/cytolytic 26–28 Kdal protein of *B. thuringiensis* subsp. *israelensis* have homology with *B. thuringiensis* subsp. *san diego* (Herrnstadt et al., 1987; Waalwijk et al., 1985).

B. thuringiensis subsp. *tenebrionis* insecticidal protein crystals consist of a major protein about 66 Kdal in mass. It appears to be also processed from a predicted 72 Kdal protein based on analysis of the cloned gene. Also, the toxin gene appears to be naturally truncated because it lacks the C-terminal fragment present in most other entomocidal protein toxins of *B. thuringiensis* (Hofte et al., 1987; McPherson et al., 1988; Sekar et al., 1987). There seems to be virtually no difference between *B. thuringiensis* subspp. *tenebrionis* and *san diego*.

Chromosomal protoxin gene sequences apparently are present in some subspecies of *B. thuringiensis* (see Table 2). Probe analysis using the cloned *B. thuringiensis* subsp. *kurstaki* HD-1 gene seem to indicate a chromosomal location in *B. thuringiensis* subsp. *dendrolimus* (Klier et al., 1982), subspecies *wuhanensis* (Kronstad et al., 1983), and subspecies *finitimus* (harbors a gene encoding a 135 Kdal protein unrelated to the other protein toxin genes of *B. thuringiensis* (Debro et al., 1986). There is also a cryptic chromosomal gene in *B. thuringiensis* subsp. *thuringiensis* (Berliner 1715) that possesses sequence homology to an active plasmid gene as well as to the *B. thuringiensis* subsp. *kurstaki* HD-1 toxin protein gene (Klier et al. 1982). Held et al. (1982) cloned a fragment of the chromosomal DNA gene of *B. thuringiensis* subsp. *kurstaki* HD-1 into a Charon phage which was toxic; the gene seems to be cryptic since plasmic cured derivatives were nontoxic.

Cloning and expression of the entomocidal protein toxin genes into *P. fluorescens*, *C. xyli* and *A. tumefaciens* (Adang et al., 1987a; Anonymous, 1987; Fischkoff et al., 1987; McPherson et al., 1988; Obukowicz, 1986a,b, 1987; Watrud et al., 1985) have been aligned with research efforts at increasing the efficacy and persistance of the toxin for field use. For further details of the protein toxin cloning investigations the reader is referred to the numerous cited papers and to the discussions in the reviews of Carlton and Gonzalez (1985b), Whiteley and Schnepf (1986), Whiteley et al. (1982), Dean (1984), Aronson et al. 1986), and Andrews et al. (1987).

NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCES AND PROTOXIN SUBDOMAINS ARE KNOWN AND HAVE BEEN COMPARED BETWEEN SUBSPECIES OF LEPIDOPTERAN- DIPTERAN- AND COLEOPTERAN-KILLING SUBSPECIES OF *BACILLUS THURINGIENSIS*

Partial or complete DNA and deduced amino acid sequences have been reported for the insecticidal crystal protein genes from 13 strains and isolates of *B. thuringiensis* subspecies *thuringiensis*, *kurstaki*, *sotto*, *aizawai*, *israelensis*, *morrisoni*, *entomocidus tenebrionis*, and *san diego* (see Table 2). In several reports there are indications that only a portion of the protoxin gene is required to code

for insecticidal activity (Adang et al., 1985; Schnepf and Whiteley, 1985; Wabiko et al., 1985, 1986; Visser et al., 1988; Sanchis et al., 1989; Donovan et al., 1988a,b; Sen et al., 1988; Lecadet et al., 1988; Sanchis et al., 1988). For example, sequential deletion subclones of the protoxin gene of a lepidopteran-killing strain (berliner 1715) of *B. thuringiensis* subsp. *thuringiensis* were constructed and analyzed by immunoassay and toxicity determinations (Wabiko et al., 1986). The results revealed that only the N-terminal segment, constituting 53% of the protoxin and corresponding to a 68 Kdal protein, was required for insecticidal activity comparable to that of the native protoxin. When the truncated protoxin lost antigenicity, toxicity was also lost. This N-terminal segment appears to be composed of three subdomains: a relatively hydrophobic domain (residues 1 to 279), a variable domain (residues 280 to 460), and the toxic boundary domain (residues 461 to 614). The entire 3,778 base pair (bp) DNA sequence of the insecticidal protoxin gene coding sequence and flanking regions encoded a protoxin of 1,155 amino acids and had a calculated mass of 130,615 daltons.

Wabiko et al. (1986) found than an amino-terminal 612 amino acid protein was toxic but a 603 amino acid protein was not toxic in bioassays. Ninety percent of the amino acid residues were homologous to the protoxins of *B. thuringiensis* subspecies *kurstaki* HD-1-Dipel and *sotto*. The differences occurred both in the amino-terminal half (toxic portion) and in the C-terminal half and were clustered in several regions.

Wabiko et al. (1986) from a comparative analyses of *B. thuringiensis* subspecies *thuringiensis* (berliner 1715) *kurstaki* HD-1 and HD-73, and *sotto* proposed a model whereby the protoxin molecule is divided into distinct structural and functional domains that may be respsonsible for the differences in specific toxicities and spectra of insect host range among the subspecies. Amino acid residues 36–38 in the relatively hydrophobic domain were entirely conserved among the subspecies [it has been suggested that this region may be involved in interactions with the midgut membrane of susceptible insects (Schnepf et al., 1985)]. Wabiko et al. (1986) contend it is likely the amino acid differences in residues 280–460 that contribute to the variability in specific toxicity among the protoxins and, possibly, to the spectra of insect host ranges. The toxic boundary domain was extremely conserved among *B. thuringiensis* subspecies *thuringiensis* (berliner 1715), *kurstaki* HD-1-Dipel, and *sotto* and was predicted to be entirely alpha-helical at the carboxyl end. This domain may be responsible for maintaining the structural and functional integrity of the insecticidal toxin because of the loss of this boundary resulted in the loss of toxicity as well as immunoreactivity.

One exception to the structural patterns characterized for the three subspecies and noted by Wabiko et al. (1986) was in the comparison of the primary DNA sequence for subspecies *kurstaki* HD-73. The strain is more toxic to *Heliothis zea* (cotton budworm) than subspecies *kurstaki* HD-1-Dipel, whereas subspecies *kurstaki* HD-1-Dipel is more toxic to *Trichoplusia ni* (cabbage looper) (Dulmage, 1981). The DNA of *B. thuringiensis* subsp. *kurstaki* HD-73 is 85% homologous to that of subspecies *thuringiensis* (berliner 1715) and subspecies *kurstaki* HD-1-Dipel. *B. thuringiensis* subsp. *kurstaki* HD-73 exhibited little conservation in the boundary domain when compared to the other two strains (berliner 1715, HD-1-Dipel) and the functional integrity of the toxic portion of the molecule was less than the protoxin as evidenced by its depressed toxicity (Adang et al., 1985). This suggested that the C-terminal segment of the protoxin is necessary for optimum toxic activity in *B. thuringiensis* subsp. *kurstaki* HD-73. There was a 26 consecutive amino acid insert in subspecies *kurstaki* HD-1-Dipel, *sotto*, and *kurstaki* HD-73 that was absent in subspecies *thuringiensis* (berliner 1715) (Wabiko et al., 1986). When comparing *B. thuringiensis* subsp. *kurstaki* HD-1-Dipel, the protoxin gene showed 99% homology to subspecies *sotto*, 91% to subspecies *thuringiensis* (berliner 1715) and 85% to subspecies *kurstaki* HD-73 (Andrews et al., 1987). The differences that existed among these genes were manifested in base changes and gaps that were clustered in several areas along the genes rather than being randomly dispersed. Most of the gaps occurred within the C-terminal halves.

Overall, the N-terminal halves of the protoxins are principally hydrophobic, whereas, the

carboxy-terminal halves are primarily hydrophilic and the majority of cysteine and lysine residues and a few of the methionine residues are harbored in the C-terminal half of the protein. Structural and functional analysis of the sequence of the cloned protein toxin gene from *B. thuringiensis* subsp. *thuringiensis* (berliner 1715) by Hofte et al. (1986) and comparison of the deduced amino acid sequence with subspecies *kurstaki* HD-1-Dipel, *kurstaki* HD-73 and *sotto* led these investigators to propose that homologous recombination between the different toxin genes had occurred during evolution. Work by Geiser et al. (1986) on the hypervariable region in the genes coding for the toxin of *kurhd* 1 gene of *B. thuringiensis* subsp. *kurstaki* HD-1, and the work of Thorne et al. (1986) on the "5.3 kb" toxin gene of *kurstaki* HD-1, strengthen the possibility that extensive recombination activities occur between the different genes.

Recently, Oeda et al. (1987) found that the nucleotide sequence of *B. thuringiensis* subsp. *aizawai* IPL7 toxin gene, when compared with the subspecies *thuringiensis* (berliner 1715) toxin gene, only had 8 nucleotide changes (99.8% homologous) in the coding regions and therefore are more closely related to each other than to subspecies *kurstaki* (90.8% homologous). The nucleotide sequence upstream from the initiation codon of the *B. thuringiensis* subspecies *aizawai* IPL7 gene (nucleotides –1 to –156) contained a putative ribosome-binding site which was completely identical to the subspecies *thuringiensis* (berliner 1715) gene. A large hairpin structure (transcription terminator) was also conserved in the 3'-flanking region of both genes.

Comparison of the toxin gene sequences of *B. thuringiensis* subsp. *kurstaki* HD-1 with sequences for the two contiguous genes of subsp. *israelensis* by Thorne et al. (1986) revealed two regions of strong homology, although the DNA homologies were not located in the structural genes. In *B. thuringiensis* subsp. *kurstaki* the gene homology lay approximately 70 to 100 bases upstream from the start point for translation while the position of the homology in the putative subspecies *israelensis* control region was approximately 80 bp upstream from the probable point of translation. There was limited homology between the structural gene sequences of the two subspecies at the nucleotide level, but there were similarities in the deduced amino acid sequences in three regions. The gene product of the *B. thuringiensis* subsp. *israelensis* cloned gene in *B. subtilis* had a molecular size of about 58 Kdal that is likely the result of processing from a 72 Kdal mosquitocidal protein (when cloned in *E. coli*).

Thorne et al. (1986) found no homology between the deduced amino acid sequence of the mosquitocidal toxin and the sequence of the 26–28 Kdal toxin and the restriction map bears no resemblance to the 26–28 Kdal protein gene clone described by Waalwijk et al. (1985). As mentioned, Thorne et al. (1986) found DNA sequence homology between the region immediately upstream from the open reading frame of the cloned *B. thuringiensis* subsp. *israelensis* gene and the promoter recognition sequences for the subspecies *kurstaki* HD-1 toxin gene (1986). The homology was described as consistent with the common pattern of regulation of the two toxin genes. It appears that there is some structure/functional conservation between the lepidopteran- and-dipteran killing *B. thuringiensis*.

Angsuthanasombat et al. (1987) partially sequenced the 130 Kdal mosquitocidal protein gene of *B. thuringiensis* subsp. *israelensis* (4Q2–72) and found no significant homology with the toxin genes of subspecies *kurstaki* HD-1-Dipel, *thuringiensis* (berliner 1715), *sotto*, and the 26–28 Kdal *israelensis* gene. However, some homologies in both regulatory and coding sequences were observed with the 72 Kdal toxin gene sequenced by Thorne et al. (1986). The Shrine-Dalgarno region of nucleotides (sequences used by ribosomes to initiate protein synthesis) was identical. The coding regions from nucleotide bases 163–177 and 196–252 coded for 20 identical amino acids suggesting that this region is highly conserved. Also, there were no significant homologies in other regions, and Thorne et al. (1986) suggested that the 72 Kdal protein gene is not part of the 130 Kdal protein gene reported by Angsuthanasmobat et al. (1987). Ward and Ellar (1987) also reported the nucleotide sequence of a strain of *B. thuringiensis* subsp. *israelensis* (IPS78) encoding a 130 Kdal protein toxin and found considerable differences in the N-terminal region from the sequence reported by

Angsuthanasombat et al. (1987), although it was almost identical with the corresponding C-terminal portions.

The nucleotide and deduced amino acid sequence of the 26–28 Kdal protein shows it to be highly hydrophobic which is consistent with the postulated cytolytic mechanism of action (Ward and Ellar, 1986). Earp and Ellar (1987) have also determined the nucleotide sequence of the gene encoding a 27 Kdal crystal protein from *B. thuringiensis* subsp. *morrisoni* PG14. The nucleotide sequence of the gene was highly homologous to the *B. thuringiensis* subsp. *israelensis* HD-1-Dipel protein; gene and the gene product (expressed in *E. coli*) showed cross-reaction with *anti-israelensis* 26–28 Kdal toxin serum as mentioned previously. A single nucleotide base-pair difference (G replacing C) that results in an alanine residue in *B. thuringiensis* subsp. *morrisoni* PG14 rather than the proline found in subspecies *israelensis* was the basic difference. There were no "silent" substitutions, and downstream regions, including the inverted repeat sequences present, were conserved.

Nucleotide sequences for the coleopteran-killing subspecies of *B. thuringiensis* (*tenebrionis* and *san diego*) also have been reported. In *B. thuringiensis* subsp. *tenebrionis* a 1,932 nucleotide bp open reading frame with a coding capacity of 73,119 dal was sequenced (Sekar et al., 1987). Transcription of the crystal protein toxin is initiated 130 base pairs upstream from the translational start site ATG codon during both early and midsporulation states and is similar to reports for the 26–28 Kdal gene of *B. thuringiensis* subsp. *israelensis* (Waalwijk et al., 1985; Ward and Ellar, 1986; Ward et al., 1986). However, Sekar et al. (1987) noted that it contrasts with the results of Wong et al. (1983) who found two different initiation sites in *B. thuringiensis* subsp. *kurstaki* HD-1 during early and midsporulation stages, but only a single site in the exponential and stationary stages of *E. coli* containing the toxin gene. Additionally, some homology (5bp out of 8 for the –35 region and 4 out of 8 for the –10 region) exists for the PBl promoter of *B. thuringiensis* subsp. *israelensis* and the BT1 promoter of subspecies *kurstaki*. Sekar et al. (1987) also reported that the *B. thuringiensis* subsp. *tenebrionis* protein crystal gene has an A+T rich region upstream from the site of RNA initiation and that similar A+T rich regions are located prior to the *B. thuringiensis* subspecies *israelensis* and *kurstaki* toxin genes. Transcription starts approximately 170 bp upstream from the initiation ATG and a consensus *E. coli* –10 promoter region (TATAAT) is located near this start site. *B. thuringiensis* subsp. *tenebrionis* contains only a single crystal protein gene in contrast to subspecies *kurstaki* HD-1(80). An inverted repeat beyond the 3′ end of the gene was identified.

Using computer analysis Sekar et al. (1987) found a 50.2% DNA homology between the *B. thuringiensis* subsp. *tenebrionis* gene and the 5′ 1,827 nucleotide of the 3,537 nucleotide of subspecies *kurstaki* HD-73 gene. When the amino acid sequences of the two protein regions were aligned, all matches occurred between the complete *B. thuringiensis* subsp. *tenebrionis* protein and the N-terminal 609 amino acids of the subspecies *kurstaki* HD-73 protein. Sekar et al. (1987) point out that it is this region of the *B. thuringiensis* subsp. *kurstaki* HD-73 protein that contains the active 60 Kdal toxin. There is 22.7% homology in this region and from amino acid 106 to 382 (33.0% homology) and from amino acid 451 to 644 (22.4% homology), the *B. thuringiensis* subsp. *tenebrionis* protein is similar to the corresponding residues in the protein toxin region of the subspecies *kurstaki* HD-73 protein gene. Sekar et al. (1987) additionally noted that after a 33-amino acid gap is inserted at the start of the *B. thuringiensis* subsp,. *kurstaki* HD-73 sequence, residues 67–94 of the subspecies *tenebrionis* protein align with residues 35–70 of the subsp. *kurstaki* HD-73 protein. These regions have similar hydropathy with a possible functional role predicted for this region of the *B. thuringiensis* subsp. *kurstaki* HD-1 toxin protein.

McPherson et al. (1988) and Hofte et al. (1987) have also reported sequences of the *B. thuringiensis* subsp. *tenebrionis* protein toxin gene. The cloned gene reported by the latter investigators encodes a protein with mass of 72 Kdal. The protein shows limited regional amino acid homology with the lepidopteran-killing protein toxins. The gene cloned into *E. coli* or *P. fluorescens* by McPherson et al. (1988) contained two functional translation initiation codons in the same reading frame which allowed for the production of both the full length protein toxin and an N-terminal

truncated form (the gene was engineered to produce both the proteins separately). A region of DNA approximately 70 bp upstream of the translational initiation site, which is highly conserved among lepidopteran-killing protein genes and found in a mosquitocidal active gene (Thorne et al., 1986), is absent in the *B. thuringiensis* subsp. *tenebrionis* gene. McPherson et al. (1988) noted that the amino acid homology exhibited between subspecies *tenebrionis* and *kurstaki* HD-1 resembles similar comparisons between the mosquitocidal protein gene and the subspecies *kurstaki* HD-1 gene (Thorne et al., 1986). They also noted that the homology between *B. thuringiensis* subsp. *tenebrionis* and subspecies *kurstaki* HD-1 is limited to the N-terminal half of the subspecies *kurstaki* HD-1 protein (Fischkoff et al., 1987).

Finally, the nucleotide and deduced amino acid sequences of *B. thuringiensis* subsp. *san diego* also showed limited regional homology with both lepidopteran- and dipteran-killing protein toxins (Herrnstadt et al., 1987). Amino acid sequence alignment revealed some homologies existing in the protein toxins of *B. thuringiensis* subspecies *san diego, israelensis* and *kurstaki* HD-1. Herrnstadt et al. (1987) noted that there were 220 identities out of 582 possible amino acid matches between residues of *B. thuringiensis* subsp. *san diego* and subspecies *kurstaki* HD-1, and 207 out of 588 with subspecies *israelensis*, supporting the contention that the sequences are distantly related. One region of short stretches of exact homology had 17 of 20 exact amino acid matches for *B. thuringiensis* subsp. *san diego* and subspecies *kurstaki* HD-1. One sequence within the latter stretch started at amino acid 281, 291, or 236 respectively for *san diego, israelensis* or *kurstaki*, and was an exact match for all published sequences of the insecticidal crystal protein, with the exception of the 26–28 Kdal hemolytic/cytolytic toxin of subspecies *israelensis*.

As with *B. thuringiensis* subsp. *tenebrionis*, neither the C-terminal half of the 130 Kdal lepidopteran-killing protein nor the cytolytic 26–28 Kdal subsp. *israelensis* protein (Waalwijk et al., 1985) had homology with the subspecies *san diego* toxin. Conservation of N-terminal hydrophilic and hydrophobic regions were also noted (Herrnstadt et al., 1987) with a region spanning 200 residues in the N-terminal half of the *B. thuringiensis* subsp. *san diego* protein toxin matching comparable regions in the subspecies *kurstaki* HD-1 130 Kdal protein and the cloned subspecies *israelensis* 72-Kdal protein (Thorne et al., 1986). This conservation of certain nucleotides among the different subspecies of *B. thuringiensis* suggests further that there was likely a common origin for the lepidopteran, dipteran, and coleopteran protein toxins. Herrnstadt et al. (1987) postulate, therefore, that this region is involved with the toxic mechanism rather than with specificity. We can detect no differences between the deduced amino acid sequences of the subspecies *san diego* and subspecies *tenebrionis* protein toxins reported by the investigations of Sekar et al. (1987) and Herrnstadt et al. (1987).

In spite of the accumulated data, the protein features that lead to the different insecticidal spectra of action of the crystal protein toxins are not clear. Indeed, Widner and Whiteley (1989) have recently demonstrated that two highly related insecticidal crystal proteins of *B. thuringiensis* subsp. *kurstaki* possess different host range specificities!

POSSIBLE REGULATORY MECHANISMS OF INSECTICIDAL CRYSTAL PROTEIN GENE EXPRESSION HAVE BEEN DESCRIBED

Normal synthesis of the insecticidal crystal protoxin in *B. thuringiensis* is apparently a sporulation-specific event because the time of appearance of the parasporal crystal and spore overlaps and they are formed in close proximity to each other. It is also known that the insecticidal crystal protein is related to the spore-coat protein since relatively large amounts of the protein toxin are present in the spore coat (Faust and Bulla, 1982). Although acrystalliferous strains do form spores, the spores are not as heat or solvent resistant as crystalliferous isolates (Aronson et al., 1982; Bechtel and Bulla, 1976). Also, in other spore forming bacteria the synthesis of spore-coat proteins is a sporulation-

specific event. Parasporal crystal toxin antigen appears in the cells during sporulation stages III to IV and cells in the logarithmic phase or in the early stages of sporulation do not contain the crystal toxin antigen (Aronson et al., 1982; Klier et al., 1983). Crystal toxin antigens begin to appear in the vegetative cells at approximately 4 hr after the onset of sporulation and corresponds closely with the appearance of the crystal inclusion (Klier et al., 1983).

Presumably, synthesis of the insecticidal crystal protein is controlled at the level of transcription; activation of transcription and translation seems to be closely coupled to protoxin synthesis and accumulation. Studies with *B. thuringiensis* subsp. *kurstaki* have demonstrated that crystal toxin-specific mRNA is present only in sporulating cells and only during periods when *de novo* crystal protein synthesis is in process (Andrews et al., 1982). Similar findings were obtained with *B. thuringiensis* subsp. *israelensis*. The vegetative mRNA from *B. thuringiensis* has a half-life of about 2 minutes, whereas the protein toxin-specific mRNA has a half-life of 11 to 13 minutes (Andrews et al., 1982). Wong et al. (1983) used S1 nuclease mapping to show that one peak of RNA hydridizing to a cloned crystal gene occurred between 1.5 and 4 hours after the onset of sporulation and that another burst of crystal toxin-specific mRNA could be detected at about T_6 (Jarrett, 1985). Wong et al. (1983) have also demonstrated that the cloned toxin gene from subspecies *kurstaki* HD-1 contains –10 and –35 regions that are highly homologous to a sporulation-specific promoter of *B. subtilis*. Klier et al. (1983) have shown that these same regions of the cloned crystal toxin gene are recognized by a sporulation-specific RNA polymerase. Two forms of RNA polymerase have been reported in sporulating cells of *B. thuringiensis*: form I contains the apparently modified RNA polymerase, whereas form II does not; form II of the enzyme is probably responsible for the synthesis of the stable mRNA fraction, 40% of which is likely crystal toxin-specific mRNA (Klier and Lecadet, 1976; Klier et al., 1983).

Regulation probably involves a number of factors such as gene dosage, unique promoter sequences, sigma submits, etc. (Aronson et al., 1986). Specific sigma factors are responsible for directing RNA polymerase to specific promoters. The expression of the cloned toxin genes in *E. coli* is low with only about 1% production of what is produced in parental isolates (20 to 30% of the total protein of the sporangium). In sporulating *B. subtilis* cells the clone of the *B. thuringiensis* subsp. *thuringiensis* (berliner 1715) toxin gene produced only about 10% of the parental isolate. Plasmid curing and transfer experiments have implicated several *B. thuringiensis* subsp. *kurstaki* HD-1 cryptic plasmids in regulation. Also, a *B. thuringiensis* subsp. *kurstaki* strain HD-1-9 produced crystal protein toxin only at 25°C, although it could sporulate well at both 25 and 32°C (Minnich and Aronson, 1984). Similar results were obtained with a *B. cereus* transcipient containing a 98-Mdal plasmid harboring the crystal gene of *B. thuringiensis* subsp. *finitimus*. Therefore, even though the nucleotide sequences of the regulatory regions may be identical, the different expression in *B. cereus* transcipients indicates that other factors (e.g., plasmid encoded determinants) may be involved (Aronson et al., 1986). Conditional synthesis of crystal toxin in certain strains of subspecies *kurstaki* HD-1 have implied that regulation of crystal toxin synthesis may involve physiological factors not required for sporulation (Aronson et al., 1986). There is probably regulatory signals specific for crystal toxin synthesis.

A comparison between the promoter of the plasmid encoded toxin protein gene (101) and the promoter of the spo VG sporulation gene (Moran et al., 1981), which shows considerable homology, has been discussed by Dean (1984) in his review. Dean (1984) noted that like the spo VG gene there seems to be two separate transcriptional start sites from the same regulatory area which may account for the "copious" production of crystal toxin protein. The abundant synthesis of the crystal protein has been reported to be due in part to mRNA stability by Petit-Glatron and Rapoport (1975). Deletion of the terminator region of the crystal protein gene markedly decreases the amount of crystal protein antigen expressed in *E. coli*, while fusion of the terminator to the 3′ end of a gene with such a deletion increases the expression (Whiteley et al., 1987). Wong and Chang (1985) obtained similar results by fusing a crystal protein gene terminator to the 3′ end of several genes cloned into *E. coli* and *B.*

subtilis. Whiteley et al. (1987) postulated that increased expression could be the result of an increase in mRNA half-life.

Wong et al. (1983) demonstrated that the crystal protein gene is transcribed from two temporally regulated, overlapping promoters (BtI and BtII). Transcription is initiated in *E. coli* from a start site located between the two *B. thuringiensis* start sites. Probably two different sigma factors are involved in the transcription (Whiteley et al., 1987). Whiteley et al. (1987) have recently isolated an RNA polymerase from sporulating *B. thuringiensis* cells which contains a new sigma subunit of about 35 Kdal. The polymerase can initiate RNA synthesis in vitro from the BtI start site (Whiteley et al., 1987).

In summary the regulatory mechanism and expression of the insecticidal crystal protein gene probably is dependent on transcription by one or more specific RNA polymerases. Expression also may be regulated or influenced by physiological factors, sporulation-regulated factors, plasmid determinants, efficient ribosome binding sites, influence of the gene terminator, and/or gene dosage.

REFERENCES

Adang, M.J., Stauer, M.J., Rochebau, T.A., Leighton, J., Basker R.F. and Thompson, D.V. 1985. Characterized full-length and truncated plasmid clones of the crystal protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-73 and their toxicity to *Manduca sexta*. *Gene* 36:289–300.

Adang, M.J., Firoozabady, E., Klein, J., DeBoer, D., Sekar, V., Kemp, J.D., Murray, E., Rochelcau, T.A., Rashka, K., Staffeld, G., Stock, C., Sutton, D. and Merlo, D.J. 1987a. Expression of a *Bacillus thuringiensis* insecticidal crystal protein gene in tobacco plants. In: Molecular Strategies for Crop Protection, Edit. C.J. Arntzen and C. Ryan, pp. 345–353, Alan R. Liss, Inc. NY.

Adang, M.J., Idler, K.F. and Rocheleau, T.A. 1987b. Structural and antigenic relationships among three insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki*. In: Biotechnology in Invertebrate Pathology and Cell Culture, Edit. K. Maramorsch, Academic Press, Inc., NY.

Andrews, R.E., Kavda, K. and Bulla, L.A., Jr. 1982. In vitro and in vivo synthesis of the parasporal crystal of *B. thuringiensis*. In; Gene Regulation in *Bacilli*, Edit. A.T. Ganesan, S. Chang and J. Hoch, p. 121, Academic Press, NY.

Andrews, R.E., Faust, R.M., Wabiko, H., Raymond, K.C. and Bulla, L.A., Jr. 1987. The biotechnology of *Bacillus thuringiensis*. In: Critical Reviews in Biotechnology, Edit. G. Stewart and I. Russell, Vol. 6, Issue 2, pp. 163–232, CRC Press, Inc., Fl.

Angsuthanasombat, C., Chungjatupornchai, W., Kertbundit, S., Luxananil, P., Settasatian, C., Wilairat, P. and Panyim, S. 1987. Cloning and expression of 130-kd mosquito-larvicidal delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis* in *Escherichia coli*. *Mol. Gen. Genet.* 208:384–389.

Anonymous. 1987. EPA Application for an Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only (Transmittal document), Crop Genetics International Corporation, Hanova, MD.

Armstrong, J.L., Rohrmann, G.F. and Beaudreau, G.S. 1985. Delta endotoxin of *Bacillus thuringiensis* subsp. *israelensis*. *J. Bacteriol.* 161:39–46.

Aronson, A.I., Tyell, D.J., Fitz-James, P.C. and Bulla, L.A., Jr. 1982. Relationship of the synthesis of spore coat proteins and the parasporal crystal protein in *Bacillus thuringiensis*. *J. Bacteriol.* 151:399–410.

Aronson, A.I., Beckman, W. and Dunn, P. 1986. *Bacillus thuringiensis* and related insect pathogens. *Microbiol. Rev.* 50:1–24.

Barton, K.A., Whiteley, H.R. and Yang, N.S. 1987. *Bacillus thuringiensis* delta-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiol.* 885:1103–1109.

Bechtel, D.B. and Bulla, L.A., Jr. 1976. Electron microscope study of sporulation and parasporal formation in *Bacillus thuringiensis*. *J. Bacteriol.* 127:1472–1481.

Bourgouin, C., Klier, A. and Rapoport, G. 1986. Characterization of the genes encoding the haemolytic toxin and the mosquitocidal delta-endotoxin of *Bacillus thuringiensis* var. *israelensis*. *Mol. Gen. Genet.* 205:390–397.

Bulla, L.A., Bechtel, D.B., Kramer, K.J., Shethna, Y.I., Aronson, A.I. and Fitz-James, P.C. 1980.

Ultrastructure, physiology and biochemistry of *Bacillus thuringiensis*. *CRC Critical Reviews in Microbiology* 8:147–204.

Carlton, B.C. and Gonzalez, J.M., Jr. 1984. Plasmid associated delta endotoxin production in *Bacillus thuringiensis*. In: Genetics and Biotechnology of Bacilli, Edit. A.T. Ganeson and J.A. Hoch, pp. 387–400, Academic Press, Inc., NY.

Carlton, B.C. and Gonzalez, J.M., Jr. 1985a. Plasmids and delta-endotoxin production in different subspecies of *Bacillus thuringiensis*. In: Molecular Biology of Microbial Differentiation, Edit. J.A. Hoch and P. Setlow, pp. 246–252, American Society of Microbiology, Washington, D.C.

Carlton, B.C. and Gonzalez, J.M., Jr. 1985b. The genetics and molecular biology of *Bacillus thuringiensis*. In: The Molecular Biology of the Bacilli, Vol. 2, Edit. D. Dubrau, pp. 211–249, Academic Press, NY.

Chak, K-F. and Ellar, D.J. 1987. Cloning and expression in *Escherichia coli* of an insecticidal crystal protein gene from *Bacillus thuringiensis* var. *aizawai* HD-133. *J. Gen. Microbiol.* 133:2921–2931.

Davidson, E.W. and Yamamoto, T. 1984. Isolation and assay of the toxic component from the crystals of *Bacillus thuringiensis* var. *israelensis*. *Curr. Microbiol.* 11:171–174.

Dean, D.H. 1984. Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*: Basic principles and prospects for genetic engineering. *Biotechnol. Gen. Engineer. Rev.* 2:341–363.

Debro, L., Fitz-James, P.C. and Aronson, A. 1986. Two different parasporal inclusions are produced by *Bacillus thuringiensis* subsp. *finitimus*. *J. Bacteriol.* 165:258–268.

Donovan, W.P., Dankocsik, C. and Gilbert, W.P. 1988a. Molecular characterization of a gene encoding a 72-kilodalton mosquito-toxic crystal protein from *Bacillus thuringiensis* subsp. *israelensis*. *J. Bacteriol.* 170:4732–4738.

Donovan, W.P., Dankocsik, C.C., Gilbert, N.P., Gawron-Burke, M.C., Groat, R.G. and Carlton, B.C. 1988b. Amino acid sequence and entomocidal activity of the P2 crystal protein. An insect toxin from *Bacillus thuringiensis* var. *kurstaki*. *J. Biol. Chem.* 263:561–567.

Dulmage, H.T. 1981. Production of bacteria for biological control of insects. In: Biological Control in Crop Production, Vol. 5, Edit. G.C. Papavizas, pp. 129–141, Allanheld, Osmun Publishers, Granada, London.

Earp, D.J. and Ellar, D.J. 1987. *Bacillus thuringiensis* var. *morrisoni* strain PG14: nucleotide sequence of a gene encoding a 27kDA crystal protein. *Nucleic Acids Res.* 15:3619.

Ellar, P.J., Thomas, W.E., Knowles, B.H., Ward, S., Todd, J., Drobniewski, F., Lewis, J., Sawyer, T., Last, D. and Nichols, C. 1985. Biochemistry, genetics, and mode of action of *Bacillus thuringiensis* delta-endotoxins. In: Molecular Biology of Microbial Differentiation, Edit. J.A. Hoch and P. Setlow, pp. 230–240, American Society of Microbiology, Washington, D.C.

Faust, R.M. 1968. In vitro chemical reaction of the delta-endotoxin produced by *Bacillus thuringiensis* var. *dendrolimus* with other proteins. *J. Invertebr. Pathol.* 11:465–475.

Fischkoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., McCormick, S.M., Niedermeyer, J.G., Dean, D.A., Kusano-kretzmer, K., Mayer, E.J., Rochester, E.E., Rogers, S.G. and Farley, R.T. 1987. Insect tolerant transgenic tomato plants. *Bio/Technology* 5:807–813.

Geiser, M., Schweitzer, S. and Grimm, C. 1986. The hypervariable region in the genes coding for entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the kurhd 1 gene of subsp. *kurstaki* HD1. *Gene* 48:109–118.

Gonzalez, J.M., Jr., Dulmage, H.T. and Carlton, B.C. 1981. Correlation between specific plasmids and δ-endotoxin production in *Bacillus thuringiensis*. *Plasmid* 5:357–365.

Gonzalez, J.M., Jr. and Carlton, B.C. 1982. Plasmid transfer in *Bacillus thuringiensis*. In: Genetic Exchange: A Celebration and a New Generation, Edit. U. Streips, S. Goodgal, W. Guild and G. Wilson, pp. 85–95, Marcel-Dekker Inc., NY.

Gonzalez, J.M., Brown, B.J. and Carlton, B.C. 1982. Transfer of *Bacillus thuringiensis* plasmids coding for delta-endotoxin among strains of *B. thuringiensis* and *B. cereus*. *Proc. Natl. Acad. Sci. USA* 79:6951–6955.

Gonzalez, J.M., Jr. and Carlton, B.C. 1984. A large transmissible plasmid is required for crystal toxin production in *Bacillus thuringiensis* variety *israelensis*. *Plasmid* 11:28–38.

Halder, M.Z., Knowles, B.H. and Ellar, D.J. 1986. Specificity of *Bacillus thuringiensis* var. *colmeri* insecticidal delta-endotoxin is determined by differential proteolytic processing of the protoxin by larvae gut proteases. *Eur. J. Biochem.* 156:531–540.

Halder, M.Z., Ward, E.S. and Ellar, D.J. 1987. Cloning and heterologous expression of an insecticidal delta

endotoxin gene from *Bacillus thuringiensis* var. *aizawai* IC1 toxic to both lepidoptera and diptera. *Gene* 52:285–290.

Hall, I.M., Arakawa, K.Y., Dulmage, H.T. and Correa, J.A. 1977. The pathogenicity of strains of *Bacillus thuringiensis* to larvae of *Aedes* and to *Culex* mosquitoes. *Mosquito News* 37:246–251.

Held, G.A., Bulla, L.A., Jr., Ferrari, E., Hoch, J., Aronson, A.I. and Minnich, S.A. 1982. Cloning and localization of the lepidopteran protoxin gene of *Bacillus thuringiensis* subsp. *kurstaki*. *Proc. Natl. Acad. Sci. USA* 79:6065–6069.

Herrnstadt, C., Soares, G.G., Wilcox, E.R. and Edwards, D.L. 1986. A new strain of *Bacillus thuringiensis* with activity against coleopteran insects. *BioTechnology* 4:305–308.

Herrnstadt, C., Gilroy, T.E., Sobleski, D.A., Bennett, B.D. and Gaertner, F.H. 1987. Nucleotide sequence and deduced amino acid sequence of a coleopteran-active delta-endotoxin gene from *Bacillus thuringiensis* subsp. *san diego*. *Gene* 57:37–46.

Hofte, H., de Greve, H., Seurinck, J., Jansens, S., Mahillon, J., Ampe, C., Vandekerckhove, J., Vanderbruggen, H., Van Montagu, M., Zabeau, M. and Vaeck, M. 1986. Structural and functional analysis of a cloned delta endotoxin of *Bacillus thuringiensis berliner* 1715. *Eur. J. Biochem.* 161:273–280.

Hofte, H., Seurinck, J., Van Houtnen, A. and Vaeck, M. 1987. Nucleotide sequence of a gene encoding an insecticidal protein of *Bacillus thuringiensis* var. *tenebrionis* toxic against Coleoptera. *Nucleic Acid Res.* 15:7183.

Honigman, A., Nedjar-Pazerini, G., Yawetz, A., Oron, V., Schuster, S., Broza, M. and Sneh, B. 1986. Cloning and expression of the lepidopteran toxin produced by *Bacillus thuringiensis* var. *thuringiensis* in *Escherichia coli*. *Gene* 42:67–77.

Hurley, J.M., Sung, G.L., Andrews, R.E., Klowden, M.J. and Bulla, L.A., Jr. 1985. Separation of the cytolytic and mosquitocidal proteins of *Bacillus thuringiensis* subsp. *israelensis*. *Biochem. Biophys. Res. Commun.* 126:961–965.

Ibarra, J.E. and Federici, B.A. 1986. Isolation of a relatively nontoxic 65 kilodalton protein inclusion from the parasporal body of *Bacillus thuringiensis* subsp. *israelensis*. *J. Bacteriol.* 165:527–533.

Jarrett, P. 1985. Potency factors in the delta-endotoxin of *Bacillus thuringiensis* var. *aizawi* and the significance of plasmids in their control. *J. Appl. Bacteriol.* 58:437–448.

Kller, A.F. and Lecadet, M.M. 1976. Arguments based on hybridization-competition experiments in favor of the *in vitro* synthesis of sporulation-specific RNA's by the RNA polymerase of *B. thuringiensis*. *Biochem. Biophys. Res. Commun.* 73:263.

Kller, A., Fargette, F., Ribier, J. and Rapoport, G. 1982. Cloning and expression of the crystal protein genes from *Bacillus thuringiensis* strain berliner 1715. *The EMBO J.* 1:791–799.

Kller, A., Parsot, C. and Rapoport, G. 1983. *In vitro* transcription of the cloned chromosomal gene from *Bacillus thuringiensis*. *Nucleic Acids Res.* 11:3975–3987.

Kller, A. and Rapoport, G. 1984. Cloning and heterospecific expression of the crystal protein genes from *B. thuringiensis*. In: Bacterial Protein Toxins, Edit. J.E. Alouf et al., pp. 65–72, Academic Press, London.

Kller, A., Lereclus, D., Ribier, J., Bourgouin, C., Menou, G., Lecadet, M.-M. and Rapoport, G. 1985. Cloning and expression in *Escherichia coli* of the crystal protein gene from *Bacillus thuringiensis* strain *aizawa* 7-29 and comparison of the structural organization of genes from different serotypes. In: Molecular Biology of Microbial Differentiation, Edit. J.A. Hoch and P. Setlow, pp. 217–224, American Society for Microbiology, Washington, D.C.

Kondo, S.-I., Tamura, N., Kunitate, A., Hattori, M., Akashi, A. and Ohmori, I. 1987. Cloning and nucleotide sequencing of two insecticidal delta endotoxin genes from *Bacillus thuringiensis* var. *kurstaki* HD-1 DNA. *Agric. Biol. Chem.* 51:455–463.

Kronstad, J.W., Schnepf, H.E. and Whiteley, H.R. 1983. Diversity of locations for *Bacillus thuringiensis* crystal protein genes. *J. Bacteriol.* 154:419–428.

Kronstad, J.W. and Whiteley, H.R. 1984. Inverted repeat sequences flank a *B. thuringiensis* crystal protein gene. *J. Bacteriol.* 160:95–102.

Kronstad, J.W. and Whiteley, H.R. 1986. Three classes of homologous *Bacillus thuringiensis* crystal protein genes. *Gene* 43:29–40.

Lecadet, M-M., Sanchis, V., Minou, Y., Rabot, P., Lereclus, D., Chaufaux, J. and Martour, D. 1988. Identification of a delta-endotoxin gene product specifically active against *Spodoptera littoralis* Bdv. among "K-60" proteolysed fractions of the insecticidal crystals of *Bacillus thuringiensis* subsp. *aizawai*

7-29. *Appl. Environ. Microbiol.* 54:2689–2698.

Lereclus, D., Lecadet, M-M., Ribier, J. and Dedonder, R. 1982. Molecular relationships among plasmids of *Bacillus thuringiensis*: Conserved sequences through 11 crystalliferous strains. *Mol. Gen. Genet.* 186:391–398.

Lereclus, D., Menou, G. and Lecadet, M.-M. 1983. Isolation of a DNA sequence related to several plasmids from *Bacillus thuringiensis* after a mating involving the *Streptococcus faecalis* plasmid pAMB1. *Mol. Gen. Genet.* 191:307–313.

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

Lereclus, D., Lecadet, M.-M., Klier, A., Ribier, J., Rapoport, G. and Dedonder, R. 1985. Recent aspects of genetic manipulation in *Bacillus thuringiensis*. *Biochimie* 67:91–100.

Lereclus, D., Arantes, O, Chaufaux, J. and Lecadet, M.-M. 1989. Transformation and expression of a cloned delta-endotoxin gene in *Bacillus thuringiensis*. *FEMS Microbiol. Lett.* 60:211–218.

Mahillon, J., Seurinck, J., Rompuy, L.V., Delcour, J. and Zabeau, M. 1985. Nucleotide sequence and structural organization of an insertion sequence element (IS231) from *Bacillus thuringiensis* strain berliner 1715. *EMBO J.* 4:3895–3899.

Mahillon, J., Seurinck, J., Delcour, J. and Zabvau, M. 1987. Cloning and nucleotide sequence of different iso-IS231 elements and their structural association with the Tm*4430* transposon in *Bacillus thuringiensis*. *Gene* 51:187–196.

McLean, K.M. and Whiteley, H.R. 1987. Expression in *Escherichia coli* of a cloned crystal protein gene of *Bacillus thuringiensis* subsp. *israelensis*. *J. Bacteriol.* 169:1017–1023.

McLinden, J.H., Sabourin, J.R., Clark, B.D., Gensler, D.R., Workman, W.E. and Dean, D.H. 1985. Cloning and expression of an insecticidal k-73 type crystal protein gene from *Bacillus thuringiensis* var. *kurstaki* into *Escherichia coli*. *Appl. Environ. Microbiol.* 50:623–628.

McPherson, S.A., Perlak, F.J., Fuchs, R.L., Marrone, P.G., Lavrik, P.B. and Fischhoff, D.A. 1988. Characterization of the coleopteran-specific protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *BioTechnology* 6:61–66.

Minnich, S.A. and Aronson, A.I. 1984. Regulation of protoxin synthesis in *Bacillus thuringiensis*. *J. Bacteriol.* 158:447–454.

Moran, C.P., Lang, N., Banner, C.D.B., Haldenwang, W.G. and Losick, R. 1981. Promoter for a developmentally regulated gene in *Bacillus subtilis*. *Cell* 15:783–791.

Nagamatsu, Y., Tsutsui, R., Ichimaru, T., Nagamatsu, M., Koya, K. and Hayaski, K. 1978. Subunit structure and toxic component of delta endotoxin from *Bacillus thuringiensis*. *J. Invertebr. Pathol.* 32:103–109.

Obukowicz, M.G., Perlak, F.J., Kusano-Kretzmer, K., Mayer, E.J., Bolten, S.L. and Watrud, L.S. 1986a. Tn5-mediated integration of the delta-endotoxin gene from *Bacillus thuringiensis* into the chromosome of root-colonizing pseudomonads. *J. Bacteriol.* 168:982–989.

Obukowitz, M.G., Perlak, F.J., Kusano-Kretzmer, K., Mayer, E.J. and Watrud, L.S. 1986b. Integration of the delta-endotoxin gene of *Bacillus thuringiensis* into the chromosome of root-colonizing strains of pseudomonads using Tn5. *Gene* 45:327–331.

Obukowicz, M.G., Perlak, F.J., Bolton, S.L., Kusano-Kretzmer, K., Mayer, E.J. and Watrud, L.S. 1987. IS50L as a non-self transposable vector used to integrate the *Bacillus thuringiensis* delta-endotoxin gene into the chromosome of root-colonizing pseudonomads. *Gene* 51:91–96.

Oeda, K., Oshie, K., Shimizu, M., Nakamura, K., Yamomoto, H., Nakayama, I. and Ohkawa, H. 1987. Nucleotide sequence of the insecticidal protein gene of *Bacillus thuringiensis* strain *aizawai* IPL7 and its high-level expression in *Escherichia coli*. *Gene* 53:113–119.

Ohba, M. and Aizawa, K. 1979. A new subspecies of *Bacillus thuringiensis* possessing 11a:11c flagellar antigenic structure. *J. Invertebr. Pathol.* 33:387–388.

Padua, L.E., Ohba, M. and Aizawa, K. 1980. The isolates of *Bacillus thuringiensis* serotype 10 with a highly preferential toxicity to mosquito larvae. *J. Invertebr. Pathol.* 36:180–186.

Padua, L.E., Ohba, M. and Aizawa, K. 1984. Isolation of a *Bacillus thuringiensis* strain (Serotype 8a:8b) highly and selectively toxic against mosquito larvae. *J. Invertebr. Pathol.* 44:12–17.

Petit-Glatron, M.-F. and Rappoport, G. 1975. In vitro and in vivo evidence for the existence of stable messenger ribonucleic acids in sporulating cells of *Bacillus thuringiensis*. In: Spores VI, Edit. P. Gerhardt, R.N. Costilow and H. Sadoff, pp. 255–264, American Society of Microbiology, Washington, D.C.

Sanchis, V., Lereclus, D., Menou, G., Chaufaux, J. and Lecadet, M-M. 1988. Multiplicity of delta endotoxin genes with different insecticidal specificities in *B. thuringiensis aizawai* 7-29. *Molec. Microbiol.* 2:393–404.

Sanchis, V., Lereclus, D., Menou, G., Chaufaux, J., Guo, S. and Lecadet, M-M. 1989. Nucleotide sequence and analysis of the N-terminal coding region of the *Spodoptera* active delta-endotoxin gene of *Bacillus thuringiensis aizawai* 7.29. *Molec. Microbiol.* 3:229–238.

Schnepf, E. and Whiteley, H.R. 1981. Cloning and expression of *Bacillus thuringiensis* crystal protein gene in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 78:2893–2897.

Schnepf, H.E. and Whiteley, H.R. 1985. Delineation of a toxin-encoding segment of a *Bacillus thuringiensis* crystal protein gene. *J. Biol. Chem.* 260:6273–6280.

Schnepf, H.E., Wong, H.C. and Whiteley, H.R. 1985. The amino acid sequence of a crystal protein from *Bacillus thuringiensis* deduced from the DNA base sequence. *J. Biol. Chem.* 260:6264–6272.

Sekar, V. 1986. Biochemical and immunological characterization of the cloned crystal toxin gene of *Bacillus thuringiensis* var. *israelensis*. *Biochem. Biophys. Res. Commun.* 137:748–751.

Sekar, V. 1987. DNA homology between the crystal toxin genes of several mosquito pathogenic *Bacillus thuringiensis* strains. *Curr. Microbiol.* 15:287–289.

Sekar, V. and Carlton, B.C. 1985. Molecular cloning of the delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis*. *Gene* 33:151–158.

Sekar, V., Thompson, D.V., Maroney, M.J., Bookland, R.G. and Adang, M.J. 1987. Molecular cloning and characterization of the insecticidal crystal protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *Proc. Natl. Acad. Sci. USA* 84:7030–7040.

Sen, K., Honda, G., Koyama, N., Nishida, M., Niki, A., Sakai, H., Himeno, M. and Komano, T. 1988. Cloning and nucleotide sequences of the two 130 kDa insecticidal protein genes of *Bacillus thuringiensis* var. *israelensis*. *Agric. Biol. Chem.* 52:873–878.

Shibano, Y., Yamagata, A., Nakamura, N., Iizuka, T., Sugisaki, H. and Takanami, M. 1985. Nucleotide sequence coding for the insecticidal fragment of the *Bacillus thuringiensis* crystal protein. *Gene* 34:234–251.

Shivakumar, A.G., Gundling, G.J., Benson, T.A., Casuto, B., Miller, M.F. and Spear, B.B. 1986. Vegetative expression of the delta-endotoxin genes of *Bacillus thuringiensis* subsp. *kurstaki* in *Bacillus subtilis*. *J. Bacteriol.* 166:194–204.

Thorne, L., Fermin, G., Thompson, T., Decker, D., Zounes, M., Wild, M., Walfield, A.M. and Pollock, T.J. 1986. Structural similarity between the lepidoptera- and diptera-specific insecticidal endotoxin genes of *Bacillus thuringiensis* subsp. *"kurstaki"* and *"israelensis"*. *J. Bacteriol.* 166:801–811.

Tyrell, D.J., Bulla, L.A., Jr., Andrews, R.E., Kramer, K.J., Davidson, L.I. and Nardin, P. 1981. Comparative biochemistry of entomocidal parasporal crystals of selected *Bacillus thuringiensis* strains. *J. Bacteriol.* 145:1052–1062.

Visser, B., Van Workum, M., Dullemans, A. and Waalwijk, C. 1986. The mosquitocidal activity of *Bacillus thuringiensis* var. *israelensis* is associated with M_r 230,000 and 130,000 crystal protein. *FEMS Microbiol. Lett.* 30:211–214.

Visser, B., van der Salm, T., van den Brink, W. and Folkers, G. 1988. Genes from *Bacillus thuringiensis entomocidus* 60.5 coding for insect-specific crystal proteins. *Molec. Gen. Genet.* 212:219–224.

Waalwijk, C., Dullemans, A.M., van Workun, E.S. and Visser, B. 1985. Molecular cloning and the nucleotide sequence of the M_r 28,000 crystal protein gene of *Bacillus thuringiensis* subsp. *israelensis*. *Nucleic Acids Res.* 13:8207–8217.

Wabiko, H., Held, G.A. and Bulla, L.A., Jr. 1985. Only part of the protoxin gene of *Bacillus thuringiensis* subsp. *berliner* 1715 is necessary for insecticidal activity. *Appl. Environ. Microbiol.* 49:706–708.

Wabiko, H., Raymond, K.C. and Bulla, L.A., Jr. 1986. *Bacillus thuringiensis* entomocidal protoxin gene sequence and gene product analysis. *DNA* 5:305–314.

Ward, E.S. and Ellar, D.J. 1983. Assignment of the delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis* to a specific plasmid by curing analysis. *FEBS Lett.* 158:45–49.

Ward, E.S., Ellar, D.J. and Todd, J.A. 1984. Cloning and expression in *Escherichia coli* of the insecticidal delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis*. *FEBS Lett.* 175:377–382.

Ward, E.S. and Ellar, D.J. 1986. *Bacillus thuringiensis* var. *israelensis* delta-endotoxin. Nucleotide sequence and characterization of the transcripts in *Bacillus thuringiensis* and *Escherichia coli*. *J. Mol. Biol.* 191:1–11.

Ward, E.S., Ridley, A.R., Ellar, D.J. and Todd, J.A. 1986. *Bacillus thuringiensis* var. *israelensis* delta-endotoxin. Cloning and expression of the toxin in sporogenic and asporogenic strains of *Bacillus subtilis*. *J. Mol. Biol.* 191:13–22.

Ward, E.S. and Ellar, D.J. 1987. Nucleotide sequence of a *Bacillus thuringiensis* var. *israelensis* gene encoding a 130 kDa delta-endotoxin. *Nucleic Acids Res.* 15:7195.

Ward, E.S. and Ellar, D.J. 1988. Cloning and expression of two homologous genes of *Bacillus thuringiensis* subsp. *israelensis* which encode 130-kilodalton mosquitocidal proteins. *J. Bacteriol.* 170:727–735.

Watrud, L.S., Perlak, F.J., Tran, M.-T., Kusano, K., Mayer, E.J., Miller-Wideman, M.A., Obukowicz, M.G., Nelson, D.R., Kreitinger, J.P. and Kaufman, R.J. 1985. Cloning of the *Bacillus thuringiensis* subsp. *kurstaki* delta endotoxin gene into *Pseudomonas fluorescens*: Molecular biology and ecology of an engineered microbial pesticide. In: Engineered Organisms in the Environment, Edit. H.O. Halvorson, D. Pramer and M. Rogul, pp. 40–46, American Society for Microbiology, Washington, DC.

Whiteley, H.R., Kronstad, J.W. and Schnepf, H.E. 1981. Cloning the delta-endotoxin gene of *Bacillus thuringiensis* in *Escherichia coli* and localization of the gene in different strains of *B. thuringiensis*. In: Basic Biology of Microbial Larvacides of Vectors of Human Diseases, Edit. F. Michal, pp. 147–156, UNDP/World Bank/WHO, Geneva.

Whiteley, H.R., Schnepf, H.E., Kronstad, J.W. and Wong, H.C. 1984. Structural and regulatory analysis of a cloned *Bacillus thuringiensis* crystal protein gene. In: Genetics and Biotechnology of Bacilli, Edit. Ganesan and Hoch, pp. 375–386, Academic Press, Inc., NY.

Whiteley, H.E., Kronstad, J.W. and Schnepf, H.R. 1985. Structure and expression of cloned *Bacillus thuringiensis* toxin genes. In: Molecular Biology of Microbial Differentiation, Edit. J.A. Hoch and P. Setlow, pp. 225–229, American Society for Microbiology, Washington, D.C.

Whiteley, H.R. and Schnepf, H.E. 1986. The molecular biology of parasporal crystal body formation in *Bacillus thuringiensis*. *Ann. Rev. Microbiol.* 40:549–576.

Whiteley, H.R., Schnepf, H.E., Tomczak, K. and Lara, J.C. 1987. Structure and regulation of the crystal protein gene of *Bacillus thuringiensis*. In: Biotechnology in Invertebrate Pathology and Cell Culture, Edit. K. Maramorosch, pp. 13–27, Academic Press, Inc. NY.

Widner, W.R. and Whiteley, H.R. 1989. Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. *J. Bacteriol.* 171:965–974.

Wong, H.C., Schnepf, H.E. and Whiteley, H.R. 1983. Transcriptional and translational start sites for the *Bacillus thuringiensis* crystal protein gene. *J. Biol. Chem.* 258:1960–1967.

Wong, H.C. and Chang, S. 1985. Identification of a positive retroregulator that functions in *Escherichia coli* and *Bacillus subtilis*. In: Molecular Biology of Microbial Differentiation, Edit. J.A. Hoch and P. Setlow, pp. 104–109, American Society of Microbiology, Washington, D.C.

Wu, D. and Chang, F.N. 1985. Synergism in mosquitocidal activity of 26 and 65KD proteins from *Bacillus thuringiensis* subsp. *israelensis* crystal. *FEBS Lett.* 190:232–236.

Yamamoto, T. and McLaughlin, R.E. 1981. Isolation of a protein from the parasporal crystal of *Bacillus thuringiensis* var. *kurstaki* toxic to the mosquito larvae, *Aedes taeniorhyncus*. *Biochem. Biophys. Res. Commun.* 103:414–421.

Yamamoto, T. 1983. Identification of entomocidal toxins of *Bacillus thuringiensis* by high performance liquid chromatography. *J. Gen. Microbiol.* 129:2595–2603.