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Prediction of Insecticidal Activity of *Bacillus thuringiensis* Strains by Polymerase Chain Reaction Product Profiles

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A rapid analysis of *Bacillus thuringiensis* strains predictive of insecticidal activity was established by using polymerase chain reaction (PCR) technology. Primers specific to regions of high homology within genes encoding three major classes of *B. thuringiensis* crystal proteins were used to generate a PCR product profile characteristic of each insecticidal class. Predictions of insecticidal activity were made on the basis of the electrophoretic patterns of the PCR products. Included in the screen were PCR primers specific for *cryI*, *cryIII*, and *cryIV* genes, which are insecticidal for lepidopterans, coleopterans, and dipterans, respectively. Known *B. thuringiensis* strains as well as unidentified strains isolated from soil and insect cadavers were analyzed by PCR. Small amounts of crude sample lysates were assayed in a single PCR reaction containing 12 to 20 primers capable of distinguishing between the different insecticidal genes. Insecticidal activity predicted by the PCR screen was found to correspond with the insecticidal activity of insect bioassays. In addition to identifying strains with known insecticidal genes, the PCR screen can identify strains with altered electrophoretic patterns containing potentially novel genes.

Bacillus thuringiensis is a gram-positive sporeforming bacterium. Various strains of *B. thuringiensis* produce different insecticidal protein toxins in parasporal crystals, which are used as insecticides (12) and which have been engineered into plants (1, 3, 16) for crop protection. These insecticidal proteins typically have a specific and narrow host range. Their increasing use and lack of mammalian toxicity have led to an intensified search for new strains with different spectra of activities. In addition, the interest in finding novel strains has been heightened by an increasing interest in the preventative management of insect resistance to *B. thuringiensis* by the use of insecticidal proteins affecting different targets in the insect gut.

The isolation and identification of novel strains of *B. thuringiensis* by bioassay can be a long and involved process. One of the major impediments in such a screening effort is the repeated isolation and rescreening of the same strains or genes, thereby reducing the number of novel strains being evaluated. Methods have been established to expedite the characterization of new isolates on the basis of crystal serotype (9), Southern blot analysis to test for homology with known genes (8), or reactivity with various monoclonal antibodies (6). Each of these procedures can speed up the evaluation process. Unfortunately, they are imprecise predictors of insecticidal activity (in the case of crystal serotype) or are expensive and time-consuming to establish and use on a routine basis (in the case of monoclonal antibodies and Southern blots).

The polymerase chain reaction (PCR) is a procedure that allows rapid determination of the presence or absence of a target DNA sequence (13). It is highly sensitive, requires very little DNA for analysis, and allows large numbers of samples to be processed in a relatively short time. We describe the use of PCR to identify novel *B. thuringiensis* strains and to predict the insecticidal activity of new isolates. The electrophoretic profile of the PCR products can be used to predict the insecticidal activity of new and known isolates. Novel strains characterized by different profiles can be identified for preferential testing by insect assay. This procedure allows the rapid evaluation of up to 100 isolates per day and can be used to streamline screening by insect bioassays.

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MATERIALS AND METHODS

Strains. Known *B. thuringiensis* strains were provided by the U.S. Department of Agriculture strain collection, Midwest Area Northern Research Center, Peoria, Ill. Unknown strains were isolated from soil and insect cadavers. *B. thuringiensis* strains were grown on nutrient agar and analyzed by PCR directly from the plate as described below.

Isolation of sporeforming *Bacillus* strains. A modified version of the acetate selection protocol of Travers et al. (15) was used to isolate sporeforming *Bacillus* strains from soil and insect cadavers (11). Approximately 0.5 g of soil or insect cadavers was added to 10 ml of LB broth (10) buffered with sodium acetate (0.25 M, pH 6.8) in a 125-ml flask. Sodium acetate was included to inhibit the germination of *B. thuringiensis* spores (15). The broth was incubated on a shaker (200 rpm) for 4 h (30°C). A 1-ml aliquot was then heated to 65°C for 10 min in a prewarmed 5-ml glass test tube, and undiluted aliquots were spread on L-agar plates and incubated for 48 h at 30°C. Anywhere from 1 to 30 different colony types were usually obtained. The cultures were examined for the presence of parasporal crystals by phase-contrast microscopy. Crystal-forming strains were purified by subculturing on nutrient agar plates until cultures were axenic as indicated by eye and microscopic examination. Non-crystal-forming colonies were restreaked on nutrient agar and checked for crystal production after 48 h, since many isolates did not produce crystals until the second or third passage following acetate treatment. Crystal-producing strains were grown on a shaker in 300 ml of T3

TABLE 1. Primers used in PCR screen

Primer	Sequence	Gene	Nucleotides	Reference
Lep1A	5'CCGGTGCTGGATTTGTGTTA3'	*B. thuringiensis* subsp. *kurstaki cryIA*(b)	310–330	4
Lep1B	5'AATCCCGTATTGTACCAGCG3'	*B. thuringiensis* subsp. *kurstaki cryIA*(b)	780–800	4
Lep2A	5'CCGAGAAAGTCAAACATGCG3'	*B. thuringiensis* subsp. *kurstaki cryIA*(b)	2158–2178	4
Lep2B	5'TACATGCCCTTTCACGTTCC3'	*B. thuringiensis* subsp. *kurstaki cryIA*(b)	3046–3066	4
Dip1A	5'CAAGCCGCAAATCTTGTGGA3'	*B. thuringiensis* subsp. *israelensis cryIV*	2551–2571	17
Dip1B	5'ATGGCTTGTTTCGCTACATC3'	*B. thuringiensis* subsp. *israelensis cryIV*	3328–3348	17
Dip2A	5'GGTGCTTCCTATTCTTTGGC3'	*B. thuringiensis* subsp. *israelensis cryIV*	740–760	17
Dip2B	5'TGACCAGGTCCCTTGATTAC3'	*B. thuringiensis* subsp. *israelensis cryIV*	2010–2030	17
Col1A	5'GTCCGCTGTATATTCAGGTG3'	*B. thuringiensis* subsp. *tenebrionis cryIIIA*	1801–1821	5
Col1B	5'CACTTAATCCTGTGACGCCT3'	*B. thuringiensis* subsp. *tenebrionis cryIIIA*	2430–2450	5
Col2A	5'AGGTGCCAACTAACCATGTT3'	*B. thuringiensis* subsp. *tenebrionis cryIIIA*	621–641	5
Col2B	5'GATCCTATGCTTGGTCTAGT3'	*B. thuringiensis* subsp. *tenebrionis cryIIIA*	1661–1681	5

medium (15) at 30°C and 200 rpm until sporulation was complete, as monitored by phase-contrast microscopy. To remove exotoxin, broths were centrifuged and pellets were resuspended in distilled water and lyophilized. The dry powders were assayed for biological activity.

Synthesis of PCR primers. Oligonucleotide primers were synthesized on an Applied Biosystems 380A synthesizer with reagents and conditions supplied by the manufacturer. The primers listed in Table 1 were used in the PCR screen. Table 1 describes the sequence of each primer and the corresponding gene and nucleotides from which the primer was derived.

PCR sample preparation. A loopful of cells from a *B. thuringiensis* colony on an overnight nutrient agar plate was transferred to a 0.5-ml GeneAmp reaction tube (Perkin Elmer Cetus) containing 50 μl of PCR reaction mix. The PCR reaction mix is as described in the GeneAmp Kit (Perkin Elmer Cetus), with the addition of 0.1 to 0.5 μM of each of the 12 *B. thuringiensis* primers and without the *Taq* polymerase. The microcentrifuge tube was transferred to a boiling water bath for 10 min to burst the cells. The tubes were spun briefly to collect condensate. AmpliTaq polymerase (0.5 to 5.0 U; Perkin Elmer Cetus) was added to each tube, and the contents were mixed and overlaid with a few drops of mineral oil. Amplification was accomplished with the DNA Thermal Cycler (Perkin Elmer Cetus) by using the Step-Cycle program set to denature at 94°C for 45 s, anneal at 45°C for 45 s, and extend at 72°C for 1 min, followed by a 4-s-per-cycle extension for a total of 35 cycles. Following amplification, the PCR reaction mix was analyzed by agarose gel electrophoresis as described by Maniatis et al. (10). A total of 20 μl of each PCR reaction mix was electrophoresed on a 0.8% agarose gel in Tris-borate buffer, stained with ethidium bromide, and photographed.

Conjugation of fluorescent dyes to PCR primers. PCR primers Lep1A, Lep1B, Col1A, and Col1B were synthesized with a 5′ primary amino group (Aminolink-2; Applied Biosystems) on an Applied Biosystems DNA Synthesizer. Applied Biosystems fluorescent NHS ester dyes 5′-carboxyfluorescein (FAM) and 6-carboxy-X-rhodamine (ROX) were conjugated to the oligonucleotides as described by Chehab and Kan (2). FAM and ROX were conjugated to the Lep and Col primers, respectively, to give green and red PCR products. Dye-labeled oligonucleotides were purified by Sephadex (G-25) gel filtration followed by high-pressure liquid chromatography purification on an Aguapore 300C-8 column (2). The labeled primers were used in the PCR reactions as described above. Fluorescent colored products were visualized on a long-wavelength UV transilluminator and photographed as described by Chehab and Kan (2).

Bioassay. Qualitative insect toxicity testing was carried out on lyophilized spore-crystal preparations grown in T3 broth. *B. thuringiensis* isolates were tested for their activities against insects of the order Lepidoptera by using the European corn borer, *Ostrinia nubilalis*, and the tobacco hornworm, *Manduca sexta*. Suspensions of 1 mg of spore-crystal mix per ml of sterile water were prepared for each isolate tested. An isolate was considered active if it caused 100% mortality while control mortality ranged from 0 to 20%. For both species, 30 μl of a stock spore-crystal suspension was pipetted onto 0.5-cm-diameter disks cut from artificial Black cutworm diet (Bioserv, Inc., Frenchtown, N.J.). Disks were placed individually in snap-cap petri dishes (50 by 10 mm), and one neonatal larva of either species was added to each disk. Twenty larvae of both species were tested for each *B. thuringiensis* isolate. Mortality was recorded after 4 days. The activities of *B. thuringiensis* isolates against a coleopteran were tested by using the Colorado potato beetle, *Leptinotarsa decimlineata*. The spore-crystal suspensions used in these assays contained 0.1% Triton X-100 as a wetting agent. Tomato leaflets were excised from 30-cm plants and dipped into stock suspensions, air dried, and placed in petri dishes (50 by 10 mm). Three replicates of five first-instar larvae were tested for each *B. thuringiensis* isolate. Mortality was recorded after 3 days. Activities of the isolates against dipterans were tested by using *Culex pipiens* mosquitoes. Ten third-instar mosquito larvae were added to 10 ml of sterile water in 30-ml plastic cups. After a 30-min adjustment period, 100 μg of a spore-crystal mix was added. Mortality was recorded after 24 h.

RESULTS

B. thuringiensis crystal-producing strains have been isolated that are insecticidal against lepidopterans, dipterans, and coleopterans. Several crystal-producing strains have no known insect toxicity. Nucleotide sequences reported for *B. thuringiensis* crystal protein genes represent 14 distinct genes (7). *cryIA* crystal protein genes exhibiting insecticidal activity against lepidopterans share extensive DNA sequence homology. Similarly, *cryIV* genes coding for crystal proteins with activities against dipterans share extensive regions of homology at their 3′ ends. Synthetic oligonucleotide primers were made corresponding to the regions of homology specific for the three insecticidal groups specific

TABLE 2. Products of different PCR primers

PCR primers	PCR products (bp) from following genes[a]						
	cryIA(a)	*cryIA*(b)	*cryIA*(c)	*cryIIIA*	*cryIIIB*	*cryIVA*	*cryIVB*
Lep1A + Lep1B	490	490	490				
Lep2A + Lep2B	986	908	986				
Col1A + Col1B				649	No product[b]		
Col2A + Col2B				1,060	1,060[b]		
Dip1A + Dip1B						797	797
Dip2A + Dip2B						1,290	No product

[a] *cryIA*(a), *cryIA*(b), and *cryIA*(c) are genes coding for crystal proteins active against lepidopterans; *cryIIIA* and *cryIIIB* code for crystal proteins active against coleopterans, and *cryIVA* and *cryIVB* code for crystal proteins active against dipterans.

[b] Expected product on the basis of sequence information.

for lepidopterans, dipterans, and coleopterans (Table 1). These group-specific primers were used to amplify and identify specific DNA segments in *B. thuringiensis* strains by using PCR technology (13).

Primers were selected such that each insecticidal group was identified by a specific set of PCR products of characteristic size and also color when using fluorescent dye-labeled primers. Two sets of primers specific for each of the three insecticidal groups were included in each PCR reaction. As shown in Table 2, lepidopteran-specific primers Lep1A and Lep1B yield a 490-bp PCR product with *cryIA*(a), *cryIA*(b), and *cryIA*(c) crystal genes. Primers Lep2A and Lep2B give two different size products depending on the type of *cryIA* gene. *cryIA*(a) and *cryIA*(c) genes are identified by a 986-bp PCR product and *cryIA*(b) is identified by a 908-bp product. With the *B. thuringiensis* subsp. *israelensis cryIV* genes, dipteran-specific primers Dip1A and Dip1B give a 797-bp product and Dip2A and Dip2B give a 1,290-bp product. *cryIVA* genes are identified by both the 797- and 1,290-bp products, whereas the *cryIVB* genes only give the 797-bp product. With the *B. thuringiensis* subsp. *tenebrionis cryIIIA* gene, coleopteran-specific primers Col1A and Col1B give a 649-bp product and Col2A and Col2B give a 1,060-bp product. The coleopteran-active *B. thuringiensis* subsp. *tolworthi cryIIIB* gene is expected to be identified by a 1,060-bp product on the basis of sequence information (14).

Known *B. thuringiensis* strains obtained from the U.S. Department of Agriculture, as well as unidentified strains isolated from soil and insect cadavers, were analyzed by PCR. Each PCR reaction contained 12 *B. thuringiensis* primers to amplify products characteristic for each of the three insecticidal groups. PCR analysis of 28 known *B. thuringiensis* strains obtained from the U.S. Department of Agriculture by using the 12 *B. thuringiensis* PCR primers gave characteristic PCR products corresponding to the insecticidal activity of each strain (Table 3). Strains with no known insecticidal activity gave no PCR products. Figure 1 shows the PCR product profile characteristic of each insecticidal group.

Sporeforming strains isolated from soils were analyzed by PCR, and insecticidal activity was predicted on the basis of their PCR product profiles. PCR analysis of soil and insect isolates gave characteristic products that allowed rapid prediction of insecticidal activity. Figure 2 shows the PCR product profiles of representative strains. Isolates were tested in bioassays against the European corn borer, the Colorado potato beetle, and mosquito larvae to confirm insecticidal predictions. Table 4 shows the PCR results of 11 soil and insect isolates and their correlation to insect bioassays. As shown in Table 4, the PCR assay was able to predict the insecticidal activity of each strain as determined by bioassay.

Novel isolates not containing known genes might give PCR products different in size relative to the standard or might completely lack PCR products.

DISCUSSION

We have used PCR technology and primers specific for *B. thuringiensis* delta-endotoxin genes to develop a rapid screen of new *B. thuringiensis* isolates that can predict their insecticidal activities. A set of 12 primers was chosen to give characteristic product profiles from the genes encoding three major classes of *B. thuringiensis* crystal proteins. Included in the screen are PCR primers specific for *cryI*, *cryIII*, and *cryIV* genes that are insecticidal for lepidopterans, coleopterans, and dipterans, respectively. Each class is distinguishable by a separate product profile, thereby allowing unknown isolates to be screened rapidly for the presence of known *B. thuringiensis* genes. Small amounts of cellular material can be assayed directly from crude sample lysates in a single PCR reaction containing 12 to 20 primers capable of distinguishing the different insecticidal classes of *B. thuringiensis* genes. A prediction of insecticidal activity can be made on the basis of the electrophoretic pattern of the PCR products. The absence of reaction products might be indicative of a novel strain of *B. thuringiensis*. Strains with unusual PCR profiles, characterized by either the presence of novel PCR products or the absence of PCR products, can be further analyzed for homology to known genes by using additional primers.

The versatility of this rapid screen can be customized by the addition of primers to identify specific regions within *B. thuringiensis* genes. Once a strain has been identified as containing a particular type of *B. thuringiensis* gene, it can be further characterized with primers specific to constant or variable regions. PCR reactions containing five to seven primer pairs specific to regions 200 bp apart can be used to analyze an unknown strain for differences to a known gene. Additional primers with homology to other regions of known crystal protein genes can easily be incorporated to further characterize novel isolates. Use of fluorescent dye-labeled primers allows for the prediction of insecticidal activity on the basis of color in addition to size. Novel strains displaying PCR products different in size could potentially be identified as lepidopteran or dipteran active on the basis of the color of the PCR product.

TABLE 3. Insecticidal activities of *B. thuringiensis* strains as determined by PCR

B. thuringiensis subspecies and strain	PCR products (bp)	Activity predicted by PCR	Insecticidal activity
kurstaki HD-1	490, 908, 986	Lepidopteran	Lepidopteran
kurstaki HD-73	490, 986	Lepidopteran	Lepidopteran
berliner B-4039	490, 908	Lepidopteran	Lepidopteran
sotto B-4042	490, 908, 986	Lepidopteran	Lepidopteran
kenyae B-4044	490, 986	Lepidopteran	Lepidopteran
morrisoni B-4049	490, 986	Lepidopteran	Lepidopteran
subtoxicus B-4057	490, 908	Lepidopteran	Lepidopteran
thompsoni B-4060	986	Lepidopteran	Lepidopteran
ostriniae HD-501	490, 908	Lepidopteran	Lepidopteran
thuringiensis HD-22	490, 986	Lepidopteran	Lepidopteran
entomocidus HD-9	490, 986	Lepidopteran	Lepidopteran
aizawai HD-11	490, 986	Lepidopteran	Lepidopteran
aizawai HD-68	490, 986	Lepidopteran	Lepidopteran
entomocidus HD-110	490, 986	Lepidopteran	Lepidopteran
entomocidus HD-198	490, 908, 986	Lepidopteran	Lepidopteran
aizawai HD-131	490, 908, 986	Lepidopteran	Lepidopteran
aizawai HD-133	490, 908, 986	Lepidopteran	Lepidopteran
kenyae HD-64	490, 908	Lepidopteran	Lepidopteran
dendrolimus HD-37	490, 986	Lepidopteran	Lepidopteran
thuringiensis HD-14	490, 986	Lepidopteran	Lepidopteran
israelensis HD-567	797, 1,290	Dipteran	Dipteran
israelensis B-4554	797, 1,290	Dipteran	Dipteran
indiana HD-521	None	Unknown	None known
dakota HD-511	None	Unknown	None known
tohokuensis HD-866	None	Unknown	None known
tochigiensis HD-868	None	Unknown	None known
kumamotoensis HD-867	None	Unknown	None known
tenebrionis BI 256-82	649, 1,060	Coleopteran	Coleopteran

This rapid method of screening allows the analysis of as many as 100 *B. thuringiensis* isolates per day per person and the prediction of insecticidal activity when known genes are detected. The identification of novel *B. thuringiensis* genes is expedited by the large number of strains that can be quickly identified. Strains with unique PCR product profiles are easily characterized by additional PCR reactions with additional gene-specific primers. PCR analysis is considerably less time-consuming and cumbersome than screening strains by DNA hybridization (Southern hybridization), enzyme-linked immunosorbent assay (ELISA), or insect assays. The selection of *B. thuringiensis* primers specific for each class of known insecticidal (crystal protein) genes makes the PCR screen a more accurate predictor of insecticidal activity than either DNA hybridization or ELISA.

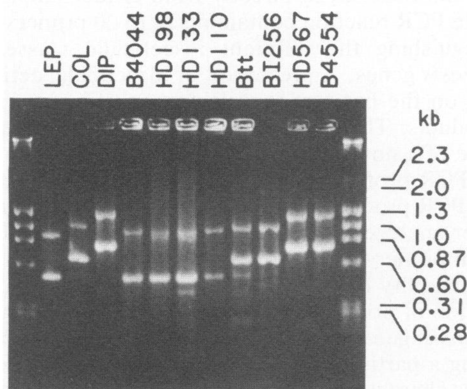

FIG. 1. Typical PCR product profiles of known *B. thuringiensis* strains. The first three lanes, labeled LEP, COL, and DIP, are the expected PCR products of strains displaying lepidopteran, coleopteran, and dipteran activity, respectively. The following lanes contain the PCR products of known strains. As described in Table 3, strains B4044, HD198, HD133, and HD110 have activities against lepidopterans and produce a typical lepidopteran PCR profile. Strains HD198 and HD133 contain both the 908- and 986-bp products, indicating the presence of at least two genes. Btt and BI256 are *B. thuringiensis* subsp. *tenebrionis* strains with activities against coleopterans. HD567 and B4554 are *B. thuringiensis* subsp. *israelensis* strains with activities against dipterans.

The PCR method of screening for novel *B. thuringiensis*

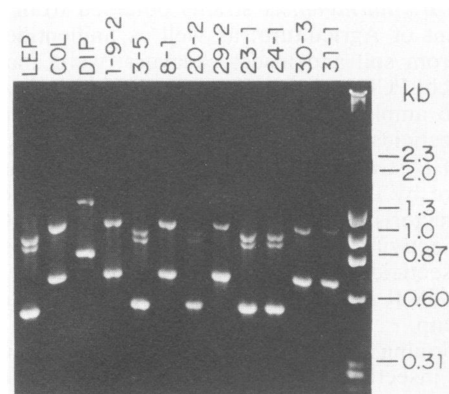

FIG. 2. PCR product profiles of representative unknown *Bacillus* strains. Strains 3-5, 20-2, 23-1, and 24-1 display the typical lepidopteran-active (LEP) PCR product profile of *B. thuringiensis*. Strains 1-9-2, 8-1, 29-2, 30-3, and 31-1 display a coleopteran-active (COL) PCR product profile. As shown in Table 4, the insecticidal activity predicted by the PCR product profile was confirmed by insect bioassay.

TABLE 4. Comparison of insecticidal activity as determined by PCR and bioassay

Unknown strain	PCR product (in bp)	Predicted activity	Bioassay results[a]		
			LEP	COL	DIP
1-9-2	649, 1,060	Coleopteran	−	+	−
3-5	490, 908, 986	Lepidopteran	+	−	−
8-1	649, 1,060	Coleopteran	−	+	−
20-2	490, 908, 986	Lepidopteran	+	−	−
23-1	490, 908, 986	Lepidopteran	+	−	−
24-1	490, 908, 986	Lepidopteran	+	−	−
27-1	490, 908, 986	Lepidopteran	+	−	−
27-3	490, 908, 986	Lepidopteran	+	−	−
29-2	649, 1,060	Coleopteran	−	+	−
30-3	649, 1,060	Coleopteran	−	+	−
31-1	649, 1,060	Coleopteran	−	+	−

[a] LEP, European corn borer; COL, Colorado potato beetle; DIP, *C. pipiens*. −, 0% mortality; +, 100% mortality.

delta-endotoxin genes is not without risk and should be used differently for different screening goals. For instance, if a new isolate contained, in addition to known genes, a novel gene that produced no PCR products, the novel gene would likely be missed. An isolate also could have a novel gene with an identical sequence in the regions of primers made from known genes but have different sequences in other regions important for determining a new insecticidal activity; again, a novel gene would probably go undetected. Also, a lack of identification of a known gene does not necessarily imply a novel strain; not all genes from all known strains have been cloned and sequenced. PCR screening provides no directly interpretable information when the goal of the screening of new *B. thuringiensis* isolates is to find a gene whose product either has a greater specific activity on a given insect or binds a different receptor in the midgut of a particular insect. However, genes shown to be novel by PCR analysis are likely to prove the best candidates for achieving each of these goals.

The PCR analysis of new isolates of *B. thuringiensis* provides a valuable prescreen that permits their prioritization for subsequent insect assays. Since new strains can be isolated more rapidly than they can be assayed on insects, the PCR assay can be used to identify strains with the greatest likelihood of containing novel genes.

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REFERENCES

1. **Barton, K. A., H. R. Whiteley, and N.-S. Yang.** 1987. *Bacillus thuringiensis* δ-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. Plant Physiol. **85**:1103–1109.
2. **Chehab, F. F., and Y. W. Kan.** 1989. Detection of specific DNA sequences by fluorescence amplification: a color complementation assay. Proc. Natl. Acad. Sci. USA **86**:9178–9182.
3. **Fischhoff, D. A., K. S. Bowdish, F. J. Perlak, P. G. Marrone, M. McCormick, J. G. Niedermeyer, D. A. Dean, K. Kusano-Kretmer, E. J. Mayer, D. E. Rochester, S. G. Rogers, and R. T. Fraley.** 1987. Insect tolerant transgenic tomato plants. Bio/Technology **5**:807–813.
4. **Geiser, M., S. Schweitzer, and C. Grimm.** 1986. The hypervariable region in the genes coding for entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the kurhdl gene of subsp. kurstaki HD-1. Gene **48**:109–118.
5. **Hofte, H., J. Seurinck, A. V. Houtven, and M. Vaeck.** 1987. Nucleotide sequence of a gene encoding an insecticidal protein of Bacillus thuringiensis var. tenebrionis toxic against Coleoptera. Nucleic Acids Res. **15**:7183–7783.
6. **Hofte, H., J. Van Rie, S. Jansens, A. Van Houtven, H. Vanderbruggen, and M. Vaeck.** 1988. Monoclonal antibody analysis and insecticidal spectrum of three types of lepidopteran-specific insecticidal crystal proteins of *Bacillus thuringiensis*. Appl. Environ. Microbiol. **54**:2010–2017.
7. **Hofte, H., and H. R. Whiteley.** 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. **53**:242–255.
8. **Kronstad, J. W., and H. R. Whiteley.** 1986. Three classes of homologous *Bacillus thuringiensis* crystal-protein genes. Gene **43**:29–40.
9. **Krywienczyk, J.** 1977. Antigenic composition of delta-endotoxin as an aid in identification of *Bacillus thuringiensis* varieties. Technical report IP-X-12. Canadian Forestry Service, Ottawa, Ontario, Canada.
10. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Meadows, M.** Personal communication.
12. **Rowe, G. E., and A. Margaritis.** 1987. Bioprocess developments in the production of bioinsecticides by *Bacillus thuringiensis*. Crit. Rev. Biotechnol. **6**:87–127.
13. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
14. **Sick, A., F. Gaertner, and A. Wong.** 1989. Nucleotide sequence of a coleopteran-active toxin gene from a new isolate of *Bacillus thuringiensis* subsp. tolworthi. Nucleic Acids Res. **18**:1305.
15. **Travers, R. S., P. A. W. Martin, and C. F. Reichelderfer.** 1987. Selective process for efficient isolation of soil *Bacillus* species. Appl. Environ. Microbiol. **53**:1263–1266.
16. **Vaeck, M., A. Reynaerts, H. Hofte, S. Jansens, M. DeBeuckeleer, C. Dean, M. Zabeau, M. Van Montagu, and J. Leemans.** 1987. Transgenic plants protected from insect attack. Nature (London) **328**:33–37.
17. **Yamamoto, T., I. A. Watkinson, L. Kim, M. V. Sage, R. Stratton, N. Akande, Y. Li, D. P. Ma, and B. A. Roe.** 1988. Nucleotide sequence of the gene coding for a 130-kDa mosquitocidal protein of Bacillus thuringiensis israelensis. Gene **66**: 107–120.