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Contribution of the 65-Kilodalton Protein Encoded by the Cloned Gene *cry19A* to the Mosquitocidal Activity of *Bacillus thuringiensis* subsp. *jegathesan*

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Two new crystal protein genes, *cry19A* and *orf2*, isolated from *Bacillus thuringiensis* subsp. *jegathesan* were cloned and characterized. The *cry19A* gene encodes a 74.7-kDa protein, and the *orf2* gene encodes a 60-kDa protein. Cry19A contains the five conserved blocks present in most *B. thuringiensis* δ-endotoxins. The ORF2 amino acid sequence is similar to that of the carboxy terminus of Cry4 proteins. The *cry19A* gene was expressed independently or in combination with *orf2* in a crystal-negative *B. thuringiensis* host. The proteins accumulated as inclusions. Purified inclusions containing either Cry19A alone or Cry19A and ORF2 together were toxic to *Anopheles stephensi* and *Culex pipiens* mosquito larvae. They were more toxic to *C. pipiens* than to *A. stephensi*. However, inclusions containing Cry19A and ORF2 together were more toxic than inclusions of Cry19A alone but less toxic than the wild-type inclusions of *B. thuringiensis* subsp. *jegathesan*.

Bacillus thuringiensis subsp. *israelensis* and *Bacillus sphaericus* are entomopathogenic bacteria. During sporulation, both produce crystals that are toxic to dipteran larvae after ingestion. *B. thuringiensis* subsp. *israelensis* crystals are composed of four major polypeptides of 135, 125, 68, and 27 kDa; crystals from *B. sphaericus* are composed of only two proteins of 51 and 42 kDa (for a review, see reference 21).

Products from these two bacteria have been successfully used in various countries as a biological control agent for mosquito and blackfly larvae (18, 32). However, in the last three years, field populations of insects have developed resistance to *B. sphaericus* (23, 27). Although no resistance to *B. thuringiensis* subsp. *israelensis* in the field has been observed yet, laboratory selections using cloned *B. thuringiensis* subsp. *israelensis* toxins showed that development of resistance is likely to occur; this is inversely correlated with the number of toxins used for selection (11). Thus, the effectiveness of existing bacteria could be improved by combining different toxins (from *B. thuringiensis* subsp. *israelensis* and/or new mosquitocidal bacteria) in a single organism (*B. sphaericus*, for example). Efforts are now focused on the identification of other mosquitocidal toxins, differing in structure and mode of action from those produced by *B. thuringiensis* subsp. *israelensis* and *B. sphaericus*.

There have been several reports of other mosquitocidal *B. thuringiensis* strains of various serotypes, including *B. thuringiensis* subsp. *jegathesan* 367 (serotype H28a28c), isolated from Malaysia (26). *B. thuringiensis* subsp. *jegathesan* is as toxic as *B. thuringiensis* subsp. *israelensis* to *Anopheles stephensi* but is slightly less toxic to *Aedes aegypti* and *Culex pipiens*. Crystals from *B. thuringiensis* subsp. *jegathesan* contain seven major polypeptides with molecular masses of 80, 70 to 72, 65, 37, 26, and 16 kDa (22). No *B. thuringiensis* subsp. *israelensis*-related gene has been detected in this strain, suggesting that the crystal proteins could be encoded by new classes of toxin genes (22). Only the gene encoding the 80-kDa polypeptide (Cry11B) has been cloned (10). Inclusions containing the Cry11B protein alone were as toxic as wild-type inclusions from *B. thuringiensis* subsp. *jegathesan* to *C. pipiens* and *A. stephensi* and were more toxic than the wild type when tested against *A. aegypti*. Cry11B is not the major component of the *B. thuringiensis* subsp. *jegathesan* inclusions, so there could be other polypeptides in the native crystals involved in toxicity, especially to *A. stephensi* and *C. pipiens*.

This paper reports the isolation, sequencing, and expression of the gene encoding the 65-kDa protein from *B. thuringiensis* subsp. *jegathesan*, hereafter referred to as *cry19A*, and a second gene, *orf2*. The deduced protein sequence of Cry19A contains the five conserved blocks present in all Cry1, Cry3, and Cry4 *B. thuringiensis* δ-endotoxins (12). The deduced ORF2 amino acid sequence is similar to that of the carboxy terminus of the Cry4 proteins. *cry19A* was expressed independently or in combination with *orf2* in a crystal-negative *B. thuringiensis* host. The toxicity of the purified inclusions obtained was tested with three mosquito species.

MATERIALS AND METHODS

Bacterial strains and plasmid. *Escherichia coli* TG1 [K-12 Δ(*lac-proAB*) *supE thi hsdD5*/F′ (*traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ*ΔM15)] was used as the host species. *B. thuringiensis* subsp. *jegathesan* 367 (from the IEBC Collection of the Unité des Bactéries Entomopathogènes) was used for purifying wild-type crystals and DNA for cloning experiments. *B. thuringiensis* subsp. *thuringiensis* SPL407 (serotype H1) was used as a recipient for transformation experiments (16). The shuttle vector pHT315 (2) was used as a cloning vector. *B. thuringiensis* SPL407 was transformed by electroporation as described by Lereclus et al. (16) except that cells were grown in Luria-Bertani medium with shaking at 37°C until the optical density at 650 nm was 0.8. *E. coli* was transformed with plasmid DNA following $CaCl_2$ treatment as previously described (15). The antibiotic concentrations for bacterial selection were 10 μg of erythromycin per ml and 100 μg of ampicillin per ml.

DNA manipulations. Restriction enzymes, Klenow fragment, T4 DNA ligase, and calf intestinal alkaline phosphatase were used as described by Sambrook et al. (25) and as recommended by the manufacturers.

Total DNA was isolated from *B. thuringiensis* subsp. *jegathesan* cells as previously described (24). Plasmid DNA was extracted from *E. coli* by a standard alkaline lysis procedure (3) and further purified with a kit from Qiagen GmbH (Hilden, Germany). The DNA fragments were purified from agarose gels with a Prep A Gene DNA purification matrix kit (Bio-Rad, Hercules, Calif.).

PCR was performed in a volume of 100 μl containing 0.1 nmol of oligonucleotide, 500 ng of DNA, 0.2 mM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), and 2 U of *Taq* polymerase (Gibco-BRL). PCR cycling conditions were as follows: 5 min at 95°C; 26 cycles of 1 min at 42°C,

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2 min at 72°C, and 1 min at 95°C; and 1 cycle of 3 min at 42°C and 4 min at 72°C, in an MJ Research PTC-100 thermocycler.

Hybridization experiments were performed with Hybond N^+ filters (Amersham, Buckinghamshire, United Kingdom). The DNA probes were labeled with peroxidase by using the ECL direct nucleic acid labeling system (Amersham). The DNA sequences were determined with an automatic sequencer (model 373A; Applied Biosystems). Genetics Computer Group sequence analysis software package programs were used (University of Wisconsin, Madison).

Cloning of the *B. thuringiensis* subsp. *jegathesan* 65-kDa toxin gene. The 65-kDa protein from *B. thuringiensis* subsp. *jegathesan* was transferred to Problot membranes (Applied Biosystems), and its amino-terminal sequence was determined (Laboratoire de Microséquençage des Protéines, Institut Pasteur, Paris, France) with an automatic sequencer (model 473; Applied Biosystems). The sequence of the first 15 amino acids (MHYYGNRNEYDILNA) was used to design a degenerate 26-mer oligonucleotide probe (26-mer jeg65 oligonucleotide), ATGCATTATTATGGIAATIGIAATGA. This oligonucleotide corresponds to the sequence between M (position 1) and E (position 9); deoxyinosine was used as a neutral base for all three- or fourfold degenerate positions. PCR was performed on *B. thuringiensis* subsp. *jegathesan* total DNA, using the 26-mer jeg65 oligonucleotide and a *cry4A*-specific oligonucleotide (positions 1128 to 1108 from the ATG initiation codon of the gene [AGGAGTAGTTTGCGCTT TTTC]). The resulting purified 3-kb PCR product was labeled with peroxidase and used as a probe in DNA hybridization experiments with restriction enzyme-digested total DNA from *B. thuringiensis* subsp. *jegathesan*. The probe specifically hybridized to an *Eco*RI-*Pst*I restriction fragment of approximately 3 kb.

Size-selected (2- to 4-kb) *Eco*RI-*Pst*I fragments of *B. thuringiensis* subsp. *jegathesan* 367 DNA were inserted into the *Eco*RI-*Pst*I site of the shuttle vector pHT315. Recombinant colonies of transformed *E. coli* TG1 were tested for hybridization with the labeled probe. The probe hybridized strongly to one colony which contained the pJEG65.1 plasmid. The restriction map of this plasmid was determined (see Fig. 1). It lacks the end of *cry19A*.

PCR was then performed with oligonucleotides D2 (GGATTTACGGGAGG AGATTTGG; positions 1827 to 1848 [see Fig. 2]) and D2R (GATTTTATCTA ATATAAGAGATGG; positions 2153 to 2130 [see Fig. 2]) and total DNA of *B. thuringiensis* subsp. *jegathesan* to obtain a 326-bp PCR probe. This probe, containing the same sequence as the *Cla*I-*Eco*RI fragment of pJEG65.1, was used to identify a 7-kb *Hin*dIII fragment from *B. thuringiensis* subsp. *jegathesan* total DNA. This fragment was then ligated into pHT315 digested with *Hin*dIII to produce the pJEG65.2 plasmid (see Fig. 1), which contains *orf2* and the end of the *cry19* gene.

Subcloning experiments. Plasmid pJEG65.1S was obtained by eliminating a 1.3-kb *Sph*I fragment from pJEG65.1.

Plasmid pJEG65.3 was constructed as follows. A 700-bp *Bam*HI fragment was eliminated from pJEG65.2 to give plasmid pJEG65.2S. Then the 1.7-kb *Hin*dIII fragment from pJEG65.1S was cloned into the pJEG65.2S *Hin*dIII site to give pJEG65.3.

Plasmid pJEG65.4 was constructed by ligating the 3.3-kb *Sph*I fragment from pJEG65.3 into the *Sph*I site of pHT315.

Plasmid pJEG65.5 was obtained by eliminating the 3.1-kb *Cla*I-*Sma*I fragment from pJEG65.3. The *Cla*I site was blunted with Klenow fragment.

Protein analysis. *B. thuringiensis* cells were grown in HCT medium (14) containing erythromycin if required, with shaking at 30°C until cell lysis. Spores and crystals were harvested and washed once in 1 M NaCl and twice in cold double-distilled water. The crystals were further purified on discontinuous sucrose gradients as previously described (29). Crystal protein concentrations were measured by the Bradford assay (Bio-Rad) after alkaline solubilization of the extracts (9). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (13).

Mosquitocidal activity assay. Mosquitoes from colonies of *C. pipiens* (strain Montpellier), *A. aegypti* (strain Bora-Bora), and *A. stephensi* (strain ST15) were reared in the laboratory at 26°C with 80% relative humidity and under a 14-h-day/10-h-night photoperiod. Larvae were reared in dechlorinated water and fed with commercial cat biscuits. Purified inclusions were diluted in glass petri dishes containing 20 ml of deionized water and tested in duplicate against 20 fourth-instar larvae of *C. pipiens* and *A. aegypti* and second-instar larvae of *A. stephensi*. Each bioassay was repeated at least three times. Larval mortality after 48 h was recorded, and concentrations causing 50% mortality were determined by probit analysis.

Nucleotide sequence accession number. The nucleotide sequence data shown below (see Fig. 2) are available in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession no. Y07603.

RESULTS

Cloning experiments. The gene encoding the 65-kDa protein from *B. thuringiensis* subsp. *jegathesan* 367 (designated *cry19A* by the Cry Gene Nomenclature Committee [6a]) was isolated as described in Materials and Methods. A positive clone, *E. coli*(pJEG65.1), was selected; plasmid pJEG65.1 contains a 3.4-kb *Pst*I-*Eco*RI DNA insert for which a restriction map was determined (Fig. 1). The restriction map, hybridization with the 26-mer jeg65 oligonucleotide, and PCR experiments using the 26-mer jeg65 oligonucleotide and universal or reverse oligonucleotide primers showed that the *cry19A* gene was present on the *Sph*I/*Eco*RI fragment. The upstream 1.3-kb *Sph*I fragment was deleted from pJEG65.1 to give plasmid pJEG65.1S (Fig. 1). Sequencing of plasmid pJEG65.1S showed that the *cry19A* gene was incomplete: no stop codon was found. The end of the gene was therefore cloned by inserting a 7-kb *Hin*dIII fragment into plasmid pJEG65.2 (Fig. 1). The complete gene was then obtained by production of plasmid pJEG65.3 (Fig. 1) as described in Materials and Methods.

Sequence analysis. The sequence of pJEG65.3 in the region containing the gene encoding the 65-kDa protein was determined on both strands. Two open reading frames oriented in the same direction and separated by 145 bp were identified (Fig. 2): *cry19A* (1,947 bp) and *orf2* (1,581 bp). A putative ribosome binding site, GGAGG, was identified 7 nucleotides upstream from the start codon of the *cry19A* gene (Fig. 2), and a sequence, AAAGGTTGTG, that could act as a ribosome binding site was identified 6 nucleotides upstream from *orf2* (Fig. 2). ΔGs, calculated by comparison with the gram-positive consensus sequence (17) as described by Tinoco et al. (31), were −60.2 (for *cry19A*) and −50.2 (for *orf2*) kJ/mol. No typical promoter or terminator sequences were identified upstream or downstream from the two coding regions.

Cry19A is a polypeptide of 648 residues with a predicted mass of 74.7 kDa. ORF2 is a protein of 526 residues with a predicted mass of 60 kDa. The deduced amino acid sequences of the Cry19A and ORF2 polypeptides were compared with those of other known *B. thuringiensis* toxins. The Cry19A protein showed no similarity to the Cry11B protein of *B. thuringiensis* subsp. *jegathesan* (10), and the only similarities to *B. thuringiensis* toxins were restricted to the five blocks conserved in most of the δ-endotoxins (12) (Fig. 2). The Cry4A and Cry10A δ-endotoxins have the sequences most similar to Cry19A, but the similarity is confined to the first 51 amino acids (60% identity). No other significant similarity was found. This protein therefore belongs to a novel class of δ-endotoxins. The deduced ORF2 amino acid sequence was 65% identical to the carboxy terminus of the Cry4 protein (Fig. 3) and 85% identical to the known partial sequence of ORF2 from *B. thuringiensis* subsp. *israelensis* (30). ORF2 from *B. thuringiensis* subsp. *jegathesan* contains a stretch of 41 amino acids absent from the carboxy terminus of the Cry4 proteins.

Expression of *cry19A* and *orf2* in a crystal-negative strain of *B. thuringiensis*. Plasmids pJEG65.5 (Fig. 1), which contains both the *cry19A* and the *orf2* genes, and pJEG65.4 (Fig. 1), which contains the entire *cry19* gene but only an incomplete *orf2* gene, were constructed as described in Materials and Methods. These two plasmids and the vector pHT315 were independently introduced by electroporation into the crystal-negative strain *B. thuringiensis* 407 to determine the toxicity of their products. Recombinant 407(pJEG65.4), 407(pJEG65.5), and 407(pHT315) cells sporulated well in HCT medium and lysed after 48 h of growth. Small inclusions were visible under the light microscope for recombinants 407(pJEG65.4) and 407(pJEG65.5). These inclusions were purified on discontinuous sucrose gradients from recombinant clones collected by centrifugation at the end of sporulation. Purified inclusions were subjected to SDS-PAGE followed by staining with Coomassie brilliant blue. The banding patterns were compared with those produced by wild-type *B. thuringiensis* subsp. *jegathesan* (Fig. 4). The major polypeptide in inclusions purified from recombinant strain 407(pJEG65.4) was approximately 65 kDa (Fig. 4, lane 2), the same size as that found in the crystals

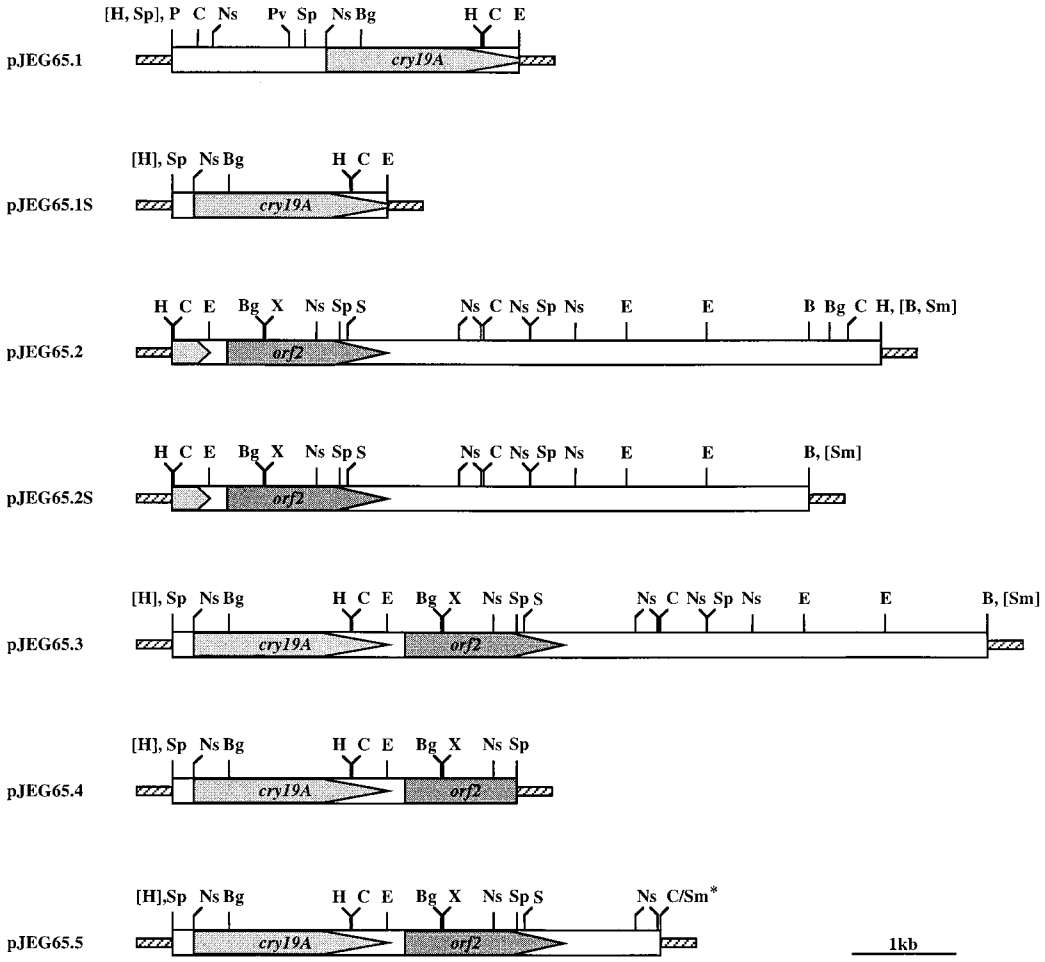

FIG. 1. Restriction map of plasmid pJEG65.1 and derivatives containing all or part of the *cry19A* gene. The positions and directions of transcription of the genes are indicated. Hatched bar, vector pHT315; asterisk, both sites lost; brackets, sites from the polylinker. The sites indicated for the polylinker are only those used for the plasmid constructions. Abbreviations: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Ns, *Nsi*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; Sm, *Sma*I; Sp, *Sph*I; X, *Xba*I.

of the toxic wild-type strain 367 (Fig. 4, lane 1). Three major products of 65, 66, and 67 kDa were detected in inclusions purified from recombinant strain 407(pJEG65.5) (Fig. 4, lane 3). The amino-terminal sequence of these polypeptides was determined by using the first five amino acids. The first five amino acids of the 65-kDa polypeptide were identical to the first five amino acids of the Cry19A predicted sequence. The two other proteins had the same amino-terminal sequence, identical to that of the *orf2* product.

Larvicidal activity of the *cry19A* and *cry19A-orf2* products. Purified inclusions from strains 407(pJEG65.4) and 407 (pJEG65.5) were assayed for activity against larvae of *A. aegypti*, *A. stephensi*, and *C. pipiens* as described in Materials and Methods. Crystals purified from wild-type strain *B. thuringiensis* subsp. *jegathesan* 367 were also tested. The activity of inclusions containing Cry19A alone or both Cry19A and ORF2 was restricted to *A. stephensi* and *C. pipiens* larvae (Table 1), with greater toxicity to *C. pipiens*. None of the recombinant inclusions were toxic to *A. aegypti* larvae at a concentration of 400 μg/ml. Inclusions containing both Cry19A and ORF2 were more toxic than those containing only Cry19A, about 24 times more toxic for *A. stephensi* and 55 times more toxic for *C. pipiens*. Inclusions containing Cry19A and ORF2 were less toxic than wild-type inclusions, about 28 times less toxic for *A. stephensi* and 9 times less toxic for *C. pipiens.*

DISCUSSION

We report the cloning and characterization of two new *B. thuringiensis* genes, *cry19A* and *orf2*, from *B. thuringiensis* subsp. *jegathesan*. The *cry19A* gene encodes a protein of 74.7 kDa, and the *orf2* gene codes for a protein of 60 kDa. The Cry19A protein contains the five blocks that are conserved in the amino-terminal region of most δ-endotoxins but has no other significant similarity to known toxins, other than the first 51 amino acids of Cry4A and Cry10A. This protein therefore belongs to a novel class of δ-endotoxins. ORF2 is very similar to the carboxy-terminal region of the 130-kDa toxins. Thus, Cry19A and ORF2 have elements of primary structure in common with the 130-kDa δ-endotoxins. Cry19A is similar to the variable amino-terminal half, and ORF2 is similar to the conserved carboxy-terminal half. This structure is comparable to those of Cry10A and ORF2 in *B. thuringiensis* subsp. *israelensis*, in which Cry10A has a sequence similar to the amino-terminal half of δ-endotoxins and contains the five conserved blocks. The partial sequence of ORF2 is similar to the carboxy-termi-

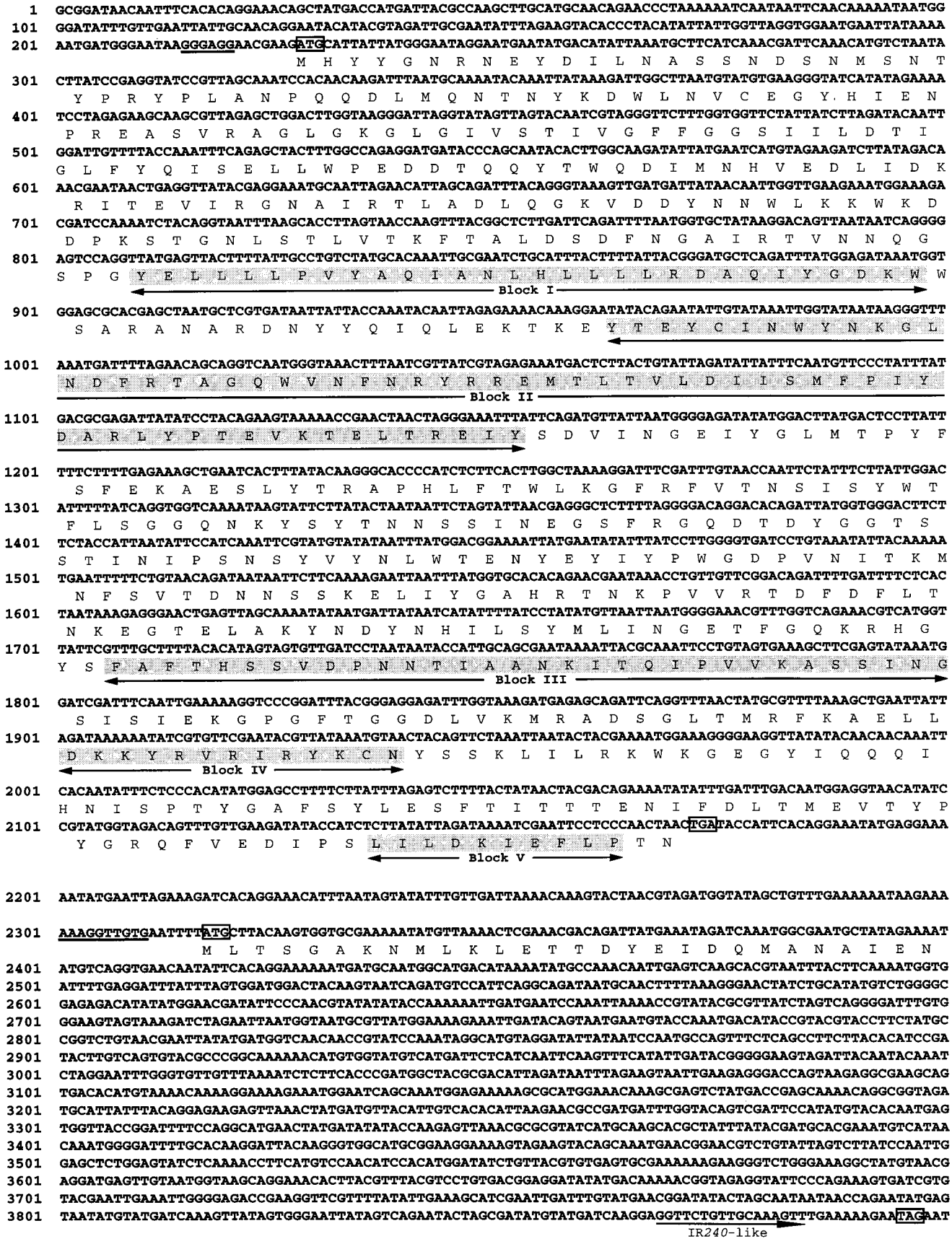

FIG. 2. Nucleotide sequences of the *cry19A* and *orf2* genes and amino acid sequence of Cry19A. The first amino acids of ORF2 are indicated. The putative ribosome sites (underlined), the start and stop codons (boxed), the five conserved blocks of Cry19A (shaded), and the inverted repeat sequence of the IS*240*-related element (arrow) are indicated.

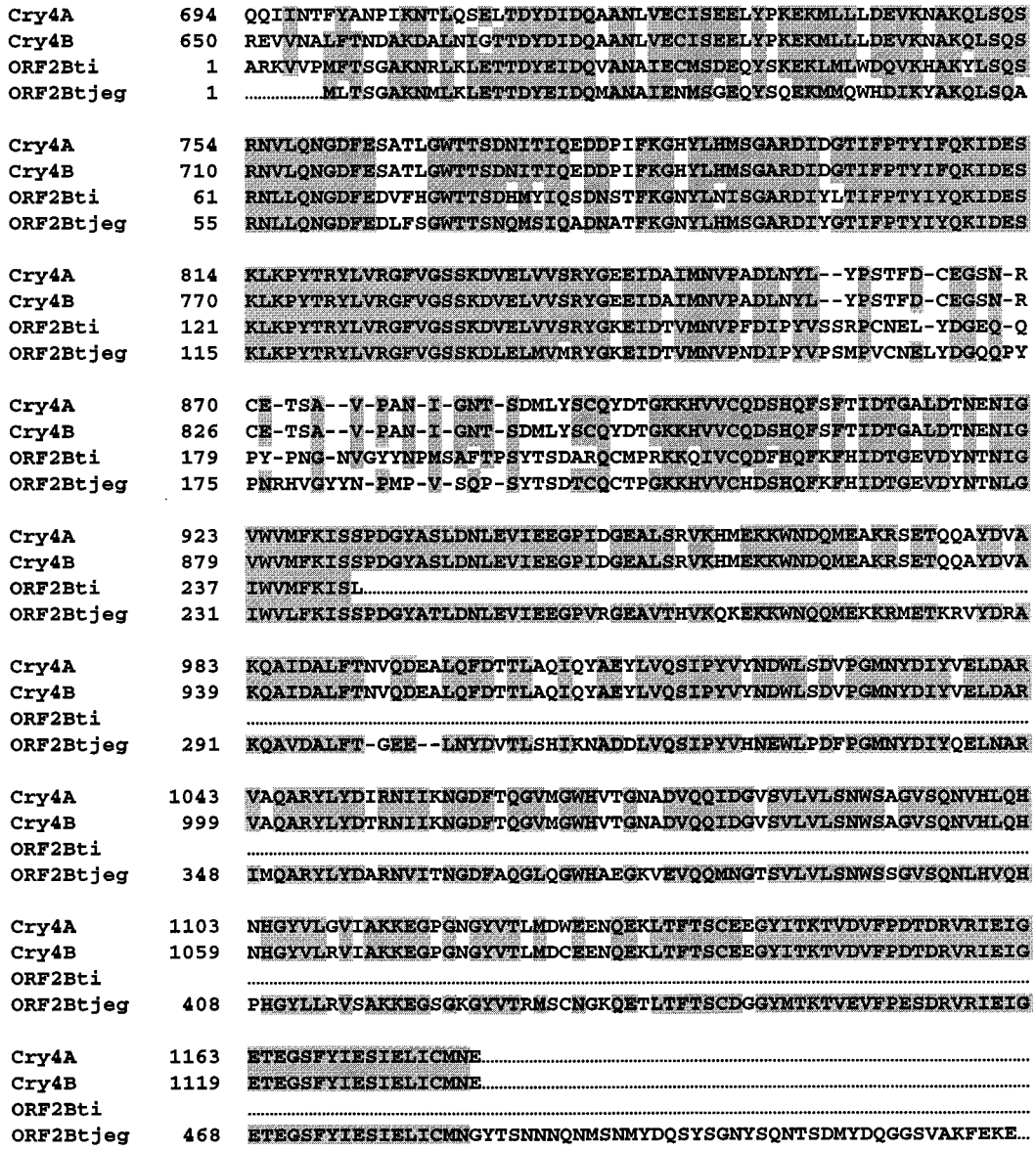

FIG. 3. Comparison of ORF2 protein sequence from *B. thuringiensis* subsp. *jegathesan* (ORF2Btjeg) with carboxy-terminal part of Cry4A and Cry4B and partial known sequence of ORF2 from *B. thuringiensis* subsp. *israelensis* (ORF2Bti). Residues which are identical and functionally similar among at least three δ-endotoxins are indicated (shaded) (conservative-replacement groupings are as follows: I, L, V, and M; D, E, Q, and N; K and R; T and S; G and A; and F and Y). Dots indicate that no sequence was available.

nal half of a 130-kDa toxin (8, 30). The events leading to this configuration are unknown, but it may have evolved through the insertion of a DNA fragment into a gene that would otherwise encode a 130-kDa protein. This insertion could be the result of a transposition of a mobile element. We identified an open reading frame oriented in the opposite direction in the vicinity of *orf2* (data not shown). Comparison of the deduced amino acid sequence with others in the Swiss-Prot data bank showed similarities with the putative transposase of insertion sequence IS*240* from *B. thuringiensis* subsp. *israelensis* (7). The inverted repeat sequence of this mobile element is present within the end of the coding frame of *orf2* (Fig. 2). This could explain the stretch of 41 amino acids present in ORF2 and absent from the carboxy-terminal region of the Cry4 proteins. Two copies of IS*240* flank the *cry4A* gene in *B. thuringiensis* subsp. *israelensis* (4, 7). One copy of the IS*240*-related insertion element was found upstream from the *cry11B* gene in *B. thuringiensis* subsp. *jegathesan* (10), and the other was found close to the *cyt1Ab1* gene from *B. thuringiensis* subsp. *medellin* (28). IS*240*-related sequences are present in all the diptera-toxic strains of *B. thuringiensis* tested (24). Although no transposition event has yet been demonstrated for this element, IS*240*-like insertion sequences may account for the dispersion of toxin genes in various *B. thuringiensis* strains.

The regulation of *cry19A* and *orf2* gene expression has not yet been studied. Sequence analysis of the region upstream from *orf2* did not detect any *B. thuringiensis* consensus promoter. The two open reading frames are separated by only 145 bp and are oriented in the same direction, so they could be part of the same operon. Experiments are in progress to determine

TABLE 1. Mosquitocidal activities of purified inclusions from *B. thuringiensis* strains

Strain	Inclusion composition	LC_{50} against[a]:	
		A. stephensi	*C. pipiens*
B. thuringiensis subsp. *jegathesan* 367	Wild type	37 (33–40)	20 (18–22)
407(pJEG65.4)	Cry19A	24,926 (19,580–31,732)	10,282 (8,010–13,521)
407(pJEG65.5)	Cry19A + ORF2	1,039 (842–1,260)	187 (164–213)

[a] LC_{50}, 50% lethal concentration (in nanograms per milliliter) after 48 h, determined by probit analysis. Confidence limits are indicated in parentheses.

the length of this putative operon and to identify the sigma factor(s) involved in its expression.

Inclusions containing either Cry19A alone or Cry19A and ORF2 together were toxic to *C. pipiens* and *A. stephensi*. However, inclusions containing only Cry19A were less toxic than inclusions containing both Cry19A and ORF2. This suggests that either Cry19A and ORF2 are both toxic and could act in synergy, as previously described for *B. thuringiensis* subsp. *israelensis* proteins Cry4A, Cry4B, Cry11A, and Cry10A (1, 6, 8, 9, 20), or Cry19A is the only toxic polypeptide and ORF2 interacts with Cry19A at a transcriptional or posttranscriptional level, increasing the toxicity. The second hypothesis is more likely, as Cry19A is poorly expressed on its own and appears degraded in SDS-PAGE analysis. More Cry19A is produced when ORF2 is produced simultaneously (data not shown). The presence of *orf2* may stabilize the mRNA, or ORF2 may act as a chaperone to increase the stability of Cry19A. The carboxy-terminal region of the 130-kDa toxins, similar to ORF2, is not essential for toxicity of Cry4B. Only the amino-terminal fragment, similar to Cry19A in terms of structure (five conserved blocks), retained full activity (5, 8, 19). This suggests that the carboxy-terminal region is involved in stability rather than toxicity. We are investigating the effect of ORF2 on the toxicity and stability of Cry19A.

Although the combination of Cry19A and ORF2 is more toxic than Cry19A alone, it is less toxic than the wild-type inclusions of *B. thuringiensis* subsp. *jegathesan*. It was also totally inactive against *A. aegypti*, which is sensitive to the wild-type crystals. This shows that several polypeptides may be responsible for *B. thuringiensis* subsp. *jegathesan* activity. Cry11B, the first characterized polypeptide from *B. thuringiensis* subsp. *jegathesan*, is highly toxic to *A. aegypti*, *C. pipiens*, and *A. stephensi* (10). The spectra and levels of activity of Cry11B and of Cry19A plus ORF2 are different. Thus, the high toxicity of the wild-type *B. thuringiensis* subsp. *jegathesan* crystals may be the result of additive or synergistic interactions between these polypeptides. Further studies are needed to investigate the nature of these interactions.

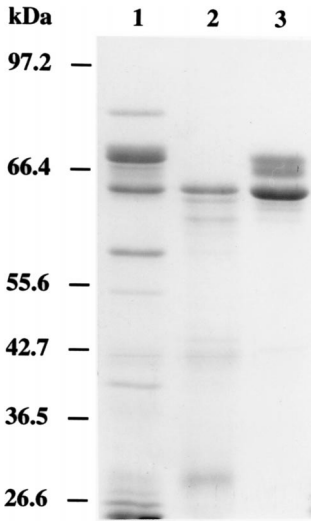

FIG. 4. Protein analysis of Cry19A inclusions and Cry19A plus ORF2 inclusions. Purified inclusions were subjected to SDS–10% PAGE and stained with Coomassie brilliant blue. Lanes: 1, wild-type *B. thuringiensis* subsp. *jegathesan* 367; 2, clone 407(pJEG65.4); 3, clone 407(pJEG65.5). The molecular masses of standard protein markers are indicated on the left.

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