RESEARCH NOTE

Cloning of a Fragment of the Gene *cryIVB* from *Bacillus thuringiensis* var. *israelensis* Coding for the Aminoterminal Segment of a 130 kDa Larvicidal Endotoxin

Jörg H Saar, Wim M Degrave*

Departamento de Bacteriologia *Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Key words: *Bacillus thuringiensis* var. *israelensis* - toxins

During sporulation, *Bacillus thuringiensis* var. *israelensis* (Bti) produces a parasporal crystal that is highly toxic to certain dipteran larvae. This finding led to the use of this microorganism as a biological pest control agent, especially in third world countries, where the control of mosquito populations limits the spread of severe human diseases, such as dengue fever, malaria, yellow fever, viral encephalitis and filariasis.

Biological vector control with *B. thuringiensis* var. *israelensis* and *B. sphaericus* strains has several advantages when compared to the commonly used chemical pesticides, as for example high selectivity of the toxic effects of the *Bacillus* crystal proteins together with low environmental hazard. Resistance problems are limited due to the presence of several different toxin proteins in the *Bacillus* crystals.

There are, however, a series of drawbacks that have so far limited the application of entomopathogenic *Bacillus* as a mosquito control agent. Amongst them, the most important factor is the rapid sedimentation of bacterial spores after their application onto the water surface (EW Davidson et al. 1984, *Appl Environm Microbiol 47:* 125-129).

With the objective to overcome this problem, insecticidal toxin genes of both *Bacillus* species have been transferred into cyanobacteria, such as *Agmenellum quadruplicatum* (RC Murphy & JR Stevens Jr 1992 *Appl Environm Microbiol 58:* 1650-1655) and *Synechococcus* sp. (E Soltes-Rak et al. 1993 *Appl Environm Microbiol 59:* 2404-2410). The gram negative bacterium *Caulobacter crescentus* was equally used as a host, with the objective to achieve toxin expression at the natural breeding sites of target larvae by microorganisms that are part of the aquatic environment (T Thanabulu et al. 1992 *Appl Environm Microbiol 58:* 905-910).

The choice of *C. crescentus* as a host for crystal protein genes was due to (1) its wide distribution in every type of aquatic environment, (2) the fact that the microorganism is found predominantly at or close to the water surface, where the target larvae feed, and (3) its ability to grow in environments with low nutrient concentration (Thanabulu et al. *loc. cit.*).

However, all studies quoted above report a low toxicity of the recombinant cells. Therefore, we considered the possibility of obtaining recombinant *C. crescentus* strains with increased toxicity to *Aedes* and *Anopheles* larvae by cloning and expression of *B. thuringiensis* var. *israelensis* toxins as fusion proteins with the microorganism's well characterized surface-layer protein (J Smit et al. 1992 *J Bacteriol 174:* 6527-6538).

We have cloned a 2058 bp fragment of the gene *cryIVB* from *B. thuringiensis* var. *israelensis* in *Escherichia coli* strain DH5αF'. This fragment (denominated *cryBF*) codes for the amino terminal segment of the Bti 135 kDa endotoxin and has been shown to retain most of its larvicidal activity (A Delécluse et al. 1988 *Mol Gen Genet 214:* 42-47).

The fragment was amplified by the polymerase chain reaction (PCR) from total DNA isolated from *B. thuringiensis* var. *israelensis* strain 4Q2-72, a derivative of reference strain Bti IPS-82 cured of all extrachromosomal elements except for a single 72 MDa plasmid coding for all of the microorganisms crystal genes.

Primers for PCR amplification were designed based on the nucleotide sequence of gene *cryIVB* from Bti 4Q2-72 (S Tungpradubkul 1988 *Nucl Acids Res 166:* 1637-1638). PCR was carried out under the following conditions: 9 cycles: 95°C, 1 min; 40°C, 2 min; and 72°C, 3 min, followed by 26 cycles: 95°C, 1 min; 40°C, 2 min; and 72°C, 7 min. The primers used were: Forward primer: 5'-CAT GCC ATG GGG AAT TCA GGC TAT CCG TTA GCG-3' Reverse primer: 5'-CGG AAT TCA AGA AAT ACA TTC CAC AAG ATT TGC GGC-3'.

The forward primer preserves the natural EcoRI site at the beginning of the coding region. An additional NcoI site was put upstream including the start codon. The reverse primer contains a TGA stop codon overlapping with a second EcoRI site that guarantees chain termination upon gene translation.

Amplification yielded a single signal at 2.1 kb, as judged by agarose gel electrophoreses. Amplicon DNA was digested with EcoRI and in-

This work was supported by CNPq
Received 24 June 1994
Accepted 2 January 1995

serted into the EcoRI site of cloning vector pBluescript II SK (+). The resulting plasmid was used to transform *E. coli* made competent by the $CaCl_2$ method. The vector's blue/white screening system was used to detect recombinant clones on LB/Amp/X-Gal/IPTG agar plates.

Two clones were identified harboring the 2.1 kb insert. By sequencing the 5'- and 3'-extremities of both inserts, we could demonstrate that the nucleotide sequence in these regions of both recombinants was identical to the corresponding published *cryIVB* sequence. One recombinant, denominated *E. coli* Q5, contained the insert *in frame* with the initial β-galactosidase sequence of the cloning vector. This strain should therefore be able to express the *B. thuringiensis* var. *israelensis* toxin fragment as a *lacZ/cryBF* fusion protein in conditions of induction of the lac-operon. The determination of the larvicidal activity of recombinant *E. coli* Q5 and the detection of its fusion protein using denaturating SDS polyacrylamide gel electrophoresis is now underway.

Our long-term objective is the expression of the *cryBF* fragment alone or in combination with other toxin genes in *C. crescentus*. The construction of translational fusion proteins of, for example, *cryBF* with the cells surface layer protein (*rsaA* gene product; J Smit et al. 1992 *J Bacteriol 174:* 6527-6538) should permit the expression of the *Bacillus* endotoxin on the cell surface. By limiting intracellular proteolysis and facilitating the accessibility of the toxin determinants to the binding sites in the larvae's midgut, such a *cryBF/rsaA* fusion protein could be a means to considerably increase the larvicidal potential of recombinant *Caulobacter* cells.