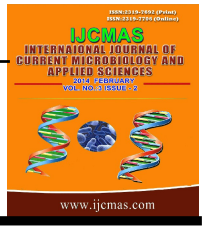
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 355-358**
http://www.ijcmas.com



Original Research Article

Insecticidal properties of Cry9Aa5 crystal spore preparations

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K e y w o r d s

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae); *Bacillus thuringiensis*; Cry9Aa5

A B S T R A C T

Cry9Aa5, produced by *Bacillus thuringiensis* is reported to be more toxic for *Spodoptera exigua* (beet armyworm) as compared to Cry9Aa1. In our previous studies we have demonstrated insecticidal properties of Cry9Aa5 produced in recombinant *E. coli* strain. However the molecular basis of Cry9Aa5 increased activity against *S. exigua* remains still unclear. In order to study insecticidal properties of Cry9Aa5 in more details, *cry9Aa5* gene was cloned and expressed in recombinant *B. thuringiensis* strain. Toxicity of recombinant Cry9Aa5 crystal-spore preparations toward *S. exigua*, was assessed and compared to the activity of crystal-spore preparations of the natural Bt isolate 8S4, harboring the *cry9Aa5* gene.

Introduction

Spodoptera exiqua (Hübner) is a destructive insect pest in many economically important crop species (Wilson 1932, Azidah and Sofian-Azirun 2006) causing significant yield loss. Long term use of chemical insecticides for *S. exigua* control, has resulted in rapid development of insect resistance to most of commercially available chemical products (Hernandez-Martinez, Ferre et al. 2009). Delta-endotoxins produced by the entomopathogenic bacterium *Bacillus thuringiensis* are among the biological alternatives for integrated insect pest management. However the number of *S. exigua*-active delta-endotoxins is limited to the Cry1C, Cry1D, Cry1F, and Cry9C classes (Lambert, Buysse et al. 1996, de Maagd, Weemen-Hendriks et al. 2000, Hernandez-Martinez, Ferre et al. 2008). A recent study (Naimov, Nedyalkova et al. 2014) demonstrated increased *S. exigua* toxicity of a novel Cry9Aa5 when produced in recombinant *E. coli*. Until recently the research on Cry9Aa5 delta-endotoxin was limited and the molecular basis of increased activity against *S. exigua* remained unclear.

In this study we have investigated crystal formation and insecticidal properties of Cry9Aa5 crystal spore preparations. Cry9Aa5 encoding gene was cloned into a shuttle vector and expressed in acrystalliferous strain of *B. thuringiensis.* Insecticidal properties of Cry9Aa5 crystal-

spore preparations were assessed and compared to activity of crystal-spore preparations produced by natural Bt isolate 8S4, harboring the same *cry* gene.

Materials and Methods

Shuttle vectors and *B. thuringiensis* transformation.

The vector pSB629, and the shuttle vector pSB634 used in this study, have been described before (de Maagd, Kwa et al. 1996, Sasaki, Asano et al. 1996) and were kindly provided by T. Yamamoto. To clone the *cry9Aa5* gene between the *cry1Ca* promoter and *cry1Ac* terminator, the *Nco*I-*BamH*I (*cry1Ca*) fragment of pSB629 was replaced by the *Nco*I-*BamH*I fragments of pCIz1, described earlier (Naimov, Nedyalkova et al. 2014). The resulting plasmid (pSB629-9Ax) was digested with *Apa*I-*Sac*I, and the expression cassette was cloned into the *Apa*I-*Sac*I sites of pSB634 giving pCIz2. The shuttle vector was introduced via *E. coli* strain GM2163 (*dam, dcm, hsdR*) into the *cry*-negative *B. thuringiensis* strain Bt51 by electroporation, as described previously (He, Liu et al. 2000). Crystal production and purification were performed as described earlier (Naimov, Martens-Uzunova et al. 2006). Protein concentrations were estimated in duplicate by SDS-polyacrylamide gel electrophoresis using a standard curve of bovine serum albumin. Protein bands density was estimated using Image Studio Software (LI-COR, USA).

Spodoptera exigua bioassay

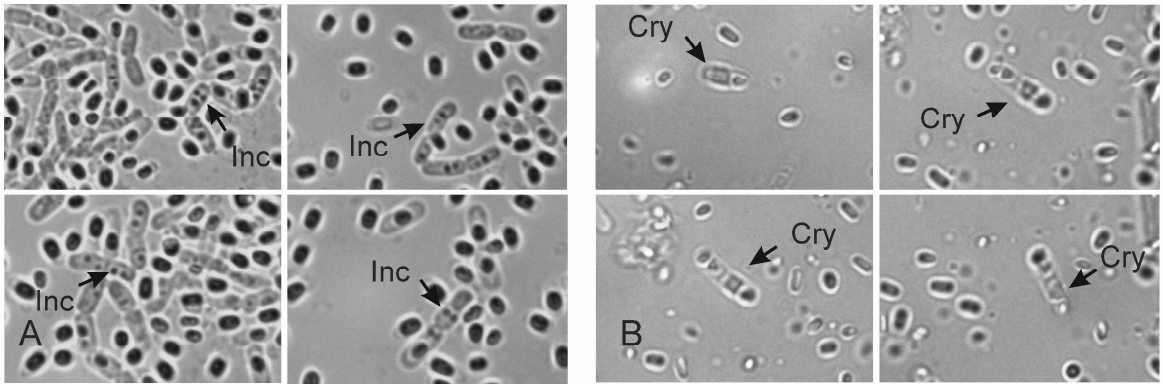
For the *Spodoptera exigua* bioassay, a diet surface contamination assay with neonate larvae was used with an artificial diet, as described earlier (Boncheva, Dukiandjiev et al. 2006). *S. exigua* eggs were purchased from Andermatt Biocontrol AG, Switzerland. The larvae mortality was scored after 4 days at 28 °C. The concentrations causing 50% mortality (LD_{50}) and their 95% fiducial limits were determined by Probit analysis of results from three or more independent experiments with five toxin dilutions each, using the PoloPC program (Russel, Robertson et al. 1977).

Results and Discussion

To assess the insecticidal potency of Cry9Aa5 crystal spore preparations against *S. exigua* a comparison of insecticidal activity of preparations of the native 8S4 isolate and the recombinant Bt strain expressing only *cry9Aa5* was conducted.

All Cry9Aa5 crystal-spore preparations used in this study were produced by small-scale fermentation in 0.5 L flasks. Both the recombinant *B. thuringiensis* strain as well as the isolate 8S4 sporulated, and formed inclusion bodies, crystalline or otherwise. As it is shown in Figure 1, when produced in a recombinant strain, Cry9Aa5 forms regular crystals (panel A), while under the same conditions the native 8S4 isolate formed inclusion bodies with no regular shape (panel B). Although the light microscopy of Bt crystal-spore preparations at magnification 1000x did not allow precise determination of the crystal shape, the Cry9Aa5 inclusions produced by isolate 8S4 were clearly different from the regular crystalline structures formed in the recombinant strain. This observation might be explained by the crystal formation dynamics and the presence, respectively

Figure.1 Light microscopy of Cry9Aa5 crystals/ inclusions produced in natural *B.thuringiensis* isolate 8S4 (panel A) and in recombinant *B. thuringiensis* Bt51 (panel B). Sp: spore; Inc: inclusion bodies; Cry: crystal



absence of additional protein components in the inclusions from the native strains, such as chaperones or other crystal proteins (Crickmore and Ellar 1992, Shi, Zeng et al. 2008, Diaz-Mendoza, Bideshi et al. 2012).

The insecticidal activity of crystal-spore preparations against *S. exigua* was tested in a diet surface bioassay. Both native and recombinant Cry9Aa5 crystal spore preparations were tested and LD50 values of 1322.4 ng/cm^2 (95% fiducial limits 825.4-1654.7 ng/cm^2) and 1227.4 ng/cm^2 (95% fiducial limits 1002.5-1649.9 ng/cm^2) respectively were calculated. Comparison between toxicity of Cry9Aa5 expressed by the native 8S4 strain and recombinant Bt51 strains showed no statistically significant difference in LD50 values for both preparations. LD50 values calculated for both Bt preparations were similar to LD50 value for *E. coli* purified Cry9Aa5 protoxin (774.0 ng/ cm2, 95% fiducial limits 657.4-954.5 ng/cm^2) reported earlier (Naimov, Nedyalkova et al. 2014). Based on this finding we conclude that Cry9Aa5 by itself is the only toxicity determinant required for insecticidal activity of the native Bt 8S4 strain against *S. exigua*.

Acknowledgement

We would like to acknowledge COST 862 action: Bt toxins for insect control. This research was partially supported by the FP7 BioSUPPORT project and the Bulgarian Science Fund.

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