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Bacillus thuringiensis ssp. *galleriae* simultaneously produces two δ-endotoxins differing strongly in primary structure and entomocidal activity

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Strain 11-67 of *B. thuringiensis* ssp. *galleriae* produces two entomocidal proteins of molecular mass 130 kDa. Limited proteolysis of these proteins – protoxins – yields the 'true' toxins of molecular mass 65 kDa which are drastically different with respect to their charges at pH 8.6, immunological properties and toxicity (host range). One of the proteins – the 'negative' component – is toxic for *Lymantria dispar* larvae and shows 65% homology when compared with *B. thuringiensis* ssp. *kurstaki* δ-endotoxins. The other – the 'positive' component – is toxic for *Galleria mellonella* larvae. Its N-terminal sequence of 11 amino acid residues is homologous with the *B. thuringiensis* ssp. *israelensis* and *san diego* endotoxins known to be toxic for Coleoptera and Diptera but not for Lepidoptera. Hence, *B. thuringiensis* subspecies may produce simultaneously δ-endotoxins differing substantially in structural features and host range.

Entomocidal crystal; δ-Endotoxin; (*B. thuringiensis*, Diptera, Lepidoptera, Coleoptera)

1. INTRODUCTION

Bacillus thuringiensis is known to be pathogenic for larvae of Lepidoptera, Diptera and Coleoptera. Its entomocidal action is caused by a protein –δ-endotoxin – that forms crystalline inclusion bodies within the cell during its sporulation. The entomocidal crystals produced by various subspecies of *B. thuringiensis* and differing in specificity of action (host range) are mainly formed by proteins (protoxins) of 130–145 kDa. These proteins possess a similar molecular organization and contain biologically active N-terminal domains ('true toxins') of ~70 kDa [1].

Comparison of the primary structures of three *B. thuringiensis* ssp. *kurstaki* δ-endotoxins as well as those produced by the subspecies *alesti, sotto, berliner* and *aizawai* revealed a common pattern of structural organization [2]: they contain a hypervariable region (residues 347–650) accumulating approx. 90% of all amino acid exchanges. The C-terminal halves of all these endotoxins as well as their first 346 residues are highly homologous.

This paper is aimed at studying in detail the entomocidal proteins produced by *B. thuringiensis* ssp. *galleriae*.

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2. MATERIALS AND METHODS

B. thuringiensis ssp. *galleriae*, strain 11-67, kept in the bacterial collection of this institute, was used throughout. Cultivation of the bacteria and isolation of the crystals as well as N-terminal domains (true toxins) have been described in [1]. The fraction containing the N-terminal domain was freed from NaCl by dialysis and the mixture was then submitted to chromatography on a DEAE-cellulose column (1.6 × 30 cm) in 10 mM sodium carbonate buffer (pH 8.6) containing 2 M urea.

Electrophoresis in 0.1% SDS was performed on 7.5% polyacrylamide slab gels (10 × 20 cm) at 150 mA and 150 V using Coomassie R-250 to visualize protein bands. Rocket immunoelectrophoresis was performed in 50 mM veronal buffer (pH 8.6) on 1% agarose containing antiserum to the solution of crystals produced by strain 11-67 of *B. thuringiensis* ssp. *galleriae*.

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Amino acid analysis and sequencing of proteins were performed as in [2].

Toxicity of preparations was evaluated by inhibition of *Galleria mellonella* larval weight gain [3] as well as being assessed from mortality of *Lymantria dispar* larvae.

3. RESULTS AND DISCUSSION

The solution of crystals formed by *B. thuringiensis* ssp. *galleriae*, strain 11-67, was found to contain one protein band as judged from SDS gel electrophoresis of molecular mass 130 kDa, whereas rocket immunoelectrophoresis (pH 8.6) vs antiserum to the initial solution of crystals gave a double peak travelling towards the anode. Tryptic digestion of this material at pH 8.5 gave, as usual, the true toxin(s) of 65 kDa, whereas the C-terminal part of the protoxin was converted into a mixture of short peptides. Rocket immunoelectrophoresis of this solution at pH 8.6 showed the presence of two peaks. One peak migrated towards the anode (the 'negative' component), the other towards the cathode (the 'positive' component).

To separate both components ion-exchange chromatography on DEAE-cellulose at pH 8.6 in 2 M urea was performed. Under these conditions the positive component appeared in the breakthrough, whereas the fraction eluted with 0.16 M NaCl contained practically pure negative component.

The isolated proteins differ drastically in host ranges. The positive component was toxic for *G. mellonella* larvae, causing 100% inhibition of larval feeding when added to the food at a concentration of 2 μg/g. The negative component produced 90% inhibition of larval weight gain only at 55 μg/g. In contrast, the negative component was found to be substantially more toxic for *L. dispar* larvae: the LC_{50} values for the negative and positive components amounted to 1.0 and 55 μg/g, respectively.

These components do not share common antigenic determinants: double immunodiffision vs antiserum to the total protein solution produced no interference. Neither toxin cross-reacted when tested with antisera to endotoxins of the *B. thuringiensis* ssp. *kurstaki* group (subspecies *alesti* and *dendrolimus*) or that of subspecies *thuringiensis* (str. *insectus*) and *israelensis*.

The N-terminal sequences shown in table 1 were

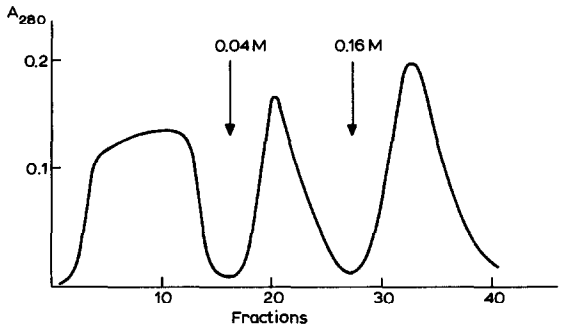

Fig.1. Chromatography of N-terminal domains of δ-endotoxin from *B. thuringiensis* ssp. *galleriae* (strain 11-67) on a DEAE-cellulose column (1.6 × 30 cm). Protein solution in 1 mM sodium carbonate buffer (pH 8.6) containing 2 M urea was applied to the column and equilibrated with the same buffer. Elution was carried out with the same buffer containing 0.04 and 0.16 M NaCl.

established for the negative and positive components by automated Edman degradation.

16 our of 25 amino acid residues determined for the N-terminal sequence of the negative component coincided with those in the N-terminal sequence of δ-endotoxins of the *B. thuringiensis* ssp. *kurstaki* group which indicates the relatedness of these structures. On the other hand, 35% of amino acid substitutions in the region which is strictly conservative in all endotoxins of the *B. thuringiensis* ssp. *kurstaki* type show substantial divergence of these structures. This casts serious doubts on the established view that the N-terminal sequences of the true toxins are strictly conservative [2].

Whereas the N-terminal sequence of the positive component showed no similarity with the structure of δ-endotoxins of the *B. thuringiensis* ssp. *kurstaki* group, it turned out to be homologous (~60%) with those of *B. thuringiensis* ssp. *israelensis* and *san diego* [4–7]. Although the homology of the latter two endotoxins is not that high when the whole sequences are aligned, all three sequences might be considered as belonging to a separate group. It should be mentioned that the positive component is the only member of this group that possesses activity against Lepidoptera.

Hence, strain 11-67 of *B. thuringiensis* ssp. *galleriae* produces simultaneously two entomocidal proteins of equal molecular mass, 130 kDa, one of which (the negative component precursor) is struc-

Table 1
N-terminal sequences of the two endotoxin components

Type of endotoxin		N-terminal sequence
ssp. *galleriae* 'negative' component		LETGNTVADISLGLTNFLYSNFVSG
ssp. *kurstaki, sotto, alesti, aizawai, berliner* endotoxins		29 IETGYTPIDISLSLTQFLLSEFVPG
ssp. *galleriae* 'positive' component		YPLANNPYSQA
ssp. *israelensis* 70 kDa	[4]	29 YPLANKP-NQPLKNTNYKDWLNVCQDNQQYG
ssp. *israelensis* 130 kDa	[5]	29 YPIENSP-KQLLQSTNYKDWLNMCQQNQQYG
ssp. *israelensis*[a] 130 kDa	[6]	5 YPLANDL-EGSMKNTNYKDWLAMCENNQQYG
ssp. *san diego*[a]	[7]	35 YPLAETP-NPTLEDLNYKEFLRMTADNNTEA

[a]Sequences apparently coded by two different genes

turally related to endotoxins of the *B. thuringiensis* ssp. *kurstaki* group, whereas the other, the precursor of the positive component, differs strongly from the former and is responsible for the toxicity of the subspecies crystals for *G. mellonella* larvae.

The coexistence of two or more active genes responsible for the synthesis of entomocidal proteins has been shown for other subspecies of *B. thuringiensis* [8,9]. The multiplicity of toxins produced by one strain increases its host range thus providing the strain with substantial ecological advantages. On the other hand, the plasmid localization of the endotoxin genes, the 'transception' mechanism shown for these bacteria and the presence of several related toxin genes within the same cell should enhance the intragenic recombination process and thus accelerate the appearance of a rather broad pattern of structurally related toxins with modified properties.

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