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Short communication

Bacillus thuringiensis δ-endotoxin Cry1Ac domain III enhances activity against *Heliothis virescens* in some, but not all Cry1-Cry1Ac hybrids

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Abstract

We investigated the role of domain III of *Bacillus thuringiensis* δ-endotoxin Cry1Ac in determining toxicity against *Heliothis virescens*. Hybrid toxins, containing domain III of Cry1Ac with domains I and II of Cry1Ba, Cry1Ca, Cry1Da, Cry1Ea, and Cry1Fb, respectively, were created. In this way Cry1Ca, Cry1Fb, and to a lesser extent Cry1Ba were made considerably more toxic.
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Keywords: *Bacillus thuringiensis*; δ-Endotoxin; Cry1Ac; Hybrid protein; *Heliothis virescens*

1. Introduction

Bacillus thuringiensis is a gram-positive soil bacterium that forms during sporulation a parasporal crystal containing insecticidal proteins (called Cry proteins or δ-endotoxins). Cry proteins are formed as protoxins, which are normally activated by proteases of the insect gut. The elucidation of the three-dimensional structure has shown that several, and probably most, activated Cry toxins share a common three-domain structure [for review, see (de Maagd et al., 2001)]. The N-terminal domain I is thought to insert into the target membrane and form part of a membrane pore. Domain II is involved in receptor binding and thereby in co-determining the insect specificity. The C-terminal domain III is also involved, among other proposed functions, in determining specificity through receptor binding. Several studies have demonstrated that the creation of hybrid Cry proteins can result in substantially improved toxins, in terms of toxicity or target spectrum [reviewed in (de Maagd et al., 2001)].

A serious pest of cotton and tobacco is Tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae). It is susceptible to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa, Cry1Ja, Cry2A, and Cry9Ca, with Cry1Ac being the most toxic (K. Van Frankenhuyzen and C. Nystrom, *B. thuringiensis* specificity database, http://www.glfc.cfs.nrcan.gc.ca/Bacillus/btsearch.cfm). Several groups have suggested a role for domain III of Cry1Ac in specificity by showing that hybrids containing domains I and II of Cry1Aa and domain III of Cry1Ac have increased activity against *H. virescens* (Ge et al., 1991; Schnepf et al., 1990). In this paper, we have studied the extent to which Cry1Ac domain III is a determinant for specificity by combining it with domains I and II of various other Cry1 proteins.

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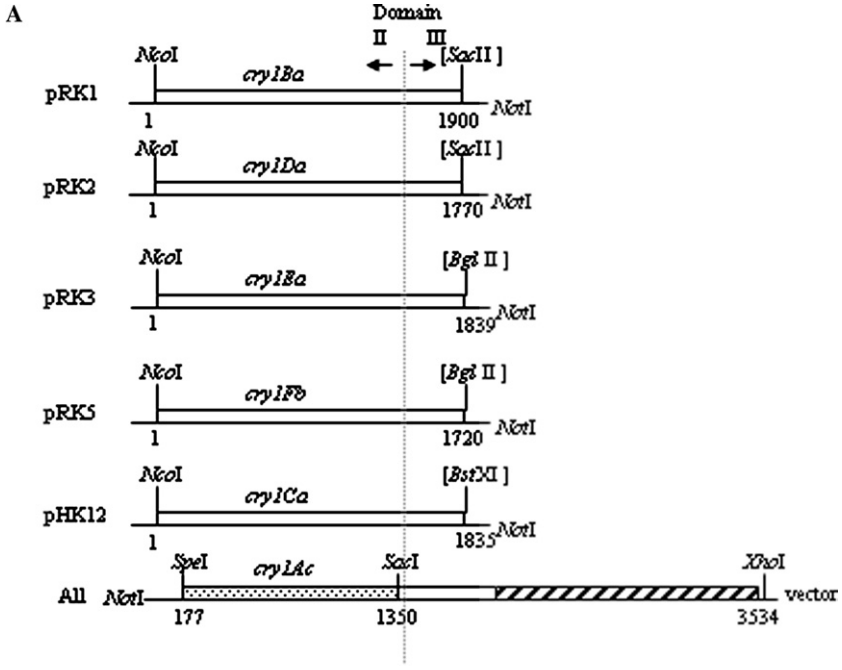
1.1. Production and selection of hybrid toxins

All used Cry protein expression vectors were based on pBD10, a derivative of pKK233-2 (Bosch et al., 1994). Expression plasmids pB03 (*cry1Ac*), pMH19 (*cry1Ba*), pBD150 (*cry1Ca*), pMH15 (*cry1Da*), pBD160 (*cry1Ea*), and pMH21 (*cry1Fa*), have been described before (Bosch et al., 1994; de Maagd et al., 2000). The *cry1Fb* gene from strain BTS00349A was obtained from Aventis CropScience NV. For production of Cry1Fb, an *Eco*NI–*Mun*I (nucleotides 35–3494) fragment of *cry1Da* in pMH15 was replaced with the corresponding fragment of *cry1Fb* (nucleotides 36–3514), resulting in *cry1Fb* expression vector pMH35.

cry1Ca-cry1Ac tandem plasmid pHK12 (Fig. 1) has been described before (de Maagd et al., 1996). Tandem plasmids pRK1 (*cry1Ba-cry1Ac*), pRK2 (*cry1Da-cry1Ac*), and pRK3 (*cry1Ea-cry1Ac*) were produced by replacing the *cry1Ca* containing *Nco*I–*Not*I fragment of pHK12 by that of the corresponding *cry1*-fragments of previously described *cry1-cry1Ca* tandem plasmids pMH22 (*cry1Ba-cry1Ca*) (de Maagd et al., 2000), pMH18 (*cry1Da-cry1Ca*) (de Maagd et al., 2000), and pBD650 (*cry1Ea-cry1Ca*) (Bosch et al., 1994), respectively. For *cry1Fb-cry1Ac* tandem plasmid pRK5, the *cry1Ca Nco*I–*Sac*II fragment from pHK12 was replaced by the *cry1Fb Nco*I–*Fsp*I fragment of pMH35 using a synthetic *Fsp*I–*Sac*II linker. The common arrangement of *cry1* 5′ fragments, followed by a polylinker and an identical 3′ fragment of *cry1Ac* for the 5 tandem plasmids is shown in Fig. 1A.

After allowing intramolecular recombination of tandem plasmids in *Escherichia coli* JM101 (recA$^+$), plasmid DNA was isolated and digested with *Sac*I and *Not*I to linearize non-recombinant plasmids. *Not*I has a unique recognition site in the polylinker of all tandem plasmids and *Sac*I has a unique recognition site at position 1350



B

	Domain II / Domain III	
Cry1Ac	[449]RAPMFSWIHRSAEFNNIIASDSITQIPAVKGNFLF	
Cry1Ba	[456]NV.VY..T....drt.t.gpnr.....m..ase.p	RK6
Cry1Ca	[445]TGVV...t....tlt.t.dper.n...l...fevw	RK15
Cry1Da	[438]G-TV...t....spt.evspsr.....w..aht.a	RK7
Cry1Ea	[442]SL.T.V.t.h..tnt.t.np.i.....l...fr.g	RK9
Cry1Ea	[442]SL.T.V.T.H..tnt.t.np.i.....l...fr.g	RK8
Cry1Ea	[442]SL.T.V.T.H..TNT.T.NP.I.....l...fr.g	RK10
Cry1Fb	[442].....T....DRT...NPNI.....ahn.h	RK12

Fig. 1. (A) Schematic representation of tandem plasmids and in vivo recombination strategy. Locations of domain III borders are indicated by a dotted vertical line. The overlapping regions of the involved genes are aligned vertically, and the polylinker between the two genes is shown as cut by *Not*I. Since recombinants were selected with *Not*I and *Sac*I, recombination within the dotted area of *cry1Ac* was not found. The hatched area indicates the protoxin-specific encoding part of the gene. (B) Amino acid alignment of Cry1Ac and other parental toxins in the area of border between domains II and III. Amino acid identity to Cry1Ac is represented by dots. The homologous area that contains the cross-over is underlined for each hybrid. For the hybrids, the parental toxin sequence is shown in lowercase beyond the crossover site.

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