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Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* Is Toxic to the Diamondback Moth, *Plutella xylostella*, and Synergizes the Activity of Cry1Ac towards a Resistant Strain

ALI H. SAYYED,[1] NEIL CRICKMORE,[2] AND DENIS J. WRIGHT[1]*

Department of Biological Sciences, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY,[1] and School of Biological Sciences, University of Sussex, Falmer, Brighton, BN1 9QG[2], United Kingdom

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The *Bacillus thuringiensis* subsp. *israelensis* cytolytic protein Cyt1Aa was found to be toxic to an insecticide-susceptible laboratory population of *Plutella xylostella*. Cry1Ac-resistant populations of *P. xylostella* showed various degrees of resistance to Cyt1Aa. Cyt1Aa/Cry1Ac mixtures showed a marked level of synergism in the Cry1Ac-resistant populations.

Until 1996, *Bacillus thuringiensis* was used only in conventional spray formulations against insect pests, and cases of field-acquired resistance have been restricted to larvae of the diamondback moth, *Plutella xylostella*, a crucifer specialist that has been sprayed intensively with products based on *Bacillus thuringiensis* subsp. *kurstaki* and *Bacillus thuringiensis* subsp. *aizawai* (12, 19). With the advent of *B. thuringiensis*-transgenic crops expressing crystal (Cry) endotoxins, many more insect species are subject to selection pressure from *B. thuringiensis* toxins (15). Resistance management strategies advocated for *B. thuringiensis* crops include the periodic rotation of plants that produce different Cry toxins, the use of mixtures of Cry toxins in the same plant (7), the combination of Cry toxins with synergists, and the use of refugia in which susceptible plants are planted along with insect-resistant plants (2). The last strategy, together with high levels of expression of the Cry toxin, is the one currently recommended for crops such as *B. thuringiensis* cotton (15).

Unlike resistance to *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai*, resistance to *Bacillus thuringiensis* subsp. *israelensis*, which is active against dipterans, has not been reported in the field (21). *B. thuringiensis* subsp. *israelensis* produces a cytolytic protein, Cyt1Aa, along with Cry4Aa, Cry4Ba, Cry10Aa, and Cry11Aa proteins. Cyt1Aa is a highly hydrophobic endotoxin that shares no sequence homology with Cry proteins and appears to have a different mode of action (14). The primary affinity for Cyt1A is for the lipid component of the membrane, specifically, unsaturated fatty acids (20), and its unique mode of action and capacity to interact synergistically with Cry proteins have suggested that it could suppress the onset of resistance to Cry4Aa, Cry4Ba, and Cry11Aa toxins in dipteran pests (21). Cyt1Aa shows no activity against the lepidopteran insects *Manduca sexta* and *Trichoplusia ni* and was recently shown to be inactive against populations of *P. xylostella* and *Pectinophora gossypiella* (9). However, in this study we demonstrate that Cyt1Aa does have activity against other *P. xylostella* populations and consider its potential for agricultural pest control.

A field population of *P. xylostella* (SERD5) was obtained from Malaysia in August 1999 (13) following reports of reduced susceptibility of the insect to commercial *B. thuringiensis* sprays. The population was divided into two subpopulations at F_2. One subpopulation was left unselected (UNSEL) while the other (Cry1Ac-SEL) was selected with activated Cry1Ac toxin from F_2 to F_9 (13). Bioassays were conducted with third-instar larvae as described previously (13). An insecticide-susceptible population of *P. xylostella* (ROTH) was obtained from the Institute of Arable Crops Research, Rothamsted (Harpenden, Hertfordshire, United Kingdom). Purified Cyt1Aa and Cry1Ac crystals were prepared from *B. thuringiensis* strain IPS78/11(cam2027) (5) and *Escherichia coli*, respectively.

Compared with the ROTH strain, the UNSEL and Cry1Ac-SEL populations showed resistance ratios of 44 and 1,165, respectively, towards Cry1Ac (Table 1). Cyt1Aa also showed activity towards ROTH, approximately 20-fold less than that of Cry1Ac. Interestingly, similar resistance ratios were observed with the two SERD5 populations and Cyt1Aa compared with the ratios observed for Cry1Ac. A mixture of Cyt1Aa and Cry1Ac (1:1, wt/wt) showed a small synergistic interaction against ROTH but an increasingly more marked one against the UNSEL and Cry1Ac-SEL populations. In the case of Cry1Ac-SEL, the combination of Cyt1Aa and Cry1Ac gave a synergistic factor of 450 (χ^2, $P < 0.05$) and reduced the resistance ratio to just 5.

The observed toxicity of Cyt1Aa towards *P. xylostella* is, to the best of our knowledge, the first reported case of this toxin showing activity against an agriculturally important lepidopteran pest. Activity against another nondipteran insect, the cottonwood leaf beetle (*Chrysomela scripta*), has been reported, although in this case the toxin had to be presolubilized before significant activity was observed (6).

Although solubilized and proteolytically activated Cyt1Aa shows cytolytic activity against a broad spectrum of insect cells in vitro (3), the in vivo activity of the toxin crystals has generally been considered to be restricted to dipteran larvae. Possi-

* Corresponding author. Mailing address: Department of Biological Sciences, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom. Phone: 44 207 594 2248. Fax: 44 207 594 2339. E-mail: d.wright@bio.ic.ac.uk.

TABLE 1. Toxicity of Cry1Ac and Cyt1Aa against susceptible (ROTH) and field-derived (UNSEL and Cry1Ac-SEL) strains of *P. xylostella*

Strain	Toxin(s)	Observed LC_{50}[a]	95% FL[b]	Slope (mean ± SE)	Expected LC_{50}[a,c]	Synergistic factor[d]	χ^2	Resistance ratio[e]	P[f]
ROTH	Cry1Ac	0.0020	0.0001–0.0085	0.69 ± 0.18					
	Cyt1Aa	0.0440	0.011–0.131	0.87 ± 0.17					
	Cyt1Aa + Cry1Aa	0.0019	0.0004–0.006	1.16 ± 0.34	0.0038	2	0.0009		0.98
UNSEL	Cry1Ac	0.0880	0.034–0.190	1.29 ± 0.24				44	
	Cyt1Aa	2.35	1.04–4.35	1.47 ± 0.33				53	
	Cyt1Aa + Cry1Ac	0.0150	0.001–0.095	0.56 ± 0.14	0.1700	11	0.141	8	0.71
SEL	Cry1Ac	2.33	0.14–29.62	0.54 ± 0.22				1,165	
	Cyt1Aa	80	61–114	3.93 ± 0.73				1,818	
	Cyt1Aa + Cry1Ac	0.0100	0.006–0.05	1.06 ± 0.19	4.5	450	4.48	5	0.03

[a] LC_{50}, concentration (in micrograms per milliliter) estimated to give 50% mortality after 5 days. Data are corrected for control mortality (1).
[b] FL, fiducial limits.
[c] Calculated according to the formula of Tabashnik (18).
[d] Ratio of the observed LC_{50} to the expected LC_{50}. If the ratio is 1, then toxin interaction is simply additive; if it is greater than 1, interaction is synergistic; if it is less than 1, interaction is antagonistic.
[e] LC_{50} ratio for given strain-toxin combination compared with ROTH.
[f] Level of significance (χ^2 test).

ble explanations for this are that the crystals require a combination of alkaline and reducing conditions in order to dissolve and that they require particular proteolytic processing at both the N and C termini in order to acquire maximum activity (3). The toxin may also be rapidly degraded by proteinases found in other orders of insect. Clearly, the conditions found within the gut of the *P. xylostella* strains used in this study favor successful activation.

Cyt1Aa has a long history of synergizing the activity of other dipteran toxins against a variety of mosquito species (5). The mechanism of this synergism is unknown, although Ravoahangimalala and Charles (10) observed altered binding of Cry toxins to the gut of *Anopheles gambiae* larvae in the presence of Cyt1Aa. The effect of Cyt1Aa in overcoming or preventing the onset of resistance of dipteran insects to the other toxins of *B. thuringiensis* subsp. *israelensis* has been well documented (21). Cyt1Aa also synergizes the activity of the *B. sphaericus* binary toxin towards various mosquito larvae (22).

The observation that the Cry1Ac-SEL population was over 30 times less sensitive to Cyt1Aa than the UNSEL population suggests a common resistance mechanism for these toxins. We have found that resistance to Cry1Ac in Cry1Ac-SEL SERD5 can be largely overcome if the insects are challenged with in vitro-activated toxin (13) and suggest that a general mechanism reducing the availability of toxin within the gut is a more likely resistance mechanism than a specific defect in binding or proteolytic activation. Such a mechanism would be consistent with the observed cross-resistance between Cry1Ac and Cyt1Aa.

P. xylostella is the only reported example of an agriculturally important pest acquiring resistance to *B. thuringiensis* in the field. The data presented above suggest that a combination of Cyt1Aa and Cry1Ac, either presented in a spray formulation or expressed in a transgenic plant, could significantly overcome this resistance and prevent or slow the onset of resistance, at least with some populations. However, despite many years of safe use as a mosquitocide, there are potential safety concerns in using Cyt1Aa due to its general cytolytic activity in vitro. This is particularly true if there is a significant risk of the toxin becoming activated (16), a likelihood that should be considered before any large-scale employment of the toxin.

Meyer et al. (9) have published results showing that Cyt1Aa is toxic to neither susceptible nor resistant strains of diamondback moth or pink bollworm. There are various possibilities to explain this apparent discrepancy, including, for example, differences in the insect strains, the bioassay procedure, or the toxin itself. Meyer et al. expressed their Cyt1Aa toxin in an acrystalliferous strain of *B. thuringiensis* subsp. *kurstaki*, whereas we used an acrystalliferous variant of *B. thuringiensis* subsp. *israelensis*. Differences in crystal solubility have been observed with different *B. thuringiensis* expression hosts that could affect relative toxicity (4). Also, different proteinases are known to be produced by the two subspecies (11), and these could affect the specificity of the toxin, as has been observed previously (17). We believe, though, that variation in the susceptibilities of different populations of *P. xylostella* to *B. thuringiensis* toxins is the single most important explanation for the observed discrepancy. It has been observed that ROTH is some 170-fold more susceptible to Cry1Ac than another nonresistant strain, Lab-V (8). A similar difference in the susceptibility towards Cyt1Aa could largely account for the observed differences.

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