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Cyt1A from *Bacillus thuringiensis* Lacks Toxicity to Susceptible and Resistant Larvae of Diamondback Moth (*Plutella xylostella*) and Pink Bollworm (*Pectinophora gossypiella*)

SUSAN K. MEYER,[1] BRUCE E. TABASHNIK,[1]* YONG-BIAO LIU,[1] MARGARET C. WIRTH,[2] AND BRIAN A. FEDERICI[2]

Department of Entomology, University of Arizona, Tucson, Arizona 85721,[1] and Department of Entomology and Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, Riverside, California 92521[2]

Received 18 August 2000/Accepted 9 October 2000

We tested Cyt1Aa, a cytolytic endotoxin of *Bacillus thuringiensis*, against susceptible and Cry1A-resistant larvae of two lepidopteran pests, diamondback moth (*Plutella xylostella*) and pink bollworm (*Pectinophora gossypiella*). Unlike previous results obtained with mosquito and beetle larvae, Cyt1Aa alone or in combination with Cry toxins was not highly toxic to the lepidopteran larvae that we examined.

The soil bacterium *Bacillus thuringiensis* produces insecticidal cytolytic (Cyt) and crystal (Cry) proteins that are useful for pest control (7). Cyt1Aa interacts synergistically with Cry4A, Cry4B, and Cry11A to reduce the resistance of mosquito larvae (*Culex quinquefasciatus*) to these proteins (13) and with Cry3A proteins to reduce the resistance of cottonwood leaf beetle larvae (*Chrysomela scripta*) to Cry3A (2). These results led to the hypothesis that Cyt proteins may be useful for managing the resistance of other pests to Cry toxins used in microbial insecticides and transgenic plants (2). To test this hypothesis, we determined the effects of Cyt1Aa on susceptible and Cry1A-resistant larvae of two major lepidopteran pests, diamondback moth (*Plutella xylostella*) and pink bollworm (*Pectinophora gossypiella*).

For diamondback moth, we tested the susceptible LAB-PS strain and the resistant NO-QA strain (4, 10). For pink bollworm, we tested the susceptible APHIS-S strain and the resistant APHIS-98R strain (5). Both susceptible strains had been reared in the laboratory for many years without exposure to toxins. The NO-QA strain was derived from a resistant field population in Hawaii and had been selected repeatedly in the laboratory with Dipel. Dipel is a formulated version of the HD-1 strain of *B. thuringiensis* subsp. *kurstaki*, which contains Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, spores, and other materials (Valent BioSciences, Libertyville, Ill.). The APHIS-98R strain was derived from the APHIS-S strain and had been selected repeatedly in the laboratory with leaf powder from transgenic cotton containing Cry1Ac and with MVPII (Dow Agrosciences, San Diego, Calif.), a liquid formulation containing Cry1Ac (3). Diamondback moth larvae were reared and tested on cabbage foliage (9). Pink bollworm larvae were reared and tested on an artificial diet (8).

We tested Cyt1Aa alone and in combination with Dipel against susceptible and resistant diamondback moth larvae. We also tested Cyt1Aa alone and in combination with Cry1Ac against susceptible and resistant pink bollworm larvae. In these experiments, Cyt1Aa was obtained from a recombinant strain of *B. thuringiensis* that produced only Cyt1Aa (14). MVPII was the source of Cry1Ac. For resistant and susceptible diamondback moth larvae, we also compared the toxicity of a lyophilized powder of *B. thuringiensis* subsp. *kurstaki* HD-1 with the toxicity of a recombinant strain that produced Cyt1Aa in addition to the Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, spores, and other materials produced by the HD-1 strain. This recombinant strain was constructed by transforming HD-1 with plasmid pWF45 (14), which encodes *cyt1Aa*, using methods described previously (6). Cyt1Aa accounted for 10 to 15% of the dry weight of the powder containing HD-1 and Cyt1Aa.

All bioassays and rearing were conducted at 27°C with a photoperiod consisting of 14 h of light and 10 h of darkness. We used a leaf residue bioassay to test diamondback moth larvae (9). Cabbage leaf disks were dipped for about 5 s in distilled water with 0.2% Triton AG-98 (a surfactant; Rohm & Haas Co., Philadelphia, Pa.) containing various concentrations of Dipel, HD-1, and Cyt1Aa. After the surfaces of the dipped leaf disks dried, each disk was put in a petri dish with a filter paper disk that had been moistened with 1 ml of water. Ten third-instar larvae were placed on each leaf disk (this was one replicate). After 2 days, fresh untreated leaves were added. Mortality was recorded after 5 days. We tested at least four replicates of each treatment for each strain of diamondback moth.

Pink bollworm larvae were tested with artificial diet bioassays (11). Cry1Ac and Cyt1Aa were blended into the diet thoroughly with a food processor to achieve the appropriate concentration. We tested groups of five neonates by using 10 to 12 g of diet per cup in sealed plastic cups (37.5 ml; Bio-Serv, Frenchtown, N.J.). For each treatment, eight cups with five neonates per cup were tested. After 1 week, the cups were put in a sealed plastic box with a small screened window in the lid for ventilation and a cup of water to maintain the moisture level. After 21 days, the number of survivors and their stage of

* Corresponding author. Mailing address: Department of Entomology, 410 Forbes, 1140 S. Campus Dr., University of Arizona, Tucson, AZ 85721. Phone: (520) 621 1141. Fax: (520) 621 1150. E-mail: brucet@ag.arizona.edu.

TABLE 1. Effects of Cyt1Aa alone and in combination with Cry protein preparations against larvae of diamondback moth and pink bollworm

Insect and resistance level	Cry prepn		% Mortality without Cyt1Aa[b]	Effect of Cyt1Aa on mortality (%)[c]
	Type	Concn[a]		
Diamondback moth				
Susceptible	Dipel	0	NA[d]	3.8
		0.256	27.2	−6.4
		1.28	36.0	5.2
Resistant	Dipel	0	NA	3.8
		25.6	4.9	0
		128	5.0	−2.6
		256	2.7	−2.7
		1,280	83.8	−16.2
Susceptible	HD-1	0.5	18.4	−10.5
		2.0	43.5	1.2
		5.0	65.8	−23.7
		20.0	81.6	15.8
Resistant	HD-1	20.0	15.3	−15.3
		50.0	5.3	−0.2
Pink bollworm				
Susceptible	Cry1Ac	0	NA	0
		0.03	0	0
		0.1	7.4	0
Resistant	Cry1Ac	0	NA	6.8
		1.0	0	17.2
		10.0	20.7	0

[a] Dipel and HD-1 concentrations are given in milligrams per liter. Cry1Ac concentrations are given in micrograms per gram of diet. The concentrations of Cyt1Aa were 40 mg per liter with Dipel and 1.6 μg per g of diet. For tests with HD-1, the concentration of Cyt1Aa varied as the concentration of HD-1 varied (see text).

[b] Mortality values were adjusted for control mortality values by using Abbott's correction.

[c] The effect of Cyt1Aa on mortality was calculated as follows: percent mortality with Cyt1Aa − percent mortality without Cyt1Aa. Overall, adding Cyt1Aa did not significantly increase mortality for diamondback moth or pink bollworm (sign test, $P > 0.05$).

[d] NA, not applicable.

development were recorded. Pupae and live fourth-instar larvae were counted as survivors.

For all bioassays, mortality was adjusted for the mortality in controls (no Cry or Cyt1Aa protein) by using Abbott's correction. The effect of Cyt1Aa was calculated as follows: percent mortality with Cyt1Aa−percent mortality without Cyt1Aa.

Cyt1Aa had little or no effect on mortality alone or in combination with various preparations of Cry toxins (Table 1). In tests performed with susceptible and resistant diamondback moth larvae, mortality was lower with Cyt1Aa than without Cyt1Aa in 8 of 12 trials with Dipel and HD-1 powder (Table 1). In tests performed with susceptible and resistant pink bollworm larvae, Cyt1Aa had no effect on mortality in three of four trials with Cry1Ac (Table 1).

Cyt1A is toxic to certain beetle larvae and to larvae of Diptera in the suborder Nematocera, which includes mosquitoes, blackflies, and craneflies (1, 2). The molecular mechanism underlying this specifity is not known, but sensitivity to Cyt1A may be related to the specific fatty acid composition of lipids in the microvillar membrane of midgut epithelial cells (12). Because the larval midguts of diamondback moth and pink bollworm are alkaline and contain proteases, we suspect that solubilization and activation of Cyt1A can occur. The results reported here show that Cyt1Aa is not effective against the larvae of two lepidopteran species belonging to different families, which suggests that this protein may not be active against many lepidopterans.

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