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Note

Deletion of the Cry11A or the Cyt1A toxin from *Bacillus thuringiensis* subsp. *israelensis*: effect on toxicity against resistant *Culex quinquefasciatus* (Diptera: Culicidae)

Bacillus thuringiensis subsp. *israelensis* is a bacterium that produces crystalline, protein toxins whose activity has been exploited to develop environmentally safe biopesticides that are important in mosquito control. *B. t.* subsp. *israelensis* has six genes encoding toxic proteins on a 137 kDa plasmid, including: *cry4A*, *cry4B*, *cry10A*, *cry11A* (formerly *cry4D*), *cyt1Aa*, and *cyt2Ba* (Ben-Dov et al., 1999). Each component toxin in *B. t.* subsp. *israelensis* is mosquitocidal and Cyt2Ba and Cyt1Aa are additionally cytolytic and hemolytic. The toxicity of the native complex of toxins is considerably higher than that of the individual toxins because activity is enhanced by synergism between the component toxins, particularly between Cyt1A and the Cry toxins (Poncet et al., 1995). Interestingly, it was reported that when the *cyt1A* gene was genetically inactivated, the recombinant strain was as active or nearly as active as native *B. t.* subsp. *israelensis* toward susceptible *Culex quinquefasciatus*, suggesting that this toxin plays little or no significant role in the activity of *B. t.* subsp. *israelensis* (Delécluse et al., 1991). Alternatively, the multi-toxin complex in *B. t.* subsp. *israelensis* may have sufficient redundancy that loss of a single toxin has little effect on activity.

Although loss of Cyt1A from *B. t.* subsp. *israelensis* did not alter toxicity against susceptible mosquito larvae, its absence was discovered to have some important consequences. Mosquito larvae selected with native *B. t.* subsp. *israelensis* failed to develop resistance, whereas larvae selected with a strain of *B. t.* subsp. *israelensis* lacking the *cyt1A* gene developed substantial resistance (Georghiou and Wirth, 1997). Further, the resistant mosquitoes remained susceptible to native *B. t.* subsp. *israelensis*, despite high levels of resistance and cross-resistance to Cry toxins (Wirth and Georghiou, 1997). Because the two bacterial strains differed solely in the presence or absence of the *cyt1A* gene, it was concluded that this toxin was responsible for suppressing resistance, which was subsequently demonstrated (Wirth et al., 1997).

Our understanding of Cyt1A's role in the activity of *B. t.* subsp. *israelensis* identifies it as important in toxin synergism, in delaying resistance development, and in suppressing resistance. The contribution of Cyt1A, particularly in synergism and suppressing resistance, suggested that the loss of one component Cry toxin would have less impact on activity against resistant *C. quinquefasciatus* than would the loss of the Cyt1A toxin. We tested this hypothesis by assaying colonies of *C. quinquefasciatus* that were selected to moderate or high levels of insecticide resistance toward single or multiple toxins from *B. t.* subsp. *israelensis* and comparing the activity of two *B. t.* subsp. *israelensis* strains in which either the *cry11A* gene or the *cyt1A* gene was disrupted by in vivo recombination (Delécluse et al., 1991; Poncet et al., 1993).

Five toxin preparations, consisting of crystal/spore mixtures of lyophilized powders were assayed. Two preparations were from recombinant strains that produced toxin(s) by expressing cloned gene(s) in acrystalliferous strains of *B. thuringiensis*, Cry11A (Chang et al., 1992) or Cry4A + Cry4B (Delécluse et al., 1993). Two bacterial strains were previously developed using homologous recombination to disrupt one component toxin, Deleted CytA, lacking the *cyt1A* gene (Delécluse et al., 1991) and Deleted Cry11A, lacking the *cry11A* gene (Poncet et al., 1993). The fifth strain was native *B. t.* subsp. *israelensis* (preparation IPS80, Pasteur Institute, Paris, France).

Five colonies of *C. quinquefasciatus* were evaluated, including a parental reference colony, CqSyn90, and four colonies derived from CqSyn90 that were resistant to one or more *B. t.* subsp. *israelensis* toxin(s). The resistant colonies were Cq80 selected with native *B. t.* subsp. *israelensis* (IPS 80, resistance ratio at LC_{95} (RR_{95}), 5.8); Cq4ABD selected with Deleted CytA (RR_{95}, 264); Cq4AB selected with Cry4A and Cry4B (RR_{95}, 101); and Cq11A selected with Cry11A (RR_{95}, >90,000).

Bacterial strains were grown on solid or liquid media and crystal/spore powders were prepared using standard methods (Chang et al., 1992; Delécluse et al., 1991; Poncet et al., 1993). Stock suspensions, in distilled water, were prepared monthly; dilutions were prepared weekly and frozen at −20 °C when not in use. Bioassays

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Table 1
Toxicity of different strains of *B. thuringiensis* expressing one or more *B. t.* subsp. *israelensis* toxin(s) against *Culex quinquefasciatus* that are susceptible or resistant to various *B. t.* subsp. *israelensis* toxins

Mosquito colony	Toxins assayed	LC_{50} (confidence interval) (μg/ml)	LC_{95} (confidence interval) (μg/ml)	Resistance ratio[a]	
				LC_{50}	LC_{95}
CqSyn90	IPS 80	0.0422 (0.0366–0.0485)	0.280 (0.220–0.377)	1.0	1.0
	Deleted Cry11A	0.0210 (0.0183–0.0241)	0.138 (0.110–0.181)	1.0	1.0
	Deleted CytA	0.0393 (0.0228–0.0627)	0.803 (0.388–2.74)	1.0	1.0
	Cry4A, Cry4B	0.0692 (0.0579–0.0823)	1.05 (0.776–1.51)	1.0	1.0
	Cry11A	0.240 (0.203–0.285)	1.18 (0.878–1.79)	1.0	1.0
Cq80	IPS 80	0.108 (0.0905–0.129)	1.61 (1.13–2.52)	2.6	5.8
	Deleted Cry11A	0.0611 (0.0518–0.0718)	0.739 (0.556–1.04)	2.9	5.4
	Deleted CytA	0.107 (0.0862–0.132)	4.10 (2.76–6.65)	2.7	5.1
Cq4ABD	IPS 80	0.0412 (0.0356–0.0478)	0.330 (0.255–0.452)	1.0	1.2
	Deleted Cry11A	0.0373 (0.0319–0.0437)	0.391 (0.294–0.554)	1.8	2.8
	Deleted CytA	0.695 (0.543–0.879)	81.1 (52.3–136)	17.7	101
Cq4AB	IPS 80	0.0282 (0.0239–0.0332)	0.275 (0.201–0.413)	0.7	1.0
	Deleted Cry11A	0.0233 (0.0159–0.0339)	0.254 (0.142–0.626)	1.1	1.8
	Deleted CytA	0.573 (0.483–0.677)	8.14 (6.08–11.6)	14.5	10.1
	Cry4A, Cry4B	1.93 (0.919–4.04)	277.5 (53.3–1475)	27.9	264
Cq4D	IPS 80	0.0728 (0.0544–0.0989)	0.551 (0.337–1.18)	1.7	2.0
	Deleted Cry11A	0.0563 (0.0475–0.0666)	0.774 (0.567–1.13)	2.7	5.6
	Deleted CytA	0.220 (0.172–0.275)	7.40 (4.89–12.6)	5.6	9.2
	Cry11A	115 (55.9–307)	110,662 (14435–53270)	479	93,781

[a] Resistance ratios were calculated by dividing the LC_{50} or LC_{95} of the resistant colony by the same value calculated for the susceptible reference colony, CqSyn90.

followed standard procedures for mosquito larvae with mortality determined after 24 h (Wirth et al., 1997). Mosquito colonies were tested concurrently to minimize variation and to enable direct comparisons of toxicity between mosquito strains. Data were analyzed using a Probit program (Raymond et al., 1993) and values with overlapping fiducial limits were not considered significantly different.

Deleted Cry11A was highly toxic with LC_{50} and LC_{95} values of 0.021 and 0.14 μg/ml against the susceptible CqSyn90 (Table 1). This deletion mutant was equally or more toxic than native *B. t.* subsp. *israelensis*, which showed LC_{50} and LC_{95} values of 0.042 and 0.28 μg/ml. Deleted Cry11A was also highly toxic to resistant mosquitoes. For example, the LC_{50} against colony Cq11A was 0.056 μg/ml, which was not significantly different than that for native *B. t.* subsp. *israelensis* (0.073 μg/ml) (Table 1). Similar toxicity patterns were observed for the other resistant mosquito strains.

Deleted Cyt1A showed significantly higher LC_{50} and LC_{95} values toward both susceptible and resistant mosquitoes than tests with Deleted Cry11A. This difference was most evident in tests against Cq4AB, which yielded an LC_{50} value of 0.023 μg/ml with Deleted Cry11A and an LC_{50} value of 0.57 μg/ml with Deleted CytA, a 24.8-fold difference in activity. The resistant mosquito colonies showed resistance ratios against their selecting toxin(s) ranging from 5-fold for *B. t.* subsp. *israelensis* (IPS80) to >90,000-fold for Cq11A. With Deleted Cyt1A, resistance levels ranged from 5.1- to 101-fold, whereas using Deleted Cry11A, resistance ratios were lower and ranged from 1.8- to 5.6-fold. These data indicate that loss of the Cyt1A toxin had a greater impact on toxicity against resistant *Culex* than loss of the Cry11A toxin.

In conclusion, a deletion mutant of *B. t.* subsp. *israelensis*, lacking the *cry11A* gene was substantially more toxic than a deletion mutant lacking the *cyt1A* gene when tested against resistant *C. quinquefasciatus*. Cyt1A, because of its capacity to synergize Cry toxins and to suppress resistance, plays a pivotal role in the activity of this material against resistant mosquito larvae and may prove important in managing resistance in mosquitoes and, possibly, other species.

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