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Variable Cross-Resistance to Cry11B from *Bacillus thuringiensis* subsp. *jegathesan* in *Culex quinquefasciatus* (Diptera: Culicidae) Resistant to Single or Multiple Toxins of *Bacillus thuringienisis* subsp. *israelensis*

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A novel mosquitocidal bacterium, *Bacillus thuringiensis* subsp. *jegathesan*, and one of its toxins, Cry11B, in a recombinant *B. thuringiensis* strain were evaluated for cross-resistance with strains of the mosquito *Culex quinquefasciatus* that are resistant to single and multiple toxins of *Bacillus thuringiensis* subsp. *israelensis*. The levels of cross-resistance (resistance ratios [RR]) at concentrations which caused 95% mortality (LC_{95}) between *B. thuringiensis* subsp. *jegathesan* and the different *B. thuringiensis* subsp. *israelensis*-resistant mosquito strains were low, ranging from 2.3 to 5.1. However, the levels of cross-resistance to Cry11B were much higher and were directly related to the complexity of the *B. thuringiensis* subsp. *israelensis* Cry toxin mixtures used to select the resistant mosquito strains. The LC_{95} RR obtained with the mosquito strains were as follows: 53.1 against *Cq*4D, which was resistant to Cry11A; 80.7 against *Cq*4AB, which was resistant to Cry4A plus Cry4B; and 347 against *Cq*4ABD, which was resistant to Cry4A plus Cry4B plus Cry11A. Combining Cyt1A with Cry11B at a 1:3 ratio had little effect on suppressing Cry11A resistance in *Cq*4D but resulted in synergism factors of 4.8 and 11.2 against strains *Cq*4AB and *Cq*4ABD, respectively; this procedure eliminated cross-resistance in the former mosquito strain and reduced it markedly in the latter strain. The high levels of activity of *B. thuringiensis* subsp. *jegathesan* and *B. thuringiensis* subsp. *israelensis*, both of which contain a complex mixture of Cry and Cyt proteins, against Cry4- and Cry11-resistant mosquitoes suggest that novel bacterial strains with multiple Cry and Cyt proteins may be useful in managing resistance to bacterial insecticides in mosquito populations.

The strategies currently used for biological control of mosquitoes depend primarily on products based on two mosquitocidal bacteria, *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* (17, 20). These bacteria have high degrees of insect specificity and environmental safety, which makes them particularly suitable for use against mosquitoes in sensitive wetlands and against mosquito populations resistant to synthetic chemical insecticides. The toxicity of these bacteria to mosquitoes is due to endotoxin proteins which are synthesized during sporulation and are assembled into parasporal crystals that are toxic when they are ingested by larvae (14). The crystals of *B. thuringiensis* subsp. *israelensis* contain four major endotoxins, designated Cry4A (125 kDa), Cry4B (134 kDa), Cry11A (67 kDa), and Cyt1Aa (27 kDa) (5, 14), whereas the *B. sphaericus* crystals are composed of two proteins with molecular masses of 51 and 42 kDa (1, 2).

While bacterial larvicides are currently very effective, resistance to *B. sphaericus* has been reported in several populations of *Culex quinquefasciatus* and *Culex pipiens* in different regions of the world; this resistance threatens the long-term viability of products based on *B. sphaericus* (22, 25, 26). Moreover, although resistance to *B. thuringiensis* subsp. *israelensis* has not been reported in field populations of mosquitoes, laboratory selection studies have demonstrated that *C. quinquefasciatus* has the potential to develop resistance to individual toxins of this bacterium, as well as combinations of toxins (11). Tactics for managing resistance to the mosquitocidal bacteria include rotating different mosquitocidal strains of *B. thuringiensis* and using genetic engineering to produce strains of *B. thuringiensis* and *B. sphaericus* that contain new combinations of toxins.

Several recently isolated novel mosquitocidal strains of *B. thuringiensis* may facilitate resistance management (8, 21). One of the recently isolated organisms is *B. thuringiensis* subsp. *jegathesan*, an organism that was originally isolated in Malaysia (24) and is highly toxic to *Aedes aegypti*, *C. pipiens*, and *Anopheles stephensi* (21). The parasporal crystals of this species are complex and contain seven major proteins that have molecular masses of 80, 70, 72, 65, 37, 26, and 16 kDa (8). The 80-kDa protein, designated Cry11B, is related to Cry11A (formerly Cry4D), which was originally isolated from *B. thuringiensis* subsp. *israelensis*; Cry11B exhibits 58% identity with Cry11A at the amino acid level (8). Cry11B is a potentially important protein for resistance management because its toxicity to mosquitoes is similar to that of intact parasporal crystals of *B. thuringiensis* subsp. *jegathesan* (8).

Although *B. thuringiensis* subsp. *jegathesan* and Cry11B have potential for integration into resistance management programs, their successful use in such programs will depend upon the degree of cross-resistance to *B. thuringiensis* subsp. *israelensis*, especially the degree of cross-resistance between the component endotoxins. Cross-resistance between the distantly related mosquitocidal Cry4 and Cry11 endotoxin proteins from *B. thuringiensis* has already been demonstrated (31), and cross-resistance among different Cry proteins toxic to lepidopterous insects has also been described (12, 13, 15, 16, 18, 28–30). Consequently, novel mosquitocidal strains and Cry proteins

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TABLE 1. Toxicities for different mosquitocidal bacterial toxins with strain *Cq*Syn90

Toxin(s)	No. of larvae	LC_{50} (μg/ml)	LC_{95} (μg/ml)	Slope	Theoretical LC_{50} (μg/ml)	SF
B. thuringiensis subsp. *israelensis*	600	0.0261 (0.0228–0.0299)[a]	0.154 (0.122–0.207)	2.1		
B. thuringiensis subsp. *jegathesan*	600	0.0707 (0.0612–0.0815)	0.441 (0.343–0.607)	2.1		
Cry11A	700	0.783 (0.681–0.899)	4.99 (3.94–6.67)	2.0		
Cry11B	700	0.0881 (0.0768–0.101)	0.480 (0.383–0.636)	2.2		
Cyt1A	500	25.6 (7.5–90.3)	Plateau[b]			
Cry11B + Cyt1A	700	0.149 (0.129–0.173)	1.07 (0.836–1.44)	1.9	0.117	0.78
Cry4A + Cry4B	900	0.185 (0.156–0.218)	2.22 (1.69–3.12)	1.5		
Cry4A + Cry4B + Cry11A	800	0.0211 (0.0185–0.0242)	0.113 (0.0907–0.147)	2.3		

[a] The values in parentheses are the fiducial limits (95% confidence interval).
[b] There was a plateau at concentrations from 100 to 1,000 μg/ml, with an average mortality of 63.5% (32).

need to be evaluated for potential cross-resistance to mosquitocidal *B. thuringiensis* strains that are already widely used.

In the present study, using strains of *C. quinquefasciatus* resistant to single or multiple toxins of *B. thuringiensis* subsp. *israelensis*, we evaluated the levels of cross-resistance to *B. thuringiensis* subsp. *jegathesan* and to Cry11B. We found that our resistant strains of *C. quinquefasciatus* exhibit levels of cross-resistance to *B. thuringiensis* subsp. *jegathesan* and Cry11B that are variable and are related primarily to the type of toxin or toxin combination used to select for resistance. In addition, we found that Cyt1A combined with Cry11B can suppress most of the cross-resistance to Cry11B in two of the resistant strains examined.

MATERIALS AND METHODS

Experimental design. Statistical accuracy in the bioassays used to evaluate cross-resistance in control and resistant mosquito populations required gram quantities of toxin preparations. As a result, crystal-spore mixtures of the bacterial strains, rather than purified toxins, were used. The test powders were evaluated with resistant mosquito strains which were maintained in the laboratory by routine selection with crystal-spore mixtures of *B. thuringiensis* subsp. *israelensis* strains that contained the selecting toxins alone or in combination.

Bacterial strains and toxins. Seven toxin preparations consisting of crystal-spore mixtures from lysed cultures were evaluated. Five of these were preparations from recombinant strains that produced toxins, alone or in combination, by expressing cloned genes in acrystalliferous strains of *B. thuringiensis*. These strains are referred to below by the name(s) of the toxin(s) which each produced, as follows: Cry11A, which produced Cry11A in *B. thuringiensis* subsp. *kurstaki* (3); Cyt1Aa (33), Cry4A-Cry4B (7), and Cry4A-Cry4B-Cry11A (6), which produced the toxin or toxin combination in an acrystalliferous strain of *B. thuringiensis* subsp. *israelensis*; and Cry11B, which produced Cry11B in a strain of *B. thuringiensis* subsp. *thuringiensis* (H1) (8). In addition to the recombinant strains, we used lyophilized powders of two wild-type strains (a *B. thuringiensis* subsp. *israelensis* strain and a *B. thuringiensis* subsp. *jegathesan* strain) that produced the toxins native to each subspecies (9, 24).

Toxin powder production, preparation, and storage. Bacterial strains producing the various toxins were grown on solid media or in liquid media as described previously (3, 6–9, 33). The sporulated cells were then washed in 1 M NaCl and/or distilled water and sedimented, and each resultant pellet was lyophilized. For mosquito selection and bioassays, stock suspensions of the powders were prepared in distilled water and homogenized by using approximately 25 glass beads. Stocks were prepared monthly, and 10-fold serial dilutions were prepared weekly as needed. All stocks and dilutions were frozen at −20°C when not in use.

Mosquito strains. Five strains of *C. quinquefasciatus* were utilized in this study. These were *Cq*Syn90, a nonresistant parental reference strain, and four highly resistant strains derived from *Cq*Syn90 by selection with strains of *B. thuringiensis* that produced single or multiple *B. thuringiensis* subsp. *israelensis* toxins (11). The resistant mosquito strains used and their current levels of resistance (resistance ratios [RR] at concentrations which caused 95% mortality [LC_{95}]) were: *Cq*4D, which was selected with Cry11A (formerly CryIVD) (RR, >7,000); *Cq*4AB, which was selected with Cry4A and Cry4B (RR, 290); *Cq*4ABD, which was selected with Cry4A, Cry4B, and Cry11A (RR, 949); and *Cq*4ABDCytA, which was selected with the wild-type preparation of *B. thuringiensis* subsp. *israelensis* (RR, 12.7).

Selection and bioassay procedures. The four strains of resistant mosquitoes have been under selection pressure since 1991. Resistance was maintained by exposing groups of 1,000 early-fourth-instar larvae in 1 liter of distilled water in an enameled metal pan to an appropriate concentration of a powder containing the selecting toxin or toxin combination. The mortality was estimated after 24 h, and survivors were then fed and maintained in the treatment pan for approximately 3 days after exposure before they were transferred to fresh water.

Standard procedures were used for the bioassays (11). Twenty early-fourth-instar larvae were placed in 237-ml plastic cups containing 100 ml of distilled water. The appropriate concentration of toxin powder was added, and mortality was determined after 24 h. At least five (but usually 10 to 12) different concentrations were used, which yielded mortality rates ranging from 0 to 100%. Tests were replicated at least five times on 4 or 5 different days. Data were analyzed by probit analysis (10, 23). RR were calculated relative to the dose-response values obtained with nonresistant parental mosquito strain *Cq*Syn90. Dose-response values with fiducial limits which overlapped were not considered significantly different from each other, nor were RR whose fiducial limits included the integer 1 considered significantly different from the RR for *Cq*Syn90. Bioassays in which a toxin or combination of toxins was used were performed concurrently with the different mosquito strains to minimize extraneous variation. In tests in which Cyt1A and Cry11B were used, the toxin powders were combined at a ratio of 1 part of Cyt1A to 3 parts of Cry11B by weight.

Evaluation of synergism. Possible synergistic interactions between Cyt1A and Cry11B were evaluated and quantified by using the procedure of Tabashnik (27). Individual LC_{50} were determined for Cry11B alone, Cyt1A alone, and combinations of Cry11B and Cyt1A by using the nonresistant parental mosquito strain and each of the four resistant mosquito strains. The theoretical LC_{50} for the mixture of the two toxins was calculated from the weighted harmonic mean of the two individual values. The synergism factor (SF), which was defined as the ratio of the theoretical LC_{50} to the observed LC_{50}, was calculated for the Cry11B-

TABLE 2. Toxicities and RR for various mosquitocidal bacterial toxins with strain *Cq*4D

Toxin(s)	No. of larvae	LC_{50} (μg/ml)	LC_{95} (μg/ml)	RR at:		Slope	Theoretical LC_{50} (μg/ml)	SF
				LC_{50}	LC_{95}			
B. thuringiensis subsp. *israelensis*	800	0.0903 (0.0774–0.105)[a]	0.811 (0.618–1.13)	3.4 (2.9–4.1)	5.3 (3.8–7.3)	1.7		
B. thuringiensis subsp. *jegathesan*	600	0.236 (0.206–0.268)	1.22 (0.984–1.61)	3.3 (2.8–3.9)	2.8 (1.9–3.9)	2.3		
Cry11A	800	5,772	—[b]	7,369	—	0.65		
Cry11B	1,400	0.808 (0.676–0.963)	25.5 (18.8–36.2)	9.2 (7.85–10.7)	53.1 (39.8–70.8)	1.1		
Cyt1A	600	23.3 (10.6–52.9)	Plateau[c]			1.3		
Cry11B + Cyt1A	900	1.06 (0.905–1.25)	18.7 (13.6–27.5)	7.1 (6.1–8.3)	17.5 (12.9–23.7)	1.3	1.06	1.0

[a] The values in parentheses are the fiducial limits (95% confidence interval).
[b] —, value not given because the predicted value was extraordinarily high.
[c] There was a plateau at concentrations from 100 to 1,000 μg/ml, with an average mortality of 51.5% (32).

TABLE 3. Toxicities and RR for various mosquitocidal bacterial toxins with strain *Cq*4AB

Toxin(s)	No. of larvae	LC_{50} (μg/ml)	LC_{95} (μg/ml)	RR at:		Slope	Theoretical LC_{50} (μg/ml)	SF
				LC_{50}	LC_{95}			
B. thuringiensis subsp. *israelensis*	500	0.0387 (0.0317–0.0468)	0.287 (0.218–0.399)[a]	1.48 (1.2–1.8)	1.86 (1.3–2.6)	1.9		
B. thuringiensis subsp. *jegathesan*	700	0.179 (0.157–0.204)	0.999 (0.800–1.31)	2.5 (2.1–3.0)	2.3 (1.6–3.2)	2.2		
Cry11A	800	4,017	—[b]	5,129	—	0.38		
Cry11B	1,200	0.855 (0.695–1.05)	38.8 (26.5–60.8)	9.7 (8.3–11.3)	80.7 (59.8–109)	1.0		
Cyt1A	500	21.8 (18.2–27.1)	Plateau[c]			1.7		
Cry11B + Cyt1A	800	0.236 (0.204–0.273)	1.75 (1.38–2.35)	1.6 (1.3–1.9)	1.6 (1.2–2.2)	1.9	1.13	4.8
Cry4A + Cry4B	1,100	7.35 (5.74–9.37)	646 (410–1,111)	39.7 (34.5–45.7)	290 (221–380)	0.85		

[a] The values in parentheses are the fiducial limits (95% confidence interval).
[b] —, value not given because the predicted value was extraordinarily high.
[c] There was a plateau at concentrations from 100 to 1,000 μg/ml, with an average mortality of 75.5% (32).

Cyt1A mixture for each strain. No SF were calculated at the LC_{95} because Cyt1A bioassay lines were not linear at higher dosage-mortality concentrations. When the ratio was greater than 1, the toxin interaction was considered synergistic as the observed toxicity was greater than predicted from the individual toxicities. When the ratio was less than 1, the interaction was considered antagonistic, whereas a ratio of 1 indicated an additive interaction.

RESULTS

Toxicity to nonresistant mosquito strain *Cq*Syn90. In our baseline studies, the *B. thuringiensis* subsp. *jegathesan* strain was less toxic to parental strain *Cq*Syn90 than the *B. thuringiensis* subsp. *israelensis* strain; the LC_{50} were 0.070 and 0.026 μg/ml, respectively (Table 1). Consistent with previous work (8), the toxicity of the Cry11B strain to *Cq*Syn90 (LC_{50}, 0.088 μg/ml) was similar to the toxicity of *B. thuringiensis* subsp. *jegathesan* to *Cq*Syn90, and the Cry11B strain was approximately 10 times more toxic than the Cry11A strain (Table 1). Importantly, Cyt1A was not synergistic with Cry11B; this combination was actually mildly antagonistic, with an SF of 0.78 (Table 1).

Resistance in mosquito strain *Cq*4D. Strain *Cq*4D was highly resistant to its selecting toxin, Cry11A (LC_{95} RR, >7,000), and exhibited significant cross-resistance to Cry11B (LC_{95} RR, 53.1), as shown in Table 2. Bioassays performed with this strain revealed a low but statistically significant level of resistance to *B. thuringiensis* subsp. *israelensis* and an even lower level of cross-resistance to *B. thuringiensis* subsp. *jegathesan* (LC_{95} RR, 5.3 and 2.8, respectively) (Table 2). Cyt1A combined with Cry11B at a 1:3 ratio resulted in an SF of 1.0, indicating that the toxicity was additive (i.e., there was no synergism), and the cross-resistance ratios obtained at LC_{50} and LC_{95} were 7.1 and 17.5, respectively (Table 2).

Resistance in mosquito strain *Cq*4AB. Strain *Cq*4AB exhibited high levels of resistance to Cry4A plus Cry4B (LC_{95} RR, 290) (Table 3) but no significant resistance or cross-resistance to either *B. thuringiensis* subsp. *israelensis* (LC_{95} RR, 1.86) or *B. thuringiensis* subsp. *jegathesan* (LC_{95} RR, 2.3). However, there was a significant level of cross-resistance to Cry11B (LC_{95} RR, 80.7), which was completely suppressed when Cyt1A was combined with Cry11B at a 1:3 ratio (LC_{95} RR, 1.6) (Table 3 and Fig. 1). The SF was 4.8 for the interaction of these toxins, indicating that the increased toxicity of the combination resulted from synergism.

Resistance in mosquito strain *Cq*4ABD. As shown in Table 4, strain *Cq*4ABD was highly resistant (LC_{95} RR, 949) to a combination of three selecting toxins and exhibited a significant level of resistance to *B. thuringiensis* subsp. *israelensis* (LC_{95} RR, 12.4), as well as an extremely low but statistically significant level of cross-resistance to *B. thuringiensis* subsp. *jegathesan* (LC_{95} RR, 3.5). The level of resistance in *Cq*4ABD to Cry11A (LC_{50} RR, >7,000) and the level of cross-resistance to Cry11B (LC_{95} RR, 347) were very high. However, when Cry11B was combined with Cyt1A, the level of cross-resistance to Cry11B was reduced substantially (LC_{95} RR, 3.7) (Table 4 and Fig. 1). The interaction between Cyt1A and Cry11B was highly synergistic, with an SF of 11.2.

Resistance in mosquito strain *Cq*4ABDCytA. Mosquito strain *Cq*4ABDCytA exhibited a moderate level of resistance (LC_{95} RR, 12.7) to the selecting bacterium, *B. thuringiensis* subsp. *israelensis*, and a low but statistically significant level of cross-resistance (LC_{95} RR, 5.1) to *B. thuringiensis* subsp. *jegathesan* (Table 5). Strain *Cq*4ABDCytA, however, exhibited a high level of resistance to Cry11A (LC_{95} RR, 567) and a moderate level of cross-resistance to Cry11B (LC_{95} RR, 11.8), as shown in Table 5. A moderate level of resistance to Cyt1A (LC_{50} RR, 8.3) was also detected in this strain. Combining Cyt1A with Cry11B resulted in a mild antagonism between these toxins and an SF of 0.72.

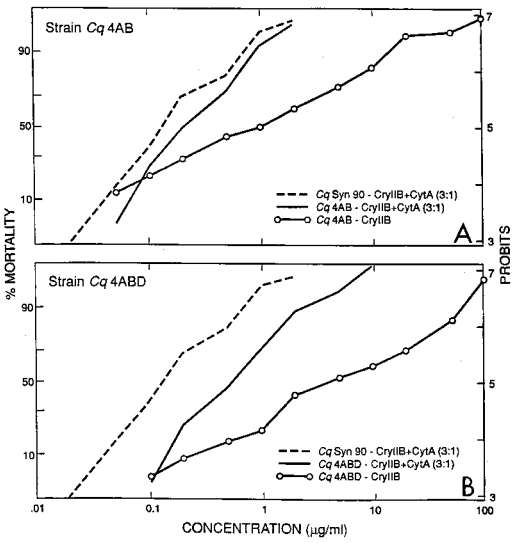

FIG. 1. Dose-response regression lines for Cry11B toxin from *B. thuringiensis* subsp. *jegathesan* in the presence or absence of Cyt1A toxin, as determined with mosquito strains susceptible or resistant to Cry toxins from *B. thuringiensis* subsp. *israelensis*. (A) Toxicity of Cry11B in the presence or absence of Cyt1A, to susceptible strain *Cq*Syn90 and resistant strain *Cq*4AB, which was selected with Cry4A and Cry4B. (B) Toxicity of Cry11B in the presence or absence of Cyt1A to susceptible strain *Cq*Syn90 and resistant strain *Cq*4ABD, which was selected with Cry4A, Cry4B, and Cry11A.

TABLE 4. Toxicities and RR for various mosquitocidal bacterial toxins with strain *Cq*4ABD

Toxin(s)	No. of larvae	LC_{50} (μg/ml)	LC_{95} (μg/ml)	RR at:		Slope	Theoretical LC_{50} (μg/ml)	SF
				LC_{50}	LC_{95}			
B. thuringiensis subsp. *israelensis*	1,100	0.122 (0.103–0.144)[a]	1.91 (1.41–2.72)	4.6 (3.9–5.5)	12.4 (9.1–16.9)	1.4		
B. thuringiensis subsp. *jegathesan*	800	0.274 (0.243–0.309)	1.56 (1.27–1.98)	3.9 (3.3–4.6)	3.5 (2.5–4.8)	2.2		
Cry11A	800	5,521	—[b]	7,049	—	0.5		
Cry11B	1,200	4.96 (4.05–6.07)	167 (114–262)	56.2 (48.3–66.7)	347 (259–484)	1.1		
Cyt1A	400	27.6 (11.8–69.0)	Plateau[c]			1.7		
Cry11B + Cyt1A	700	0.555 (0.479–0.642)	3.95 (3.08–5.38)	3.7 (3.1–4.4)	3.7 (2.7–5.1)	1.9	6.24	11.2
Cry4A + Cry4B + Cry11A	1,100	1.44 (1.13–1.82)	107 (70.2–176)	68.1 (58.1–79.9)	949 (707–1,272)	0.88		

[a] The values in parentheses are fiducial limits (95% confidence interval).
[b] —, value not given because the predicted value was extraordinarily high.
[c] There was a plateau at concentrations from 100 to 1,000 μg/ml, with an average mortality of 56.3% (32).

DISCUSSION

We found that strains of the mosquito *C. quinquefasciatus* selected for high levels of resistance to single and multiple toxins of *B. thuringiensis* subsp. *israelensis* exhibit only low levels of cross-resistance to *B. thuringiensis* subsp. *jegathesan*. In addition, we found that the same resistant mosquito strains exhibited moderate to high levels of cross-resistance to the Cry11B toxin from *B. thuringiensis* subsp. *jegathesan*, but this cross-resistance could be markedly reduced in two of the strains by combining Cry11B with Cyt1A.

Our observation that there were only low levels of cross-resistance to wild-type *B. thuringiensis* subsp. *jegathesan* in our resistant mosquito strains is consistent with prior work (31). Previously, we showed that the same resistant mosquito strains were highly sensitive to *B. thuringiensis* subsp. *israelensis* provided that all of the toxins were present in the test preparations. The lack of any substantial resistance to the toxin complex of *B. thuringiensis* subsp. *israelensis* was shown to result from highly synergistic interactions between the three Cry toxins and Cyt1A (32) and, to a lesser extent, from interactions among the Cry toxins (19, 31). Although synergism between Cyt1A and the Cry toxins against the nonresistant mosquito strain was demonstrated, the synergism against the resistant mosquito strains was much more pronounced. These results suggest that the low levels of cross-resistance to *B. thuringiensis* subsp. *jegathesan* in the resistant mosquito strains observed in the present study were due to interactions among the complex of seven toxins (the 80-, 72-, 70-, 65-, 37-, 26-, and 16-kDa proteins) present in this new mosquitocidal bacterium (8).

The levels of cross-resistance to Cry11B exhibited by the mosquito strains increased with the complexity of the Cry toxin mixture used for selection. The lowest level of cross-resistance was exhibited by strain *Cq*4D (LC_{95} RR, 53.1), whereas higher levels of cross-resistance were exhibited by strains *Cq*4AB (LC_{95} RR, 80.7) and *Cq*4ABD (LC_{95} RR, 347) (Tables 2 to 4). This finding is in direct contrast to the pattern of Cry11A resistance and cross-resistance reported previously for the same mosquito strains (31). The levels of resistance to Cry11A were highest in the strain selected with a single Cry toxin from *B. thuringiensis* subsp. *israelensis* and declined with increasing complexity of the selecting mixture. Although Cry11B and Cry11A are similar, they differ in many amino acids whose roles in toxicity are not known. One explanation for the observed differences in cross-resistance patterns is the possibility that Cry11A and Cry11B bind to different receptors or with different affinities. Identification of the receptors for these two proteins, as well as the mechanism of resistance in the mosquito strains, would facilitate understanding these toxicity patterns. The contrasting patterns of resistance and cross-resistance between toxins with a significant degree of structural similarity suggest that these differences may provide information concerning toxin characteristics which are important for high mosquitocidal activity.

Another interesting observation that emerged from the present study concerned the interaction of Cry11B with Cyt1A, which varied from antagonistic to highly synergistic depending on the mosquito strain with which the combination was tested. No synergism at the LC_{50} was observed when the Cyt1A-Cry11B combination was tested against *Cq*4D. However, a threefold decline in resistance at the LC_{95} suggests that this combination may, in fact, have some impact on cross-resistance. When it was tested against *Cq*Syn90 or *Cq*4ABDCytA, the combination was slightly antagonistic. However, against *Cq*4AB and *Cq*4ABD, the combination was moderately and highly synergistic, respectively, and resulted in elimination of cross-resistance to Cry11B in strain *Cq*4AB and reduction of the RR to 3.7 for strain *Cq*4ABD. It is particularly notable that the Cyt1A-Cry11B combination resulted in no enhanced toxicity to nonresistant parental mosquito strain *Cq*Syn90 because high levels of synergism were observed with combinations of

TABLE 5. Toxicities and RR for various mosquitocidal bacterial toxins with strain *Cq*4ABDCytA

Toxin(s)	No. of larvae	LC_{50} (μg/ml)	LC_{95} (μg/ml)	RR at:		Slope	Theoretical LC_{50} (μg/ml)	SF
				LC_{50}	LC_{95}			
B. thuringiensis subsp. *israelensis*	700	0.164 (0.138–0.195)[a]	1.96 (1.44–2.87)	6.3 (5.3–7.4)	12.7 (9.1–17.7)	1.5		
B. thuringiensis subsp. *jegathesan*	800	0.245 (0.212–0.282)	2.28 (1.79–3.07)	3.5 (2.9–4.1)	5.1 (3.8–7.0)	1.7		
Cry11A	1,000	19.9 (15.3–26.4)	2,831 (1,528–6,088)	25.5 (21.9–29.7)	567 (410–784)	0.76		
Cry11B	1,100	0.268 (0.222–0.322)	5.67 (4.16–8.21)	3.0 (2.6–3.6)	11.8 (8.7–16)	1.2		
Cyt1A	600	211 (158–307)	NA[b]	8.3		0.99		
Cry11B + Cyt1A	900	0.497 (0.418–0.589)	6.93 (5.07–10.1)	3.3 (2.8–3.9)	6.5 (4.7–8.8)	1.4	0.356	0.72

[a] The values in parentheses are the fiducial limits (95% confidence interval).
[b] NA, the average mortality was 35% at a concentration of 1,000 μg/ml.

Cyt1A plus Cry11A or Cyt1A with Cry4 against the same mosquito strain in previous studies (32). The lack of synergism between Cyt1A and Cry11B against the nonresistant parental mosquito strain may have been due to the high toxicity of the latter toxin, which is approximately 10 times more toxic than Cry11A (8). The antagonistic interaction between Cyt1A and Cry11B in strain *Cq*4ABDCytA is more likely due to the eightfold level of resistance to Cyt1A detected in this strain. The mechanism of synergism between Cyt and Cry toxins is not known, but it has been postulated that Cyt1A may act by enhancing the binding to or insertion of Cry toxins into the mosquito microvillar membrane (32). If Cry11B's high toxicity compared to the toxicity of Cry11A is due to higher binding affinity or ability to insert into the microvillar membrane, then this may account for the lack of synergism between Cyt1A and Cry11B in the sensitive strain.

The focus of this study was to assess cross-resistance to Cry11B and *B. thuringiensis* subsp. *jegathesan* in mosquito strains resistant to the mosquitocidal toxins of *B. thuringiensis* subsp. *israelensis*. However, it is noteworthy that the level of resistance reported here (LC_{95} RR, 12.7) (Table 5) in *C. quinquefasciatus* to Cry4ABDCytA (the wild-type strain of *B. thuringiensis* subsp. *israelensis*) was a level that would be of concern in mosquito control programs. Nevertheless, substantial levels of resistance to *B. thuringiensis* subsp. *israelensis* were not detected until after 60 generations of selection, whereas resistance to single or multiple mosquito Cry toxins appeared as early as generation 16 (11). A key difference between *B. thuringiensis* subsp. *israelensis* and the various bacterial strains used to select resistance to Cry4 and Cry11 toxins is that the wild-type bacterium produces Cyt1A. These results, in conjunction with our finding of a low level of cross-resistance to *B. thuringiensis* subsp. *jegathesan*, which also produces a mixture of Cry and Cyt proteins (4), suggest that bacterial strains with combinations of Cry and Cyt proteins may be useful in management of resistance in mosquito populations.

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