

MOLECULAR STUDY OF INTERACTIONS BETWEEN Cry4Ba AND Cyt2Aa2
TOXINS FROM *Bacillus thuringiensis*

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ABSTRACT

The functional importance of the β_2 - β_3 and β_4 - β_5 hairpin-loops in the receptor-binding domain of Cry4Ba toxin from *Bacillus thuringiensis* subsp. *israelensis* has been clearly identified. These two critical surface-exposed loops of Cry4Ba toxin are also involved in synergistic interactions with Cyt2Aa2 toxin from *B. thuringiensis* subsp. *darmstadiensis*. In this study, Thr³²⁸ in β_2 and Thr³⁶⁹ in β_5 of the receptor-binding domain of Cry4Ba toxin were investigated for their essential roles in H-bonded stabilisation of β_2 - β_3 and β_4 - β_5 hairpins. Alanine substitution was employed to generate T328A-RQ and T369A-RQ mutants using Cry4Ba-RQ as a template. Bioassays revealed that these two mutants displayed a drastic decrease in toxicity against *Aedes aegypti* mosquito-larvae. Additionally, ELISA-based binding assays revealed a complete loss in binding of both T328A-RQ and T369A-RQ mutants to Cyt2Aa2 toxin. These results suggested that both Thr³²⁸ and Thr³⁶⁹ are involved in interactions between Cry4Ba and Cyt2Aa2. Moreover, structural analysis disclosed the side-chain H-bonding between Thr³²⁸ and Thr³⁶⁹ which is critical for stability of the β_2 - β_3 and β_4 - β_5 hairpins of Cry4Ba. In contrast, the E417A/Y455A-RQ double-mutant, containing mutations at β_6 - β_7 and β_8 - β_9 loops of cry4Ba toxin, showed partial loss in toxicity against *A. aegypti* mosquito-larvae. In addition, E417A/Y455A-RQ still retained a high affinity for binding to Cyt2Aa2. Additionally, toxicity restoration assays revealed that in combination with Cyt2Aa2, E417A/Y455A-RQ exhibited greatly-enhanced toxicity against *A. aegypti* larvae at a level comparable to that of the wild-type toxin. These results indicate that β_6 - β_7 and β_8 - β_9 loops were involved in binding to *Aedes* receptors, but not involved in interactions with Cyt2Aa2. Additionally, Cyt2Aa2 toxin can act as an alternative receptor for Cry4Ba. Moreover, these two toxins showed a synergistic effect on toxicity against *A. aegypti* mosquito larvae.

KEY WORDS: *Bacillus thuringiensis* / Cry4Ba / Cyt2Aa2 / δ -ENDOTOXIN /
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