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Isolation of *Bacillus thuringiensis* llp29 and its mechanism on *Aedes albopictus*

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Many *Bacillus thuringiensis* isolates were recovered from the phylloplanes of plants and Bryophyte in this study. Among them, the new strain *LLP29* isolated from phylloplanes of *Magnolia denudata* was the most toxic to mosquitoes according to the results of the preliminary screening. To learn more about the characteristics of this novel mosquitocidal isolate, phase-contrast microscopy, insecticidal activity and PCR analysis were performed. The LC50 values of LLP29 against *Aedes albopictus* was 0.33 μg of protein/mg. A new gene, *cyt1Aa6*, was detected, cloned, named and expressed successfully. Bioassays on *A. albopictus* showed that *LLP29* and the expressed *BL21* were both toxic to the 3rd-instar larvae. Meanwhile, the purified Cyt1Aa6 protein was highly toxic against Ae. albopictus larvae and C6/36 cells.

In order to further understand the novel isolates the mechanism against Ae. albopictus was studied. In indirect immunofluorescence assay, Cyt1Aa6 protein infected target cells by attaching to various cell receptors located on the cell surface. Immunohistochemistry showed that Cyt1Aa6 protein was detected in the midgut of *A. albopictus*. It was consistent with previous studies that midgut was the target site for *Bacillus thuringiensis*.

It was reported that Cyt1 might prolong high-level resistance in the mosquito management. However, the resistance ratio was 19.16 fold when the resistant C6/36 cells were selected *in vitro* activated *Cyt1Aa6* at generation 10. In order to elucidate the resistance mode of the novel toxin on cultured insect cells, receptor binding properties of *Cyt1Aa6* towards the susceptible and resistant C6/36 cells were studied as well. More susceptible cells were detected with goat-anti-rabbit-FITC-labelled antibody, and the quantity of vitro-activated Cyt1Aa6 toxin bound to resistant cells reduced greatly. Ligand western blot assays showed that the disappearance of the 26 kD protein and the weakness of the positive bands of 67 kD and 52 kD from resistant cells might lead to the resistance of C6/36 cells to Cyt1Aa6 toxin.

This study informed us of the mechanism and characterization of resistance to this new toxin. It would be useful for designing management strategies delaying or counteracting the resistance to *Bacillus thuringiensis*.

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