CONSTRUCTION OF CHROMOSOMAL ENGINEERED *BACILLUS THURINGIENSIS* SEROVAR *AIZAWAI* EXPRESSING A TRANSCRIPTIONALLY FUSED CHITINASE GENE DURING SPORULATION

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ABSTRACT

A transcriptionally fused chitinase gene comprising the *P19* gene from the *Bacillus thuringiensis cry11Aa* operon fused with a promoterless *chiBlA* gene from *Bacillus licheniformis* was integrated into the *B. thuringiensis* subsp. *aizawai* BTA1 genome by homologous recombination. The resulting *B. thuringiensis* subsp. *aizawai* strain (INT1) showed growth and sporulation comparable to that of the wild type strain. INT1 produced four chitinases of different molecular masses (i.e., 66, 55, 39 and 36 kDa). Three of these (66, 55 and 36 kDa) were derived from the cloned *chiBlA* gene whereas the 39 kDa chitinase originated from BTA1. Using surface contamination bioassays, the LC_{50} of lyophilized whole culture broth of INT1 against *Spodoptera exigua* neonate larvae was 12.2 $\mu g/cm^2$ compared to 30.8 $\mu g/cm^2$ for BTA1. Bioassays using filtered culture supernatant of INT1 (110 $\mu g/cm^2$) together with trypsin-activated purified Cry1C protein of *B. thuringiensis* (1,280 ng/cm^2) showed 75.0% mortality compared to 56.7% mortality for Cry1C combined with BTA1 at the same concentration. Using scanning electron microscopy, clear perforations were observed in *S. exigua* 5th instar peritrophic membranes incubated with either crude or purified chitinase, or isolated from 5th instar *S. exigua* fed purified chitinase since the first instar. These results help clarify the role of chitinase in enhancing *B. thuringiensis* δ-endotoxin insecticidal activity and suggest that expressing heterologous proteins chromosomally may provide a method for increasing the insecticidal activity of commercial applications of *B. thuringiensis*-based products.

KEY WORDS: CHITINASE / *BACILLUS THURINGIENSIS SUBSP. AIZAWAI* INSECTICIDAL ACTIVITY / BIOASSAY /

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