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Evaluation of chitinase activity of Sri Lankan *Bacillus thuringiensis* strains

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Bacillus thuringiensis (*Bt*) is widely used as a bio-pesticide owing to the toxic proteins*Cry*, *Cyt* and *Vip* produced during their growth stages. Many strains of *Bt* produce *chitinase* enzyme, which can enhance the insecticidal activity by degrading the chitin in exoskeleton of insect pests as well as by degrading the chitin component in the peritrophic membrane of the insect midgut, which facilitates the entrance of toxic proteins to the midgut. The objective of the current study was to evaluate the chitinase activity of native *Bt* strains. Seventeen chitinase producing *Bt* strains of subspecies *Bt kurstaki* (AB8, AB15, AB20, AB49), *Bt graciosensis* (AB6, AB7, AB10, AB11, AB12, AB13, AB14 and AB16), *Bt poloniensis* (AB17), *Bt canadensis* (AB19), *Bt konkukian* (AB23), and *Bt israelensis* (AB22, AB24), selected from a qualitative disk dip assay, were used in this study. Fresh *Bt* cultures (1 mL) were inoculated separately into 10 mL of LB medium (containing 1% colloidal chitin) and incubated overnight at 37°C with shaking at 200 rpm. The cultures were transferred into 250 mL chitinase inducing broth (supplemented with 1% colloidal chitin) and incubated at 37°C on a rotary shaker at 200 rpm for 3 days. Proteins in the culture supernatant fluid were precipitated with ethyl alcohol at −20°C overnight. The precipitate was recovered by centrifugation and dissolved in 1.5 mL of 0.1M Na-phosphate buffer (pH 7.0). Chitinase activity was measured by the reduction of N-acetyl-D-glucosamine released by enzymatic hydrolysis of chitin using DNS assay. The absorbance was recorded at 535 nm. Readings were compared with a standard curve prepared with a series of dilutions of NAG (0–10 mg/mL). The chitinase activity was assayed in triplicate for each strain. One chitinase activity unit (U) was defined as the amount of enzyme required producing 1 µmol of NAG in 1 h. Standard *Bt aizawi* strain exhibited the highest chitinase activity of 3.345 U/mL. Of the 17 *Bt* strains screened, all strains exhibited chitinase activity ranging from 1.717 to 2.558 U/mL. Two *Bt kurstaki* strains (AB15 and AB20) exhibited higher chitinase activity of 2.558 U/mL, which is the highest recorded out of the tested strains. Further, these *Bt* strains contained a mixture of lepidopteran, coleopteran, and dipteran toxic genes as characterized by PCR analysis previously. This study demonstrated that these *Bt* strains could enhance the insecticidal activity of *Bt* due to the production of chitinase enzyme.

Keywords:*Bacillus thuringiensis*, Chitinolytic strains, DNS assay

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