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KEY WORD : *BACILLUS LICHENIFORMIS*, *BACILLUS THURINGIENSIS*, CHITINASE, delta-ENDOTOXIN, *SPODOPTERA EXIGUA*
SRISURANG TANTIMAVANICH : CLONING OF CHITINASE GENE FROM *BACILLUS LICHENIFORMIS* TP-1 IN *BACILLUS THURINGIENSIS* SUBSP. *AIZAWAI* AND STUDY OF ITS EFFECT TOWARDS *SPODOPTERA EXIGUA*. THESIS ADVISOR : SOMSAK PANTUWATANA, Ph.D., AMARET BHUMIRATANA, Ph.D., WATANALAI PANBANGRED, D. Eng. 179 p. ISBN 974-589-167-3

A *Bacillus* sp. strain TP-1 produced the highest amount of extracellular chitinase (2,250 μU/ml) when compared to another 199 *Bacillus* isolates, including 195 isolates of *B. thuringiensis* and 4 isolates of *B. subtilis*. The strain TP-1 was identified as *B. licheniformis* (TP-1). The chitinase gene from the TP-1 strain was cloned and subcloned in *E. coli* DH5α and the gene was sequenced. The DNA region required for full expression of chitinase activity contained a single open reading frame of 1,815 bp which encoded a polypeptide of 604 amino acid residues. This polypeptide comprised a characteristic signal sequence of 35 amino acid residues including a concensus recognition sequence of signal peptidase, Ala-X-Ala. The deduced N-terminal amino acid residues 36 to 334 showed the highest homology (69.5%) to *B. circulans* ChiA1. The 299 amino acid residues from N-terminus of TP-1 chitinase corresponded to the catalytic domain which contained 2 conserved amino acids, Asp and Glu, of the active site of chitinase. The C-terminus contained conserved amino acid residues which were homologous to those found in chitin binding domain of chitinases from other bacteria. *E. coli* harboring a complete chitinase gene (pCHIL3) expressed chitinases of 3 different molecular masses, 68, 62, and 50 kDa. By contrast, the TP-1 strain produced only a single 50 kDa chitinase which might be a processed form of an enzyme precursor. The gene was subcloned into pBC16 to yield pCHIL3-16 which was subsequently transferred into a chitinase non-producing *B.t. aizawai* (*B.t.a.*). The specific activity of chitinase from *B.t.a.* (pCHIL3-16) was 480 mU/mg which was about 80 and 4 times higher than those of *E. coli* harboring pCHIL3 (7 mU/mg) and *B. licheniformis* TP-1 (102 mU/mg). *B.t.a* (pCHIL3-16) showed 10 fold higher in insecticidal activity and killed *S. exigua* larvae 2 days more rapidly than the parental *B.t.a.* An enhanced effect of exogenous chitinase was also demonstrated. In the presence of 10 mU of chitinase, the LD_{50} of *B.t.a.* could be reduced 7.6, 13.8 and 15 folds, as determined on days 3, 5, and 7, respectively. A remarkable retardation on larval growth and development was observed using a combination of chitinase (10 mU) and a sublethal dose of *B.t.a.*. These results indicate that chitinase in combination with *B.t.a.* can be effective in controlling lepidopteran pests.