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SURANG THAMTHIANKUL : CHARACTERIZATION AND PRODUCTION OF CHITINASE FROM *BACILLUS THURINGIENSIS* SUBSP. *PAKISTANI* AND ITS TRANSFORMANTS. THESIS ADVISORS : WATANALAI PANBANGRED, D.Eng, VITHAYA MEEVOOTISOM, Ph.D., CHUENCHIT BOONCHIRD, Ph.D. 148 P, ISBN 974-663-473-9

The purpose of this study is to characterize chitinase enzyme from *Bacillus thuringiensis* subsp. *pakistani* (*B.t.p.*). The chitinase (*chi*) encoding gene from *B.t.p.* is composed of 1,905 nucleotides encoding a protein of 635 amino acids. Comparison of deduced amino acid sequence of *B.t.p.* chitinase with those of other bacterial chitinases revealed 4 domain structures from N-terminus to C-terminus. Domain 1 from N-terminus, contained a characteristic signal peptide of 34 amino acids with the recognition sequence of signal peptidase, Ala-X-Ala, implying that the gene encoded a pro-enzyme of chitinase. Domain 2 consisted of 315 amino acids, and was identified as the catalytic domain. It contains Asp and Glu, which are the two conserved amino acids found at the active site of family 18 glycosyl hydrolases. Domain 3 (94 amino acids) showed homology with type III homology units of fibronectin and was designated as fibronectin like domain (FLD). Domain 4, at C-terminus (192 amino acids) was the chitin binding domain (CBD), since it exhibited identity with several CBDs of other chitinases. pCHIPIII/R-02 harboring *B.t.p. chi* gene was transformed into *B.t.p.* parental strain and two chitinase negative mutants *B.t.p.*CAT1 and *B.t.p.*CAT8. In nutrient broth-salt medium with 0.2% colloidal chitin (NYSMC), transformed *B.t.p.*, *B.t.p.*CAT1 and *B.t.p.*CAT8 all produced chitinase activity (at 24 hr) at 8.12, 9.82 and 14 mU/ml, respectively, which were 4-7 times higher than that produced by the *B.t.p.* wild type (2.1 mU/ml). Chitinase produced by *B.t.p.*, *B.t.p.* (pCHIPIII/R-02), and *B.t.p.*CAT8 (pCHIPIII/R-02) showed 4 chitinase activity bands when detected with renaturing SDS-PAGE but different activity was found for each band. Their estimated molecular masses were 66-, 60-, 47- and 32 kDa. All 4 chitinases had the same N-terminal amino acid sequence (D-S-P-K-Q), suggesting that the 60-, 47- and 32 kDa chitinases were derived from the 66 kDa chitinase by processing from the C-terminus. Growth, sporulation and enzyme production of *B.t.p.*, *B.t.p.* (pCHIPIII/R-02) and *B.t.p.*CAT8 (pCHIPIII/R-02) were studied using LBC (Luria-Bertani broth containing 0.2% colloidal chitin), NYSMC, NBC (nutrient broth containing 0.2% colloidal chitin), NBC/2 (half strength nutrient broth containing 0.2% colloidal chitin). *B.t.p.*, *B.t.p.*CAT8 (pCHIPIII/R-02) and *B.t.p.* (pCHIPIII/R-02) grown in NYSMC produced the highest enzyme activity at 1.8, 13.97 and 7.81 mU/ml, respectively. *B.t.p.*CAT8 (pCHIPIII/R-02) was found to sporulate poorly in all media tested (less than 20 CFU/ml). Fructose (0-2.0%) supplemented to NYSMC medium enabled *B.t.p.*CAT8 (pCHIPIII/R-02) to sporulate better and produced higher chitinase activity, in which at 1.0% and 0.3% fructose were the most suitable concentration. Cell grown in NYSMC plus 1.0% and 0.3% fructose could produce enzyme at 17.57 mU/ml and spore count 2.64 X 10^9 CFU/ml, respectively. This study proposes the possible improvement of other *B.t.* strain with higher chitinase activity.