

OB 4 - Molecular Characterization of a Novel Chitinase from *Bacillus thuringiensis* subsp. *kurstaki*

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BUPM255 is a chitinase-producing strain of *Bacillus thuringiensis*, characterized by its high chitinolytic and antifungal activities. The cloning and sequencing of the corresponding gene chi255, showed an open reading frame of 2031 bp, encoding a protein of 676 amino acid residues. Both nucleotide and amino acid sequences similarity analysis revealed that the chi255 is a new chitinase gene, presenting several differences from the published chi genes of *B. thuringiensis*. Identification of chitin hydrolysis products issued from the activity exhibited by Chi255 by heterologous expression in *E. coli*, revealed that this enzyme should be a chitobiosidase. The mature form of the corresponding chitinase Chi255 consists of an N-terminal domain corresponding to the catalytic domain, two fibronectin-like (type III) and a chitin-binding domain at the C-terminal end. In order to determine the minimal sequence required for the chitinolytic activity, various deletions derivatives encoded by modified chi255 were constructed by oligonucleotide-directed mutagenesis. Nonsense codons were created to stop elongation at target positions that generate truncated chitinases lacking, the binding domain, one or two fibronectin-like domains, or all of them. The modified chi255 genes were expressed in *Escherichia coli* and the gene products were analysed for their colloidal chitin-hydrolysing activities. Only the form lacking the C-terminal domain corresponding to the chitin-binding domain preserves its chitinolytic activity. Thus, it was concluded that the C-terminal domain is not essential for the chitinolytic activity and therefore a deletion of 300 pb from chi255 gene does not affect the activity of the corresponding truncated chitinase.