Characterization of the chitinase gene in Bacillus thuringiensis Mexican isolates

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Abstract

The chitinase gene was molecularly characterized in five *Bacillus thuringiensis* Mexican isolates,MR10,MR11, MR21, MR33, and RN52. The proteins derived from these genes were tested for their chitinase activity using fluorogenic chitin derivatives. In order to verify if chitinase genes were functional, they were cloned, and enzymatic activity of recombinant chitinases was also tested. Results indicated that enzymes exhibited endochitinase activity. The highest hydrolytic activity shown against the chitin tetrameric derivative occurred at pH value of 6.5, and the optimum activity temperature was around 60 °C. The recombinant endochitinases showed a molecular mass of ∼77 kDa with isoelectric points from 6.5 to 7.0. Analysis of the nucleotide sequences showed highly conserved sequences among all isolates (97–99 %). Gene sequence analysis revealed a putative promoter (−35 TTGAGA and −10 TTAATA) and a Shine–Dalgarno sequence (5´-AGGAGA-3´) upstream from the open reading frame. The deduced amino acid sequence revealed that the proteins are modular enzymes composed by a family 18 glycosyl hydrolase domain located between amino acids 134 and 549, a fibronectin-binding domain (580 through 656), and a chitin-binding domain (664 through 771). The deduced amino acid sequences of our isolates showed a similarity close to 100 % respect to the sequences reported in the GenBank database.