An endochitinase A from *Vibrio carchariae*: cloning, expression, mass and sequence analyses, and chitin hydrolysis

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Abstract

We provide evidence that chitinase A from *Vibrio carchariae* acts as an endochitinase. The *chitinase A* gene isolated from *V. carchariae* genome encodes 850 amino acids expressing a 95-kDa precursor. Peptide masses of the native enzyme identified from MALDI-TOF or nanoESIMS were identical with the putative amino acid sequence translated from the corresponding nucleotide sequence. The enzyme has a highly conserved catalytic TIM-barrel region as previously described for *Serratia marcescens* ChiA. The M_r of the native chitinase A was determined to be 62,698, suggesting that the C-terminal proteolytic cleavage site was located between R^{597} and K^{598}. The DNA fragment that encodes the processed enzyme was subsequently cloned and expressed in *Escherichia coli*. The expressed protein exhibited chitinase activity on gel activity assay. Analysis of chitin hydrolysis using HPLC/ESI-MS confirmed the endo characteristics of the enzyme.

Keywords: Endochitinase; Gene isolation; Cloning; Gene sequence; TIM barrel; Gel activity assay; Chitin hydrolysis; HPLC-ESI MS