No. 0820

Cloning and Expression of Halotolerant Proteases from *Bacillus subtilis* Strain FP-133

Shinji Takenaka

Kobe University, Graduate School of Agriculture

Summary

Bacillus subtilis strain FP-133, isolated from a fermented fish paste, synthesizes two halotolerant extracellular proteases (Expro-I and Expro-II), showing protease activity and stability at concentrations of 0 - 20% (w/v) NaCl. In this study reported here, we cloned and analyzed the genes encoding Expro-I and Expro-II. We also examined the expression of protease gene in *Escherichia coli*.

The gene encoding Expro-I was cloned and sequenced. The amino acid sequence predicted from the open reading frame (1,149 base pairs) contained the NH_2-terminal amino acid sequence of purified Expro-I. The Expro-I amino acid sequence exhibited 98% identity to that of sublitisin like serine proteases from *Bacillus* spp. and *Brevibacillus latrosporus* G4. Most of previously reported proteases showed killing nematodes and the nematocidal activity and these proteases reported as a pathogenic factor. The deduced amino acid sequence of Expro-I contains all the characteristics conserved subtilisin functional motifs found in sublitisin like serine proteases characterized to date, including the conserved protease catalytic triad residues (Asp, His, Ser). Expro-I have a high hydrophilic amino acid, such as Asp and Glu, compared with a typical subtilisin, Carlsberg from *Bacillus licheniformis*. These hydrophilic amino acids probably would contribute to NaCl resistance. The cloned Expro-I gene without signal peptide region was expressed in *Escherichia coli*.

The gene encoding Expro-II was cloned and sequenced. The amino acid sequence predicted from the open reading frame (1,566 base pairs) contained the NH_2-terminal amino acid sequence of purified Expro-II. The Expro-II amino acid sequence exhibited 98% identity to that of Zinc-metalloproteases from *Bacillus* spp. and *Brevibacillus latrosporus* G4. The deduced amino acid sequence of Expro-II contains all the characteristics conserved metalloprotease functional motifs found in Zinc metalloproteases characterized to date, including the conserved protease catalytic triad residues (Glu, Asp, His) and the Zinc-binding motif (HEXXH).