E-7

Cloning, Expression and Purification of Chitosanases from *Bacillus subtilis* CH1 and CH2

Chulhong Oh and Jehee Lee*
*Cheju National University

Introduction

Chitosans are high molecular weight polysaccharides consisting of 1,4-β-linked D-glucosamine residues and are hydrolyzed by chitosanase. Chitosan and its partially degraded oligosaccharides are becoming important because of their potential usefulness and novel application in the field of functional foods, medical aids, pharmaceuticals, and agricultural agents (Li et al., 1992). Chitosanase activity has been detected in a variety of prokaryotes, fungi, plants and viruses. Among these, bacterial chitosanases would appear to be especially useful for obtaining large amounts of chitosan oligomers because the enzyme can be easily prepared. Most bacteria and fungi secrete chitosanases extracellularly. We performed the cloning of chitosanase genes from *Bacillus subtilis* CH1 and CH2 in pET vector system and the protein was expressed in *E. coli* BL21(DE3). The pET system is one of the commonly used systems for the cloning and expression of recombinant proteins in *E. coli*. This is based on the strong T7 promoter-driven system originally developed by Studier and colleagues (Studier et al., 1990).

Materials and Methods

PVDF blotting from purified chitosanase obtained from our previous study was used to know N-terminal amino acid sequence of two chitosanases. Long and Accurate Polymerase Chain Reaction (LA-PCR) was used in order to amplify the unknown chitosanase sequence that primers were based on the N-terminal amino acid sequence. Coding sequence including signal sequence of two chitosanases were amplified and ligated into pET11a expression vector after digestion with *Nde*I and *Bam*HI. The ligated products were transformed into *E. coli* BL21 (DE3) cell and expression of chitosanases were induced by IPTG addition. Purification of expressed chitosanases were carried out by new osmotic pressure method. SDS-PAGE was used to identify protein and activity was checked by Rondle method.

Results and Summery

Fifteen amino acids of the N-termius of mature chitosanases were identified as AGLNKDQKRRAEQLT. Chitosanase coding sequence of *B. subtilis* CH1 was 834 bp (277 amino acid) and mature sequence was 729 bp (242 amino acid). Chitosanase coding sequence of *B. subtilis* CH2 was 816 bp (272 amino acid) and mature sequence was 729 bp (242 amino acid). The difference between two chitosanases coding region was 32 bases (signal sequences differed by 20 bases and mature sequences differed by 12 bases). However, it was observed that the mature sequences are same when converted into amino acid sequence from nucleotide sequence. The predicted number of cysteine residues of the amino acid sequence was only one out of the total amino acid residues. Two chitosanases tested in this study do not have disulfide bonds. IPTG induced chitosanases lysissed through ultra-sonication on ice. It was identified by SDS-PAGE and was tested for chitosanase activity. IPTG was used to induce chitosanase expression and temperatures used for induction were 18, 25 and 30℃. In 25 and 30℃ insoluble proteins were made. However, at 18℃ high amount of active proteins were observed. Some amount of expressed chitosanases were moved to periplasm by signal peptide of the chitosanase and it was purified with NaCl solution by our new method.

References

Li, Q., E.T. Dunn, E.W. Grandmasion and M.F.A. Goosen, 1992. Applications and properties of chitosan. J. Bioactive and Compatible Polymers., 7: 370-397.

Studier, F.W., A.H. Rosenberg, J.J. Dunn and J.W. Dubendorff, 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. 185: 60-89.