Synopsis of Original Research Paper

Application of Microbial Plysac-charide-degradingenzymes to Cosmetology

Satoshi Nakamura

Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology

β-1,4-Xylans, major components of plant hemicelluloses, are heterogeneous polysaccharides that have a backbone of β-1,4-linked xylopyranose units. Xylanases (1,4-β-D-xylan xylanohydrolase; EC3.2.l.8) catalyze the hydrolysis of xylan to xylooligosaccharides and xylose. Recently, we have isolated alkaliphilic *Bacillus* sp. strain 41M-l from soil. Strain 41M-l secreted multiple xylanases and one major form of them, termed xylanase J. had an alkaline pH optimum. In this study, we describe cloning, sequencing and specific mutagenesis of the gene encoding xylanase J from strain 41M-1.

A genomic library of strain 41M- l was screened for xylanase activity to obtain a 2.2-kb Eco RI-Sph I fragment containing the xylanase J gene. Then, the nucleotide sequence of the 2.2-kb fragment was determined. The putative xylanase J gene contained an open reading frame of 1,062 bp and encoded a 27-aa leader peptide followed by a 327-aa mature enzyme. The promoter-like sequence and typical Shine-Dalgarno sequence were observed upstream from the possible TTG start codon. A perfect 14-bp inverted repeat, corresponding to a transcriptional terminator, occurred downstream from the TAG stop codon.

The xylanase J gene was expressed in *Escherichia coli*. More than 90% of xylanase activity was located in the periplasmic space. Characteristics of the *E. coli*-produced xylanase J were quite equal to those of the enzyme from strain 41M-1.The deduced amino acid sequence of xylanase J was compared with the sequences of other bacterial xylanases. The potential catalytic domain of xylanase J was located at the N-terminus and had strong similarity to family G xylanases, suggesting that the enzyme also belonged to the family G hydrolases. A linker sequence rich in Ser, Thr and Pro occurred between the catalytic domain and an additional domain at the Cterminus. This (-terminal domain of unknown function showed no significant similarity to any other proteins.

Two Glu residues, previously identified as essential for catalytic activity in the family G xylanase from *Bacillus purnilus*, are conserved in xylanase J at positions 93 and 183. These two Glu residues were targeted for mutational analysis. Substitution of Glu-93 or Glu-183 by Gln (mutants E93Q and El83Q, respectively) drastically reduced xylanase activity. The carboxylic residues of these two Glu would probably act by general acid catalysis as has been shown for other hydrolytic enzymes such as lysozyme. The enzyme activity of xylanase J was inhibited by N-bromosuccinimide, suggesting that Trp and/or Tyr was involved in catalysis. Some of Trp and Tyr in xylanase J were replaced by Phe. The large decrease in activity were observed with the mutant enzymes Wl8F, W86F, Y84F and Y95F. These results suggest that Trp-18, Trp-86, Tyr-84 and Tyr-95 play important roles in binding of the substrate.