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SONGSAK ROEKRING : ISOLATION, ANALYSIS, AND EXPRESSION OF CEPHALOSPORIN C DEACETYLASE GENE FROM *BACILLUS CEREUS* STRAIN BT-24. THESIS ADVISORS : CHUENCHIT BOONCHIRD, Ph.D., VITHAYA MEEVOOTISOM, Ph.D., SUTHEP WIYAKRUTTA, Ph.D., p160. ISBN 974-663-807-6

Cephalosporin deacetylase (CAH) is an enzyme used for removal of the side chain at C-3 position of cephalosporin C. The product obtained is deacetylcephalosporin C that is useful for production of cephalosporin derivatives. In this study, high esterase producing strains isolated from soil samples were screened by the chemical and microbiological methods. Positive colonies were confirmed by HPLC for their ability to convert cephalosporin C to deacetylcephalosporin C. One strain showing highest amount of cephalosporin C deacetylase activity was chosen for the source of CAH gene cloning. The strain was identified as *Bacillus cereus*. The gene was cloned from *Sau*3AI digested genome using *Bam*HI dephosphorelated pBS II (SK+) as a cloning vector. A positive clone was detected by both the chemical and microbiological methods. Ability of the clone to remove the acetate group at C-3 position was also confirmed by HPLC. Study on the localization of the esterase gene with CAH activity in the cloned fragment showed that the gene was located on a 1.085 Kb *Xba*I-*Eco*RI fragment. DNA sequence of this gene fragment revealed that it contained one open reading frame (ORF) containing 903 nucleotides, which encoded for a polypeptide consisting of 301 amino acids. Analysis of the deduced amino acid sequence of the ORF showed that the cloned gene contained the common sequence Gly-X-Ser-X-Gly found in many esterases, lipases and serine proteases. The putative hexanucleotide sequence (-10 region, TATAAT) recognized by *Bacillus* RNA polymerase was found in the 5′ flaking region of the esterase gene. A Shine-Dalgarno sequence (GAGG) was also recognized. Study on expression of the cloned esterase using pKK223-3 vector showed that the level of expression increased about 3 folds with IPTG induction. Analysis by SDS-PAGE revealed that the cloned gene encoded for protein with MW 34.6 kDa. Activity staining of the renatured SDS-PAGE and Native-PAGE confirmed that the expressed protein possessed esterase activity. Comparison of the deduced amino acid sequence with those reported in the GenBank showed that the gene had low homology with many esterases. This gene was suggested to be a novel esterase which could convert cephalosporin C to deacetylcephalosporin C.