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APANCHANID THEPOUYORN : STUDY OF NUCLEOTIDE SEQUENCE
AND EXPRESSION OF CHITINASE GENE FROM *BACILLUS CIRCULANS* No.4.1
IN *ESCHERICHIA COLI*. THESIS ADVISORS: CHANPEN WIWAT Ph.D., AROME
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A chitinase encoding gene from *Bacillus circulans* was cloned and expressed in *Escherichia coli*. The recombinant was designated as pCHI43. Its nucleotide sequence revealed a single open reading frame containing 1,794 bp and encoding 598 amino acids. The deduced molecular weight was 65.7 kDa. G+C and A+T residues accounted for 45.57 mol% and 56.37 mol% of the base composition of the coding sequence, respectively. The nucleotide sequence showed the putative initiation codon (ATG), -10 (TATAAA), -35 (TGCAGC), Shine-Dalgarno (SD) sequence (GAAA), and termination sequence (TAA). The signal peptide containing 35 amino acids were the recognized sequence of signal peptidase for Ala-X-Ala. The chitinase gene was subcloned in various plasmid vectors : pHY300PLK, pKK223-3 and pBluescript II KS (-/+), designated as pHY43, pKK43 and pKS43. Subsequently, the nucleotide fragment from the C-terminal and N-terminal domains of the gene were sequentially deleted in pCHI43. The deletion clones were designated as pC66, pC6S, pSS6 and pEVS. The results demonstrated that deletion of the C-terminal domain did not affect enzyme activity for soluble chitins 4-MU(GlcNAc)₂, and glycol chitin. Thus, it was concluded the C-terminal domain was a chitin binding domain required for the specific binding to chitin. The chitin binding domain and the catalytic domain of the gene were investigated and founded to contain 40 and 57 conserved amino acid residues respectively. The chitinase activities of various clones were detected using 4-MU(GlcNAc)₂ as substrate after SDS-PAGE. All showed that chitinolytic activity, the highest activity, was found in pCHI43 at 60.80 mU/mg. The others, pHY43, pKK43, pKS43, pC66, pC6S, pSS6 and pEVS expressed the chitinase activities at 28.49 mU/mg, 19.54 mU/mg, 3.70 mU/mg, 22.53 mU/mg, 36.10 mU/mg, 5.02 mU/mg, 0.96 mU/mg respectively.