C726864 MAJOR BIOTECHNOLOGY
KEY WORD:
CLONING / PROTEASE / *Bacillus subtilis* TISTR25 / GST GENE FUSION SYSTEM

KALYAKORN WONGKALASIN : CLONING OF PROTEASE GENE FROM *Bacillus subtilis* TISTR25 TO *E. coli* BY GST GENE FUSION SYSTEM. THESIS ADVISOR : ASSIST. PROF. NAPA SIWARUNGSON. THESIS CO-ADVISOR : ASSIST. PROF. WILAI ANOMASIRI, Ph. D. 114 pp. ISBN 974-637-122-3

Bacillus subtilis TISTR25 was isolated from soil in Thailand and produced both neutral and alkaline proteases.Chromosomal DNA of *B. subtilis* TISTR25 was inserted into expression vector pGEX-2T of GST Gene Fusion system and transformed into *E. coli* HB101 and shown gene expression of the fusion protein. The transformants were screened on skim milk plus IPTG and ampicillin plate. The result of colony hybridization showed signal of neutral protease gene. Succinate containing basal medium was used as a carbon source for the bacterial growth in 24 hours to assay protease activity from transformant No.97. The enzyme showed an optimum activity in buffer pH 8.5 with 305.08 unit/ mg protein. This enzyme was completely inhibited by 5 mM EDTA and remained the activity in 1 mM PMSF.This results confirms that the neutral protease were definitely produced from the transformant No.97. The fusion protein was purified under mild condition by using glutathione Sepharose 4B affinity chromatography column. The specific activity of free neutral protease was 1,289.18 unit/mg protein at pH 8.5 after thrombin cleavage on the fusion protein. The purification fold was 4.23 and the molecular weight of the free neutral protease was approximately 44,000.

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