3936477 SCBT/M : MAJOR : BIOTECHNOLOGY ; M.Sc. (BIOTECHNOLOGY)
KEY WORDS : ACETATE KINASE / PHOSPHOTRANSACETYLASE / RAMBACH / *SALMONELLA TYPHIMURIUM*

CHAYAPORN SARANPUETTI : MOLECULAR CLONING AND SEQUENCING OF PHOSPHOTRANSACETYLASE GENE FROM *SALMONELLA TYPHIMURIUM*. THESIS ADVISORS : WATANALAI PANBANGRED, D.Eng., CHAUFAH THONGTHAI, Ph.D., SKORN MONGKOLSUK, Ph.D. 175 P. ISBN 974-662-604-3

Plasmid pDU1 enables *E.coli* transformants to become pink colonies on Rambach agar (RA). Plasmid pDU1 harbored a 10 kb specific DNA probe for *Salmonella* detection which was cloned from *S. typhimurium* 23566. Acetate kinase (*ack*) gene without promoter was found on pDU6, a deleted plasmid derived from pDU1. Analysis of a 3 kb upstream region of *ack* from a 10 kb resubcloned fragment did not reveal any possible promoter sequence but instead showed a complete 384 bp ORF of another gene encoding a 14.37 kDa protein. This ORF might be phosphotransacetylase (*pta*) gene which is usually located in operon with *ack* gene in several bacteria. *E.coli* transformants harboring different plasmids, *ack* gene with its upstream region (pDU19), only *ack* gene (pDU12, pDU8-12R) and only *ack* upstream region (pDU16) were analyzed for acetate kinase and phosphotransacetylase enzymes. The result showed that the levels of enzyme activity among these transformants were not significantly different by analysis of varience.

Since *E. coli* transformants harboring pDU17 and pDU18 do not contain *ack* gene, but show red color colony, *ack* gene is not involved in red colony formation on RA. pDU18 turned colorless colonies of *E.coli*, *S.typhi* and *S.paratyphi* on RA into red colonies. The results indicate that a gene residing in pDU18 is responsible for acid accumulation which turns colorless colonies to red. BLAST searching of amino acid sequence showed that this fragment contains gene encoding for DNA gyrase inhibitory protein homolog. Mixed organic acids and inorganic phosphate analysis by using HPLC do not indicate the acids or substances responsible for red colony formation. There might be another acid or substance involved in red colony formation which was not detected by using the HPLC columns in this analysis system.