Thesis Title	**Cloning and Sequencing of Acetate Kinase Gene (*ack*), a Gene Involved in Propanediol Utilization From *Salmonella typhimurium*.**
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ABSTRACT

The plasmid pDU1 harboring a 10 kb fragment from *Salmonella typhimurium* 23566, a gene specific DNA probe for *Salmonella* spp., enables transformed *E. coli* cells becoming pink colonies on Rambach agar. The pDU6 and pDU8 plasmids derived from that cloned fragment also turned colorless colonies of *E. coli*, *S. typhi* and *S. paratyphi* on such medium into red colonies. This result indicated that the genes on pDU6 and pDU8 had an ability to overproduce acid. Nucleotide sequence analysis of a 5 kb inserted fragment in pDU6 showed that at least 2 genes reside in that fragment. One gene is acetate kinase gene (*ack*) which is located on one end closed to *Eco*RI site of the fragment. The other end was found to harbor only half of an open reading frame of a hydrogen sulfide F (*phs*F) producing gene. *S. typhimurium* acetate kinase gene consists of 1,206 nucleotides which is deduced to a 43.8 kDa protein. Two specific active sites of this acetate kinase enzyme family were also found residing in this deduced amino acid sequence. This gene sequence was submitted to Genbank

database as the first published *Salmonella ack* gene sequence. Southern blot DNA hybridization of chromosomal DNA from *Salmonella typimurium*, *S. enteritidis*, *S. typhi* and *S. paratyphi* with *S. typhimurium ack* gene serotypes revealed the same size of hybridized fragments when the DNA was cut with the same restriction enzyme, *Nsi*I, *Cla*I, and *Cla*I/*Eco*RI. Whereas hybridization of *S. typhimurium ack* gene to DNA of other bacterial strains did not show hybridization signal under the same hybridization condition. Since *ack* promoter sequence was not resided within the inserted fragment in pDU6 and pDU8, a 300 bp upstream region of *ack* was subsequently cloned in pDU8, and it nucleotides were analyzed. The sequence analysis of this fragment did not show any possible promoter sequence but instead it revealed an incomplete open reading frame of another gene which might be phosphotransacetylase (*pta*) gene. The *pta* gene is usually located in operon with *ack* gene in several bacteria such as *ack/pta* operon found in *C. acetobutylicum* and *M. thermophila.* To test any possibility of *ack* gene expression, several *E. coli* transformants harboring *ack* gene with or without its upstream region were analyzed for acetate kinase enzyme. The result showed that the level of enzyme activity in both transformant groups were not significantly different. Therefore, it is suggested that the *ack* gene is not involved in red colony formation. *Sac*I deletion of *phs*F region was performed. The transformant contained a deleted plasmid which lacks most part of *phs*F gene, still displayed red colony color on Rambach agar. Whereas the other transformant lacks all parts of *phs*F gene and its downstram region displayed a colorless colony on Rambach agar. Hence, it is possible that another gene may exist in the region between *phs*F and *ack* gene and the gene might be responsible for acid accumulation and turning colorless colonies to red. Further experiment to identify the gene responsible for the red color colony is suggested.