F330 Cloning and Expression of Catechol 1,2-dioxygenase Gene from *Flavimonas oryzihabitans*

홍성갑*,[1]박용근, 이영록
고려대학교 생물학과, [1]고려대학교 생명공학원

PCR for cloning of C120(catechol1,2-dioxygenase) gene from chromosomal DNA of *Flavimonas oryzihabitans* KH1 was performed and earned approxymately 1 kb DNA as PCR product. This PCR product was inserted into pT7Blue vector and designated as pTB10. The recombinated plasmid, pTB10 was transformed into *E. coli*. 1kb DNA cloned was also identified as a origin from chromosomal DNA of *F. oryzihabitans* KH1. Nucleotide sequences of cloned C120 gene were determined as 933 nucleotide sequences and examined for homology with the genes from the other origines that already reported. As this result, C120 gene of *F. oryzihabitans* KH1 showed high homology of 96% with *catA* of *A. calcoaceticus* and homology of 67-62% with *catA* of *Pseudomonas*. Amino acid sequences deduced from C120 gene of *F. oryzihabitans* KH1 showed 94% homology with *A. calcoaceticus* and 33-28% homology with TcbC and ClcA. Considering the low amino acid homology with TcbC and ClcA and negative degradation activity of chlorocatechol, C120 from *F. oryzihabitans* is considered to be C120 I type. To express effectively the cloned C120 gene, it was isolated from transformant(C120 within pTB10) and transformed into *E. coli* GI724 using pTrxFus, fusion vector and designated as pTF12. Expression from a fused protein including C120 was identified on PAGE and Western blot hybridization. Molecular weight of expressed fusion protein represented about 50 kDa showing that the C120 gene product(33.6 kDa) was fused with thioredoxin(16.4 kDa). C120 activities from cell free extract of *E. coli* GI724 carrying recombinant pTF12 increased successively during the induction period of tryptophane, in accordance with the fused protein contents. This indicates that the C120 activity represents as status of the fused protein.

F331 Charicterization and Isolation of *Salmonella typhimurium* Encoding the Gene of the Anaerobiosis-Inducible

신은아*, 방일수, 박용근
고려대학교 생물학과, 고려대학교 생명공학원

Salmonella typhimurium is facultative so that it has pathogenesis in anaerobic condition, inside of intestine. *S. typhimurium* is a tool for studying molecular pathogenesis of anaerobiosis. As the first step to understand the adaptation of anaerobiosis in intestine, anaerobic condition, the present study was performed to generate mutants of *S. typhimurium* which contain genes encoding anaerobiosis-inducible proteins. By using MudJ (Kan, lac)-directed operon fusion technology, mutants of *S. typhimurium* whose gene expression is induced under anaerobic growth conditions were isolated. Through further study of this topic, these results will suggest that anaerobiosis-inducible genes play a role in *S. typhimurium* pathogenesis later.