

- F305** Molecular cloning and characterization of *mucK*, a gene determining growth of *Acinetobacter calcoaceticus* 93A₂ on exogenous *cis*, *cis*-muconate

Kye-Heon Oh^{1*} and Peter A. Williams²

¹Department of Life Science, Soonchunhyang University; ²Department of Biological Sciences, University of Wales, United Kingdom

The DNA sequence of a 1.68 kilobase-pair *XbaI/SalI* *Acinetobacter calcoaceticus* 93A₂ restriction fragment carrying *mucK*, encodes a member of a superfamily of membrane transport proteins and within a subgroup involved in the uptake of *cis*, *cis*-muconate, was determined. Comparison of the *mucK* sequences from *A. calcoaceticus* ADP1 and *A. calcoaceticus* 93A₂ demonstrated 8 base-pair substitution. Mutants from naturally transformed *A. calcoaceticus* 93A₂ were isolated and the lysated DNA was prepared. Using polymerase chain reaction, the DNA fragments containing *mucK* were amplified and subcloned by pCR-blunt vector. As a result of sequencing DNA, a 29 base-pair deletion of the regulatory gene of *mucK* was determined. The deletion appeared to be functionally related to the utilization of *cis*, *cis*-muconate as an exogenous carbon source.

- F306** Characterization of the *pcbE* Gene Encoding 2-Hydroxypenta-2,4-dienoate Hydratase from *Pseudomonas* sp. DJ-12

Jong-Chul Lim*, Kyung-Rak Min¹, Youngsoo Kim¹ and Chi-Kyung Kim
Department of Microbiology, ¹Department of Pharmacy, Chungbuk National University

Pseudomonas sp. DJ-12 is able to degrade 4-chlorobiphenyl via the meta-cleavage pathway. We have sequenced the downstream region of *pcbC* of pCU1 which was cloned from the DJ-12 strain. The *pcbE* gene encoding 2-hydroxypenta-2,4-dienoate hydratase was identified, which is required for the conversion of 2-hydroxypenta-2,4-dienoate to 4-hydroxy-2-oxovalerate in the degradation of biphenyl and 4-chlorobiphenyl. The *pcbE* gene is apart about 1.5 kb from *pcbC* gene. A deduced amino acid sequence of the hydratase encoded in *pcbE* gene exhibited about 45-55% identities with those of 2-hydroxypenta-2,4-dienoate hydratases from *Pseudomonas* sp. KKS102, *Burkholderia cepacia* LB400, *Pseudomonas putida* F1, *Pseudomonas* sp. DJ77, *Pseudomonas pseudoalcaligenes* KF707.