F334 Cloning and Nucleotide Sequencing of *xylE* Gene Encoding Catechol 2,3-Dioxygenase from *Pseudomons* sp. S-47

Sang-Jin Noh, Song-I Park, and Chi-Kung Kim
Department of Microbiology, Chungbuk National University

Catechol 2,3-dioxygenase (C23O), which is an extradiol type dioxygenase cleavaging the C-C bond of dihydroxylated aromaitc compounds at *meta* positon, catalyzes the conversion of catechol to 2-hydroxymuconic semialdehyde. The *xylE* gene encoding C23O was cloned from chromosomal DNA of *Pseudomonas* sp. S-47, a strain degrading 4-chlorobenzoate, and its nucleotide sequence was analyzed. The *xylE* gene localized in an 1.2 kb *Sac*II fragment was well expressed in *E. coli* JM109 by using pBluescript II SK(+) as a vector. The *xylE* gene was composed of 924 bp with ATG initiation codon and TGA termination codon, and encoded polypeptide of molecular weight 35 kDa containing 307 amino acids. A deduced amino acid sequence of the C23O exhibited the highest 99.7% and 43.5% identity, respectively, comparing with those of related enzymes from TOL plasmid and *Pseudomonas pseudoalcaligenes*. The structurally and functionally important amino acid residues of C23O are thought to be very conserved when the amino acids of C23O in the strain S-47 were comparatively analyzed with those of other extradiol type dioxygenases.

F335 Molecular cloning and characterization of *rfb* gene cluster of *Escherichia coli* O157:H7

Mi soo Kim* and Jai Yun Lim
Department of microbiology, Chungbuk National University

O antigen is a major structural component of the lipopolysaccharide of Gram-negative bacteria and encoded by *rfb* gene cluster that directs the synthesis of O polysaccharide portion of lipopolysaccharide (LPS). The *Sal* I -digested DNA fragment of chromosomal DNA encoding the O-antigen selected by southern hybridization with O157 *rfb*E PCR product probe were ligated with cosmid vector pWE15. The ligated DNA was transformated into *E. coli* LE392. Clone was selected by slide agglutination test with O157 antiserum and PCR test with *rfb*E primers. The clone was named *E. coli* JS833 and had strong agglutination activity. Restriction endonuclease mapping of *E. coli* JS833 demonstrate that this clone has about 20 kb insert DNA carring the genes encoding O antigen of *E. coli* O157:H7. LPS extract of *E. coli* JS833 and *E. coli* O157:H7 showed low-molecular-weight O antigen by western blotting with polyvalent O157 specific antiserum, but *E. coli* LE392 showed negative results.