F340 Trans-acting regulation of ArsR and ArsD from pHM12 in Klebsiella oxytoca

Soo-Chan Lee, Kyung Seon Choi, Yo Hun Aum, and Ho Sa Lee Department of Biology, KyungHee University, Seoul, Korea

The arsenic resistance operon from pMH12 in *Klebsiella oxytoca* contains two regulatory genes. The first open reading frame for arsR extend up to 348 bp and has a translational product corresponding to a protein of 116 amino acid residue polypeptide with a molecular mass of 13 kDa. And the second ORF for arsD extend up to 360 bp and express a protein of 120 amino anid residue polypeptide with 13kDa. ArsR and ArsD have both metal binding domain and DNA binding helix-turn-helix. For revealing the regulation of ArsR and ArdD. We constructed promoter::lacZ vector which was complemented by ArsR or ArsD experiment repression of transcription by β -galactosidase assay. Sodium-m-arsenite shifts 100 fold of β -galactosidase activity. This result demonstrate that ArsR and ArsD have arsenite binding domain and DNA binding domain.

F341 Study on nickel resistance determinant of *K. oxytoca* CCUG 15788

Minwon Kang*, H. G. Schlegel¹ and Ho Sa Lee
Department of Biology, Kyunghee University, Seoul, Korea, 130-701

¹Institut für Mikrobiologie und Genetik, Universität Göttingen, Germany

Klebsiella oxytoca strain CCUG 15788 is nickel resistance bacterium. This strain is resistance to 3mM NiCl₂. 4.2kb, KOHI4 fragment was cloned from the genomic DNA of K.oxytoca. Ligated into the vector pKK232-8, the fragment tolerate 3mM NiCl₂ in E. coli and nickel resistance is an inducible property. After cloning into the promoterless vectorpKK232-8 in E. coli JM109, the strain is resistance to chloramphenicol. When 0.8kb of 5' region of KOHI4 is ligated into pUJ10, β -galactosidase activity is higher than the control vector pUJ10. Therefore we consider 0.8kb of 5' region of KOHI4 as promoter region. For determination of nucleotide sequence, seven subfragment is obtained. Both subfragment pMW101 (2.7kb) and pMW102(3.4kb) are resistance to 2mM NiCl₂. The nucleotide sequence of the 4.2kb fragment were determined. The sequence share strong similarity with enterobacterium Hafnia alvei 5-5.