fractionation, Sephadex G-75 gel filtration, and Affi-gel blue chromatography. Partially purified and immobilized cephalosporin 7a-hydroxylase with resins were used to synthesize 7a-hydroxy cephalosporin C from the substrate, cephalosporin C. The molecular weights of the product isolated from the reaction mixture were determined by ESI-Mass to be 431. ^{1}H NMR also support the conversion of cephalosporin C to 7a-hydroxycephalosporin C by immobilized enzyme.

H313

Neural Networks and Molecular Analyses of Recycling Piggery Slurry Treatment System

Jung-Hye Choi*[1], Jun-Il Sohn[2], Min-ho Lee[2] and Sung-Cheol Koh[1]

Division of Civil and Environmental Engineering, Korea Maritime University, Pusan 606-791[1]; Department of Sensor Engineering, Kyungpook National University, Taegu 702-701[2]

A recycling reactor system operated under sequential anoxic and oxic conditions for the swine wastewater has been developed, in which piggery slurry is fermentatively and aerobically treated and then part of the effluent is recycled to the pigsty. The most dominant heterotrophs were *Alcaligenes faecalis* (TSA-3), *Brevundimonas diminuta* (TSA-1) and *Abiotrophia defectiva* (TSA-2) in order whereas lactic acid bacteria (MRS-1, *etc.*) were dominantly observed in the anoxic tank. One of the most dominant aerobes was *Alcaligenes faecalis* (TSA-3). The most dominant species of LAB was strain MRS-1 that was yet to be characterized. In this study, we tried to model the treatment process for each tank in the system based on population densities of heterotrophic and lactic acid bacteria. Principal component analysis (PCA) was first applied to identify a relation between input (microbial densities and parameters for the treatment such as population densities of heterotrophic and lactic acid bacteria, suspended solids (SS), COD, NH_4^+-N, *ortho*-P, and total-P) and output. Multi-layer neural networks using error back propagation learning algorithm were then employed to model the treatment process for each tank. PCA filtration of input data as microbial densities was found to facilitate the modeling procedure for the system monitoring even with a relatively lower number of input. Neural networks independently trained for each treatment tank and their subsequent combined data analysis allowed a successful prediction of the treatment system for at least two days. The isolated heterotrophs were found to uptake ammonium as a sole nitrogen source for their growth. The molecular analysis data (PCR and Southern hybridization) showed that glutamine synthetase (GS) could be involved in ammonium utilization by the heterotrophic bacteria isolated from the treatment system.

H801

Overexpression and Purification of *Listeria grayi* p60 Protein in *Escherichia coli.*

Sang-Hoon Han*[1], Ji-Hye Lee[1], Yong-Soon Lee[2], Yoon-Kyu Lim[3], Sung-Il Kim[4] and Byoung-Su Yoon[1]

Department of Biology, College of Natural Science, Kyonggi University Suwon 442-760[1]; College of Veterinary Medicine, Seoul National University Suwon 441-744[2]; College of Veterinary Medicine, Cheju National University Chejudo 690-756[3]; National Veterinary Research and Quarantine Service Anhyang 430-824[4]

The *iap*(invasion associated protein) gene from *Listeria grayi* encoding listerial major extracellular protein p60 was cloned and expressed with expression vector

pMAL-c2(Novagen) in *E. coli*. Unique 1,536 bps *iap* gene of *Listeria grayi* was amplified with specific primer using polymerase chain reaction(PCR) and then the amplified gene was cloned with expression vector pMAL-c2. Recombinated pMAL-*iap*/*grayi* was induced with IPTG and overexpressed recombinant p60 that fused with maltose binding protein(MBP) in *E. coli* strain DH5αF'. Optimum concentration of IPTG and induction time were estimated 0.5mM and 4hrs respectively. Overexpressed p60/*grayi* was analysed by SDS-PAGE and was purified by amylose resin based affinity chromatography. Purified recombinant p60 protein will be useful in rapid detection and production of monoclonal antibody against *Listeria grayi*.

H802

Selective Isolation of Mammalian Genes by TAR cloning

Jung-Hyun Kim*, hyung-Jin Jang and Sun-Hee Leem

Dept. of Biology, Faculty of Natural Sciences, Dong-A University, Pusan 604-714

Mammalian genome analysis has been advanced considerably by the development of YAC and BAC cloning system. These traditional methods of isolation of a specific gene from a YAC or BAC genomic library have typically involved a long and laborious process of identification of the region of interest among thousands random YAC or BAC clones. Using the recently developed TAR cloning technique in S. cerevisiae, which allows entire genes and large chromosomal regions to be specifically and accurately isolated from total genomic DNA. In spite of this usefulness of TAR cloning, the frequency of capture of the recombinant insert was less than 1% of transformants. To improve the frequency of positive clones, the non-homologous end-joining pathway is essential for repair of specific classes of double strand breaks termini in cells of S. cerevisiae. To know the effects of the NHEJ in homologous recombination of TAR cloning, several isogenic strains with defects in NHEJ pathway genes are examined in TAR cloning. To examine the essential region of targeting Hook, we mutagenized each 20 bp of the 60 bp of Hook and examined the frequency of the homologous recombination in TAR cloning using of the SV40 model system.