

F017

Development of a Reporter Vector for the Analysis of Promoter by Beta-galactosidase Assay in *Candida albicans*.

So Jeong Park* and Won Ja Choi

Department of Life Science, College of Natural Sciences, Ewha Womans University

Several virulence factors of pathogenic *Candida albicans* are known including morphological transition, adhesion to host cells and its evasion of the host immune system. Over past years a variety of genes involved directly or indirectly in morphological transition have been identified. However functional studies of such genes have been hampered by lack of extrachromosomal replication-competent vectors and proper reporter vectors in contrast to *Saccharomyces cerevisiae*. In this study we constructed a vector that can be used for promoter functional analysis of the gene ECE1 (Extent of Cell Elongation 1), the promoter of which is known to be well regulated in two distinct cell types with no detection in budding yeast cell but abundant expression in cells within 30min after induced to form hyphae. Location of the LacZ open reading frame of *Streptococcus thermophilus* immediately downstream of the cloning sites for insertion of exogenous promoters enabled us to monitor LacZ expression in situ and to quantitate promoter activity by liquid beta galactosidase assay.

F018

A Bacteriocin-mediated Competitive Growth Advantage in *Pseudomonas aeruginosa* Strains

Yun-Jeong Heo¹, Jung-Eun Kim, and You-Hee Cho

Department of Life Science, Sogang University

R-type pyocin is a bacteriophage tail-shaped bacteriocin produced by *Pseudomonas aeruginosa*, but its physiological roles are relatively unknown. Here we describe a role for R-type pyocin in the competitive growth advantages of *P. aeruginosa* strains, PAO1 and PA14 over PAK strain. The culture supernatant of PA14 and PAO1 contains an activity that kills PAK. Both the killing activity and the competitive growth advantage of PA14 over PAK were completely abolished by disruption of the R pyocin gene cluster. These findings may provide insight into the forces that drive strain diversity in *P. aeruginosa* populations.

F019

Interaction between SigR and its Redox-sensitive Antisigma Factor RsrA in *Streptomyces coelicolor*

Min-Sik Kim*, Joo-Hong Park, Jae-Bum Bae, and Jung-Hye Roe

Laboratory of Molecular Microbiology, School of Biological Sciences and Institute of Microbiology, Seoul National University

The activity of SigR, one of extracytoplasmic function (ECF) sigma factors, is regulated by an anti-sigma factor RsrA, whose binding activity is regulated through thiol-disulfide exchange. RsrA contains seven cysteines and coordinates one Zn²⁺ in its reduced state. In oxidized state, a disulfide bond is formed between C11 and C44, and the bound Zn²⁺ is released. Zinc is coordinated to three cysteines (C3, C41, and C44) and one histidine (H7). Substitution of cysteine 41 or 44 to histidine didn't affect SigR binding, while substitution to serine caused loss of binding activity. A C41/44H variant of RsrA with histidines in place of cysteines 41 and 44, binds SigR under both reducing and oxidizing conditions *in vitro*. The mutant or wild type *rsrA* gene in *sigR* operon was introduced into the chromosome of $\Delta sigRrsrA$ mutant. The transcripts from *sigRp2* promoter recognized by SigR itself increased upon oxidative treatment in the strain with wild type *rsrA* gene. However, in the strain with C41/44H mutant *rsrA* gene, the amount of RNA did not increase upon oxidative treatment. The C41/44H variant form of RsrA constitutively binds to SigR *in vitro* and *in vivo* regardless of redox conditions.

F020

Expression, Purification and Characterization of Trimethylamine Dehydrogenase of *Methylophaga aminosulfidovorans* SK1 in *Escherichia coli*

Hee Gon Kim^{1*} and Si Wouk Kim²¹Department of Bio-Materials, Chosun University, ²Department of Environmental Engineering, Chosun University

Trimethylamine dehydrogenase (TMADH) is an iron-sulfur flavoprotein that catalyzes the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde. *Methylophaga aminosulfidovorans* SK1, isolated from seawater at Mokpo, could be grown aerobically on trimethylamine as a source of carbon and energy. 437 bp fragment of TMADH was generated by using degenerated PCR. 2983 bp genomic fragment which contain entire TMADH ORF was isolated by colony hybridization with 437 bp fragment as a probe. Depending on the nucleotide sequence analysis, putative promoter region and ORF of TMADH were revealed in the 2983 bp fragment. We isolated *tmd* gene encoding TMADH and designed a recombinant construct containing T5 promoter sequence instead of native one with N-terminal 6 histidine tag. It seems that the recombinant protein displays the same absorption spectra as the protein isolated from *M. aminosulfidovorans* SK1. Also the recombinant TMADH showed similar catalytic properties to its wild type TMADH isolated from *M. aminosulfidovorans* SK1.