region of the rDNA were amplified using the PCR and digested with 10 restriction enzyme, RFLP patterns were analyzed. The PCR primer CNL12 and CNS1 were used to amplify the IGS region. The restriction enzyme used were BglII, HincII, HindIII, EcoRI, NruI, SalI, SmaI, PstI, XbaI, XhoI. The size of amplification product showed the interspecific polymorphism is approximately 2350 bp for strains of *F. sambucinum*, 2600 bp for formae speciales of *F. oxysporum*, approximately 2700 bp for strains of *F. graminearum* except *F. gra.* UBC830 (2600 bp) and for *F. solani* except *F. sol. piperis* (2600 bp) and *F. sol. pisi* (2900 bp). Cluster analysis based on the presence and absence of comigrating restriction fragments devided into two major groups. One group is included *F. oxysporum* formae speciales, *F. gra.* UBC830, and *F. sol. piperis*. A second group is included strains of *F. graminearum*, *F. sambucinum*, and *F. solani*.

F305

Pseudomonas syringae pv. actinidiae Strains Isolated from Korea Produce the Phytotoxin Coronatine instead of Phaseolotoxin

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Pseudomonas syringae is divided into 57 pathovars and produces a wide spectrum of phytotoxic compounds. *P. syringae* pv. actinidiae was known to cause bacterial canker on kiwifruit. Many research have already confirmed that pv. actinidiae produces phaseolotoxin as a phytotoxic compound. *P. syringae* pv. actinidiae strains were collected from the major field of kiwifruit cultivation areas in Korea. These strains, together with type strain of this pathovar, were tested for the presence of several toxin related genes by using PCR. All Korean strains contained the gene involved in coronatine biosynthesis, whereas type strain of this pathovar possessed the gene for phaseolotoxin. The nucleotide sequence of PCR product of Korean strains was identical to that of *P. syringae* pv. glycinea. The location of the gene cluster was determined for coronatine producing Korean strains by subjecting their DNA to pulsed-field electrophoresis and Southern blot analysis with a hybridization probe from the coronatine gene cluster. The coronatine gene cluster was contained in plasmids with different size in all Korean strains tested. These results suggested that *P. syringae* pv. actinidiae isolated from Korea is not closely related to type strain of this pathovar.

F306

Nucleotide Sequence, Mutational Analysis and Expression of the Inducible Nickel Resistance Determinant from pEJH501 of *Hafnia alvei* 5-5

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The nucleotide sequence of the 4.8 kb *Sal* I -*Eco*R I fragment encoding nickel and cobalt resistance on plasmid pEJH501 in *Hafnia alvei* 5-5 was determined. The promoter region was located at the upstream of inserted 0.8 kb *Sma* I fragment of plasmid 4.8 kb and detected by b-galactosidase assay. The plasmid with the putative promoters were nickel inducible. Five open reading frames were assigned to five polypeptides which were expressed from this determinant in *Escherichia coli*. The roles of the polypeptides from the open reading frames were analyzed with transposon mutagenesis.

The proposed translation products of the *ncr*A show a strong homology with *nre*B from pTOM9 of *Alcaligenes xylosoxydans* 31A, there was 77 % amino acid identity.

F307

Analysis of Reverse Transcriptase Gene (pol) of the Korean-type Bovine Leukemia Virus (BLV)

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Bovine leukemia virus (BLV) is an etiological agent of chronic diseases in cows worldwide. The BLV is one of retroviruses that contain a multi-functional enzyme, reverse transcriptase produced from the pol gene in its genome. We have sequenced some regions in the pol gene of 13 BLV proviruses found in the Southern province of Korea from samples that turned out to be BLV positives by a PCR analysis. On the 5' side of the BLV pol gene (polymerase region), it was found that there were four leucines located at every 7 amino acid. They can form a leucine zipper motif that was not same as the pol gene of Japanese BLV isolate. The sequencing result of the proviral pol gene in Korean-type BLV also revealed some mutations leading to amino acid changes such as AAT (Asn)→AAA(Lys), CCT(Pro)→CTC(Leu), and non-sensible variations i.e., TCT(Ser)→TCC(Ser) and ACG (Thr)→ACA(Thr). On the 3' side of the pol gene (integrase region), some nucleotide sequences were mutated and led to amino acid changes. Among them, a mutation, GAA(Glu)→GAC(Asp) occurred in many Korean-type BLV proviruses was very interesting because the amino acid was regarded as one of the most conserved amino acids in the retroviral integrase. It was also notable that the mutation on any leucine residue did not occur, inspite of its frequent appearance. Furthermore, it was found that the mutation rates in the analyzed regions of the Korean-type BLV pol gene were variable from BLV10C (2.01%-2.81%) to BLV6C (3.21%-4.02%) although the values were far less than those of other countries' BLV.

F308

Single Point Mutation at the 26th Amino Acid in Spp2p of *Saccharomyces cerevisiae* is Responsible for the Dominant Negative Phenotype

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The Spp2p of *Saccharomyces cerevisiae* is known to promote the first transesterification reaction of the pre-mRNA splicing and interacts with the Prp2p which is also involed in the pre-mRNA splicing. As an approach to searching for the functional domain of the Spp2p, we have tried to isolate dominant negative mutations of the *SPP2* gene of *S. cerevisiae*. First, we constucted a YEp-GAL1-*SPP2* plasmid in which *SPP2* gene expression is inhibited by glucose but induced by galactose. Then, libraries of mutant *spp2* genes were generated by subculturing the *dnaQ E. coli* strains CGSC 6485 and CGSC 6862 harboring the YEp-GAL1-*SPP2* plasmid for 500 generations at 37° C. Because the *E. coli* strains have no proof-reading activity of the DNA polymerase III at nonpermissive temperatures, mutations would be randomly generated in the *SPP2* DNA during the generations. Ura^- yeast cells were transformed to Ura^+ with mutant library DNAs isolated from the *E. coli* cells. We then screened yeast transformants which cannot grow upon shifting to a galactose-containing