medium. The mutant plasmid DNA designated M3 was retrieved from one of the screened transformants, and its base sequence was analyzed. We found that only one base had been altered at the 76th nucleotide of the *SPP2* reading frame from thymine to cytosine, resulting in a codon change of Ser26Pro. Currently, we are isolating mutant *spp2* genes which have other amino acid codons at the 26th codon.

F309

The Autonomously Replicating Sequence (ARS) of the *Coriolus versicolor*

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Fragments containing ARSes were cloned from the genomic DNA library of the fungus *Coriolus versicolor*. Fifteen recombinant plasmids from the library showed high frequencies of transformation in yeast *Saccharomyces cerevisiae*. They were maintained stably under selective conditions, but were gradually lost from yeast cells at different rates under nonselective conditions, indicating that they contain eukaryotic origins of DNA replication and exist as extrachromosomal plasmids. Base sequences of three ARS DNAs among the 15 cloned fragments revealed that all of them contain the 11 bp [(A/T)TTTA(T/C)(A/G)TTT(A/T)] consensus sequence of the budding yeast ARS. and the other elements. In order to construct a shuttle vector for *E. coli*, *S. cerevisiae*, and *C. versicolor* we generated recombinant plasmids consisting of one of these ARS fragmants, the origin sequence for ColE1 replication, the yeast *URA3* marker, and the phosphinothricin resistance gene. We are currently focusing on the isolation of phosphinothricin resistant transformants of *C. versicolor* by introducing the ARS-containing recombinant plasmids into the protoplasts of *C. versicolor*.

F310

Genomic cloning and characterization of *HpaD* gene encoding 2,3-dioxygenase from *Pseudomonas* sp.DJ-12.

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2,3-dioxygenase (*Hpa*D gene) catalyzes the ring-fission reaction of aromatic substrate. *Hpa*D gene was cloned from the chromosomal DNA of *Pseudomonas* sp. DJ-12 and it's nucleotide sequence analyzed. The 40kb fragment containing *Hpa*D gene was inserted into the BamH1 site of pWE15 cosmid vector and designated as pKC1. The 3.4 kb fragment containing *Hpa*D gene was made from pKC1 with subcloning. The open reading frame (ORF) corresponding to the *Hpa*D gene consists 864 base pair with ATG initiation codon and TGA termination codon. The putative RBS gene fragment is located on the 10bp upstream of the initiation codon. The gene analysis was carried out by comparison of the gene with another strains. Sequence homology was revealed 55.6% with another strain such as *Escherichia, Klebsiella* and *Salmonella*.

F311

Molecular Cloning and Expression of Fe-Containing Superoxide Dismutase from *Neospora caninum*

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The superoxide dismutase (SOD) gene fragment was amplified by reverse transcription polymerase chain reaction (RT-PCR) using degenerate oligonucleotide primers derived from amino acid sequences conserved in the Fe-SODs. An *N. caninum* cDNA library was screened with the SOD gene fragment as a probe. As a result, a complete gene encoding the Fe-SOD was identified. The gene had an open reading frame of 606 bp and deduced amino acid sequence of 202. Sequence analysis showed that the gene had conserved metal binding residues and conserved amino acid residues which were found in Fe-SODs. Comparison of the deduced amino acid sequence of the enzyme with previously reported Fe-SOD amino acid sequences revealed considerably high homo- logies. The coding region of the *N. caninum* Fe-SOD was cloned and expressed in *E. coli*. The molecular weight of expressed protein was approximately 24 kDa. Staining of native polyacrylamide gel for SOD activity of the expressed protein revealed SOD activity that was inactivated by hydrogen peroxide but not by sodium azide and potassium cyanide. This means that the presence of the recombinant fusion protein is indicative of Fe-SOD.

F312

Regulation of Histidine Biosynthetic Genes from *Corynebacterium glutamicum*

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The genes of *Corynebacterium glutamicum* involved in histidine biosynthesis were cloned and characterized by complementation of *Escherichia coli* mutants. Complementation and sequence analysis showed the existence of 9 genes which are fragmented in three regions (*hisDCBd*, *hisHAFI*, and *hisEG*). Transcription initiation site of the *C. glutamicum his* genes was determined by primer extension analysis. The start site is 196bp upstream of *hisD* gene. The region corresponding to the untranslated 5′ end of the transcript, named the *his* leader region, displays the typical features of the Gram-positive synthetase gene family, including the terminator and antiterminator. Deletion and mutational analysis of the *his* leader region was performed to identify regions and particular nucleotides important for its function.

F313

Cloning of the *nahAb,Ac,Ad* Gene Encoding Naphthalene Dioxygenase from *Pseudomonas fluorescens* SMEII

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The *P. fluorescens* SMEII can utilize naphthalene as its sole carbon. Naphthalene dioxygenase (encoded *nahA*), the first step of naphthalene degradation pathway, converts naphthalene to *cis*-dihydrodiol naphthalene. Naphthalene dioxygenase is composed of 4 subunits, reductase (*nahAa*), ferredoxin(*nahAb*), oxygenase large subunit(*nahAc*), and small subunit(*nahAd*) in *Pseudomonas* system. To study the naphthalene dioxygenase, 3kb PCR product was cloned from genomic DNA of *P. fluorescens*. The cloned DNA fragment has restriction sites of *KpnI*, *SalI*, and *PstI*. By means of unidirectional ExoIII deletion and dideoxynucleotide chain termination, the