was assayed with tolidine and ABTS as substrate and its amino acid compositions and N-terminal amino acid sequence was determined.

E315

Characterization of the Gene Family Encoding Alternative Oxidase from Candida albicans

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Candida albicans possesses a cyanide-resistant respiratory pathway mediated by alternative oxidase, which seems to be encoded by a gene family with two members. Cloning and expression of one of the genes encoding alternative oxidase from *C. albicans*, *AOX1*, has previously been reported (W.-K. Huh and S.-O. Kang, J. Bacteriol. 181: 4098-4102, 1999). Here we report isolation of another gene coding for alternative oxidase, designated *AOX2*. *AOX2* contained a continuous open reading frame that encodes a polypeptide consisting of 365 amino acids. Interestingly, *AOX2* and *AOX1* were found to be located in tandem on one of the chromosomes of *C. albicans*. b-Galactosidase reporter assay indicated that, whereas *AOX1* was expressed constitutively, the expression of *AOX2* was dependent on growth phase and induced by treatment of cyanide, antimycin A, hydrogen peroxide, menadione, and paraquat. Growth of the cells in the media with non-fermentable carbon sources also enhanced the expression of *AOX2*. The presence of cyanide in medium remarkably retarded the growth of the *aox1/aox1* mutants. The growth of the *aox2/aox2* mutants and the *aox1/aox1 aox2/aox2* double mutants was almost completely inhibited in the same medium. Interestingly, the activity of cyanide-resistant respiration and the expression level of alternative oxidase were found to be significantly low in the *sln1/sln1* mutants under normal conditions, suggesting that *SLN1*, a histidine kinase gene, may be involved in regulation of the basal expression of alternative oxidase in *C. albicans*.

E316

Regulation of Manganese-Containing Superoxide Dismutase Expression in *Bacillus subtilis*

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Bacillus subtilis was found to possess a single superoxide dismutase, manganese containing superoxide dismutase (MnSOD) and the SOD activity increased by manganese supplementation in growth media. Western and Northern analyses revealed that manganese ion at micromolar concentration in LB⁺ media induced expression and transcription of *sodA* encoding MnSOD, while Ferrous ion did not. To study the molecular mechanisms of transcriptional activation of *sodA* by manganese ion, a set of 5′-flanking region deletions was generated in *sodA* promoter fragment that had been previously fused to the reporter gene *lacZ*. Gel mobility shift assays of *sodA* promoter fragment with cell extracts indicated the presence of manganese-responsive DNA-binding protein, which can play a role as a transcriptional activator.

E317

Characterization of Aerobic Repressor PpsR from *Rhodobacter*

sphaeroides

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PpsR from the facultative photoheterotroph *Rhodobacter sphaeroides* is involved in représsion of photosynthesis gene expression under aerobic condition. PpsR was heterologously overexpressed and purified to homogeneity. Gel mobility shift assays showed that the purified PpsR is biologically active. The activity of PpsR in wild type was higher in aerobic condition than in photosynthetic condition. The activity was not detected in null mutant and was amplified in the exconjugant with *ppsR* carrying plasmid. Both cysteins in PpsR exist in their reduced form under the oxidation-reduction potential that is estimated in living cell. The amount of PpsR in aerobic and photosynthetic conditions in wild type maintained almost constant level by Western analyses. PpsR was not detected in null mutant and increased in the exconjugant with *ppsR* carrying plasmid by Western analyses.

E318

Characterization of Repressor Regulating the Expression of Pyruvate Dehydorgenase Gene in *Streptomyces seoulensis*

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Pyruvate dehydrogense repressor gene (pdhR) is located within PDH (pyruvate dehydrogenase complex) operon and shows homology with gluconate repressor (GntR) family, which is well known as DNA binding protein family. PdhR was overexpressed using pIJ702 vector in *Streptomyces seoulensis* and the growth of the overexpressed cells was retarded compared with wild type. PdhR was partially purified by heparin-sepharose and ion exchange chromatography from *S. seoulensis*. The native enzyme had a molecular mass of 45 kDa and SDS-PAGE revealed that the enzyme consists of two subunits , each with a molecular mass of 23 kDa. Electrophoretic mobility shift assay showed that PdhR binds to both promoters, Ppdh and Pace. This result indicates that PdhR acts as a repressor inhibiting transcription of PDH complex by binding to Ppdh as well as to Pace.

E319

Calcium-Induced Conformational Changes of the Recombinant CBP3 Protein and its Domains in *Dictyostelium discodeum*

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In order to characterize calcium affinity features of the CBP3 protein (Y.-H. Han and S.-O. Kang (1998) FEBS Lett. 441, 302-306) which has four EF-hand motifs in *Dictyostelium discodeum*, the protein was overexpressed in *Escherichia coli* and purified by Nickel-column using His 쟣Bind Buffer Kit (Novagen) under denaturing condition. After refolding and removal of histidine tag, this recombinant protein was further characterized by fluorescence, cysteine titration with 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB), and circular dichroism (CD) spectroscopy in the presence or absence of Ca^{2+}. The fluorescent intensity for CBP3 increased with 3-5 nm maximal wavelength