change, as the concentration of calcium ion in the solution increased. In the apo- and Ca^{2+}-binding states of the protein, the number of cysteine residues titrated with DTNB was different. The far-UV CD spectrum was sensitive to calcium binding. These results pointed out that the calcium binding induces certain conformational changes. Also we prepared the N- and C-domain of CBP3 protein. Conformational changes induced by calcium were also showed in the N- and C-domain. It seems that the CBP3 has calcium sensor character and calcium affinity of the N-terminal domain is higher than C-terminal domain. CBP3 protein and its domains have low solubility because of aggregation depending on the concentrations of protein and calcium, reducing agent, pH, etc.

E320

Functional Expression of Alternative Oxidase from *Candida albicans* in *Escherichia coli*

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In addition to the cytochrome-involved respiratory pathway, *Candida albicans* is known to possess an alternative, cyanide-resistant respiratory pathway that is mediated by alternative oxidase (AOX). But, only little information concerning the molecular structure and enzymatic features of AOX have been available. In this study, the *AOX1* and *AOX2* genes were cloned into the expression vector pGEX-4T-1 and pET 15b/ pET3a, respectively, and were expressed in *Escherichia coli*. The growth of the transformant carrying pET15b-AOX2 was cyanide-resistant. · Polypeptides with molecular masses of 69 kDa and 44 kDa were found in the cytoplasmic membrane of *E. coli* carrying pGEX-4T-1-AOX1 and pET 15b-AOX2, respectively, and were recognized by antibody against plant-type AOX from *Sauromatum guttatum*. The ubiquinol oxidase activity found in the membrane of the transformant was insensitive to cyanide, while that of the control strain, which contained vector alone, was inhibited. These results clearly showed the functional expression of *C. albicans* AOX in *E. coli*. When *C. albicans AOX* genes (*AOX1/AOX2*) were expressed in alternative oxidase-deficient *Saccharomyces cerevisiae*, it could also confer cyanide-resistant respiration on *S. cerevisiae*.

E321

Identification of a Protein that Interacts with Calcium-Binding Protein 3 (CBP3) in *Dictyostelium discoideum*

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Dictyostelium discoideum, at least eight small, four-EF hand calcium-binding proteins respectively are expressed at specific stages during development. One of these proteins, calcium-binding protein 3 (CBP3), first appears just prior to cell aggregation and then maintains relatively constant levels throughout development. To determine the role of CBP3 during development, the protein was used as a bait in a yeast two-hybrid screen to reveal putative CBP3-interacting proteins. Of 7.0×10^6 independent transformants, one positive transformant carrying Actin8, which is the major component of the cellular microfilament system or actin cytoskeleton, was identified. Thus CBP3 might function to

help regulate the reorganization of *Dictyostelium* actin cytoskeleton during cell aggregation.

E322

Responses to Explosive TNT and Temperature Stress Shocks in *Pseudomonas* sp. HK-6 Isolated from Explosive Contaminated Sites

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The purpose of this work was to examine the induction of stress shock proteins in *Pseudomonas* sp. HK-6 isolated from explosive contaminated sites in the response to explosive 2, 4, 6-trinitrotoluene (TNT) and temperature as stress agents. The stress shock proteins, which contribute to the resistance of the cytotoxic effect of TNT, were induced at different TNT concentrations and exposure period in exponentially growing cultures of *Pseudomonas* sp. HK-6. Synthesis of heat shock proteins in the strain occurred by a heat shock at temperature from 30 to 42℃ for 20 min. Heat shock proteins were maximally induced in the cells exposed for 2 hrs. Also this organism was capable of a cold shock (shifted 30 to 4℃ for 5 min) response similar to that of heat shock, and the maximal level of induced cold shock proteins was detected in the cells exposed for 1 hr. Heat/cold shock proteins disappeared as normal after 10 hrs of temperature shocks. These responses involved the induction of a 70-kDa DnaK and a 60-kDa GroEL proteins, characterized by SDS-PAGE and Western blot by use of anti-DnaK and anti-GroEL monoclonal antibodies.

E323

Purification and Characterization of Metal Dependent Serine Proteinase from the Dermatophytic Fungus *Trichophyton mentagrophytes*

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The metal dependent serine proteinase from *T. mentagrophytes* was purified by ammonium precipitation (80%), DEAE-Sepharose CL6B, Arginine-Sepharose 4B and Superose 6 column chromatography. The molecular weight of the purified proteinase was estimated to be 44.5 kDa by SDS-Polyacrylamide gel electrophoresis and approx. 190 kDa in non-reduced condition. The optimal pH was 8.0 and stable in pH 7.5 and 9.0. Proteinase activity was optimum at 40℃ and remained high level in 25℃ and 37℃. The activity of purified enzyme was increased by adding Ca^{++}, Mn^{++}, Mg^{++} and strongly inhibited by PMSF, chymostatin and chelating agents (EDTA, EGTA, 1,10-phenanthroline). N-terminal sequence was similar to allergen from *Trichophyton rubrum*, alkaline serine protease from *Penicillium citrinum* and allergen from *Penicillium chrysogenum*.

E324

Synergism between Cellulases from *Tricoderma* sp. C-4

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Hydrolysis of cellulose is done by the synergistic reaction of cellulase family enzymes. Exo-1,4-β-D-glucan cellobiohydrolase and endo-1,4-β-D- glucan glucanohydrolase can directly solubilize