change, as the concentration of calcium ion in the solution increased. In the apo- and Ca<sup>2+</sup>-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 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 pET3a, respectively, and were expressed in Escherichia coli. The growth of the transformant carrying pET15b-AOX2 was cyanide-resistant. · Polypeptides molecular masses of 69 kDa and 44 kDa were

found in the cytoplasmic membrane of E. coli pGEX-4T-1-AOX1 carrying 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 confer cyanide-resistant could also 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. 7.0x10° Of independent transformants, one positive transformant carrying Actin8, which is the component of the 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 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°C and remained high level in 25°C and 3 7℃. The activity of purified enzyme was increased by adding Ca++, Mn++, Mg++ and strongly inhibited by PMSF, chymostatin and (EDTA, agents chelating 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- $\beta$ -D-glucan cellobiohydrolase and endo-1,4- $\beta$ -D- glucan glucanohydrolase can directly solubilize