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

crystalline cellulose and mainly generate cellobiose, which is a product inhibitor for these enzymes. We have isolated 4 endoglucanases, 2 exoglucanases, and β-glucosidase from *Trichoderma* sp. C-4, a strain with high cellulolytic activity to determine an optimum hydrolytic condition for cellulose. Combination of endoglucanase 1 and 2 gave highest hydrolytic rate. Addition of exoglucanase 2, exoglucanase 1, and β-glucosidase showed the increase of activity. The presence of 2 endoglucanases, 2 exoglucanases, and β-glucosidase is enough for solubilization of cellulose in vitro, although the roles of remaining 2 endoglucanases are not elucidated clearly.

E325

Investigation on the Physiological Role of Maltogenic Amylase in *B. subtilis*

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The *bbma* gene isolated from *B. subtilis* SUH4-2, an isolate from Korean soil, encoded a maltogenic amylase and it found to be a homologue of the *yvdF* gene of *B. subtilis* 168 with unknown function, which located in a gene cluster involved in maltose/maltodextrin utilization. *bbma* promoter-*lacZ* gene fusion study in *B. subtilis* 168 suggested that the promoter was most active when the cells were cultured in the medium containing β-cyclodextrin (β-CD), moderately active in the maltose medium, and relatively less active in the starch medium. The promoter was under catabolite repression. Based on the results, the *bbma* gene product was likely to be involved in maltose and β-CD utilization when other sugars, which are readily usable as energy source, are not available. In oder to test this hypothesis, mutagenesis of the *bbma* gene was carried out by Campbell type recombination in both *B. subtilis* 168 and SUH4-2 strains using an internal bbma gene fragment. The resulting mutants grew poorly in culture medium containing either 2% β-CD or 2% maltose, but grew as well as wild type strains in 2% starch medium. The β-CD hydrolyzing activities of maltogenic amylase decreased significantly in both strains when they were grown in β-CD or maltose medium. However, temporal expression of the β-CD hydrolyzing activity was slightly different in the two *Bacillus* strains investigated. The results obtained in this study support our working hypothesis on the physiological function of maltogenic amylase in *Baciilus*.

E326

한국산 버섯 추출물로부터 a-glucosidase의 저해제 탐색과 분리

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버섯 추출물에서 a-glucosidase (당분해효소)의 활성을 저해하는 추출물을 선별한 후 그 추출물로부터 저해제를 분리하였다. 한국의 야산에서 채취한 버섯을 MeOH, 65° C H_2O, 100° C H_2O에서 추출한 후 이 추출물을 이용하여 a-glucosidase의 활성을 억제하는 추출물을 선별하였고, 이로부터 충분리, Silica 크로마토그래피, C-18 MPLC, TLC, 이온교환 크로마토그래피 (NaCl 농도구배, pH 구배), SEPHADEX G-10 겔여과 크로마토그래피, C-18 HPLC등을 통해 저해제를 분리하였다. 그 결과 Phallus impudicus (말뚝버섯)에서 a-glucosidase의 저해활성이 가장 높았으며, 여러 단계의 크로마토그래피법을 통해 순수한 물질로 분리해 내었다. 더 많은 노력과 연구를 통하여 아직 밝혀지지 않은 이 물질의 확실한 구조를 밝혀냄으로써 확실한 당뇨병 치료제의