

at pH 8.7 and 50 °C. The PHA_{MCL} depolymerase was capable of hydrolyzing polycaprolactone as well as various PHA_{MCL} and *para*-nitrophenyl esters of fatty acids. However, poly (L-lactic acid) and substrates for lipase such as *para*-nitrophenylpalmitate and triolein were not hydrolyzed by this enzyme. The enzyme was insensitive to phenylmethylsulfonyl fluoride and dithiothreitol, indicating that serine residue and disulfide bonds do not play an important role in the active site of the enzyme. On the other hand, this enzyme was completely inactivated by 5 mM *N*-bromosuccinimide that is a specific reagent for tryptophan residue. These results suggest that the PHA_{MCL} depolymerase from *Streptomyces* sp. KJ-72 is a novel enzyme which is significantly different from PHA depolymerases of other bacteria in many properties.

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Purification and Structural Elucidation of a New Laminin Adhesion Inhibitor, Cytometryrin from a Fungal strain F-70912

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Tumor cell interaction with extracellular matrix (ECM) is defined as the critical event of tumor invasion that signals the initiation of the metastatic cascade. Laminin, one of major adhesive proteins of basement membrane (BM), is known to play a key role in cell adhesion, spreading, and movement. In the course of screening fungal metabolites

for the inhibitors of cell adhesion to laminin in a whole cell assay, strong inhibitory activity was detected in fermentation extract of a fungal strain F-70912. The active compound was isolated from culture broth by bioassay-guided isolation procedures composed of ethylacetate extraction, and chromatographies on silica gel, Sephadex LH-20, and YMC-ODS-A. The purified compound was found to be a new type of cytochalasin, which is cytochalasin E derivative with one additional methoxyl group linked to its phenylalanine moiety and designated cytometryrin. Cytometryrin inhibited the adhesion of B16 melanoma cells to laminin with an IC₅₀ value of 4 mg/ml.

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진균에 대한 Methyl 2-Benzimidazole Carbamate의 항균 효과

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항진균제로 사용되는 methyl 2-benzimidazole carbamate (carbendazim, CBZ)를 대상으로 하여 동물독성조사를 실시한 결과 0.001% 이상의 농도에서 세포 성장이 크게 억제되었다. Broth microdilution test를 실시하여 fungi 16주에 대한 CBZ의 MIC를 측정하였다. 대부분의 균주에 대한 MIC가 1.95 x 10⁻⁴ µg/ml로 측정되었으며 *Penicillium pinophilum*, *Aspergillus niger*, *Aspergillus funigatus*, *Paecilomyces farinosus*에 대한 agar disk diffusion test 결과 0.1% CBZ 농도에서 30~60 mm의 억제대가 형성되었다. 그러나 CBZ에 대해 내성이 유도된 내성주 *P. farinosus*는 1% CBZ에서도 내성이 형성되지 않았으며 비내성주보다 MIC 값이 증가되었다 (내성주의 MIC; 5.12 x 10³ µg/ml). *Aspergillus niger*의 포자와 균사시기에 CBZ의 항균활성 test를 실시하였을 때 CBZ가 발아관의 형성을 억제하는 것으로 나타났다.

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p-Hydroxyphenyl acrylate의 항세균 효과 및 항균 기작

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p-Hydroxyphenyl acrylate를 합성하여 그람 음성세균 7주와 그람 양성세균 5주에 대한 항균성을 조사한 결과 MIC는 625 $\mu\text{g}/\text{ml}$ ~ 1250 $\mu\text{g}/\text{ml}$ 로 측정되어 기존에 사용되어 왔던 chloroxyphenol, 2-phenylphenol, nitromide, homosulfanilamide hydrochloride의 항균력을 고려하였을 때 비교적 좋은 항균력을 보였다. *E. coli*를 대상으로 세포호흡활성, 용균현상, 세포구성성분의 유출을 조사하였다. p-Hydroxyphenyl acrylate를 MIC 농도로 처리하였을 때 *E. coli*의 용균이 일어났고, 세포구성성분의 유출이 관찰되었다. MIC 보다 낮은 농도에서도 *E. coli*의 세포 호흡 활성이 현저히 감소하였으며, MIC 이상의 농도에서는 인산 수용액내에서의 호흡활성이 완전히 억제되었다. 이 결과로부터 p-Hydroxyphenyl acrylate는 *E. coli*의 세포막에 작용하여 항균기작을 나타낸 것을 보여주었다.

E313

A Study on the Molecular Genetic Response to Copper Ion in *Salmonella enterica* serovar Typhimurium

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Since copper ions are both essential cofactors and cytotoxic agents, the net accumulation of this element in a cell must be carefully balanced. Copper ion-induced gene was screened in virulent *Salmonella enterica* serovar Typhimurium UK1 using technique of P22-*MudJ* (Km, lacZ) directed lacZ operon

fusion, LF153 *cuiD::MudJ* that was induced by copper was selected. The *cuiD* mutant was showed copper sensitivity but not to other metals. Therefore we suggest that *cuiD* is important gene for copper homeostasis. The copper sensitive phenotype was complemented by pLJ4.2 and carrying *cuiD*. In the result of sequence analysis, *CuiD* contains one open reading frame (ORF) and was showed homology with multicopper oxidases in other bacteria, plant, and human. This ORF contains conservative 12 copper-binding site(type1,2,3); Histidine, cysteine and Methionine.

E314

Biochemical Characterization of Laccase Isozymes in the White Rot Basidiomycete *Ganoderma lucidum*

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Ganoderma lucidum, a medicinal white rot basidiomycete, produces three laccase isozymes in a liquid culture. The isozymes have been isolated from culture filtrates and one of these has been purified through an anion exchange chromatography and a preparative gel electrophoresis. The isozyme is a monomeric glycoprotein containing 21% carbohydrate and has a molecular weight of approximately 68kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It has an isoelectric point of 3.0. With toluidine as the substrate, its optimal reaction pH is 3.5 and its optimum temperature is 20°C. It is relatively stable in a pH range from 4 to 7 and in temperature range from 10°C to 40°C, retaining 92% activity after 4h at 40°C. Its activity was strongly inhibited by FeSO₄ but not by CuSO₄, MgCl₂, MnCl₂ and HgCl₂. Also, Km