p-Hydroxyphenyl acrylate의 항세균 효과 및 항균 기작

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p-Hydroxyphenyl acrylate를 합성하여 그람음성세균 7주와 그람양성세균 5주에 대한 항균성을 조사한 결과 MIC는 625 ㎍/㎖ ~ 1250 ㎍/㎖ 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 인산수용액내에서의 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 preperative gel electrophoresis. The isozyme is a monomeric glycoprotein containing 21% carbohydrate and has a molecular weight of approximately 68kDa as detrmined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It has an isoelectric point of 3.0. With tolidine as the substrate, its optimal reaction pH is 3.5 and its optimum temperature is 20℃. It is relatively stable in a pH range from 4 to 7 and in temperature range from 10℃ to 40℃, retaining 92% activity after 4h at 40℃. Its activity was strongly inhibited by $FeSO_4$ but not by $CuSO_4$, $MgCl_2$, $MnCl_2$ and $HgCl_2$. Also, Km

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*