

E009

Purification and Characterization of an NAD(P)H: Quinone Oxidoreductase from White Rot Basidiomycete *Trametes versicolor*Sang-Soo Lee*, Hyoung-Tae Choi, and Hong-Gyu Song
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White rot basidiomycete *Trametes versicolor* can degrade not only lignin, but also various aromatic compounds. Quinones are derived from the degradation of aromatic compounds, and some quinones damage to fungal hyphae, therefore they should be removed. However, intracellular enzyme for quinone transformation has not yet been reported in white rot fungi. We performed isolation and characterization of an intracellular reductive enzyme, NAD(P)H:quinone oxidoreductase from *T. versicolor*. This enzyme was purified by using weak anion exchange, hydrophobic interaction, strong anion exchange and gel filtration chromatography, and single band of enzyme protein was confirmed by SDS-PAGE analysis. Purified quinone reductase had molecular weight of approximately 41 kDa. Its activity was inhibited by CuSO_4 , HgCl_2 , MgSO_4 , MnSO_4 , AgNO_3 , dicumarol, KCN, NaN_3 , and EDTA. When NADH was used as an electron donor, its K_m was 23.02 μM and a maximal specific utilization rate was 101 mM/mg/min. Purified quinone reductase was able to reduce *p*-benzoquinone to hydroquinone. Induction of this enzyme was higher by 1,4-benzoquinone than those of other quinone compounds.

E010

Identification of Putative Transcriptional Regulator for Purine Biosynthetic Genes in *Corynebacterium ammoniagenes*.Seok-Myung Lee¹, Younhee Kim², and Heung-Shuck Lee¹
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In order to isolate transcriptional regulatory protein involved in purine nucleotide-dependent repression in *Corynebacterium ammoniagenes*, proteins binding to the putative promoter region upstream of the *guaB* (IMP dehydrogenase) and *purA* (SAMP synthetase) genes were isolated by DNA-binding *in vitro* assay. Internal amino acid sequence of the bound proteins were analyzed by ESI-MS and showed high sequence similarity with polynucleotide phosphorylase (PNPase, 81 kDa) and predicted hydrolase of the metallo-beta-lactamase superfamily (77 kDa) of *C. glutamicum*, respectively. Scrutiny of the protein sequence identified helix-turn-helix DNA-binding motifs as well as RNA binding regions. Among them, PNPase has been known as RNA processing enzyme of degradosome complex in *E. coli* and *B. subtilis*. According to recent studies, PNPase was identified as an enzyme which is recycling transcriptional regulatory protein by sequestration of limiting regulatory protein from unnecessary RNA strand when leader sequence is terminated in *trp* operon of *B. subtilis*. Therefore, with all results we tested, it could be assumed that PNPase has its role in purine nucleotide-dependent regulation of *guaB* and *purA*.

E011

Purification and Characterization of Aromatic Nitroreductase from White Rot Fungus *Irpex lacteus*Eun-Hye Shin* and Hong-Gyu Song
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White rot fungus *Irpex lacteus* isolated from Korea can transform 2,4,6-trinitrotoluene (TNT) via two different initial reduction pathways. Since there has not been a report on fungal enzymes involved in the initial reduction of nitroaromatic compounds, characterization of fungal nitroreductase can be very important in the investigation of precise metabolism of nitroaromatic. Its nitroreductase was purified by phenyl hydrophobic interaction chromatography, DEAE anion exchange chromatography and size exclusion chromatography. Two bands were shown in sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis, but just one band was reported by non-denaturing polyacrylamide gel electrophoresis. Therefore, aromatic nitroreductase seemed to be a dimeric protein. Purified enzyme has a molecular weight of approximately 50 kDa. This purified enzyme utilizes NADPH as an electron donor and FMN as a cofactor. Its optimum pH is 4 and temperature optimum is 20°C. The enzyme activity was inhibited by CuSO_4 , HgCl_2 and MnSO_4 . Other enzymatic properties were characterized. This is the first report on the characterization of membrane bound aromatic nitroreductase of fungi.

E012

1.35 Å Crystal Structure of PedB: A Bacterial Immunity Protein Conferring Immunity to the Antimicrobial Activity of the Type IIa Bacteriocin, Pediocin PP-1Min-Kyu Kim^{1*}, In-Kwon Kim¹, Sun-Shin Cha², and Sa-Ouk Kang¹¹Laboratory of Biophysics, School of Biological Sciences, and Institute of Microbiology, Seoul National University, ²Beamline Division, Pohang Accelerator Laboratory

Bacteriocins produced by lactic acid bacteria are ribosomally synthesized antimicrobial peptides. Among them, pediocin-like bacteriocins (Type IIa bacteriocins) constitute an important and well-studied class of antimicrobial peptides. In general, genes encoding pediocin-like bacteriocins are cotranscribed with, or in close vicinity to, a gene encoding a cognate immunity protein which protects the bacteriocin-producer from their own bacteriocin. We report here the crystal structure of 112-amino acid immunity protein (PedB) for Pediocin PP-1 from *Pediococcus pentosaceus*. The crystal structure of PedB revealed that it consists of an antiparallel 4-helix bundle. The fact that the immunity proteins conferring immunity to carnobacteriocin B2 and enterocin A, which are members of the Type IIa bacteriocin, also consists of a 4-helix bundle strongly indicates that this is a conserved structural motif in all pediocin-like immunity proteins.

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