E013

Purification and Characterization of Aromatic Nitroreductase from TNT-Degrading *Klebsiella* sp. C1

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Klebsiella sp C1 can degrade 2,4,6-trinitrotoluene (TNT) by simultaneous utilization of two initial reduction pathways, and shows a high mineralization rate For the investigation of precise metabolism of TNT by this bacterium, analysis of enzymes involved in initial reduction is necessary Three different aromatic nitroreductases were detected in cell free extract of *Klebsiella* sp C1 These nitroreductases showed some different reduction capabilities, and designated as enzyme I, II and III. Among these, enzyme II was purified to homogeneity by sequential chromatographies of ion exchange, hydrophobic interaction and gel filtration Enzyme II is an oxygen-insensitive nitroreductase and catalyzes TNT and nitroblue tetrazolium Purified enzyme II has molecular weight of approximately 50 kDa. Its activity was inhibited by $CuSO_4$, $HgCl_2$, $FeSO_4$, $MgCl_2$, $MnSO_4$, $AgNO_3$, dicumarol, dephenyliodonium, acetate, lactate and benzoate. Purified enzyme II could transform TNT by the reduction of nitro groups of TNT, and the metabolites from direct ring reduction could not be detected It catalyzes reduction of TNT to 2-hydroxylamino-4,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene.

E014

Functions of Nitrogen PTS Genes and Their Nearby Genes in *Escherichia coli*

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The phosphoenolpyruvate(PEP). sugar phosphotransferase system(PTS) phosphorylates sugars and regulates cellular metabolic processes using a phosphoryl transfer and protein-protein interaction This transfer system includes the general energy coupling proteins, Enzyme I(EI) and HPr as well as the sugar specific enzyme II complexes The *ptsP* gene encodes an EI paralogue designated Enzyme I^{Ntr} (EI^{Ntr}), and two genes located in the *rpoN* operon encode PTS paralogues, NPr and IIA^{Ntr}. Here, we show that the dephospho-form $EIIA^{Ntr}$ of is required for derepression of *ilvBN* operon, and the product of *YgdP* gene which is in an operon with the *ptsP* gene provide same function as dephospho-form $EIIA^{Ntr}$ does

E015

The iso-Glucose-6-Phosphate Dehydrogenase of *Deinococcus radiophilus*

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Deinococcus radiophilus, an extremely resistant bacterium to UV, ionizing radiation,and oxidative stress, metabolizes glucose via Embden-Meyerhof-Parnas (EMP) and oxidative pentose phosphate (PP) pathways. Such resistance of *D radiophilus* seems to be attributed to efficient scavenging system of reactive oxygen species. Glucose 6-phosphate dehydrogenase (G6PDH, EC 1 1.1.49) is the first key enzyme of PP pathway of glucose providing intracellular reducing power which is required to biosynthetic machinery as well as ROS scavenging system *D radiophilus* seems to possess two isoforms of G6PDH, which are specific with both NAD and NADP. The iso-G6PDHs of *D radiophilus* were purified by steps of DEAE cellulose ion-exchange and 2'5' ADP Sepharose 4B affinity chromatography These iso-G6PDHs seems to be very similar in their enzyme kinetics although they differ in molecular sizes and pIs The sizes of subunit for G6PDH-1 and G6PDH-2 are ca. 35.2 kD and 60 kD, respectively. The isoelectric points of G6PDH-1 and G6PDH-2 are ca. 6.4 and 5 7, respectively. And a significant increase in activity of G6PDH-1 at stationary phase suggests us that this enzyme is an inducible enzyme.

E016

Partial Cloning of a Laccase and Manganese Peroxidase Gene in *Merulius tremellosus.*

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A white rot basidiomycete *Merulius tremellosus* secretes laccases and peroxidases which are involved in the degradation of polymeric lignin. Monokaryon is required which is used as the genomic DNA source and as the recipient host in the genetic transformation experiment It is possible to isolate monokaryon from dikaryotic culture through the protoplast generation. To establish conditions for the protoplast isolation and regeneration from the mycelia of *M tremellosus*, strength of Novozyme 234, and pH of medium were examined Laccases which have the conserved copper-binding domains can be amplified using the DNA fragment of the domain as the PCR primers Using this technique we have cloned a laccase gene fragment which shows high similarity with the reported fungal laccases. We have also cloned manganese peroxidase gene fragment using the PCR primers which represent the DNA regions of metal-binding domains