E017

E. coli Genes Associated with Methylglyoxal Metabolism

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Methylglyoxal (MG), a typical 2-oxaldehyde, is a ubiquitous metabolite derived from glycolysis. We showed that lactaldehyde, one of the metabolic products of MG, inhibits growth of *E coli* Under aerobic conditions, L-lactaldehyde is oxidized to L-lactate by *aldA*, while under anaerobic conditions, L-lactaldehyde is reduced to L-1,2-PD by *fucO* ^{1}H-NMR analysis of crude proteins of corresponding mutant strains showed absence of other genes compensating *aldA* and *fucO*. Our ^{1}H-NMR studies showed that *gldA* of *E coli* converts MG into lactaldehyde and DHA into glycerol in the presence of NADH or NADPH. Substrate specificity for this enzyme was highest for DHA followed by MG, acetol and glyceraldehyde respectively. DHA was found to be toxic to *E coli* cells with the LC_{50} value of 25-30 mM but no significant toxicity was observed for HA. We also screened other MG-detoxifying genes by transforming genomic library in *gloA* deletion background. As a result, we were able to isolate several clones conferring resistance to MG, including the *yqhD* gene Overproduction of *yqhD* also conferred resistance to DHA up to 10 mM concentration Metabolic role of *yqhD* is currently under investigation

E018

Transformation of *Trametes versicolor* by Restriction Enzyme Mediated Integration (REMI) and Analysis of Transformants.

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A white rot basidiomycete *Trametes versicolor* secretes laccases and peroxidases which involved in the degradation of polymeric lignin In order to examine over expression of the lignin degrading enzymes in this fungus at the molecular level, the genetic transformation of the gene related in the lignin degradation has been carried out Genetic transformation of *T versicolor* was successfully carried out by restriction enzyme mediated integration (REMI). We have constructed a pBARGPMRP which has the phosphinothricin resistance gene(*bar*) and a manganese-repressed gene(*mrp*) A mono-karyotic strain of *T versicolor* was transformed to *bar* using the pBARGPMRP In order to performed REMI-transformation in *T versicolor*, pBARGPMRP was linearized by restriction enzyme *cla*I and they were mixed with the fungal protoplast for integration of the vector into the fungal chromosome The integration of the plasmid in *T versicolor* chromosomal DNA is confirmed by Southern blot analysis using probe containing *bar* gene The transformants showed a correlation between the decolorization of dyes (poly-R, RBBR) and ligninonytic ability of microorganism

E019

Funtional Analysis of an Acidic Laccase of *Coprinellus congregatus* through the Heterologous Expression in Yeast.

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C. congregatus, a mushroom forming basidiomycete secreted high amount of a laccase when transferred into an acidic liquid medium (pH 4 1, YpSs), and the laccase synthesis was controlled at the transcription level When *C congregatus* was grown in the acidic liquid medium, pH of medium is neutralized to higher than the pH 5 0 after day 1. This laccase is designated an acidic laccase (*lac2*), and cDNA and genomic DNA have been cloned. We have constructed an acidic laccase expression cassette consisted of the laccase promoter (1 2 kb promoter) and *lac2* cDNA, and it has been transformed to *S cerevisiae* in order to determine the expression mechanism of the promoter When we have analysed the expression of the acidic laccase promoter, *S. cerevisiae* transformed to *lac2* showed highly increased survival rate under the peroxide stresses. We have also constructed the promoter analysis system consisted of the laccase promoter (2 kb promoter) and the same cDNA which is transformed to *S. cerevisiae*. We will discuss the regulation of *lac2* promoter (2 kb promoter) gene under diverse stress conditions.

E020

Stereospecificity of Microbial Hydroxylation of Linoleic acid to 10-hydroxy-12(Z)-octadecenoic acid.

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This report describes the stereospecific microbial hydroxylation of linoleic acid to 10-hydroxy-12(Z)-octadecenoic acid by *Pseudomonas* sp. NRRL B-2994 The bioconversion of 10-hydroxy-12(Z)-octadecenoic acid was achieved by an anaerobic oxidation by microbial lipoxygenase of *Pseudomonas* sp NRRL B-2994 on hydrolysed safflower oil (over 75% linoleic acid) Safflower oil from Uiseong, Gyeongsangbuk-do that contains >75% of linoleic acid, was hydrolyzed using lipase Cultures were grown by using our standard two-stage fermentation protocol The product was extracted by ethyl acetate, and its structure was determined by GC mass spectral analysis Maxium production of 10-hydroxy-12(Z)-octadecenoic acid with 49 2% conversion of the substrate was reached after 4 days of reaction This is the first report of a chiral specific 10-hydroxy-12(Z)-octadecenoic acid production by microbial transformation

Key words Hydroxylation, bioconversion, *Pseudomonas* sp NRRL B-2994
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