Analytical approach for lipid oxidative mechanism of white rot fungi

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Recently wood biomass conversion systems drew attention as one of solutions for environmental problems becuase cyclic utilization of wood is essentially carbon-neutral. White rot fungi are the most efficient lignin degraders in nature and play an important role in carbon recycling.

The fungi secrete extracellular ligninolytic enzymes including manganese peroxidase (MnP). It was reported that MnP is capable of decomposing recalcitrant aromatic compounds such as nonphenolic lignin models in the presence of Mn(II) and unsaturated lipids. This indicates that the MnP/lipid system generates active oxidants leading to radical production involved lignin degradation.

In this study, analytical approaches for lipid oxidation were investigated by genetic engineering and biochemical procedure to elucidate the oxidation mechanism.

i) Suppression of *ku70* gene in *Pleurotus ostreatus*

To establish gene targeting system of white rot fungi, *Poku70*, which encodes protein that functions in nonhomologous end-joining of double-stranded breaks, was targeted for RNAi mechanism.

In basidiomycetes, two pathways have identified, as repairing mechanism of double-strand break (DSB). One is homologous recombination (HR), involves interaction between homologous sequences. The other is non-homologous end-joining (NHEJ), involves direct ligation of the strand ends independent of DNA homology. Homologous recombination is the most efficient method of gene targeting system. However, many organisms, without *Saccharomyces cerevisiae*, seem to use NHEJ preferentially in DSB repair. As a result, exogenous DNA can be integrated randomly in the genome. Under hypothesis that the rate of homologous recombination can be increased by blocking NHEJ, RNAi vector aimed at repression of *Poku70* expression was introduced into protoplasts of *P. ostreatus* with drug-resistant plasmids by co-transformaton.

PCR analyses of DNA of the drug-resistant isolates obtained from co-transformation experiments demonstrated specific amplification of the marker fragment in dikaryon and monokaryon transformants each. The resulting mutants showed slower growth than wild types. By comparison with other transformants which RNAi vector couldn't insert, it seemed not to be influence on growth whether it inserted or not.

ii) Analysis of products formed by lipid peroxidation by lignin-degrading enzyme

At an incipient stage of wood decay, a selective white rot fungus *Ceriporiopsis subvermispora* produced MnP and large amounts of unsaturated fatty acids. MnP oxidized the lipids to produce hydroperoxides and aldehydes. Although, reactions of lipids with MnP have been studied, reaction intermediates produced by the oxidation of lipids with MnP are not fully elucidated. In this study, products formed by the reactions of linoleic acid with MnP were profiled by GC/MS.