Genetic Transformation and Its Application in Basidiomycete Fungus, *Pleurotus ostreatus*

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Pleurotus ostreatus, oyster mushroom, is a popular edible mushroom cultivated world wide, and also a focus of research interests for its high ability of degrading plant cell wall lignin and numerous environmental pollutants. At the moment, the genome sequencing project is undergoing and the results are going to be open to public via JGI (Joint Genome Institute), Department of Energy, USA (http://www.jgi.doe.gov/sequencing/why/50009.html). We set sail for development of a genetic transformation system in this fungus, constructing a new transformation marker gene. A mutant allele with site-directed base substitution was constructed and demonstrated to work as a dominant drug resistant marker to a systemic fungicide, carboxin[1)]. A genetic transformation system renders us functional analysis of particular gene(s), overexpression of enzyme(s) of interest, and breeding of strains with suitable properties, such as higher ligninolytic activity.

Lignin degradation by *P. ostreatus* is thought to proceed by successive oxidative decomposition triggered by its extracellular oxidizing enzyme(s). This fungus contains at least four genes encoding extracellular peroxidases with Mn^{2+}-oxidizing activity, namely, MnP1-4. Among them, two major isozymes, MnP2 and MnP3, were extensively studied in substrate specificity, catalytic property and expression control using non-synthetic media[2-4)]. Especially, a versatile peroxidase MnP2 was characterized for its unique property of oxidizing activity of high-molecular-weight compounds. We developed a homologous gene expression system in *P. ostreatus*, and succeeded in overproduction of the recombinant MnP2 protein without concomitant expression of endogenous *mnp2*. A series of mutant MnP2 were produced by *P. ostreatus* transformants containing a recombinant *mnp2* with site-directed base substitutions. Analyses of their reactivity for low- and high-molecular weight substrates demonstrated that MnP2 directly oxidizes aromatic substrates at an external active site, W170, and that its surrounding environment has much impacts on the unique reactivity with polymeric substrates.

Cohen *et al*. showed that amendment of Mn^{2+} to the medium containing cotton stalks accelerated lignin decomposition and that Mn^{2+} caused differential transcriptional control of *mnp* gene family[5,6)]. It is plausible that Mn^{2+} plays a significant role in lignin decomposition by the fungus. In our experiments with a synthetic medium, *P. ostreatus* showed extracellular MnP activity, depending on the presence of Mn^{2+}. RT-PCR analyses revealed that transcription of *mnp3* was totally dependent on Mn^{2+}, whereas *mnp2* was transcribed even in the

absence of Mn^{2+} and its transcription level was not affected by the addition of Mn^{2+}. Elucidation of the mechanism for differential expression of MnPs in *P. ostreatus* is now under way.

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