

## SI-1-1

### REACTION MECHANISM OF PEROXIDASE FROM A STRAIN OF POLYPORACEAE AND CLONING OF THE GENE

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An extracellular peroxidase (PoP) from a fungal strain of Polyporaceae was studied in relation to lignin biodegradation. PoP oxidized  $\beta$ -O-4 lignin model dimeric compound, 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1,3-dihydroxypropane to produce 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-oxo-3-hydroxypropane, 2,6-dimethoxyhydroquinone, 2-(2-methoxyphenoxy)-3-hydroxypropanal, 2-(2-methoxyphenoxy)-ethanol, 2-(2-methoxyphenoxy)-3-hydroxypropanoic acid, 2,6-dimethoxy-1,4-benzoquinone and guaiacol. Radicals were identified as reaction intermediates. From these results, reaction mechanism for the degradation of  $\beta$ -O-4 lignin model compound by PoP was proposed. The reaction was initiated by one-electron oxidation leading to free radical intermediate. This radical may undergo  $C_{\alpha}$ - $C_{\beta}$  cleavage,  $C_{\alpha}$ -oxidation and alkyl-phenyl cleavage. cDNA and genomic DNA clones for PoP were isolated and characterized. The cDNA clone encoded a protein of 499 amino acid residues which shows little homology to other peroxidases. The regions of proximal and distal histidine conserved in other peroxidases were not found in PoP. Multiple alignment of PoP with other peroxidases revealed that PoP is a new type of peroxidase. Six introns which have consensus sequences were found. Expression of cDNA in *S. cerevisiae* resulted in the production of recombinant peroxidase (rPoP) glycosylated at various level. Site-directed mutagenesis study revealed that His364 is the possible proximal histidine residue of PoP.

## SI-1-2

### DEVELOPMENTAL STUDIES IN *ALLOMYCES MACROGYNUS*

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*Allomyces macrogyrus*, a member of the Class Chytridiomycetes, is an aquatic fungus living in fresh water. This fungus exhibits a definite alternation of generations, haploid gametothalli alternating with diploid sporothalli, and produces four types of motile cells, two zoospores and two gametes.

Following the encystment, zoospores form germ tubes. It was found that the linear growth rate of germlings was proportional to the density of inoculated zoospores. Depletion of atmospheric oxygen also stimulated the linear growth of germlings. However, Spitzenkorper was not observed in the tips of fast growing germlings.

Two days after inoculation of zoospores, sporangia or gametangia are formed at the tips of the hyphae, and they release zoospores and gametes, respectively, when submerged in salt solution. We observed that even the cysts could be induced to release zoospores and gametes, even within several hours. The cysts formed from diploid zoospores released a single zoospore while those formed from haploid zoospores released four male gametes. These two mini cycles of reproduction might be an excellent model system for the studies of the regulation of asexual and sexual reproduction.