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Screening for lignin peroxidase genes in natural isolates of white rot fungi

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Abstract

To find lingin peroxidase genes similar to those of *Phanerochaete chrysosporium*, we have studied several species of white rot fungi collected in nature. The methodology has been DNA hybridization techniques using two synthetic oligonucleotides with a sequence that corresponds to a fragment of the H8 lignin peroxidase gene from *P. chrysosporium* (Schalch et al. 1989. Mol. Cell. Biol. 9:2743-2747) controlling the structure of the predominant form of the enzyme in this fungus.

Screening for lignin peroxidase genes in natural isolates of white rot fungi.

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The white rot fungi *Pycnoporus cinnabarinus* (strain Pc58), *Pycnoporus sanguineus* (strain Ch470), *Bjerkandera adusta* (strain Ch1080), *Coriolus versicolor* (strain Ch730) and *Pleurotus sp*. (strain Ch1040) were isolated from carpophores collected in the rain forest of southern Chile, and now are kept in our laboratory collection.

In the laboratory, these strains were grown for 10 days at 30 C in liquid medium containing 1.5% malt extract, 0.75% yeast extract and 0.4% glucose, on a rotary shaker at 120 rpm. Mycelium was harvested on Whatman #1 paper and washed with distilled water, frozen at -20 C and ground in a mortar, DNA was prepared by the rapid lithium chloride method (Leach et al. 1987. FGN 34:32-33), treated with 30 ug/ml RNAase A at 37 C, followed by three phenol:chloroform:isoamyl alcohol (25:24:1) extractions as an additional step. DNA was dialyzed against TE buffer (10 mM Tris-HCl, 1 mM EDTA) for 24 h. DNA samples, 4 ug, were digested with *Bam*HI at 37 C for 3 h. Then, two phenol: chloroform:isoamyl alcohol (25:24:1) extractions were performed, DNA samples, in 100 ul of TE buffer, were denatured, transferred to a nylon membrane (Pall), treated with UV light for 5 min and prehybridized for 10 h at 48 C. The 50-base synthetic oligonucleotides used as probes were:

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I.    AGCTCCAGAA GCCATTCGTT CAGAGGCACG GTGTCACCCC TGGTGACTTC
II.   ATCGCCTTCG CTGGTGCTGT CGCGCTCAGC AACTGCCCTG GTGCCCCGCA
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The oligonucleotides were mixed and end labeled with polynucleotide kinase and [32P]-gamma-ATP. Hybridization was carried out at 48 C for 24 h. An additional probe was pAT153 containing a cDNA for H8 lignin peroxidase labeled with biotin.

Table 1 shows the results of hybridization experiments using *Phanerochaete chrysosporium* and plasmid DNA as positive hybridization controls and *Neurospora crassa* DNA as a negative control, respectively.

Only two species exhibited hybridization with the probes used. These species were *Bjerkandera adusta* (Ch1080) and *Coriolus versicolor* (Ch730). In contrast, *Pycnoporus cinnabarinus* (Pc58) and *Pycnoporus sanguineus* (Ch470), which have significant lignin degrading capacities, did not show hybridization with these two oligonucleotides.

Other fungi, such as *Pleurotus sp*. (Ch1040) and *S. commune* (Ch29), gave only faint hybridization signals that were insufficient to suggest the presence of lignin peroxidase genes. Although both *Pyconoporus* and *Pleurotus* species are classified as white rot fungi, these results suggest the presence of a different lignin degrading system in these fungi. *P. cinnabarinus* and *P. sanguineus* have high levels of phenol oxidases, such as laccase and when grown in a medium containing ground wood of *Nothofagus dombeyi* plus malt extract, they utilize such substrate efficiently.

Table 1. Dot blot analysis of Chilean natural isolates of lignolytic fungi.

	DNA probe	
	Oligonucleotides	pAT153-H8
Phanerochaete chrysosporium BKM-1767	+++	+++
Pycnoporus cinnabarinus (Pc58)	–	–
Pycnoporus sanguineus (Ch470)	–	–
Coriolus versicolor (Ch730)	++	++
Bjerkandera adusta (Ch1080)	+	+
Schizophyllum commune (Ch29)	–	–
Pleurotus sp. (Ch1040)	–	–
Neurospora crassa 74A	–	–
pAT153-H8	+++	+++