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Recent advances in the organization and regulation of lignin peroxidase genes of *Phanerochaete chrysosporium*

Jill Gaskell and Daniel Cullen

Institute for Microbial and Biochemical Technology, Forest Products Laboratory, Madison, Wisconsin, USA

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Summary

Lignin depolymerization is catalyzed by extracellular peroxidases of white-rot basidiomycetes such as *Pherochaete chrysosporium.* In submerged culture, production of multiple lignin peroxidase (LiP) isozymes is derepressed under carbon, nitrogen, or sulfur limitation. The roles of the individual isozymes in lignin degradation and their genetic regulation are poorly understood (reviewed by Kirk and Farrell, 1987).

Lignin peroxidase; Genomic organization; *Phanerochaete chrysosporium*

The lignin peroxidases of *P. chrysosporium* are encoded by a family of structurally related genes, but their total number is still unclear. Clones from *P. chrysosporium* strain BKM-1767 include the cDNA encoding isozyme H8 (Tien and Tu, 1987), the corresponding genomic clone (Smith et al., 1988), cDNA's designated CLG4 and CLG5 (de Boer et al., 1987), genomic clone GLG2 which is nearly identical to CLG5 (Zhang, 1987), genomic clones V4 and O282 (Schalch et al., 1989), genomic and cDNA clones designated ML4 (Andrawis et al., 1989), and most recently, genomic clone LPOB (Huoponen et al., 1990) which is closely linked to H8 (=LPOA). A large family of structurally similiar genes has also been

Correspondence to: D. Cullen, Inst. Microbial and Biochemical Technology, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705, USA.

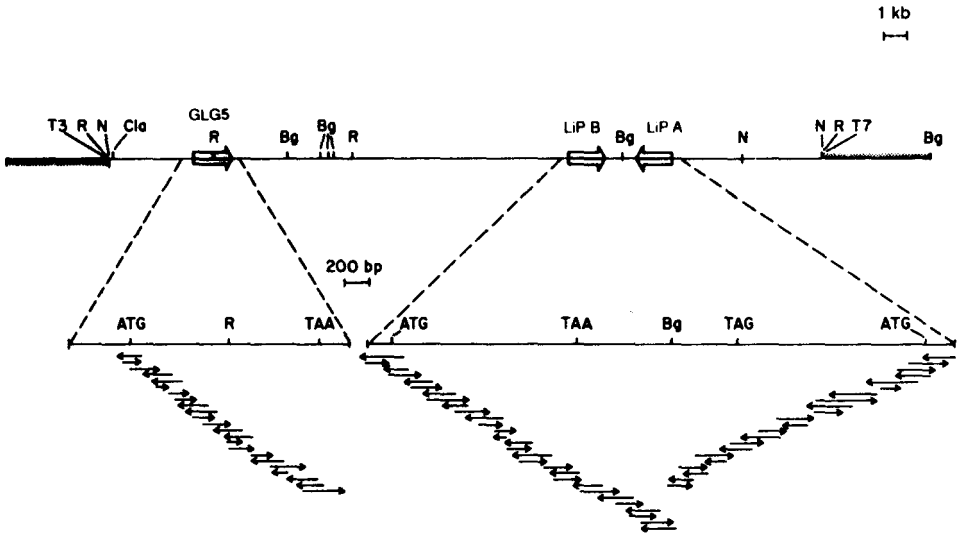

Fig. 1. Partial restriction map of cosmid pQQ24 containing LiP gene clusters. Sequenced regions and strategy shown below. Open arrows show position and transcriptional orientation of LiP genes GLG5, LiPB and LiPA. Translational start and stop codons are indicated. Shaded area is cosmid pWE15. The distance between GLG5 and LiPB is approximately 14 kb. Abbreviations: R, *Eco* RI; N, *Not* I; Cla, *Cla* I; Bg, *Bg/* II.

identified in *P. chrysosporium* strain ME446 (Brown et al., 1988). Recently, we described three closely linked LiP genes (Gaskell et al., 1991; Fig. 1).

Excluding the intron sequences, the nucleotide sequence of GLG5 (EMBL Accession No. X54256) is identical to a previously published cDNA clone, CLG5 (de Boer et al., 1987), and it is remarkably similiar to another genomic clone,

TABLE 1

Summary of sequence homology among LiP clones

Nucleotide	Amino acid							
	H8	LiPA	O282	LPOB	LiPB	GLG5	GLG2	CLG4
HB		99.5	96.5	91.1	91.4	82.6	82.8	72.1
LiPA	99.0 (97.8)		96.5	91.4	91.9	82.6	82.6	71.8
O282	88.1 (79.8)	87.5 (79.9)		89.5	89.8	81.2	81.2	70.5
LPOB	86.6 (73.6)	86.2 (76.6)	84.3 (72.8)		99.5	81.0	81.0	69.7
LiPB	86.9 (76.1)	86.7 (76.3)	84.4 (72.3)	98.5 (96.3)		81.2	81.2	69.4
GLG5	80.8 (70.5)	80.9 (70.4)	78.4 (70.2)	79.3 (69.0)	79.9 (71.4)		100	66.8
GLG2	80.5 (70.5)	80.6 (70.0)	78.1 (69.2)	79.3 (69.0)	79.9 (70.4)	99.2 (97.5)		66.8
CLG4	73.7	73.2	71.1	73.3	73.1	71.8	72.1	

Nucleotide homology based upon optimized alignment of nucleotides from –100 bp of Translational start codon to +100 bp beyond the translation stop is shown in parenthesis following coding sequence homology. Only cDNA sequence is available for CLG4 (de Boer et al., 1987). All sequences are from BKM-1767.

GLG2. The nucleotide and amino acid alignments of the two genomic clones are 97.7% and 100% identical, respectively (Table 1). All nucleotide mismatches are located in the third positions of codons or within introns. Both GLG2 and GLG5 contain nine introns as opposed to eight found in other LiP clones. GLG2 and GLG5 may be allelic forms of the same gene.

Two transcriptionally convergent LiP-like clones, LiPA and LiPB, are located approximately 14 kb downstream of GLG5 (Fig. 1). The nucleotide sequence of the entire region was determined (EMBL Accession No. X54257). This region is strikingly similar in structure to a recently reported λ EMBL3 subclone which contains two lignin peroxidase genes designated LPOA and LPOB (Huoponen et al., 1990). The overall nucleotide sequence identity between the LPO and the LiP clones, including the intergenic region, is 93%. The translational stop codons of the LiP and LPO genes are separated by 1360 bp and 1322 bp, respectively. Within this region the nucleotide identity is 87%. lntron numbers and their relative positions are conserved between the homologous LPO and LiP genes.

Sequence comparisons reveal LiPA to be identical to ML-4 (Andrawis et al., 1989) and LPOA identical to LiPH8 (Smith et al., 1988). The nucleotide sequence identity between the LiPA and LPOA coding regions is 99%. Their deduced amino acid sequences are identical except for a single amino acid change in the signal sequence. In contrast, LiPB and LPOB nucleotide and amino acid sequences are 96.5% and > 99% identical, respectively. The single amino acid difference is at position 298 with a Leu residue in LiPB and a His in LPOB.

The close stuctural similarities between LPO and LiP clones suggest allelism. This was formally tested by Southern blot analysis of single basidiospore cultures, which are homokaryotic (Alic and Gold, 1985; Thompson and Broda, 1987). The LPO and LiP clones were shown to be allelic variants (Gaskell et al., 1991). Thus, as previously proposed (Andrawis et al., 1989), ML4 is an allelic variant of H8 (= LPOA).

The genomic organization of LiP genes is also poorly understood. This is in part due to the lack of a well-defined recombination system in *P. chrysosporium* (Alic and Gold, 1985). Analyses of single basidiospore cultures have been used to generate a genetic map using restriction fragment length polymorphisms (RFLP's; Raeder et al., 1989). The homokaryotic recombinants vary substantially with respect to pigmentation, sporulation, and lignin peroxidase activity (Raeder et al., 1989, unpublished). In these studies, the LiP genes were mapped to two clusters, and another locus, unlinked to LiP genes, exerted a significant effect on LiP activity.

Electrophoretic analysis of *Phanerochaete chrysosporium* karyotype

To further investigate the chromosomal organization of the entire LiP gene family, we have resolved *P. chrysosporium* chromosomes using clamped homogeneous electrical field (CHEF) electrophoresis (Gaskell et al., 1991). At least seven chromosome bands were resolved from *P. chrysosporium* DNA (Fig. 2). At least

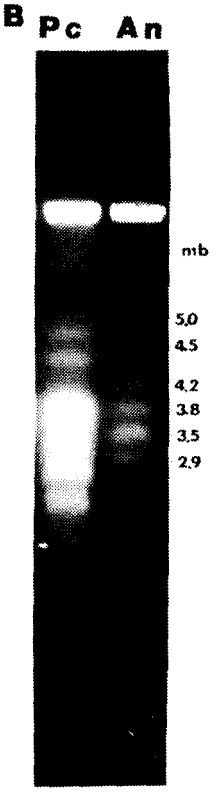

Fig. 2. CHEF chromosome separations *P. chrysosporium* (Pc) and *A. nidulans* (An) run as standard. Chromosomal plugs prepared and run as described by Gaskell et al. (1991). Estimated size of *A. nidulans* chromosome standards in mb shown in right margin.

two of these are doublets, as judged by the relative intensity of ethidium bromide staining. Based upon comparisons with *Aspergillus nidulans,* the *P. chrysosporium* bands are approximately 2.0, 2.8, 3.2, 3.6, 3.8, 4.4, 4.8 mb. The band pattern differs substantially among homokaryons because of meiotic recombination and chromosome polymorphisms. For example, basidiospore culture No. 2 (Fig. 3, lane 3) has bands at approx. 2.0, 2.8, 3.0, 3.5, 3.7, and 4.3 mb. Assuming doublets at 2.8, 3.0, and 3.7 mb the total genome size is about 29 mb, somewhat lower than the previous estimate of 36.5 mb for the haploid genome (Raeder et al., 1989).

Six different LiP clones from BKM-1767 hybridized to electrophoretic bands at 3.7 and 3.5 mb. The clones were H8 (= LPOA), O282, V4 (pFFF62), and the three genes residing on pQQ24 (Fig. 1). In addition, the 33-mer which hybridizes to eight of the eleven LiP clones of ME446 (Raeder et al., 1989), hybridizes to the same two bands. These results suggest that homologous chromosomes in the dikaryon are dimorphic with respect to electrophoretic mobility. The latter explanation is supported by the presence of single bands in homokaryotic derivatives (Fig. 3). This, in turn, is entirely consistent with the identification of specific alleles in these cultures (Gaskell et al., 1992). Additional dimorphic chromosomes have been

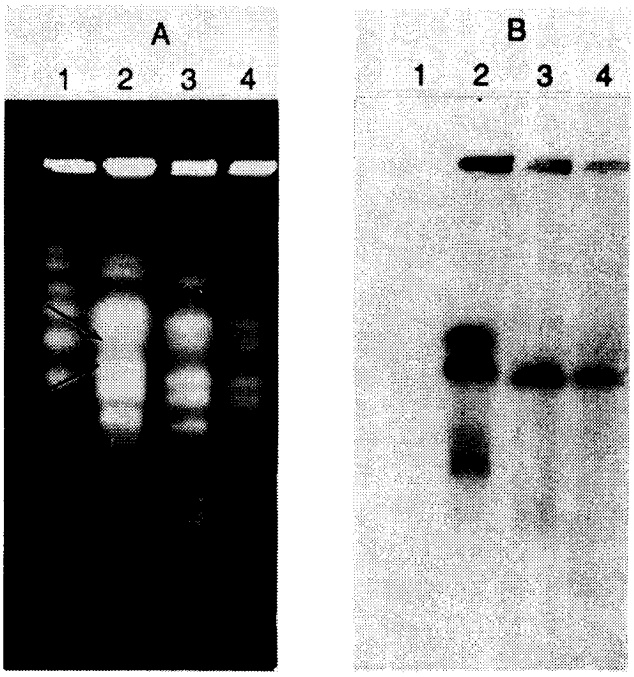

Fig. 3. Segregation of homologous chromosomes in single basidiospore cultures. Ethidium bromide stained gel (Panel A) and Southern blot probed with nick-trmrslated pQQ24 (Panel B). Lane 1 contains *A. nidulans* as molecular weight standard. Lanes 2, 3, and 4 contain DNA from dikaryotic BKM-1767, single basidiospore culture No. 2 and No. 10, respectively. Arrows in lane 2 indicate position of the 3.5 and 3.7 mb hybridizing bands. Taken from Gaskell et al. (1991).

identified for *P. chrysosporium* gene encoding lignin peroxidase CLG4 (Stewart et al., 1992), cellobiohydrolases (Covert et al., 1992), and glyoxal oxidase (Kersten, personal communication).

Chromosome length polymorphisms have been observed in *Plasmodium falciparum* (Corcoran et al., 1988), *Sacharomyces cerevisiae* (Ono and Ishino-Arao, 1988), *Candida albicans* (Rustehenko-Bulgac et al., 1990), *Ustilago maydis* (Kinscherf and Leong, 1988), and *Coprinus cinerus* (M. Zolan, personal communication). The latter two fungi belong to the same taxonomic class as *P. chrysosporium,* the Basidiomycetes. The mechanism(s) giving rise to chromosome length polymorphisms may be related to variations in LiP activity. In *P. falicurum,* polymorphisms are caused by homologous recombination in distal regions of chromosomes, and it has been suggested that recombination in these dynamic regions might be related to antigenic variation (Corcoran et al., 1988). We are currently mapping the LiP-containing homologies to determine if a similar mechanism is operative in *P. chrysosporium.*

Acknowledgements

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