Expression of *Phanerochaete chrysosporium* Genes Encoding Lignin Peroxidases, Manganese Peroxidases, and Glyoxal Oxidase in Wood

BERNARD J. H. JANSE, JILL GASKELL, MASOOD AKHTAR, and DANIEL CULLEN

Department of Microbiology, University of Stellenbosch, Stellenbosch, 7600 South Africa, 1 and Institute of Microbial and Biochemical Technology, USDA Forest Products Laboratory, 2 and University of Wisconsin Biotechnology Center, 3 Madison, Wisconsin 53706

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Expression of *Phanerochaete chrysosporium* genes encoding ligninolytic enzymes was assessed in wood. Poly(A) RNA was extracted from colonized wood chips by magnetic capture, and specific transcripts were quantified by competitive reverse transcriptase PCR. mRNA levels varied substantially among lignin peroxidase genes, and transcript patterns were dramatically different from those in previous studies with defined media.

Lignin depolymerization is catalyzed by extracellular enzymes of white rot basidiomycetes such as *Phanerochaete chrysosporium*. Major components of this system include lignin peroxidases (LiPs), manganese-dependent lignin peroxidases (MnPs), and a peroxide-generating enzyme, glyoxal oxidase (GLOX) (for review, see references 6, 11, and 17). Under nutrient limitation in defined media, multiple peroxidase and GLOX isoforms are secreted.

The peroxidases of *P. chrysosporium* are encoded by families of structurally related genes. Ten LiP genes, designated *lipA* through *lipJ*, have been characterized and shown to be distributed on three linkage groups (reviewed in reference 12). The three known MnP genes (*mnp* genes) are unlinked to each other or to any LiP genes (reference 28 and unpublished data). In contrast, GLOX is encoded by a single gene (*glox*) with two alleles (20, 23). The precise roles and interactions of these genes in lignin degradation and in commercial processes such as biomechanical pulping (for review, see reference 24) are poorly understood.

Numerous studies have demonstrated differential regulation of LiP and MnP genes in response to culture conditions. Northern blots showed *lipD* transcripts dominating in carbon-starved cultures (18) and in defined media supplemented with balled-mill straw (19). In contrast, *lipA* transcripts were relatively more abundant in nitrogen-limited media (18). Nuclease protection assays identified *lipA* as the major transcript in both carbon- and nitrogen-starved cultures (30). Quantitative reverse transcriptase-mediated PCR (RT-PCR) techniques largely confirmed Northern blots and also showed dramatic upregulation of *lipC* and *lipD* under nitrogen starvation (31). All LiP gene transcripts except *lipF* were detected in anthracene-contaminated soil cultures (3). The MnP genes of *P. chrysosporium* exhibit complex regulation by nutrient limitation (15, 29), Mn concentration (7, 9, 14), culture agitation, heat shock (8), H2O2 concentration, and other chemical stresses (25). *mnp3* appears not to be regulated by Mn. In contrast, *mnp1* and *mnp2* respond strongly to Mn and are differentially regulated in response to culture agitation (15). The three MnP genes are coordinately transcribed in soil cultures (4). Nothing is known of the regulation of *P. chrysosporium* peroxidase genes in woody tissue, the natural substrate.

To assess transcript levels of all known LiP, MnP, and GLOX genes in *P. chrysosporium*-colonized wood, 2.5 kg of aspen wood chips was steam sterilized and inoculated by standard biomechanical pulping methods (1) (reviewed in reference 2). Poly(A) RNA was extracted from 10-g samples as described elsewhere (32), with minor modifications. Specifically, the initial extract buffer was squeezed through Miracloth (Calbiochem, Inc., La Jolla, Calif.) filters, and following incubation with Dynabeads oligo(dT)25 (Dynal, Great Neck, N.Y.), the hybridization buffer was twice extracted with a model MPC-1 magnetic concentrator. Poly(A) RNA levels were too low to accurately quantify (<1 μg/10 g), but yields were adequate for a minimum of 600 separate RT-PCRs. The competitive RT-PCR protocol was adapted from the work of Gilliland et al. (16) with gene-specific primers (Table 1). Competitive templates, in the form of full-length genomic subclones, were added to 50-μl PCR mixtures as 10-fold serial dilutions ranging from 10 ng to 0.1 fg. Preliminary experiments quantifying *lipA*, *lipC*, *lipE*, and *lipF* transcripts with various amounts of poly(A) template in RT-PCRs showed no evidence for RT inhibition (10).

PCR products were size fractionated on 1.5% agarose gels and ethidium bromide stained, and the image was recorded with a Foto/Analyst digital camera (Fotodyne, Inc., Hartland, Wis.). The image was digitized with NIH Image software (version 1.61). Linear regressions were determined by plotting ratios of genomic competitor to cDNA target against the concentration of competitive template. Adjusting for length differences, equivalence points were determined on linear regressions where the ratios were 1.5 for *lip* genes, 1.3 for *mnp* genes, and 1.19 for *glox*. Results were expressed in picograms of cDNA (Fig. 1). Independent analysis for *lipC* and *mnp2* transcript levels in separate wood chip cultures varied less than 12%.

Differences in transcript levels ranged up to 10,000 fold (Fig. 2), and transcript patterns in aspen were unlike the patterns previously observed in defined media or in soil cultures. Transcripts of *lipF*, absent in soil cultures, were abundant. *lipD* and *lipE*, major transcripts in soil and defined media, ranked lowest among LiP gene transcripts in aspen. Transcripts of *lipJ*, rep-
to *lip* genes, the *mnp* genes showed less difference in transcript levels.

The identification of *glx* transcripts in wood was consistent with a close physiological connection between extracellular peroxidases and GLOX (21, 22). The simultaneous detection of *lip* and *glx* transcripts was reported in defined media (20, 23) and in soil cultures (3).

Transcript levels generally declined by 8 weeks of incubation, although it is unclear whether transcription was reduced or whether mRNA was partially degraded. *lipA* and *glx* transcripts decreased more than 100-fold, while *lipB*, *lipE*, and *lipF* transcripts increased 3- to 4-fold. Temporal shifts in transcription have been observed in defined media (3, 5, 25).

No clear relationship between genomic organization and transcription emerges from these and previous results. Within the two LiP gene clusters (*lipA*, *lipB*, *lipC*, *lipE* and *lipF*, *lipG*, *lipH*, *lipI*), no patterns are evident. The unlinked LiP genes, *lipD* and *lipE*, show patterns very different from those of one another and from those of most other *lip* genes. In comparing all genes on all substrates, *lipD* and *lipE* transcript patterns are most alike, although the *lipD* transcript levels are consistently 5- to 10-fold higher than those of *lipE*.

The substantial differences in transcript levels probably reflect enzyme activity in aspen, as has been demonstrated in extracellular peroxidases and GLOX (21, 22). The simultaneous detection of *lip* and *glx* transcripts was reported in defined media (20, 23) and in soil cultures (3).

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