Appl Microbiol Biotechnol (2004) 65: 559–565
DOI 10.1007/s00253-004-1645-z

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Differential regulation and xenobiotic induction of tandem P450 monooxygenase genes *pc*-1 (CYP63A1) and *pc*-2 (CYP63A2) in the white-rot fungus *Phanerochaete chrysosporium*

Received: 31 December 2003 / Revised: 8 April 2004 / Accepted: 23 April 2004 / Published online: 23 July 2004

Abstract The two tandem P450 monooxygenase genes (*pc*-1 and *pc*-2) reported by us earlier in *Phanerochaete chrysosporium* were investigated for their regulation under nutrient-limited and nutrient-rich culture conditions. Transcript analysis based on real-time quantitative RT-PCR showed higher expression of *pc*-1 in defined low-nitrogen and *pc*-2 in defined high-nitrogen media, with maximum expression on day 4, indicating that the two genes, though tandemly linked, are regulated in a non-coordinate manner. Transcript levels of *pc*-1 and *pc*-2 were differentially influenced by the type of carbon source, incubation temperature, and oxygenation. Both genes were inducible by organic xenobiotic chemicals. Of the 42 xenobiotics tested in nutrient-rich cultures, *pc*-1 transcription was induced 2.12(±0.40)-fold to 6.27(±0.48)-fold in the presence of 19 compounds and *pc*-2 transcription was induced 2.00(±0.73)-fold to 29.39(±9.40)-fold in the presence of 22 compounds. Particularly, it is significant that both *pc*-1 and *pc*-2 are induced by polycyclic aromatic hydrocarbons (PAHs) of varying ring size, including naphthalene (4.35±0.09, 6.02±1.39), phenanthrene (2.82 ±0.12, 2.14±0.61), pyrene (3.93±0.01, 1.0±0.12), benzanthracene (1.67±0.03, 6.08±1.50), and benzo(a)pyrene (1.55±0.01, 5.54±2.75) respectively. This study constitutes the first report on the identification of P450 genes in a fungus that are responsive to environmentally significant pollutant chemicals (PAHs, DDT, long-chain alkyl phenols) and lignin derivatives.

Introduction

Phanerochaete chrysosporium is extensively used as a model to study the physiology, biochemistry, and genetics of the biodegradation of lignin and chemical pollutants by white-rot fungi (Gold and Alic 1993; Paszczynski and Crawford 1995; Reddy 1995; Cullen 1997). The initial depolymerization of lignin (Kirk and Farrell 1987; Orth and Tien 1995) is catalyzed by extracellular peroxidases, releasing a range of chemical compounds that are internalized and further degraded by diverse intracellular enzymes, possibly P450 monooxygenases. However, there is little information available on the role of P450s in lignin biodegradation. *P. chrysosporium* has an extraordinary ability to degrade and mineralize a broad spectrum of aromatic, alicyclic and aliphatic chemical pollutants, such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), dioxins, polychlorinated biphenyls (PCBs), herbicides, pesticides, wood preservatives, chlorinated aliphatic solvents, detergent components, azo dyes, and nitroaromatics, among others (Bumpus and Aust 1987; Paszczynski and Crawford 1995; Reddy 1995). Degradation of many of these pollutant chemicals occurs under both nutrient-limited (ligninolytic) and nutrient-sufficient (non-ligninolytic) conditions. Initially, extracellular peroxidases of the lignin-degrading enzyme system were shown to catalyze the biodegradation of pollutants structurally related to lignin substructures (Bumpus and Aust 1987; Gold and Alic 1993). However, subsequent studies using high-nitrogen- and high-carbon-containing media, which do not induce peroxidase system, demonstrated the involvement of alternate oxygenases, particularly P450 monooxygenases, in several xenobiotic degradation reactions in *P. chrysosporium* (Sutherland et al. 1991; Reddy 1995; Kullman and Matsumura 1996; Yadav et al. 2001). This led to an increasing interest in the characterization of cytochrome P450 systems in this model organism.

Initial efforts to characterize the P450 monooxygenase systems of *P. chrysosporium* led to the first isolation of two full-length P450 monooxygenase genes belonging to the CYP63 family, CYP63A1 (Kullman and Matsumura 1997; Yadav et al. 2003) and CYP63A2 (Yadav et al. 2003), and the P450 reductase gene (Yadav and Loper 2000) that is responsible for electron transfer to multiple

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P450 proteins. Subsequently, whole-genome sequencing of *P. chrysosporium* by the Joint Genome Institute (http://genome.jgi-psf.org/whiterot) of the United States Department of Energy led to the identification of over 100 full-length P450 genes (Yadav and Doddapaneni 2003) in this model white-rot fungus, reflecting its enormous biodegradation capacity and functional diversity. This warrants the functional characterization of these diverse novel P450 genes. Several P450 monooxygenases are induced by their substrates (Anzenbacher and Anzenbacherova 2001), a characteristic that may help identify the endogenous substrates for inducible P450 enzymes. Here, we describe the physiological regulation and xenobiotic induction of the two tandem P450 genes, CYP63A1 (*pc*-1) and CYP63A2 (*pc*-2), cloned in our laboratory, as a first step towards understanding their physiological role in this organism.

Materials and methods

Strain and culture conditions *P. chrysosporium* strain BKM-F-1767 (ATCC 24725) was used in the study and was maintained on malt extract agar (Difco Laboratories). The fungus was grown as shaken cultures (100 ml) in 250-ml Erlenmeyer flasks in duplicate at 37°C and 180 rpm for a given time in defined low-nitrogen (LN) medium (2.4 mM N, 1% glucose), defined high-nitrogen (HN) medium (24 mM N, 1% glucose) and malt extract (ME) medium (8 mM N, 2% glucose), as described by Yadav et al. (2003). Mycelia were grown using a 10% blended inoculum prepared in the respective media. Cultures were oxygenated every 24 h under aseptic conditions (1 min at 82.75 kPa) and the harvested mycelia were quickly frozen and stored at −80°C for RNA extraction, as described by Yadav et al. (2003). Five different carbon sources, namely glucose, sucrose, raffinose, corn starch, and carboxymethyl cellulose (CMC) were added at 1% (w/v) each to the growth medium (LN or HN), providing the following test concentrations for simpler carbohydrates: glucose (55.6 mM), raffinose (16.8 mM), and sucrose (29.2 mM). The effects of temperature (37°C vs 22°C) and oxygenation on transcription were studied in regular LN and HN cultures (containing 1% glucose). To study the effect of oxygen, one set of cultures was flushed with 100% oxygen for 1 min (82.75 kPa) at 24 h intervals (Yadav et al. 2003), while a second set was grown under normal air atmosphere.

Xenobiotic induction of pc-1 and pc-2 A total of 42 xenobiotics, representing aliphatic, alicyclic, and aromatic (both synthetic and natural) compounds, were tested for P450 transcriptional induction in ME cultures. The synthetic aromatics included common environmental pollutant chemicals, whereas the complex natural aromatics included compounds mimicking naturally occurring terpenes and lignin derivatives. Xenobiotic chemicals used were of analytical grade and purchased from different commercial sources. ME cultures grown for 24 h as above were spiked with individual chemicals at a pre-tested non-inhibitory concentration and mycelia were harvested after 24 h. Details of the xenobiotics tested and their molar concentrations used are given in Table 2. A parallel control culture treated with an equal volume of the solvent [dimethyl sulfoxide (DMSO) for solids, water for liquids] was also included.

Transcript analysis by real-time quantitative RT-PCR Gene transcripts for *pc*-1 and *pc*-2 in fungal cultures were quantified by real-time quantitative RT-PCR, using the 7900 HT ABI Prism (Applied Biosystems, Forset city, Calif.) and Brilliant SYBR green QRT-PCR master mix kit (Stratagene, La Jolla, Calif.) as per the manufacturer's specifications. Total RNA (75 ng) from frozen mycelia was used for RT-PCR amplification using gene-specific primers (Table 1). Reverse transcription was carried out at 50°C followed by PCR amplification (37 cycles) as follows. For *pc*-1, each amplification cycle consisted of heat denaturation at 95°C for 30 s, followed by annealing and extension at 60°C for 1 min. For *pc*-2, each cycle involved denaturation at 95°C for 30 s and annealing at 60°C for 30 s, followed by extension at 72°C for 1 min. Quantification was based on a ca.300-bp amplicon (Table 1). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control in RT-PCR amplifications (Table 1).

Gene transcript number was quantified based on cycle threshold,as described byYadav et al. (2003). Each sample was amplified twice and the mean value and standard deviation of the replications were calculated. The data were analyzed with either Student's *t*-test or analysis of variance. A probability level of $P \leq 0.05$ was regarded as significant. The *x*-fold difference was calculated separately for each replicate by dividing the transcript number of the experimental sample by that of the control. More than two-fold induction over the control was considered significant.

Results

Physiological regulation of pc-1 and pc-2 Transcription levels of *pc*-1 varied with the nutrient status of the media, with nutrient-limited cultures showing significantly higher ($P \leq 0.05$) overall expression than nutrient-rich cultures. *pc*-1 expression in nutrient-rich cultures (HN, ME) showed a gradual increase as nutrients depleted, beginning on day 4 (Fig. 1, top panel), which was sustained in older cultures. In contrast, LN cultures showed a surge in expression on day 4 (which coincided with the onset of the secondary metabolic phase) followed by a decline thereafter. *pc*-2 showed a different pattern of expression under varying nutrient conditions, with an overall level of expression being significantly higher ($P \leq 0.05$) in HN cultures than in LN and ME cultures (Fig. 1, bottom panel). The time-course showed maximal expression on day 4 in the optimal nitrogen-rich medium (HN). In contrast, expression levels remained relatively unchanged in LN and ME cultures throughout the culture period (Fig. 1, bottom

Table 1 Oligonucleotide primers used in this study

Target gene	Forward primer	Reverse primer	Amplicon (bp)
pc-1	PC-1 UP3 [5′-GAGACTCTGCGGCTTTATCCTCC-3′]	PC-1 DN3 [5′-GTATGCGAACTGCTGCCCCAAGC-3′]	297
pc-2	PC-2 UP1 [5′-AGCCCGAACCCGTTCATCTTCCTC-3′]	PC-2 DN1 [5′-GCAGCAGGGCACATCCACTAGG-3′]	382
Promega RNA	UP pri [5′-GCCATTCTCACCGGATTCAGTCGTC-3′]	DN pri [5′-AGCCGCCGTCCCGTCAAGTCAG-3′]	323
GAPDH	GPD UP1 [5′-GGCATTGTGCAGGGTCTCATG-3′]	GPD DN1 [5′-GAGTAGCCCCACTCGTTGTC-3′]	458

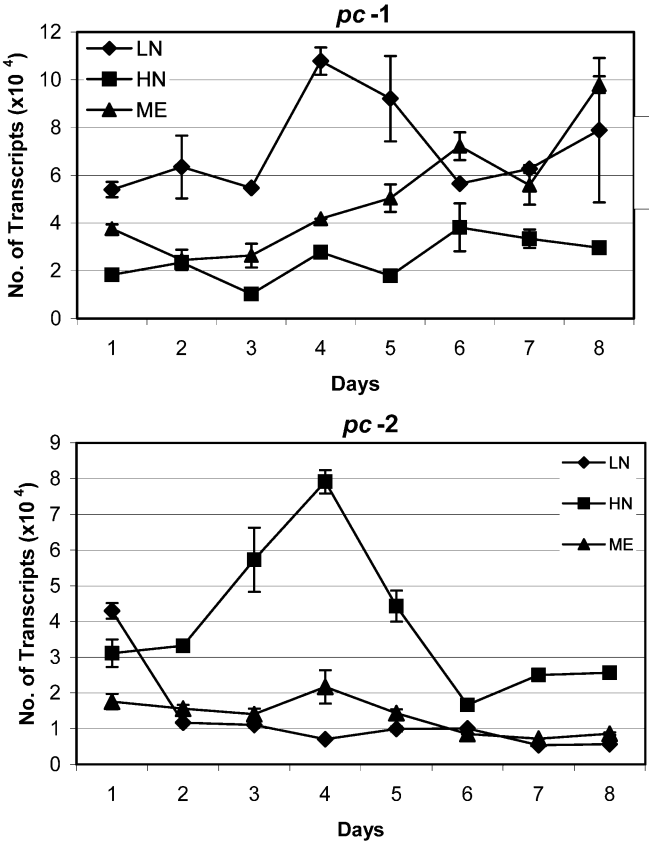

Fig. 1 Time-course of transcription of *pc*-1 (CYP63A1) and *pc*-2 (CYP63A2) genes under varying nutrient conditions. Quantification was based on real-time quantitative RT-PCR using gene-specific primers (Table 1). Values plotted are means ± standard deviations of two replicates

panel).

pc-1 transcription was affected by the carbon source, as studied in 4-day-old LN cultures, while the transcript levels were comparable in the three simple (monosaccharide, disaccharide, trisaccharide) carbon sources, glucose, sucrose, raffinose) and expression was lowered in the polysaccharide substrates (two-fold in starch, four-fold in CMC; Fig. 2). The expression of *pc*-2 also varied with the carbon source used, albeit in a different manner. While the expression levels of *pc*-2 in LN cultures were comparable for glucose and starch, expression was higher for raffinose [2.44(±0.37)-fold], sucrose [2.02(±0.16)-fold], and CMC [1.64(±0.14)-fold], as compared with glucose (Fig. 2). In contrast, in 4-day-old HN cultures, *pc*-2 was differentially upregulated in glucose as compared with the other carbon sources (Fig. 2). The transcript numbers were 6- to 18-fold higher in glucose than in the other carbon sources tested; and transcript levels were comparable for sucrose, raffinose, CMC, and starch (Fig. 2).

The physical factors temperature and oxygenation differentially affected the transcription levels of *pc*-1 and *pc*-2. For *pc*-1, expression was 2.13(±0.04)-fold higher at 37°C versus the ambient temperature (22°C). While temperature did not affect *pc*-2 expression in LN cultures, its expression level in HN cultures was 7.51(±0.60)-fold

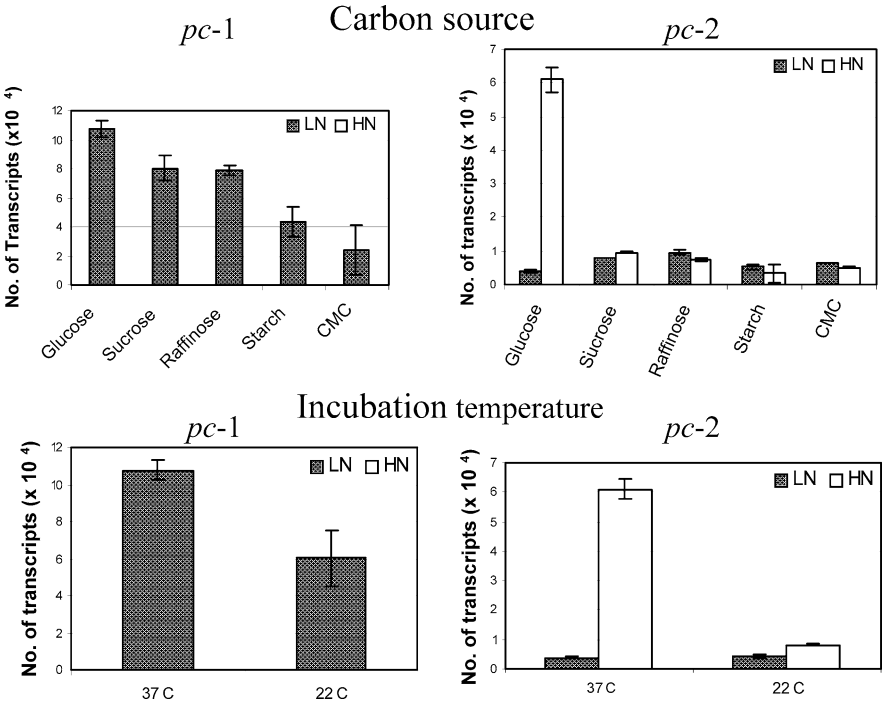

Fig. 2 Effect of carbon source and incubation temperature on *pc*-1 expression in LN cultures and *pc*-2 expression in LN and HN cultures. Values plotted are means ± standard deviations of two replicates. Transcript numbers (*y*-axis) were measured by real-time quantitative RT-PCR in 4-day-old cultures. **Top panel** *x*-axis: effect of different carbon sources (glucose, sucrose, raffinose, starch and CMC).**Bottom panel** *x*-axis: effect of incubation temperature (37°C vs 22°C)

higher at 37°C as compared to 22°C (Fig. 2). Transcript levels, in general, were higher in oxygenated cultures than in non-oxygenated cultures for both *pc*-1 and *pc*-2 (data not shown). The oxygenation effect was more pronounced for *pc*-2 than *pc*-1. While *pc*-1 transcript levels were 3.67 (±0.10)-fold higher in LN cultures flushed with oxygen, *pc*-2 transcript levels were elevated 17.69(±2.80)-fold under these conditions and 25.27(±2.35)-fold in HN cultures.

Xenobiotic induction of transcription of pc-1 and pc-2
Transcription of *pc*-1 was induced 2.12(±0.40)-fold to 6.27(±0.48)-fold over the control by 19 different chemicals, including both aliphatics (*n*-alkanes, fatty acids) and aromatics (monoaromatics, polyaromatics; Table 2). Induction over five-fold ($P \leq 0.05$) occurred in the presence of *m*-hydroxy benzoic acid (6.24±0.24), dodecyl benzene sulfonic acid (6.18±1.14), dodecylaldehyde (5.11±0.16), and estradiol (4.97±0.65), in that order. In contrast, induction of *pc*-2 was overall higher [2.0(±0.73)- to 29.39(±9.4)-fold] in the presence of 22 of the tested compounds, including both aliphatics and aromatics (Table 2). Over five-fold ($P \leq 0.05$) induction of *pc*-2 occurred in the presence of 1-dodecene (29.39±9.4), decane (26.72±4.4), nonylphenol (23.63±7.5), dodecanol (12.86±4.1), linoleic acid (10.21±4.2), phenyldodecane (7.02±0.41), benzanthracene (6.08±1.5), naphthalene (6.02±1.39), and benzo(a)pyrene (5.54±2.75), in that order.

Discussion

Our initial studies showed that the *pc*-1 gene is upregulated under nitrogen-limited conditions (Yadav et al. 2003). Here, we report the results in undefined nutrient-rich ME medium and further information for defined LN and HN media, using inocula grown in the respective media. Time-course expression of *pc*-1 under both nutrient conditions showed the responsiveness of this gene to nutrient-depletion. This effect is more pronounced in LN cultures, which showed a surge on day 4. However, the increased expression was not sustained beyond day 4 in LN cultures, unlike in HN and ME cultures. It is possible that transcripts are degraded in older LN cultures due to the onset of secondary metabolism that triggers the release of non-specific ribonucleases and proteases in fungal organisms, such as that reported for *Schizosaccharomyces pombe* (Nakashima et al. 2002) and *P. chrysosporium* (Dosoretz et al. 1990). However, further functional studies need to be done to support this hypothesis.

In contrast, the *pc*-2 gene showed a divergent pattern of expression under nutrient-limited and nutrient-rich conditions. This gene was expressed at a relatively higher level in HN than in LN cultures, showing its upregulation under high-nitrogen conditions. However, ME cultures (high-nitrogen, high-carbon) showed a relatively lower expression, indicating upregulation of this gene in presence of low carbon and high nitrogen (inorganic) as present in HN medium rather than in high carbon and high nitrogen (organic) as present in ME medium. The expression of both *pc*-1 and *pc*-2 is not tightly regulated by nitrogen or carbon levels, unlike that observed for ligninolytic peroxidases in this organism (Gold and Alic 1993; Orth and Tien 1995; Cullen 1997). Non-coordinate expression of the two tandem genes *pc*-1 and *pc*-2 indicates the involvement of different promoters and/or regulatory elements, as predicted in our earlier study (Yadav et al. 2003).

Table 2 Xenobiotic induction of the P450 genes *pc*-1 and *pc*-2 in *P. chrysosporium*, as measured by real-time quantitative RT-PCR. Values given are means ± standard deviations of two replicates. Cultures were grown in ME medium as described in the Materials and methods. Chemicals originally in liquid form (*liq.*) were used at 1%. All other chemicals (originally in solid form) were used at 20 mg l^{-1}. These concentrations were non-inhibitory to the fungus

Xenobiotic	Concentration (mM)	*pc*-1		*pc*-2	
		Transcript number ($\times 10^4$)	*x*-Fold change over control	Transcript number ($\times 10^4$)	*x*-Fold change over control
DMSO		2.00±0.28		2.07±0.86	
H_2O		2.36±0.63		1.19±0.2	
Aliphatics					
n-Tetradecane*liq.*	50.51	1.77±0.03	0.78±0.2	3.42±0.45	2.94±0.86
n-Hexadecane*liq.*	44.16	6.59±0.7	2.94±1.08	2.47±0.25	2.11±0.56
n-Decane*liq.*	70.28	3.25±0.25	1.41±0.27	31.48±0.32	26.72±4.4
n-Dodecane*liq.*	58.71	3.99±0.45	1.78±0.66	1.90±0.01	1.60±0.29
1-Dodecene*liq.*	59.42	5.63±0.82	2.53±1.02	34.15±5.45	29.39±9.4
1-Tetradecanol	0.09	1.74±0.1	0.88±0.17	3.84±0.22	2.00±0.73
1-Dodecanol	0.11	2.01±0.59	1.03±0.44	24.85±2.56	12.86±4.1
Dodecylaldehyde	0.11	10.21±1.1	5.11±0.16	0.76±0.12	0.40±0.16
Linoleic acid*liq.*	35.65	1.33±0.87	0.63±0.54	11.77±3.01	10.21±4.2
Levulinic acid*liq.*	86.12	1.30±0.22	0.56±0.06	0.31±0.03	0.26±0.01
1,2-Hydroxy-dodecanoic acid	0.09	4.50±0.59	2.25±0.02	2.68±1.21	1.55±1.23
1,2-Dodecane dicarboxylic acid	0.08	2.53±0.13	1.27±0.11	3.34±0.1	1.76±0.69
Aromatics					
Benzoate	0.14	2.69±0.01	1.36±0.19	1.27±0.29	0.64±0.13
m-Hydroxybenzoic acid	0.14	12.46±1.27	6.24±0.24	3.39±1.53	1.62±0.06
Resorcinol	0.18	4.18±0.2	2.12±0.4	1.86±0.53	1.04±0.69
Nitrophenol	0.14	6.27±0.34	3.17±0.61	1.81±0.07	0.96±0.4
Phenoxy acetic acid	0.13	5.68±0.61	2.84±0.09	1.70±0.38	0.86±0.17
Polyaromatics					
Naphthalene	0.16	8.73±1.39	4.35±0.09	11.87±2.32	6.02±1.39
Phenanthrene	0.06	5.64±0.55	2.82±0.12	4.17±0.59	2.14±0.61
Pyrene	0.10	7.85±0.9	3.93±0.01	2.05±0.62	1.02±0.12
Benzo(a)pyrene	0.08	3.10±0.46	1.55±0.01	10.29±0.9	5.54±2.75
1,2-Benzanthracene	0.09	3.34±0.42	1.67±0.03	11.93±2.09	6.08±1.5
3-Methyl cholanthrene	0.10	12.49±0.8	6.27±0.48	2.06±0.22	1.12±0.57
1-Naphthol	0.08	3.61±0.34	1.81±0.08	9.32±1.4	4.77±1.31
p-p'-Biphenol	0.11	4.63±0.03	2.33±0.31	6.48±0.69	3.5±1.79
DDT	0.06	2.75±0.25	1.38±0.07	8.19±0.56	4.31±1.72
PCB (Aroclor 1254)	0.09	5.21±0.86	2.24±0.23	2.45±0.39	1.34±0.75
Alkyl-substituted aromatics					
Dodecyl benzene sulfonate	0.06	12.21±0.55	6.18±1.14	6.05±1.17	3.33±1.96
1-Phenyl dodecane	40.58	14.00±0.7	6.12±1.33	8.34±0.89	7.02±0.41
Octylphenol	0.10	1.29±0.97	0.62±0.58	7.74±0.63	4.16±2.04
Nonylphenol*liq.*	45.45	1.45±0.9	0.69±0.56	27.46±4.3	23.63±7.5
Cumene*liq.*	83.20	0.75±0.68	0.37±0.39	2.20±0.05	1.87±0.35
Limonene*liq.*	73.40	5.11±1.05	2.31±1.06	2.96±0.7	2.57±1.01
Eugenol*liq.*	60.90	1.29±0.3	0.55±0.02	4.73±0.38	3.99±0.35
Cinnamaldehyde*liq.*	75.67	0.66±0.32	0.34±0.21	0.92±0.24	0.76±0.08
Alicyclics					
Cyclohexanone*liq.*	101.90	0.50±0.57	0.27±0.32	0.29±0.122	0.24±0.06
P450 inducers					
Phenobarbital	0.08	1.23±0.64	0.64±0.41	3.69±0.31	1.99±0.98
Estradiol	0.07	10.04±2.69	4.97±0.65	3.81±0.68	2.09±1.2
Lignin derivatives					
Lignin alkali		2.38±0.22	1.21±0.28	1.30±0.31	0.72±0.45

Table 2 (continued)

Xenobiotic	Concentration (mM)	*pc*-1		*pc*-2	
		Transcript number ($\times 10^4$)	*x*-Fold change over control	Transcript number ($\times 10^4$)	*x*-Fold change over control
Lignin alkali carboxylated		1.73±0.67	0.89±0.46	1.75±0.99	1.03±0.91
Lignin alkali, 2-hydroxy propyl ether		1.98±0.88	1.03±0.58	1.53±0.29	0.78±0.18
Lignosulfonic acid		8.61±1.35	4.39±1.29	8.44±0.84	4.56±2.31

The type of carbon source affected *pc*-1 and *pc*-2 expression. Interestingly, expression of both *pc*-1 and *pc*-2 under the respective optimal nitrogen conditions was higher on glucose than on disaccharide, trisaccharide, or polysaccharide. This indicates a preference for a readily available carbon source for expression of *pc*-1 and *pc*-2. A higher transcript number for *pc*-1 and *pc*-2 at 37°C versus 22°C is consistent with the optimal growth temperature (ca.37°C) of this organism (Keyser et al. 1978). The effect of oxygenation was more pronounced for *pc*-2 transcription (18- to 25-fold) than for *pc*-1 transcription (four-fold), indicating a differential regulation of the two genes by oxygen.

Xenobiotic induction was studied under non-ligninolytic nutrient-rich culture conditions using ME medium because P450 catalysis of xenobiotics in this organism has been reported under these culture conditions (Sutherland et al. 1991). While *pc*-1 was highly induced by readily biodegradable monocyclic aromatics (alkyl-substituted aromatics, hydroxylated aromatics) and lower molecular weight PAHs (2–4 rings), *pc*-2 was induced by environmentally recalcitrant chemicals, such as high molecular weight PAHs (4–5 rings), DDT, and long-chain alkylphenols (octyl-, nonyl-phenols; Table 2). Interestingly, the majority of these environmentally significant inducer chemicals are known to be degradable by *P. chrysosporium* in laboratory studies (Paszczynski and Crawford 1995; Reddy 1995).

The induction of *pc*-1 by alkyl-substituted aromatics in nutrient-rich ME medium is consistent with the ability of the whole fungus to generate P450-type reaction products from dodecyl benzene sulfonate (also called linear alkyl benzene sulfonate) as reported by Yadav et al. (2001). The responsiveness of *pc*-1 and *pc*-2 to chemicals with long alkyl side-chains is consistent with the presence of the conserved motif (RDTTAG) in the I-helix of these genes, as seen in the alkane-hydroxylating P450s (Yadav et al. 2003). However, the substrate responsiveness of *pc*-1 and *pc*-2 extended beyond chemicals with alkane substructures.

Notable examples of fungal P450 gene families with substrate-inducibility are CYP52 in *n*-alkane and fatty acid hydroxylation from species of the yeasts *Candida* (Ohkuma et al. 1995), *Debaryomyces* (Yadav and Loper 1999), and *Yarrowia* (Lida et al. 2000), CYP53 in benzoate *p*-hydroxylation from *Aspergillus niger* (Van Gorcom et al. 1990) and *Rhodotorula minuta* (Fukuda et al. 1994), and CYP57 from *Nectria haematococca* in detoxification of pisatin (Maloney and VanEtten 1994). This study identifies a new group of fungal P450s responsive to more complex pollutants, including long-chain alkyl substituted aromatics (alkyl benzenes,alkyl phenols) and polyaromatics (PAHs, DDT, biphenol). Our results are particularly significant in light of the fact that past efforts over the years to isolate PAH-oxidizing P450 from pollutant-oxidizing fungi including white-rot fungi have eluded researchers worldwide (Van den Brink et al. 1998).

Identification of the inducing chemicals for *pc*-1 and *pc*-2 suggests possible substrates for these P450s. Future efforts will include functional expression of these genes and investigation of their substrate-specificity for pollutant degradation. Our ongoing efforts include heterologous expression of these white-rot fungal P450s in microbial hosts for understanding their catalytic role in pollutant metabolic pathways and bioremediation.

Acknowledgement This work was supported by the NIH's National Institute of Environmental Health Sciences grant R01-ES10210 (to J.S.Y.).

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