



Genome-to-function characterization of novel fungal P450 monooxygenases oxidizing polycyclic aromatic hydrocarbons (PAHs)

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ABSTRACT

Fungi, particularly the white rot basidiomycetes, have an extraordinary capability to degrade and/or mineralize (to CO₂) the recalcitrant fused-ring high molecular weight (≥ 4 aromatic-rings) polycyclic aromatic hydrocarbons (HMW PAHs). Despite over 30 years of research demonstrating involvement of P450 monooxygenation reactions in fungal metabolism of HMW PAHs, specific P450 monooxygenases responsible for oxidation of these compounds are not yet known. Here we report the first comprehensive identification and functional characterization of P450 monooxygenases capable of oxidizing different ring-size PAHs in the model white rot fungus *Phanerochaete chrysosporium* using a successful genome-to-function strategy. In a genome-wide P450 microarray screen, we identified six PAH-responsive P450 genes (*Pc-pah1*–*Pc-pah6*) inducible by PAHs of varying ring size, namely naphthalene, phenanthrene, pyrene, and benzo(a)pyrene (BaP). Using a co-expression strategy, cDNAs of the six *Pc-pah* P450s were cloned and expressed in *Pichia pastoris* in conjunction with the homologous P450 oxidoreductase (*Pc-POR*). Each of the six recombinant P450 monooxygenases showed PAH-oxidizing activity albeit with varying substrate specificity towards PAHs (3–5 rings). All six P450s oxidized pyrene (4-ring) into two monohydroxylated products. *Pc-pah1* and *Pc-pah3* oxidized BaP (5-ring) to 3-hydroxyBaP whereas *Pc-pah4* and *Pc-pah6* oxidized phenanthrene (3-ring) to 3-, 4-, and 9-phenanthrol. These PAH-oxidizing P450s (493–547 aa) are structurally diverse and novel considering their low overall homology (12–23%) to mammalian counterparts. To our knowledge, this is the first report on specific fungal P450 monooxygenases with catalytic activity toward environmentally persistent and highly toxic HMW PAHs.

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1. Introduction

Fused-ring polycyclic aromatic hydrocarbons (PAHs) constitute an important group of highly toxic and significant environmental chemicals that are generated from different anthropogenic and industrial processes and accidents such as crude oil spills [1]. The persistence and genotoxicity of PAHs increase with increasing aromatic rings. Consequently, high molecular weight (HMW) PAHs (≥ 4 benzene rings) are particularly a significant problem both from the point of view of environmental clean up as well as human health as these are recalcitrant to biodegradation [2] and are muta-

genic and/or carcinogenic to living systems [3]. Among the microorganisms (bacteria, yeasts, and filamentous fungi) investigated for their ability to break down these hazardous chemicals [1,4,5], only a few species belonging to actinomycetes and fungi have the ability to oxidize high molecular weight (HMW) PAHs. In particular, white rot group of basidiomycete fungi have shown an extraordinary capability to completely degrade (to CO₂) both low molecular weight (LMW) and HMW PAH compounds [5–7].

Phanerochaete chrysosporium (henceforth abbreviated as *PC*) is the most intensively studied model white rot fungus for mechanisms to degrade lignin and a wide range of xenobiotics including PAHs [6]. Originally, its aromatic ring-oxidizing activity was ascribed to the non-specific extracellular peroxidases [8,9] that are differentially expressed under nutrient-limited (ligninolytic) culture conditions [10]. Subsequent studies by us and others have led to increasing evidences on peroxidase-independent degradation of several aromatics including PAHs under nutrient-sufficient (non-ligninolytic) culture conditions and involvement of P450 monooxygenation reactions [11–17]. Role of P450 monooxygenation in the rate-limiting initial oxidation of HMW PAHs and

Abbreviations: PAH, polycyclic aromatic hydrocarbon; CYP, cytochrome P450; POR, cytochrome P450 oxidoreductase; PC, *Phanerochaete chrysosporium*; PP, *Pichia pastoris*.

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certain LMW PAHs with high ionization potential (>7.35 eV) such as phenanthrene has also been reported in other basidiomycete and non-basidiomycete fungi [4,18]. However, despite the past over 30 years of research on PAH biodegradative fungal species [1,4,5], the specific P450 monooxygenase enzymes responsible for this oxidation activity have not been reported. Collectively considering the above discussion, there is a critical need for identification of specific fungal P450 genes and enzymes responsible for the oxidation of HMW and/or both LMW and HMW PAH compounds, in order to understand the P450-mediated mechanisms and to facilitate development of improved biotransformation processes and applications.

Cytochrome P450 monooxygenases are a superfamily of heme-thiolate proteins that catalyze a broad range of reactions such as carbon hydroxylation, heteroatom oxygenation, dealkylation, epoxidation, reduction, dehalogenation [19]. The typical eukaryotic P450 monooxygenase system contains a P450 monooxygenase and a P450 oxidoreductase (POR), both of which are normally membrane-associated. This family of proteins has seen a tremendous growth in the genomic era [20], <http://drnelson.uthsc.edu/CytochromeP450.html>. However, post-genomic functional characterization of orphan P450s in fungal and other genomes has been a challenging task due to their poor amenability to heterologous expression in simpler hosts. Whole genome sequencing of *PC* [21] has uncovered the presence of a large P450 diversity, comprised of about 150 P450 genes [22]. While functional genomic studies on these P450s are emerging [16,23,24], majority remain orphan with virtually unknown function. Hence, there is a great need to characterize the role of individual P450s in this model organism. Our recent studies using the first custom-designed genome-wide P450 microarray [25,26], have led to a working hypothesis that substrate specific inducibility could be key to identifying the xenobiotic substrates for orphan P450 enzymes in this organism [16,23,25]. In this study, we therefore employed a two-stage genome-to-function strategy, including a genome-wide (microarray-based) transcriptional induction profiling to identify candidate P450 genes responsive to PAHs of varying ring-size followed by co-expression and catalytic characterization of the identified recombinantly expressed P450 proteins. These efforts led to identification of a set of six PAH-responsive P450 monooxygenase genes in the *PC* genome (designated *Pc-Pah1* through *Pc-Pah6*). Subsequent co-expression along with the reductase partner and catalytic analysis revealed their diverse PAH substrate specificity particularly towards HMW (4–5 ring) PAHs. Part of the data reported in this manuscript was presented at the 104th annual general meeting of the American Society for Microbiology, New Orleans, LA [27].

2. Materials and methods

Microbial strains, chemicals, media, and culture conditions are detailed under [Supplementary information](#). P450 inhibitor studies and induction of P450s in *PC* were performed as described in our re-

cent study [16]. Preparation of fungal microsomes, CO-difference spectrum analysis, and calculation of total P450 concentration were performed using established methods [28,29]. Differential gene expression profiling was performed using a genome-wide custom-designed 70 mer oligos-based P450 microarray developed in our previous studies [25,26]. Full-length cDNAs for the identified PAH-inducible P450 genes (designated *Pc-Pah1* through *Pc-Pah6*) were cloned using gene-specific RT-PCR as described previously [26]. cDNA sequences are available in the GenBank with accession numbers listed in [Table 1](#). Heterologous co-expression of the *Pc-Pah* genes along with the homologous *Pc-POR* [30] in the yeast *P. pastoris* (PP) was performed using the pPICZB expression vector system (Invitrogen). The experimental strategy used for creating the binary constructs is depicted in [Fig. S1](#). Expression analysis and PAH compound oxidation in yeast whole cell assays were performed as detailed in [Supplementary information](#). P450 hydroxylated metabolites of the PAH compounds were analyzed by HPLC followed by liquid chromatography-electrospray mass spectrometry (LC-ESI/MS) (Thermo Finnigan) as described in [Supplementary information](#).

3. Results and discussion

3.1. Involvement of P450 enzyme system in biodegradation of HMW PAH compounds in *PC*

Addition of the P450 inhibitor piperonyl butoxide (PB) in the nutrient-rich malt extract (ME) cultures of *PC* led to a significant abrogation of the degradation activity towards pyrene and benzo(a)pyrene ([Fig. 1](#)). This suggested a key role of P450 monooxygenase(s) in initial oxidation of these HMW PAH compounds in *PC*, analogous to that reported for the lower PAH phenanthrene [11]. Comparison between the chemically-killed (CK) control cultures and uninoculated control revealed that unextractable mycelial adsorption partially contributed toward the apparent total disappearance in case of pyrene (~16.6%) but not in benzo(a)pyrene (BaP) degradation. The PB attenuation effect on biodegradation was relatively higher for BaP ([Fig. 1](#)), implying the involvement of divergent sets of P450s for the two PAHs. Role of P450s in PAH oxidation was further evidenced by our initial P450 enzyme induction studies on the whole fungus grown under similar conditions. Results showed about two fold induction of total P450 enzyme content (based on typical CO-difference spectrum) in response to 10 ppm pyrene ([Table S2](#)). Considering this, we performed a genome-wide induction profiling for identification of specific PAH-inducible P450 genes as part of our genome-to-function strategy to characterize those catalyzing PAH oxidation.

3.2. Microarray-based identification of PAH-responsive P450 monooxygenases (*Pc-Pah* P450s)

Genome-wide P450 transcriptional profiling using the P450ome microarray first developed in our previous studies [25,26] showed

Table 1
Multiple PAH-responsive P450 genes identified using genome-wide P450 microarray analysis on *P. chrysosporium*.^a

P450 ID	CYP name	NCBI Gene Accession number	Protein ID (JGI WGS Ver. 2)	Transcriptional induction (fold-change) ^b			
				Naphthalene	Phenanthrene	Pyrene	Benzo(a)pyrene
Pc-pah1	CYP5136A2	AY515588	5852		7.8 ± 4.64	3.24 ± 1.95	
Pc-pah2	CYP5145A3	GU270838	6327	2.72 ± 0.04	3.28 ± 0.73	1.67 ± 0.65	1.71 ± 0.11
Pc-pah3	CYP5144A7	AY515589	133311	7.34 ± 0.01	2.87 ± 1.24		15.12 ± 7.19
Pc-pah4	CYP5136A3	GU270839	5001		9.52 ± 2.38	1.32 ± 0.08	
Pc-pah5	CYP5142A3	AY515590	28946	7.37 ± 0.26			
Pc-pah6	CYP5144A5	AY515591	132481	4.43 ± 0.6			

^a PAH treatments and transcriptional profiling were performed using nutrient-rich (malt extract) cultures as described under (Section 2).

^b The fold-change values are means ± standard errors obtained by evaluating eight independent spots for each gene and are statistically significant ($P \leq 0.05$ and FDR of 0.1). Abbreviations: CYP, Cytochrome P450; JGI, Joint Genome Institute of the US Department of Energy; WGS, Whole genome sequence.

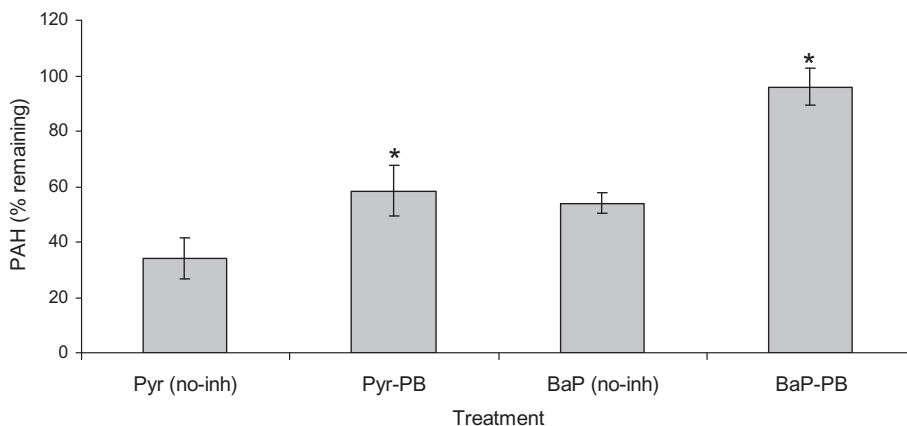


Fig. 1. Effect of P450 inhibitor piperonyl butoxide (PB) on biodegradation of pyrene and benzo(a)pyrene by *P. chrysosporium*. Nutrient-rich malt extract broth cultures and negative controls were prepared as described under (Section 2). Final concentrations of the test PAH and PB in the cultures were 20 ppm and 0.5 mM, respectively. Asterisk indicates statistically significant ($P \leq 0.05$) effect of the inhibitor. The plotted values represent means \pm standard deviations for three biological replicates. Abbreviations: Pyr, pyrene; BaP, benzo(a)pyrene; no-inh, no inhibitor.

significant induction (up to ~ 15 -fold) of multiple P450 genes in response to the four individual representative PAHs of increasing ring size (2–5 rings) in nutrient-rich ME cultures (Table 1 and Fig. S2). A total of six candidate PAH-responsive P450 genes (designated *Pc-Pah1* through *Pc-Pah6*) were selected, based on a cut off criterion of ≥ 3 -fold induction by at least one of the inducer PAHs. Subsequent qRT-PCR analysis on selected genes and treatments verified the microarray results. For instance, qRT-PCR based fold-change values for *Pc-Pah2* were 2.45 ± 0.03 (naphthalene), 4.21 ± 0.44 (phenanthrene), and 2.47 ± 0.11 (benzo(a)pyrene) as against 2.72 ± 0.04 , 3.28 ± 0.73 , and 1.71 ± 0.11 , respectively in microarray analysis. The house-keeping gene GAPDH remained unchanged. Of the six PAH-responsive genes, a set of four genes each was inducible by naphthalene (*Pc-Pah2*, *Pc-Pah3*, *Pc-Pah5* and *Pc-Pah6*) and phenanthrene (*Pc-Pah1* through 4) whereas a set of two genes each was inducible by pyrene (*Pc-Pah1* and *Pc-Pah4*), and BaP (*Pc-Pah2* and *Pc-Pah3*) (Table 1). The two pyrene-inducible genes (*Pc-Pah1* and *Pc-Pah4*) were also inducible in the presence of the lower PAH phenanthrene. The two BaP-inducible genes (*Pc-Pah2* and *Pc-Pah3*) were also inducible by the lower PAHs naphthalene and phenanthrene. Taken together, a *Pc-Pah* gene inducible by the higher PAH (4- or 5- ring) was also inducible by a lower PAH (2- and/or 3- ring).

Interestingly, five of the six PAH-responsive P450 genes identified here also showed upregulation under defined high nitrogen (HN) culture conditions in our earlier study [26], further confirming their optimal expressibility under non-ligninolytic conditions. We also observed inducibility of two of the genes (*Pc-Pah4* and *Pc-Pah5*) in response to alkylphenols [16], indicating their broader substrate range against environmental xenobiotics.

3.3. Recombinant co-expression of *Pc-Pah* P450s and *Pc-POR* in *Pichia pastoris*

Bioinformatic analyses on the six *Pc-Pah* genes and their corresponding cDNAs, cloned and sequenced in this study for recombinant expression, revealed variable structural features such as 8 to 12 introns with variable distribution (Fig. S3) and genome-wide localization. Interestingly, two of the genes *Pc-Pah3* and *Pc-Pah6*, are part of a tandem gene cluster of 12 P450 members in the *PC* genome. *Pc-Pah5* is also on the same genome scaffold and is separated from the upstream 4 tandem P450s cluster by the intervening 3 non-P450 genes.

The co-expression strategy enabled a successful active expression of the individual *Pc-Pah* P450 proteins. This strategy assumes

significance considering the expression difficulties encountered in our previous efforts on independent expression of *Pc-Pah* P450s without the homologous *POR*. SDS-PAGE analysis of the six microsomal preparations showed a clear band coinciding with *Pc-POR* when compared to the control (Fig. 2A). Western blot analysis using anti-His antibody showed full-length expression of all six *Pc-Pah* P450 proteins (Fig. 2B). Active nature of the expressed recombinant P450 proteins was evident from their characteristic CO-difference spectra (Fig. 2C). The observed maximum absorption peaks were at 448 nm (*Pc-Pah4*), 450 nm (*Pc-Pah1* and *Pc-Pah3*), 451 nm (*Pc-Pah2*), and 452 nm (*Pc-Pah5* and *Pc-Pah6*). P450 and *POR* expression levels varied for the six *Pc-Pah* clones (Fig. 2D), with *Pc-Pah5* and 6 showing the highest P450 concentration. *Pc-Pah6* had the highest co-expressing *POR* activity, an observation consistent with the corresponding band intensity in the SDS-PAGE gel (Fig. 2A).

3.4. Catalytic analysis of the recombinant fungal P450 enzymes

3.4.1. Substrate specificity

Whole-cell yeast biocatalysts are considered ideal for screening of hydrophobic compounds such as PAHs as the use of cells allows ready uptake as well as alleviates the need for isolation of microsomes and *in vitro* reconstitution of the P450 assay [31]. In whole cell assays, the six recombinant *Pc-Pah* enzymes showed catalytic activity toward PAHs, albeit with varying activity and substrate specificity (Fig. 3). No activity was observed in the empty vector control yeast. While all six *Pc-Pah* P450s showed pyrene oxidation, quantitative differences in the activity were observed among the individual enzymes; *Pc-Pah5* and *Pc-Pah4* showed relatively higher pyrene oxidation activity as compared to the other four *Pc-Pah* P450s. In comparison, a tighter substrate specificity was observed for phenanthrene as only *Pc-Pah4* ($\sim 65\%$) and *Pc-Pah6* ($\sim 14\%$) could oxidize this compound (Fig. 3). This is significant considering that phenanthrene is not a substrate for lignin peroxidases because of its high ionization potential (>7.35 eV). Anthracene, another 3-ring PAH, was, however, not detectably oxidized by any of the six *Pc-Pah* P450s in this study. Only *Pc-Pah1* and *Pc-Pah3* showed activity towards BaP, with the former showing relatively higher levels of oxidation (32.6% vs. 14.3%). The co-expressed *POR* levels (Fig. 2D) did not seem to quantitatively correlate either with the relative P450 expression levels or with the PAH oxidation activity (Fig. 3) of the individual *Pc-Pah* P450s. Common specificity of *Pc-Pah4* and *Pc-Pah6* towards phenanthrene, albeit to a varying extent, may be because of their evolution from a common progenitor

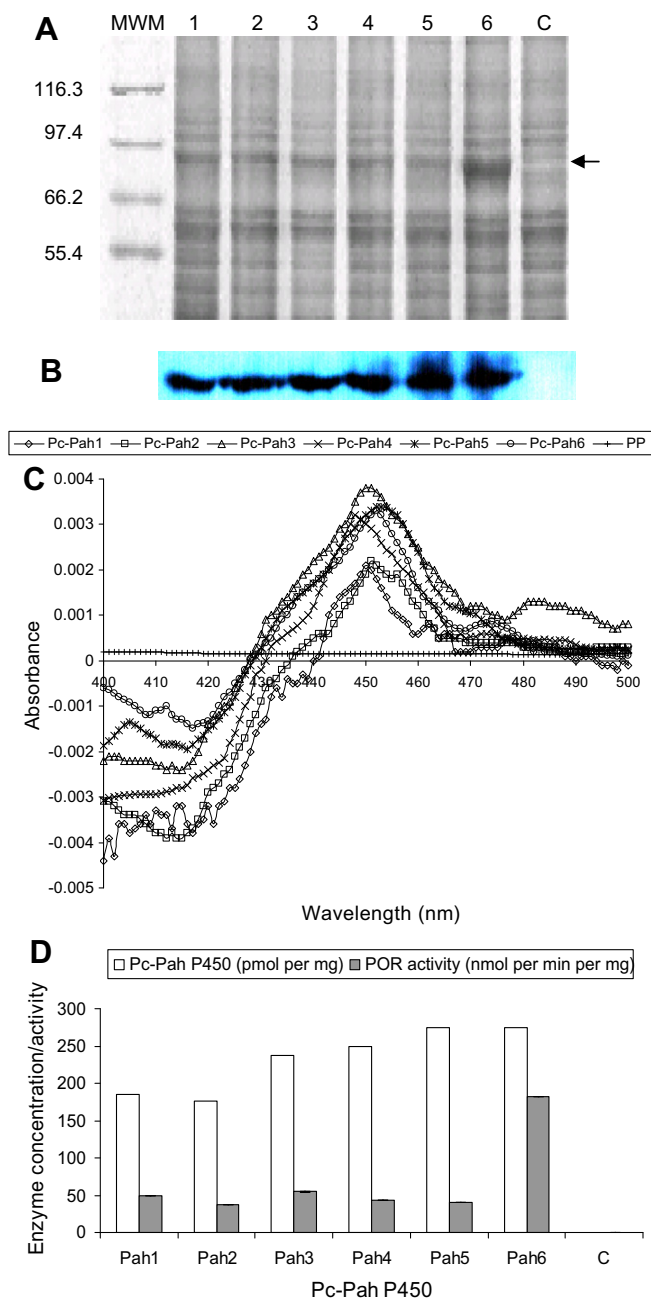


Fig. 2. Co-expression and activity characterization of Pc-Pah P450 monooxygenases and the homologous P450 reductase partner (Pc-POR) in *P. pastoris*. The expressed enzymes were assessed in the yeast microsomes prepared as described under (Section 2) (A) SDS-PAGE analysis of the microsomal protein preparations from recombinant P450-POR yeast clones Pc-Pah1 through Pc-Pah6 (lanes 1–6) and negative control yeast (lane c). Proteins were separated on 7% SDS-PAGE and detected by staining with SYPRO Ruby (BioRad). The protein band coinciding with Pc-POR (estimated 81.6 kDa) is shown by an arrow. (B) Western blot analysis of Pc-Pah proteins using anti-his antibody. (C) Characteristic CO-difference spectra for the individual Pc-Pah proteins (Pc-Pah 1 through 6) and negative control. (D) P450 enzyme concentrations and POR activity levels of the individual Pc-Pah clones versus the negative control (C). P450 concentration values are based on the CO-difference spectra shown in panel C. POR activity values are the means for three biological replicates (the SD values varied between 0.07 and 0.23).

gene as has been hypothesized for members of the same P450 genomic cluster in our previous genome-wide bioinformatic study on P450ome of this organism [22].

The 450 inhibitor PB almost completely abrogated the observed PAH-oxidizing activity of all six Pc-Pah enzymes in the recombi-

nant yeast cultures (Table S3) confirming their role in the observed PAH catalysis. PB did not exert any growth inhibition, confirming the enzymatic basis of the observed activity inhibition. Comparison of the P450 inhibitor effect on the whole fungus versus the recombinant Pc-Pah P450 yeast clones showed complete inhibition of BaP-oxidizing P450 activity in both the biological systems. In contrast, only partial inhibition of activity was observed for pyrene in the whole fungus (unlike the yeast clones), indicating the likely presence of additional pyrene-oxidizing P450s (non-responsive to PB) in the PC genome. This is not unexpected considering the observed general amenability of pyrene to oxidation by all six P450s in this study.

3.4.2. Catalytic specificity

Initial HPLC screening on the PAH oxidation reaction extracts for the individual reactive P450s showed variable number of metabolite peaks for individual PAHs. No such peaks were observed in the empty vector control yeast. Subsequent LC-ESI/MS analysis (Fig. S4) confirmed the observed HPLC patterns of the metabolite peaks and allowed identification of the oxidation metabolites by accurate mass measurement on the whole reaction extracts using authentic standards (Table S4). Phenanthrene reaction extracts for the Pc-Pah4 and Pc-Pah6 clones showed three peaks (I, II, III) with same measured monoisotopic mass (m/z) but different retention time (R_t). The R_t values of the three peaks exactly matched with those of the authentic standards (Table S4). Based on the R_t and m/z comparisons, the peaks were thus identified as 3-phenanthrol (peak I), 4-phenanthrol (peak III), and 9-phenanthrol (peak II). Among the three metabolites, 9-phenanthrol was the most abundant followed by 4- and 3-phenanthrols, respectively. In contrast, pyrene reaction extracts for each of the 6 Pc-Pah P450s showed two peaks exhibiting the same m/z but different R_t . The second peak (II) showed R_t coinciding with that of the authentic standard 1-hydroxypyrene. In contrast with phenanthrene and pyrene, a single peak was observed in the benzo(a)pyrene reaction extracts from Pc-Pah1 and Pc-Pah3. As the R_t and m/z values of the peak were the same as those of the authentic standard, this peak was identified as 3-hydroxybenzo(a)pyrene (Table S4).

Taken together, catalytic activity of the Pc-Pah P450s towards varying ring-size PAHs revealed their functional diversity albeit with some redundancy. While all Pc-Pah P450s catalyzed the oxidation of pyrene, specificity was observed in respect of phenanthrene and BaP (Fig. 4). It is interesting that the multifunctional Pc-Pah P450s (those catalyzing oxidation of more than one PAH) showed a pattern of ring-size specificity. For instance, the P450s showed activity either with phenanthrene and pyrene (3 and 4 ring PAH) or pyrene and BaP (4 and 5 ring PAH) but none of them oxidized both phenanthrene and BaP (3 and 5 ring PAH) (Fig. 4). While the substrate responsiveness (induction) profiling strategy proved critical in successful identification of relevant PAH-oxidizing P450s (Table 1), the induction patterns did not show absolute correlation with the respective substrate specificities (Fig. 3).

Formation of oxidized products in the single P450 enzyme-based oxidation reactions confirmed that Pc-Pah P450s are involved in the initial oxidation step of the PAH biodegradation process. P450-mediated oxidation of the PAH ring in eukaryotes occurs via formation of an epoxide which in turn is converted into hydroxylated products by non-enzymatic isomerization and/or by the action of epoxide hydrolase [4]. The relative abundance of the three phenanthrols was consistent with the regioselectivity profile observed for the whole fungus [1]; 9-phenanthrol was found to be the most abundant whereas 3-phenanthrol the least. In this context, it is interesting that recent whole fungus-based studies using nutrient-limited (defined low nitrogen) cultures showed the formation of a single monohydroxylated product, 9-phenanthrol [17]. In previous PC studies [11], it was unclear whether a single

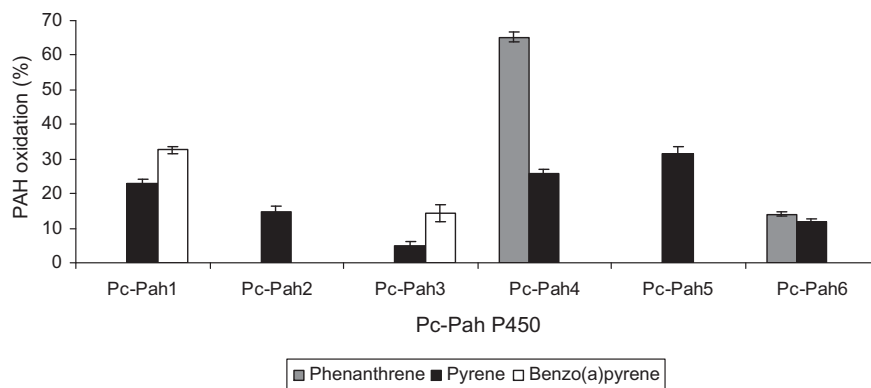


Fig. 3. Relative PAH-oxidizing activities of the six recombinant Pc-Pah P450 enzymes towards varying ring-size PAH compounds in whole cell assays. Each culture was spiked with a test PAH and the oxidation activity was assessed at 36 h of incubation. The values represent means \pm standard deviations for three biological replicates and are statistically significant ($P \leq 0.05$).

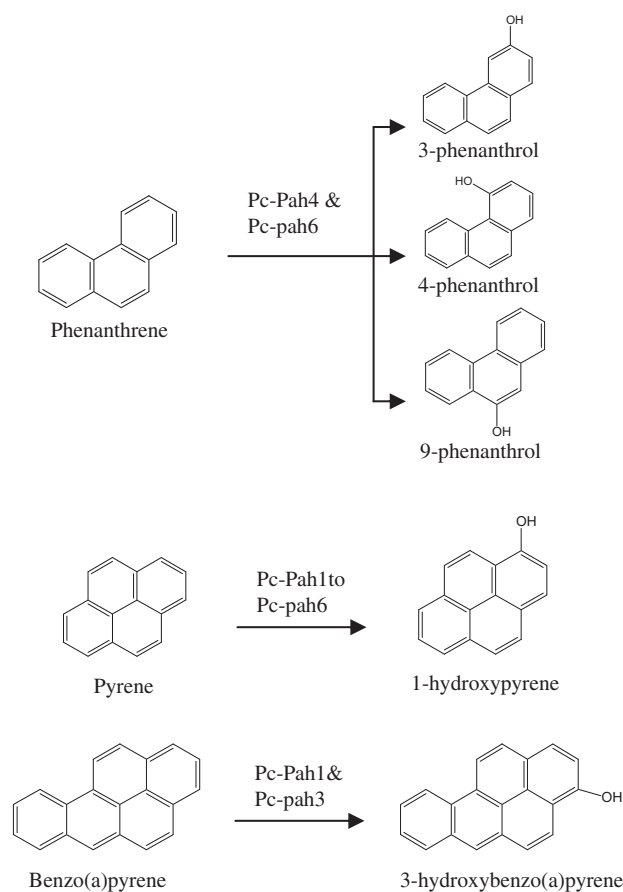


Fig. 4. PAH oxygenation reactions catalyzed by the individual recombinant Pc-Pah enzymes (Pc-Pah1 to Pc-Pah6) of *P. chrysosporium*.

P450 or multiple P450s are involved in the formation of multiple phenanthrols via formation of different epoxides (9, 10-oxide versus 3, 4-oxide). In this context, our results showed involvement of a single P450 in the formation of all three phenanthrols, suggesting that individual P450 (Pc-Pah4 or Pc-Pah6) can oxidize phenanthrene at both positions (9,10 or 3,4). Each of the six Pc-Pah P450s oxidized pyrene into two mono hydroxylated products, of which one was identified as 1-hydroxypyrene. BaP was oxidized to 3-hydroxybenzo(a)pyrene by Pc-Pah1 and Pc-Pah3. This is consistent with the whole fungus study on PC wherein

3-hydroxybenzo(a)pyrene was identified as the sole oxidation product [15].

Taken together, matching regiospecificity of the recombinant Pc-Pah P450s and the parent whole fungus (PC) towards oxidation of different PAHs and gene inducibility under *in vivo* conditions confirm the authenticity and physiological relevance of the identified P450s in PC. Considering that the white rot fungi possess a comprehensive enzymatic machinery to completely degrade (to CO₂) PAHs, the P450 monooxygenases may link up with the PAH mineralization pathway components under appropriate conditions; such common pathway components (non-ligninolytic and ligninolytic) for cycling of PAH metabolites have been indicated in previous studies [18,32]. Hence, the presence of P450s in white rot fungi assumes greater significance than in other biological systems that have a standalone P450 enzyme system for PAH metabolism such as in non-basidiomycete fungi [4] and animals [19]. Interestingly, there was a partial overlap in catalytic regiospecificity [33,34] between the fungal Pc-Pah P450 enzymes and mammalian PAH-oxidizing P450s (human/rat/mouse CYP1A1, 1A2, and 1B1), possibly because of low overall protein level homology (12–23%) (Table S5). This phenomenon holds promise for future basic structure-activity relationship studies to generate improved versatile biocatalysts for detoxification and biotransformation applications.

4. Conclusions

We report a successful genome-to-function approach to functionally characterize orphan P450s in the *P. chrysosporium* genome, a strategy that could be extendable to other genomes (prokaryotes and other eukaryotes such as fungi, insects, plants, and animals) with orphan P450s. To our knowledge, this report constitutes the first genome-wide comprehensive identification and functional characterization of fungal P450 monooxygenases catalyzing oxidation of varying ring-size PAH compounds, namely phenanthrene, pyrene and BaP. This study opens up avenues for investigating similar PAH-oxidizing P450 enzymes in other fungal systems and for exploiting these structurally and catalytically diverse P450 enzymes for future basic and applied research on P450-mediated mechanisms and processes in bioremediation and biotechnological applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbrc.2010.07.094](https://doi.org/10.1016/j.bbrc.2010.07.094).

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