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Gene Expression Profiling and Enzymatic Function of *Phanerochaete Chrysosporium* Cytochrome P450s Involved in the Metabolism of Benzo(a)pyrene

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Abstract The completed genome sequences and genomics databases currently available for plants, animals and fungi provide a scaffold for addressing the biological role of the impressive gene collection in these organisms. The white-rot fungus *Phanerochaete chrysosporium* encodes 149 cytochrome P450 (CYP) genes in its genome. The functions of many of these genes or their target substrates are still unknown. This study aimed at elucidating the functions of *P. chrysosporium*'s cytochrome P450 (PcCYP) gene repertoire using the polyclic aromatic hydrocarbon (PAH) benzo(a)pyrene (BaP) as a substrate. The set of complementary expression systems used in this study was pivotal in assigning function to the PcCYPs investigated. A cDNA microarray system targeting 133 *P. chrysosporium* P450 cDNAs as probes was used to investigate the differential expression patterns of PcCYPs gene diversity in response to this PAH. BaP was able to elicit a response of 12 cytochrome P450 genes strongly suggesting that it was a potential substrate of these PcCYPs. A functional analysis of the 12 PcCYP genes targeting the coding sequences of these *P. chrysosporium* P450 cDNAs as probes was conducted. These PcCYPs 1a, 5b, 24s, 30d, 59a, 59c and 66a were proved to be functional with a heterologous *Saccharomyces cerevisiae* expression system. These findings strongly suggest that species that gave a physiological response and metabolized the substrate are key to the metabolism of this PAH. This knowledge can be applied to make improved predictions on the cellular systems optimized for aromatic degradation by this fungus applicable in bioremediation.

Keywords: *phanerochaete chrysosporium, benzo(a)pyrene, cytochrome P450, differential expression, elucidation of function*

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that are widespread in various ecosystems and are pollutants of great concern due to their persistence and potential toxicity, mutagenicity and carcinogenicity [2]. Their persistence in the environment is due chiefly to molecular stability, hydrophobicity and low aqueous solubility ([4,7]). Benzo(a)pyrene (BaP) is a PAH with a five ring recalcitrant structure that has been found in almost all environmental media and food items ingested by humans and animals [27], and has been identified as one of the most important environmental contaminants because of its mutagenesis and carcinogenesis that is well recognized ([11,12]). It is therefore an important target PAH to study and devise efficient methods that may prove beneficial for controlling its concentration in the environment during bioremediation and for limiting exposure. Though methods such as volatilization, photo-oxidation, chemical oxidation and bioaccumulation [49] have been utilized for eliminating BaP from the environment, microbial degradation represents the major method used for the ecological recovery of PAH-contaminated sites. The biodegradation of PAHs by brown and white-rot basidiomycetes has been extensively studied. White-rot fungi such as *P. chrysosporium* ([9,17]), *Pleurotus ostreatus* [5] or *Trametes versicolor* [16] and a host of other species have the ability to degrade PAHs. There is substantial and conclusive evidence linking the participation of ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, and laccase in PAH mineralization by white-rot fungi ([6,7,16,25,26]). However, fungal metabolism of aromatic compounds under ligninolytic conditions has focused less attention on cytochrome P450 (CYP) involvement due to the overwhelming past interest on ligninolytic peroxidases.

The diverse cytochrome P450 (CYP) mixed function oxidase system plays a major role in the metabolism of important endogenous substrates as well as in the biotransformation of xenobiotics [50]. The extent of diversity has only recently become apparent with the emergence of data from whole genome sequencing projects ([19,42,43-51]). 149 cytochrome P450 genes that were found in the genome of white-rot fungus *P. chrysosporium* suggest their importance in fungal ligninolytic and aromatic metabolism systems. Its completed genome database provides a framework for addressing the biological role of the impressive gene collection among cytochrome P450s ([18,37]). A strategy for the high level screening of PcCYPs using the PAH anthracene with the goal of establishing function was established [15]. From the results of the study [15] it was suggested that cDNA microarray analysis complemented with the *S. cerevisiae* heterologous expression system can be a powerful tool to elucidate function and to point out PcCYP species substrate specificity. The data on specific CYP enzymes to PAH metabolism contribute to the understanding of PAH biotransformation in this fungus, which is important in attempting to understand the differences in sensitivity towards PAHs among fungi. Furthermore, with this knowledge, an improved prediction on the cellular systems optimized for aromatic degradation by this fungus applicable in bioremediation can be made.

Therefore, the main objective of this study is to identify cytochrome P450 monoxygenases from the fungus *P. chrysosporium* specific to BaP metabolism using cDNA microarray analysis complemented with the *S. cerevisiae* heterologous expression system.

2. Materials and Methods

2.1. Chemicals

Benzo(a)pyrene and piperonyl butoxide were purchased from Wako pure chemicals (Osaka, Japan). All solvents used in extraction and HPLC analytical procedures were of the purest grade available from suppliers. Water for HPLC and molecular biology procedures was purified using Millipore's MilliQ purification system (Millipore, Bedford, Mass).

2.2. Fungal Conversion of BaP by *P. chrysosporium*

P. chrysosporium (ATCC 34541) was grown from conidia inocula at 37℃ in a stationary culture under air (10 mL of medium in a 100-mL Erlenmeyer flask). The medium used in this study was as previously described with 1% glucose and 1.2 mM ammonium tartrate at pH 4.5 [31]. After a 3-day incubation, the substrate (in 2.5 mM acetone solution) was added to the cultures to a final concentration of 0.025 mM. After additional incubation, the metabolic products were analyzed either by HPLC after homogenization in acetone (the same amount as that of medium), centrifugation (1000 x g), and filtration (0.45 mm) or by GC-MS after extraction with ethyl acetate (10 mL x 3) at pH 2, drying over Na_2SO_4, and evaporation under N_2.

2.3. Effect of P450 Inhibitor

The effect of Piperonyl butoxide (PB) dissolved in acetone on PAH metabolism was assessed in incubations containing 0.025 mM of substrate. PB was added to the cultures 30 minutes prior to the addition of substrates to final concentrations of 0, 0.025, 0.25 and 2.5 mM. Quantitative analysis was then conducted as described for fungal conversions.

2.4. Metabolic Analysis

HPLC analysis was carried out using a Shimadzu STR ODS-II column with a linear gradient from 20% acetonitrile in water (0.05% phosphoric acid) (isocratic for 5 min) to 100% acetonitrile (21-30 min) at a flow rate of 1.0 mL/min. Detection was monitored at 254 nm. Products and substrates were quantified by calibration curves of standards. GCMS was performed at 70 eV on a *Shimadzu QP 5000* system equipped with Rtx-5MS (30 m x 0.25 mm I.D., 0.25 µm film thickness, RESTEK). The oven temperature was programmed from 80 to 300℃ at 8℃/min. Mass spectra were obtained with electron impact ionization at 70 eV with a split ratio of 25:1. The injection temperature was 280℃. Products were identified by a comparison of their retention times on HPLC and GC. Further identification of mass fragmentation patterns was through spectral matching of standards with the commercial NIST 2005 library database, and or independently by interpreting the fragmentation patterns.

2.5. Microarray Analysis

A set of cDNA clones containing full length gene sequences of 133 PcCYPs and five positive control genes (b-actin, ATPase, elongation factor g, enolase, glyceraldehydes 3-phosphate dehydrogenase (GAPDH)) were amplified by PCR using M13 primers from a cDNA library, previously constructed with pUC or pGEM plasmids. Amplified cDNAs were separated by agarose gel electrophoresis, precipitated with ethanol and glycogen and resuspended in 50% DMSO to make 0.5-1 µg/µL solutions. PcCYPs, positive controls and negative controls (pUC, CotI, 50% DMSO, 50% DMSO + glycogen) were spotted on high-density amino group-coated slides (Matsunami Glass Co. Ltd.) with GTMAS Stamp (Moritex Inc.) using 16 pins. Before hybridization, slides were processed by UV crosslinking (600 KJ) with a UV Crosslinker XL-1500 (BM Equipment) and by blocking as previously described [41].

After 3-days incubation either in the absence or presence of exogenous substrate, total RNA was isolated from *P. chrysosporium* by phenol-guanidinium thiocyanate-chloroform extraction method [46]. Complementary DNA synthesis was carried out in the presence of 2-aminoallyl-dUTP using 55 µg of total RNA, $Oligo(dT)_{18}$ primer and Powerscript Reverse Transcriptase (BD Bioscience) at 42˚C for 1.5 h. RNA degradation was performed with 25 µL of 100 mM EDTA (pH 8.0) and 10 µL of 1 M NaOH. Samples were purified using a Microcon-YM30 (Millipore) column, dried and coupled to Cy3 and Cy5 monofunctional dyes (Amersham Biosciences) for 1 h in the dark. Before hybridization, free dyes were removed and labeled cDNAs

were purified using a QIA quick PCR purification kit (QIAGEN). Efficiency of cDNA synthesis and dye incorporation was measured spectroscopically using an Ultraspec 3300 Pro (Amersham Biosciences).

Each sample was mixed with the same dye concentration and the same volume of hybridization buffer (BD Biosciences) was added. Samples were hybridized for 12-15 h at 60℃. The hybridized slides were washed 3 times with 2xSSC buffer in 0.2% SDS at room temperature for 5 min, twice with 0.2xSSC buffer in 0.2% SDS at room temperature for 5 min, twice with 2xSSC buffer in 0.2% SDS at 60℃ for 20 min, twice with 0.2xSSC buffer in 0.2% SDS at room temperature for 5 min, and finally 3 times with 0.2xSSC buffer at room temperature for 5 sec. Slides were scanned in an FLA8000 (FUJI FILM). Image analysis, including a Lowess normalization, was carried out using with Array Vision. Each competitive hybridization was performed 4 times including a dye swap experiment. At least 32 data points were used for statistical calculation for each gene.

2.6. Functional analysis of PcCYPs

Full-length cDNAs amplified by PCR were cloned into the *Nco*I, *Xho*I or *Hind*III restriction sites of the pGYR expression plasmid [28]. Briefly, 120 full-length PcCYP cDNAs were cloned into pGYR vector carrying a NADPH-cytochrome P450 oxidoreductase from yeast *Saccharomyces cerevisiae*, and transformed into *S. cerevisiae [28]*. From this library termed the reaction array, all the 12 PcCYPs (see Table 2) that were induced by BaP in cDNA microarrays were selected and analyzed for their ability to metabolize BaP in vivo. Transformants of *Saccharomyces cerevisiae* AH22 cells were grown in a dextrose medium (8% glucose, 5.4% yeast nitrogen base w/o amino acids, 160 mg/L *L*-histidine) in 96-deep-well plates. Inoculated plates were incubated for 2 days in the dark (30℃, 1400 rpm) followed by the addition of 0.5 mM benzo(a)pyrene. After further incubation, the reactions were stopped with methanol (equal volume to the medium). The liquid phase was recovered after centrifugation (150 rpm, 4℃, and 10 min) and filtration with a 0.45 µM membrane (Millipore). Aliquots were analyzed by HPLC at 254 nm. Intermediates were further analyzed for identification using GC-MS. For scaled-up reactions, yeast transformants were cultured in dextrose medium in 500 ml conical flasks.

3. Results

3.1. Fungal Metabolism of BaP by *P. chrysosporium* in Vivo

During fungal metabolism, substrate disappearance and product formation were monitored by HPLC and GC-MS. BaP was significantly metabolized by the fungus under nitrogen limiting conditions as observed in Figure 1.

BaP dihydrodiol was identified by GCMS analysis as a metabolite from fungal conversions of BaP in vivo. Mass spectrum had the base peak at *m/z* 286 [M^+] and fragment ions at *m/z* 268 [M^+-H_2O] and 239 [M^+-H_2O-HCO], being consistent with a benzo(a)pyrene dihydrodiol [44] (see Table 1).

Furthermore, a decrease in the fungal metabolism of BaP in the presence of piperonyl butoxide, a potent cytochrome P450 inhibitor, was observed in 6-day cultures., confirming that endocellular cytochrome P450 enzymes are involved in their degradation (Figure 2). In further studies, the relevant P450 enzymes of the fungus *P. chrysosporium* involved in the metabolism of this PAH compound were determined using cDNA microarray analysis.

Table 1. Products of BaP Biotransformation by Biological Systems

R_t (min)	*m/z* (%) of fragment ions (Relative intensity)	Identification
31.29	M^+252 (100), 224 (4), 126 (19), 113 (13)	Benzo(a)pyrene
32.79	M^+282 (41), 267 (100), 252 (1), 250 (2), 239 (34), 119 (20)	[a]Methoxy-BaP[32]
34.33	M^+ 286 (33.3) 268 (100) 252 (16.7) 239 (91.7) 119 (75.0) 87 (25.0)	[b]4,5-dihydroxy -BaP[44]

BaP metabolites were identified via NIST library and cross referenced with spectra available from published literature [a]Recombinant *S. cerevisiae* expressing PcCYs and [b]*P. Chrysosporium.*

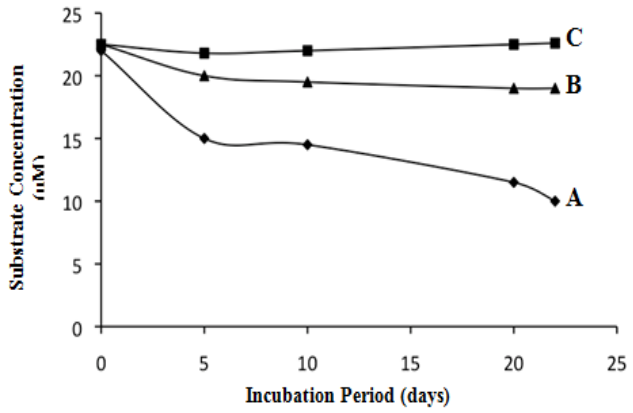

Figure 1. BaP biodegradation by the white-rot fungus *P. chrysosporium* in vivo. Key **A.** Fungal culture, **B.** Abiotic control:uninocculated with fungus, and **C.** Sodium azide treated control. Each data point is a mean value of three replicates.

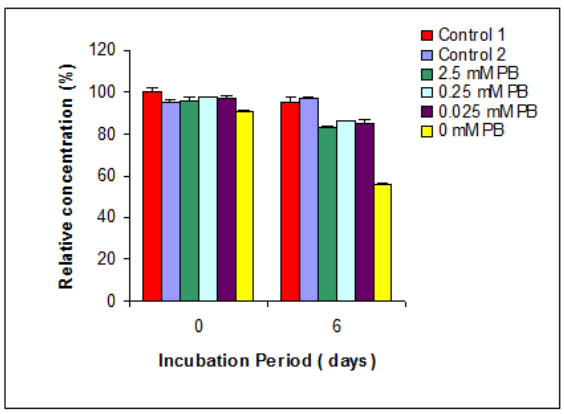

Figure 2. BaP % recovery from *P. chrysosporium* cultures incubated with various PB concentrations. Control 1- uninoculated cells, Control 2- Sodium azide treated cells. Each data point is a mean value of three replicates

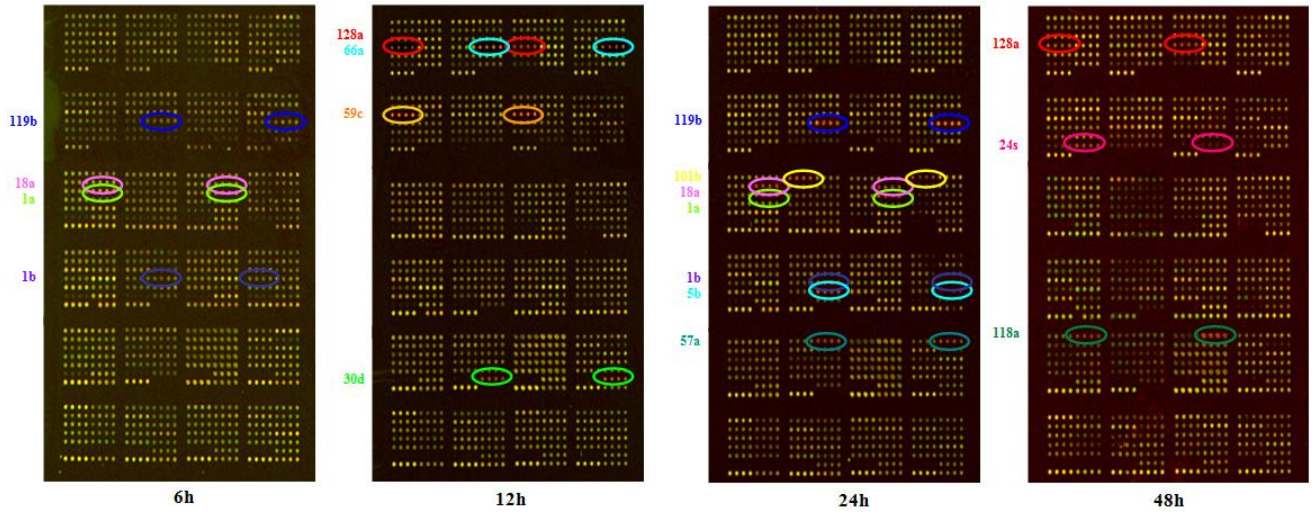

Figure 3. Differential expression of *P. chrysosporium* cytochrome P450 genes in response to BaP addition**.** Colour coded circles on the microarray grid indicate the position of spots corresponding to the PcCYP genes (adjacent numbers) up-regulated. Fungal cultures were harvested at the indicated time intervals after BaP addition and used for probing gene response. The first and third columns carried similar cDNA genes whilst the same was true for the second and fourth columns

3.2. Transcriptional Regulation of PcCYPs Responding to BaP

In order to understand the differential regulation of PcCYPs by BaP, *P. chrysosporium* cultures were treated with this PAH under nutrient limiting conditions. The transcriptional profiles of PcCYPs were examined using the cDNA microarray. Using four time points for data sampling, 12 PcCYP species out of the 133 investigated showed differential expression in response to this substrate as shown in Figure 3.

The images show the level of test to reference cDNA that was hybridized to spotted probe cDNA. In the derived ratio image, the red spots indicate that the test cDNA was expressed at a higher level to the reference and the green spots are an indication that the expression level was lower, whilst it was unchanged for spots that appeared yellow. Furthermore, the relative intensities of the 12 PcCYPs showing physiological responses to the substrate were extrapolated to generate a quantifiable intensity based ratio analysis, the results of which are shown in Table 2.

Based on preliminary experiments, it has been verified that the constructed cDNA microarray system is able to detect transcriptional regulation when gene expression is up-regulated more than two fold or down-regulated less than half fold [41]. PcCYPs 1a, 1b, 18a,101b, and 119b were induced as early as 6 hours after substrate addition, whilst PcCYPs (30d, 59c, 66a, 128a) were induced 12 hours later with the rest of the PcCYPs (5b, 24s, 57a,) differentially expressing 24 hours later. Interestingly, all the 12 PcCYPs were induced between the 12h and 24 h time points. The PcCYPs responsive to BaP were induced at least twice, with the exception of PcCYPs 5b, 30d and 57a.

3.3. Functional Analysis of PcCYPs Induced by BaP

PcCYP activities against BaP were also investigated with recombinant *S. cerevisiae* expressing the 12 PcCYPs that were induced by BaP in cDNA microarrays. These genes were tested for their ability to metabolize BaP. Recombinant *S. cerevisiae* expressing PcCYPs18a, 101b, 119b and 128a did not metabolize BaP. PcCYPs, 1a, 5b, 24s, 30d, 59a, 59c and 66a all showed activity on BaP degrading it to metabolites designated M1 to M5 detected using HPLC (Figure 4).

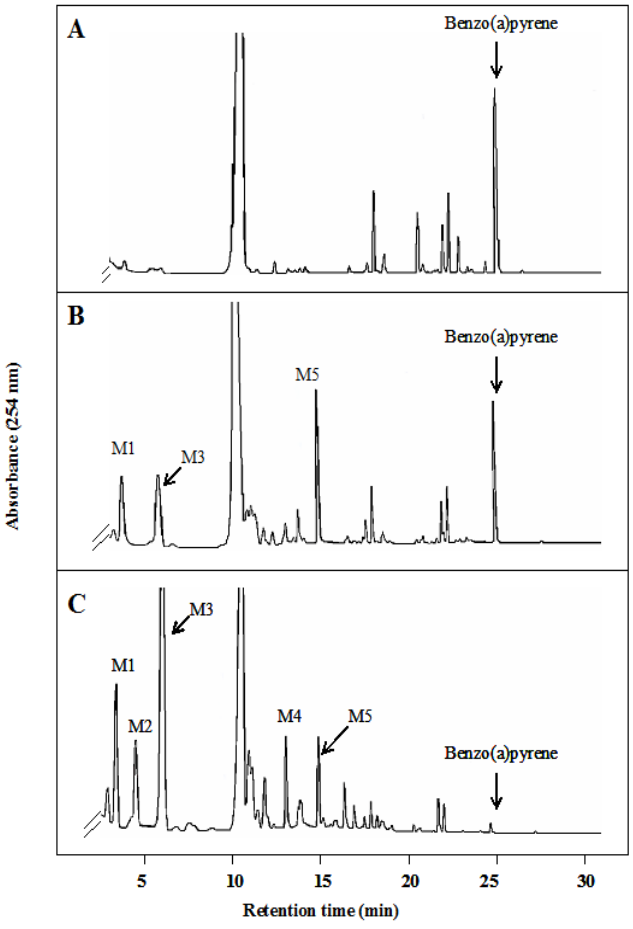

Figure 4. HPLC analysis of BaP metabolism by heterologous *S. cerevisiae* Key: Chromatograms at 254 nm **A**. –P450 + substrate; empty pGYR vector **B.** PcCYP59c + substrate **C.** PcCYP66a + substrate. M1-M5 are metabolites produced during metabolism

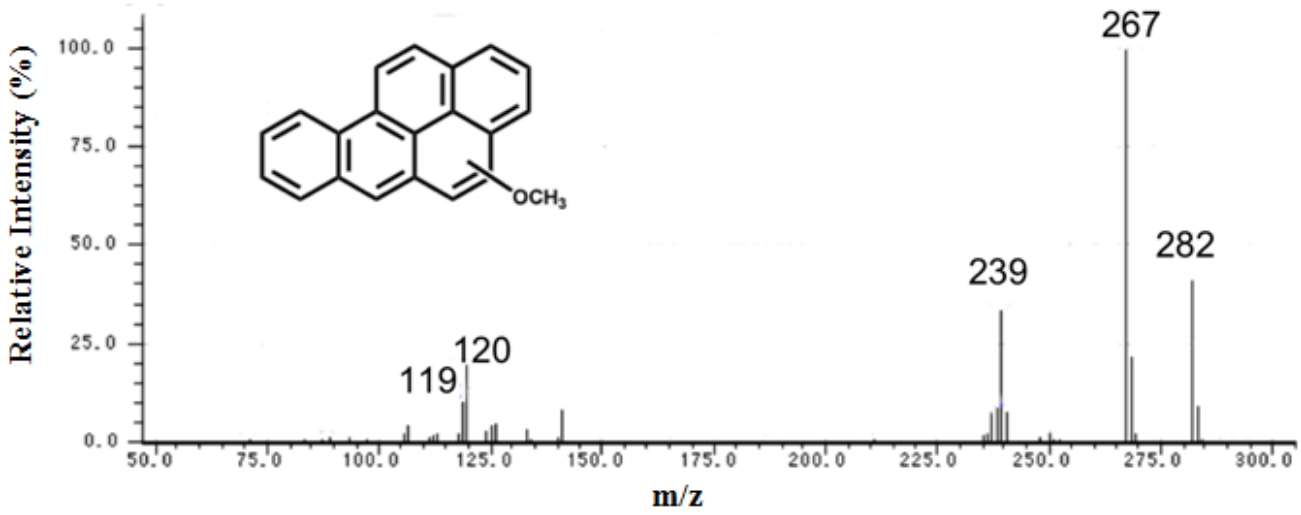

Figure 5. Mass spectra of the metabolite of BaP obtained using heterologous *S. cerevisiae* cells. The metabolite was identified as Methoxy-BaP [32]

Table 2. Expression Profiles of PcCYP Genes Induced by BaP

PcCYPs	Incubation Period (hours)			
	6.0	12.0	24.0	48.0
1a	2.0 ± 0.1	2.0 ± 0.3	3.0 ± 0.5	1.0 ± 0.6
1b	2.0 ± 0.3	2.0 ± 0.2	2.0 ± 0.6	1.0 ± 0.3
5b	1.3 ± 0.2	1.2 ± 0.3	2.5 ± 0.9	1.0 ± 0.4
18a	2.0 ± 0.3	1.3 ± 0.2	2.3 ± 0.5	1.3 ± 0.6
24s	1.0 ± 0.1	1.4 ± 0.5	2.0 ± 0.4	3.0 ± 1.5
30d	0.7 ± 0.1	3.0 ± 1.0	1.0 ± 0.3	1.2 ± 0.3
57a	1.2 ± 0.2	1.3 ± 0.3	3.0 ± 0.8	1.0 ± 0.2
59c	1.1 ± 0.4	2.0 ± 0.6	1.0 ± 0.1	2.0 ± 0.3
66a	1.1 ± 0.2	4.0 ± 1.0	1.2 ± 0.2	2.0 ± 0.2
101b	2.0 ± 0.2	2.0 ± 0.8	3.0 ± 0.8	2.0 ± 0.6
119b	2.0 ± 0.2	2.0 ± 0.4	3.0 ± 0.7	2.0 ± 0.3
128a	0.9 ± 0.2	2.2 ± 0.6	2.0 ± 0.3	2.0 ± 0.2

Gene expression levels ± SD from 2 independent biological replicates and are means of these independent assays. Hybridization experiments were done in two channels (Cy3 and Cy5), then the intensity ratio between the two channels quantified. Genes that were ascribed as significantly changed were those exhibiting ≥2.0 and or ≤ 0.5 fold change.

Incubations with control *S. cerevisiae* not expressing PcCYPs were negative for BaP metabolism. The PcCYPs did not degrade BaP to the same extent. As shown in Figure 4, PcCYP66a was more efficient in degrading the substrate when compared to PcCYP59c cultures. GCMS analysis of the culture extract identified methoxy-BaP as a metabolite produced in vivo (Figure 5). It produced a mass spectrum that had a molecular ion at m/z 282, and the intensive fragments ions at m/z 267 [M^+-CH_3] and m/z 239 [$M^+-CO-CH_3$].

4. Discussion

P. chrysosporium metabolized BaP significantly in this study (Figure 1). The metabolism of the potent carcinogen BaP by fungal cells has previously been reported ([5,6,8,13,14,25-35]). An analysis of the fungal extracts using GCMS revealed BaP was metabolized to BaP 4,5-dihydrodiol as one of the metabolites in this study. This is the same product reported for BaP metabolism by *Pleurotus pulmonarius* [39]. Corresponding microsomal P450s were suggested for *P. pulmonarius*. It was therefore hypothesized that an intracellular cytochrome P-450 monooxygenase system was eventually involved in BaP metabolism, alongside other enzymes. In order to check the involvement of such enzymes, the effect [33] of a specific inhibitor of cytochrome P-450, piperonyl butoxide was tested. The inhibition of fungal metabolism of BaP in the presence of the P-450 inhibitor was observed in 6-days cultures (Figure 2). This result suggested that a cytochrome P-450 is one of the enzymes involved in the metabolism of BaP by *P.chrysosporium* under nutrient limiting conditions known to favor peroxidase activity. Similar results have been reported on various PAHs metabolized by fungi ([4,5]). Substrate metabolism slowed down in the presence of inhibitor and was still observed at high concentrations of 2.5 mM PB. Murray et al., [40] suggested that PB is not uniformly potent against the activity of all P450s hence it is possible to observe metabolism at high PB concentration levels. Moreover, two routes of metabolism exist under ligninolytic conditions with one remaining predominantly active when the other has been made dysfunctional by the inhibitor [33,40]. Two main enzyme groups are involved in the initial attack on PAHs by fungi in the two routes, namely the intracellular cytochrome P-450 monooxygenases and the extracellular lignin peroxidases ([25,26,39-48]).

In *P. chrysosporium* 149 cytochrome P450 genes (P450ome) were annotated from the genomic sequence of this white-rot basidiomycete [52]. The endogenous substrates of most of them remain unknown. In addition, there is limited knowledge on the contribution of the relevant cytochrome P450 isozymes in metabolic processes. The results of the cDNA microarray revealed several differentially expressed PcCYP genes likely to be involved in the metabolism of this compound as shown in Table 2. It is well known that inducers for eukaryotic P450 monooxygenases can also be the substrates that these enzymes can oxidize [1]. Therefore, it can be assumed that BaP is a potential substrate for the 12 PcCYPs up-regulated under nutrient limiting conditions utilized in this study. PcCYPs 1a, 1b, 18a, 101b, and 119b were induced as early as 6 hours after substrate addition

implying that they may be the first to recognize BaP as a potential substrate and in so doing initiate attack on the PAH. PcCYPs 30d, 59c, 66a, 128a were induced 12 hours later with the rest of the PcCYPs 5b, 24s, 57a, differentially expressing 24 hours later. Interestingly, all the 12 PcCYPs were induced between the 12h and 24 h time points. It can be speculated that compositional differences occurring in the culture media with prolonged incubation resulted in the formation of endogenous substrates such as fatty acids and vitamins, besides the xenobiotic compound BaP that later become inducers of more PcCYPs as they themselves become substrates for these species. Furthermore, BaP exposure prepares the fungal system for assimilating alternate carbon sources [54] to sustain the nutrient environment required for P450 mediated biodegradation.

The next focus of the study was to determine PcCYPs function using heterologous *S. cerevisie*.. *S cerevisiae* expressing PcCYPs (PcCYPs, 1a, 5b, 24s, 30d, 59a, 59c and 66a) showed activity on BaP. The substrate disappearance and product formation were monitored by HPLC. Figure 4 shows a typical chromatogram of BaP metabolites from the culture extracts. The degradation of this substrate was accompanied by the formation of several metabolite peaks. When the culture extract was examined by GC-MS, the substance recognized as a degradation product of BaP with PcCYPs produced a mass spectrum that is typical of methoxy-BaP (Figure 5), which is a hydroxy benzo(a)pyrene adduct. It had a molecular ion at m/z 282, and the intensive fragment ions at m/z 267 $[M^{+}-CH_3]$ and m/z 239 $[M^{+}-CO-CH_3]$. The fragmentation pattern of this compound was identical to that reported previously for methoxy-BaP ([32,36]). Hydroxy BaP is a major metabolite of the environmental procarcinogen BaP produced in a wide variety of biological systems. In this study, the introduction of a methyl group to hydroxy- BaP could have occurred in the biological system via a methyltransferase reaction or during analysis in the GCMS column showing that the metabolite was relatively less stable. In one study [30], hydroxymethoxy and dimethoxy metabolites of BaP were also identified and corroborated a report that demonstrated a catechol-*O*-methyltransferase reaction. In fungal cultures of *P.chrysoporium* the production of dihydroxy-BaP is made possible by the presence of the epoxide hydratase enzyme [4] which is not found in *S. cerevisiae* recombinant systems that may rely on the non enzymatic rearrangement of the arene oxides [53] known to be the initial products of cytochrome P450 enzyme mediated reactions.

Cytochromes P450 enzymes are involved in a variety of metabolic and biosynthetic processes and have considerable potential in bioremediation. Several reports have reviewed and emphasized this potential and discussed the use of natural microbial and mammalian P450s, in addition to engineered P450 mutants, for the biodegradation of environmentally harmful compounds such as insecticides, pesticides and other agrochemicals ([24,29]). In this study, we report on fungal P450s relevant to BaP metabolism by the fungus *P. chrysosporium*. The demand for highly active enzymes that are capable of degrading environmental waste has been constantly growing. Most advantageous bioremediation strategies for treating contaminated sites can thus possibly be planned and applied using novel strains with desirable properties for bioremediation applications based on the knowledge of PAH degradation by microorganisms.

5. Conclusion

In this study, a time dependent comprehensive analysis of cytochrome P450s, induction and function was conducted in order to understand cellular systems optimized for BaP degradation by the basidiomycete *P.chrysosporium*. Differential gene expression patterns were observed with cDNA microarray indicating responses to BaP as a potential substrate for the 12 PcCYPs up-regulated under nutrient limiting conditions. PcCYPs 1a, 5b, 24s, 30d, 59a, 59c and 66a were proved to be functional with the *S. cerevisiae* heterologous expression system. A role for PcCYPs in the biodegradation of the specific compound BaP was demonstrated. Development of designer CYPs which may be useful in removal of toxins, pollutants, and engineering CYPs to extend their catalytic capabilities are likely to further extend the activities of this versatile family of enzymes. Furthermore, conducting MOE analysis will give a clearer picture of the active site of these enzymes in relation to aromatic substrates. Overall, the findings in this study are important as they help to clarify the presence of such a large contingent of P450 genes in *P. chrysosporium* and to assign function.

Statement of Competing Interests

The authors confirm that the content of this article has no competing interests.

List of Abbreviations

BaP Benzo(a)pyrene
CYP Cytochrome P450
PcCYP *Phanerochaete chrysosporium* cytochrome P450
PAH Polycyclic aromatic hydrocarbon
GAPDH Glyceraldehyde 3-phosphate dehydrogenase
NIST National Institute of Standards and Technology
NCBI National Centre for Biotechnology Information
SSC Saline sodium citrate

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