Bio function of Cytochrome P450 on fungus: a review

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Abstract. Cytochrome P450 is the superfamily of proteins involved in the metabolism of organisms, including fungi. Fungal have more diverse P450 families than plants, animals, or bacteria. Research on fungal P450 has blossomed and become an important area in biology and ecology. Cytochrome P450 could be detoxifying natural and environmental contaminants to survive in several ecological niches. Furthermore, the presence of the fungal Cytochrome P450 as an antifungal drug target is a promising approach for the controlling of pest and plant pathogenic fungi. To date, numerous studies have revealed the annotation of diverse P450 followed by an elucidation of P450 functions. This mini-review starts with some basic information of P450s on fungi, then discusses the incredible bio function of characterized fungal P450.

1. Introduction
Cytochrome P450 (P450) is the superfamily of proteins involved in the metabolism of organisms, including fungi [1, 2]. The discovery of metabolic activity in rabbit liver microsomes was the main key for the introduction of the first P450 (Axelrod (1955). The definition of cytochrome was introduced in 1958 by Klingenberg as a carbon monoxide-binding pigment (P) with a spectral absorbance maximum at 450 nm, and Omura and Sato [3] confirmed that P450 is a heme protein [3, 4]. In the following years' research progress of P450 on bacteria, animals, plants, and fungi expanded widely. The early research on fungal P450 was reported by Ferris & Jeffrey 1973, this paper successfully demonstrated a fungal system similar to liver microsomes indicating the presence of P450 enzymes on fungus Cunninghamella bainieri [5]. During the past three decades, Studies of molecular diversity and metabolic activity depending on fungal P450 reactions have grown rapidly. Ichinose undertook a series of studies on the metabolic diversity and function of cytochrome P450. The result describes that the divergence molecular and functional diversity of fungal P450 is greater than that of animal, plant, or bacteria [6, 7]. The statement above is supported by three papers describing that P450 on fungi are the most abundant and versatile than other organisms [2, 8, 9].

Research on fungal P450 has blossomed and become an important area in biology and ecology. P450 possessed by fungi has been organized into 805 families [10]. Numerous research has noted fungal is applying P450 as a strategy to survival in several ecological niches. The evolution of fungal P450 diversity as a weapon for survival strategy to provide superior metabolic requirements is a strategy of fungi to adapt rapidly [2, 11, 12]. To date, numerous studies have revealed the annotation of diverse P450 followed by an elucidation of P450 functions. A functionomic approach is critically studied to create a research space between biology and future biotechnology application, for example, Phanerochaete chrysosporium can degrade wood compounds and as potential biodegradation of
xenobiotics compounds [13]. This mini-review starts with some basic information of P450s on fungi, then discusses the incredible bio function of characterized fungal P450.

2. The incredible bio function of fungal P450 reactions

2.1 Fungal P450 as a biodegrade aromatic compounds

The cytochrome P450 activities were identified in plant cell wall degradation processes by fungus species [14–16]. The function of fungal P450 in wood degradation is involved in metabolic activities, signaling networks [17], and lignin degradation [11]. The following are the results of investigating the role of enzyme P450 in wood degradation by fungi.

Fungi in basidiomycetes employ various CYP53 families to colonize and degrade wood components (17,18). The presence of the CYP53 family in basidiomycete biotrophic plant pathogens, for example, Armillaria mellea has been reported. Interestingly, the results showed CYP53 successfully colonized plants through involvement in detoxification of antifungal agents and degradation of wood [18]. A study has described the CYP53 family as the three main keys to successfully degrade components of wood. Firstly, CYP53 is an enzyme that can detoxify wood-derived components, including benzoic acid (BA) derivatives is a key intermediate in aromatic compound metabolism in fungi. Secondly, CYP53 genes play a role in the synthesis of aryl-metabolites, including veratryl alcohol, which involves the formation of benzoic and para-hydroxybenzoic acid as intermediate molecules. And Thirdly, demethylation of stilbene, a class of molecule found in plants [19].

The early research, Ide et al. (2012) conducted functional screening to discover potential P450s from Postia placenta. The results showed clear insight that CYP53D2 is involved in degrading aromatic compounds to catalyzing the O-demethylation of stilbene derivatives during the decomposition of woody biomass [20].

The ability of P450 possesses P. chrysosporium involved in the biodegradation of natural aromatic polymer lignin has been reported. Expression of the CYP53 family PcCYP1f (CYP53A15) encodes has shown the ability to catalyze the hydroxylation of benzoic acid into 4-hydroxybenzoic acid, 2- and 4-hydroxybenzoic acid, and 3-methoxy-4-hydroxybenzoic acid [21, 22]. Furthermore, the number of CYP5138A1 genes is capable to catalyze the oxidation of naringenin yielding eriodictyol (5,7,3',4'-tetrahydroxyflavanone) by the recombinant Saccharomyces cerevisiae [23].

The presence of CYP53 was also detected in the phylum ascomycota, for example Cochliobolus lunatus, Aspergillus niger, and Fusarium oxysporum. The pathogenic filamentous ascomycete C. lunatus was identified posses CYP53A15 gene [24] and A. niger posses CYP53A1 [25, 26] are involved in the formation of para hydroxylation of benzoate. A study has reported that The benzoate hydroxylase catabolism from F. oxysporum was successfully identified through FoCYP53A19 gene expression [27]. The results suggest that the CYP53A subfamily has O-demethylation activity of 3-methoxybenzoate derivative, which is important in detoxification of other antifungal substances. Biodegrade aromatic compounds by Fungal P450 are summarized in Table 1.

Recent studies have reported that fungal P450 are enzymes with high potential as biocatalysts for industrial applications, through versatile catalytic potentials and functional diversity [8]. Research progress of fungal P450-mediated whole-cell biotransformations is key to industrial implementation. Whole-cell biocatalysis is suggested to alleviate the problems often encountered with cytochrome P450-based applications by identifying suitable hosts for heterologous expression of P450s [28]. In recent years, several researchers have reported recombinant Arxula adenisinivorans was found to be the most promising host, which is capable of expressing the heterologous p-Hydroxybenzoic acid (pHBA), a CYP53B1 product from Rhodotorula minuta [29]. Another study showed that Bacterial Glucose Dehydrogenase (BsGDH) from Bacillus subtilis in Saccharomyces cerevisiae is a host for the heterologous expression and biotransformation of the benzoate hydroxylase FoCYP53A19 from F. Oxysporum [30].
Tabel 1 Biofunction of fungal P450 in wood degradation

| P450 sources | CYP       | Metabolic Activity                                      | Target                  | References |
|--------------|-----------|--------------------------------------------------------|-------------------------|------------|
| P. placenta  | CYP53D2   | Oxidizes stilbene derivatives to O-demethylation       | benzoate derivatives    | [20]       |
| P. Chrysosporium | PcCYP1f* | Oxidizes naringenin to eriodictyol (5,7,3',4'-tetrahydroxyflavanone) | naringenin              | [23]       |
| C. lunatus   | CYP53A15  | Catalyze the para-hydroxylation of benzoic acid        | benzoate derivatives    | [24]       |
| A. niger     | CYP53A1   | Catalyze the para-hydroxylation of benzoic acid        | benzoate derivatives    | [25]       |
| F. oxysporum | FoCYP53A19** | Catalyze the para-hydroxylation of benzoic acid | benzoate derivatives    | [27]       |

* The coding of P. chrysosporium’s cytochrome P450 (PcCYP) genes
** The coding of F. oxysporum’s cytochrome P450 (PcCYP) genes

2.2 Fungal P450 involved in bioremediation

The numerous studies of bioremediation focused on chemical and solid waste management by applying natural biological processes. A previous study reported that fungi play a major role in bioremediation owing to their diverse metabolic capacity [13].

Fungi produce a variety of metabolites processed by intracellular enzymes, including fungal P450 enzymes. A series of papers by Ichinose has demonstrated the significance of fungal P450s as mediators for the degradation of a range of endogenous and exogenous compounds [11]. The process of degradation of exogenous compounds correlated with xenobiotic chemicals. Definition of xenobiotics is chemical compounds found within an organism or ecosystem that are not produced by that organism or ecosystem. Population explosion, industrial and agricultural activities have created xenobiotic compounds that are persistent in the environment with toxic effects on living organisms.

2.2.1 Fungal P450 as biodegradation of industrial pollutants

As mentioned above, the development studies of biological function on fungal P450 play a key role in bioremediation applications for industrial pollutants. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins (PCDDs) are classified as global industrial pollutants [31].

PAHs are persistent organic pollutants and widespread distribution in the environment. The first publication in 1991 reported that the possible involvement of P450 enzymes in PAH metabolism by the fungus P. chrysosporium [32]. Over the past three decades, several studies have highlighted and reported that fungal P450 is involved in degrading PAHs [33–35].

P. chrysosporium has been studied extensively and is most commonly used for the degradation of pollutant compounds due to the production of intracellular and extracellular enzymes, including P450 enzymes. The presence of an extraordinarily large contingent of P450s in P. Chrysosporium as potential biodegradation of xenobiotics compounds have been reported in a series of papers of fungal P450 functional diversity [35–37]. The study of the genome to function of novel fungal P450 In P. Chrysosporium have identified six genes of fungal P450 (CYP5136A2, CYP5145A3, CYP5144A7, CYP5136A3, CYP5142A3, CYP5144A5) that can catalyze the oxidation of varying ring-size PAH
compounds (phenanthrene, pyrene, and benzo(a)pyrene) [35], and CYP5136A3 plays a key role in the oxidative activity of endocrine-disrupting alkylphenols (APs) and benzo[a]pyrene (BaP) [36].

Two papers authored by Doddapaneni et al., have reported that the CYP63 genes (CYP63A1, A2, and A3) have the ability to degrade PAHs and substituted alkanes via P450-type reactions [38, 39]. Similar results have been reported that CYP63A2 has the capability to oxidized the 4-ring PAHs pyrene and fluoroanthene, Benzo(a)pyrene, benzo(ghi)perylene, alkylphenols (AP), and alkanes [40, 41]. Functional analysis of PcCYPs, 1a, 5b, 24s, 30d, 59a, 59c, and 66a has been shown height responsive to degradation BaP [42]. Furthermore, CYP617D1 is found in Aspergillus nidulans and has been identified to play a functional role in degrading BaP [43]. Similar results investigation of P450 on Postia placenta showed catalytic activity and capability to oxidize a series of PAHs. The results would suggest that CYP5150D1, CYP5027B1, and CYP5350B2v1 have the capability to oxidize anthracene, carbazole, phenanthrene, and pyrene compounds [20].

Two papers have noted that fungal P450 has the capability to bioremediate dioxins [44, 45]. Dioxins or polychlorinated dibenzo-p-dioxins (PCDDs) are known as one of the global industrial pollutants. Gene expression of CYP5145A3 posses P. Chrysosporium showed the highest PCDDs degradation activity compared to other PcCYPs, such as 1,2-monochloro-dibenzo-p-dioxin (1,2-MCDD) and 2,3-dichlorodibenzo-p-dioxin (2,3-DCDD) [44, 46]. Another study investigated the CYP5138A1 gene has capability to exhibit catalytic activities against chlorinated dioxins [47]. Previous study by Kasai et al., have noted that CYP5138A1 is involved the degradation of dibenzo-p-dioxin (DD), 2-monochloro-DD, biphenyl, and naphthalene by the recombinant Saccharomyces cerevisiae [23]. Studies investigating the biodegradation of industrial pollutants by fungal P450 are summarized in Table 2.

| P450 sources | CYP          | Metabolic Activity                                                                 | Target                  | References |
|--------------|--------------|------------------------------------------------------------------------------------|-------------------------|------------|
| P. chrysosporium | CYP5136A2   | oxidize of phenanthrene, pyrene and benzo(a)pyrene                                  | PAH                     | [35]       |
|              | CYP5145A3   |                                                                                    |                         |            |
|              | CYP5144A7   |                                                                                    |                         |            |
|              | CYP5136A3   |                                                                                    |                         |            |
|              | CYP5142A3   |                                                                                    |                         |            |
|              | CYP5144A5   |                                                                                    |                         |            |
| P. chrysosporium | CYP5136A3   | oxidizes phenanthrene, benzo[a]pyrene, pyrene and endocrine-disrupting alkylphenols | PAHs and APs            | [36]       |
|              |             |                                                                                    |                         |            |
| P. chrysosporium | CYP63A1     | Oxidizes PAHs and substituted alkanes                                               | PAHs and substituted alkanes | [38, 39]  |
|              | CYP63A2     |                                                                                    |                         |            |
|              | CYP63A3     |                                                                                    |                         |            |
*P. chrysosporium*  
*CYP63A2*  
Oxidizes pyrene and fluoranthene, Benzo(a)pyren, benzo(ghi)perylen, alkylenphenols (AP), and alkanes  
Higher molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs), (APs), and alkanes  
[40, 41]

*P. chrysosporium*  
PcCYPs, 1a, 5b, 24s, 30d, 59a, 59c, and 66a*  
Oxidized BaP to BaP 4,5-dihydrodiol  
PAHs  
[42]

*A. nidulans*  
*CYP617D1*  
Oxidizes BaP  
PAHs  
[43]

*P. placenta*  
*CYP5150D1*  
*CYP5027B1*  
*CYP5350B2v1*  
Oxidizes anthracene, carbazole, phenantrrene, and pyrene.  
PAHs  
[20]

*P. chrysosporium*  
*CYP5145A3*  
Catalyzes 1,2-monochlorodibenzo-p-dioxin (1,2-MCDD) and 2,3-dichlorodibenzo-p-dioxin (2,3-DCDD)  
PCDDs  
[44, 46]

*P. chrysosporium*  
*CYP5138A1*  
Catalyzes chlorinated dioxins  
Chlorinated Dioxins  
[47]

*P. chrysosporium*  
*CYP5138A1*  
Catalyzes dibenzo-p-dioxin (DD), 2-monochloro-DD, biphenyl, and naphthalene  
PCDDs  
[23]

*The coding of* *P. chrysosporium* *’s cytochrome P450 (PcCYP) genes*

### 2.2.2 Fungal P450 as a biodegrader of pesticides

The fungal P450 approach as a biological agent to degrade pesticides was extensively studied for improving the safety of foods and protection of ecology. Pesticides have been used and accumulated in the environment and caused negative impacts on humans and non-targets. The metabolic diversity of fungal P450 is suggested as a mediator for the degradation of pesticides, including insecticides [48].

A series paper has to describe that Phanerochaete sp. P450 plays a critical role in the degradation of a neonicotinoid insecticide [49–52]. Acetamiprid (ACET) has been widely used for the last two decades, persistence in environments, potential risks to non targets, and one of the first-generation neonicotinoid
insecticides. In 2019, a study reported that *P. Chrysosporium* posses fungal cytochrome P450 encoded CYP5147A3 that is responsible for the degradation and detoxification of ACET, and two ACET metabolites (N'-cyano-N-methyl acetamide and 6-chloro-3-pyridinemethanol) [50]. Another study on *P. chrysosporium* showed that the genes CYP5037B3 and CYP5147A3 were identified as the key isozymes involved in the metabolism of acetamiprid, imidacloprid, and thiacloprid by an N-dealkylation, resulting in 6-chloro-3-pyridinemethanol [53]. Similar results indicated the presence of P450 activity in *Trametes versicolor*, P450 showed involvement in the degradation of acetamiprid and imidacloprid [54].

As mentioned above, besides being involved in PAH and APs degradation, the CYP63A2 gene in the *P. Chrysosporium* has been reported to be responsive to biodegradable synthetic insecticide. Dichloro Diphenyl Trichloroethane (DDT) [39]. DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) is one of the insecticides that are hazardous for the environment and human health. In addition, P450 on brown-rot fungus *Fomitopsis pinicola* has been reported to be involved in the degradation of DDT [55].

2.3 Fungal P450 as an antifungal drug targets

The presence of the fungal Cytochrome P450 as an antifungal drug target is a promising approach for the controlling of pest and plant pathogenic fungi. The discovery of the antifungal amphotericin B in 1940 and azoles several decades later have been widely used for the treatment of fungal infections [56], specifically targeting the fungal CYP51 (14α-demethylase) [57]. Fungal CYP51 encodes the ergosterol biosynthetic enzyme [58,59], ergosterol is an essential plasma membrane and responsible for fungal growth and as a target of antifungal compounds [2, 60]. Azoles as an agricultural fungicide have been applied extensively, the mechanism of action of azoles is to block the demethylation of lanosterol to ergosterol biosynthesis, in particular, binding and inhibiting 14α-methylated precursors [61]. Thus, azoles cause changes in the fluidity of fungal membranes and the activity of several membrane-bound enzymes [56, 60].

To date, numerous studies have reported the critical role of CYP51 as an antifungal target. A study on the fungus *Puccinia striiformis* has identified the expression of 14α-demethylase activity, which we know to be a target forazole antifungal drugs [62]. The statement above is supported by research on *Fusarium graminearum* posses CYP51A, CYP51B, and CYP51C genes are paralogs of CYP51 have been investigated encodes an ergosterol biosynthetic [57, 63], the CYP51A and CYP51B gene showed sensitivity to azole fungicides [11, 64, 65]. Gene expression in *F. graminearum* Similar to *F. oxysporum*, *F. oxysporum* poses FoCYP51A and FoCYP51B are responsible for sensitivity to different azoles [66].

In humans, *Aspergillus flavus* is one of genus causing invasive infections. A. flavus has CYP51A, CYP51B, and CYP51C genes which are the target ofazole drugs [67]. The result of gene expression analysis showed that CYP51A and CYP51B expression levels were greater than that of CYP51C. Interestingly, numerous studies have reported azole resistance among *A. fumigatus* isolates, which is mainly related to mutations in the CYP51A gene [68, 69]. Further research of this case is a critical challenge to design new treatments with an eco-friendly approach.

Previous studies have reported CYP53 plays a critical role in degradation of plant cell wall, through the hydroxylation of benzoic acid (BA) and its derivatives. CYP53 has been investigated as an antifungal drug target BA product, p-Hydroxybenzoic acid (pHBA) [18, 29]. The CYP53 family has been linked to persistence and biotrophic plant pathogens. A study inhibiting the growth of the fungi *C. Lunatus, A. Niger* and *Pleurotus ostreatus* via CYP53A15 activity has been reported. This study used cinnamic acid derivatives as antifungal drugs, Cinnamic acid derivatives are plant defense compounds with antimicrobial and antifungal properties. The results showed that cinnamic acid derivatives could inhibit BA hydroxylation by CYP53A15 activity [70]. The summary of the fungal Cytochrome P450 as an antifungal drug target for the controlling of pest and plant pathogenic fungi in Table 3.
Table 3 Bio function of Fungal P450 as an antifungal drug targets

| P450 sources | Gene target | Antifungal drug | Reference |
|--------------|-------------|-----------------|-----------|
| *F. graminearum* | CYP51A      | Azole fungisides | [57, 63]  |
|               | CYP51B      |                 |           |
| *F. oxysporum* | CYP51A      | Azole fungisides | [66]      |
|               | CYP51B      |                 |           |
| *A. flavus*   | CYP51A      | Azole fungiside | [67–69]   |
|               | CYP51B      |                 |           |
|               | CYP51C      |                 |           |
| *C. lunatus*  | CYP53A15    | Cinnamic acid derivatives | [70] |
| *A. niger*    |             |                 |           |
| *P. ostreatus*|             |                 |           |

3. Conclusions and future perspectives

Genome-wide, structure, and functional annotations of fungal P450 are currently being explored and exploited. As mentioned above, fungi continue to evolve conquering the limited environment as a living resource through the diversity of P450 [18]. Likely fungal P450 could be detoxifying natural and environmental contaminants to survive in several ecological niches. Furthermore, the presence of the fungal Cytochrome P450 as an antifungal drug target is a promising approach for the controlling of pest and plant pathogenic fungi.

In the last decade reports on the evolution of azole resistance in plant pathogenic fungi have been in the spotlight. Resistance is closely related to overexpression of the CYP51 family [69, 71, 72]. Furthermore, CYP51 becomes a major target for controlling pests and diseases in crop plants. In this case, the discovery of Host-induced gene silencing (HIGS) employing RNA silencing mechanisms as a strategic approach to control pests and diseases was successfully applied [73]. For example: to fight *F. graminearum* (Fg) and *Fusarium Culmorum* in cereal crops is to apply a spray of double-stranded (ds)RNA called CYP3RNA, which is targeting the three fungal CYP51 genes CYP51A, CYP51B, and CYP51C. The results showed that Fusarium sp. is sensitive to dsRNA derived from homologous fungal CYP51 genes [73–75]. Furthermore, RNA-based technology is a strategic approach to replace azole fungicides for the control of fungal diseases in an eco-friendly way.

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