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Article in *Genes & genomics* · January 2014

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Genome-wide identification, annotation and characterization of novel thermostable cytochrome P450 monooxygenases from the thermophilic biomass-degrading fungi *Thielavia terrestris* and *Myceliophthora thermophila*

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Received: 5 November 2013 / Accepted: 24 December 2013
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Abstract Cytochrome P450 monooxygenases (P450s) are ubiquitous heme-thiolate proteins that have potential biotechnological application. Thermostable-P450s that can withstand hostile industrial conditions, such as high temperatures, extremes of pH and organic solvents, are needed for biotechnological usage. Here, for the first time, we report a large number of thermostable-P450s from two thermophilic biomass-degrading fungi, *Myceliophthora thermophila* and *Thielavia terrestris*. Genome-wide P450 analysis revealed the presence of 79 and 70 P450s (P450ome) in *T. terrestris* and *M. thermophila*. Authentic P450s containing both the P450 signature domains (EXXR

and CXG) were classified as follows: *T. terrestris* (50 families and 56 subfamilies) and *M. thermophila* (49 families and 53 subfamilies). Bioinformatics analysis of P450omes suggested the presence of a large number of thermostable-P450s. Based on aliphatic index cut-off (>90), 14 and 11 P450s were determined to be thermostable in *T. terrestris* and *M. thermophila*. Among the thermostable P450s, six P450s from *T. terrestris* and three from *M. thermophila* had a melting temperature (T_m) of >65 °C, suggesting their hyperthermal tolerance. Analysis of the instability index of two ascomycete P450omes revealed the presence of 12 and 19 in vitro stable P450s in *T. terrestris* and *M. thermophila*. Overall, six P450s from *T. terrestris* and four from *M. thermophila* showed both thermal tolerance and in vitro stability. Thermophilic ascomycetes P450s are of potential interest from a structural, mechanistic and biotechnological point of view, as five P450s showed higher thermal tolerance and five showed higher in vitro stability compared to the well-characterized thermostable-P450s CYP175A1 (bacteria) and CYP119 (archaea).

Electronic supplementary material The online version of this article (doi:10.1007/s13258-013-0170-9) contains supplementary material, which is available to authorized users.

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Keywords Aliphatic index · CYP119 · CYP175A1 ·
Fungi · Protein melting temperature · Thermostable P450s

Introduction

Cytochrome P450 monooxygenases (P450s) are heme-thiolate proteins distributed across the biological kingdoms (Nelson 2013). P450s perform a wide variety of reactions, such as ω -hydroxylation of hydrocarbon chains, epoxidation, deamination and dehalogenation, aromatic hydroxylation and *N*-oxidation, as well as *N*-, *O*- and *S*-dealkylation (Sono et al. 1996; Bernhardt 2006). P450s also perform

atypical reactions including cleavage of C–C bonds (Shyadehi et al. 1996), C–C and C–O phenol coupling (Woithe et al. 2007), Baeyer–Villiger oxidation (Isin and Guengerich 2007) and rearrangement reactions (e.g. ring formation and oxidative aryl migration) (Ortiz de Montellano and Nelson 2011). Diverse catalytic reactions performed by P450s suggest that these enzymes are capable of accepting diverse substrates. The last five decades of P450 research revealed that chemical compounds belonging to different categories, such as aliphatic, aromatic, poly/polycyclic-aromatic, poly-chlorinated, and hetero-aromatic compounds, can serve as P450s substrates.

Immense catalytic activities and diverse substrate acceptance of P450s prompted the use of these enzymes as potential catalysts (Grogan 2011) for the production of fine chemicals (Guengerich 2002) pharmaceutical compounds (Ingelman-Sundberg 2004; Guengerich 2006), antibiotics, fragrances and detoxification of carcinogenic and/or mutagenic compounds (Urlacher and Eiben 2006; Urlacher and Girhard 2011). Biotechnological utilization of enzymes on an industrial scale requires robust enzymes that can withstand industrial reaction conditions, including thermostability (Niehaus et al. 1999; Zeikus et al. 1998). However, the thermal and chemical instability of P450 enzymes hinders their practical applications (O'Reilly et al. 2011). Considering the fact that P450s are weak and less stable enzymes, research has focused on identifying thermostable P450s (Nishida and Ortiz de Montellano 2005; Li et al. 2007). Thermostable P450s can serve as a potential biocatalyst for practical industrial applications, such as the synthesis of important organic intermediates. Thermal stability also increases the stability of the protein in hostile environments, such as extremes of pH and organic solvents. Thermostable P450s furthermore give an advantage to setup reaction in a two-liquid phase system where P450 substrates (mostly organic in nature) can react with P450s in the organic phase and products (hydrophilic in nature) can enter into the water phase. This method facilitates the generation and purification of the valuable P450 products in bulk. Successful use of P450s in a two-liquid phase system has been reported (Schula et al. 2005).

To date, only a few thermostable P450s have been described in the literature and most of them are of archaeal origin. The first identified and the best studied thermostable P450 CYP119 was isolated from the acidothermophilic archaeon *Sulfolobus sulfataricus* (Wright et al. 1996; Koo et al. 2000, 2002). The best characterized thermostable P450 of bacterial origin is CYP175A1 from *Thermus thermophilus* (Blasco et al. 2004). In addition to the natural thermostable P450s, a large number of thermostable P450s with new catalytic activities were created using the well-characterized *Bacillus megaterium* P450 CYP102A1 (Li et al. 2007).

No thermostable P450s from other biological kingdoms have been reported. In this paper we report thermostable P450s from the large and diverse lower eukaryotic organisms containing kingdom, fungi. In this study we identified a large number of thermostable P450s of biotechnological potential from the two thermophilic biomass-degrading ascomycetes, *M. thermophila* (*Sporotrichum thermophile*) and *T. terrestris* (Berka et al. 2011).

Materials and methods

Fungal genome data mining for P450s

A search was conducted for publicly available genomes [JGI MycoCosm (Grigoriev et al. 2012)] of the thermophilic biomass-degrading fungi *M. thermophila* (*S. thermophile*) v2.0 (<http://genome.jgi.doe.gov/Spoth2/Spoth2.home.html>) and *T. terrestris* v2.0 (<http://genome.jgi-psf.org/Thite2/Thite2.home.html>) (Berka et al. 2011), using the term “P450”. All the hit protein sequences were downloaded and subjected to ClustalW analysis (multiple and pairwise alignment) using Molecular Evolutionary Genetics Analysis (MEGA 5.0.5) (Tamura et al. 2011). The aligned protein sequences were searched for the presence of the P450 signature motifs, namely the oxygen-binding motif (FXXGXXXCXG) and the heme-binding motif (EXXR). Sequences that showed both conserved P450 signature motifs were retained and the remaining sequences were subjected to BLAST analysis (blastp at NCBI). In BLAST analysis, the sequences that showed homology to P450s were retained and the remaining sequences that showed homology to other protein families were excluded from the analysis. The sequences that showed both P450 conserved signature motifs were regarded as “authentic P450s” and the remaining sequences that showed one of the two conserved domains or showed homology to P450 proteins were grouped as “pseudo-P450s”. Authentic P450s were selected for further analysis.

Annotation and phylogenetic analysis of P450s

Annotation and phylogenetic analysis of P450s were performed in the same way as described by Dr Syed in his recent publication (Syed et al. 2013a). For each of the authentic P450s a homologous P450 was identified by performing BLAST analysis using “against all named P450s” criteria at Cytochrome P450 Homepage (<http://drnelson.uthsc.edu/P450seqs.dbs.html>) (Nelson 2009). Based on the BLAST analysis, a table showing homolog P450 and percentage homology to each of the authentic P450s was constructed (data not shown). Each authentic P450 was assigned a family and subfamily following the

standard P450 nomenclature rule, i.e. >40 % sequence identity for assigning a family and >55 % for a subfamily (Nelson 2004). Any P450 that showed less than 55 % homology was assigned to a new subfamily (NS). Furthermore, the assigned family and subfamily naming for each of the authentic P450s was classified based on their phylogenetic placement (Nelson 2009), i.e. P450s belonging to the same family and subfamily should group together in the phylogenetic tree.

The minimum evolution method (Rzhetsky and Nei 1992) chooses the smallest value of the sum of all branches as an estimate of the tree, resulting in pairing of the true neighbor taxa (e.g. proteins) reflecting the true evolutionary aspect. This method has also been widely used in P450 research for phylogenetic analysis of P450s (Chen et al. 2012). Considering its accuracy and wide use in P450 research, in this study we used the minimum evolution method for phylogenetic analysis of P450s. Minimum evolution method based phylogenetic analysis of P450s was carried out using Molecular Evolutionary Genetics Analysis (MEGA 5.05) software (Tamura et al. 2011). The evolutionary distances were computed using the Poisson correction method (Zuckerandl and Pauling 1965) and are in the units of the number of amino acid substitutions per site. The minimum evolution tree was searched using the close-neighbor-interchange algorithm (Nei and Kumar 2000) at a search level of 1. The neighbor-joining algorithm (Saitou and Nei 1987) was used to generate the initial tree.

Synteny analysis of P450s in thermophilic genomes

The physical localization of P450 genes on the chromosomes was carried out as described elsewhere (Nazir et al. 2010). Chromosomes and their sequences were downloaded from the respective thermophilic fungi databases at JGI. The position of P450s was marked on each chromosome and presented as a synteny figure (Figs. 2, 3). Considering the position of each authentic P450 gene on the chromosome, a synteny picture was generated (Nazir et al. 2010). *Thielavia terrestris* has six chromosomes (Fig. 2) whereas *M. thermophila* has seven (Fig. 3) (Berka et al. 2011).

Ascomycete species P450s for comparative analysis

For comparative P450 analysis, annotated P450 sequences from 14 ascomycete species, *Mycosphaerella fijiensis*, *Uncinocarpus reesii*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Aspergillus clavatus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Neurospora crassa*, *Neurospora discrete*, *Fusarium graminearum* and *Fusarium oxysporum*, were

downloaded from the publicly available P450 database, Cytochrome P450 Homepage (<http://drnelson.uthsc.edu/P450seqs.dbs.html>) (Nelson 2009). Family-level comparative analysis was carried out to check the presence of common and unique P450s in thermophilic biomass-degrading ascomycetes compared to the 14 mesophilic ascomycetes. Family-level comparison was limited to the families identified in *T. terrestris* and *M. thermophila*, considering the ascomycetes P450omes are highly diverse and comprise a large number of P450 families (Nelson 2011).

Measuring P450s thermostability

The aliphatic index (Ikai 1980) is taken as an indicator of the thermostability of P450 proteins. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The index is regarded as a positive factor for the increase of thermostability of globular proteins (Ikai 1980). The aliphatic index of P450 proteins was calculated using the ProtParam programme (Gasteiger et al. 2005) available at ExPASy Bioinformatics Resource Portal (<http://www.expasy.org/>). Each P450 sequence is analyzed individually with ProtParam and P450s showing an aliphatic index of more than 90 °C is selected as thermostable P450s. For comparative analysis of thermostability, homologous P450 proteins from mesophilic ascomycete species were used. Two thermophilic P450 proteins, CYP119 (archaeon) and CYP175A1 (bacterial), are the only P450s that are well-characterized for their biochemical and biophysical properties with respect to thermostability (McLean et al. 1998; Park et al. 2002; Yano et al. 2000, 2003; Blasco et al. 2004). For this reason, in the present study, these P450 proteins were used as reference protein for comparison of thermostable properties.

Measuring P450s melting temperature

Protein melting temperature (T_m) is the temperature at which 50 % of the protein is unfolded. T_m is an inherent property of a protein towards thermal tolerance. P450 proteins' T_m is calculated directly using protein sequences by means of the bioinformatics program T_m predictor available at <http://tm.life.nthu.edu.tw/> (Ku et al. 2009).

Assessing the in vitro stability of P450 proteins

The instability index (II) is used as an indication of the in vitro stability of P450s. The instability index is a measure of proteins, used to determine whether they will be stable in a test tube (Guruprasad et al. 1990). If the index is less than 40, the protein is regarded as stable in the test tube and if it is greater than 40, it is regarded as not stable. The

instability index of P450 proteins was calculated using the ProtParam program (Gasteiger et al. 2005) available at ExPASy Bioinformatics Resource Portal (<http://www.expasy.org/>). The instability index of functionally characterized thermal P450s CYP119 (McLean et al. 1998) and CYP175A1 (Blasco et al. 2004) is used as a reference.

Results and discussion

Fungi represent large and diverse lower eukaryotes. Recent genome sequencing of fungal species revealed the presence of a large number of P450s in their genomes, with some

exceptions (Moktali et al. 2012; Nelson 2011). Functional analysis of fungal P450s showed the presence of P450s displaying extraordinary catalytic activities (Syed et al. 2013b) and recently a fungal P450 has been engineered to oxidize carcinogenic and/or mutagenic compounds (Syed et al. 2013c). However, to date, P450s with a thermostable nature have not been reported from this kingdom. Genome sequencing of thermophilic biomass-degrading ascomycete species *T. terrestris* and *M. thermophila* revealed the presence of thermostable enzymes in their genomes (Berka et al. 2011). In this study we aimed to identify thermostable P450s in the two biomass-degrading ascomycetes *T. terrestris* and *M. thermophila*.

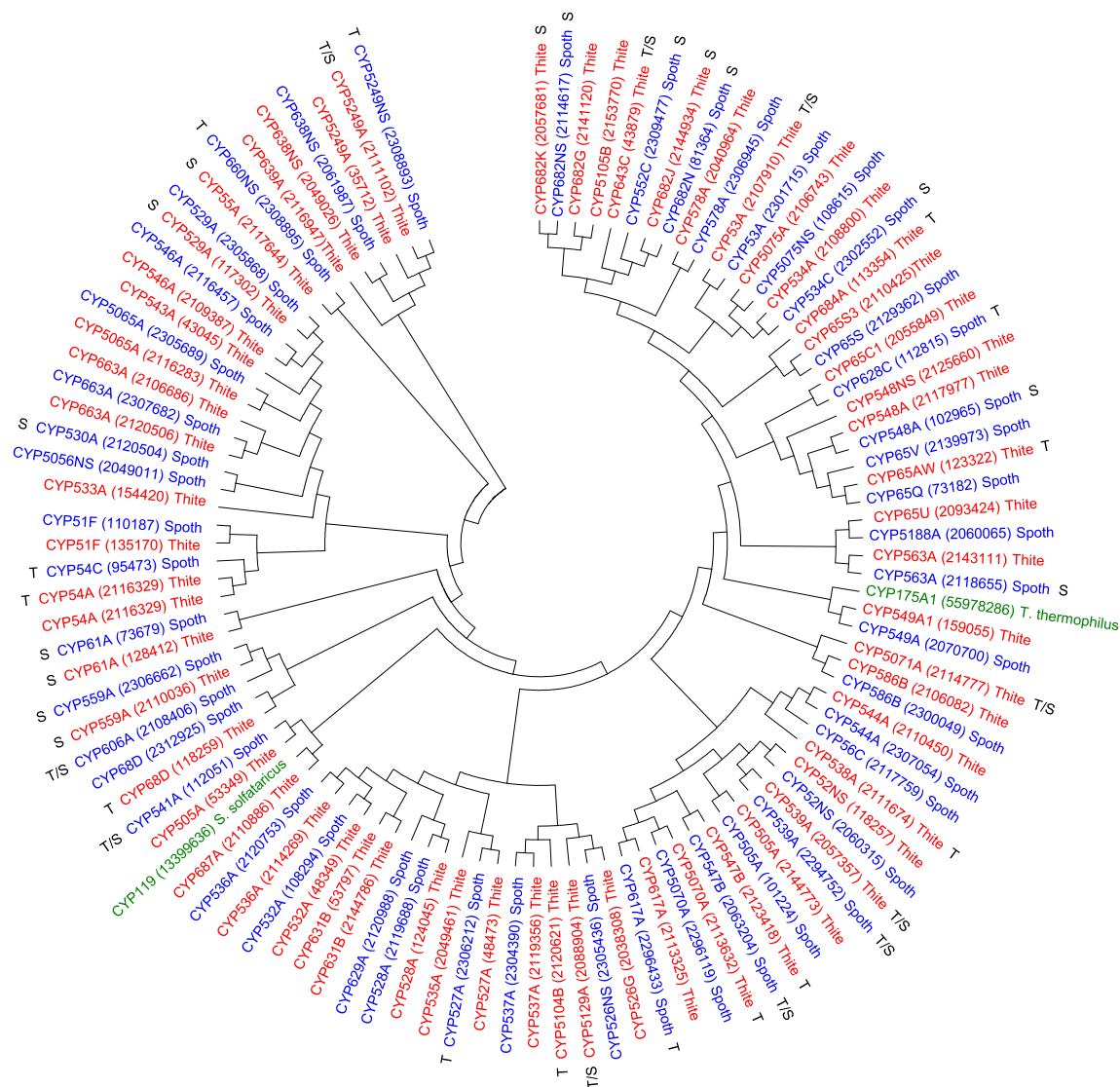
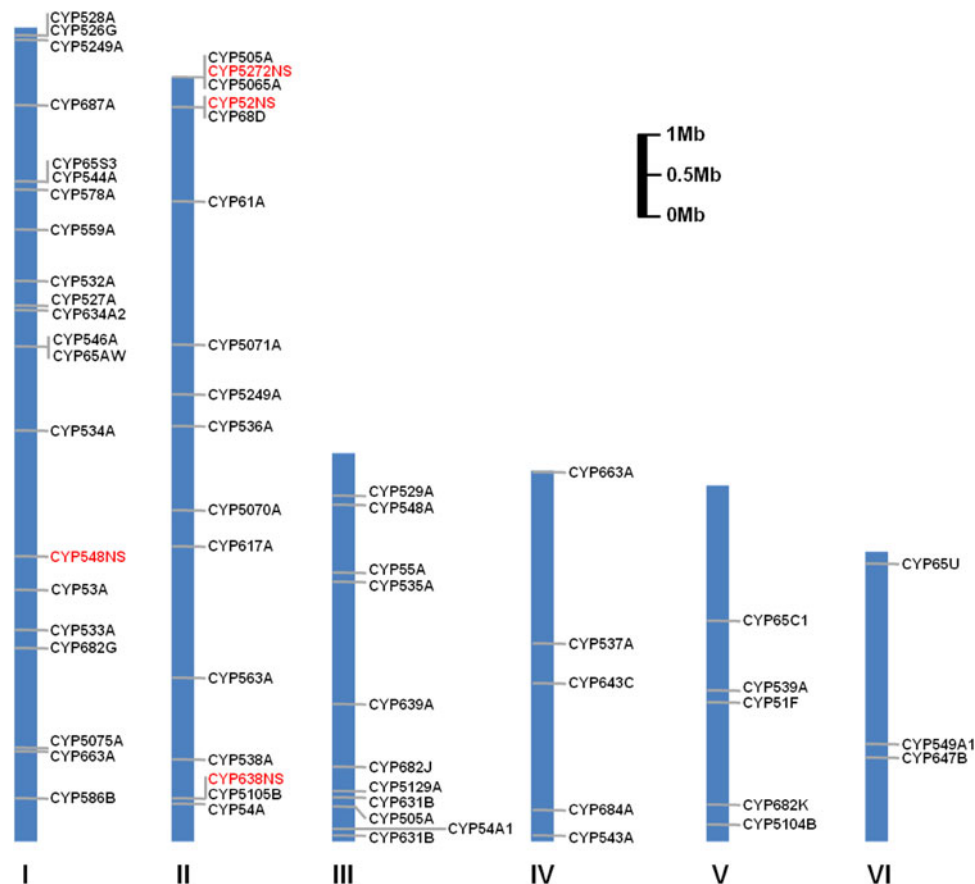


Fig. 1 Phylogenetic analysis of the P450ome of *T. terrestris* (Thite) and *M. thermophila* (Spath). In total 108 P450 sequences from two thermophilic ascomycetes were included in the tree. Thermostable P450s CYP119 and CYP175A1 were also included for evolutionary analysis. A minimal evolution tree was constructed using the close-

neighbor-interchange algorithm in MEGA (version 5.05). For ease of visual identity, P450s along with their protein IDs (parenthesis) were presented in red (*T. terrestris* P450s) and blue (*M. thermophila* P450s). Symbols next to P450s, S and T indicate stable (in vitro in a test tube) P450 and thermostable P450. (Color figure online)

Fig. 2 Chromosomal localization of P450s in *T. terrestris*. New P450 subfamilies identified in this fungus are shown in red font. (Color figure online)



Thielavia terrestris P450ome

Genome-wide search using the term “P450” with gene ontology selection criteria yielded 96 hits. Among the 96 hits, 61 protein sequences showed both P450 signature motifs and were regarded as authentic P450s (Fig. 1; Supplementary Table 1). Eighteen hit sequences showed one of the P450 signature domains or high homology to P450s, hence they were grouped under pseudo-P450s (Supplementary Table 1). The remaining 17 sequences showed homology to non-P450 proteins, hence these were considered as non-P450s. A comprehensive list of *T. terrestris* P450s containing the CYP name, protein ID and location is presented in Supplementary Table 1.

Based on the international P450 nomenclature criteria (Nelson 2004), the authentic 61 P450s were grouped into 50 families and 56 subfamilies (Fig. 1; Supplementary Table 1). One P450 protein (ID: 53349) showed high homology to CYP505 family proteins known as fusion P450s (P450-CPR) (Nakayama et al. 1996). Interestingly, JGI automated annotation did not yield any redox (CPR) domain for this protein. We successfully predicted the redox domain and grouped this protein under the CYP505A1 family. Furthermore, phylogenetic analysis of named P450 sequences revealed family-specific grouping

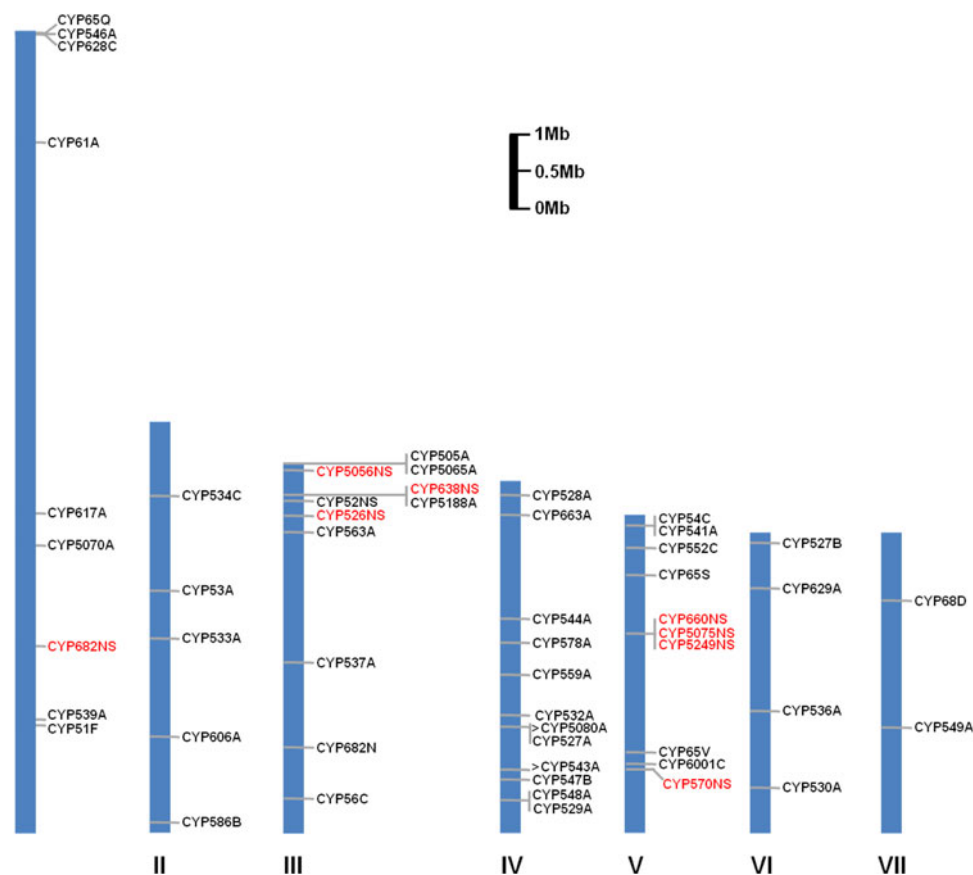
of P450s (Fig. 1), suggesting correct assignment of the family and subfamily to the P450 sequences. Among the 56 subfamilies, *T. terrestris* P450ome showed three new subfamilies. New subfamilies were found in P450 families CYP52, CYP548, CYP638 and CYP5272 (Supplementary Table 1).

Synteny analysis of P450s suggested that the P450 genes are well-distributed among the six chromosomes (Fig. 2). Twenty-one P450s were localized on chromosome I. The four new subfamilies are located on chromosome II (Fig. 2).

Myceliophthora thermophila P450ome

The *M. thermophila* genome showed 88 hits for the term “P450”. Among these 88 hits, 53 sequences were regarded as authentic P450s (Fig. 1; Supplementary Table 2), 17 were pseudo-P450s (Supplementary Table 2) and 18 were non-P450 sequences. According to the international P450 nomenclature criteria, 53 authentic P450s were grouped into 49 families and 53 subfamilies (Fig. 1; Supplementary Table 2). A comprehensive list of *M. thermophila* P450s containing the CYP name, protein ID and location is presented in Supplementary Table 2. *M. thermophila* P450ome showed the presence of eight new subfamilies in the P450

Fig. 3 Chromosomal localization of P450s in *M. thermophila*. New P450 subfamilies identified in this fungus are shown in red font. (Color figure online)



families, CYP526, CYP570, CYP638, CYP660, CYP682, CYP5056, CYP5075 and CYP5249 (Supplementary Table 2). Similar to *T. terrestris*, *M. thermophila* P450 genes are well-distributed among seven chromosomes (Fig. 3). Most of the eight new subfamilies are located on chromosome III and V (Fig. 3). Genome-wide distribution of P450s is considered a characteristic of ascomycetes, as these fungi display fewer P450 gene duplications and high diversity compared to basidiomycetes (Nelson 2011).

Comparative P450ome analysis

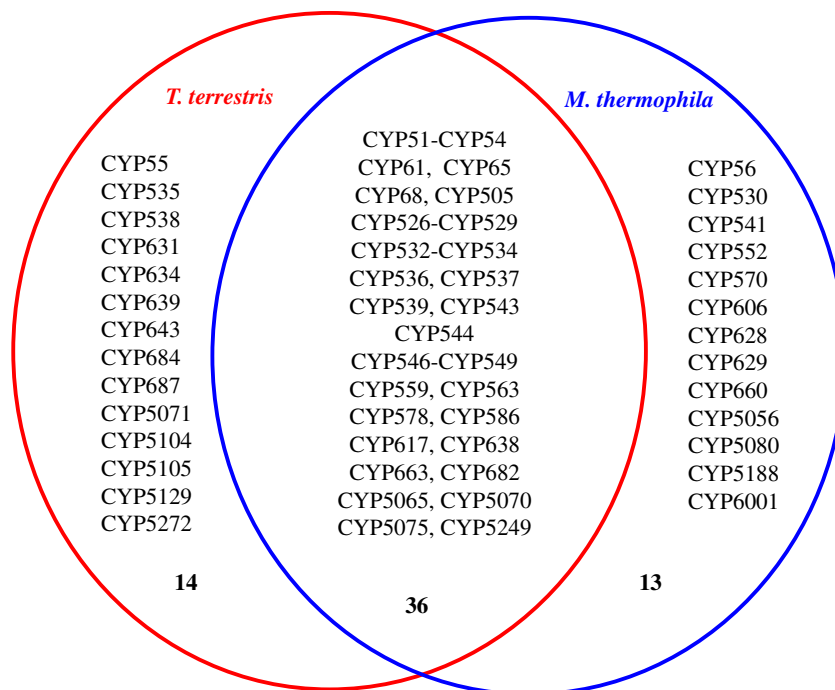
P450ome comparison between two thermophilic biomass degraders revealed the presence of almost the same number of P450s, 79 in *T. terrestris* and 70 in *M. thermophila*. Family-level comparison of P450omes revealed the presence of 36 common P450 families between two thermophile ascomycetes (Fig. 4). The presence of such a large number of P450 families common in both strains is understandable, considering that the thermophilic ascomycetes belong to the same family, i.e. chaetomiaceae. A total of 13 and 14 unique P450 families were found in *M. thermophila* and *T. terrestris* P450omes (Fig. 4). A comprehensive comparison of *T. terrestris* and *M. thermophila* P450omes with 14 mesophilic ascomycete species P450omes (Table 1) revealed that thermophilic ascomycete species P450ome is smaller than the

Fusarium species, *Aspergillus* species and *M. fijiensis*. However, *T. terrestris* and *M. thermophila* P450omes are largest when compared to the P450omes from the *Neurospora* species (*N. crassa* and *N. discrete*) and *U. reesii*, *H. capsulatum*, and *Coccidioides immitis* (Table 1). Family-level P450ome comparison revealed the presence of three unique P450 families CYP563, CYP5071 and CYP5129, in two thermophilic ascomycetes, compared to 14 mesophilic ascomycetes. Interestingly, P450ome of two thermophilic ascomycetes showed diversity in terms of P450 families. *T. terrestris* and *M. thermophila* P450omes showed P450 families that are widespread across the 14 mesophilic ascomycetes (Table 1). For example, P450 families CYP634 and CYP638 found in *Fusarium* species are present in the two thermophile ascomycetes. Apart from the CYP51 and CYP61 P450 families that are well-conserved across fungi, we identified three more families (CYP53, CYP539 and CYP548) conserved across the 16 ascomycetes (Table 1).

Thielavia terrestris and *Myceliophthora thermophila* genomes as a reservoir for thermostable P450s

Genome sequencing analysis of the two thermophilic biomass-degrading ascomycetes *T. terrestris* and *M. thermophila* revealed the presence of a large number of thermostable enzymes (Berka et al. 2011). However, in this

Fig. 4 Family-level comparative analysis of the P450omes of *T. terrestris* and *M. thermophila*. Numbers in boldface indicate the number of P450 families



study the cytochrome P450 enzyme superfamily is completely disregarded. Considering the thermophilic nature and identification of thermostable enzymes (Berka et al. 2011), one can expect the presence of thermostable P450s in the two thermophiles. In this study, we investigated the thermophilic ascomycetes P450omes for thermostable P450s. We followed two criteria to select thermostable P450s, i.e. aliphatic index and T_m .

Analysis of the aliphatic index for two thermophilic ascomycete P450omes revealed the presence of a large number of thermostable P450s (Table 2). Based on the aliphatic index cut-off (>90) we found 14 and 11 P450s as thermostable P450s in *T. terrestris* and *M. thermophila* (Table 2). All selected thermostable P450s showed a higher aliphatic index compared to the P450s from mesophilic ascomycetes (Table 2), suggesting that the identified P450s are thermostable. Furthermore, to authenticate the identified thermostable P450s, the aliphatic index of the ascomycete P450s was compared with thermostable P450s from bacteria (CYP175A1) and archaea (CYP119). Three P450s, CYP5129A1 (100.43), CYP5071A (96.83) and CYP547 (102.34) from *T. terrestris*, had a higher aliphatic index compared to CYP119 (aliphatic index: 96.63). *M. thermophila* P450ome featured four P450s, CYP547B (101.3), CYP606A (97.49), CYP54C (98.56) and CYP61 (96.74), with a higher aliphatic index compared to CYP119. In comparison to CYP175A1's aliphatic index (97.20), two and three P450s from *T. terrestris* and *M. thermophila* had a higher aliphatic index (Table 2). This is the first report on the identification of thermostable P450s enzymes from fungi with an aliphatic index higher than the

well-characterized thermostable P450s CYP175A1 and CYP119.

T_m is an inherent property of a protein and it indicates the thermal tolerance of the protein. A higher T_m improves thermal tolerance. According to the measurement (Ku et al. 2009), a T_m value <55 °C indicates non-thermostable protein, a T_m value between 55 and 65 °C indicates thermostable protein and a T_m value >65 °C indicates hyper-thermostable protein (Ku et al. 2009). An analysis of T_m for the identified thermostable P450s of the two ascomycetes showed that all of the P450s (except CYP538A1 from *T. terrestris*) have a T_m value in the range of thermostable protein (55–65 °C) or hyper-thermostable protein (>65 °C) (Table 2). In order to authenticate the T_m value, we also measured the T_m for CYP119 and CYP175A1. As shown in Table 2, CYP119 and CYP175A1 showed a T_m between 55 and 65 °C and above 65 °C. This indicates that the software program prediction of T_m value is accurate. Based on a T_m of above 65 °C (the same observed for CYP175A1), we conclude that six thermostable P450s from *T. terrestris* and three thermostable P450s from *M. thermophila* are hyper-thermostable P450s (Table 2).

Thielavia terrestris and *Myceliophthora thermophila* genomes are reservoirs for in vitro stable P450s

In order to identify stable P450s, two ascomycetes P450omes were subjected to instability index measurement (Guruprasad et al. 1990). The instability index is a direct measure of protein stability under in vitro conditions, such as in a test tube. It is based on the dipeptide composition of

Table 1 Comparative analysis of the P450omes of thermophilic and mesophilic ascomycetes

P450 family	Thermophilic pezizomycetes		Mesophilic pezizomycetes													
	TT	MT	MF	UR	HC	CI	AC	AN	AF	AO	AT	AFu	NC	ND	FG	FO
CYP51	1	1	1	2	2	1	2	2	4	3	3	2	1	1	3	3
CYP52	1	1	1	1			2	5	4	5	4	3				
CYP53	1	1	1	1		1	1	1	1	2	1	1	1	1	3	3
CYP54	2	1											1	1	1	1
CYP55	1			1	1			1	1		1		1	1	1	4
CYP56		1		2		1	1		1	1						
CYP61	1	1	1	1	1	1	2	2	2	2	3	1		1	2	2
CYP65	4	3	2		7	1	5	14	9	10	8	3	2	2	4	2
CYP68	1	1			2		1	3	1	2	3	1	1	1	5	2
CYP505	2	1	3				2	3	4	3	2	1	1	1	2	4
CYP526	1	1									1		1	1	2	1
CYP527	1	2						1					2	2	1	3
CYP528	1	1	1										1	2	1	
CYP529	1	1											1	1		
CYP530		1		1		1					1	1	1	1	1	1
CYP532	1	1	1					2	1	1	1		1	1	4	3
CYP533	1	1											1	1		
CYP534	1	1	1										1	1	1	
CYP535	1								1	1			1	1		
CYP536	1	1	1										1	1		
CYP537	1	1							1	1	1		1		1	2
CYP538	1												1	1		
CYP539	1	1	1	1	2	1	2	1	1		3	2	1	1	2	1
CYP541		1					1	1	1	1	1	1	1	1		
CYP543	1	1	1										1	1		
CYP544	1	1											1	1	1	1
CYP546	1	1											1	1	1	
CYP547	1	1	1					1	1	1	2	1	1	1	1	1
CYP548	2	1	3	2	1	2	4	6	2	3	3	1	1	1	2	6
CYP549	1	1											1	1		
CYP552		1							1		1		1	1	3	3
CYP559	1	1	2										1	1		1
CYP563	1	1														
CYP570		1	2	1		1									3	5
CYP578	1	1	3	2	1	2	2	3	3	2	2	2				2
CYP586	1	1			1			2			1	1				
CYP606		1					1				1				1	1
CYP617	1	1	1	1			5	3	1	1	1	3			3	3
CYP628		1			1			1	1	1	1				1	4
CYP629		1			1										1	1
CYP631	2								2	1					1	
CYP634	1														1	1
CYP638	1	1			1										1	1
CYP639	1														1	1
CYP643	1							1	1						4	1
CYP660		1				1	1	1	2	2	1	1				
CYP663	2	1	1				1	1	1		1					

Table 1 continued

P450 family	Thermophilic pezizomycetes		Mesophilic pezizomycetes														
	TT	MT	MF	UR	HC	CI	AC	AN	AF	AO	AT	AFu	NC	ND	FG	FO	
CYP682	3	2	1	4	1	6	2	2	3	2	2	1					1
CYP684	1		1					2	1	1	3						1
CYP687	1								1								
CYP5056		1															
CYP5065	1	1															1
CYP5070	1	1							1	1							
CYP5071	1																
CYP5075	1	1						1	2	2							
CYP5080		1															
CYP5104	1					1		2			1						
CYP5105	1							1	1								
CYP5129	1																
CYP5188		1	1														
CYP5249	2	1			1												
CYP5272	1							1								1	
CYP6001		1	1		1	1	2	2	3		3					1	1
Total no. of P450s in the genome	61	53	89	38	47	40	91	154	162	142	124	74	41	43	109	140	

P450omes of the thermophilic ascomycetes *T. terrestris* (TT) and *M. thermophila* (MT) were compared with P450omes of the 14 mesophilic ascomycete species, *Mycosphaerella fijiensis* (MF), *Uncinocarpus reesii* (UR), *Histoplasma capsulatum* (HC), *Coccidioides immitis* (CI), *Aspergillus clavatus* (AC), *Aspergillus niger* (AN), *Aspergillus flavus* (AF), *Aspergillus oryzae* (AO), *Aspergillus terreus* (AT), *Aspergillus fumigatus* (AFu), *Neurospora crassa* (NC), *Neurospora discrete* (ND), *Fusarium graminearum* (FG) and *Fusarium oxysporum* (FO). P450s belong to CYP6001 family was found in different strains of the same species. Hence, as a representative, the CYP6001 count is included in the species. Considering the presence of a large number of P450 families in ascomycetes P450omes (Nelson 2011) the comparison is limited to P450 families present in thermophilic ascomycetes. For comparative purposes the total number of P450s present in each species is also shown at the bottom of the table

a protein. An instability index of less than 40 indicates that a protein is stable and higher than 40 indicates that the protein is unstable in a test tube. Analysis of two ascomycete P450omes revealed the presence of a large number of stable P450s (Table 3). Twelve P450s from *T. terrestris* and 19 P450s from *M. thermophila* had an instability index of less than 40, suggesting their high stability in a test tube (Table 3). The software predictions were authenticated by measuring the instability index of CYP119 and CYP175A1 (Table 2). Both P450s had an instability index lower than 40. This suggests that the software predictions are accurate and the stable P450s that were identified are possibly true stable proteins. CYP643C from *T. terrestris* had the lowest instability index (31.62) compared to the stable P450s identified in two ascomycetes and CYP119 and CYP175A1, suggesting that this P450 is highly stable.

Overall, considering the thermostability and instability index, a total of 10 P450s, six P450s from *T. terrestris* and four from *M. thermophila* (Fig. 1) are biotechnologically valuable, as they showed both thermal and in vitro stability. Furthermore, five P450s showed highest thermal tolerance (Table 2) and five showed the highest in vitro stability (Table 3) in comparison to the well-characterized CYP119 and CYP175A1, suggesting

these P450s are of potential interest from a structural, mechanistic and biotechnological point of view.

Functional analysis of thermophilic ascomycete P450s

Based on the homologous P450s in other fungi, the functional data of some of the thermophilic ascomycetes P450s can be predicted. CYP51 and CYP61 perform lanosterol-14 α -demethylation (Lepesheva and Waterman 2004) and C-22 sterol desaturase activity (Kelly et al. 1995) in cell wall ergosterol biosynthesis. CYP52 oxidizes *n*-alkanes and fatty acids (Van Bogaert et al. 2011) and CYP53 is involved in xenobiotic compound benzoate hydroxylation (Faber et al. 2001). CYP55 is a nitric oxide reductase that performs the denitrification step in the global nitrogen cycle (Tomura et al. 1994). CYP56 synthesizes *N*, *N'*-bisformyl dityrosine, a component of the outer spore wall layer, by joining the two molecules of *N*-formyl tyrosine (Melo et al. 2008). CYP65, CYP68 and CYP526 are involved in trichothecenes, a group of sesquiterpens (mycotoxins) biosynthesis (reviewed in Kimura et al. 2007). CYP68 is also involved in plant hormone gibberellin biosynthesis (Rojas et al. 2001). CYP532 and CYP535 perform pisatin demethylation, an important

Table 2 Analysis of thermostability of the P450omes of *T. terrestris* and *M. thermophila*

P450	Aliphatic index	Melting temperature (T _m)		Homolog P450 from mesophilic ascomycetes	
		T _m index	Predicted T _m (°C)	Aliphatic index	P450 and species name
<i>T. terrestris</i>					
CYP538A	92.67	−0.1	<55	87.72	CYP538A1 NC
CYP539A	91.17	0.6	55–65	86.63	CYP539A8P AT
CYP643C	93.35	1.4	>65	89.09	CYP643C2 AF
CYP684A	92.63	0.7	55–65	83.71	CYP684A4 AT
CYP5070A	90.83	0.1	55–65	83.37	CYP5070A1 AF
CYP5129A	100.43	1.21	>65	96.08	CYP5129A PN
CYP65AW	92.64	0.78	55–65	88.00	CYP65AW1 FO
CYP53A	95.1	0.8	55–65	85.13	CYP53A1 AN
CYP5249A	91.09	1.17	>65	98.91	CYP5249A HC
CYP5104B	91.33	–	<55	86.22	CYP5104B1 AN
CYP68D	92.31	0.5	55–65	88.31	CYP68D3 AN
CYP5071A	96.83	1.2	>65	88.53	CYP5071A FG
CYP547B	102.34	1.61	>65	100.8	CYP547B3 FO
CYP54A	94.57	1.1	>65	86.05	CYP54A1 ND
<i>M. thermophila</i>					
CYP628C	93.3	0.5	55–65	89.66	CYP628C1 AF
CYP617A	91.12	0.5	55–65	85.34	CYP617A3 FO
CYP547B	101.3	1.07	>65	78.66	CYP547A5 FO
CYP539A	92.33	0.8	55–65	86.63	CYP539A8P AT
CYP5249NS	92.78	1.5	>65	99.84	CYP5249A1P HC
CYP541A	91.88	1.0	55–65	92.89	CYP541A1 NC
CYP606A	97.49	0.7	55–65	86.23	CYP606A3P AT
CYP527A	93.25	0.7	55–65	81.03	CYP527A1 ND
CYP54C	98.56	0.96	55–65	86.88	CYP54C5 FO
CYP660NS	92.21	0.7	55–65	88.24	CYP660A4 AT
CYP61A	96.74	1.3	>65	83.01	CYP61A1 AT
<i>T. thermophilus</i>					
CYP175A1	97.20	1.39	>65		
<i>S. Solfataricus</i>					
CYP119 ^a	96.63	0.94	55–65		

Two parameters, viz. aliphatic index and protein melting temperature (T_m), were used to measure the thermostability of P450. P450s that had an aliphatic index of >90 (indicates thermostable P450) were shown in the table along with their T_m. Thermostable P450s' aliphatic index is compared with their homologous P450s from mesophilic ascomycetes (presented as reference species). Ascomycete thermostable P450s' aliphatic index and T_m were also compared with thermostable P450s CYP119 of *S. Solfataricus* and CYP175A1 of *T. thermophilus*. The aliphatic index and T_m are calculated as described in “Materials and methods” section

Abbreviations: PN: *Phaeosphaeria nodorum*; HC: *Histoplasma capsulatum*; AN: *Aspergillus niger*; AF: *Aspergillus flavus*; AT: *Aspergillus terreus*; NC: *Neurospora crassa*; ND: *Neurospora discrete*; FG: *Fusarium graminearum*; FO: *Fusarium oxysporum*; NS: new subfamily

^a The difference in T_m value predicted by the T_m calculator and wet laboratory experiment (McLean et al. 1998; Yano et al. 2000) is due to the fact that the T_m predictor considers the primary sequence of the protein for measuring the thermostability. The wet laboratory experiment performed using purified protein involves a three-dimensional structure where the critical amino acids responsible for proteins' thermostability form a protective fold and increase its thermostability. This is the critical factor resulting in an increase of T_m in wet laboratory conditions. Despite the difference, the T_m predictor places CYP119 under thermostable P450s

reaction in the detoxification of antifungal toxicants (Maloney and VanEtten 1994). CYP682 is a phenol oxidase involved in the biosynthesis of conidiophore pigment (McCorkindale et al. 1983). The P450–CPR fusion protein (CYP505) was found to hydroxylate fatty acids (Nakayama

et al. 1996), and also to be involved in the synthesis of the mycotoxin fumonisin (Proctor et al. 2003). CYP6001 family P450s are involved in the synthesis of hormone-like, fatty acid-derived oxylipins collectively called “psi” (precocious sexual inducer) signaling factors (Brodhun et al. 2009).

Table 3 Analysis of in vitro stability of the P450omes of *T. terrestris* and *M. thermophila*

P450	Instability index (II)
<i>T. terrestris</i>	
CYP682K	37.99
CYP539A	34.35
CYP643C	31.62
CYP682J	38.57
CYP5129A	36.72
CYP534A	39.17
CYP5249A	37.75
CYP529A	37.99
CYP55A	38.49
CYP559A	35.68
CYP61A	39.73
CYP5071A	39.12
<i>S. solfataricus</i>	
CYP119	39.28
<i>T. thermophilus</i>	
CYP175A1	34.55
<i>M. thermophila</i>	
CYP682N	34.65
CYP534C	33.49
CYP682NS	33.75
CYP547B	35.5
CYP61A	37.23
CYP552C	34.35
CYP548A	37.02
CYP563A	34.39
CYP539A	37.7
CYP541A	36.88
CYP530A	37.96
CYP606A	36.54
CYP559A	39.91
CYP5217A	39.68
CYP52A	38.28
CYP56E	38.51
CYP61A	36.79
CYP533A	38.25
CYP570NS	36.51

The stability of P450s was measured using the Instability index (II) as described in “Materials and methods” section. P450s with an instability index lower than 40 (considered as stable protein) are shown in the table. The instability index of thermostable P450s, CYP119 of *S. solfataricus* and CYP175A1 of *T. thermophilus* is taken as reference
Abbreviation: NS: new subfamily

Transcriptome profiling showed up regulation of several P450s during the fungal growth on different straws as carbon source (Berka et al. 2011). CYP5075NS, CYP586B, CYP526NS, CYP5065A, CYP663A, CYP5249NS and

CYP660NS were up-regulated during *M. thermophila* growth on alfalfa straw compared to growth on glucose (Berka et al. 2011). CYP663A also showed up-regulation in response to barley straw. *T. terrestris* P450s, CYP586B and CYP5065A showed up-regulation during growth on alfalfa straw and barley straw compared to growth on glucose. CYP5075A was up-regulated during *T. terrestris* growth on barley straw. Up-regulation of P450s during fungal growth on a straw carbon source suggests the potential role of P450s in wood degradation. However, the specific role(s) of P450s in wood degradation is yet to be identified. Among the P450s (function predicted/known), CYP52A1 from *M. thermophila* is of potential interest, as this P450s showed in vitro stability (Table 3) and can be used for the production of dicarboxylic acids, compounds of potential commercial value (Picataggio et al. 1992).

In this genomic era, bioinformatic programs have become critical in sorting enzymes of potential biotechnological value in an easy and accurate manner. This minimizes laborious and costly laboratory methods of screening etc. Overall, bioinformatic programs are useful to sort potential biotechnologically valuable candidates, but further validation needs wet laboratory experimentation. In this study we performed systematic genome-wide P450 analysis in the two thermophilic biomass-degrading ascomycetes, *T. terrestris* and *M. thermophila*, and identified thermostable P450s using different bioinformatics programs. Based on the aliphatic index, protein melting temperature (T_m) and instability index (II) we identified a large number of thermostable and in vitro stable P450s from two thermophilic ascomycetes. Our results were authenticated by using two well-characterized thermostable P450s, CYP119 and CYP175A1. Six P450s from *T. terrestris* and four from *M. thermophila* are biotechnologically valuable, considering their thermal and in vitro stability. Future work includes experimental characterization of *in silico* predicted thermostable P450s. Furthermore, identified fungal thermostable P450s (this study) can serve as novel tools for understanding the structural and mechanical aspects of protein thermostability.

Acknowledgments Syed thanks the Central University of Technology (CUT) for a grant from the University Research and Innovation Fund. Shale thanks the Medical Research Council (MRC) of South Africa. Krasevec thanks the Slovenian Research Agency Program P1-0104: Functional genomics and biotechnology for health. The authors thank Ms Barbara Bradley, Pretoria, South Africa for English language editing.

Conflict of interest The authors declare that there are no conflict of interest.

References

- Berka RM, Grigoriev IV, Otilar R, Salamov A, Grimwood J, Reid I, Ishmael N, John T, Darmond C, Moisan MC et al (2011) Comparative genomic analysis of the thermophilic biomass-

- degrading fungi *Myceliophthora thermophila* and *Thielavia terrestris*. *Nat Biotechnol* 29:922–927
- Bernhardt R (2006) Cytochromes P450 as versatile biocatalysts. *J Biotechnol* 124:128–145
- Blasco F, Kauffmann I, Schmid RD (2004) CYP175A1 from *Thermus thermophilus* HB27, the first β -carotene hydroxylase of the P450 superfamily. *Appl Microbiol Biotechnol* 64:671–674
- Brodhun F, Göbel C, Hornung E, Feussner I (2009) Identification of PpoA from *Aspergillus nidulans* as a fusion protein of a fatty acid heme dioxygenase/peroxidase and a cytochrome P450. *J Biol Chem* 284:11792–11805
- Chen S, Xu J, Liu C, Zhu Y, Nelson DR, Zhou S, Li C, Wang L, Guo X, Sun Y et al (2012) Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. *Nat Commun* 3:913
- Faber BW, van Gorcom RF, Duine JA (2001) Purification and characterization of benzo *para*-hydroxylase, a cytochrome P450 (CYP53A1), from *Aspergillus niger*. *Arch Biochem Biophys* 394:245–254
- Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A (2005) Protein identification and analysis tools on the ExPASy server. In: Walker JM (ed) *The proteomics protocols handbook*. Humana Press, Totowa, pp 571–607
- Grigoriev IV, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kuo A, Minovitsky S, Nikitin R, Ohm RA et al (2012) The genome portal of the Department of Energy Joint Genome Institute. *Nucleic Acids Res* 40:D26–D32
- Grogan G (2011) Cytochrome P450: exploiting diversity and enabling application as biocatalysts. *Curr Opin Chem Biol* 15:241–248
- Guengerich FP (2002) Cytochrome P450 enzymes in the generation of commercial products. *Nat Rev Drug Discov* 1:359–366
- Guengerich FP (2006) Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS J* 8:E101–E111
- Guruprasad K, Reddy BV, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Eng* 4:155–161
- Ikai A (1980) Thermostability and aliphatic index of globular proteins. *J Biochem* 88:1895–1898
- Ingelman-Sundberg M (2004) Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol Sci* 25:193–200
- Isin EM, Guengerich FP (2007) Complex reactions catalyzed by cytochrome P450 enzymes. *Biochim Biophys Acta* 1770:314–329
- Kelly SL, Lamb DC, Corran AJ, Baldwin BC, Parks LW, Kelly DE (1995) Purification and reconstitution of activity of *Saccharomyces cerevisiae* P450 61, a sterol Δ^{22} -desaturase. *FEBS Lett* 377:217–220
- Kimura M, Tokai T, Takahashi-Ando N, Ohsato S, Fujimura M (2007) Molecular and genetic studies of *Fusarium trichothecene* biosynthesis: pathways, genes, and evolution. *Biosci Biotechnol Biochem* 71:2105–2123
- Koo LS, Tschirret-Guth RA, Straub WE, Moënn-Loccoz P, Loehr TM, Ortiz de Montellano PR (2000) The active site of the thermophilic CYP119 from *Sulfolobus solfataricus*. *J Biol Chem* 275:14112–14123
- Koo LS, Immoos CE, Cohen MS, Farmer PJ, Ortiz de Montellano PR (2002) Enhanced electron transfer and lauric acid hydroxylation by site-directed mutagenesis of CYP119. *J Am Chem Soc* 124:5684–5691
- Ku T, Lu P, Chan C, Wang T, Lai S, Lyu P, Hsiao N (2009) Predicting melting temperature directly from protein sequences. *Comput Biol Chem* 33:445–450
- Lepesheva GI, Waterman MR (2004) CYP51—the omnipotent P450. *Mol Cell Endocrinol* 215:165–170
- Li Y, Drummond DA, Sawayama AM, Snow CD, Bloom JD, Arnold FH (2007) A diverse family of thermostable cytochrome P450s created by recombination of stabilizing fragments. *Nat Biotechnol* 25:1051–1056
- Maloney AP, VanEtten HD (1994) A gene from the fungal plant pathogen *Nectria haematococca* that encodes the phytoalexin-detoxifying enzyme pisatin demethylase defines a new cytochrome P450 family. *Mol Gen Genet* 243:506–514
- McCorkindale NJ, Hayes D, Johnston GA, Clutterbuck AJ (1983) *N*-acetyl-6-hydroxytryptophan—a natural substrate of monophenoloxidase from *Aspergillus nidulans*. *Phytochemistry* 22:1026–1028
- McLean MA, Maves SA, Weiss KE, Krepich S, Sligar SG (1998) Characterization of a cytochrome P450 from the acidothermophilic archaea *Sulfolobus solfataricus*. *Biochem Biophys Res Commun* 252:166–172
- Melo NR, Moran GP, Warrilow AG, Dudley E, Smith SN, Sullivan DJ, Lamb DC, Kelly DE, Coleman DC, Kelly SL (2008) CYP56 (Dit2p) in *Candida albicans*: characterization and investigation of its role in growth and antifungal drug susceptibility. *Antimicrob Agents Chemother* 52:3718–3724
- Moktali V, Park J, Fedorova-Abrams ND, Park B, Choi J, Lee YH, Kang S (2012) Systematic and searchable classification of cytochrome P450 proteins encoded by fungal and oomycete genomes. *BMC Genomics* 13:525
- Nakayama N, Takemae A, Shoun H (1996) Cytochrome P450foxy, a catalytically self-sufficient fatty acid hydroxylase of the fungus *Fusarium oxysporium*. *J Biochem* 119:435–440
- Nazir KHMNH, Ichinose H, Wariishi H (2010) Molecular characterization and isolation of cytochrome P450 genes from the filamentous fungus *Aspergillus oryzae*. *Arch Microbiol* 192:395–408
- Nei M, Kumar S (2000) *Molecular evolution and phylogenetics*. Oxford University Press, New York
- Nelson DR (2004) Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* 14:1–18
- Nelson DR (2009) The cytochrome P450 homepage. *Hum Genomics* 4:59–65
- Nelson DR (2011) Progress in tracing the evolutionary paths of cytochrome P450. *Biochim Biophys Acta* 1814:14–18
- Nelson DR (2013) A world of cytochrome P450s. *Philos Trans R Soc B Biol Sci*. doi:10.1098/rstb.2012.0430
- Niehaus F, Bertoldo C, Kahler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. *Appl Microbiol Biotechnol* 51:711–729
- Nishida CR, Ortiz de Montellano PR (2005) Thermophilic cytochrome P450 enzymes. *Biochem Biophys Res Commun* 338:437–445
- O'Reilly E, Köhler V, Flitsch SL, Turner NJ (2011) Cytochromes P450 as useful biocatalysts: addressing the limitations. *Chem Commun (Camb)* 47:2490–2501
- Ortiz de Montellano PR, Nelson SD (2011) Rearrangement reactions catalyzed by cytochrome P450s. *Arch Biochem Biophys* 507:95–110
- Park SY, Yamane K, Adachi S, Shiro Y, Weiss KE, Maves SA, Sligar SG (2002) Thermophilic cytochrome P450 (CYP119) from *Sulfolobus solfataricus*: high resolution structure and functional properties. *J Inorg Biochem* 91:491–501
- Picataggio S, Rohrer T, Deanda K, Lanning D, Reynolds R, Mielenz J, Eirich LD (1992) Metabolic engineering of *Candida tropicalis* for the production of long-chain dicarboxylic acids. *Bio/Technology* NY 10:894–898
- Proctor RH, Brown DW, Plattner RD, Desjardins AE (2003) Co-expression of 15 contiguous genes delineates a fumonisins biosynthetic gene cluster in *Gibberella moniliformis*. *Fungal Genet Biol* 38:237–249

- Rojas MC, Hedden P, Gaskin P, Tudzynski B (2001) The P450-1 gene of *Gibberella fujikuroi* encodes a multifunctional enzyme in gibberellin biosynthesis. *Proc Natl Acad Sci USA* 98:5838–5843
- Rzhetsky A, Nei M (1992) A simple method for estimating and testing minimum evolution trees. *Mol Biol Evol* 9:945–967
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Schula F, Leca F, Hollmann F, Reetz MT (2005) Towards practical Baeyer-Villiger-Monooxygenases: applying a thermostable enzyme in the gram-scale synthesis of optically active lactones in a two-liquid-phase system. *Beilstein J Org Chem* 1:10
- Shyadehi AZ, Lamb DC, Kelly SL, Kelly DE, Schunck WH, Wright JN, Corina D, Akhtar M (1996) The mechanism of the acyl-carbon bond cleavage reaction catalyzed by recombinant sterol 14 alpha-demethylase of *Candida albicans* (other names are: lanosterol 14 alpha-demethylase, P-45014DM, and CYP51). *J Biol Chem* 271:12445–12450
- Sono M, Roach MP, Coulter DE, Dawson JH (1996) Heme-containing oxygenases. *Chem Rev* 96:2841–2887
- Syed K, Nelson DR, Riley R, Yadav JS (2013a) Genomewide annotation and comparative genomics of cytochrome P450 monooxygenases (P450s) in the polypore species *Bjerkandera adusta*, *Ganoderma sp.* and *Phlebia breviaspora*. *Mycologia* 105:1445–1455
- Syed K, Porollo A, Lam YW, Grimmer PE, Yadav JS (2013b) CYP63A2, a catalytically versatile fungal P450 monooxygenase capable of oxidizing higher-molecular-weight polycyclic aromatic hydrocarbons, alkylphenols, and alkanes. *Appl Environ Microbiol* 79:2692–2702
- Syed K, Porollo A, Miller D, Yadav JS (2013c) Rational engineering of the fungal P450 monooxygenase CYP5136A3 to improve its oxidizing activity toward polycyclic aromatic hydrocarbons. *Protein Eng Des Sel* 26:553–557
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Tomura D, Obika K, Fukamizu A, Shoun H (1994) Nitric oxide reductase cytochrome P-450 gene, CYP55, of the fungus *Fusarium oxysporum* containing a potential binding-site for FNR, the transcription factor involved in the regulation of anaerobic growth of *Escherichia coli*. *J Biochem* 116:88–94
- Urlacher VB, Eiben S (2006) Cytochrome P450 monooxygenases: perspectives for synthetic application. *Trends Biotechnol* 24:324–330
- Urlacher VB, Girhard M (2011) Cytochrome P450 monooxygenases: an update on perspectives for synthetic application. *Trends Biotechnol* 30:26–36
- Van Bogaert INA, Groeneboer S, Saerens K, Soetaert W (2011) The role of cytochrome P450 monooxygenases in microbial fatty acid metabolism. *FEBS J* 278:206–221
- Woithe K, Geib N, Zerbe K, Li DB, Heck M, Fournier-Rousset S, Meyer O, Vitali F, Matoba N, Abou-Hadeed K, Robinson JA (2007) Oxidative phenol coupling reactions catalyzed by OxyB: a cytochrome P450 from the vancomycin producing organism. *J Am Chem Soc* 129:6887–6895
- Wright RL, Harris K, Solow B, White RH, Kennelly PJ (1996) Cloning of a potential cytochrome P450 from the archaeon *Sulfolobus solfataricus*. *FEBS Lett* 384:235–239
- Yano JK, Koo LS, Schuller DJ, Li H, Ortiz de Montellano PR, Poulos TL (2000) Crystal structure of a thermophilic cytochrome P450 from the archaeon *Sulfolobus solfataricus*. *J Biol Chem* 275:31086–31092
- Yano JK, Blasco F, Li H, Schmid RD, Henne A, Poulos TL (2003) Preliminary characterization and crystal structure of a thermostable cytochrome P450 from *Thermus thermophilus*. *J Biol Chem* 278:608–616
- Zeikus JG, Vieille C, Savchenko A (1998) Thermozyms: biotechnology and structure–function relationships. *Extremophiles* 2:179–183
- Zuckerklund E, Pauling L (1965) Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ (eds) *Evolving genes and proteins*. Academic Press, New York, pp 97–166