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Comparative Genomics and Evolutionary Analysis of Cytochrome P450 Monooxygenases in Fungal Subphylum Saccharomycotina

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Cytochrome P450 monooxygenases (P450s) are heme-thiolate enzymes and play an important role in the primary and secondary metabolism of living organisms. Genome sequencing analysis of fungal organisms revealed the presence of numerous P450s in their genomes, with few exceptions. P450s in the fungal subphylum Saccharomycotina, which contains biotechnologically important and opportunistic human pathogen yeasts, have been underexplored because there are few P450s in their genomes. In the present study we performed comparative analysis of P450s in 25 yeast species. A hundred and seventy-two P450s were found in 25 yeast species and these are grouped into 13 P450 families and 27 subfamilies. P450s ranged from a minimum of three (Saccharomyces species) to a maximum of 21 (Candida species) in the yeast genomes. Among the P450 families, the CYP52 family showed the highest number of member P450s (71) followed by CYP51 (27), CYP61 (25), CYP56 (20) and CYP501 (11). *Pichia pastoris* and *Dekkera bruxellensis* showed a novel P450 family, CYP5489, in their genome. Based on the functional properties of characterized P450s, we conclude that P450s in Saccharomycotina species possibly play a role in organisms' physiology either in the synthesis of cellular components or in the utilization of simpler organic molecules. The ecological niches of yeast species are highly enriched with simpler organic nutrients and it is well known that yeast species utilize simpler organic nutrients as carbon source efficiently. This might have played a role in compacting yeast genomes and possibly losing a considerable number of P450s during evolution.

Key words: Adaptation, Comparative genomics, Cytochrome P450 monooxygenases, Ecological niches, Evolution, Fungi, Saccharomycotina, Yeast.

The fungal kingdom is the largest biological kingdom and consists of diverse organisms that are adapted to diverse ecological niches. This kingdom is classified into four phyla, namely Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota[1]. The phylum Ascomycota is further classified into three subphyla, namely Pezizomycotina, Saccharomycotina and Taphrinomycotina[2]. Our interest lies in the Saccharomycotina subphylum, which comprises of yeast organisms that are well known for their potential biotechnological value, as well as opportunistic human pathogens. Table 1 shows a list of Saccharomycotina species, provides general information and indicates the importance of these species.

Genome sequencing analysis of fungal species revealed the presence of numerous cytochrome P450 monooxygenases (P450s) in their genomes, with few exceptions[3]. P450s are heme-thiolate enzymes and their role in organisms' primary and secondary metabolism and their potential use in biotechnology, bioremediation, pharmacology and biofuel generation has been

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documented[4]. Fungal P450s occupy a special place because of their diverse catalytic activities compared to the P450s from other biological kingdoms[5]. Furthermore, the presence of large numbers of P450s and diverse families in fungi makes fungal P450s ideal for the study of P450s' evolution. Since the genome sequence of fungi became available[6], study on fungal P450s, particularly on functional and evolutionary aspects, has gained momentum. Study on fungal P450s revealed that basidiomycetes enriched P450s in their genome owing to duplication to help the organism to adapt to diverse ecological niches, such as wood degradation[3,7]. Study also revealed that ascomycetes, particularly species belonging to the subphylum Pezizomycotina, contain diverse P450 families in their genome[8]. Furthermore, fungal P450s were used as model P450s to identify P450 family-specific signature sequences[9]. Authors have suggested that these signature sequences evolved during the evolution of P450 families from a common P450 ancestor. Recent studies also revealed the presence of a large number of thermostable P450s in fungi[10]. P450 evolutionary studies or genome-wide comparative P450 studies on fungal P450s were based on P450s from species belonging to the phyla Basidiomycotoa and Ascomycota. In the phylum Ascomycota only P450s from the subphylum Pezizomycotina have been explored for both evolutionary and functional characterization[3,8]. P450s from Saccharomycotina species are under explored and to date no study on comparative P450s in Saccharomycotina species has been carried out. Studies have been limited to describing the number of P450s in Saccharomycotina species and detailing one or two P450 families. Furthermore, genome sequencing of new Saccharomycotina species necessitated the performance of comparative P450 profiling in their genomes. Considering the importance of P450s in general and the use of Saccharomycotina species in biotechnology and their pathogenicity towards animals, particularly humans, it is important to understand the role of P450s in yeast species. In the present study we performed genome-wide comparative P450s analysis in 25 yeast species and P450s in three yeast species, *Pichia pastoris*, *Dekkera bruxellensis* and *Pichia anomala,* were identified and annotated as per International Cytochrome P450 Nomenclature Committee[11].

MATERIALS AND METHODS

Saccharomycotina species

A total of 25 Saccharomycotina species whose genomes have been sequenced and are publicly available were used in the study. A list of the yeast species used in this study is shown in Table 1.

Genome-data mining of Saccharomycotina species for P450s

The 22 Saccharomycotina species P450s were downloaded from the publicly available Cytochrome P450 Homepage[12]. The downloaded P450s were cross checked at the web pages: http://www.yeastgenome.org/[13] and http://www.candidagenome.org/[14]. P450s in the remaining three yeast species,i.e. *P. pastoris*, *D. bruxellens* and *P. anomala*, were annotated following the standard procedure described in our recent studies[7,10]. Briefly, the proteome of yeast species was downloaded from their genome data base (*Dekkera bruxellensis* CBS 2499 v2.0: http://genome.jgi.doe.gov/ Dekbr2/Dekbr2.home.html; *Pichia Pastoris*: http://bioinformatics.psb.ugent.be/orcae/;*Pichia anomala*[currently named *Wickerhamomyces anomalus*]NRRL Y-366-8 v1.0: http://genome.jgi.doe. gov/Wican1/Wican1.home.html) and the whole proteome was subjected to functional annotation using NCBI Batch Web CD-search tool[15]. The proteins grouped under the P450 superfamily were selected and analyzed for the presence of the P450 family signature motifs, namely EXXR and CXG. The proteins that showed both motifs were considered authentic P450s and used in this study.

Annotation and classification of P450s

The proteins identified as P450 were subjected to BLAST analysis against all named fungal species at the Cytochrome P450 Homepage[12]. For each P450, the closest homolog was identified and based on the homology percentage, family and subfamily names were assigned. For assigning the family and subfamily names, the standard rule set by the International P450 Nomenclature Committee was followed, i.e. P450s within a family share more than 40% amino acid homology and members of subfamilies share more than 55% amino acid homology[11]. Furthermore, P450s that showed less than 40% homology with known P450s were assigned to a

new family with the help of the P450 nomenclature of Dr David R Nelson, University of Tennessee Health Science Center, Memphis, Tennessee, USA.

Phylogenetic analysis of P450s

Phylogenetic analysis of P450s was carried out in the same way as described in our recent publications[7,10]. Briefly, evolutionary analysis was carried out using the minimum evolution method[16]. The phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis (MEGA 5.05) software[17].

Functional analysis of P450s

Considering the large number of P450s used in this study and the availability of functional data, we performed a literature survey on the functional analysis of Saccharomycotina species P450s and their role in organisms' physiology.

RESULTS AND DISCUSSION

P450ome of *P. pastoris*, *D. bruxellens* and *P. anomala*

The yeast species *P. pastoris* and *D. bruxellens* showed four P450s and *P. anomala* showed six P450s in their genome (Table 2). Among the P450 families found in these species CYP51, CYP61 and CYP501 are common in three species. Compared to two other yeast species, P450s belonging to CYP56, CYP5205 and CYP5217 families are only present in *P. anomala*. Surprisingly, *P. pastoris* and *D. bruxellens* showed a novel P450 family, i.e. CYP5489, in their genome. However, a difference was found in the CYP5489 subfamily type in yeast species. *P. pastoris* contained P450 belonging to subfamily A and *D. bruxellens* contained P450 belonging to subfamily B. It is noteworthy that this P450 family is not found in other yeast species (Table 2).

Comparative analysis of P450ome in yeast species

A hundred and seventy-two P450s were found in 25 yeast species (Table 2). The P450s ranged from a minimum of three to a maximum of 21 in the yeast genomes; 172 P450s were grouped under 13 P450 families and 27 subfamilies (Figure 1 and Table 2). Among the P450 families, CYP52 family showed the highest number of member P450s (71) followed by CYP51 (27), CYP61 (25), CYP56 (20) and CYP501 (11) (Figure 2). The remaining P450 families showed less than 6% of member P450s (Figure 2).

Fig. 1. Phylogenetic analysis of the P450s in subphyla Saccharomycotina. In total, 172 P450 sequences were included in the tree. A minimal evolution tree was constructed using the close-neighbor-interchange algorithm in MEGA (version 5.05). For ease of visual identity, P450s belonging to different families were presented in unique colors.

Table 1. List of yeast species selected for the study and general information and importance of the species.

Species name	General information and importance	Reference
Saccharomyces cerevisiae	The most useful yeast, which has been instrumental in winemaking, baking and brewing since ancient times. It is well known as baker's yeast and it was the first eukaryote ever to have its genome completely sequenced. It is the microorganism behind the most common type of fermentation. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium. It is the organism employed most in industry in terms of production volumes.	24,25
Saccharomyces paradoxus	Non-domesticated yeast living on the bark of deciduous trees. Closest relative of *S. cerevisiae* hence it is an attractive model organism for the study of the population genetics and genomics of wild yeast.	26
Saccharomyces mikatae	Belongs to Saccharomyces sensustricto complex. Shows high conservation of synteny with *S. cerevisiae*.	26
Saccharomyces castellii	Model organism for telomere research since many of the features of the telomere biology of this yeast are closer to those of humans than other yeast.	27
Saccharomyces bayanus	Used in winemaking, cider fermentation and making distilled beverages. It is also used extensively for comparative genomic studies, including expression patterns and nucleosome profile.	26, 27
Saccharomyces kudriavzevii	Belongs to Saccharomyces sensustricto complex. Used in making beer and wine. It can grow at low temperatures.	27, 28
Saccharomyces kluyveri	More efficient user of glucose than model yeast *S. cerevisiae.* It is used in industrial applications, such as the production of proteins, as its biomass yield is greater than that of *S. cerevisiae*. Lives in diverse environments, for example this yeast is a plant pathogen and has been isolated from drosophila species and tissues of an HIV-infected patient.	27
Candida albicans	It is one of the major opportunistic pathogenic fungi causing systemic infection (candidiasis) in immuno-compromised and immuno-competent hosts. It is a diploid fungus that grows both as yeast and filamentous cells and a casual agent of opportunistic oral and genital infections (superficial infections) in humans.	29, 30, 31
Candida tropicalis	The most prevalent opportunistic pathogenic yeast species of the *Candida*-non-*albicans* group. Human pathogen that is well known as medical yeast pathogen. It causes *Candida*-non-*albican* candidiasis. It is a glucose and maltose fermenting yeast. The industrial strain of *C. tropicalis* is engineered to produce long-chain-dicarboxylic acids.	19, 31, 32
Candida guilliermondii (anamorph of *Pichia guilliermondii*)	The model organism of a group of so-called "flavinogenic yeasts" capable of riboflavin oversynthesis. It often causes onychomycosis and is rarely involved in invasive fungal infections.	32, 33
Candida glabrata	It was thought to be a primarily non-pathogenic organism of the normal flora of healthy individuals. However, with the increasing population of immuno-compromised individuals, trends have shown *C. glabrata* to be a highly opportunistic pathogen of the urogenital tract and of the bloodstream.	34
Candida parapsilosis	A fungal species of the yeast family that has become a major cause of sepsis and of wound and tissue infections in immuno-compromised patients. Among Candida species this yeast is a particular problem in neonates, transplant recipients and patients	31

Table 1 Continue

	receiving parenteral nutrition.	
Candida dubliniensis	It is the most closely related species to *C. albicans* and causes superficial infections. Compared to *C. albicans*, this yeast has low virulence and a longer survival rate in hosts.	35
Candida lusitaniae	Responsible for less than 5% of invasive infections among Candida species. Patients who undergo bone marrow transplantation and high-dose cytoreductive chemotherapy have been identified as being at risk of infections caused by this organism.	31, 36
Kluyveromyces lactis	Best alternative yeast for genetics and physiology. Its name comes from the ability to assimilate lactose and convert it to lactic acid. *K. lactis* is one of the main organisms grown in industry in fermenters to produce chymosin on a large scale. Chymosin is used for cheese production. This yeast has GRAS (generally regarded as safe) status.	37
Kluyveromyces polysporus	Whole genome duplicated yeast. Distant lineage to *S. cerevisiae* and considered as best yeast to study comparative whole genome duplication event.	38
Kluyveromyces waltii (recently named as *Lachancea waltii)*	This yeast is a protoploid or pre whole genome duplication budding yeast descended from a lineage and did not undergo the whole genome duplication event. Genome sequencing of this yeast revealed how the genome of *S. cerevisiae* evolved *via* the whole genome duplication event.	39
Pichia stipites	Capable of both aerobic and oxygen-limited fermentation and has the highest known natural ability of any microbe to ferment xylose directly, converting it to ethanol.	40
Lodderomyces elongisporus	Causes blood stream infection in humans. It is the second or third most common yeast species isolated from patients with bloodstream infections in Europe, Canada, Asia and Latin America.	31
Ashbya gossypii	Contains the smallest genome of a free-living eukaryote and because of this is considered as model organism to study filamentous growth. It infects plants such as cotton and citrus. It is a natural overproducer of riboflavin (vitamin B2), which protects its spores against ultraviolet light. This makes it an interesting organism for industries, where genetically modified strains are still used to produce this vitamin.	41
Debaryomyces hansenii	Metabolically versatile, osmotolerant and oleaginous. It is used for surface ripening of cheese and meat products. It one of the most halotolerant species capable of growing in media containing NaCl as high as 4 M.	31
Yarrowia lipolytica	Most extensively studied “non-conventional” yeast (strictly aerobic) and currently used as model organism for study of protein secretion, peroxisome biogenesis, dimorphism, degradation of hydrophobic substrates. It produces important metabolites and has developed intense secretory activity. Capable of growing on organic compounds and producing bio surfactants. This yeast has been used in industry (as a biocatalyst) and in academic studies. This yeast has GRAS status.	4, 43
Dekkera bruxellensis	Although this yeast is distantly related to *S. cerevisiae*, is commonly found in the same habitat. It competes with *S. cerevisiae* in fermentation, can produce high amounts of ethanol and grow without oxygen. It plays a key role in flavor development in lambic beer. However, in the wine industry, this yeast is considered spoilage yeast owing to wine spoilage by the generation of a high amount of phenolic compounds (4-ethylguaiacol and 4-ethylphenol).	44

Table 1 Continue

Pichia pastoris	This methylotrophic yeast is the most commonly used yeast species in the production of recombinant proteins and is widely used to produce proteins for basic research and medical applications. It is a model organism for the study of peroxisomal proliferation and methanol assimilation.	45
Wickerhamomyces anomalus (formerly *Pichia anomala* and *Hansenula anomala*)	It exhibits a multitude of biotechnologically important characteristics in flavor enhancement, food processing, biopreservation, dairy fermentation and waste water treatment.	46

Among the 13 P450 families, CYP51 and CYP61 were conserved across the 25 yeast species. A single copy of CYP61 members were found in the yeast species, whereas CYP51 members were found in more than a single copy in two yeast species , *Candida guillermondii* and *Kluyveromyces polysporus* (Table 2). The presence of more than one copy of CYP51 members is not rare and species belonging to other biological kingdoms, such as plants, also showed more than once copy of CYP51 members in their genomes[18]. CYP52 family members are enriched in species belonging to the genus Candida and in *Yarrowia lipolytica*. Among candida species *C. tropicalis* showed the maximum number of CYP52 members (17 members) in its genome and *Y. lipolytica* showed 12 members in its genome (Table 2). Phylogenetic analysis of CYP52 members (Figure 1) resulted in the grouping of *Y. lipolytica* CYP52 members together, suggesting possible duplication of member P450s in its genome after speciation. Among yeast species only *P. stipites*, *Lodderomyces elongisporus* and *Debaryomyces hansenii* showed CYP52 members in their genome. It is interesting to note that *P. anomala* did not contain CYP52 members compared to *P. stipites*. The presence of CYP52 members in selected yeast species suggested that these P450s play a role in

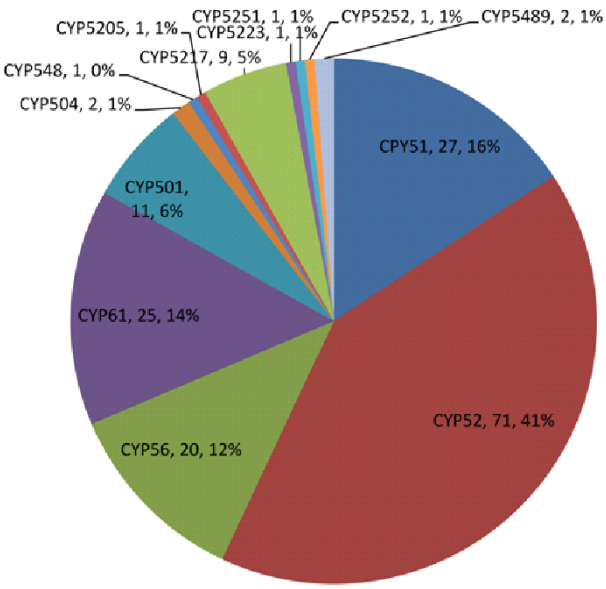

Fig. 2. Comparative analysis of P450s in subphyla Saccharomycotina. A total of 172 P450s representing all P450 families found in Saccharomycotina were used for analysis. The P450 family, number of member P450s in a family and percentage compared to overall P450 count (172 P450s) are also shown in the pie chart.

Table 2. Genome-wide comparative family and sub-family level analysis of cytochrome P450 monooxygenases (P450s) in subphylum Saccharomycotina.

CYP family	SF	SC	SP	SM	SK	SCa	SKl	SB	CA	CT	CG	CGu	CP	CD	CL	KL	KW	KP	PS	LE	AG	DH	YL	DB	PP	PA	TP
CPY51	F	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	27
CYP52	A								4	14		4	7	4	3				5	5		5					71
	B									1																	
	C								1	1			2	1						1							
	D									1																	
	F																						11				
	S																						1				
CYP56	A	1	1	1	1	1	1	1			1					1	1	1			1						20
	D														1												
	E								1	1			1	1					1	1						1	
CYP61	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	25
CYP501	A								1	1			1	1													11
	B														1												
	C											1															
	D																		1			1				1	
	E																								1		
	F																							1			
CYP504	A																						1				2
	G											1															
CYP548	P																						1				1
CYP5205	NS																									1	1
CYP5217	A								1			1	1	1	1				1	1		1				1	9
CYP5223	A																						1				1
CYP5251	A															1											1
CYP5252	A															1											1
CYP5489	A																								1		2
	B																							1			
TP		3	3	3	3	3	3	3	10	21	3	10	14	10	8	5	3	4	10	10	3	9	17	4	4	6	172

Abbreviations: SC, *Saccharomyces cerevisiae*; SP, *Saccharomyces paradoxus*; SM, *Saccharomyces mikatae*; SK, *Saccharomyces kudriavzevii*; SCa, *Saccharomyces castellii*; Ski, *Saccharomyces kluyveri*; SB, *Saccharomyces bayanus*; CA, *Candida albicans*; CT, *Candida tropicalis*; CG, *Candida glabrata*; CGu, *Candida guillermondii*; CP, *Candida parpsilosis*; CD, *Candida dublinensis*; CL, *Candida lusitaniae*; KL, *Klyveromyces lactis*; KW, *Kluyveromyces waltii*; KP, *Kluyveromyces polysporus*; PS, *Pichia stipites*; LE, *Lodderomyces elongisporus*; PA, *Pichia anomala*; AG, *Ashbya gossypii*; DH, *Debaryomyces hansenii*; YL, *Yarrowia lipolytica*; DB, *Dekkera bruxellensis*; PP, *Pichia pastoris*; TP, total P450 count; NS, new subfamily.

the adaptation of yeast species to particular ecological niches, e.g. utilization of specific nutrients, such as alkanes, as carbon source[19,20]. The distribution of other P450 families and the number of member P450s across the selected yeast species can be obtained in Table 2. One interesting observation is that the CYP548 P450 family is present only in *Y. lipolytica*.

The number of P450s in the yeast genomes was lower compared to the fungal species from other fungal phyla, Basidiomycota, Zygomycota and Chytridiomycota, and even compared to the subphylum Pezizomycotina[12], with few exceptions, suggesting the significant loss of P450s in saccharomycotina species. The phenomenon of the presence of a low number of

Table 3. General function and substrate specificity of fungal P450s. P450s that are functionally characterized and present in Saccharomycotina species are shown in the table.

P450	General function	Substrate	Product(s)	Reference
CYP51	Cell membrane sterol biosynthesis	Lanosterol	4,4-dimethylcholesta-8,14,24-trienol	47
		24-methylene-24,25-dihydrolanosterol	4,4-dimethylfecosterol	
CYP52	Alkanes and fatty acid hydroxylation	Arachidonic acid	20-Hydroxy-5,8,11,14-icosatetraenoic acid	48
		Dodecane	1-Dodecanol 2-Dodecanol	
		Hexadecane	1-Hexadecanol 2-Hexadecanol	
		Octadecane	1-Octadecanol 2-Octadecanol	
		Decane	1-Decanol 2-Decanol	
		Tetradecane	1-Tetradecano 2-Tetradecanol	
		Lauric acid	12-Hydroxydodecanoic acid 11-Hydroxydodecanoic acid	
		Myristic acid	14-Hydroxytetradecanoic acid 13-Hydroxytetradecanoic acid	
		Palmitoleic acid	16-Hydroxy-9-hexadecenoic acid 15-Hydroxy-9-hexadecenoic acid	
		Palmitic acid	16-Hydroxyhexadecanoic acid 15-Hydroxyhexadecanoic acid	
		alpha-Linoleic acid	18-hydroxy-alpha-linoleic acid 17-hydroxy-alpha-linoleic acid	
		Linoleic acid	18-hydroxy-linoleic acid 17-hydroxy-linoleic acid	
		Oleic acid	18-Hydroxy-9-octadecenoic acid 17-Hydroxy-9-octadecenoic acid	
		Stearic acid	18-Hydroxyoctadecanoic acid 17-Hydroxyoctadecanoic acid	
CYP56	Synthesis of component of outer spore wall layer	*N*-formyl tyrosine	*N*,*N*'-bisformyl dityrosine	49
CYP61	Cell membrane sterol biosynthesis	Ergosta 5,7,24(28)-trienol	Ergosta 5,7,22,24(28)-tetraenol	50
CYP504	Phenylacetate degradation	Phenylacetate	2-Hydroxyphenylacetate	51
		3-Hydroxyphenylacetate	Homogentisate	52
		3,4-Dihydroxyphenylacetate	2,4,5-Trihydroxyphenylacetate	

P450s in Saccharomycotina species is mentioned in earlier studies[3,21,22]. It has been shown that a large number of P450s, especially certain P450 families that are highly enriched in Basidiomycota, play a key role in the adaptation of Basidiomycetes to different ecological niches, especially in wood degradation, by performing extraordinary catalytic activities[5,7]. This suggests that P450 numbers in an organism can be directly linked to the physiology/ecological niches of the organism. Significant loss of P450s and the role of enrichment of certain members of P450 families in few Saccharomycotina species physiology are discussed in the next section.

Role of P450s in Saccharomycetes physiology

Based on the literature available on the functional characterization of P450s (Table 3) and the characteristic life style of yeast species (Table 1), we conclude that Saccharomycotina species retained P450s that are critical in their physiology. For example, CYP51 and CYP61 P450s play a key role in the synthesis of membrane components and CYP56 is involved in the synthesis of the outer spore wall layer (Table 3). CYP52 family members that are enriched in Candida species and *Y. lipolytica* are involved in organisms' primary (oxidation of fatty acids) and secondary (oxidation of alkanes) metabolism. CYP52 P450s of *C. tropicalis* play a key role in the production of biotechnologically valuable long-chain dicarboxylic acids[23]. The presence of the highest number of CYP52 members in both yeast species gives the species an advantage to utilize alkanes as a carbon source (adaptation to different ecological niches). A deletion mutant of *Y. lipolytica*, where 12 CYP52 genes were deleted, was unable to utilize *n*-alkanes (10-16 carbon length) as a carbon source[20]. This strongly supports the argument that P450s play a key role in the adaptation of yeast species to diverse ecological niches. CYP504 oxidizes simple aromatic compounds such as phenylacetate and helps organisms to grow on this organic compound. Considering the functional properties of characterized P450s, we conclude that the orphan P450s in Saccharomycotina possibly play a role in organisms' physiology, either in synthesis of cellular components or utilization of simpler organic molecules.

CONCLUSIONS

The ecological niches of yeast species are highly enriched with simpler organic nutrients, unlike other fungal species such as Basidiomycetes that degrade wood, a complex polymer, to gain access to nutrients. Because of the complex metabolic process involved in degradation of wood components, Basidiomycetes are enriched with catalytically diverse P450s[5,7]. This clearly suggests that ecological niches of organisms play a critical role in shaping their genome content. Further evidence of this phenomenon can be obtained from the fact that *Cryptococcus neoformans* and *Tremella mesentrica* contain eight and 10 P450s in their genomes compared to other basidiomycetes[7]. *C. neoformans* is a well-known human pathogen and *T. mesenterica* is a parasite fungus. It is shown that these non-wood-degrading Basidiomycetes lost P450s owing to their different ecological niches (life style pattern) compared to the wood-degrading basidiomycetes[7]. It has been suggested that yeast-forming fungi (Saccharomycotina and Taphynomycotina) have small P450omes, while mycorrhizal relationships and complex nutrient degradation seem to enhance P450 diversification[21]. Considering the type of ecological niches adapted by yeast species, it is clear that these species do not need a large number of P450s. The presence of abundant and simpler organic nutrients and adaptation of yeast species for efficient utilization of these simpler nutrients further compacted their genomes and they lost a considerable number of P450s in their genome during evolution.

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