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A comparative genomic analysis of the oxidative enzymes potentially involved in lignin degradation by *Agaricus bisporus*

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ABSTRACT

The oxidative enzymatic machinery for degradation of organic substrates in *Agaricus bisporus* (Ab) is at the core of the carbon recycling mechanisms in this fungus. To date, 156 genes have been tentatively identified as part of this oxidative enzymatic machinery, which includes 26 peroxidase encoding genes, nine copper radical oxidase [including three putative glyoxal oxidase-encoding genes (GLXs)], 12 laccases sensu stricto and 109 cytochrome P450 monooxygenases. Comparative analyses of these enzymes in Ab with those of the white-rot fungus, *Phanerochaete chrysosporium*, the brown-rot fungus, *Postia placenta*, the coprophilic litter fungus, *Coprinopsis cinerea* and the ectomychorizal fungus, *Laccaria bicolor*, revealed enzyme diversity consistent with adaptation to substrates rich in humic substances and partially degraded plant material. For instance, relative to wood decay fungi, Ab cytochrome P450 genes were less numerous (109 gene models), distributed among distinctive families, and lacked extensive duplication and clustering. Viewed together with P450 transcript accumulation patterns in three tested growth conditions, these observations were consistent with the unique Ab lifestyle. Based on tandem gene arrangements, a certain degree of gene duplication seems to have occurred in this fungus in the copper radical oxidase (CRO) and the laccase gene families. In Ab, high transcript levels and regulation of the heme-thiolate peroxidases, two manganese peroxidases and the three GLX-like genes are likely in response to complex natural substrates, including lignocellulose and its derivatives, thereby suggesting an important role in lignin degradation. On the other hand, the expression patterns of the related CROs suggest a developmental role in this fungus. Based on these observations, a brief comparative genomic overview of the Ab oxidative enzyme machinery is presented.

1. Introduction

Lignocellulose, the most abundant biopolymer on earth, and its degradation is an essential component of the global carbon cycle. This substrate is composed of lignin, hemicellulose and cellulose and is degraded in nature by fungi, most efficiently by basidiomycetous fungi. Based on patterns of decay, these fungi are often classified as white-rots, brown-rots or litter-rots (Eriksson, 1990; Floudas et al., 2012; Kirk and Farrell, 1987; Martinez et al., 2005). The white-rot fungi have the enzymatic machinery required to completely breakdown all plant cell wall components including cellulose, hemicelluloses and the more recalcitrant lignin. Brown-rot fungi possess the capacity for rapid and efficient cellulose depolymerization through the action of highly reactive hydroxyl radicals, while leaving lignin as a substantially modified residue (Martinez et al., 2011; Yelle et al., 2008). In contrast to these wood decay fungi, litter decomposers (litter-rots) of the Agaricomycotina degrade cellulose via hydrolytic mechanisms. The ability to remove or modify lignin has been found in some litter decomposing basidiomycete fungi; although genome-based evidence is still lacking (Steffen et al., 2000). This variation in decay patterns is likely due to the distinctive nature of the oxidative systems present in these fungi.

The oxidative machinery generally attributed to ligninolytic fungi includes peroxidases, glyoxal oxidases (GLX), copper radical oxidases (CRO), laccases and cytochrome P450 monooxygenases (P450s). The peroxidases, belonging to the class II type, are heme-containing proteins and are typically secreted enzymes. They include lignin peroxidases (LiPs), manganese peroxidases (MnPs), and versatile peroxidases (VPs) (Hatakka, 1994; Hofrichter et al., 2010). Heme-thiolate peroxidases (HTPs) are a different class of peroxidase enzymes, which catalyze the transfer of peroxide-oxygen to substrate molecules (Hofrichter et al., 2010). Another

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class of heme-peroxidases, dye-decolorizing peroxidases (DyPs, Kim and Shoda, 1999; Zubieta et al., 2007), are capable of oxidizing various anthraquinone derivatives and phenolic dyes (Sugano et al., 2009).

The common feature of these peroxidases is the requirement of H_2O_2 as the electron acceptor. Generation of extracellular H_2O_2 likely involves CROs, of which GLX has been most intensively studied (Hammel et al., 1994; Leuthner et al., 2005; Whittaker et al., 1996). Catalytically distinct from CROs, laccases are also produced by most, but not all, wood decay fungi (Cullen, 1997). Finally, cytochrome P450 enzymes are a superfamily of heme-thiolate proteins that are involved in a wide range of catalytic reactions, most important of which is the hydroxylation of substrates (Anzenbacher and Anzenbacherova, 2001; Ichinose, 2012). These enzymes are widely distributed in both prokaryotic and eukaryotic organisms (more than 11,000 P450 enzymes identified) and have highly diversified during the course of evolution (Nelson, 2009).

Information about the organisms and the mechanisms involved in degradation of lignocellulose has recently grown, especially with the published genome analyses for basidiomycetous fungi, such as *Phanerochaete chrysosporium* (Pc), *Postia placenta* (Pp), *Schizophyllum commune* (Sc), *Serpula lacrymans*, and *Coprinopsis cinerea* (Cc, Eastwood et al., 2011; Martinez et al., 2009, 2004; Ohm et al., 2010; Stajich et al., 2010). Genome analysis of several wood decay fungi was recently reported (Fernandez-Fueyo et al., 2012; Floudas et al., 2012). In contrast to these wood decayers, there is a dearth of similar information regarding those fungi that are involved in metabolizing partially degraded organic and humic substances; thus considered as secondary, litter-decomposing fungi. Augmenting the analysis of *Agaricus bisporus* (Ab) class II peroxidases, HTPs and laccases (Morin et al., 2012), here we present detailed genome-wide analysis of oxidase families, P450s and CROs. Based on comparisons with other sequenced Agaricomycotina and gene expression profiles, a brief overview of the Ab oxidative enzyme machinery is presented.

2. Materials and methods

The oxidase enzymes belonging to the basidiomycete fungus, Ab var. *bisporus* namely LiPs, MnPs, VPs, HTPs, DyPs, GLXs and CROs were identified previously (Morin et al., 2012). The classification of peroxidases has been performed in line with earlier publications (Hofrichter et al., 2010; Liers et al., 2012; Ruiz-Duenas et al., 2009). The mRNA-to-genomic DNA alignment program, Spidey, was used to predict the intron–exon junctions. Transcript levels were determined using microarray data derived from mycelium grown in casing, in compost, and in fruiting bodies. A detailed description of the methods used for growth of the fungus Ab (commercial strain A15), RNA extraction and microarray analyses can be found in the main genome paper (Morin et al., 2012). Cluster analyses presented in this study was generated using the MultiExperiment viewer (MEV) software (Saeed et al., 2003).

A total of 125 predicted cytochrome P450 DNA and protein sequences were downloaded from the Joint Genome Institute's (JGI) website (http://genome.jgi.doe.gov/Agabi_varbisH97_2/Agabi_varbisH97_2.home.html) using the search word "P450". These sequences were next scanned for two conserved cytochrome P450 protein domains, namely the ERR triad motif (EXXR) and the oxygen binding motif (CXG) to identify a subset of 114 P450 proteins. Of these, five proteins were later identified as non-P450 proteins using BLAST search and hence were not included in the subsequent analyses. The remaining 109 proteins were used for family/clan classification and phylogenetic tree construction as described previously (Doddapaneni et al., 2005). The P450 proteins were assigned family/clan names based on protein homology to other known fungal P450 proteins. The mRNA-to-genomic DNA alignment program Spidey was used to predict the intron–exon junctions. Comparative analyses was performed for Ab var *bisporus* (H97) strain, Pp, Pc, Cc and *Laccaria bicolor* (Lb) P450 sequences using BLAST and results were compared using custom perl scripts. All publicly accessible fungal genomes, including those mentioned here, are available through the JGI's interactive MycoCosm web portal (http://genome.jgi.doe.gov/programs/fungi/index.jsf).

3. Results and discussion

3.1. Cytochrome P450 proteins and their redox partners

In fungi, the P450 enzymes play important roles in secondary metabolic pathways. In particular, P450s in basidiomycetous fungi are known to be involved in the breakdown of various xenobiotics and lignin metabolites released by the action of extracellular peroxidases (Ide et al., 2012; Kullman and Matsumura, 1996; Subramanian and Yadav, 2009; Sutherland et al., 1991; Syed et al., 2010).

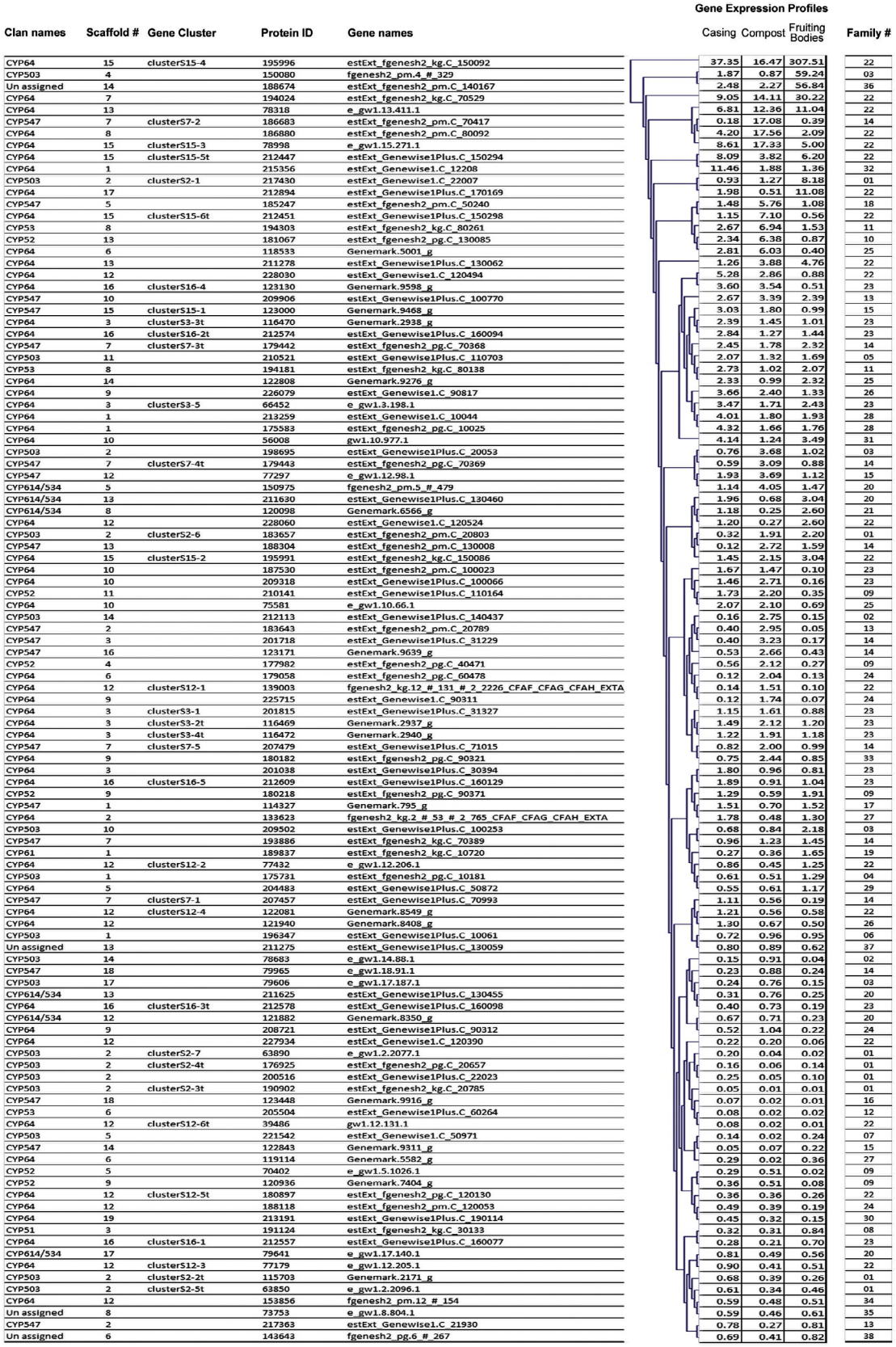
3.1.1. Gene characteristics, classification, phylogenetic relationships and comparative genomics

The Ab genome has 109 P450 genes varying in length from 0.5 to 4.1 kb with introns in individual genes ranging from two in gw1.10.977.1 (Protein ID 56008) to 23 introns in fgenesh2_pg.6_#_267 (Protein ID 143643). Based on the established criterion for grouping of cytochrome P450 enzymes (>40% sequence similarity being classified as members of a family), the 109 P450 sequences fall under 38 CYP families. Of these, 87 proteins are grouped into 16 families of 2–19 P450s and the remaining 22 are assigned as single member families. Additionally, 105 of these P450 proteins were further classified into 8 fungal clans namely CYP51 (1 protein), CYP52 (6 proteins), CYP53 (3 proteins), CYP61 (1 protein), CYP64 (51 proteins), CYP503 (18 proteins), CYP547 (19 proteins) and CYP614/534 (6 proteins) (Fig. 1).

Genome scaffolds 2 and 12 have the highest concentration of P450s (12 and 14, respectively) while the rest of the P450s are scattered across 17 other scaffolds (Figs. 1 and 2). There are 6 genomic clusters on 6 different scaffolds (Figs. 1 and 2). Each cluster contains 5–7 P450 genes within a 18–58 Kb region. In total, there are 34 clustered genes: scaffold 2 (7 genes spanning 58.5 Kb), scaffold 3 (5 genes spanning 18.2 Kb), scaffold 7 (5 genes, spanning 51.6 Kb), scaffold 12 (6 genes, spanning 29.4 Kb), scaffold 15 (6 genes spanning 45.2 Kb) and scaffold 16 (5 genes spanning 53.6 Kb) (Figs. 1 and 2). With the exception of one gene (Genemark.9468_g), members of individual clusters belong to the same family. Of the gene clusters only 4 genes on scaffolds 2, 3 genes on scaffold 3 and 2 genes each on the rest of the 4 scaffolds are tandem repeats (total 15 genes). There are two striking examples of gene duplication events (Protein IDs 211278 and 195991, 179058 and 225715), where proteins are not only more than 90% identical at the amino acid level, but their 5′ and the 3′ UTR sequences (100 bp upstream and downstream) were highly conserved (~90%).

Comparative analyses of P450s in Ab with those of the white-rot, Pc, brown rot, Pp, the other litter fungus, Cc and its closest phylogenetic relative and ectomychorizal fungus, Lb revealed enzyme diversity that is indicative of both lifestyle adaptation as well as phylogenetic relevance. The P450omes of Pp and Pc contain 254 genes and 152 genes, respectively, whereas Ab contains only 109 P450 genes (Martinez et al., 2009, 2004).

Ab features a unique protein (ID 143643, Gene model fgenesh2_pg.6_#_267) that is 1021 aa long with 23 introns and is not grouped under the previously known clans in white-rots,



Clan names	Scaffold #	Gene Cluster	Protein ID	Gene names	Gene Expression Profiles: Casing	Compost	Fruiting Bodies	Family #
CYP64	15	clusterS15-4	195996	estExt_fgenesh2_kg.C_150092	37.35	16.47	307.51	22
CYP503	4		150080	fgenesh2_pm.4_#_329	1.87	0.87	59.24	03
Un assigned	14		188674	estExt_fgenesh2_pm.C_140167	2.48	2.27	56.84	36
CYP64	7		194024	estExt_fgenesh2_kg.C_70529	9.05	14.11	30.22	22
CYP64	13		78318	e_gw1.13.411.1	6.81	12.36	11.04	22
CYP547	7	clusterS7-2	186683	estExt_fgenesh2_pm.C_70417	0.18	17.08	0.39	14
CYP64	8		186880	estExt_fgenesh2_pm.C_80092	4.20	17.56	2.09	22
CYP64	15	clusterS15-3	78998	e_gw1.15.271.1	8.61	17.33	5.00	22
CYP64	15	clusterS15-5t	212447	estExt_Genewise1Plus.C_150294	8.09	3.82	6.20	22
CYP64	1		215356	estExt_Genewise1.C_12208	11.46	1.88	1.36	32
CYP503	2	clusterS2-1	217430	estExt_Genewise1.C_22007	0.93	1.27	8.18	01
CYP64	17		212894	estExt_Genewise1Plus.C_170169	1.98	0.51	11.08	22
CYP547	5		185247	estExt_fgenesh2_pm.C_50240	1.48	5.76	1.08	18
CYP64	15	clusterS15-6t	212451	estExt_Genewise1Plus.C_150298	1.15	7.10	0.56	22
CYP53	8		194303	estExt_fgenesh2_kg.C_80261	2.67	6.94	1.53	11
CYP52	13		181067	estExt_fgenesh2_pg.C_130085	2.34	6.38	0.87	10
CYP64	6		118533	Genemark.5001_g	2.81	6.03	0.40	25
CYP64	13		211278	estExt_Genewise1Plus.C_130062	1.26	3.88	4.76	22
CYP64	12		228030	estExt_Genewise1.C_120494	5.28	2.86	0.88	22
CYP64	16	clusterS16-4	123130	Genemark.9598_g	3.60	3.54	0.51	23
CYP547	10		209906	estExt_Genewise1Plus.C_100770	2.67	3.39	2.39	13
CYP547	15	clusterS15-1	123000	Genemark.9468_g	3.03	1.80	0.99	15
CYP64	3	clusterS3-3t	116470	Genemark.2938_g	2.39	1.45	1.01	23
CYP64	16	clusterS16-2t	212574	estExt_Genewise1Plus.C_160094	2.84	1.27	1.44	23
CYP547	7	clusterS7-3t	179442	estExt_fgenesh2_pg.C_70368	2.45	1.78	2.32	14
CYP503	11		210521	estExt_Genewise1Plus.C_110703	2.07	1.32	1.69	05
CYP53	8		194181	estExt_fgenesh2_kg.C_80138	2.73	1.02	2.07	11
CYP64	14		122808	Genemark.9276_g	2.33	0.99	2.32	25
CYP64	9		226079	estExt_Genewise1.C_90817	3.66	2.40	1.33	26
CYP64	3	clusterS3-5	66452	e_gw1.3.198.1	3.47	1.71	2.43	23
CYP64	1		213259	estExt_Genewise1.C_10044	4.01	1.80	1.93	28
CYP64	1		175583	estExt_fgenesh2_pg.C_10025	4.32	1.66	1.76	28
CYP64	10		56008	gw1.10.977.1	4.14	1.24	3.49	31
CYP503	2		198695	estExt_Genewise1Plus.C_20053	0.76	3.68	1.02	03
CYP547	7	clusterS7-4t	179443	estExt_fgenesh2_pg.C_70369	0.59	3.09	0.88	14
CYP547	12		77297	e_gw1.12.98.1	1.93	3.69	1.12	15
CYP614/534	5		150975	fgenesh2_pm.5_#_479	1.14	4.05	1.47	20
CYP614/534	13		211630	estExt_Genewise1Plus.C_130460	1.96	0.68	3.04	20
CYP614/534	8		120098	Genemark.6566_g	1.18	0.25	2.60	21
CYP64	12		228060	estExt_Genewise1.C_120524	1.20	0.27	2.60	22
CYP503	2	clusterS2-6	183657	estExt_fgenesh2_pm.C_20803	0.32	1.91	2.20	01
CYP547	13		188304	estExt_fgenesh2_pm.C_130008	0.12	2.72	1.59	14
CYP64	15	clusterS15-2	195991	estExt_fgenesh2_kg.C_150086	1.45	2.15	3.04	22
CYP64	10		187530	estExt_fgenesh2_pm.C_100023	1.67	1.47	0.10	23
CYP64	10		209318	estExt_Genewise1Plus.C_100066	1.46	2.71	0.16	23
CYP52	11		210141	estExt_Genewise1Plus.C_110164	1.73	2.20	0.35	09
CYP64	10		75581	e_gw1.10.66.1	2.07	2.10	0.69	25
CYP503	14		212113	estExt_Genewise1Plus.C_140437	0.16	2.75	0.15	02
CYP547	2		183643	estExt_fgenesh2_pm.C_20789	0.40	2.95	0.05	13
CYP547	3		201718	estExt_Genewise1Plus.C_31229	0.40	3.23	0.17	14
CYP547	16		123171	Genemark.9639_g	0.53	2.66	0.43	14
CYP52	4		177982	estExt_fgenesh2_pg.C_40471	0.56	2.12	0.27	09
CYP64	6		179058	estExt_fgenesh2_pg.C_60478	0.12	2.04	0.13	24
CYP64	12	clusterS12-1	139003	fgenesh2_kg.12_#_131_#_2_2226_CFAF_CFAG_CFAH_EXTA	0.14	1.51	0.10	22
CYP64	9		225715	estExt_Genewise1.C_90311	0.12	1.74	0.07	24
CYP64	3	clusterS3-1	201815	estExt_Genewise1Plus.C_31327	1.15	1.61	0.88	23
CYP64	3	clusterS3-2t	116469	Genemark.2937_g	1.49	2.12	1.20	23
CYP64	3	clusterS3-4t	116472	Genemark.2940_g	1.22	1.91	1.18	23
CYP547	7	clusterS7-5	207479	estExt_Genewise1Plus.C_71015	0.82	2.00	0.99	14
CYP64	9		180182	estExt_fgenesh2_pg.C_90321	0.75	2.44	0.85	33
CYP64	3		201038	estExt_Genewise1Plus.C_30394	1.80	0.96	0.81	23
CYP64	16	clusterS16-5	212609	estExt_Genewise1Plus.C_160129	1.89	0.91	1.04	23
CYP52	9		180218	estExt_fgenesh2_pg.C_90371	1.29	0.59	1.91	09
CYP547	1		114327	Genemark.795_g	1.51	0.70	1.52	17
CYP64	2		133623	fgenesh2_kg.2_#_53_#_2_765_CFAF_CFAG_CFAH_EXTA	1.78	0.48	1.30	27
CYP503	10		209502	estExt_Genewise1Plus.C_100253	0.68	0.84	2.18	03
CYP547	7		193886	estExt_fgenesh2_kg.C_70389	0.96	1.23	1.45	14
CYP61	1		189837	estExt_fgenesh2_kg.C_10720	0.27	0.36	1.65	19
CYP64	12	clusterS12-2	77432	e_gw1.12.206.1	0.86	0.45	1.25	22
CYP503	1		175731	estExt_fgenesh2_pg.C_10181	0.61	0.51	1.29	04
CYP64	5		204483	estExt_Genewise1Plus.C_50872	0.55	0.61	1.17	29
CYP547	7	clusterS7-1	207457	estExt_Genewise1Plus.C_70993	1.11	0.56	0.19	14
CYP64	12	clusterS12-4	122081	Genemark.8549_g	1.21	0.56	0.58	22
CYP64	12		121940	Genemark.8408_g	1.30	0.67	0.50	26
CYP503	1		196347	estExt_Genewise1Plus.C_10061	0.72	0.96	0.95	06
Un assigned	13		211275	estExt_Genewise1Plus.C_130059	0.80	0.89	0.62	37
CYP503	14		78683	e_gw1.14.88.1	0.15	0.91	0.04	02
CYP547	18		79965	e_gw1.18.91.1	0.23	0.88	0.24	14
CYP503	17		79606	e_gw1.17.187.1	0.24	0.76	0.15	03
CYP614/534	13		211625	estExt_Genewise1Plus.C_130455	0.31	0.76	0.25	20
CYP64	16	clusterS16-3t	212578	estExt_Genewise1Plus.C_160098	0.40	0.73	0.19	23
CYP614/534	12		121882	Genemark.8350_g	0.67	0.71	0.23	20
CYP64	9		208721	estExt_Genewise1Plus.C_90312	0.52	1.04	0.22	24
CYP64	12		227934	estExt_Genewise1.C_120390	0.22	0.20	0.06	22
CYP503	2	clusterS2-7	63890	e_gw1.2.2077.1	0.20	0.04	0.02	01
CYP503	2	clusterS2-4t	176925	estExt_fgenesh2_pg.C_20657	0.16	0.06	0.14	01
CYP503	2		200516	estExt_Genewise1Plus.C_22023	0.25	0.05	0.10	01
CYP503	2	clusterS2-3t	190902	estExt_fgenesh2_kg.C_20785	0.05	0.01	0.01	01
CYP547	18		123448	Genemark.9916_g	0.07	0.02	0.01	16
CYP53	6		205504	estExt_Genewise1Plus.C_60264	0.08	0.02	0.02	12
CYP64	12	clusterS12-6t	39486	gw1.12.131.1	0.08	0.02	0.01	22
CYP503	5		221542	estExt_Genewise1.C_50971	0.14	0.02	0.24	07
CYP547	14		122843	Genemark.9311_g	0.05	0.07	0.22	15
CYP64	6		119114	Genemark.5582_g	0.29	0.02	0.36	27
CYP52	5		70402	e_gw1.5.1026.1	0.29	0.51	0.02	09
CYP52	9		120936	Genemark.7404_g	0.36	0.51	0.08	09
CYP64	12	clusterS12-5t	180897	estExt_fgenesh2_pg.C_120130	0.36	0.36	0.26	22
CYP64	12		188118	estExt_fgenesh2_pm.C_120053	0.49	0.39	0.19	24
CYP64	19		213191	estExt_Genewise1Plus.C_190114	0.45	0.32	0.15	30
CYP51	3		191124	estExt_fgenesh2_kg.C_30133	0.32	0.31	0.84	08
CYP64	16	clusterS16-1	212557	estExt_Genewise1Plus.C_160077	0.28	0.21	0.70	23
CYP614/534	17		79641	e_gw1.17.140.1	0.81	0.49	0.56	20
CYP64	12	clusterS12-3	77179	e_gw1.12.205.1	0.90	0.41	0.51	22
CYP503	2	clusterS2-2t	115703	Genemark.2171_g	0.68	0.39	0.26	01
CYP503	2	clusterS2-5t	63850	e_gw1.2.2096.1	0.61	0.34	0.46	01
CYP64	12		153856	fgenesh2_pm.12_#_154	0.59	0.48	0.51	34
Un assigned	8		73753	e_gw1.8.804.1	0.59	0.46	0.61	35
CYP547	2		217363	estExt_Genewise1.C_21930	0.78	0.27	0.81	13
Un assigned	6		143643	fgenesh2_pg.6_#_267	0.69	0.41	0.82	38

Fig. 1. Cytochrome P450 enzymes in *Agaricus bisporus* var *bisporus* H97. From left to right, column 1 – assigned P450 Clan names per the established nomenclature; column 2 – genomic scaffold number on which a P450 is located; column 3 – indicates if a P450 is part of the 6 identified CYP clusters (for e.g., ClusterS15-5t represents a gene that is part of the P450 cluster on scaffold# 15 and is the fifth gene in the cluster, and 't' indicates that it is a tandem gene); Genes in these clusters are numbers from left to right on the scaffold. Column 4 – Protein IDs; column 5 – JGI Gene model; columns 6–8 – showing gene expression profile of individual P450 genes in casing, compost and fruiting bodies (FB). Column 9 indicates the family numbers (total 38 families) to which a particular P450 belongs. Hierarchical gene expression clustering under the three conditions, casing, compost and FB are shown adjacent to the casing expression profile. Analyses were done using the MEV software with default settings.

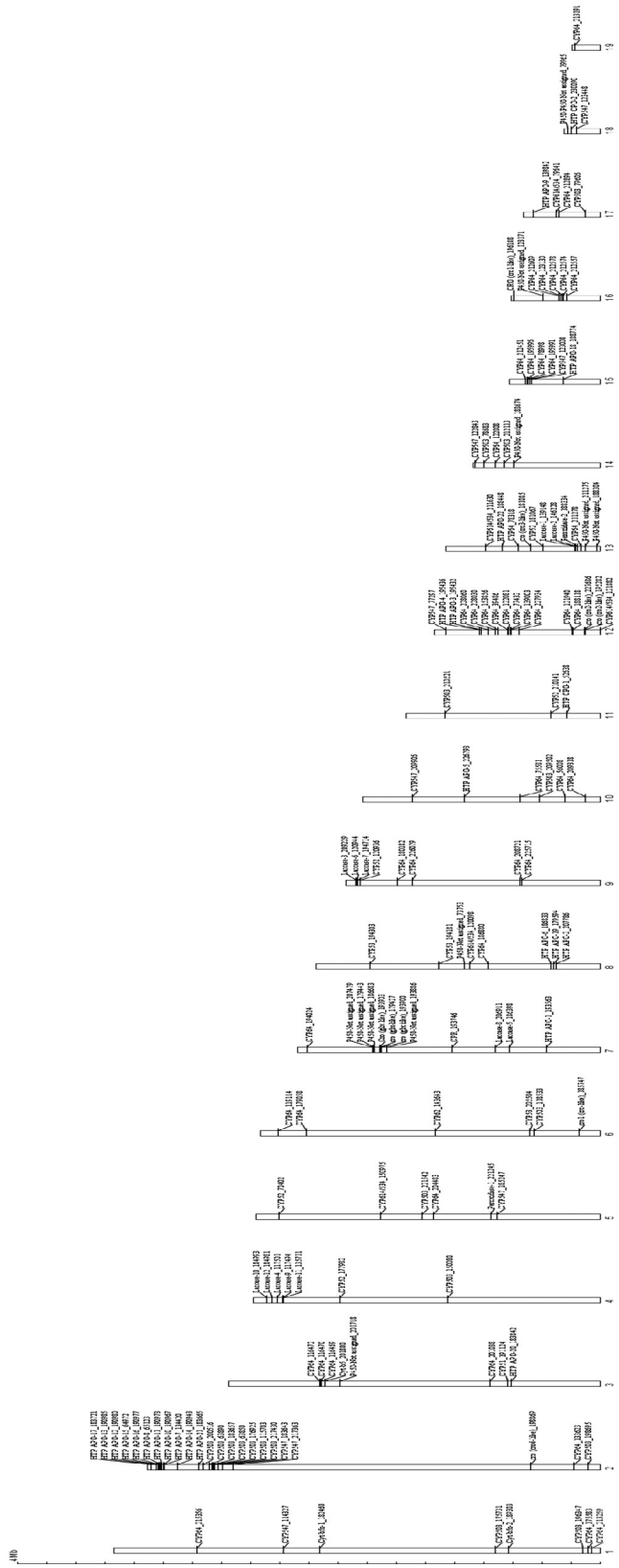


Fig. 2. Schematic diagram of genomic locations of the oxidative enzymatic machinery genes in *Agaricus bisporus*. There are a total of 156 genes distributed across 19 genomic scaffolds (represented by vertical bars and numbered at the bottom from left to right). These genes are grouped under 5 functional classes and identified in the figure using different prefixes (MnP – manganese peroxidase; Laccase – laccases; HTP – Heme-thiol peroxidases; CRO – copper radical oxidases and CYP – cytochrome P450 monooxygenases). GMC oxidoreductases are not mapped, in large part due to uncertainties assigning functional classes based on sequence (Hernandez-Ortega et al., 2012).

brown-rots or the other humiphilic fungus, Cc. However, its closest relative, Lb, has two homologs of this gene (putative linoleate diol synthase coding gene) that are known to be important for oxilipin biosynthesis and sporulation in mushrooms (Andreou et al., 2009). Similarly, Ab has a unique protein (ID 221542, Gene model estExt_Genewise1.C_50971) that is part of the CYP503 clan, but classified under a new family seen only in Lb among the compared genomes.

In contrast to the above two examples of P450 genes that Ab shares only with Lb, this fungus also contains two unique P450 proteins, Protein IDs 183657 (Gene model estExt_fgenesh2_pm.C_20803) and 185247 (Gene model estExt_fgenesh2_pm.C_50240). These fall under two new CYP families, from two different clans, CYP503 and CYP547, respectively that are absent in Lb.

Noticeably absent in this fungus are three P450 families, two P450 families CYP5035, CYP5036 belonging to the CYP67 clan and a third family, CYP505 belonging to the CYP505 clan. The CYP67 clan includes the CYP67 family of proteins that are known to be involved in secondary metabolite production, including aphidicolin and sterigmatocystin production, in *Phoma betae* and *Emericella nidulans*, respectively (Doddapaneni et al., 2005). On the other hand, a P450 encoded by estExt_Genewise1Plus.C_60264 (Protein ID 205504) is similar only to the Pc P450 family, CYP58. This family of P450s is known to be involved in synthesis of toxins in *Fusarium* sp. (Hohn et al., 1995). The absence of the CYP67 family combined with the presence of a CYP58 family protein might influence survival potential of this fungus relative to the pollutant-degrading basidiomycete fungus, Pc.

Seven P450 proteins are classified into five families and three fungal clans in Ab (5 from CYP64 and one each from CYP53 and CYP503) that are absent in white- and brown-rots (Fig. 1). Interestingly, the CYP53 clan protein (ID 205504, Gene model estExt_Genewise1Plus.C_60264) is more divergent when compared to the Pp protein (only 13.7% similar) than to the corresponding Pc protein (39.1% similarity); thus constituting a new P450 family in Ab.

In contrast, Ab model proteins ID 175583 (Gene model estExt_fgenesh2_pg.C_10025), ID 176925 (Gene model estExt_fgenesh2_pg.C_20657) and ID 213259 (Gene model estExt_Genewise1.C_10044), have homologs with more than 40% similarity to white-rot and brown-rot models. Of the three P450s, the former two belong to the CYP64 clan and the latter belongs to the CYP503 clan. Interestingly, the three proteins showed high transcript levels under the three growth conditions (Fig. 1).

The P450, protein ID 217430 (Gene model estExt_Genewise1.C_22007), which is part of the clusterS2–1 (Fig. 1) and belongs to the CYP503 clan (family # 3, Fig. 1), has the closest homolog (family level similarity) only in Cc suggesting that this protein may be specific to humic lifestyle. Supporting this observation this P450 also showed high expression levels (~8-fold up-regulation) in fruiting bodies (FBs) (Fig. 1).

3.1.2. Gene expression and functional inference

Hierarchical clustering results of gene expression showed up-regulation (at least 2-fold) of P450 genes in the three studied conditions in the following order: Compost (35 genes) > Casing (27 genes) > FB (23 genes) (Fig. 1). Among multi-gene families, two from the CYP64 clan (family # 22 and 25), one family of the CYP 547 clan (#13) and one of the CYP53 clan (#11) were consistently up-regulated in all the three conditions, while family # 23 (CYP64 clan) was up-regulated in casing and compost only (Fig. 1). Five other families (#s 1, 3, 9, 14 and 28) showed stage specific up-regulation (Fig. 1), the most significant of which being family #14 (CYP547). Five out of 10 genes in this family showed induction under compost-grown conditions. With few exceptions, the genes in the six genomic clusters discussed in Section 3.1.1 are not tightly

co-regulated (Figs. 1 and 2). Moreover, even tandemly positioned genes were not tightly co-regulated. In short, members of the same family show diverse expression pattern and tandemly arranged genes in Ab have presumably diverged functionally. This is different from white-rots and brown-rots where an overlap of functional responses was observed in response to P450 metabolizing xenobiotics.

The Ab genome carries a single homolog (one member family, Protein ID 153856) of the *eln2* gene of the mushroom, Cc. Previous studies have shown that mutation in the Cc gene could result in altered mushroom morphogenesis (Muraguchi and Kamada, 2000). Similar to its constitutive expression in Cc, it is also constitutively expressed in Ab suggesting this gene is structurally and functionally conserved.

Another P450 designated as fbg14 from *Lentinula edodes* (Le) showed very high homology (*E* value 0.0) to Ab protein encoded by the P450 gene model, estExt_Genewise1Plus.C_100253 (Protein ID 209502). The expression of this gene is specifically observed in the FB of the basidiomycete, Le (Hirano et al., 2004). Interestingly, a similar higher level of expression was also observed in Ab indicating structural and functional conservation between the two species.

Le. CYP1 and *Le*. CYP2, belonging to the CYP510A1 and CYP510A3 families of P450s in Le have been found to have higher levels of transcripts in the stipe of the premature FB as compared to the whole pileus and gill tissue (Akiyama et al., 2002). A homolog (*E* value >1e−098) of these two P450 genes is also found in Ab and is encoded by a single gene model, estExt_Genewise1Plus.C_190114 (Protein ID 213191). Expression of this gene is very low in compost, casing and FB, in comparison to the agar-grown culture in this fungus. This expression pattern is similar to Le to a certain extent, where higher expression was found in the primordium (i.e., initial stages of FB formation), suggesting a similar role of this P450 in this fungus (Akiyama et al., 2002).

Two P450s, Cox1 and Cox2, from the basidiomycete fungus, Cc, have been shown to have terpene oxidizing properties (Agger et al., 2009). Specifically, these proteins are involved in oxidation of the sequiterpene, -cuprenene. Terpenes are secondary metabolites that are synthesized by fungi as defense chemicals or as pheromones. Interestingly, Ab contains protein sequences that are homologous to both Cox1 and Cox2, which are encoded by gene models estExt_Genewise1.C_120524 (Protein 228060) and estExt_Genewise1Plus.C_130062 (Protein ID 211278), respectively. Both these proteins belong to the CYP64 clan of P450s. Whereas transcripts of the latter accumulate in all the three growth conditions, albeit to different extents, the former was found to be up-regulated only in FB in comparison to agar-grown cultures in Ab. The observed high level of homology (*E* value of e−139 with Cox1 and e−134 with Cox2) and similar induction in FB suggest a similar role in both fungi.

The other cytochrome P450 families conserved in fungi include CYP51 and CYP53. Ab contains a single gene model, estExt_fgenesh2_kg.C_30133 (Protein ID 191124) that is homologous to a lanosterol 14-alpha-demethylase (CYP51) from Cc (*E* value of 0.0). CYP51 typically functions in sterol biosynthesis in both prokaryotes and eukaryotes, including yeasts and fungi (Waterman and Lepesheva, 2005). Two gene models [estExt_fgenesh2_kg.C_80138 (Protein ID 194181), estExt_fgenesh2_kg.C_80261 (Protein ID 194303)] were identified as CYP53 that showed high homology to a cytochrome P450 encoding benzoate para-hydroxylase, in other fungi (Fukuda et al., 1996; Matsuzaki and Wariishi, 2005; Shiningavamwe et al., 2006; van Gorcom et al., 1990).

A search for potential cytochrome P450 NADPH redox partners, specifically NADPH cytochrome P450 reductase, cytochrome b5 and b5 reductase identified four homologs of these proteins in the Ab genome. This included a single cytochrome P450 reductase (Protein ID 193746, Gene model estExt_fgenesh2_kg.C_70243) on scaffold 7, a single cytochrome b5 (Protein ID 201800, Gene model estExt_Genewise1Plus.C_31312) on scaffold 3 and two cytochrome b5 reductase proteins on scaffold 1 (Protein ID 182460, Gene model estExt_fgenesh2_pm.C_10659 and Protein ID 189303, Gene model estExt_fgenesh2_kg.C_10173). In white-rots and brown-rots, presence of a single NADPH cytochrome P450 reductase to supply electrons to P450s was reasoned to be compensated by presence of multiple fused CYP505 family of proteins. On the other hand, in *Aspergillus nidulans*, eight NADPH cytochrome P450 reductase proteins are found, some in the extreme vicinity of the P450 proteins suggesting that they may be specific to the respective P450 cluster (Kelly et al., 2009). When compared to these two examples, it is surprising that the genome of Ab not only has a single NADPH cytochrome P450 reductase, but also lacks the fused CYP505 family of proteins. In this aspect, Ab resembles Lb and *Cryptococcus cinerea* whose genomes are also devoid of these fused proteins, suggesting existence of alternatives for these proteins in these genomes; possibly cytochrome b5 and cytochrome b5 reductases. In Ab, the four redox partners are not only scattered in the genome but also none of them are present in the vicinity of P450 proteins. Further, all redox genes showed minimal transcriptional regulation under the different growth conditions (data not shown). It would therefore be interesting to understand how electron transfer proceeds efficiently in this fungus.

3.2. Copper radical oxidases (CROs)

CROs are enzymes that contain two distinct redox centers, one of which is a metal ion, and the other a stable protein-free radical. GLXs are a type of CRO enzyme with broad substrate specificity. GLXs catalyze the two-electron oxidation of simple aldehydes such as glyoxal and methylglyoxal to the corresponding carboxylic acids (Whittaker et al., 1996). The protein free radical in this case is localized on a covalently modified tyrosine residue that is cross-linked to cysteine, forming a tyrosine–cysteine dimer (Whittaker, 1994). These are extracellular H_2O_2-producing enzymes that are produced in conjunction with the peroxidases.

3.2.1. Gene characteristics and comparative genomics

Three GLX genes were identified in Ab, estExt_fgenesh2_pg.C_70353 (Protein ID 179427), estExt_fgenesh2_kg.C_70406 (Protein ID 193903) and estExt_fgenesh2_kg.C_70403 (Protein ID 193900), each of which is ~2200 bp long, and are located on scaffold 7 of the Ab genome (Figs. 2 and 3). The GLX genes are interrupted by 8 introns each. GLX Protein IDs 179427 and 193903 contained 561 aa each, whereas Protein ID 193900 was short by 5 aa (i.e., 557 aa). GLX gene models estExt_fgenesh2_pg.C_70353 (Protein ID 179427) and estExt_fgenesh2_kg.C_70403 (Protein ID 193900) are located in tandem on scaffold 7 (Figs. 2 and 3) and have 91% aa identity. The third gene model, estExt_fgenesh2_kg.C_70406, is located on the same scaffold but separated by a single intervening gene, estExt_fgenesh2_kg.C_70404 (Protein ID 193901) that encodes a metal-dependent phosphoesterase. Based on aa identity, Protein ID 193901 (third gene) is 88% identical to Protein ID 179427 and 90% identical to Protein ID 193900.

The other CRO genes (6 gene models) are distributed among scaffolds 2, 6, 12, 13, and 16 (Figs. 2 and 3). Only scaffold 12 contains two CRO genes, estExt_Genewise1.C_120051 (Protein ID 227686) and estExt_fgenesh2_kg.C_120028 (Protein ID 195282), as compared to the other scaffolds, which contain one CRO gene each (Figs. 2 and 3). The scaffold 12 genes are tandemly arranged (Fig. 2), are ~2700 bp long, and encode proteins that are 790 aa (Protein ID 227686) and 757 aa (Protein ID 195282) long. However they are only 60% identical at the aa level suggesting that despite being positioned in tandem, they are not recently duplicated genes.

Enzyme class	Scaffold #	Protein ID	Gene names	Introns	Gene Size (bp)	Protein Size (aa)	Gene Expression Profiles: Casing	Compost	Fruiting Bodies
HTP APO	7	193563	estExt_fgenesh2_kg.C_70055	4	1486	382	299.64	1492.14	15.79
HTP APO	17	139842	fgenesh2_kg.17_#_78_#_3557_1_CFAF_CFAG_CFAH_EXTA	3	1550	428	278.63	15.61	2.64
HTP APO	2	134420	fgenesh2_kg.2_#_850_#_2_986_CFAF_CFAG_CFAH_EXTA	4	2066	466	94.65	23.90	2.38
cro (cro3-like)	13	181085	estExt_fgenesh2_pg.C_130103	19	5702	1018	91.49	35.32	23.23
Laccase	13	146228	fgenesh2_pg.13_#_40	14	2302	521	1.90	94.68	0.87
Laccase	13	139148	fgenesh2_kg.13_#_30_#_2_1575_CFAF_CFAG_CFAH_EXTA	14	2404	521	1.71	108.08	0.78
HTP APO	8	207786	estExt_Genewise1Plus.C_80196	4	1333	338	0.92	109.31	1.85
HTP APO	12	195432	estExt_fgenesh2_kg.C_120181	4	1525	378	37.99	90.52	1.24
HTP APO	12	195436	estExt_fgenesh2_kg.C_120185	4	1414	383	26.84	58.95	1.44
Cro (glx like)	7	193903	estExt_fgenesh2_kg.C_70406	8	2263	561	1.18	47.71	0.98
HTP APO	2	65123	e_gw1.2.2299.1	4	1379	390	44.86	15.86	1.86
HTP APO	2	190967	estExt_fgenesh2_kg.C_20852	4	2056	470	42.32	15.33	0.63
HTP APO	10	226793	estExt_Genewise1.C_100552	4	1482	382	3.20	34.98	1.91
HTP APO	8	186833	estExt_fgenesh2_pm.C_80038	4	1364	374	0.86	26.57	1.14
HTP CPO	11	52638	gw1.11.650.1	1	810	249	0.67	15.17	0.33
Laccase	9	209229	estExt_Genewise1Plus.C_91003	12	2780	522	1.43	12.51	2.01
HTP APO	2	190973	estExt_fgenesh2_kg.C_20858	4	1591	390	3.43	14.51	1.03
HTP APO	2	190980	estExt_fgenesh2_kg.C_20865	4	1681	426	4.07	13.40	0.98
Peroxidase	5	221245	estExt_Genewise1.C_50584	12	2067	355	2.28	17.98	3.39
cro (glx-like)	7	179427	estExt_fgenesh2_pg.C_70353	8	2118	561	0.96	21.03	0.79
cro (glx iike)	7	193900	estExt_fgenesh2_kg.C_70403	8	2320	557	1.17	20.83	1.06
HTP APO	2	64972	e_gw1.2.2296.1	4	1383	391	24.23	11.55	1.15
HTP APO	2	190943	estExt_fgenesh2_kg.C_20827	5	1957	407	17.35	12.09	1.04
Laccase	4	117501	Genemark.3969_g	11	2241	536	12.77	9.45	0.10
HTP APO	2	190985	estExt_fgenesh2_kg.C_20870	4	2173	539	11.97	12.36	0.75
HTP APO	2	190977	estExt_fgenesh2_kg.C_20862	4	1622	446	9.39	9.97	0.75
Laccase	4	117494	Genemark.3962_g	10	2283	537	1.77	0.76	5.17
cro1 (cro-like)	6	185747	estExt_fgenesh2_pm.C_60028	10	2320	588	0.70	0.28	4.51
cro (cro6-like)	2	198869	estExt_Genewise1Plus.C_20228	1	2273	741	0.76	0.59	6.56
Laccase	9	120944	Genemark.7412_g	11	2220	525	3.72	1.98	0.11
Laccase	9	194714	estExt_fgenesh2_kg.C_90339	11	2383	525	4.09	1.71	0.07
HTP CPO	18	230090	estExt_Genewise1.C_180108	1	1063	270	0.65	2.34	0.08
HTP APO	2	183721	estExt_fgenesh2_pm.C_20868	4	1353	376	0.95	4.14	0.33
HTP APO	15	188774	estExt_fgenesh2_pm.C_150036	4	1349	375	0.60	4.12	0.23
HTP APO	8	179594	estExt_fgenesh2_pg.C_80068	4	1342	358	0.21	4.04	0.29
HTP APO	3	183842	estExt_fgenesh2_pm.C_30119	5	2604	372	0.19	3.45	0.37
Laccase	4	184993	estExt_fgenesh2_pm.C_40715	13	2440	518	0.73	0.65	0.25
Laccase	4	135711	fgenesh2_kg.4_#_647_#_2_498_CFAF_CFAG_CFAH_EXTA	11	2583	529	0.99	0.28	0.03
Laccase	4	184981	estExt_fgenesh2_pm.C_40702	11	2329	532	0.30	0.20	0.01
HTP APO	13	188448	estExt_fgenesh2_pm.C_130158	4	1357	375	0.12	0.06	0.01
HTP APO	2	183665	estExt_fgenesh2_pm.C_20811	4	1431	374	2.17	1.58	2.57
Peroxidase	13	188334	estExt_fgenesh2_pm.C_130039	12	2134	256	1.22	1.65	1.84
Laccase	7	186398	estExt_fgenesh2_pm.C_70127	11	2217	516	1.22	2.01	1.28
Laccase	7	206911	estExt_Genewise1Plus.C_70444	11	2393	527	1.05	1.15	0.92
CRO (cro1-like)	16	196108	estExt_fgenesh2_kg.C_160094	10	2810	637	0.64	1.01	1.56
cro (cro2-like)	12	227686	estExt_Genewise1.C_120051	3	2750	790	0.51	0.42	2.48
cro (cro2-like)	12	195282	estExt_fgenesh2_kg.C_120028	3	2708	757	0.61	0.27	2.59

Fig. 3. Lignin-modifying enzymes, copper-radical oxidases and laccases in *Agaricus bisporus* var *bisporus* H97. From left to right, column 1 – enzyme class; column 2 – genomic scaffold number on which a gene is located; column 3 – Protein IDs; column 4 – JGI Gene models; columns 5 – # of introns; column 6 – gene sizes, column 7 – protein sizes, columns 8–10 – gene expression proliles of individual genes under three conditions, casing, compost and fruiting bodies (FB). Hierarchical gene expression clustering under the three conditions casing, compost and FB are shown adjacent to the casing expression profile. Analyses were done using the TMEV software with default settings.

The other CRO genes (4 gene models) range in size from 2273 bp in estExt_Genewise1Plus.C_20228 (Protein ID 198869) to 5702 bp in estExt_fgenesh2_pg.C_130103 (Protein ID 181085). Whereas, three gene models, estExt_fgenesh2_pm.C_60028 (Protein ID 185747), estExt_fgenesh2_kg.C_160094 (Protein ID 196108) and estExt_Genewise1Plus.C_20228 (Protein ID 198869), were between 2273 and 2810 bp long, gene model estExt_fgenesh2_pg.C_130103 (Protein ID 181085) was unusually long with 5702 bp. This gene (estExt_fgenesh2_pg.C_130103) was interrupted by 19 introns, as opposed to 10 introns in estExt_fgenesh2_pm.C_60028 and estExt_fgenesh2_kg.C_160094, and only one in estExt_Genewise1Plus.C_20228. The protein ID 181085 (estExt_fgenesh2_pg.C_130103) was predicted to be 1018 aa, whereas the other three CRO proteins ranged from 588 to 741 aa.

While the CROs are found in varying extents in white-rot fungi and brown-rot fungi, their numbers seem to be the highest in the ectomycorrhizal fungus, Lb (Table 1). Ab contains 9 CRO genes, out of which 3 belong to the GLX class (Table 1). The brown-rot fungi in general, the ectomycorrhizal fungus, Lb, and the coprophilous fungus, Cc are not known to contain GLX genes (Table 1). On the other hand, Lb is known to have 10 CRO genes, which is the highest among the sequenced basidomycete genomes (Table 1).

The three GLX paralogs are closely related, but distinct from other GLX-like genes including those identified in another member of the Agaricales, *Pleurotus ostreatus* (Po) (Fig. 4). Consistent with an important role in lignin degradation, the GLX-encoding genes are observed only in those genomes that also harbored at least one ligninolytic peroxidase (LiP, MnP or VP). Brown-rot fungi, Sc, Cc and Lb genomes does not carry GLX genes. Nevertheless, the white rot fungi *Ceriporiopsis subvermispora*, *Heterobasidion annosum*, and *Fomitiporia mediterranea* genomes possess class II peroxidases but no GLX-like genes. Possibly, the presence of related CRO genes compensates by generating peroxide from yet unknown substrates.

Table 1
Summary of oxidase genes in the different Basidiomycete fungal categories.

Enzyme type	Basidiomycete fungal category				
	Humicolous (Ab)	Coprophilic (Cc)	White-rot (10 species)	Brown-rot (7 species)	Ectomycorrhizal (Lb)
LiP	0	0	0–10	0	0
MnP	2	0	0–13	0	0
VP	0	0	0–3	0	0
HTP	24	8	3–16	2–6	5
DyP	0	4	0–11	0–2	2
GLX	3	0	0–5	0	0
CRO	6	5	2–7	2–6	10
Laccase	12	17	0–15	0–6	9
CYP	109	139	115–249	126–238	101

Note: This table is adapted from Morin et al. (2012) and Fernandez-Fueyo et al. (2012). Only published genomes are considered.
LIP, lignin peroxidase; MnP, manganese-dependent peroxidase; VP, versatile peroxidase; HTP, heme-thiolate peroxidase; DyP, dye-decolorizing peroxidase; GLX, glyoxal oxidase; CRO, copper-radical oxidase; CYP, cytochrome P450 monooxygenase; Ab, *Agaricus bisporus*; Cc, *Coprinopsis cinerea*.

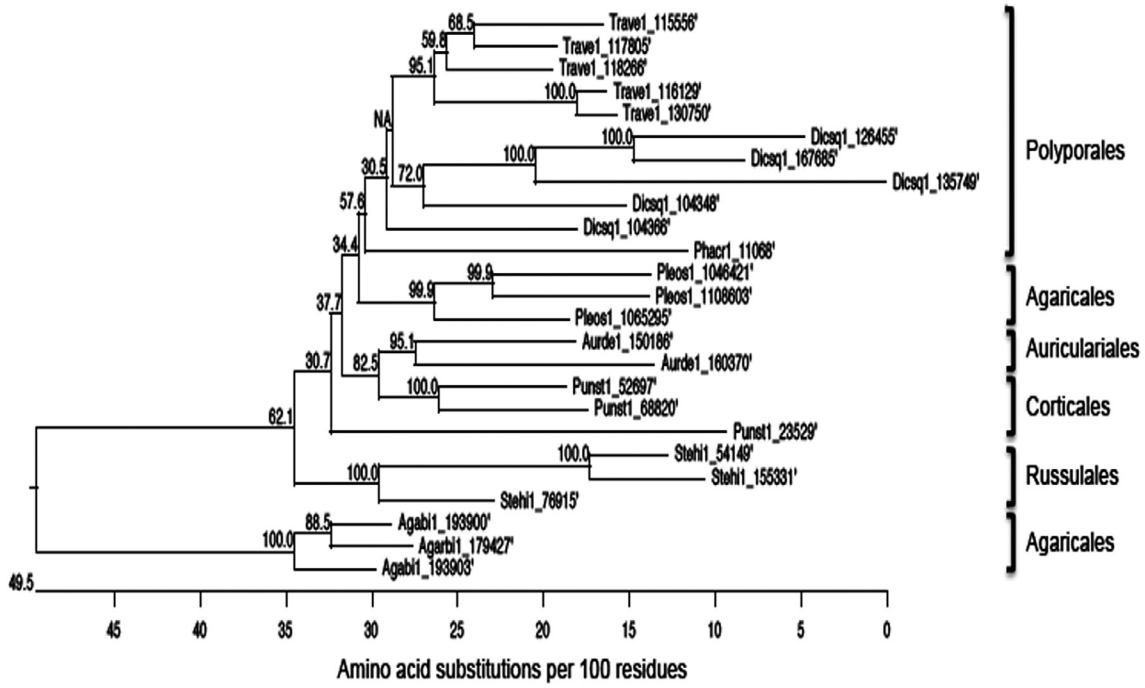


Fig. 4. Relationship among GLX proteins. Protein ID numbers are derived from the JGI's genome portals (http://genome.jgi.doe.gov/programs/fungi/index.jsf) for *T. versicolor* (Trave1), *Dichomitus squalens* (Dicsq1), *P. chrysosporium* (Pchcr1), *Pleurotus ostreatus* (Pleos1), *Auricularia delicata* (Aurde1), *Punctularia strigosozonata* (Punst1), *Stereum hirsutum* (Stehi1) and *A. bisporus* (Agabi1). Predicted secretion signals were trimmed prior to Clustal W alignment and tree construction. Bootstrap values (1000 trials) are indicated at nodes and the Kimura distance formula used to calculate distances (substitutions).

3.2.2. Gene expression and functional inference

Microarray data revealed that all three GLX genes [estExt_fgenesh2_pg.C_70353 (Protein ID 179427), estExt_fgenesh2_kg.C_70406 (Protein ID 193903), and estExt_fgenesh2_kg.C_70403 (Protein ID 193900)] showed up-regulation in compost conditions (between 20- and 40-fold) when compared to the agar grown cultures (Fig. 3). In this connection, the CRO gene estExt_fgenesh2_pg.C_13010 (Protein ID 181085) also showed up-regulation (35-fold) under the same condition (Fig. 3). On the other hand, only one CRO gene [estExt_fgenesh2_pg.C_13010 (Protein ID 181085)] showed up-regulation (~90-fold) under casing condition (Fig. 3). Interestingly, the same gene also showed induction in the FB stages (~23-fold, Fig. 3). Four additional genes [estExt_fgenesh2_pm.C_60028 (Protein ID 185747), estExt_Genewise1Plus.C_20228 (Protein ID 198869), estExt_Genewise1.C_120051 (Protein ID 227686) and estExt_fgenesh2_kg.C_120028 (Protein ID 195282)] also showed up-regulation in FB, albeit to low levels (2- to -6-fold, Fig. 3). The observed induction of these four CRO genes in FB, suggests their possible involvement in FB development in this fungus. Gene model estExt_fgenesh2_pg.C_130103 (Protein ID 181085) was the only CRO gene that showed induction under all the three growth/nutrient stages, albeit in the order casing > compost > FB (Fig. 3). The observed induction of all three GLX genes under compost-grown conditions supports the physiological connection of GLXs and peroxidases in the breakdown of partially decomposed organic matter. Relatively high transcript levels of gene model estExt_fgenesh2_pg.C_130103 under all the growth conditions, suggest an important but unknown role in this fungus.

3.3. Other oxidases (LiPs, MnPs, VPs, HTPs and laccases)

Among the so-called lignin modifying enzymes, peroxidases are known to be key players in breaking down lignin, among fungal species. These enzymes typically are heme-containing proteins and belong to the class II peroxidase superfamily (Hammel and Cullen, 2008; Martinez, 2002). Categorized as LiPs, MnPs or VPs,

all are extracellular enzymes that require H_2O_2 as a substrate. Another family of peroxidases, the HTPs, catalyze the transfer of peroxide-oxygen to substrate molecules. In fungi, two types of HTPs are found, namely the chloroperoxidases (CPOs) and the aromatic peroxygenases (APOs). Whereas CPOs are involved in the oxidation of chloride ion into hypochlorous acid at low pH (pH < 5), which in turn acts as a strong oxidant and can chlorinate organic compounds, APOs are involved in the peroxygenation of aromatic chemicals (Hofrichter and Ullrich, 2010; Morris and Hager, 1966; Shaw and Hager, 1959; Ullrich and Hofrichter, 2005; Ullrich et al., 2004). Laccases are four-copper containing metalloenzymes catalyzing the oxidation of phenolic and lower-redox potential compounds with the simultaneous reduction of molecular oxygen to water (Baldrian, 2006; Hilden et al., 2009; Leonowicz et al., 2001; Mayer and Staples, 2002; Thurston, 1994).

3.3.1. Gene characteristics and comparative genomics

Genome sequencing of Ab revealed that among the class II peroxidases, the Ab genome contains only two Mnp genes (Figs. 2 and 3). No LiPs or VPs were identified. Based on the protein sequence similarity, the isolated MnP1 (Lankinen et al., 2005), seems most closely related to Protein ID 221245 (Gene model estExt_Genewise1.C_50584) in the sequenced genome. On the other hand, there are 24 HTP genes in this organism, two of which are CPOs and the rest are APOs. This is the highest number of HTPs (24 genes) in comparison to the white-rots (3–16 genes), brown-rots (2–6 genes), ectomycorrhizal (5 genes), and the coprophilous fungi (8 genes, Table 1). The Ab genome also contains 12 laccase genes, which is among the highest laccase-containing genomes in basidiomycetous fungi and is only second to the coprophilic fungus, Cc (Table 1). Two laccase gene models estExt_fgenesh2_kg.C_90339 (Protein ID 194714) and Genemark.7412_g (Protein ID 120944) are located in tandem on scaffold 9 (Figs. 2 and 3). Similarly, gene models, fgenesh2_kg.13_#_30_#_2_1575_CFAF_CFAG_CFAH_EXTA (Protein ID 139148) and fgenesh2_pg.13_#_40 (Protein ID 146228) are also tandemly positioned on scaffold 13 (Figs. 2 and 3). The two laccase genes (Protein IDs 139148 and 146228) were previously identified as *lcc2* (GenBank Accession Number L10663) and *lcc1* (GenBank Accession Number L10774), respectively, and were predicted to be in tandem (Perry et al., 1993; Smith et al., 1998).

3.3.2. Gene expression and functional inference

Of the two MnP genes, estExt_Genewise1.C_50584 (Protein ID 221245) showed an ~18.0-fold (*p*-value 0.0848) induction when grown in compost in comparison to agar grown cultures (Fig. 3). The observed induction when grown in partially degraded organic substrate (compost) as opposed to defined, readily available nutrient conditions further supports the important role of MnPs in the growth of Ab in natural humic environments. It has been shown earlier that the lignin degradation by Ab occurs primarily in the vegetative stage and decreases at the onset of FB formation (Durrant et al., 1991; Waksman and Nissen, 1932). Further, Bonnen et al. also showed that lignin degradation, and MnP and lacccase activities coincide thereby suggesting the role of MnP in lignin degradation by this fungus (Bonnen et al., 1994). Both CPOs showed >2-fold up-regulation in compost grown cultures (Fig. 3). Similarly, transcripts of 20 APOS accumulated >2-fold under compost grown conditions. The three most up-regulated genes were closely related to an APO gene of *Agrocybe aegerita* (Ae, Gene Accession Number FM872457). The Ae APO gene is induced ~500-fold after 20 days of growth on soybean-based medium when the pH of the medium increased beyond pH 8 (Pecyna et al., 2009). Of the 20 APO genes, 14 of them also showed induction of >2-fold under casing conditions (Fig. 3). There is a high level of overlap observed in the induction profile of HTPs in compost- and casing-grown conditions suggesting a possible redundancy in function under these conditions. Only four APO genes showed induction (2–15-fold) during FB formation (Fig. 3). Interestingly, the four genes also showed induction under the other tested conditions (i.e. casing, compost or both, Fig. 3). Low expression (~2–15-fold) of the three genes (Protein IDs 193563, 134420 and 139842) in FB (Fig. 3), relative to the other tested conditions (~15–1500-fold) suggests minimal involvement in FB development.

Laccase genes are expressed to varying extents in the different growth phases. Particularly, two laccase genes [fgenesh2_kg.13_#_30_#_2_1575 (Protein ID 139148) and fgenesh2_pg.13_#_40 (Protein ID 146228)] showed ~100-fold induction in compost conditions when compared to agar-grown cultures (Fig. 3). Based on RT-PCR analysis, the former (also called *lcc2*) was found to be nearly 7000-fold up-regulated in compost-grown cultures as opposed to the latter (*lcc1*) by Smith et al. (Smith et al., 1998). The observed induction of laccases, similar to MnPs, under compost-grown conditions is consistent with the higher lignin degradation potential of this organism under this growth condition (Bonnen et al., 1994; Durrant et al., 1991; Waksman and Nissen, 1932). Laccases are known to be developmentally regulated in this fungus, as evidenced by their abundance during vegetative phase as opposed to FB stage (Turner, 1974; Wood, 1980; Wood and Goodenough, 1977). However, the observed transcript accumulation of estExt_Genewise1Plus.C_91003 and Genemark.3962_g in FB suggests a developmental role similar to laccases of Lb, Po and Le (Courty et al., 2009; Lettera et al., 2010; Sakamoto et al., 2009).

4. Conclusions

Among the compared basidiomycete fungi, the sequenced litter rot fungus Ab contains a lower number of P450 genes. Noticeably absent are the genes of the CYP505 clan (with fused CYP domain and the NADPH P450 reductase domain, which catalyzes electron transfer). In addition, Ab lacks extensive P450 gene duplication and clustering as seen in the other sequenced basidiomycete white-rot and brown-rot genomes. The P450 gene expression profiles under the studied conditions are more closely tied to family assignment as opposed to physical location. These observations suggest an already established functional divergence in these clusters, a characteristic more noticeable in cases where gene duplication is not a recent event. Retention of co-regulation at the family level also suggests involvement of multiple gene family members in a common pathway as seen in the case of CYP64 clan families.

In comparison to white-rots and brown-rots, Ab retained a minimal set of the typical heme-containing peroxidases (i.e., only 2 MnPs). Relative to white rot fungi, the reduced number of heme-containing peroxidases may reflect a different lignin composition and accessibility in compost compared to woody substrates. Such high oxidation potential peroxidases are absent in brown rots, coprophilic and ectomycorrhizal fungi, which modify or circumvent lignin. On the other hand, Ab contains the greatest number of HTPs known so far among the sequenced basidiomycete fungi and this expansion is well complemented by a high number of H_2O_2-producing enzymes, namely the GLXs and the CROs. This unique repertoire of genes may enhance survival in humic- and partially degraded organic matter. In particular, a supportive role of three GLXs in peroxidase-mediated breakdown of organic compounds is bolstered by their induction under compost-grown conditions. On the other hand, the related CROs seem to have a more developmental role in this fungus. Further, most of the HTPs showed induction under compost-grown and/or casing conditions; only a selected few were found to be expressed under FB conditions, implying a higher involvement of HTPs in nutrient acquisi-

tion as opposed to cellular development. It could further be predicted that high numbers of HTPs, which are considered a hybrid of peroxidases and monooxygenase enzymes, compensate for the almost complete absence of typical class II peroxidases in this fungus. Whereas the so-far sequenced basidiomycetous fungal categories contain DyPs to varying extents, the humicolous fungi, Ab does not contain any DyPs in its genome. Based on tandem gene arrangements, it could be concluded that a certain degree of gene duplication has occurred, especially in the CRO and the laccase gene families. In terms of laccases, Ab also contains higher number of laccase-encoding genes, in comparison to other basidiomycete fungi. The number and expression profiles suggest both developmental and degradative roles.

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