

Transcriptome of an *Armillaria* root disease pathogen reveals candidate genes involved in host substrate utilization at the host–pathogen interface

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Summary

Armillaria species display diverse ecological roles ranging from beneficial saprobe to virulent pathogen. *Armillaria solidipes* (formerly *A. ostoyae*), a causal agent of Armillaria root disease, is a virulent primary pathogen with a broad host range of woody plants across the Northern Hemisphere. This white-rot pathogen grows between trees as rhizomorphs and attacks sapwood as mycelial fans under the bark. Armillaria root disease is responsible for reduced forest productivity due to direct tree mortality and non-lethal infections that impact growth. Here, we characterize a transcriptome of a widespread, virulent genet (vegetative clone) of *A. solidipes* isolated from a mycelial fan on a natural grand fir (*Abies grandis*) sapling in northern Idaho, USA. cDNA from polyA⁺-purified total RNA was sequenced using a single-end read approach on the Illumina GAIIx platform which generated 24 170 384 reads. A BLASTx search against the NCBI nr database using 39 943 *de novo* assembled contigs resulted in 24 442 sequences with significant hits ($e\text{-value} < 1e^{-3}$), predominantly to fungi (85%). A filtered data set of 20 882 assembled transcripts that encoded putative homologous fungal proteins was created and used for all subsequent analyses. Signal P identified 10 668 putative signal peptides from these fungal transcripts, and 14 360 were annotated with gene ontology terms. Several sequences showed strong homology to annotated genes with functions in pathogenesis, specifically those involved in plant cell wall degradation and response to the post-infection host environment. This transcriptome contributes to the growing body of resources for studies on fungal pathogens of woody plants, and our results provide useful insights towards identifying specific genes with potential roles associated with pathogenesis and other metabolic functions.

1 Introduction

Armillaria (Fr.) Staude (Agaricales, Basidiomycota) is a genus of facultative necrotrophs that is globally distributed in natural and planted forests in temperate, boreal and tropical ecosystems (Baumgartner et al. 2011). This genus includes approximately 40 morphological species (Volk and Burdsall 1995), although work is ongoing to resolve biological (Korhonen 1978; Anderson and Ullrich 1979) and phylogenetic species, with a recent focus on using nuclear gene translation elongation factor 1- α sequences to facilitate species identification (Maphosa et al. 2006; Antonin et al. 2009; Hasegawa et al. 2010; Brazee et al. 2011; Ota et al. 2011; Mulholland et al. 2012; Ross-Davis et al. 2012; Tsykun et al. 2012). Currently recognized *Armillaria* species vary in virulence and host range (Brazee and Wick 2009; Hasegawa et al. 2011; Keča and Solheim 2011; Travadon et al. 2012) and play important roles in carbon and mineral cycling in forest ecosystems (Hicke et al. 2012; Sierota 2012). The complex lifestyle associated with facultative necrotrophism, coupled with heavy production of rhizomorphs (root-like, mycelial cords), creates the potential for *Armillaria* species to form extensive ecological networks encompassing multiple host species. Relationships within these networks may be epiphytic, mycorrhizal, saprophytic or pathogenic. Plant exudates, living root tissue and dead plant tissue are nutrition sources for the fungus, and the rhizomorph network may function as active conduits passing signals, nutrients, carbon and perhaps water. These characteristics make *Armillaria* species important organisms to include in the search for novel genes involved in pathogenesis, biodegradation, network signalling and ecosystem carbon dynamics.

One of the most virulent species of *Armillaria* is *A. solidipes* (formerly *A. ostoyae*, Burdsall and Volk 2008; pending a motion for nomenclatural conservation, Hunt et al. 2011), a causal agent of Armillaria root disease and one of the largest living organisms on earth (Ferguson et al. 2003). It is a pathogen of primarily timber species across the Northern Hemisphere (Kile et al. 1991), and it may act either as a primary pathogen, killing healthy young trees in the span of a few years or older trees over a period of several years in active disease centres, or as a secondary pathogen, infecting already compromised trees (Klutsch et al. 2012; Lehtijärvi et al. 2012; Westwood et al. 2012). *Armillaria solidipes* can form mats of dense mycelia (mycelial fans) beneath the bark of roots and basal stems, infecting sapwood and heartwood. In addition, *A. solidipes* forms melanized, rhizomorphs associated with the host tissue and surrounding soil that facilitate underground spread so that a single genet

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may actively connect to thousands of hosts. *Armillaria* root disease is responsible for reduced forest productivity due to direct tree mortality and non-lethal infections that impact growth (Filip et al. 2009, 2010; Cruickshank 2011; Cruickshank et al. 2011; Morrison 2011). Thus, effective management strategies are needed to reduce the large impact of *A. solidipes* on diverse forest hosts across wide ranges of the Northern Hemisphere (Filip et al. 2009, 2010; Chapman et al. 2011; Shaw et al. 2012).

Our understanding of root-, butt- and wood-rot fungi is improving with the increasing availability of curated genomes of several wood-decay fungi (e.g., Martínez et al. 2004, 2008, 2009; Ohm et al. 2010; Ramírez et al. 2011; Chen et al. 2012; Floudas et al. 2012; Olson et al. 2012). Furthermore, recent advances in comparative genomics (Soanes et al. 2008; Fernandez-Fueyo et al. 2012; Suzuki et al. 2012), transcriptomics (Sato et al. 2009; Wymelenberg et al. 2009, 2010; MacDonald et al. 2011; Yu et al. 2012), proteomics (Mahajan and Master 2010; Ryu et al. 2011) and secretomics (Wymelenberg et al. 2005, 2010; Ravalason et al. 2008; Martínez et al. 2009) allow the detection of peptides associated with plant-pathogen interactions and the post-translational modifications to those proteins during pathogenesis. Only a small proportion of known fungal species are pathogenic (Hawksworth 2012) and these fungal pathogens are most often not closely related (Hibbett et al. 2007; Humber 2008), suggesting that genes associated with the pathogenic lifestyle have evolved independently and repeatedly over several lineages. Such genes include those involved in the formation of infection structures, cell wall degradation, overcoming or avoiding host defences, responding to the host environment, production of toxins and signal cascades (Idnurm and Howlett 2001; Yoder and Turgeon 2001). The objective of this study is to characterize a transcriptome of a mycelial fan of a widespread, virulent genet of *A. solidipes*, with a focus on identifying genes involved in degrading plant cell wall components and responding to the host environment.

2 Material and methods

2.1 Fungal material

Fungal material was collected at the host-pathogen interface from an active mycelial fan of *A. solidipes*, named RNA1, growing between the inner bark and sapwood of a live 15- to 20-year-old grand fir (*Abies grandis*) sapling in a naturally regenerating stand near Elk River, Idaho, USA (latitude: 46.80252°, longitude: -116.16225°, 881 masl). Weather conditions at the time of sampling (10 June 2010) were overcast with a mean temperature of 13.9°C (based on nearest weather station data from the Dworshak Fish Hatchery, Orofino, ID, USA). To preserve the gene expression profile, small sections of the mycelial fan (approximately 5 × 5 mm) were isolated from the infected tree and immediately stabilized in 1.5 ml RNeasy lysis reagent (Qiagen Inc. USA, Valencia, CA, USA). This material was stored at -20°C, and RNA was isolated from this material using the RNeasy Plant Mini Kit (Qiagen Inc.) according to the manufacturer's protocol. A subsample of the mycelial fan was established in culture (3% malt, 3% dextrose, 1% peptone and 1.5% agar) and used in vegetative incompatibility pairings (Mallett et al. 1989) against *Armillaria* genets previously documented at the site.

2.2 *De novo* assembly and annotation

cDNA was generated from polyA⁺-purified total RNA according to the manufacturer's protocol (Illumina mRNA Sequencing Sample Preparation Guide, part #1004898 rev. D; Illumina, Inc., San Diego, CA, USA) and sequenced using an 80-bp single-end read approach on the Illumina GAIIx platform at the Oregon State University Center for Genome Research and Biocomputing. *De novo* assembly of the resulting sequencing reads was performed using CLC Genomics Workbench Version 4.7.2 (CLC bio, Cambridge, MA, USA). The mismatch cost for the nucleotides was set at 2, while the insertion and deletion costs for nucleotides among reads were each set at 3. The length fraction and the similarity of the sequences were set at 0.5 and 0.8, respectively. Any conflicts among the individual bases across reads were resolved by voting for the most abundant base. A minimum length of 200 bp was set for an assembled sequence to be recorded as a contig.

Contig homologies were identified by querying assembled transcripts against the NCBI non-redundant database using BLASTx with an expectation value of $\leq 1e^{-3}$ in BLAST2GO PRO V.2.6.4 (Conesa et al. 2005), which was also used to retrieve gene ontology (GO) terms associated with hits following the search and assign them to fungal transcripts (i.e., assembled contigs with fungal homologies). The distribution of assigned GO terms among three domains [i.e., biological process (BP), cellular component (CC) and molecular function (MF)] was visualized using BLAST2GO. SignalP 4.1 (Petersen et al. 2011) was used to identify putative signal peptides (i.e., short peptides that allow the translocation of proteins from the cell via constitutive or regulated secretion) among the fungal transcripts. Carbohydrate-active enzymes (CAZymes; glycoside hydrolases, glycosyltransferases, polysaccharide lyases and carbohydrate esterases) present in the transcriptome were identified using the CAZymes Analysis Toolkit (CAT; Park et al. 2010). Homologs of genes involved in plant cell wall degradation as well as detoxification enzymes were identified via enzyme codes assigned during the annotation procedure in BLAST2GO. This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession GAHM00000000. The version described in this paper is the first version, GAHM01000000.

3 Results and discussion

3.1 Genet identification and extent

Cultured segments of the mycelial fan revealed no evidence of other culturable microbes in association with *A. solidipes*. Unlike obligate saprotrophic systems in which decaying logs are known to harbour complex microbial communities

(Kubartová et al. 2012), initial infection processes of facultative necrotrophs, like *A. solidipes*, are not known to involve other microbial species. Mycelial fusion with a previously mapped genet confirmed that RNA1 belongs to genet ID001 (*A. solidipes*; partial 3' large subunit – intergenic spacer 1 sequences deposited in GenBank: AY968176.1 and AY968109.1), which was originally collected in 1986 at widespread locations across the 3-ha, 'Pete's Creek' site in northern Idaho, USA (data on file: Moscow Forestry Sciences Laboratory). Genet ID001 is the most abundant and virulent of the six *A. solidipes* genets occupying the site. The genet covered almost the entire 190 × 150 m area and was found in association with 197 trees and shrubs comprising grand fir, Douglas-fir (*Pseudotsuga menziesii*), western white pine (*Pinus monticola*), western service berry (*Amelanchier alnifolia*), redosier dogwood (*Cornus stolonifera*), alder (*Alnus* spp.) and huckleberry (*Vaccinium globulare*). This genet displays diverse ecological behaviour including lethal infections of 78 grand fir seedlings and three Douglas-fir saplings, non-lethal infections of three Douglas-fir trees and four grand fir trees in the overstorey, new infections on 101 stumps of western white pine within 3 months of felling, occasional basidiocarp formation and epiphytic rhizomorphs on diverse tree and shrub species. Despite the overwhelming prevalence of western white pine regeneration at the site, this genet only produced disease on grand fir and Douglas-fir. Although this genet extends beyond the study site, its documented distribution suggests a minimum age of more than 500 years based on an estimated rhizomorph growth rate of 0.22 m per year (van der Kamp 1993).

3.2 *De novo* assembly and transcriptome characterization

Sequencing the cDNA library generated 24 170 384 reads containing 1 933 630 720 bases. After the removal of adapter dimers and failed reads, 24 166 534 reads were assembled *de novo* into 39 943 contigs containing a total of 22 027 774 bases, with an average length of 551 bp and an N50 of 717 bp (Table 1). Of the 39 943 assembled contig sequences, 24 442 (61%) encoded homologous proteins in the NCBI non-redundant database according to BLASTx analysis (cut-off e-value < 1e⁻³). Two sequences exceeded the length limit for analysis in BLAST2GO and were manually aligned in GenBank: the first manually aligned transcript, 'Seq4597' (8 747 bp), aligned with a predicted protein (accession XP_001877728.1) from *Laccaria bicolor* S238N-H82 (96% coverage, e-value: 0.0, 66% maximum identity). Putatively conserved domains were detected within this transcript including UBA [cd00194] ubiquitin-associated domain (e-value: 7.08e⁻⁰⁴). The second manually aligned transcript, 'Seq22655' (11 494 bp), aligned with a predicted protein (accession XP_001878731) from *Laccaria bicolor* S238N-H82 (22% coverage, e-value: 3e⁻⁵⁵, 73% maximum identity). Of the 24 442 alignments, most aligned best to fungal sequences (n = 20 882, 85%). Of these, 20 337 (97%) fell within the phylum Basidiomycota, with over half of these within the order Agaricales (n = 10 592) in which *A. solidipes* is classified. Only 89 alignments were to sequences from *Armillaria* species, although this result likely reflects the limited availability of *Armillaria* sequences within the database.

This analysis resulted in 3 031 enzyme codes that were assigned to 2 788 fungal transcripts, including 1 076 hydrolases, 861 transferases, 634 oxidoreductases, 218 ligases, 148 lyases and 94 isomerases. At least one GO term was assigned to 14 360 contigs, including 8 500 in the MF domain, 6 501 in the BP domain and 3 523 in the CC domain. As a means to organize transcripts into putative functional groups and facilitate cross-study comparisons, the distribution among these three, non-mutually exclusive GO domains was sorted based on level 2 classifications (Fig. 1). Within the metabolic function (MF) domain, the most abundant terms were catalytic activity (6 206) and binding (4 926). For the BP domain, metabolic process (4 703), cellular process (2 253) and localization (1 266) were the most abundant terms. Cell (3 422) and organelle (1 364) were the most abundant terms in the CC domain.

3.3 Plant cell wall degradation

The ability to use the host as a carbon source plays an essential role in pathogenesis. White-rot fungi are capable of efficiently depolymerizing, degrading and mineralizing all components of plant cell walls including recalcitrant lignin, pectin, cellulose and hemicellulose (Eriksson et al. 1990). Evidence for the use of an array of enzymes involved in plant cell wall degradation was detected in the transcriptome (Table 2).

Lignin is a hydrophobic, aromatic biopolymer that serves as an important component of plant secondary cell walls. Ligninolytic enzymes (FOLymes; Levasseur et al. 2008) include laccase (EC 1.10.3.2) and heme peroxidases such as manganese peroxidase (MnP; EC 1.11.1.13), lignin peroxidase (LiP; EC 1.11.1.14) and versatile peroxidase (VP; EC 1.11.1.16). The cata-

Table 1. Assembly, BLASTx and annotation statistics.

	Count	Average length	Total bases
Reads	24 166 534	76.77	1 855 146 290
Matched ¹	20 281 443	76.77	1 556 994 617
Not matched ¹	3 885 091	76.74	298 151 673
Contigs	39 943	551	22 027 774
BLASTx hits to nr database (1e ⁻³)	24 442	–	–
Fungal homologs	20 882	–	–
Annotated	14 360	–	–

¹Matched refers to those reads that were matched to a contig during the assembly process; not matched refers to those reads that were not matched to a contig during the assembly process.

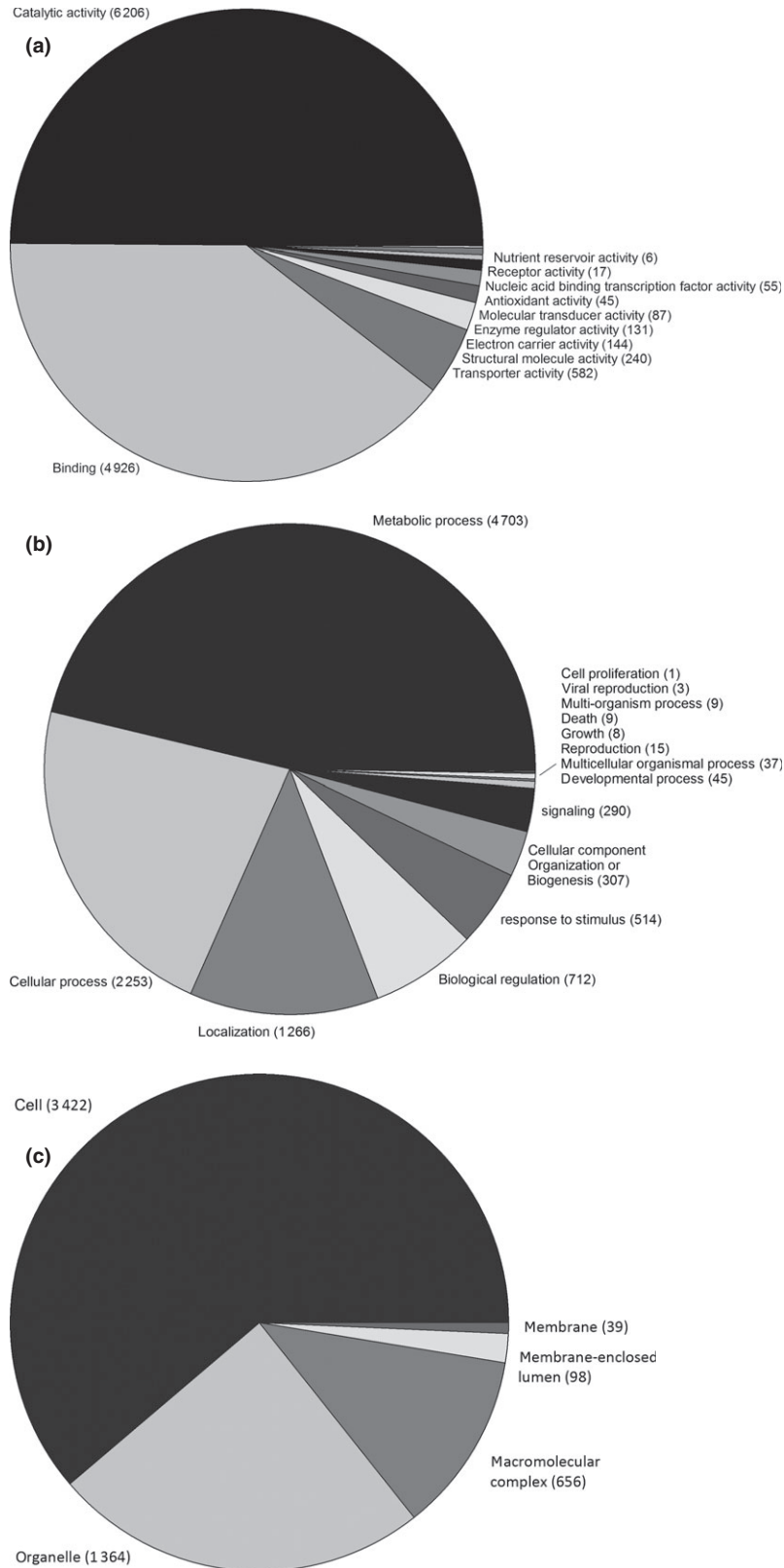


Fig. 1. Classification of sequences from *de novo* transcriptome assembly of *Armillaria solidipes* RNA1 based on predicted gene ontology (GO) terms. (a) Molecular function, (b) Biological process and (c) Cellular component. GO terms were determined using BLAST2GO PRO V.2.6.4 with an *e*-value cut-off of $1e^{-3}$, filtered by fungal homology and are sorted based on level 2 classifications.

Table 2. Enzymes involved in plant cell wall degradation expressed in the transcriptome of *Armillaria solidipes* RNA1.

Enzymes	Homologs
Ligninolytic and related enzymes	
Laccase (EC 1.10.3.2)	3
Manganese peroxidase (EC 1.11.1.13)	5
Versatile peroxidase (EC 1.11.1.16)	1
Aryl alcohol oxidase (EC 1.1.3.7)	3
Alcohol oxidase (EC 1.1.3.13)	2
D-Arabinono-1,4-lactone oxidase (EC 1.1.3.37)	4
Cytochrome P450s	5
Pectinolytic enzymes	
Pectinesterase (EC 3.1.1.11)	2
Feruloyl esterase (EC 3.1.1.73)	2
Polygalacturonase (EC 3.2.1.15)	15
Pectate lyase (EC 4.2.2.2)	9
Cellulolytic, hemicellulolytic and related enzymes	
Cellulase (EC 3.2.1.4)	4
β -Glucosidase (EC 3.2.1.21)	2
Cellulose 1,4- β -cellobiosidase (non-reducing end) (EC 3.2.1.91)	7
Feruloyl esterase (EC 3.1.1.73)	2
β -Galactosidase (EC 3.2.1.23)	2
α -Mannosidase (EC 3.2.1.24)	6
Mannan endo-1,4- β -mannosidase (EC 3.2.1.78)	4
Endo-1,4- β -xylanase (EC 3.2.1.8)	5
Xylan 1,4- β -xylosidase (EC 3.2.1.37)	1
α -N-Arabinofuranosidase (EC 3.2.1.55)	3
Xylan α -1,2-glucuronosidase (EC 3.2.1.131)	2
Xyloglucan-specific exo- β -1,4-glucanase (EC 3.2.1.155)	1
Cellobiose dehydrogenase (EC 1.1.99.18)	2

lytic activation of these ligninolytic enzymes also requires H₂O₂-generating oxidases such as aryl alcohol oxidase (EC 1.1.3.7), alcohol oxidase (EC 1.1.3.13) and D-arabinono-1,4-lactone oxidase (EC 1.1.3.37). Transcript-level expression of laccase, MnP, VP and several H₂O₂-generating oxidases was evident in the transcriptome (Table 2). Similar to the enzyme assays of Stoytchev and Nerud (2000), no evidence of LiP transcription was detected in this mycelial fan sample from *A. solidipes*. Lignin-modifying enzyme systems are known to differ among white-rot fungi (Hatakka 1994; Tuor et al. 1995), and it is believed that peroxidases expanded in the evolution of the Agaricomycetes and then contracted in multiple independent lineages of brown-rot and mycorrhizal species (Floudas et al. 2012). The genome of the white-rot fungus *Schizophyllum commune* lacks genes encoding peroxidases altogether (Ohm et al. 2010), while that of *Phanerochaete chrysosporium*, also a white-rot fungus, lacks genes encoding laccase (Wymelenberg et al. 2010).

Cellobiose dehydrogenase (CDH; EC 1.1.99.18), which plays a role in cellulose degradation via Fenton chemistry as discussed below, is also involved in lignin degradation by mediating the conversion of non-phenolic lignin to phenolic lignin through the production of hydroxyl radicals, thus allowing oxidation by MnP (Henriksson et al. 2000; Hildén et al. 2000). In addition to Fenton reactions and extracellular peroxidases, aromatic molecules such as lignin can be oxidized by the cytochrome P450 system of enzymes involved in metabolizing exogenous compounds (Thuillier et al. 2011). Cytochrome P450s comprise a large superfamily of heme-containing monooxygenases with the greatest diversity of all kingdoms observed among fungi: currently, 2 487 fungal cytochrome P450 species have been identified and assigned to 399 families (see: Ichinose 2012 for a review) with an apparently greater diversity among brown-rot fungi relative to white-rot fungi (Ide et al. 2012). Expression of five putative cytochrome P450 transcripts was observed in this transcriptome (Table 2), suggesting that *A. solidipes* may also rely on the cytochrome P450 system for lignin degradation, although the expression of these enzymes may also be associated with metabolizing other compounds.

Found in the primary cell walls and middle lamellae of plant tissue, pectin is a complex set of polysaccharides (homogalacturonan, xylogalacturonan, rhamnagalacturonans I and II, arabinogalactans I and II and arabinan; Benoit et al. 2012). Known pectinolytic enzymes are classified in the glycoside hydrolase (GH), polysaccharide lyases (PL) and carbohydrate esterases (CE) CAZy (i.e. carbohydrate-active enzymes) families with the expression of several transcripts evident in the transcriptome (Table 2; Fig. 2). As reported for *Phanerochaete chrysosporium* and *Schizophyllum commune* (van den Brink and de Vries 2011), two pectinesterase (EC 3.1.1.11) transcripts were detected (Table 2); however, while *Phanerochaete chrysosporium* lacked both pectin and pectate lyases, nine homologs of pectate lyase (EC 4.2.2.2) were expressed in this *A. solidipes* transcriptome, a result consistent with that reported for *Schizophyllum commune*. Further, the *A. solidipes* transcriptome contained 15 homologs of polygalacturonase (EC 3.2.1.15), while *Phanerochaete chrysosporium* contained four and *Schizophyllum commune* contained three (van den Brink and de Vries 2011).

Cellulose is a polymer of β -(1,4)-linked glucose with individual chains linked via hydrogen bonds. Fungal degradation of cellulose is mediated by the synergistic activity of endoglucanases, cellobiohydrolases and β -glucosidases (Persson et al. 1991; Baldrian and Valášková 2008). Hemicellulose is more complex than cellulose and is classified according to the main sugar in the backbone of the polymers (xylan, mannan and xyloglucan) that have branches composed of a variety of differ-

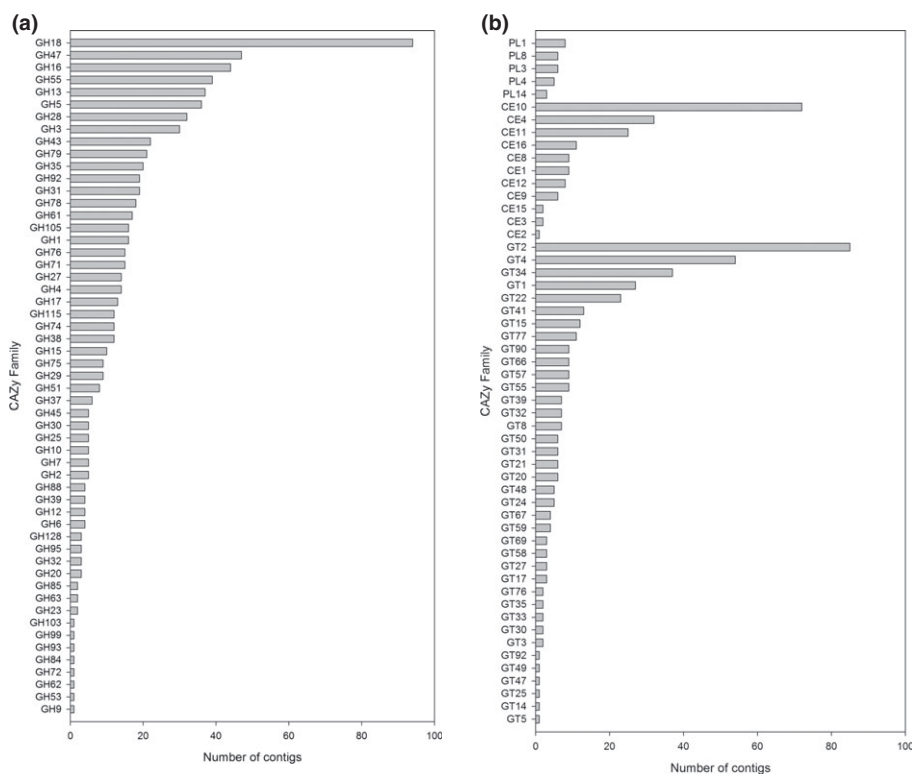


Fig. 2. CAZYome (i.e. the gene complement coding for carbohydrate-active enzymes) of *Armillaria solidipes* RNA1 transcriptome: the distribution of homologs within (a) Glycoside hydrolases (GH) and (b) Polysaccharide lyases (PL), Carbohydrate esterases (CE) and Glycosyltransferases (GT) CAZY families.

ent monomers (galactose, xylose, arabinose and glucuronic acid; Scheller and Ulvskov 2010; van den Brink and de Vries 2011). Enzymes that hydrolyse the glycosidic bonds or the ester linkages of the side groups are employed in fungal degradation of hemicellulose (van den Brink and de Vries 2011). Consistent with other fungal CAZYomes (i.e., gene complements coding for carbohydrate-active enzymes; Floudas et al. 2012), the *A. solidipes* transcriptome contained 1 652 CAZY homologs, most of which fell within the GH class of enzymes responsible for hydrolysis of glycosidic bonds and thus essential for hydrolytic cleavage of cellulose and hemicellulose ($n = 748$; Fig. 2). Similar to CAZymes of other fungi (Martínez et al. 2004, 2008; Ohm et al. 2010; Battaglia et al. 2011), transcripts of a number of glycosidase homologs (EC 3.2.1.-) were expressed in the *A. solidipes* transcriptome (Table 2). Specifically, putative homologs of cellulase (EC 3.2.1.4; $n = 4$), β -glucosidase (EC 3.2.1.21; $n = 2$), cellulose 1,4- β -cellobiosidase (non-reducing end) (EC 3.2.1.91; $n = 7$), β -galactosidase (EC 3.2.1.23; $n = 2$), α -mannosidase (EC 3.2.1.24; $n = 6$), mannan endo-1,4- β -mannosidase (EC 3.2.1.78; $n = 4$), endo-1,4- β -xylanase (EC 3.2.1.8; $n = 5$), xylan 1,4- β -xylosidase (EC 3.2.1.37; $n = 1$), α -N-arabinofuranosidase (EC 3.2.1.55; $n = 3$), xylan α -1,2-glucuronosidase (EC 3.2.1.131; $n = 2$) and xyloglucan-specific exo- β -1,4-glucanase (EC 3.2.1.155; $n = 1$) were evident in this *A. solidipes* transcriptome. In addition to these hydrolytic enzymes, the extracellular oxidative enzyme cellobiose dehydrogenase (CDH; EC 1.1.99.18; $n = 2$) is known to play a role in lignocellulose degradation via Fenton chemistry (Henriksson et al. 2000; Baldrian and Valášková 2008; MacDonald et al. 2012), a process that has been demonstrated for brown-rot fungi as well as for some white-rot fungi (Arantes et al. 2011; Žifčáková and Baldrian 2012). Additionally, two feruloyl esterase (CE-1; EC 3.1.1.73) homologs were expressed (Table 2), which are known to hydrolyse the phenolic groups involved in the cross-linking of arabinoxylan to other polymeric structures, an important step for opening the cell wall structure for hydrolysis. Thus, several putative hydrolytic enzymes as well as enzymes that act by direct oxidation of cellulose (e.g., CDH) were expressed in this *A. solidipes* transcriptome (Table 2). These results suggest that *A. solidipes*, like other white-rot fungi (Arantes et al. 2011; Wymelenberg et al. 2011; Žifčáková and Baldrian 2012), relies on both hydrolytic and oxidative decomposition of cellulose and hemicellulose. Because this transcriptome was characterized from a composite sample of both the advancing edge of the active mycelial fan growing between the inner bark and sapwood and the inner, older sections of the fan, it likely represents the genetic complement of both necrotrophic parasitism and saprotrophic wood decay, trophic strategies with known differences in gene expression as demonstrated for the pathogenic wood-decay fungus *Heterobasidion irregulare* (Olson et al. 2012). Relative to the mean gene content of the eight white-rot fungal genomes detailed in Floudas et al. (2012), comparable representation in this *A. solidipes* transcriptome was observed for families GH7 (cellobiohydrolases that act from the reducing ends of cellulose chains to generate cellobiose), GH10 (xylanases), GH12 (endoglucanase and xyloglucan hydrolase), AA9 (i.e., auxiliary activities 9; formerly GH61; copper-dependent lytic polysaccharide monoxygenases) and CE16 (acetyl esterase). No transcripts were observed for GH11 (xylanase) or CE5 (acetyl xylan esterase and cutinase), although these CAZY families are known to be rare in the Basidiomycota (Floudas et al.

2012). Finally, a greater abundance was observed within families GH3, GH5, GH6, GH28, GH43, GH74, CE1, CE8 and CE12, containing enzymes involved in the degradation of cellulose, hemicellulose and pectin.

3.4 Detoxification enzymes

For pathogenic fungi to ultimately cause disease, they must be able to evade, suppress or tolerate the host's defence responses, including the oxidative burst associated with localized cell stress or death at the infection site and the production of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Several fungal ROS-detoxifying enzymes have been documented (Aguirre et al. 2006), including catalase (EC 1.11.1.6; n = 10) and superoxide dismutase (SOD; EC 1.15.1.1; n = 4). Glutathione S-transferases (GSTs; EC 2.5.1.18) comprise a complex and widespread enzyme superfamily that function in the detoxification of xenobiotics and peroxides (McGoldrick et al. 2005; Morel et al. 2009; Thuillier et al. 2011). A single GST homolog was detected in the transcriptome, with a conserved domain corresponding to the GST N family, class Phi subfamily composed of plant-specific class Phi GSTs and related fungal and bacterial proteins. Fungal ATP-binding cassette (ABC) transporters are perhaps the most well-characterized transmembrane proteins involved in cellular detoxification (e.g. Sipos and Kuchler 2006), with a total of 39 homologs expressed in this *A. solidipes* transcriptome.

3.5 Conclusions

A number of gene homologs with known roles in plant cell wall degradation and detoxification of the post-infection host environment were identified from this *A. solidipes* transcriptome. Capturing gene expression profiles *in planta* improves our understanding of pathogenesis and contributes to baseline information for comparative transcriptomics, although some limitations are also associated with environmental samples, such as ensuring that transcripts are derived from a single organism (Cairns et al. 2010). Comparisons among the transcriptomes of *A. solidipes* and other root-, butt- and wood-rot pathogens will be facilitated as these studies are further developed and as genomic sequences become available. For example, differential gene expression among isolates grown under diverse conditions and at various time points during the infection process can be examined to identify the role of specific genes. Further, cross-species comparisons of host-infecting fungal transcriptomes will help to identify suites of core genes essential for the infection process. Identification of genes involved in plant cell wall degradation specifically, either via intracellular systems like the cytochrome P450 or via extracellular oxidases, is critical not only to understand the organism's biology, but also for industrial applications, such as biofuel production or biopulping (e.g., Isroi et al. 2011) as well as bioremediation (e.g., Harms et al. 2011). Furthermore, improved understanding of gene expression related to pathogenesis can facilitate the development of forest management strategies to reduce the impact of *Armillaria* root disease and other related diseases.

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References

- Aguirre, J.; Hansberg, W.; Navarro, R., 2006: Fungal responses to reactive oxygen species. *Med. Mycol.* **44**, S101–S107.
- Anderson, J. B.; Ullrich, R. C., 1979: Biological species of *Armillaria mellea* in North America. *Mycologia* **71**, 402–414.
- Antonín, V.; Tomšovský, M.; Sedlák, P.; Májek, T.; Jankovský, L., 2009: Morphological and molecular characterization of the *Armillaria cepistipes* – *A. gallica* complex in the Czech Republic and Slovakia. *Mycol. Prog.* **8**, 259–271.
- Arantes, V.; Milagres, A. M. F.; Filley, T. R.; Goodell, B., 2011: Lignocellulosic polysaccharides and lignin degradation by wood decay fungi: the relevance of nonenzymatic Fenton-based reactions. *J. Ind. Microbiol. Biotechnol.* **38**, 541–555.
- Baldrian, P.; Valášková, V., 2008: Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol. Rev.* **32**, 501–521.
- Battaglia, E.; Benoit, I.; van den Brink, J.; Wiebenga, A.; Coutinho, P. M.; Henrissat, B.; de Vries, R. P., 2011: Carbohydrate-active enzymes from the zygomycete fungus *Rhizopus oryzae*: a highly specialized approach to carbohydrate degradation depicted at genome level. *BMC Genomics* **12**, 38. doi: 10.1186/1471-2164-12-38.
- Baumgartner, K.; Coetzee, M. P. A.; Hoffmeister, D., 2011: Secrets of the subterranean pathosystem of *Armillaria*. *Mol. Plant Pathol.* **12**, 515–534.
- Benoit, I.; Coutinho, P. M.; Schols, H. A.; Gerlach, J. P.; Henrissat, B.; de Vries, R. P., 2012: Degradation of different pectins by fungi: correlations and contrasts between the pectinolytic enzyme sets identified in genomes and the growth on pectins of different origin. *BMC Genomics* **13**, 321.
- Brazee, N. J.; Wick, R. L., 2009: *Armillaria* species distribution on symptomatic hosts in northern hardwood and mixed oak forests in western Massachusetts. *For. Ecol. Manage.* **258**, 1605–1612.
- Brazee, N. J.; Hulvey, J. P.; Wick, R. L., 2011: Evaluation of partial tef1, rpb2, and nLSU sequences for identification of isolates representing *Armillaria calvescens* and *Armillaria gallica* from northeastern North America. *Fungal Biol.* **115**, 741–749.
- van den Brink, J.; de Vries, R. P., 2011: Fungal enzyme sets for plant polysaccharide degradation. *Appl. Microbiol. Biotechnol.* **91**, 1477–1492.
- Burdsall Jr, H. H.; Volk, T. J., 2008: *Armillaria solidipes*, an older name for the fungus called *Armillaria ostoyae*. *North Am. Fungi* **3**, 261–267.
- Cairns, T.; Minuzzi, F.; Bignell, E., 2010: The host-infecting fungal transcriptome. *FEMS Microbiol. Lett.* **307**, 1–11.
- Chapman, W. K.; Schellenberg, B.; Newsome, T. A., 2011: Assessment of *Armillaria* root disease infection in stands in south-central British Columbia with varying levels of overstorey retention, with and without pushover logging. *Can. J. For. Res.* **41**, 1598–1605.
- Chen, S.; Xu, J.; Liu, C.; Zhu, Y.; Nelson, D. R.; Zhou, S.; Li, C.; Wang, L.; Guo, X.; Sun, Y.; Luo, H.; Li, Y.; Song, J.; Henrissat, B.; Levasseur, A.; Qian, J.; Li, J.; Luo, X.; Shi, L.; He, L.; Xiang, L.; Xu, X.; Niu, Y.; Li, Q.; Han, M. V.; Yan, H.; Zhang, J.; Chen, H.; Lv, A.; Wang, Z.; Liu, M.;

- Schwartz, D. C.; Sun, C., 2012: Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. *Nat. Commun.* **3**, 913. doi: 10.1038/ncomms1923.
- Conesa, A.; Götz, S.; García-Gómez, J. M.; Terol, J.; Talón, M.; Robles, M., 2005: Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**, 3674–3676.
- Cruickshank, M. G., 2011: Yield reduction in spruce infected with *Armillaria solidipes* in the southern interior of British Columbia. *Forest Pathol.* **41**, 425–428.
- Cruickshank, M. G.; Morrison, D. J.; Lalumière, A., 2011: Site, plot, and individual tree yield reduction of interior Douglas-fir associated with non-lethal infection by *Armillaria* root disease in southern British Columbia. *For. Ecol. Manage.* **261**, 297–307.
- Eriksson, K.-E. L.; Blanchette, R. A.; Ander, P., 1990: *Microbial and Enzymatic Degradation of Wood and Wood Components*. Berlin, Germany: Springer-Verlag.
- Ferguson, B. A.; Dreisbach, T. A.; Parks, C. G.; Filip, G. M.; Schmitt, C. L., 2003: Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Can. J. For. Res.* **33**, 612–623.
- Fernandez-Fueyo, E.; Ruiz-Dueñas, F. J.; Ferreira, P.; Floudas, D.; Hibbett, D. S.; Canessa, P.; Larrondo, L. F.; James, T. Y.; Seelenfreund, D.; Lobos, S.; Polanco, R.; Tello, M.; Honda, Y.; Watanabe, T.; Watanabe, T.; Ryu, J. S.; Kubicek, C. P.; Schmoll, M.; Gaskell, J.; Hammel, K. E.; St John, F. J.; Wymelenberg, A. V.; Sabat, G.; BonDurant, S. S.; Syed, K.; Yadav, J. S.; Doddapaneni, H.; Subramanian, V.; Lavín, J. L.; Oguiza, J. A.; Perez, G.; Pisabarro, A. G.; Ramirez, L.; Santoyo, F.; Master, E.; Coutinho, P. M.; Henrissat, B.; Lombard, V.; Magnuson, J. K.; Kües, U.; Hori, C.; Igarashi, K.; Samejima, M.; Held, B. W.; Barry, K. W.; LaButti, K. M.; Lapidus, A.; Lindquist, E. A.; Lucas, S. M.; Riley, R.; Salamov, A. A.; Hoffmeister, D.; Schwenk, D.; Hadar, Y.; Yarden, O.; de Vries, R. P.; Wiebenga, A.; Stenlid, J.; Eastwood, D.; Grigoriev, I. V.; Berka, R. M.; Blanchette, R. A.; Kersten, P.; Martinez, A. T.; Vicuna, R.; Cullen, D., 2012: Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 5458–5463.
- Filip, G. M.; Fitzgerald, S. A.; Chadwick, K. L.; Max, T. A., 2009: Thinning ponderosa pine affected by *Armillaria* Root Disease: 40 years of growth and mortality on an infected site in central Oregon. *West. J. Appl. For.* **24**, 88–94.
- Filip, G. M.; Maffei, H. M.; Chadwick, K. L.; Max, T. A., 2010: *Armillaria* root disease-caused tree mortality following silvicultural treatments (shelterwood or group selection) in an Oregon mixed-conifer forest: insights from a 10-year case study. *West. J. Appl. For.* **25**, 136–143.
- Floudas, D.; Binder, M.; Riley, R.; Barry, K.; Blanchette, R. A.; Henrissat, B.; Martínez, A. T.; Otilar, R.; Spatafora, J. W.; Yadav, J. S.; Aerts, A.; Benoit, I.; Boyd, A.; Carlson, A.; Copeland, A.; Coutinho, P. M.; de Vries, R. P.; Ferreira, P.; Findley, K.; Foster, B.; Gaskell, J.; Glotzer, D.; Górecki, P.; Heitman, J.; Hesse, C.; Hori, C.; Igarashi, K.; Jurgens, J. A.; Kallen, N.; Kersten, P.; Kohler, A.; Kües, U.; Arun Kumar, T. K.; Kuo, A.; LaButti, K.; Larrondo, L. F.; Lindquist, E.; Ling, A.; Lombard, V.; Lucas, S.; Lundell, T.; Martin, R.; McLaughlin, D. J.; Morgenstern, I.; Morin, E.; Murat, C.; Nagy, L. G.; Nolan, M.; Ohm, R. A.; Patyshakuliyeva, A.; Rokas, A.; Ruiz-Dueñas, F. J.; Sabat, G.; Salamov, A.; Samejima, M.; Schmutz, J.; Slot, J. C.; St. John, F.; Stenlid, J.; Sun, H.; Sun, S.; Syed, K.; Tsang, A.; Wiebenga, A.; Young, D.; Pisabarro, A.; Eastwood, D. C.; Martin, F.; Cullen, D.; Grigoriev, I. V.; Hibbett, D. S., 2012: The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336**, 1715–1717.
- Harms, H.; Schlosser, D.; Wick, L. Y., 2011: Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat. Rev. Microbiol.* **9**, 177–192.
- Hasegawa, E.; Ota, Y.; Hattori, T.; Kikuchi, T., 2010: Sequence-based identification of Japanese *Armillaria* species using the elongation factor-1 alpha gene. *Mycologia* **102**, 898–910.
- Hasegawa, E.; Ota, Y.; Hattori, T.; Sahashi, N.; Kikuchi, T., 2011: Ecology of *Armillaria* species on conifers in Japan. *Forest Pathol.* **41**, 429–437.
- Hatakka, A., 1994: Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiol. Rev.* **13**, 125–135.
- Hawksworth, D. L., 2012: Global species numbers of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? *Biodivers. Conserv.* **21**, 2425–2433.
- Henriksson, G.; Zhang, L.; Li, J.; Ljungquist, P.; Reitberger, T.; Pettersson, G.; Johansson, G., 2000: Is cellobiose dehydrogenase from *Phanerochaete chrysosporium* a lignin degrading enzyme? *Biochim. Biophys. Acta* **1480**, 83–91.
- Hibbett, D. S.; Binder, M.; Bischoff, J. F.; Blackwell, M.; Cannon, P. F.; Eriksson, O. E.; Huhndorf, S.; James, T.; Kirk, P. M.; Lücking, R.; Lumbsch, H. T.; Lutzoni, F.; Matheny, P. B.; McLaughlin, D. J.; Powell, M. J.; Redhead, S.; Schoch, C. L.; Spatafora, J. W.; Stalpers, J. A.; Vilgalys, R.; Aime, M. C.; Aptroot, A.; Bauer, R.; Begerow, D.; Benny, G. L.; Castlebury, L. A.; Crous, P. W.; Dai, Y.-C.; Gams, W.; Geiser, D. M.; Griffith, G. W.; Gueidan, C.; Hawksworth, D. L.; Hestmark, G.; Hosaka, K.; Humber, R. A.; Hyde, K. D.; Ironside, J. E.; Kõljalg, U.; Kurtzman, C. P.; Larsson, K.-H.; Lichtwardt, R.; Longcore, J.; Miądlikowska, J.; Miller, A.; Moncalvo, J.-M.; Mozley-Standridge, S.; Oberwinkler, F.; Parmasto, E.; Reeb, V.; Rogers, J. D.; Roux, C.; Ryvarden, L.; Sampaio, J. P.; Schußler, A.; Sugiyama, J.; Thorn, R. G.; Tibell, L.; Untereiner, W. A.; Walker, C.; Wang, Z.; Weir, A.; Weiss, M.; White, M. M.; Winka, K.; Yao, Y.-J.; Zhang, N., 2007: A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* **111**, 509–547.
- Hicke, J. A.; Allen, C. D.; Desai, A. R.; Dietze, M. C.; Hall, R. J.; Hogg, E. H.; Kashian, D. M.; Moore, D.; Raffa, K. F.; Sturrock, R. N.; Vogelmann, J., 2012: Effects of biotic disturbances on forest carbon cycling in the United States and Canada. *Glob. Change Biol.* **18**, 7–34.
- Hildén, L.; Johansson, G.; Pettersson, G.; Li, J. B.; Ljungquist, P.; Henriksson, G., 2000: Do the extracellular enzymes cellobiose dehydrogenase and manganese peroxidase form a pathway in lignin biodegradation? *FEBS Lett.* **477**, 79–83.
- Humber, R. A., 2008: Evolution of entomopathogenicity in fungi. *J. Invertebr. Pathol.* **98**, 262–266.
- Hunt, R. S.; Morrison, D. J.; Bérubé, J., 2011: *Armillaria solidipes* is not a replacement name for *A. ostoyae*. *Forest Pathol.* **41**, 253–254.
- Ichinose, H., 2012: Molecular and functional diversity of fungal cytochrome P450s. *Biol. Pharm. Bull.* **35**, 833–837.
- Ide, M.; Ichinose, H.; Wariishi, H., 2012: Molecular identification and functional characterization of cytochrome P450 monooxygenases from the brown-rot basidiomycete *Postia placenta*. *Arch. Microbiol.* **194**, 243–253.
- Idnurm, A.; Howlett, B. J., 2001: Pathogenicity genes of phytopathogenic fungi. *Mol. Plant Pathol.* **2**, 241–255.
- Isroi; Millati, R.; Syamsiah, S.; Niklasson, C.; Cahyanto, M. N.; Lundquist, K.; Taherzadeh, M. J., 2011: Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. *Bioresources* **6**, 5224–5259.
- van der Kamp, B. J., 1993: Rate of spread of *Armillaria ostoyae* in the central interior of British Columbia. *Can. J. For. Res.* **23**, 1239–1241.
- Keča, N.; Solheim, H., 2011: Ecology and distribution of *Armillaria* species in Norway. *Forest Pathol.* **41**, 120–132.
- Kile, G. A.; McDonald, G. I.; Byler, J. W., 1991: Ecology and disease in natural forests. Chapter 8 In: *Armillaria Root Disease*. Eds by Shaw III, C. G.; Kile, G. A. Washington, DC: USDA Forest Service, Agriculture Handbook No. 691, pp. 233.
- Kluttsch, J. G.; Kallas-Richlefs, M. A.; Reich, R. M.; Harris, J. L.; Jacobi, W. R., 2012: Relationship of site and stand characteristics to *Armillaria* root disease incidence on ponderosa pine in the Black Hills, South Dakota. *Forest Pathol.* **42**, 160–170.
- Korhonen, K., 1978: Infertility and clonal size in the *Armillariella mellea* complex. *Karstenia* **18**, 31–42.
- Kubartová, A.; Ottosson, E.; Dahlberg, A.; Stenlid, J., 2012: Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. *Mol. Ecol.* **21**, 4514–4532.

- Lehtijärvi, A.; Doğmuş-Lehtijärvi, H. T.; Aday, A. G., 2012: *Armillaria ostoyae* associated with dying 60-year-old Scots pines in northern Turkey. *Forest Pathol.* **42**, 267–269.
- Levasseur, A.; Piumi, F.; Coutinho, P. M.; Rancurel, C.; Asther, M.; Delattre, M.; Henrissat, B.; Pontarotti, P.; Delattre, M.; Henrissat, B.; Pontarotti, P.; Asther, M.; Record, E., 2008: FOLy: an integrated database for the classification and functional annotation of fungal oxidoreductases potentially involved in the degradation of lignin and related aromatic compounds. *Fungal Genet. Biol.* **45**, 638–645.
- MacDonald, J.; Doering, M.; Canam, T.; Gong, Y.; Guttman, D. S.; Campbell, M. M.; Master, E. R., 2011: Transcriptomic responses of the softwood-degrading white-rot fungus *Phanerochaete carnosae* during growth on coniferous and deciduous wood. *Appl. Environ. Microbiol.* **77**, 3211–3218.
- MacDonald, J.; Suzuki, H.; Master, E. R., 2012: Expression and regulation of genes encoding lignocellulose-degrading activity in the genus *Phanerochaete*. *Appl. Microbiol. Biotechnol.* **94**, 339–351.
- Mahajan, S.; Master, E. R., 2010: Proteomic characterization of lignocellulose-degrading enzymes secreted by *Phanerochaete carnosae* grown on spruce and microcrystalline cellulose. *Appl. Microbiol. Biotechnol.* **86**, 1903–1914.
- Mallett, K. I.; Hopkin, A. A.; Blenis, P. V., 1989: Vegetative incompatibility in diploid isolates of *Armillaria* North American biological species I and V. *Can. J. Bot.* **67**, 3083–3089.
- Maphosa, L.; Wingfield, B. D.; Coetzee, M. P. A.; Mwenje, E.; Wingfield, M. J., 2006: Phylogenetic relationships among *Armillaria* species inferred from partial elongation factor 1-alpha DNA sequence data. *Australas. Plant Pathol.* **35**, 513–520.
- Martínez, D.; Larrondo, L. F.; Putnam, N.; Gelpke, M. D. S.; Huang, K.; Chapman, J.; Helfenbein, K. G.; Ramaiya, P.; Detter, J. C.; Larimer, F.; Coutinho, P. M.; Henrissat, B.; Berka, R.; Cullen, D.; Rokhsar, D., 2004: Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nat. Biotechnol.* **22**, 695–700.
- Martínez, D.; Berka, R. M.; Henrissat, B.; Saloheimo, M.; Arvas, M.; Baker, S. E.; Chapman, J.; Chertkov, O.; Coutinho, P. M.; Cullen, D.; Danchin, E. G. J.; Grigoriev, I. V.; Harris, P.; Jackson, M.; Kubicek, C. P.; Han, C. S.; Ho, I.; Larrondo, L. F.; de Leon, A. L.; Magnuson, J. K.; Merino, S.; Misra, M.; Nelson, B.; Putnam, N.; Robertson, B.; Salamov, A. A.; Schmoll, M.; Terry, A.; Thayer, N.; Westerholm-Parvinen, A.; Schoch, C. L.; Yao, J.; Barabote, R.; Nelson, M. A.; Detter, C.; Bruce, D.; Kuske, C. R.; Xie, G.; Richardson, P.; Rokhsar, D. S.; Lucas, S. M.; Rubin, E. M.; Dunn-Coleman, N.; Ward, M.; Brettin, T. S., 2008: Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nat. Biotechnol.* **26**, 553–560.
- Martínez, D.; Challacombe, J.; Morgenstern, I.; Hibbett, D.; Schmoll, M.; Kubicek, C. P.; Ferreira, P.; Ruiz-Duenas, F. J.; Martínez, A. T.; Kersten, P.; Hammel, K. E.; Wymelenberg, A. V.; Gaskell, J.; Lindquist, E.; Sabat, G.; BonDurant, S. S.; Larrondo, L. F.; Canessa, P.; Vicuna, R.; Yadav, J.; Doddapaneni, H.; Subramanian, V.; Pisabarro, A. G.; Lavin, J. L.; Oguiza, J. A.; Master, E.; Henrissat, B.; Coutinho, P. M.; Harris, P.; Magnuson, J. K.; Baker, S. E.; Bruno, K.; Kenealy, W.; Hoegger, P. J.; Kües, U.; Ramaiya, P.; Lucas, S.; Salamov, A.; Shapiro, H.; Tu, H.; Chee, C. L.; Misra, M.; Xie, G.; Teter, S.; Yaver, D.; James, T.; Mokrejs, M.; Pospisek, M.; Grigoriev, I. V.; Brettin, T.; Rokhsar, D.; Berka, R.; Cullen, D., 2009: Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1954–1959.
- McGoldrick, S.; O'Sullivan, S. M.; Sheehan, D., 2005: Glutathione transferase-like proteins encoded in genomes of yeasts and fungi: insights into evolution of a multifunctional protein superfamily. *FEMS Microbiol. Lett.* **242**, 1–12.
- Morel, M.; Ngadin, A. A.; Droux, M.; Jacquot, J.-P.; Gelhaye, E., 2009: The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete *Phanerochaete chrysosporium*. *Cell Mol. Life Sci.* **66**, 3711–3725.
- Morrison, D. J., 2011: Epidemiology of Armillaria root disease in Douglas-fir plantations in the cedar-hemlock zone of the southern interior of British Columbia. *Forest Pathol.* **41**, 31–40.
- Mulholland, V.; MacAskill, G. A.; Laue, B. E.; Steele, H.; Kenyon, D.; Green, S., 2012: Development and verification of a diagnostic assay based on EF-1 α for the identification of *Armillaria* species in Northern Europe. *Forest Pathol.* **42**, 229–238.
- Ohm, R. A.; de Jong, J. F.; Lugones, L. G.; Aerts, A.; Kothe, E.; Stajich, J. E.; de Vries, R. P.; Record, E.; Levasseur, A.; Baker, S. E.; Bartholomew, K. A.; Coutinho, P. M.; Erdmann, S.; Fowler, T. J.; Gathman, A. C.; Lombard, V.; Henrissat, B.; Knabe, N.; Kües, U.; Lilly, W. W.; Lindquist, E.; Lucas, S.; Magnuson, J. K.; Piumi, F.; Raudaskoski, M.; Salamov, A.; Schmutz, J.; Schwarze, F. W. M. R.; vanKuyk, P. A.; Horton, J. S.; Grigoriev, I. V.; Wösten, H. A. B., 2010: Genome sequence of the model mushroom *Schizophyllum commune*. *Nat. Biotechnol.* **28**, 957–1010.
- Olson, Å.; Aerts, A.; Asiegbu, F.; Belbahri, L.; Bouzid, O.; Broberg, A.; Canbäck, B.; Coutinho, P. M.; Cullen, D.; Dalman, K.; Deflorio, G.; van Diepen, L. T. A.; Dunand, C.; Duplessis, S.; Durling, M.; Gonthier, P.; Grimwood, J.; Fossdal, C. G.; Hansson, D.; Henrissat, B.; Hietala, A.; Himmelstrand, K.; Hoffmeister, D.; Högberg, N.; James, T. Y.; Karlsson, M.; Kohler, A.; Kües, U.; Lee, Y.-H.; Lin, Y.-C.; Lind, M.; Lindquist, E.; Lombard, V.; Lucas, S.; Lundén, K.; Morin, E.; Murat, C.; Park, J.; Raffaello, T.; Rouzé, P.; Salamov, A.; Schmutz, J.; Solheim, H.; Ståhlberg, J.; Véléz, H.; de Vries, R. P.; Wiebenga, A.; Woodward, S.; Yakovlev, I.; Garbelotto, M.; Martin, F.; Grigoriev, I. V.; Stenlid, J., 2012: Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. *New Phytol.* **194**, 1001–1013.
- Ota, Y.; Kim, M.-S.; Neda, H.; Klopfenstein, N. B.; Hasegawa, E., 2011: The phylogenetic position of an *Armillaria* species from Amami-Oshima, a subtropical island of Japan, based on elongation factor and ITS sequences. *Mycoscience* **52**, 53–58.
- Park, B. H.; Karpins, T. V.; Syed, M. H.; Leuze, M. R.; Uberbacher, E. C., 2010: CAZymes Analysis Toolkit (CAT): web service for searching and analyzing carbohydrate-active enzymes in a newly sequenced organism using CAZY database. *Glycobiology* **20**, 1574–1584.
- Persson, I.; Tjerneld, F.; Hahn-Hägerdal, B., 1991: Fungal cellulolytic enzyme production: a review. *Process Biochem.* **26**, 65–74.
- Petersen, T. N.; Brunak, S.; von Heijne, G.; Nielsen, H., 2011: SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Methods* **8**, 785–786.
- Ramírez, L.; Oguiza, J. A.; Pérez, G.; Lavín, J. L.; Omarini, A.; Santoyo, F.; Alfaro, M.; Castanera, R.; Parenti, A.; Muguerza, E.; Pisabarro, A. G., 2011: Genomics and transcriptomics characterization of genes expressed postharvest at 4 degrees C by the edible basidiomycete *Pleurotus ostreatus*. *Int. Microbiol.* **14**, 111–120.
- Ravalason, H.; Jan, G.; Mollé, D.; Pasco, M.; Coutinho, P. M.; Lapiere, C.; Pollet, B.; Bertaud, F.; Petit-Conil, M.; Grisel, S.; Sigoillot, J.-C.; Asther, M.; Herpoël-Gimbert, I., 2008: Secretome analysis of *Phanerochaete chrysosporium* strain CIRM-BRFM41 grown on softwood. *Appl. Microbiol. Biotechnol.* **80**, 719–733.
- Ross-Davis, A. L.; Hanna, J. W.; Kim, M.-S.; Klopfenstein, N. B., 2012: Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1 alpha gene. *Mycoscience* **53**, 161–165.
- Ryu, J. S.; Shary, S.; Houtman, C. J.; Panisko, E. A.; Korripally, P.; St. John, F. J.; Crooks, C.; Siika-aho, M.; Magnuson, J. K.; Hammel, K. E., 2011: Proteomic and functional analysis of the cellulase system expressed by *Postia placenta* during brown rot of solid wood. *Appl. Environ. Microbiol.* **77**, 7933–7941.
- Sato, S.; Feltus, F. A.; Iyer, P.; Tien, M., 2009: The first genome-level transcriptome of the wood-degrading fungus *Phanerochaete chrysosporium* grown on red oak. *Curr. Genet.* **55**, 273–286.
- Scheller, H. V.; Ulvskov, P., 2010: Hemicelluloses. *Ann. Rev. Plant Biol.* **61**, 263–289.
- Shaw, C. G.; Omdal, D. W.; Ramsey-Kroll, A.; Roth, L. F., 2012: Inoculum reduction measures to manage Armillaria root disease in a severely infected stand of ponderosa pine in south-central Washington: 35-year results. *West. J. Appl. For.* **27**, 25–29.

- Sierota, A., 2012: Effect of fungi decomposing roots of forest trees on CO₂ release – an attempt of evaluation. *Sylwan* **156**, 128–136.
- Sipos, G.; Kuchler, K., 2006: Fungal ATP-binding cassette (ABC) transporters in drug resistance & detoxification. *Curr. Drug Targets* **7**, 471–481.
- Soanes, D. M.; Alam, I.; Cornell, M.; Wong, H. M.; Hedeler, C.; Paton, N. W.; Rattray, M.; Hubbard, S. J.; Oliver, S. G.; Talbot, N. J., 2008: Comparative genome analysis of filamentous fungi reveals gene family expansions associated with fungal pathogenesis. *PLoS One* **3**, e2300.
- Stoytchev, I.; Nerud, F., 2000: Ligninolytic enzyme complex of *Armillaria* spp. *Folia Microbiol.* **45**, 248–250.
- Suzuki, H.; MacDonald, J.; Syed, K.; Salamov, A.; Hori, C.; Aerts, A.; Henrissat, B.; Wiebenga, A.; vanKuyk, P. A.; Barry, K.; Lindquist, E.; LaButti, K.; Lapidus, A.; Lucas, S.; Coutinho, P.; Gong, Y.; Samejima, M.; Mahadevan, R.; Abou-Zaid, M.; de Vries, R. P.; Igarashi, K.; Yadav, J. S.; Grigoriev, I. V.; Master, E. R., 2012: Comparative genomics of the white-rot fungi, *Phanerochaete carnosae* and *P. chrysosporium*, to elucidate the genetic basis of the distinct wood types they colonize. *BMC Genomics* **13**, 444.
- Thuillier, A.; Ngadin, A. A.; Thion, C.; Billard, P.; Jacquot, J.-P.; Gelhaye, E.; Morel, M., 2011: Functional diversification of fungal glutathione transferases from the Ure2p class. *Int. J. Evol. Biol.* **2011**, 938308. doi:10.4061/2011/938308.
- Travadon, R.; Smith, M. E.; Fujiyoshi, P.; Douhan, G. W.; Rizzo, D. M.; Baumgartner, K., 2012: Inferring dispersal patterns of the generalist root fungus *Armillaria mellea*. *New Phytol.* **193**, 959–969.
- Tsykun, T.; Rigling, D.; Nikolaychuk, V.; Prospero, S., 2012: Diversity and ecology of *Armillaria* species in virgin forests in the Ukrainian Carpathians. *Mycol. Prog.* **11**, 403–414.
- Tuor, U.; Winterhalter, K.; Fiechter, A., 1995: Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay. *J. Biotechnol.* **41**, 1–17.
- Volk, T. J.; Burdsall, H. H. Jr, 1995: A nomenclatural study of *Armillaria* and *Armillariella* species. *Synop. Fungorum* **8**, 1–121.
- Westwood, A. R.; Conciatori, F.; Tardif, J. C.; Knowles, K., 2012: Effects of *Armillaria* root disease on the growth of *Picea mariana* trees in the boreal plains of central Canada. *For. Ecol. Manage.* **266**, 1–10.
- Wymelenberg, A. V.; Sabat, G.; Martinez, D.; Rajangam, A. S.; Teeri, T. T.; Gaskell, J.; Kersten, P. J.; Cullen, D., 2005: The *Phanerochaete chrysosporium* secretome: database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium. *J. Biotechnol.* **118**, 17–34.
- Wymelenberg, A. V.; Gaskell, J.; Mozuch, M.; Kersten, P.; Sabat, G.; Martinez, D.; Cullen, D., 2009: Transcriptome and secretome analyses of *Phanerochaete chrysosporium* reveal complex patterns of gene expression. *Appl. Environ. Microbiol.* **75**, 4058–4068.
- Wymelenberg, A. V.; Gaskell, J.; Mozuch, M.; Sabat, G.; Ralph, J.; Skyba, O.; Mansfield, S. D.; Blanchette, R. A.; Martinez, D.; Kersten, P. J.; Cullen, D., 2010: Comparative transcriptome and secretome analysis of wood decay fungi *Postia placenta* and *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **76**, 3599–3610.
- Wymelenberg, A. V.; Gaskell, J.; Mozuch, M.; BonDurant, S. S.; Sabat, G.; Ralph, J.; Skyba, O.; Mansfield, S. D.; Blanchette, R. A.; Grigoriev, I. V.; Kersten, P. J.; Cullen, D., 2011: Significant alteration of gene expression in wood decay fungi *Postia placenta* and *Phanerochaete chrysosporium* by plant species. *Appl. Environ. Microbiol.* **77**, 4499–4507.
- Yoder, O. C.; Turgeon, B. G., 2001: Fungal genomics and pathogenicity. *Curr. Opin. Plant Biol.* **4**, 315–321.
- Yu, G.-J.; Wang, M.; Huang, J.; Yin, Y.-L.; Chen, Y.-J.; Jiang, S.; Jin, Y.-X.; Lan, X.-Q.; Wong, B. H. C.; Liang, Y.; Sun, H., 2012: Deep insight into the *Ganoderma lucidum* by comprehensive analysis of its transcriptome. *PLoS One* **7**, e44031.
- Žifčáková, L.; Baldrian, P., 2012: Fungal polysaccharide monoxygenases: new players in the decomposition of cellulose. *Fungal. Ecol.* **5**, 481–489.