The foliar endophyte *Phialocephala scopiformis* DAOMC 229536 secretes enzymes supporting growth on wood as sole carbon source

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Abstract

The conifer needle endophyte, *Phialocephala scopiformis*, was cultivated in media containing ground *Pinus contorta* wood as sole carbon source. After five and seven days growth, concentrated extracellular fluids were subjected to LC-MS/MS analyses. A total of 590 proteins were identified of which 99 were assigned to glycoside hydrolase families within the Carbohydrate Active Enzyme (CAzyme) system. Multiple isozymes of exo- and endo-acting cellulases were among the most abundant proteins, and oxidative degradation of cellulose was supported by the presence of lytic polysaccharide monooxygenases, glucooligosaccharide

oxidase and cellobiose dehydrogenase. Oxidoreductases were also plentiful and included GMC oxidoreductases, alcohol dehydrogenases, laccases, copper radical oxidases, tyrosinases and catalase. The expression and diversity of extracellular oxidoreductases indicates a capacity to metabolize alcohols and aromatic compounds.

Introduction

Microbial degradation of wood, a pivotal process in terrestrial carbon cycling, involves the activities of a diverse array of fungi and bacteria (1-3). A common assumption is that wood is decomposed by free-living saprotrophic fungi, primarily wood rot species in the fungal phylum Basidiomycota (4). Among these wood rot fungi, certain members of the subdivision Agaricomycotina, particularly the white-rot fungi, efficiently depolymerize and mineralize lignin. Extracellular class II peroxidases, and perhaps laccases, have been repeatedly implicated in ligninolysis by these Basidiomycetes. Low efficiency lignin degraders *Jaapia argillacea* and *Botryobasidium botryosum* lack class II peroxidases (5) and are unable to oxidize lignin model compounds. Still, these slow-growing fungi erode wood cell walls and lignin is removed. Other members of the Agaricomycotina, collectively referred to as brown rot fungi, rapidly depolymerize the cellulose within plant cell walls while leaving the lignin as a modified residue (6).

While most research has focused on the lignin-degrading abilities of the Basidiomycota, evidence is emerging that fungal species in the other dominant fungal phylum, Ascomycota,

may also play a significant role in decay of lignified wood. Evidence supports lignin degradation by several members of the Ascomycete family, Xylariaceae. This capability was most convincingly demonstrated for *Daldinia concentrica* which efficiently degrades hardwood lignin as well as ¹⁴C-labeled synthetic lignins (i.e. DHPs) (7, 8). Other Ascomycete wood decayers, collectively referred to as ‘soft rot’, have received relatively little attention (9). Some capacity to degrade cellulose and hemicellulose is presumed, but their role in ligninolysis, if any, is unknown. This is a significant knowledge gap, as the Ascomycetes are a diverse and abundant fungal group on land.

In particular, Ascomycete fungi comprise most fungal species that associate with live plant tissues, including endophytes (10). Dark septate endophytes (DSEs) are a large and widely distributed group considered nutritionally distinct from the free-living wood decay saprophytes (11). However, accumulating evidence shows that many DSEs exhibit unexpectedly broad substrate utilization patterns (12-16) and gene complements (17, 18) suggesting significant contributions to carbon cycling via latent saprotrophy. Nevertheless, the enzymes these organisms produce, and biochemical mechanism(s) they use to depolymerize plant biopolymers, have not been identified.

Phialocephala scopiformis and *Phialocephala subalpina* are well known endophytes of conifer needles and roots, respectively. Nursery inoculation of conifer seedlings with *P. scopiformis* limits spruce budworm damage, and persists in mature trees (19). Recently sequenced, the genome sizes/predicted gene numbers for *P. scopiformis* and closely related *P. subalpina*, are estimated to be 48.9Mb/18,613 (20) and 69.7 Mb/20,173 (17), respectively. Schlegel et al (17) noted an impressive repertoire of predicted genes in *P. subalpina*,

particularly the diversity and number of those encoding Carbohydrate Active Enzymes (CAzymes; (21)). The authors suggested that this gene expansion might confer the capability of saprophytic growth utilizing plant-derived substrates. Metatranscriptome examination of extensively decayed *Pinus contorta* logs (SRA accessions SAMN07573382, SAMN07573383 and SAMN07573384) have shown that transcripts most closely related to *P. scopiformis*, followed by *P. subalpina*, constitute the most abundant Ascomycetes present.

Here, we show that the previously sequenced endophyte of conifer needles, *P. scopiformis* DAOMC 229536, is indeed capable of utilizing *P. contorta* wood as a sole carbon source and, in doing so, secretes an array of hydrolytic and oxidative enzymes.

Materials and methods

P. scopiformis DAOMC 229536 was obtained from the Canadian Collection of Fungal Cultures. Two-liter flasks containing 250 ml of basal salt media (22) were supplemented with 1.25 g of *P. contorta* wood ground in a Wiley-mill (30 mesh screen) as the sole carbon source. Cultures were inoculated with mycelium scraped from the surface of malt extract agar (2% w/w malt extract, 2% glucose w/w, 0.5% peptone, 1.5% agar) and placed on a rotary shaker (150 RPM). After five and seven days incubation at 22-24C, cultures were filtered successively through Miracloth (Calbiochem) and Whatman filter paper #50 and #541. Trichloroacetic acid was added 10% (wt/vol) to the cold filtrate which was stored overnight at 0C. The precipitate was centrifuged at 12,000 x g for 20 min and the pellet washed three times in cold acetone before air drying. Total proteins from the pellet fraction were further purified via

90 methanol/chloroform/water partitioning, where chloroform and methanol were added to
 91 pellet first, followed by water and allowed to partition with protein interphase formed between
 92 polar and non-polar fraction. After multiple methanol washes, these finely purified protein
 93 preps were ultimately resolubilized in 8M Urea / 50mM NH_4HCO_3 (pH8.5) / 1mM TrisHCl and a
 94 fraction taken for protein concentration determination (PIERCE™ 660nm Protein Assay kit,
 95 ThermoFisher Scientific). NanoLC-MS/MS was used to identify proteins in culture filtrates as
 96 described (23-25). In short, equal amounts of total protein per sample were trypsin/LysC
 97 digested, OMIX C18 SPE purified (Agilent Technologies) and finally 2μg loaded for nanoLC-
 98 MS/MS analysis using an Agilent 1100 nanoflow system (Agilent Technologies) connected to a
 99 hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap Elite™, ThermoFisher
 100 Scientific) equipped with an EASY-Spray™ electrospray source. Chromatography of peptides
 101 prior to mass spectral analysis was accomplished using capillary emitter column (PepMap® C18,
 102 3μM, 100Å, 150x0.075mm, ThermoFisher Scientific) onto which 2μl of purified peptides was
 103 automatically loaded. NanoHPLC system delivered solvents A: 0.1% (v/v) formic acid , and B:
 104 99.9% (v/v) acetonitrile, 0.1% (v/v) formic acid at 0.50 μL/min to load the peptides (over a 30
 105 minute period) and 0.3μl/min to elute peptides directly into the nano-electrospray with gradual
 106 gradient from 3% (v/v) B to 20% (v/v) B over 154 minutes and concluded with 12 minute fast
 107 gradient from 20% (v/v) B to 50% (v/v) B at which time a 5 minute flash-out from 50-95% (v/v) B
 108 took place. As peptides eluted from the HPLC-column/electrospray source survey MS scans
 109 were acquired in the Orbitrap with a resolution of 120,000 followed by MS2 fragmentation of
 110 20 most intense peptides detected in the MS1 scan from 380 to 1800 m/z; redundancy was
 111 limited by dynamic exclusion. Raw MS/MS data were converted to mgf file format using

MSConvert (ProteoWizard: Open Source Software for Rapid Proteomics Tools Development) for downstream analysis. Resulting mgf files were used to search against *Phialocephala scopiformis* protein database containing 18,573 entries via the JGI portal (<https://genome.jgi.doe.gov/portal/pages/dynamicOrganismDownload.jsf?organism=Phisc1>) with a list of common lab contaminants and decoy sequences to establish False Discovery Rate (37,222 total entries) using in-house *Mascot* search engine 2.2.07 [Matrix Science] with variable methionine oxidation, asparagine and glutamine deamidation plus fixed cysteine carbamidomethylation. Scaffold (version Scaffold_4.7.5, Proteome Software Inc., Portland, OR) was used for spectral based quantification and to validate MS/MS peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 80.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (26). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. The raw mass spectrometry data was deposited to Chorus repository (chorusproject.org, ID#1478) for public sharing and visualization.

Proteins were classified by blastp queries of NCBI NR and EMBL-EBI predictions of Interpro domains and secretion signals. Carbohydrate active enzyme (CAzyme) family assignments were as described (21)

Results and discussion

We found that the spruce endophyte *P. scopiformis* DAOMC 229536 will utilize lodgepole pine wood as a sole carbon source and, to do so, a diverse set of extracellular enzymes are expressed. Peptides corresponding to 590 proteins were identified from this species and broadly classified (Fig 1). Those categorized as CAzymes included glycoside hydrolases (GHs), auxiliary activities (AAs) and carbohydrate esterases (CEs), and accounted for 24% of the total proteins. Highly expressed ribosomal and ‘housekeeping’ proteins involved in central metabolism made up 56% of the total. These are typically intracellular proteins, and their detection is likely a result of hyphal lysis. Although function could not be predicted, the 84 sequences classified as ‘hypothetical’ were generally conserved in other fungi and 32 featured clear secretion signals. One of these, model Phisc_220767, had the highest protein abundance score in both samples. Interestingly, the sequence contains InterPro domain IPR021054 that is predicted to bind hydrophobic surfaces. In *Aspergillus oryzae* cultures, binding to the polymer polybutylene succinate-co-adipate mobilizes degradation by cutinase (27). In this connection, we also detected a cutinase protein in the *P. scopiformis* cultures (Phisc_572142). Most (82%) of the identified proteins were present at both time points, and emPAI values were generally highest after seven days growth. Detailed information for all 590 proteins is listed in supporting information (S1 File).

Fig 1. Distribution of 590 *P. scopiformis* proteins detected in media containing ground lodgepole pine as the sole carbon source after five or seven days incubation. Of these, 482

proteins were identified at both time points, 10 were exclusively detected at day five and 98 were found only in day seven cultures (see S1 File for details).

The *P. scopiformis* genome is predicted to encode 387 glycoside hydrolases (<https://genome.jgi.doe.gov/mycocosm/annotations/browser/cazy/summary?f4VTMj?p=Phisc1>) and 99 were identified here. These were distributed among 39 families or subfamilies (Fig 2). For most families, a fraction of the predicted proteins was observed, but all representatives of GH36, GH39, GH5_7, GH53, GH54 and GH74 were identified. No clear relationship was observed between the number of genes per family and their detection (Fig 2) or their abundance (Table 1). Cellulases were among the most abundant enzymes and included the ‘exo’-acting cellobiohydrolases (GH6s, GH7s), endoglucanases (GH5_5) and beta-glucosidase (GH3) (Table 1). All had secretion signals and family one carbohydrate binding modules (CBM1s) were predicted in six of the cellulases. Other GHs were more likely involved in the degradation of hemicellulose which, in conifers such as lodgepole pine, is primarily composed of O-acetylglactoglucomannans and nonacetylated arabinoglucuronoxylans (28). The identified hydrolases (Table 1) are well suited for debranching and depolymerizing these complex substrates (reviewed in (29)). Inspection of the 14 predicted GH78s, including the three identified by mass spectrometry, failed to identify a feruloyl esterase domain in addition to the expected rhamnosidase. Such a bifunctional enzyme has been implicated in lignocellulose degradation by the soft rotter *Xylaria polymorpha* (30). No secretion signal was predicted for an Alpha-arabinofuranosidase (Phisc_760840) and Alpha-galactosidase (Phisc_659201), but

alternative models Phisc_597046 and Phisc_659199, respectfully, produced high-confidence eukaryote signals.

Fig 2. Predicted genes and LC-MS/MS detected glycoside hydrolases. The number of predicted genes <https://genome.jgi.doe.gov/Phisc1/Phisc1.home.html> and detected proteins are shown by brown and blue bars, respectively.

Table 1. Proteins classified as CAzyme Glycoside Hydrolases and Carbohydrate Binding Modules¹

Protein	5 Day	7 Day	CAzyme	Putative function	comment
668883	14.220	27.784	GH7-CBM	Cellobiohydrolase (CBH1)	SS
709446	9.673	22.500	GH5_5-CBM1	Endo-1,4-beta-glucanase	SS
573362	5.670	21.688	GH54	Alpha-N-arabinofuranosidase	SS
650591	4.158	13.740	GH7	Cellobiohydrolase (CBH1)	SS
703845	2.129	6.525	GH5_5	Endo-1,4-beta-glucanase	SS
749482	0.791	4.340	GH5_7	Endo-beta-1,4-mannanase	SS
617769	1.831	4.083	GH27	Alpha galactosidase	SS
760840	1.464	3.633	GH62	Alpha-L-arabinofuranosidase	
591570	1.418	3.425	GH15-CBM20	1,4-alpha-D-glucan glucohydrolase	SS
785300	1.254	3.213	GH39	Beta-xylosidase	SS
580672	0.715	2.853	GH5_7-CBM1	Endo-beta-1,4-mannanase	SS
118029	0.976	2.356	GH93	Alpha-L-arabinofuranosidase	SS
613046	1.917	2.149	GH6	Cellobiohydrolase (CBH2)	SS
706718	0.717	1.950	GH6-CBM1	Cellobiohydrolase (CBH2)	SS
706461	0.484	1.864	GH7-CBM	Cellobiohydrolase (CBH1)	SS
574388	0.241	1.818	GH78	Alpha-L-rhamnosidase	SS
639775	0.348	1.641	GH53	Arabinogalactan endo-1,4-beta-galactosidase	SS
539647	0.316	1.616	GH11	Endo-1,4-beta-xylanase	SS
701593	0.536	1.475	GH13-CBM20	Alpha-amylase	SS
783091	1.193	1.372	GH28	Endopolygalacturonase	SS
595918	0.928	1.347	GH7	Cellobiohydrolase (CBH1)	SS
593362	0.110	1.312	GH131	Exo/Endo glucanase	SS
725142	0.447	1.296	GH51	Alpha-N-arabinofuranosidase	SS
659201	0.791	1.261	GH27	Alpha-galactosidase	
679634	0.144	1.096	GH5_9	Glucan 1,3-beta-glucanase	SS
687038	0.039	1.080	GH3	Beta-glucosidase	SS
756372	0.130	1.078	GH11	Endo-1,4-beta-xylanase	SS
588206	0.242	1.061	GH10-CBM1	Endo-1,4-beta-xylanase	SS
758257	0.719	0.969	GH11	Endo-1,4-beta-xylanase	SS
709032	0.287	0.961	GH53	Endo-1,4-beta-galactanase	SS

¹Thirty CAzymes (31) with highest exponentially modified protein abundance indexes (emPAI) (32) after 5 and 7 day growth on ground lodgepole pine. Abbreviations: GH, glycoside hydrolase; CBM, carbohydrate binding module; SS, secretion signal

In addition to conventional hydrolases long known to degrade cellulose and hemicellulose, an array of extracellular oxidative enzymes and hypothetical proteins were unambiguously detected. The precise role of some of these enzymes remains uncertain, but the impressive diversity suggests complex strategies involved in lignocellulose degradation (Table 2). A glucose-methanol-choline (GMC) oxidoreductase (Phisc_171447) was the second most abundant protein in cultures. A putative alcohol oxidase, the sequence is highly conserved among a wide range of taxa. Other AA3 family members include putative glucose oxidases which, together with the copper radical oxidases (AA5_1s), may generate extracellular H₂O₂. Peroxide production is also observed in brown rot Basidiomycetes in which cellulose depolymerization is generally attributed to hydroxyl radical production via Fenton chemistry (33-39). Benzoquinone reductase (Phisc_680385) and oxalate decarboxylase (Phisc_490473; Table 3) have also been implicated in this wood decay process (38, 40). In addition to the glycoside hydrolases (Table 1) and the possibility of Fenton-generated hydroxyl radical, *P. scopiformis* may directly attack cellulose (41, 42) and xylans (43, 44) with Lytic polysaccharide monooxygenases (LPMO; AA9s), Cellobiose dehydrogenase (CDH; AA3_1) and Glucanase oxidases (GOOX; AA7). Peptides corresponding to nine model LPMO proteins were detected and, in line with the abundance of AA3s and AA5s, these copper enzymes are now known to be dependent upon H₂O₂ (45, 46). In short, *P. scopiformis* employs both hydrolytic and oxidative strategies for the utilization of *P. contorta* wood.

Table 2. Proteins classified as members of Auxiliary Activities families¹

Protein	5 Day	7 Day	Description	Blast hit	Accession	E-Value	Len	Similar	Score
612778	0.166	0.506	AA1 MCO	<i>Marssonina brunnea</i>	EKD21147	0.00E+00	550	80.36%	818.535
584830	0.154	0.154	AA1 MCO	<i>Coniochaeta ligniaria</i>	OIW22846	0.00E+00	528	75.00%	676.396
696679	0.353	0.739	AA1_3 Laccase	<i>Marssonina brunnea</i>	XP007288341	0.00E+00	582	82.65%	865.914
785626	0.166	0.166	AA1_3 Laccase	<i>Rhynchosporium agropyri</i>	CZT07190	0.00E+00	590	75.25%	746.888
590475	0.564	1.108	AA1_3 Laccase	<i>Marssonina coronariae</i>	OWO98956	0.00E+00	593	84.99%	932.554
319832	0.140	0.301	AA3 GMC OR	<i>Coniosporium apollinis</i>	EON61651	0.00E+00	675	77.04%	860.136
349182	0.259	0.514	AA3 GMC OR	<i>Glarea lozoyensis</i>	EPE33453	0.00E+00	650	74.62%	805.438
739447	0.112	0.644	AA3_1 CDH	<i>Oidiodendron maius</i>	KIM97752	0.00E+00	875	79.77%	1207.97
578381	0.163	0.739	AA3_2 glc oxidase	<i>Pseudogymnoascus sp.</i>	KFY35299	0.00E+00	589	79.12%	773.081
635790	0.050	0.276	AA3_2 glc oxidase	<i>Rhynchosporium secalis</i>	CZT50873	0.00E+00	581	84.51%	898.271
171447	15.199	57.808	AA3_2 GMC OR	<i>Cenococcum geophilum</i>	OCK94107	3.60E-173	563	66.25%	512.686
608108	0.138	0.044	AA3_3 Alc oxidase	<i>Glarea lozoyensis</i>	XP008087078	0.00E+00	668	97.16%	1307.74
189635	0.100	0.000	AA4 VAO	<i>Hypoxylon sp.</i>	OTA53856	0.00E+00	594	88.72%	979.163
151448	0.404	0.889	AA5_1 GLOX-WSC	<i>Oidiodendron maius</i>	KIN08912	0.00E+00	675	88.30%	1128.24
738058	0.504	1.122	AA5_1 WSCs-GLOX	<i>Glarea lozoyensis</i>	EPE26650	0.00E+00	907	79.82%	1254.58
634098	0.551	0.839	AA5_1 WSCs-GLOX	<i>Oidiodendron maius</i>	KIN08912	0.00E+00	1276	74.37%	1574.3
688262	0.159	0.447	AA5_1 WSCs-GLOX	<i>Marssonina brunnea</i>	XP007297057	0.00E+00	816	86.27%	1308.89
741374	0.000	0.061	AA5_1 WSCs-GLOX	<i>Botrytis cinerea</i>	CCD53798	0.00E+00	1040	76.06%	1345.1
680385	0.770	1.355	AA6 BQR	<i>Rhynchosporium commune</i>	CZS94681	2.30E-122	200	90.00%	355.525
674401	0.258	0.734	AA7 FAD oxidase	<i>Coniochaeta ligniaria</i>	OIW31430	0.00E+00	631	74.64%	789.26
608078	0.125	1.032	AA7 GOOX	<i>Marssonina brunnea</i>	EKD20480	0.00E+00	482	75.73%	627.091
702965	0.000	0.097	AA8 cytochr CDH-like	<i>Glomium stellatum</i>	OCL01754	0.00E+00	666	86.19%	1072.38
205464	0.000	1.174	AA9 LPMO	<i>Rhynchosporium secalis</i>	CZT45874	1.70E-115	230	82.61%	338.191
632963	0.111	0.884	AA9 LPMO	<i>Oidiodendron maius</i>	KIM99039	1.10E-120	265	73.96%	355.14
712823	0.102	0.215	AA9 LPMO	<i>Glarea lozoyensis</i>	XP008082509	1.10E-133	312	76.28%	391.734
315829	2.816	4.964	AA9 LPMO	<i>Botrytis cinerea</i>	EMR86785	9.90E-148	269	85.50%	422.935
708693	0.480	1.190	AA9 LPMO	<i>Pseudogymnoascus sp.</i>	KFZ07833	7.70E-142	231	90.91%	404.831

781244	0.000	0.287	AA9 LPMO	<i>Glarea lozoyensis</i>	XP008086416	2.10E-122	241	80.91%	356.681
772659	0.300	1.197	AA9 LPMO	<i>Glarea lozoyensis</i>	EPE29707	1.70E-101	206	82.04%	302.753
707156	0.327	1.567	AA9 LPMO-CBM1	<i>Sclerotinia sclerotiorum</i>	APA13672	2.10E-128	335	69.25%	378.637
582205	0.204	0.449	AA9 LPMO-CBM1	<i>Glarea lozoyensis</i>	XP008084797	2.60E-144	323	75.85%	419.083
640901	0.000	0.435	AA9 LPMO	<i>Glonium stellatum</i>	OCL03334	3.10E-132	237	87.34%	381.719

¹Exponentially modified protein abundance indexes (emPAI) (32) after 5 and 7 days growth on ground lodgepole pine. CAzymes classifications (31). Abbreviations: MCO, Multicopper oxidase; GMC OR, Glucose-methanol-choline Oxidoreductase; CDH, Cellobiose dehydrogenase; glc, Glucose; alc, alcohol; VAO, vanillyn alcohol oxidase; GLOX, Glyoxal oxidase; WSC, domain of unknown function possibly involved in carbohydrate binding; GOOX, Glucooligosaccharide oxidase; LPMO, Lytic polysaccharide monooxygenase.

Table 3. Mass spectrometry identified proteins with predicted secretion signals and redox activity¹.

Protein	5 Day	7 Day	Putative function	Blast Hit	Accession	E-Value	Score
729673	0.304	0.489	Heme peroxidase-WSCs	<i>Glarea lozoyensis</i>	XP_008077688	0.00E+00	682.945
766309	0.278	0.445	Catalase	<i>Botrytis cinerea</i>	AAK77951	0.00E+00	1135.55
706407	0.000	0.146	FAD-binding oxidoreductase	<i>Botrytis cinerea</i>	EMR83325	0.00E+00	884.789
490473	1.010	1.761	Oxalate decarboxylase	<i>Marssonina coronariae</i>	OWO98734	0.00E+00	749.969
576987	0	0.148	Chloroperoxidase	<i>Marssonina coronariae</i>	OWP06232	0.00E+00	687.567
588357	0.145	0.226	Chloroperoxidase	<i>Rhynchosporium secalis</i>	CZT46035	0.00E+00	737.643
54274	0.588	1.614	Quinoprotein-ADH-like WSC	<i>Marssonina brunnea</i>	EKD19016	4.30E-179	569.311
682730	0.799	1.275	Quinoprotein-ADH-like WSC	<i>Macrophomina phaseolina</i>	EKG22475	0.00E+00	1586.24
447552	0.065	0.159	Quinoprotein-ADH-like WSC	<i>Sclerotinia sclerotiorum</i>	APA14815	0.00E+00	1806.96
412163	0.000	0.055	Quinoprotein-ADH-like WSC	<i>Marssonina brunnea</i>	EKD19016	0.00E+00	671.774
701506	0.000	0.640	Tannase and feruloyl esterase	<i>Oidiodendron maius</i>	KIM93823	0.00E+00	677.552
686719	0.350	0.966	Tyrosinase	<i>Marssonina coronariae</i>	OWP00047	0.00E+00	581.637
579440*	0.091	0.841	Tyrosinase	<i>Rhynchosporium commune</i>	CZT11605	8.20E-160	461.07
595981	0.000	0.492	Tyrosinase	<i>Oidiodendron maius</i>	KIN02411	0.00E+00	543.117
50069	0.000	0.482	Tyrosinase	<i>Colletotrichum gloeosporioides</i>	EQB46731	4.10E-148	431.409
657273	1.625	1.878	WSCs-galactose binding domain	<i>Glarea lozoyensis</i>	XP_008079841	1.20E-88	301.597

¹Exponentially modified protein abundance indexes (emPAI) (32) after five and seven days growth on ground lodgepole pine. Abbreviations: ADH, Alcohol dehydrogenase; WSC, domain of unknown function possibly involved in carbohydrate binding.

*Extended protein model 54274 preferred.

Additional extracellular redox enzymes, not classified within the CAzyme system, are listed in Table 3. Perhaps involved in melanin synthesis by DSEs such as *P. scopiformis*, four tyrosinases were identified after seven days incubation. The roles of chloroperoxidases (Phisc_576987; Phisc_588357) and heme peroxidase WSC (Phisc_729673) are more difficult to discern as these enzymes catalyze diverse and often non-specific reactions. The WSC domain ([PF01822](#)) is widely distributed among fungi and often assumed to be involved in the carbohydrate binding of various glycoside hydrolases and copper radical oxidases. The *P. scopiformis* genome contains at least 38 gene models with WSC domains, of which 15 functionally diverse proteins were identified (five copper radical oxidases, a heme peroxidase, a GH18 chitinase, a GH16 mixed-link glucanase, an AA1 multicopper oxidases and six hypotheticals (Tables 2, 3, and S1 File).

Gene expression data is not yet available for related DSEs, but genome comparisons of Schlegel et al (17) and Knapp et al (18) suggested that the expansion of CAzyme-encoding genes in DSEs genomes, including the root endophyte *P. subalpina*, may confer the ability for saprophytic growth. In this connection, other studies demonstrated that *Phialocephala fortinii* can hydrolyze carboxymethylcellulose (12, 13) and colonize the tracheids of softwoods (14). In addition, it has been shown that other root- and foliage-associated DSEs will utilize a wide spectrum of substrates (15, 16). Thus, latent saprotrophy may be widespread among DSEs including, at least in the initial stages, the decomposition of forest litter (47), twigs (48, 49) and logs (50). Considering this enormous carbon pool (51), DSEs likely play an important role in the carbon cycle and merit consideration in current ecosystem models (52-54).

References

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Supporting Information

S1 File. Detailed information for 590 *P. scopiformis* proteins. Protein abundance, putative function, CAzyme assignments, InterPro, GO and enzyme codes for 590 *P. scopiformis* proteins.