

# Methionine oxidation of Carbohydrate-Active enZymes during white-rot wood decay

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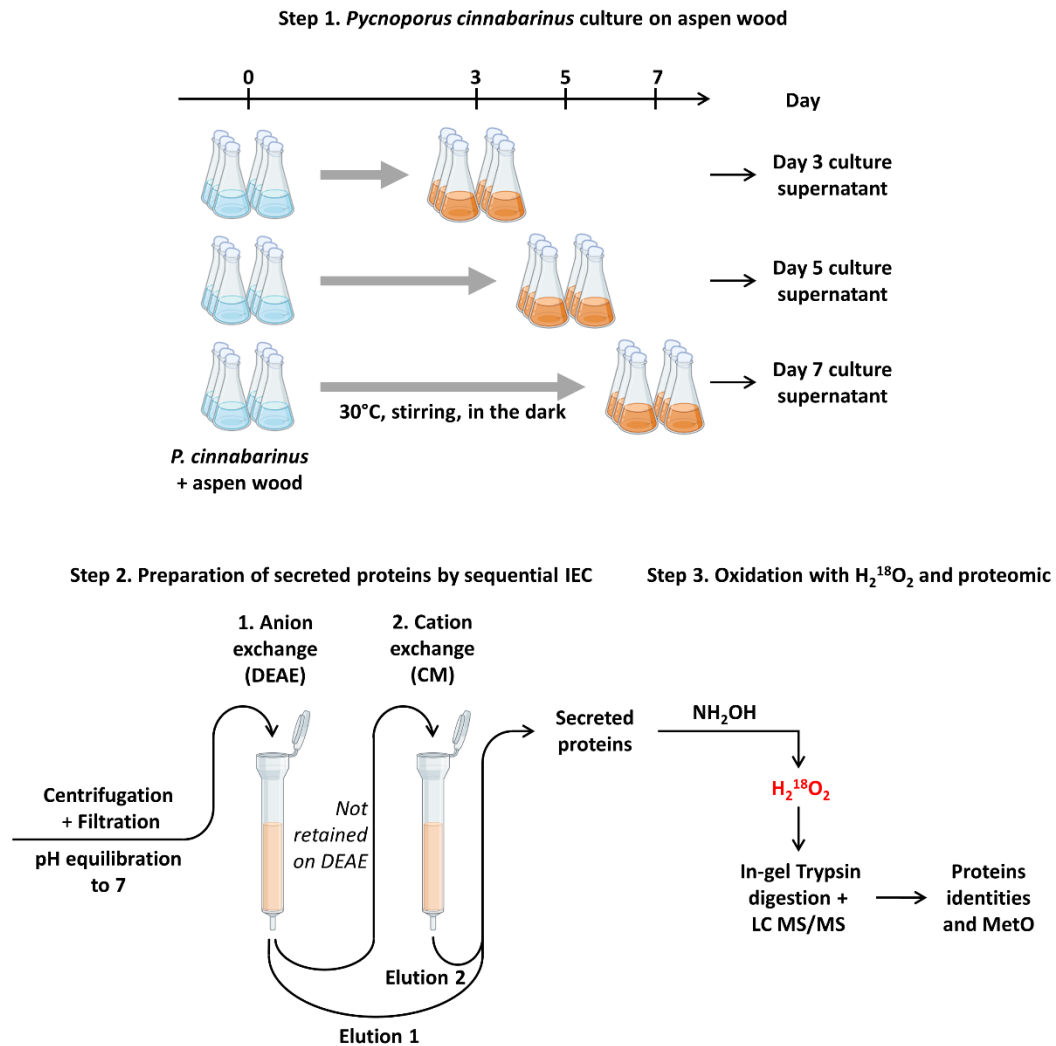
## SUPPLEMENTARY INFORMATION

### List of supplementary data

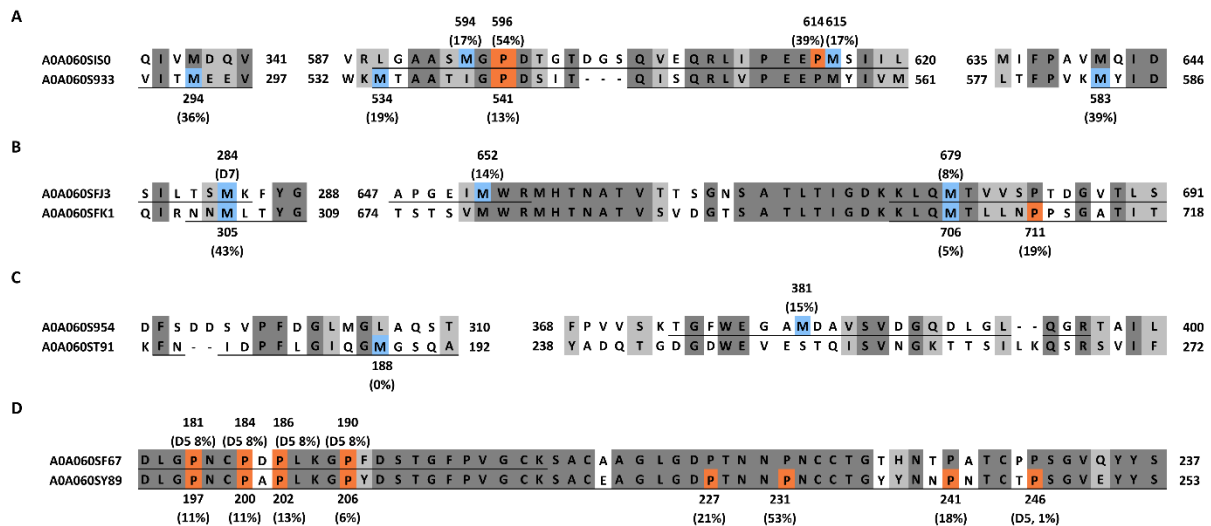
<b>Figure S1.</b> Secretome preparation and protein oxidation.	Page 2
<b>Figure S2.</b> Partial sequence alignment of GH16_2 (A), PL35 (B), protease (C) and GH152 (D) with identified oxidized residues.	Page 3
<b>Figure S3.</b> 3D model of GH7 (A) and GH18 (B) highlighting oxidized residues.	Page 4
<b>Table S1.</b> Number of secreted proteins identified. <i>Related to Figure 1</i>	Page 5
<b>Table S2.</b> Met content in secreted and non-secreted proteins of <i>P. cinnabarinus</i> , fungi, metazoans, and plants. <i>Related to Figure 2</i>	Page 6
<b>Table S3.</b> Levels of Met oxidation in secretomes obtained at day 3, 5 and 7. <i>Related to Figure 3</i>	Page 6
<b>Table S4.</b> Levels of Pro oxidation in secretomes obtained at day 3, 5 and 7. <i>Related to Figure 3</i>	Page 7
<b>Table S5.</b> Number of predicted secreted catalases in fungi.	Page 7

### List of additional datasets

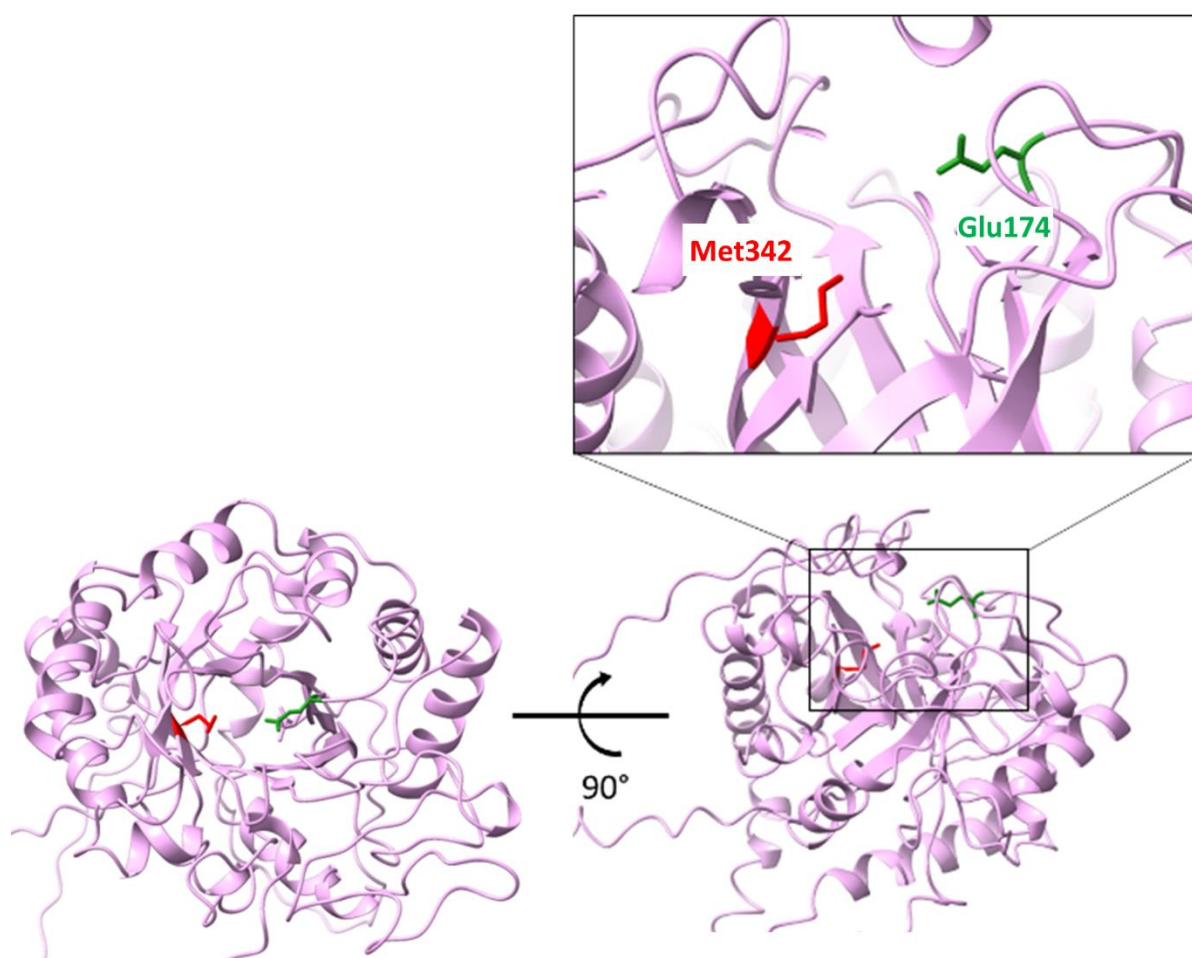
<b>Dataset 1.</b> List of identified secreted proteins and their spectral counts. <i>Related to Figure 1, Figure 2 and Table S1.</i>
<b>Dataset 2.</b> List of predicted secreted and non-secreted proteins of <i>P. cinnabarinus</i> and experimentally validated secreted and non-secreted proteins from fungi, metazoans and plants. <i>Related to Figure 2 and Table S2.</i>
<b>Dataset 3.</b> Percentage of the different forms of Met for the identified proteins. <i>Related to Figure 2, Figure 3 and Table S3.</i>
<b>Dataset 4.</b> Percentage of the different forms of Pro for the identified proteins. <i>Related to Figure 2, Figure 5 and Table S4.</i>
<b>Dataset 5.</b> Conservation of secreted proteins and their oxidized residues in a representative set of fungal species. <i>Related to Table 1.</i>



**Figure S1. Secretome preparation and protein oxidation.** The preparation of *P. cinnabarinus* secretomes and the oxidation of the secreted proteins were performed in 3 steps: *Step 1.* *P. cinnabarinus* was cultivated in the presence of *Populus tremuloides* sawdust (particle size < 2 mm) ( $15 \text{ g}\cdot\text{L}^{-1}$ ). Cultures were initiated in 18 flasks and 6 were used to prepare the secretomes of time point (day 3, 5 or 7). For each time point, the supernatants of 2 flasks were pooled to obtain one of the 3 replicates. *Step 2.* The supernatants were centrifuged, filtered and the pH adjusted to 7 with NaOH. The supernatant was then loaded onto an anion exchange column (DEAE). The solution containing the proteins not bound to the column during loading nor washing was loaded onto a cation exchange column (CM). The proteins retained on each column were eluted with NaCl (1M), pooled, desalted in 50 mM sodium acetate (pH 5.2) and concentrated by ultrafiltration (3 kDa cut-off). *Step 3.* The proteins ( $70 \mu\text{g}$ ) were incubated with 1 mM hydroxylamine ( $\text{NH}_2\text{OH}$ ) to inhibit  $\text{H}_2\text{O}_2$ -consuming activity, desalted, and incubated with  $\text{H}_2^{18}\text{O}_2$  (100 mM, 1 h). After desalting,  $10 \mu\text{g}$  were used for in-gel trypsin digestion and peptide identification by LC-MS/MS.



**Figure S2. Partial sequence alignment of GH16\_2 (A), PL35 (B), protease (C) and GH152 (D) with identified oxidized residues.** Met and Pro found oxidized are on *blue* and *orange* background, respectively. *Dark* and *light grey* backgrounds represent strictly conserved and similar amino acids, respectively. Position and percentage of oxidation of the residues are indicated above or under the sequence. For Met, the percentage presented correspond to sum of Met<sup>16</sup>O and Met<sup>16</sup>O<sub>2</sub> found at day 3, excepted indicated with 'D7', corresponding to Met found oxidized with <sup>18</sup>O at day 7. For Pro, the percentage presented correspond to the sum of all oxidized forms, either <sup>16</sup>O or <sup>18</sup>O at day 3, excepted indicated with 'D5', corresponding to Pro oxidation found at day 5. Underlined sequences represent the peptides detected by LC-MS/MS. Accession numbers are on the left of the sequences. All data are presented in **Datasets 3 and 4**.



**Figure S3. 3D model of GH18 highlighting oxidized residues.** 3D model of the GH18 A0A060SC99 was downloaded from AlphaFold database (<https://alphafold.ebi.ac.uk/entry/A0A060SC99>). The Met 342 found oxidized and the catalytic Glu 174 are represented as *red* and *green* sticks, respectively.

**Table S1. Number of identified secreted proteins.** The list of individual proteins and their abundance is provided in the **Dataset 1**.

<i>Type</i>	<i>Day 3</i>	<i>Day 5</i>	<i>Day 7</i>	<i>Total count</i>		
<b>Total proteins</b>	212 ± 7	231 ± 7	225 ± 25	308		
<b>Protease</b>	21 ± 1	25 ± 2	23 ± 6	30		
<b>Other</b>	20 ± 3	21 ± 2	20 ± 2	35		
<b>Unknown</b>	72 ± 3	81 ± 3	78 ± 8	118		
<b>CAZymes</b>	99 ± 3	105 ± 4	104 ± 10	125		
	<i>Families</i>				<i>Target substrate</i>	
	AA1_1; AA1_2	4 ± 0	4 ± 1	4 ± 0	5	Lignin; Iron
	AA2	1 ± 0	2 ± 1	2 ± 2	5	Lignin
	AA3_2	3 ± 0	3 ± 0	3 ± 0	4	Aryl-Alcohol/Glucose
	AA5_1	3 ± 1	3 ± 0	3 ± 1	4	Glyoxal
	AA8	1 ± 1	1 ± 0	0 ± 1	1	Iron
	AA9	1 ± 1	0 ± 1	1 ± 2	4	Cellulose
	CBM1; CBM13; CBM50 <sup>a</sup>	3 ± 0	3 ± 0	3 ± 0	3	Cellulose / Hemicellulose / Fungal cell wall
	CE1	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	CE4	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	CE8	0 ± 1	1 ± 0	1 ± 1	1	Pectin
	CE15	0 ± 1	1 ± 0	1 ± 0	1	Hemicellulose
	CE16	2 ± 0	2 ± 0	2 ± 1	2	Hemicellulose
	GH2	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	GH3	3 ± 1	3 ± 0	3 ± 0	3	Cellulose
	GH5_5; GH5_7; GH5_9	8 ± 1	9 ± 2	9 ± 1	11	Cellulose
	GH7	3 ± 0	3 ± 0	3 ± 0	3	Cellulose
	GH9	1 ± 1	1 ± 1	0 ± 1	1	Cellulose
	GH10	4 ± 1	3 ± 1	4 ± 1	5	Hemicellulose
	GH12	2 ± 0	1 ± 1	1 ± 1	2	Cellulose
	GH13_1; GH13_32	2 ± 0	2 ± 1	2 ± 1	2	Starch
	GH15	1 ± 0	1 ± 0	1 ± 0	1	Starch
	GH16_1; GH16_2	9 ± 1	9 ± 0	9 ± 1	9	Hemicellulose / Unknown
	GH17	2 ± 0	2 ± 0	2 ± 0	2	Hemicellulose
	GH18	8 ± 0	8 ± 0	8 ± 1	10	Fungal cell wall
	GH28	4 ± 1	4 ± 1	4 ± 0	5	Pectin
	GH30_3	3 ± 0	2 ± 0	2 ± 0	3	Glycoproteins
	GH31	2 ± 0	2 ± 0	2 ± 0	2	Glycoproteins
	GH32	1 ± 0	1 ± 0	1 ± 0	1	Disaccharides
	GH35	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	GH37	1 ± 0	1 ± 0	1 ± 0	1	Disaccharides
	GH43_6; GH43_24	1 ± 1	2 ± 0	2 ± 0	2	Hemicellulose
	GH45	1 ± 0	1 ± 0	1 ± 0	1	Cellulose
	GH47	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	GH51	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	GH55	2 ± 0	2 ± 0	2 ± 0	2	Hemicellulose
	GH72	1 ± 0	1 ± 0	1 ± 0	1	Fungal cell wall
	GH74	1 ± 0	1 ± 0	1 ± 0	1	Cellulose
	GH76	2 ± 1	2 ± 1	2 ± 1	2	Glycoproteins
	GH79	0 ± 1	2 ± 0	2 ± 0	2	Glycoproteins
	GH88	0 ± 0	1 ± 0	1 ± 0		Pectin
	GH92	1 ± 1	1 ± 0	1 ± 1	2	Glycoproteins
	GH95	1 ± 0	1 ± 0	1 ± 0	1	Hemicellulose
	GH125	0 ± 0	1 ± 0	1 ± 0	1	Fungal cell wall
	GH128	2 ± 0	2 ± 0	1 ± 1	2	Fungal cell wall
	GH131	2 ± 0	1 ± 1	2 ± 1	3	Cellulose
	GH135	0 ± 1	1 ± 0	1 ± 0	1	Fungal cell wall
	GH152	3 ± 0	3 ± 0	3 ± 0	3	Fungal cell wall
	PL8_4	1 ± 0	1 ± 0	1 ± 0	1	Potentially Animals/Algae
	PL14	1 ± 0	1 ± 0	1 ± 0	1	Potentially Animals/Algae
	PL35	1 ± 0	1 ± 0	1 ± 0	2	Chondroitin
	PL42	1 ± 0	1 ± 0	1 ± 0	1	Potentially Bacterial envelope

<sup>a</sup> CBM correspond to proteins for which only a CBM domain has been assigned. It should be noted that other CAZymes possess a CBM associated with a catalytic domain.

**Table S2. Met content in secreted and non-secreted proteins of *P. cinnabarinus*, fungi, metazoans, and plants.** The list of individual proteins is provided in the **Dataset 2**.

Dataset	Description	Total nb. of organisms	Total nb. of prot.	Met percentage				
				Average	P-value (t.test)	Min	Max	Med.
2A	<i>P. cinnabarinus</i> predicted secreted proteins	1	666	1.55 ± 0.82	2.30x10 <sup>-49</sup>	0.24	5.88	1.43
2B	<i>P. cinnabarinus</i> predicted non secreted proteins	1	9649	2.07 ± 0.96		0.18	23.16	1.97
2C	Fungal secreted proteins	121	5542	1.52 ± 0.74	0 <sup>a</sup>	0.11	7.41	1.44
2D	Fungal non secreted proteins	400	15506	2.17 ± 0.99		0.18	16.67	2.04
2E	Metazoan secreted proteins	1884	22164	2.68 ± 1.40	1.53x10 <sup>-132</sup>	0.03	21.85	2.44
2F	Metazoan non secreted proteins	738	30832	2.41 ± 1.07		0.07	18.03	2.27
2G	Plant secreted proteins	69	2960	2.59 ± 1.55	0.125	0.15	16.46	2.28
2H	Plant non secreted proteins	1172	21013	2.55 ± 1.14		0.22	17.16	2.38

<sup>a</sup> The P-value being very close to 0, the calculation software cannot display the precise value.

**Table S3. Levels of Met oxidation in the secretomes obtained at day 3, 5 and 7.** The levels of non-oxidized and oxidized Met were calculated using all detected spectra (**Dataset 3B**).

	Day 3	Day 5	Day 7
Number of identified proteins with detected Met	75 ± 4	87 ± 3	86 ± 11
Total spectral counts of detected Met	749 ± 14	859 ± 73	913 ± 118
<b>Spectral count</b>			
Not modified Met	136 ± 25	535 ± 34	542 ± 67
Met <sup>16</sup> O	122 ± 11	307 ± 40	351 ± 53
Met <sup>16</sup> O <sub>2</sub>	15 ± 3	8 ± 3	12 ± 2
Met <sup>18</sup> O	459 ± 25	8 ± 2	5 ± 4
Met <sup>18</sup> O <sub>2</sub>	17 ± 3	1 ± 1	2 ± 1
<b>Percentage</b>			
Not modified Met	18.1 ± 3.0	62.3 ± 1.3	59.4 ± 0.4
Met <sup>16</sup> O	16.3 ± 1.5	35.6 ± 1.6	38.4 ± 0.9
Met <sup>16</sup> O <sub>2</sub>	2.0 ± 0.3	1.0 ± 0.3	1.4 ± 0.1
Met <sup>18</sup> O	61.3 ± 3.9	0.9 ± 0.2	0.6 ± 0.5
Met <sup>18</sup> O <sub>2</sub>	2.3 ± 0.4	0.2 ± 0.1	0.3 ± 0.1

SC, spectral counts; Met<sup>16</sup>O, methionine sulfoxide with <sup>16</sup>O; Met<sup>16</sup>O<sub>2</sub>, methionine sulfone with <sup>16</sup>O; Met<sup>18</sup>O, methionine sulfoxide with <sup>18</sup>O; Met<sup>18</sup>O<sub>2</sub>, methionine sulfone with <sup>18</sup>O.

**Table S4. Levels of Pro oxidation in the secretomes obtained at day 3, 5 and 7.** The levels of non-oxidized and oxidized Pro were calculated using all detected spectra (**Dataset 4B**).

	Day 3	Day 5	Day 7
Number of identified proteins with detected Pro	130 ± 3	147 ± 3	143 ± 15
Total spectral counts of Pro	3099 ± 145	3150 ± 197	2899 ± 196
<b>Spectral count</b>			
<b>Not modified Pro</b>	2866 ± 146	3017 ± 193	2794 ± 195
Glutamic 5-semialdehyde- <sup>16</sup> O (SC)	75 ± 9	61 ± 3	57 ± 3
Pyroglutamic acid- <sup>16</sup> O (SC)	47 ± 7	50 ± 3	35 ± 7
<b>Sum-<sup>16</sup>O (SC)</b>	<b>122 ± 5</b>	<b>112 ± 1</b>	<b>92 ± 10</b>
Glutamic 5-semialdehyde- <sup>18</sup> O (SC)	104 ± 8	16 ± 4	11 ± 4
Pyroglutamic acid- <sup>18</sup> O (SC)	7 ± 4	5 ± 2	3 ± 1
<b>Sum-<sup>18</sup>O (SC)</b>	<b>111 ± 6</b>	<b>20 ± 5</b>	<b>14 ± 4</b>
<b>Percentage</b>			
Not modified Pro	92.4 ± 0.4	95.8 ± 0.2	96.4 ± 0.4
<b>Glutamic 5-semialdehyde-<sup>16</sup>O (%)</b>	<b>2.4 ± 0.3</b>	<b>2.0 ± 0.2</b>	<b>2.0 ± 0.1</b>
Pyroglutamic acid- <sup>16</sup> O (%)	1.5 ± 0.2	1.6 ± 0.1	1.2 ± 0.2
<b>Sum-<sup>16</sup>O (%)</b>	<b>3.9 ± 0.1</b>	<b>3.6 ± 0.2</b>	<b>3.2 ± 0.3</b>
Glutamic 5-semialdehyde- <sup>18</sup> O (%)	3.4 ± 0.3	0.5 ± 0.1	0.4 ± 0.1
Pyroglutamic acid- <sup>18</sup> O (%)	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
<b>Sum-<sup>18</sup>O (%)</b>	<b>3.6 ± 0.3</b>	<b>0.6 ± 0.1</b>	<b>0.5 ± 0.2</b>

**Table S5. Number of predicted secreted catalases in fungi.** The protein sequence of *P. cinnabarinus* secreted catalase (A0A060STN2) was used to search for homologs by BLASTP against all fungal genomes. Among the 9492 hits, 1630 were predicted to be secreted.

Phyla	Nb. Genome	Nb. Secreted Catalase	Secreted catalase per genome
Ascomycota	1069	1182	1.11
Basidiomycota	256	374	1.46
<b>White rot</b>	<b>85</b>	<b>101</b>	<b>1.19</b>
<i>Ectomycorrhizal</i>	59	73	1.24
<i>Other saprotroph</i>	40	69	1.73
<i>Phytopathogen</i>	34	85	2.50
<i>Brown rot</i>	24	29	1.21
<i>Other</i>	14	17	1.21
Mucoromycota	48	70	1.46
Chytridiomycota	3	3	1.00
Zoopagomycota	1	1	1.00
Cryptomycota	0	0	0.00
<b>Total</b>	<b>1377</b>	<b>1630</b>	<b>1.20 ± 0.24</b>