

19 **Abstract**

20 White-rot fungi employ secreted carbohydrate-active enzymes (CAZymes) along with reactive oxygen
21 species (ROS), like hydrogen peroxide (H_2O_2), to degrade lignocellulose in wood. H_2O_2 serves as a co-
22 substrate for key oxidoreductases during the initial decay phase. While the degradation of
23 lignocellulose by CAZymes is well-documented, the impact of ROS on the oxidation of the secreted
24 proteins remains unclear and the identity of the oxidized proteins is largely unknown. Methionine
25 (Met) can be oxidized to Met sulfoxide (MetO) or Met sulfone (MetO₂) with potential deleterious,
26 antioxidant, or regulatory effects. Other residues, like proline (Pro), can undergo carbonylation. Using
27 the white-rot model *Pycnoporus cinnabarinus* grown on aspen wood, we analyzed the Met content of
28 the secreted proteins and their susceptibility to oxidation combining labelled $H_2^{18}O_2$ with proteomics.
29 Strikingly, their overall Met content was significantly lower (1.4%) compared to intracellular proteins
30 (2.1%), a feature conserved in fungi but not in other eukaryotes. We also evidenced that a catalase,
31 widespread in white-rot fungi, protects the secreted proteins from oxidation. Our redox proteomics
32 approach allowed identification of 49 oxidizable Met and 40 oxidizable Pro residues within few
33 secreted proteins, mostly CAZymes. Interestingly, many of them had several oxidized residues localized
34 in hotspots. Some Met, including those in GH7 cellobiohydrolases, were oxidized up to 47%, with a
35 substantial percentage of sulfone (13%). These Met are conserved in fungal homologs, suggesting
36 important functional roles. Our findings reveal that white-rot fungi safeguard their secreted proteins
37 by minimizing their Met content and by scavenging ROS and pinpoint redox-active residues in
38 CAZymes.

39

40 **Importance**

41 The study of lignocellulose degradation by fungi is critical for understanding the ecological and
42 industrial implications of wood decay. While carbohydrate-active enzymes (CAZymes) play a well-
43 established role in lignocellulose degradation, the impact of hydrogen peroxide (H₂O₂) on secreted
44 proteins remains unclear. This study aims at evaluating the effect of H₂O₂ on secreted proteins,
45 focusing on the oxidation of methionine (Met). Using the model white-rot model *Pycnoporus*
46 *cinnabarinus* grown on aspen wood, we showed that fungi protect their secreted proteins from
47 oxidation by reducing their Met content and utilizing a secreted catalase to scavenge exogenous H₂O₂.
48 The research identified key oxidizable Met within secreted CAZymes. Importantly, some Met like those
49 of GH7 cellobiohydrolases, undergone substantial oxidation levels suggesting important roles in
50 lignocellulose degradation. These findings highlight the adaptive mechanisms employed by white-rot
51 fungi to safeguard their secreted proteins during wood decay and emphasize the importance of these
52 processes in lignocellulose breakdown.

53

54 Introduction

55 Filamentous fungi are crucial in the carbon cycle because of their ability to decompose organic
56 matter and release carbon back into the ecosystem (1). Wood-decay fungi extract carbon from
57 recalcitrant polymers like cellulose and hemicelluloses embedded in a lignin matrix, by secreting
58 enzymes into the extracellular environment (2). The secreted proteins, commonly referred to as the
59 "secretome", contains the carbohydrate-active enzymes (CAZymes), including large sets of glycoside
60 hydrolases (GH) and auxiliary activity (AA) enzymes (3, 4). The GH class contains the endo- and exo-
61 acting enzymes targeting cellulose and hemicelluloses. The AA class contains laccases (AA1) and class
62 II peroxidases (AA2) involved in lignin degradation, and lytic polysaccharide monooxygenases (LPMO,
63 AA9) proposed to play an important role in the early phase of wood degradation (5–7). Reactive oxygen
64 species (ROS), particularly hydrogen peroxide (H_2O_2), play a crucial role in the early phase of wood
65 decay (8–10). H_2O_2 is generated by glucose-methanol-choline oxidoreductases (AA3) and copper
66 radical oxidases (AA5), that target carbohydrate or aliphatic/aromatic alcohols (11, 12). In white-rot
67 fungi, H_2O_2 acts as a co-substrate to fuels different types of LPMOs and AA2 peroxidases (13–15). While
68 the roles of CAZymes and H_2O_2 during the degradation of lignocellulose is well-documented, the impact
69 of ROS on the oxidation of CAZymes remains unclear. Fenton-generated ROS were shown to decrease
70 enzymatic activity of some GHs in white-rot secretomes, through the oxidation of the secreted
71 proteins, and the most abundant form of oxidation found was the oxidation of Met residues (16). Met
72 can be converted by H_2O_2 into Met sulfoxide (MetO) or Met sulfone (MetO₂) and this has been shown
73 to play critical roles in many organisms (17–20). For instance, Met oxidation can lead either to a loss
74 of function or act as a regulatory switch, to activate enzymes (21, 22). The presence of oxidizable Met
75 in proteins can also be associated to an antioxidant role by serving as a protection to avoid the
76 oxidation of residues critical for the protein function (23). MetO can be reduced back to Met by the
77 methionine sulfoxide reductases, but, as these enzymes are most likely not secreted they cannot
78 reduce oxidized Met of secreted proteins (24). Regarding the importance of H_2O_2 during wood decay,
79 our hypothesis is that some Met of secreted proteins can be oxidized and play antioxidant or regulatory

80 roles. Interestingly, the treatment of an α -galactosidase (GH27) from *Trichoderma reesei* with H_2O_2
81 strongly increased its activity due to the oxidation of a Met located near the active site, suggesting that
82 Met oxidation of CAZymes can have a positive effect on their activity (25). To identify secreted proteins
83 with oxidizable Met, we selected the white-rot *Pycnoporus cinnabarinus* grown on aspen wood as a
84 model. *P. cinnabarinus* secretes H_2O_2 -producing enzymes in the early phase of wood degradation, to
85 provide co-substrate for the H_2O_2 -consuming CAZymes (26), but also as potential oxidant of Met. We
86 characterized the secreted proteins and investigated the sensitivity of their Met to oxidation by using
87 a redox proteomics approach based on the use of ^{18}O -labeled hydrogen peroxide ($H_2^{18}O_2$) (27–30). Our
88 investigations reveal the extend of Met oxidation in fungal secretomes and the precise localization of
89 oxidizable residues in secreted proteins and CAZymes, suggesting potential antioxidant or regulatory
90 roles.
91

92 **Material and methods**

93 ***Culture of P. cinnabarinus on aspen wood.***

94 The dikaryotic strain *Pycnoporus cinnabarinus* CIRM-BRFM 145 was grown for 3, 5 or 7 days at
95 as described (26). The media contained maltose (2.5 g.l⁻¹) and *Populus tremuloides* aspen sawdust
96 particles of size < 2 mm (15 g. l⁻¹). Six replicates were mixed in pairs to obtain three replicate samples
97 per time-point.

98 ***Preparation of P. cinnabarinus secretomes and protein oxidation with H₂¹⁸O₂.***

99 The culture supernatants containing the secreted proteins were centrifugated (1 h, 30,000xg,
100 10 °C) and sterilized by filtration (0.22 µm; Express Plus; Merck Millipore). The supernatants were
101 equilibrated to pH 7 with 0.5 mM NaOH and the proteins were purified using two ion exchange
102 chromatographies (**Fig. S1**): first, an anion exchange on DEAE Sephadex A-25 (Cytiva) and a cation
103 exchange on CM Sephadex A-25 (Cytiva) for proteins not retained on the anion exchanger. Both resins
104 were initially equilibrated with 20 mM sodium phosphate buffer (pH 7). Elution of the secreted
105 proteins was performed with 1 M NaCl. Fractions from both anion and cation exchangers were pooled,
106 desalted on Sephadex G-25 (PD-10 Desalting columns, Cytiva) in 50 mM sodium acetate (pH 5.2) and
107 concentrated using Vivaspin polyethersulfone membrane (3 kDa cut-off, Sartorius). Protein
108 concentrations were determined using the Bradford method (Bio-Rad) and the secretomes were used
109 immediately. Secreted proteins (50 µg) were incubated 30 min with 1 mM hydroxylamine, desalted on
110 Sephadex G-25 (PD SpinTrap, Cytiva), then incubated with 100 mM of H₂¹⁸O₂ for 1 h in the dark, then
111 desalted. Protein concentrations were determined, and the samples were stored at -20°C.

112 ***Proteomics analysis.***

113 Proteins (15 µg) were precipitated trichloroacetic acid and processed for trypsin proteolysis as
114 described (31). Peptides (5 µl) were injected into a nanoscale Easy-Spray™ PepMap™ Neo 2 µm C18
115 (75 µm x 750 mm) capillary column (Thermo Scientific) operated with a Vanquish Neo UHPLC system
116 (Thermo Scientific) and resolved with a 65-min gradient of CH₃CN (5-40%), 0.1% formic acid, at a flow

117 rate of 0.25 μ L per min. Data-dependent acquisition analysis of the peptides was performed with an
118 Orbitrap Exploris 480 tandem mass spectrometer (Thermo) as described (32). Each full scan of peptide
119 ions in the high-field Orbitrap analyzer was acquired from m/z 350 to 1800 at a resolution of 120,000,
120 selecting only 2+ and 3+ charged precursors for high-energy collisional dissociation and with a dynamic
121 exclusion of 10 seconds. MS/MS spectra of fragmented precursors were acquired at a resolution of
122 15,000 and assigned to peptide sequences by the MASCOT Server 2.5.1 search engine (Matrix Science)
123 using the *P. cinnabarinus* annotated proteome, with fixed and variable modifications (**Datasets 3; 4**).
124 Proteins were validated when at least two different peptides (p-value <0.01) were detected, resulting
125 in a protein identification false discovery rate below 1%. Protein abundance was estimated using the
126 Normalized Spectral Abundance Factor (NSAF) (33). The Met content of the proteins was calculated
127 using the entire sequence. The Met content corrected by the NSAF was calculated using the following
128 formula:

$$129 \frac{\sum(\%Met_{protein\ n} \times NSAF_{protein\ n})}{\sum NSAF_{protein\ n}}$$

130 With $\%Met_{protein\ n}$ and $NSAF_{protein\ n}$ corresponding to the percentage of Met and the NSAF value of the
131 individual proteins identified, respectively. The percentage of modification of Met or Pro residues was
132 calculated by dividing the number of spectral counts (SC) of the residue with the modification by the
133 total number of SC for the residue and then multiplying the result by 100. The mass spectrometry
134 proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (34) partner
135 repository with the dataset identifier PXD046271 and 10.6019/PXD046271.

136

137 ***H₂O₂-consuming activity***

138 Secreted proteins (20 μ g) were incubated 1 h with H₂O₂ (30 mM) in sodium acetate buffer, pH
139 5.2. A control without proteins was done. Then, the samples were filtered using Vivaspin 3 kDa cut-off

140 and the remaining H₂O₂ in filtrates was titrated by absorbance measurement at 240 nm and compared
141 to H₂O₂ concentration in controls.

142

143 ***Analysis of Met content in P. cinnabarinus, fungi, metazoans, and plants proteins.***

144 *P. cinnabarinus* proteins were predicted secreted if they fulfilled three conditions: (i) presence
145 of a secretion signal peptide, (ii) absence of endoplasmic reticulum retention motif and (iii) absence of
146 transmembrane helix outside the signal peptide, as described (26). Experimentally validated fungal
147 secreted proteins were retrieved from proteomics analyses (7, 35–49), from Uniprot
148 (www.uniprot.org) and FunSecKB2 (50). Metazoans experimentally validated secreted were retrieved
149 from Uniprot, MetazSecKB (51) and the Human Protein Atlas (52, 53). Experimentally validated plant
150 secreted proteins were retrieved from Uniprot, PlantSecKB (54) and the SUBA database (55). The
151 datasets of fungal, metazoans and plants non-secreted proteins were made using proteins retrieved
152 from Uniprot, the Human Protein Atlas and the SUBA database. For all datasets, only manually curated
153 proteins, and those found with least 5 SC in proteomics, were conserved. Membrane proteins, proteins
154 shorter than 50 amino acids, or not starting with Met were discarded. Statistical analyses were
155 unpaired t-test with Welch's correction.

156

157 ***Conservation of proteins and oxidized residues.***

158 A hundred and twenty fungal genomes were selected based on their public availability and
159 their representativity in the 9 fungal phyla (56, 57). Targets of the oxidized proteins were searched
160 using BLASTP (v 2.6.0+) (58). The 100 sequences with the highest e-values were aligned using MAFFT
161 tool (version 6.864b) (59). The conservation of individual residues was made by counting its occurrence
162 in all the sequences.

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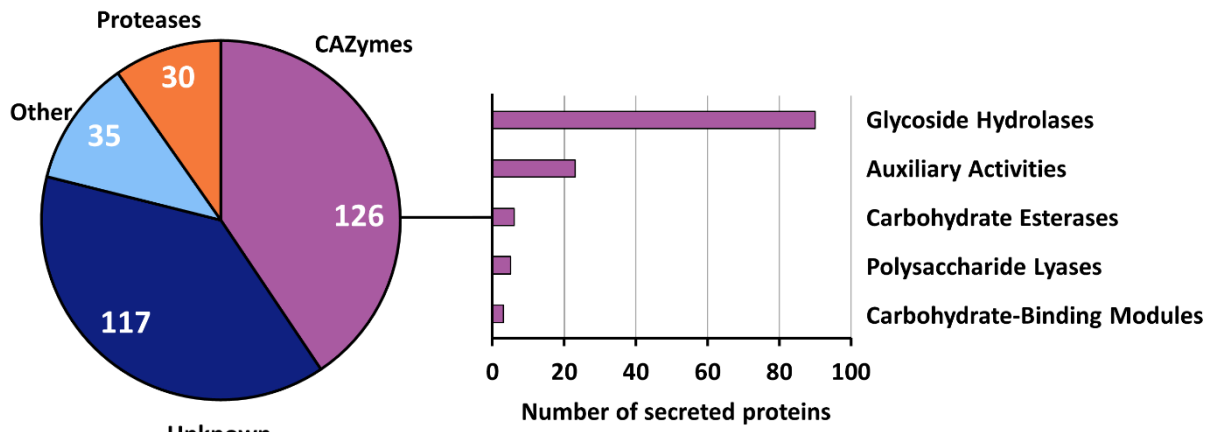
164 Results

165 ***Optimized methods allowed to obtain secreted proteins representative of early days decay.***

166 To identify oxidizable Met in the secreted proteins of *P. cinnabarinus*, we focused on the early
167 days of wood decay, during which production and consumption of H₂O₂ are likely to occur (42). We
168 performed a time-course growth of the fungus on aspen wood and purified the proteins from the
169 culture supernatants at days 3, 5 and 7 on successive anion and cation exchange chromatography to
170 avoid artifactual oxidation. Then, we oxidized the secreted proteins with H₂¹⁸O₂ and identified them
171 and their potential oxidative Met modifications using proteomics (**Fig. S1**). We identified 308 secreted
172 proteins, among which CAZymes and proteases represented 41% and 10%, respectively (**Fig. 1; Table**
173 **S1; Dataset 1**). Among the CAZymes, GH was by far the most represented class, with 89 proteins (71%)
174 spread over 34 families (**Fig. 1; Table S1**). We identified most of the CAZymes expected during wood
175 degradation, i.e. cellulases (GH3, GH5_5, GH5_9 and GH7, GH12, GH45), hemicellulases (GH10, GH43,
176 GH51, CE15, CE16) (4), as well as pectinases (GH28, CE8) and also some CAZymes targeting fungal cell
177 wall components (e.g. GH18 chitinases) (**Table S1**). Out of 23 identified AAs (18%), we found laccases
178 (AA1) and class II peroxidases (AA2), LPMOs (AA9) and H₂O₂-generating enzymes (AA3, AA5). We
179 estimated the abundance of the individual proteins using the Normalized Spectral Abundance Factor
180 (NSAF) (**Dataset 1**). The mean %NSAF of all identified proteins was ~ 0.3% and 52 proteins were above
181 this value. The most abundant protein categories were CAZymes (52%), proteins of unknown function
182 (35%) and proteases (8%). Among the most abundant CAZymes, we found the three cellobiohydrolases
183 (GH7), three chitinases (GH18) and two laccases (AA1). Interestingly, three H₂O₂-generating enzymes
184 (two AA3_2 and one AA5_1) were among these most abundant CAZymes (**Dataset 1**). Overall, the
185 identity of the secreted proteins and their abundance validated our approach, indicating that the
186 growth conditions, the proteins preparation method, and the oxidative treatment used were suitable
187 for studying Met sensitivity to oxidation.

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189



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191

192 **Figure 1. Distribution of the identified secreted proteins and CAZymes.** Values shown are the
193 aggregate of all replicates for each day (**Table S1; Dataset 1**). Bar graphs represent the number of
194 CAZymes per class.

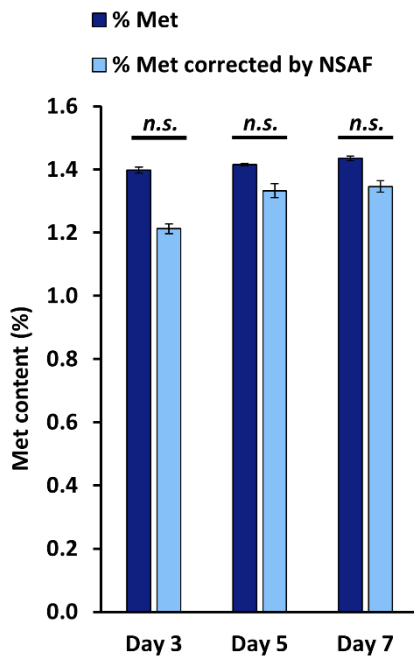
195

196 ***Met content is strongly reduced in fungal secreted proteins.***

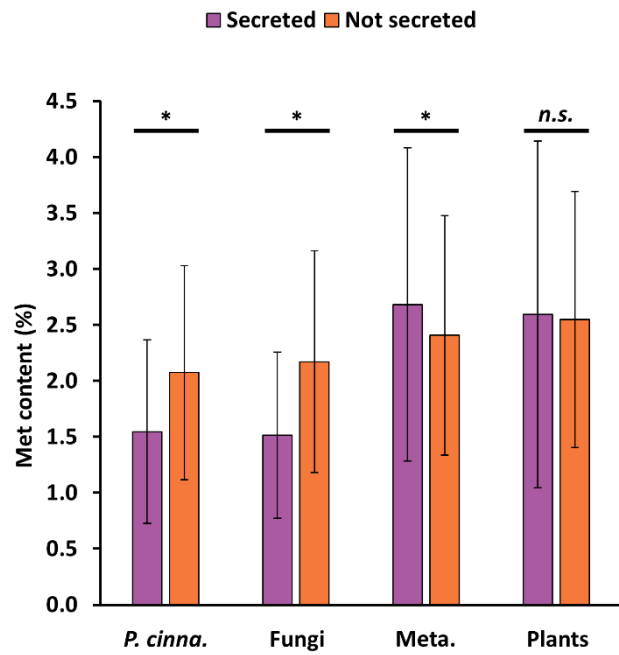
197 First, we evaluated the Met content of the secreted proteins, which was ~1.4% for each time-
198 point (**Fig. 2A**). Then, to determine whether some abundant proteins with specific Met content could
199 influence the overall content, we corrected the values by the proteins NSAF. The corrected Met
200 contents were 1.21 ± 0.02 , 1.33 ± 0.02 , and 1.35 ± 0.02 , for days 3, 5, and 7, respectively (**Fig. 2A**).
201 These results indicate that the Met content in the proteins secreted by *P. cinnabarinus* is comprised
202 between 1.2 to 1.4%. Strikingly, this value is twice lower than the Met content of proteins in general,
203 i.e. 2.4% (60). We investigated whether this characteristic may apply to all secreted proteins of *P.*
204 *cinnabarinus*. The theoretical *P. cinnabarinus* secretome consists of 666 proteins with an average Met
205 content of $1.55 \pm 0.82\%$, while the fungus non-secreted proteins have a higher mean of $2.07 \pm 0.96\%$
206 Met. (**Fig. 2B; Table S2; Dataset 2**). These results show that *P. cinnabarinus* secreted proteins contain
207 significantly less Met than the non-secreted. Then, we compared the Met content of experimentally
208 validated secreted proteins from fungi, metazoans, and plants with the Met content of intracellular
209 proteins (**Fig. 2B; Table S2; Dataset 2**). Fungal secreted proteins have a mean Met content of $1.52 \pm$
210 0.74% , significantly lower than that of non-secreted proteins ($2.17 \pm 0.99\%$). In contrast, the Met
211 content of metazoans secreted proteins ($2.68 \pm 1.40\%$) is significantly higher than that of the non-
212 secreted proteins ($2.41 \pm 1.07\%$). No statistical difference exists between the Met content in secreted
213 ($2.59 \pm 1.55\%$) and non-secreted ($2.55 \pm 1.14\%$) proteins of plants (**Fig. 2B; Table S2; Dataset 2**).
214 Altogether, these results show that the secreted proteins of fungi contain less Met than the
215 intracellular proteins, and that this feature is not conserved in other eukaryotes.

216

A



B



217

218 **Figure 2. Met content of *P. cinnabarinus* secreted proteins and comparison with fungal, metazoan,**
 219 **and plant secreted and intracellular proteins. A)** Met content of the identified *P. cinnabarinus*
 220 secreted proteins calculated for each protein and averaged for the three secretomes at each time
 221 point. Alternatively, the Met content for each protein was normalized by the protein NASF and
 222 averaged. Values shown are the mean of the 3 replicates \pm SD. **B)** Met content of intracellular proteins
 223 and of secreted in the *P. cinnabarinus* theoretical proteome versus the Met content of experimentally
 224 validated intracellular and secreted proteins from fungi, metazoans, and plants (**Table S2; Dataset 2**).
 225 Values shown are the mean of all proteins in each set \pm SD. Statistical analysis performed by unpaired
 226 t-test with Welch's correction ($*P \leq 0.001$; *n.s.*, not significant).

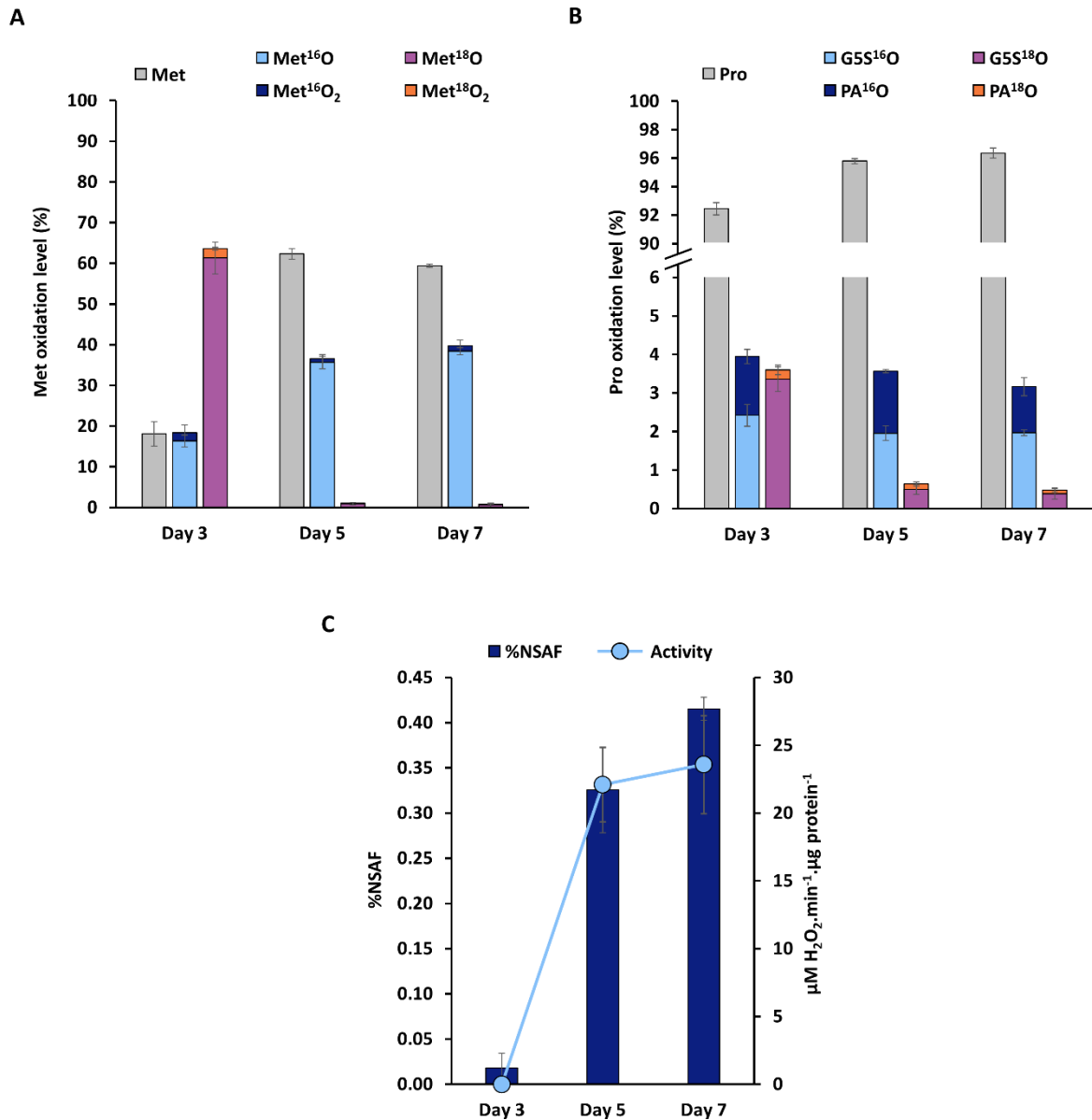
227

228 ***A catalase protects secreted proteins from oxidation.***

229 The identification of oxidizable Met in *P. cinnabarinus* secreted proteins required accurate
230 quantification of their oxidation levels. H₂¹⁸O₂ was used to prevent artifactual Met oxidation potentially
231 occurring during sample preparation by blocking the Met reduced in the samples and converting them
232 to Met sulfoxide or sulfone containing ¹⁸O atom (Met¹⁸O and Met¹⁸O₂, respectively). The use of a
233 concentrated H₂¹⁸O₂ solution (100 mM) should allow the oxidation of all the reduced Met and the
234 reliable quantification of the initial levels of Met¹⁶O and Met¹⁶O₂ (28, 29). For the day 3 secretome, the
235 levels of Met carrying ¹⁸O or ¹⁶O, either as sulfoxide or sulfone, were ~64% and ~18%, respectively.
236 Although, ~18% of Met in reduced form were still present, this indicated that most Met from the
237 identified proteins were oxidized after the treatment with H₂¹⁸O₂. Of note, ¹⁶O or ¹⁸O sulfone levels
238 reached ~2% (**Fig. 3A; Table S3; Dataset 3**). On the contrary, in day 5 and day 7 secretomes, the levels
239 of MetO and MetO₂ carrying ¹⁸O were extremely low (~1%), indicating that the blocking strategy failed
240 for these samples (**Fig. 3A; Table S3; Dataset 3**). To evaluate whether this lack of oxidation by H₂¹⁸O₂
241 in secretomes of days 5 and 7 affected only Met residues, we reanalyzed our data by searching for two
242 main forms of Pro carbonylation bearing ¹⁸O. For the day 3 secretome, we found slightly less than 4%
243 of Pro oxidized with ¹⁸O. A similar content of ¹⁶O was found, indicating that the large majority of Pro
244 were not oxidized (~92%) (**Fig. 2B; Table S4; Dataset 4**). This low level of Pro oxidation was anticipated
245 due to its low reactivity with H₂¹⁸O₂ (61). The percentage of ¹⁸O oxidation decreased to ~0.5% in the
246 day 5 and day 7 secretomes, while ¹⁶O oxidation levels remained comparable to those observed for
247 the day 3 secretome. Thus, the trend for Pro is the same as for Met indicating that the lack of oxidation
248 observed for Met was not specific to this residue. Proteins secreted during the days 5 and 7 of decay
249 were not oxidized by exogenous H₂¹⁸O₂, unlike those secreted at day 3. These results prompted us to
250 search for secreted enzymes that scavenge H₂O₂. A catalase stood out: almost absent at day 3 (0.02 ±
251 0.02 %NSAF), but more abundant at days 5 and 7, with %NSAF values of 0.33 ± 0.05 and 0.42 ± 0.01,
252 respectively (**Fig. 2C; Dataset 1**). When we measured the H₂O₂-consuming activity of the secretomes,
253 we observed no activity at day 3, but strong activities at days 5 and 7 secretomes, in a perfect

254 consistency with the catalase abundance (**Fig. 2C**). Although few potential peroxidases and LPMOs
255 were present, there was no difference in their abundance during the time course (**Dataset 1**). These
256 results indicated that this catalase was very likely responsible for most, if not all, of the H₂O₂-consuming
257 activity. Altogether, these results show that, under our experimental conditions, *P. cinnabarinus*
258 secreted a catalase at days 5 and 7, which, by consuming potential exogenous H₂O₂, protected the
259 secreted proteins from oxidation. On the contrary, at day 3, the secreted proteins are left without any
260 protection against exogenous oxidant. On the other hand, this highlight that the strategy consisting of
261 using high concentrations of H₂¹⁸O₂ to block reduced Met can hardly be applied to the secretomes
262 collected at days 5 and 7, at least in conditions where hydroxylamine was not an efficient inhibitor of
263 H₂O₂-consuming activity. Nonetheless, since Met oxidized with ¹⁸O were detected, we analyzed the
264 nature of the oxidized proteins to identify Met residues with potential antioxidant or regulatory roles.

265



266

267 **Figure 3. Levels of Met and Pro oxidation in the *P. cinnabarinus* secretomes and H₂O₂-consuming**
 268 **activity. A)** Total levels of Met oxidation as sulfoxide and sulfone, either ¹⁶O or ¹⁸O calculated using the
 269 spectral counts of all Met-containing peptides and compared to the level of non-oxidized Met for each
 270 time point (**Table S3; Dataset 3**). **B)** Total levels of Pro oxidation as glutamic 5-semialdehyde (G5S) and
 271 pyroglutamic acid (PA), either ¹⁶O or ¹⁸O calculated using the spectral counts of all Pro-containing
 272 peptides and compared to the level of non-oxidized Pro for each day (**Table S4; Dataset 4**). **C)** %NSAF
 273 of the catalase A0A060STN2 (**Dataset 2**) and total H₂O₂-consuming activity at each time point.

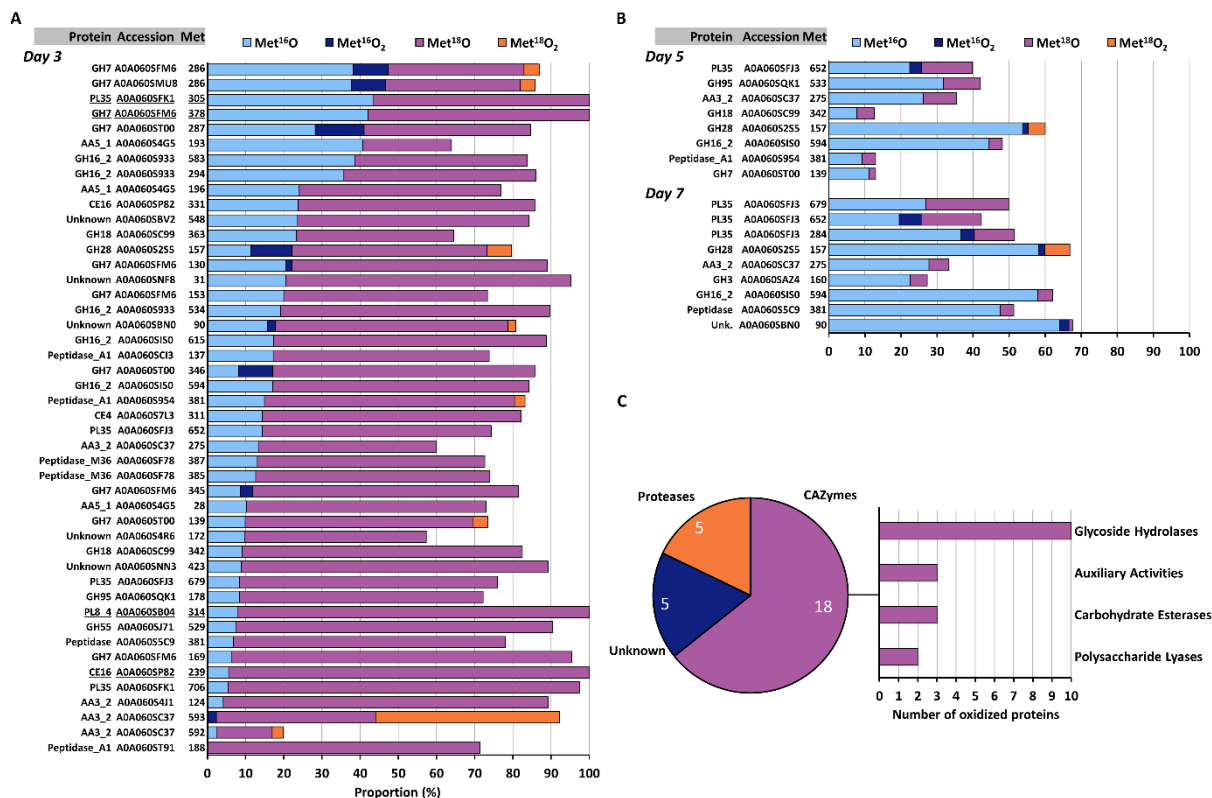
274

275 ***Most of the oxidations occur on a few secreted CAZymes.***

276 Among the 308 secreted proteins identified, we detected 265 Met belonging to 118 proteins
277 **(Table S3; Dataset 3)**. We selected only the Met-containing peptides present in all replicates for each
278 secretome, with at least five spectral counts, in at least two out of the three replicates. At day 3, all
279 selected Met were found oxidized with ¹⁸O **(Fig. 4A; Dataset 3)**. The total oxidation (¹⁶O and ¹⁸O) ranged
280 from ~ 20% to 100% for the 46 identified Met **(Fig. 4A)**. Four Met, all from CAZymes, were fully oxidized
281 and thus saturated with ¹⁸O: Met305 of a PL35, Met378 of a GH7, Met314 of a PL8, and Met239 of a
282 CE16 **(Fig. 4A; Dataset 3)**. Twenty-four proteins had oxidation levels ranging from ~81% to 97% and
283 17 from 57% to ~80%. Only the Met592 from an AA3_2 displayed a much lower value of total oxidation
284 (~20%) **(Fig. 4A; Dataset 3)**. The quantification of the ¹⁶O-oxidation levels appeared clear for the four
285 ¹⁸O-saturated Met and should correspond to the levels "naturally" found during the wood decay.
286 However, for all other Met with an oxidation value less than 100%, the saturation with ¹⁸O was
287 incomplete and some artifactual oxidation may have occurred during the sample preparation, and thus
288 the level of ¹⁶O might be overestimated. For these proteins, we assumed that the percentage of ¹⁶O
289 found during wood decay was necessarily equal to or lower than the measured one. The percentage
290 of oxidation as ¹⁶O ranged from 0% to 47% with a mean of 18% **(Fig. 4A; Dataset 3)**. This value is
291 consistent with the mean percentage of Met¹⁶O oxidation calculated considering all Met containing-
292 peptides found **(see Fig. 3A)** and indicated that most of the oxidation was concentrated on these few
293 secreted proteins. Concerning day 5 and day 7 secretomes, the very low levels of ¹⁸O oxidation
294 precluded the use of the blocking strategy. However, to find proteins with Met¹⁸O may allow to
295 consider H₂¹⁸O₂ as a probe for highly reactive residues, because of the low H₂¹⁸O₂ concentration they
296 were exposed to, due to the catalase activity. We found eight and nine Met bearing ¹⁸O in the proteins
297 secreted at day 5 and day 7, respectively, and five were common to both samples **(Fig. 4B; Dataset 3)**.
298 The percentages of ¹⁸O ranged from ~2% to ~14%, and from ~1% to ~23%, for the secreted proteins
299 of day 5 and day 7, respectively. Among the 11 oxidized Met, only three were uniquely found at day 5
300 or day 7: Met533 of a GH95 at day 5, and Met284 and Met160 belonging to a PL35 and a GH3,

301 respectively, at day 7 (**Fig. 4B; Dataset 3**). This indicates that most of the proteins found oxidized with
302 ^{18}O at day 5 or 7 were also oxidized at day 3, highlighting their high reactivity to H_2O_2 . We found a total
303 number of 49 oxidizable Met belonging to 28 proteins, representing ~24% and ~19% of all the
304 identified Met and proteins from the secretome, respectively (**Fig. 4C; Dataset 3**). Interestingly, the 18
305 identified CAZymes represented most of them (64%), while proteases and proteins of unknown
306 function both represented 18% with five identified proteins. The most represented CAZymes were GHs
307 with 10 identified proteins. These results indicates that the proportion of oxidized CAZymes was
308 enriched compared to the different types of proteins present in the secretomes (**compare Fig. 4C with**
309 **Fig. 1**).

310



312

313 **Figure 4. Identity of oxidized Met and their levels of oxidation in *P. cinnabarinus* secretomes. A)**
 314 **Proportion of the different forms of oxidation for the Met identified in the secreted proteins at day 3.**
 315 **Proteins are ranked based on the percentage of ¹⁶O (MetO and MetO₂). The four Met fully oxidized are**
 316 **underlined. Values shown are the mean of oxidation without standard deviation, omitted for clarity.**
 317 **All values are in Dataset 3. B) Proportion of the different forms of oxidation for the Met identified in**
 318 **the secreted proteins at day 5 and day 7. Proteins are ranked based on the percentage of ¹⁸O (MetO**
 319 **and MetO₂). All values are in Dataset 3. C) Distribution of the identified secreted proteins and CAZymes**
 320 **with oxidized Met. Values shown are the aggregate of all replicates for each time point (Dataset 3).**
 321 **Bar graphs represent the number of CAZymes per class.**

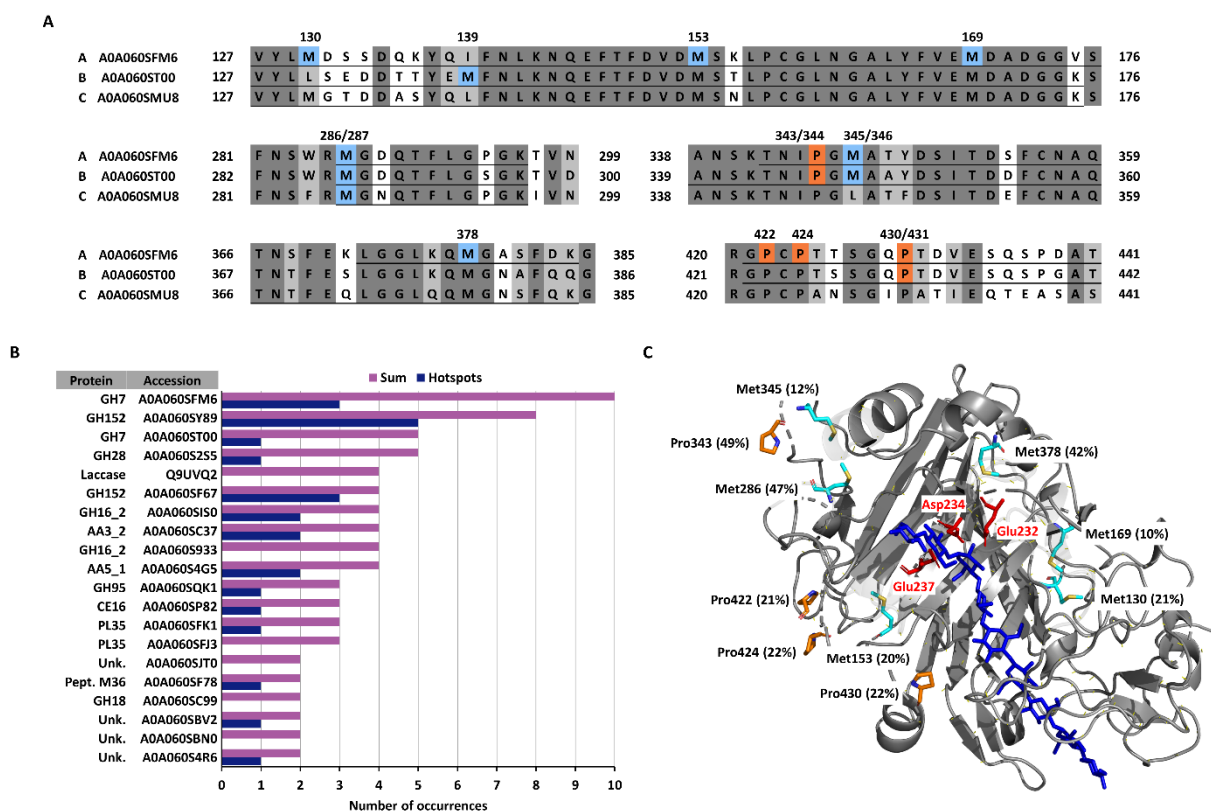
322

323 ***Oxidation occurs in multiple sites and hotspots of homologous proteins.***

324 Interestingly, among the five most oxidized, three Met belong to the three GH7 (A0A060SFM6,
325 A0A060SMU8, A0A060ST00, named A, B, and C, respectively), at position 286 of the GH7 A and B, and
326 at position 287 of the GH7 C (**Fig. 4A**). A sequence alignment showed that these positions are
327 conserved between these GH7 homologs (**Fig. 5A**). These Met were oxidized with similar levels of
328 Met¹⁶O (between 28% and 38%) and Met¹⁶O₂ (between 9% and 13%). We also detected seven other
329 oxidized Met on these GH7s, four were uniquely on the GH7 A, one on the GH7 B, and two were in a
330 position conserved in GH7 A and B. Since we had assessed the overall level of Pro oxidation, we
331 quantified the percentage of oxidation of individual Pro, considering only those labeled with ¹⁸O
332 (**Dataset 4**). GH7 A had four oxidized Pro, two of which were in positions conserved into the GH7 B
333 (**Fig. 5A**). Interestingly, we found that oxidation of Met and/or Pro occurred on homologs of GH16_2,
334 PL35, proteases and GH152 (**Fig. 4; Fig. S2**). This represented 11 proteins among the 33 proteins
335 identified having oxidized residues (28 with Met with or without Pro and 5 with Pro only) (**Fig. 4; Fig.**
336 **S2; Datasets 3; 4**). Moreover, 19 out of the 33 identified proteins were oxidized on multiple residues,
337 from two to 10. A GH152 was oxidized on 8 Pro, while the GH7 A was the protein with the highest
338 number of oxidation sites (10), including six Met and four Pro (**Fig. 4; Fig. 5; Fig. S2; Datasets 3; 4**).
339 Finally, several oxidation sites were located close to each other in the protein sequences, forming
340 “hotspots” like the Pro343 and Met345, and three other Pro of the GH7 A (**Fig. 5A**). In total, we counted
341 24 occurrences of sites containing two oxidized residues in the protein sequences (**Fig. 5B**). Analysis of
342 a tridimensional model of GH7 A revealed that oxidized residues distant on the sequence may be close
343 on the structure, as the Met130 and Met160, the Met153 in the vicinity of three Pro, and the Met286
344 clustered with the Pro343 and Met345 (**Fig. 5C**). Altogether, these results indicate that most of the
345 oxidation affects a relatively small set of CAZymes that can be oxidized at multiple positions, with some
346 hotspots. Moreover, the fact that oxidized residues were found at similar positions within the
347 homologs of *P. cinnabarinus* secreted proteins suggests that these residues have been conserved to
348 fulfill antioxidant or regulatory roles.

349

350



351

352 **Figure 5. Partial sequence alignment of GH7, numbers of oxidation sites and hotspots in identified**
 353 **secreted proteins and tridimensional model of GH7. A)** Oxidized Met and Pro residues are on *blue*
 354 and *orange* background, respectively. *Dark* and *light grey* backgrounds represent strictly conserved
 355 and similar amino acids, respectively. Position and percentage of oxidation of the residues are
 356 indicated above the sequence. For Pro, the percentage presented correspond to the sum of all oxidized
 357 forms, either ^{16}O or ^{18}O at day 3, excepted the one indicated with 'D5', corresponding to Pro oxidation
 358 at day 5. Underlined sequences represent peptides detected by LC-MS/MS. Accession numbers are on
 359 the left of the sequences. **B)** Values correspond to the total number of oxidizable Met and Pro sites
 360 found per protein. Hotspots correspond to the number of times that two oxidizable sites were located
 361 within a window of ten amino acids on the protein sequence. All data are presented in **Datasets 3 and**
 362 **4.** **C)** 3D model of the GH7 A0A060SFM6 downloaded from AlphaFold database (62). The position of
 363 the cellonanoase substrate (in *blue*) was modeled using the experimentally determined structure of
 364 *Trichoderma reesei* Cel7A (PDB #4C4C). Met and Pro found oxidized are represented as *cyan* and
 365 *orange* sticks, respectively. Catalytic residues are represented as *red* sticks. Percentage of oxidation at
 366 day 3 are in brackets. Some parts of the protein were rendered transparent for clarity.

367

368 **Conservation suggests a list of residues with antioxidant or regulatory roles.**

369 To determine whether oxidized residues found in *P. cinnabarinus* proteins were widely
370 conserved, we searched for homologs of the 33 proteins having oxidized residues in homologs from
371 other fungi and quantified the conservation of the 89 oxidized residues. Our hypothesis was that
372 oxidizable residues playing antioxidant or regulatory roles are more likely to be conserved among
373 distantly related organisms, as shown for Met of actin or of the ffh/SRP proteins (63–65). To avoid
374 overrepresentation of sequences coming from closely related organisms, the search was made in 120
375 representative fungal species, covering the nine fungal phyla (**Dataset 5**). Overall, the conservation of
376 Met or Pro ranged from 1% to 100%, with a mean of 49%. Considering only residues with conservation
377 values above 75%, and found in more than 80 sequences, we obtained a list of nine Met and nine Pro
378 at conserved positions in 14 secreted proteins (**Table 1**). The GH7 A has four conserved Met, among
379 which Met 169 conserved in 99% of the homologs, and a conserved Pro. Other Met are conserved in
380 the GH7 B and C, and in two GH16_2, one GH18, one AA3_2 and a peptidase M36, present in all fungi
381 (**Table 1; Dataset 5**). Regarding Pro, three are conserved in all homologs, at positions 614, 143 and 441
382 of a GH16_2, a GH28 and a laccase, respectively. Conserved Pro were also found in a AA3_2, an
383 unknown protein and a GH152 (**Table 1; Dataset 5**). These results allowed identification of proteins
384 with oxidizable Met and Pro residues strongly conserved across the fungal phyla.

385

386 **Table 1. *P. cinnabarinus* secreted proteins with oxidized residues conserved across fungal proteins.**
 387 Only proteins for which the number of identified homolog sequences identified was higher than 80
 388 were considered. The complete list of identified secreted proteins is presented in **Dataset 5**.

<i>Accession Uniprot (JGI)</i>	<i>Description</i>	<i>Repartition (Nb. of sequences)</i>	<i>Residue</i>	<i>Conservation (%)</i>
AOA060SFM6 (8640)	GH7	Dikarya (98)	Met 130	80
			Met 169	99
			Met 286 ^a	76
			Met 378	83
			Pro 430 ^b	99
AOA060ST00 (6809)	GH7	Dikarya (98)	Met 287 ^a	76
AOA060SMU8 (5845)	GH7	Dikarya (98)	Pro 431 ^b	99
AOA060SIS0 (301)	GH16_2	Basidiomycota (100)	Met 286 ^a	76
			Met 615	92
AOA060S933 (3097)	GH16_2	Basidiomycota (91)	Pro 614	100
AOA060S933 (3097)	GH16_2	Basidiomycota (91)	Met 583	83
AOA060SC99 (6004)	GH18	Dikarya, Mucor., Zoo. (99)	Met 342	86
AOA060S4J1 (1984)	AA3_2	Agaricomycetes (100)	Met 124	83
AOA060SF78 (8374)	Pep. M36	All fungi (100)	Met 387	81
AOA060S2S5 (8001)	GH28	Dikarya (99)	Pro 143	100
			Pro 284	96
Q9UVQ2 (8672)	Laccase	Basidiomycota (100)	Pro 441	100
AOA060SC37 (3147)	AA3_2	Dikarya (98)	Pro 594	97
AOA060S4R6 (3419)	Unknown	Dikarya, Mucor., Chytridio., Neo. (100)	Pro 170	83
AOA060SF67 (3180)	GH152	Dikarya, Mucoromycota (99)	Pro 184 ^c	94
AOA060SY89 (5455)	GH152	Dikarya, Mucor., Chytridio. (99)	Pro 200 ^c	96

389 ^{a, b, c} Conserved positions between the *P. cinnabarinus* homologs. *Mucor.*, *Mucoromycota*; *Zoo.*, *Zoopagomycota*,
 390 *Chytridio.*, *Chytridiomycota*, *Neo.*, *Neocallimastigomycota*.

391

392 Discussion

393 Taking a white-rot wood decayer as model, we showed that fungal secreted proteins have a
394 strongly reduced Met content (~1.4%) compared to the non-secreted (~2.1%) and that this feature is
395 not conserved in metazoans and plants. This decreased Met content suggests that protection of
396 proteins secreted by fungi is achieved by avoiding oxidation, in line with the previously proposed
397 mechanism (16). Interestingly, these data contrast with those obtained from animal studies which
398 showed that proteins encoded by the mitochondrial genome may contain up to 6-10% Met. This high
399 content could act as an endogenous antioxidant with the exposed Met scavenging ROS which are then
400 removed by methionine sulfoxide reductases in a cycle of oxidation and reduction (66). A similar
401 mechanism was proposed in bacteria (67, 68). The absence of external system of MetO reduction
402 preclude the use of such mechanism with fungal secreted proteins (24). This protection is most likely
403 accompanied by the secreted catalase we found (**Fig. 3**), in line with previous assumptions (16, 69–71).
404 The secretion of a catalase by *P. cinnabarinus* was unexpected, as it has not been previously detected
405 (41, 42), nor has extracellular activity been measured for the white-rot *Phanerochaete chrysosporium*
406 (72). However, searching for secreted catalase homologs, we found that it is widespread in
407 basidiomycota, and particularly in white-rot fungi (**Table S5**), contrary to previous phylogenetic
408 analysis (73). Lack of detection of the protein or its activity may be due to specific expression
409 conditions. In white-rot, it is surprising that a catalase was expressed along with H₂O₂ producing and
410 consuming enzymes. However, catalases have Km values in the mM range (70), and their action could
411 result in lowering the H₂O₂ concentration below a levels that is damaging to proteins, but allows the
412 activity of H₂O₂-driven oxidoreductases.

413 Using H₂¹⁸O₂-based proteomics, we identified oxidizable Met and highlighted that CAZymes
414 were the most oxidized secreted proteins during wood-decay, and that Met oxidation could occur on
415 several residues, sometimes clustered in hotspots. Clustering was also observed in bacterial proteins
416 (74), suggesting a universal phenomenon. The blocking strategy we used is based on a saturation of

417 reduced Met with ^{18}O . However, saturation was difficult to achieve, with only 4 Met saturated with ^{18}O
418 in the day 3 secretome (**Fig. 4**). An initial denaturation step would have probably allowed $\text{H}_2^{18}\text{O}_2$ to
419 access the Met buried in the protein core (28). However, as our goal was to identify secreted proteins
420 with potential oxidizable Met we chose not to denature them. Our idea was that an oxidizable Met
421 should be accessible to an oxidant, either natural or $\text{H}_2^{18}\text{O}_2$. The results at day 3 argue for the
422 accessibility of the Met to $\text{H}_2^{18}\text{O}_2$, as the percentage of ^{18}O was higher than that of ^{16}O for 43 Met out
423 of 46. As artifactual ^{16}O -oxidation cannot be ruled out, the percentage of ^{16}O was necessarily lower or
424 equal to those measured. In our study, the mean percentage of ^{16}O was 18%, while in studies on
425 animals using similar approaches, it was ~4% (27, 28), arguing for potential artifactual oxidation.
426 However, while intracellular proteins are protected by methionine sulfoxide reductases and ROS
427 scavengers, the MetO in secreted proteins cannot be reduced, and no H_2O_2 -consuming was detected
428 in the secretome at day 3. Thus, to find high levels of MetO in fungal secreted proteins is expected.
429 Interestingly, nine Met were oxidized as ^{16}O sulfone among which five were also oxidized as $\text{Met}^{18}\text{O}_2$
430 (**Fig. 4**), confirming their propensity to form sulfone during wood decomposition.

431 Some of the Met we found oxidized in *P. cinnabarinus* are conserved throughout the fungal
432 kingdom, suggesting potentially important roles (**Table 5**). They could act as antioxidants, as for the
433 GH7 whose Met and Pro are located at the surface of the proteins and in the substrate cleft (**Fig. 5C**),
434 potentially preventing critical residues from being oxidized, as proposed for a glutamine synthetase
435 (23). Oxidizable Met could also have a functional role altering enzyme activity, like for a GH18 in which
436 a conserved oxidized Met is located near the catalytic residue (**Fig. S3**), as shown for a GH27
437 galactosidase of *T. reesei* (25). In conclusion, our findings highlight a common mechanism in fungi
438 preventing oxidation of secreted proteins and, pinpoint redox-regulated CAZymes to be further
439 characterized.

440

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449

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460

461 **References**

- 462 1. Fabian J, Zlatanovic S, Mutz M, Premke K. 2017. Fungal–bacterial dynamics and their
463 contribution to terrigenous carbon turnover in relation to organic matter quality. *ISME J*
464 11:415–425.
- 465 2. Baldrian P, Valášková V. 2008. Degradation of cellulose by basidiomycetous fungi. *FEMS*
466 *Microbiol Rev* 32:501–521.
- 467 3. Drula E, Garron M-L, Dogan S, Lombard V, Henrissat B, Terrapon N. 2022. The carbohydrate-
468 active enzyme database: functions and literature. *Nucleic Acids Res* 50:D571–D577.
- 469 4. Hage H, Rosso M-N. 2021. Evolution of Fungal Carbohydrate-Active Enzyme Portfolios and
470 Adaptation to Plant Cell-Wall Polymers. 3. *J Fungi* 7:185.
- 471 5. Couturier M, Ladevèze S, Sulzenbacher G, Ciano L, Fanuel M, Moreau C, Villares A, Cathala B,
472 Chaspoul F, Frandsen KE, Labourel A, Herpoël-Gimbert I, Grisel S, Haon M, Lenfant N, Rogniaux
473 H, Ropartz D, Davies GJ, Rosso M-N, Walton PH, Henrissat B, Berrin J-G. 2018. Lytic xylan
474 oxidases from wood-decay fungi unlock biomass degradation. *Nat Chem Biol* 14:306–310.
- 475 6. Harris P V., Welner D, McFarland KC, Re E, Navarro Poulsen J-C, Brown K, Salbo R, Ding H,
476 Vlasenko E, Merino S, Xu F, Cherry J, Larsen S, Lo Leggio L. 2010. Stimulation of Lignocellulosic
477 Biomass Hydrolysis by Proteins of Glycoside Hydrolase Family 61: Structure and Function of a
478 Large, Enigmatic Family. *Biochemistry* 49:3305–3316.
- 479 7. Navarro D, Rosso M-N, Haon M, Olivé C, Bonnin E, Lesage-Meessen L, Chevret D, Coutinho PM,
480 Henrissat B, Berrin J-G. 2014. Fast solubilization of recalcitrant cellulosic biomass by the
481 basidiomycete fungus *Laetisaria arvalis* involves successive secretion of oxidative and
482 hydrolytic enzymes. *Biotechnol Biofuels* 7:143.

- 483 8. Hori C, Gaskell J, Igarashi K, Kersten P, Mozuch M, Samejima M, Cullen D. 2014. Temporal
484 Alterations in the Secretome of the Selective Lignolytic Fungus *Ceriporiopsis subvermispora*
485 during Growth on Aspen Wood Reveal This Organism's Strategy for Degrading Lignocellulose.
486 *Appl Environ Microbiol* 80:2062–2070.
- 487 9. Presley GN, Panisko E, Purvine SO, Schilling JS. 2018. Coupling secretomics with enzyme
488 activities to compare the temporal processes of wood metabolism among white and brown rot
489 fungi. *Appl Environ Microbiol* 84.
- 490 10. Zhang J, Presley GN, Hammel KE, Ryu J-S, Menke JR, Figueroa M, Hu D, Orr G, Schilling JS. 2016.
491 Localizing gene regulation reveals a staggered wood decay mechanism for the brown rot fungus
492 *Postia placenta*. *Proc Natl Acad Sci* 113:10968–10973.
- 493 11. Cleveland ME, Mathieu Y, Ribeaucourt D, Haon M, Mulyk P, Hein JE, Lafond M, Berrin J-G,
494 Brumer H. 2021. A survey of substrate specificity among Auxiliary Activity Family 5 copper
495 radical oxidases. *Cell Mol Life Sci CMLS* 78:8187–8208.
- 496 12. Sützl L, Laurent CVFP, Abrera AT, Schütz G, Ludwig R, Haltrich D. 2018. Multiplicity of enzymatic
497 functions in the CAZy AA3 family. *Appl Microbiol Biotechnol* 102:2477–2492.
- 498 13. Bissaro B, Várnai A, Røhr ÅK, Eijsink VGH. 2018. Oxidoreductases and Reactive Oxygen Species
499 in Conversion of Lignocellulosic Biomass. *Microbiol Mol Biol Rev* 82:1–51.
- 500 14. Hiner ANP, Ruiz JH, López JNR, Cánovas FG, Brisset NC, Smith AT, Arnao MB, Acosta M. 2002.
501 Reactions of the Class II Peroxidases, Lignin Peroxidase and *Arthromyces ramosus* Peroxidase,
502 with Hydrogen Peroxide. *J Biol Chem* 277:26879–26885.
- 503 15. Mattila H, Österman-Udd J, Mali T, Lundell T. 2022. Basidiomycota Fungi and ROS: Genomic
504 Perspective on Key Enzymes Involved in Generation and Mitigation of Reactive Oxygen Species.
505 *Front Fungal Biol* 3.

- 506 16. Castaño J, Zhang J, Zhou M, Tsai C-F, Lee JY, Nicora C, Schilling J. 2021. A Fungal Secretome
507 Adapted for Stress Enabled a Radical Wood Decay Mechanism. *mBio*
508 <https://doi.org/10.1128/mBio.02040-21>.
- 509 17. Lim JM, Kim G, Levine RL. 2019. Methionine in Proteins: It's Not Just for Protein Initiation
510 Anymore. *Neurochem Res* 44:247–257.
- 511 18. Lourenço Dos Santos S, Petropoulos I, Friguet B. 2018. The Oxidized Protein Repair Enzymes
512 Methionine Sulfoxide Reductases and Their Roles in Protecting against Oxidative Stress, in
513 Ageing and in Regulating Protein Function. *Antioxid Basel Switz* 7:E191.
- 514 19. Rey P, Tarrago L. 2018. Physiological Roles of Plant Methionine Sulfoxide Reductases in Redox
515 Homeostasis and Signaling. *Antioxid Basel Switz* 7:114.
- 516 20. Tarrago L, Kaya A, Kim H-Y, Manta B, Lee B-C, Gladyshev VN. 2022. The selenoprotein
517 methionine sulfoxide reductase B1 (MSRB1). *Free Radic Biol Med* 191:228–240.
- 518 21. Erickson JR, Joiner M-LA, Guan X, Kutschke W, Yang J, Oddis C V, Bartlett RK, Lowe JS, O'Donnell
519 SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham A-JL, Weiss RM, Spitz DR, Shea MA,
520 Colbran RJ, Mohler PJ, Anderson ME. 2008. A Dynamic Pathway for Calcium-Independent
521 Activation of CaMKII by Methionine Oxidation. *Cell* 133:462–474.
- 522 22. Hou L, Kang I, Marchant RE, Zagorski MG. 2002. Methionine 35 Oxidation Reduces Fibril
523 Assembly of the Amyloid A β -(1–42) Peptide of Alzheimer's Disease. *J Biol Chem* 277:40173–
524 40176.
- 525 23. Levine RL, Mosoni L, Berlett BS, Stadtman ER. 1996. Methionine residues as endogenous
526 antioxidants in proteins. *Proc Natl Acad Sci U S A* 93:15036–15040.

- 527 24. Hage H, Rosso M-N, Tarrago L. 2021. Distribution of methionine sulfoxide reductases in fungi
528 and conservation of the free-methionine-R-sulfoxide reductase in multicellular eukaryotes. *Free*
529 *Radic Biol Med* 169:187–215.
- 530 25. Kachurin AM, Golubev AM, Geisow MM, Veselkina OS, Isaeva-Ivanova LS, Neustroev KN. 1995.
531 Role of methionine in the active site of α -galactosidase from *Trichoderma reesei*. *Biochem J*
532 308:955–964.
- 533 26. Miyauchi S, Hage H, Drula E, Lesage-Meessen L, Berrin JG, Navarro D, Favel A, Chaduli D, Grisel
534 S, Haon M, Piumi F, Levasseur A, Lomascolo A, Ahrendt S, Barry K, LaButti KM, Chevret D, Daum
535 C, Mariette J, Klopp C, Cullen D, de Vries RP, Gathman AC, Hainaut M, Henrissat B, Hildén KS,
536 Kües U, Lilly W, Lipzen A, Mäkelä MR, Martinez AT, Morel-Rouhier M, Morin E, Pangilinan J,
537 Ram AFJ, Wösten HAB, Ruiz-Dueñas FJ, Riley R, Record E, Grigoriev I V., Rosso MN. 2020.
538 Conserved white-rot enzymatic mechanism for wood decay in the Basidiomycota genus
539 *Pycnoporus*. *DNA Res Int J Rapid Publ Rep Genes Genomes* 27:1–14.
- 540 27. Bettinger JQ, Simon M, Korotkov A, Welle KA, Hryhorenko JR, Seluanov A, Gorbunova V,
541 Ghaemmaghami S. 2022. Accurate Proteomewide Measurement of Methionine Oxidation in
542 Aging Mouse Brains. *J Proteome Res* 21:1495–1509.
- 543 28. Bettinger JQ, Welle KA, Hryhorenko JR, Ghaemmaghami S. 2020. Quantitative Analysis of in
544 Vivo Methionine Oxidation of the Human Proteome. *J Proteome Res* 19:624–633.
- 545 29. Liu H, Ponniah G, Neill A, Patel R, Andrien B. 2013. Accurate determination of protein
546 methionine oxidation by stable isotope labeling and LC-MS analysis. *Anal Chem* 85:11705–
547 11709.
- 548 30. Shipman JT, Go EP, Desaire H. 2018. Method for Quantifying Oxidized Methionines and
549 Application to HIV-1 Env. *J Am Soc Mass Spectrom* 29:2041–2047.

- 550 31. Hartmann EM, Allain F, Gaillard J-C, Pible O, Armengaud J. 2014. Taking the shortcut for high-
551 throughput shotgun proteomic analysis of bacteria. *Methods Mol Biol Clifton NJ* 1197:275–285.
- 552 32. Lozano C, Grenga L, Gallais F, Miotello G, Bellanger L, Armengaud J. 2023. Mass spectrometry
553 detection of monkeypox virus: Comprehensive coverage for ranking the most responsive
554 peptide markers. *Proteomics* 23:e2200253.
- 555 33. Paoletti AC, Parmely TJ, Tomomori-Sato C, Sato S, Zhu D, Conaway RC, Conaway JW, Florens L,
556 Washburn MP. 2006. Quantitative proteomic analysis of distinct mammalian Mediator
557 complexes using normalized spectral abundance factors. *Proc Natl Acad Sci* 103:18928–18933.
- 558 34. Perez-Riverol Y, Bai J, Bandla C, García-Seisdedos D, Hewapathirana S, Kamatchinathan S,
559 Kundu DJ, Prakash A, Frericks-Zipper A, Eisenacher M, Walzer M, Wang S, Brazma A, Vizcaíno
560 JA. 2021. The PRIDE database resources in 2022: a hub for mass spectrometry-based
561 proteomics evidences. *Nucleic Acids Res* 50:D543–D552.
- 562 35. Arfi Y, Chevret D, Henrissat B, Berrin J-G, Levasseur A, Record E. 2013. Characterization of salt-
563 adapted secreted lignocellulolytic enzymes from the mangrove fungus *Pestalotiopsis* sp. *Nat*
564 *Commun* 4:1810.
- 565 36. Arntzen MØ, Bengtsson O, Várnai A, Delogu F, Mathiesen G, Eijsink VGH. 2020. Quantitative
566 comparison of the biomass-degrading enzyme repertoires of five filamentous fungi. *Sci Rep*
567 10:20267.
- 568 37. Couturier M, Navarro D, Chevret D, Henrissat B, Piumi F, Ruiz-Dueñas FJ, Martinez AT, Grigoriev
569 IV, Riley R, Lipzen A, Berrin J-G, Master ER, Rosso M-N. 2015. Enhanced degradation of
570 softwood versus hardwood by the white-rot fungus *Pycnoporus coccineus*. *Biotechnol Biofuels*
571 8:216.

- 572 38. Couturier M, Navarro D, Olivé C, Chevret D, Haon M, Favel A, Lesage-Meessen L, Henrissat B,
573 Coutinho PM, Berrin J-G. 2012. Post-genomic analyses of fungal lignocellulosic biomass
574 degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*. *BMC*
575 *Genomics* 13:57.
- 576 39. Daou M, Farfan Soto C, Majira A, Cézard L, Cottyn B, Pion F, Navarro D, Oliveira Correia L, Drula
577 E, Record E, Raouche S, Baumberger S, Faulds CB. 2021. Fungal Treatment for the Valorization
578 of Technical Soda Lignin. *J Fungi Basel Switz* 7:39.
- 579 40. Filiatrault-Chastel C, Navarro D, Haon M, Grisel S, Herpoël-Gimbert I, Chevret D, Fanuel M,
580 Henrissat B, Heiss-Blanquet S, Margeot A, Berrin J-G. 2019. AA16, a new lytic polysaccharide
581 monooxygenase family identified in fungal secretomes. *Biotechnol Biofuels* 12:55.
- 582 41. Levasseur A, Lomascolo A, Chabrol O, Ruiz-Deñás FJ, Boukhris-Uzan E, Piumi F, Kües U, Ram
583 AFJ, Murat C, Haon M, Benoit I, Arfi Y, Chevret D, Drula E, Kwon MJ, Gouret P, Lesage-Meessen
584 L, Lombard V, Mariette J, Noirot C, Park J, Patyshakuliyeva A, Sigoillot JC, Wiebenga A, Wösten
585 HAB, Martin F, Coutinho PM, de Vries RP, Martínez AT, Klopp C, Pontarotti P, Henrissat B,
586 Record E. 2014. The genome of the white-rot fungus *Pycnoporus cinnabarinus*: a basidiomycete
587 model with a versatile arsenal for lignocellulosic biomass breakdown. *BMC Genomics* 15:486.
- 588 42. Miyauchi S, Hage H, Drula E, Lesage-Meessen L, Berrin J-G, Navarro D, Favel A, Chaduli D, Grisel
589 S, Haon M, Piumi F, Levasseur A, Lomascolo A, Ahrendt S, Barry K, LaButti KM, Chevret D, Daum
590 C, Mariette J, Klopp C, Cullen D, de Vries RP, Gathman AC, Hainaut M, Henrissat B, Hildén KS,
591 Kües U, Lilly W, Lipzen A, Mäkelä MR, Martinez AT, Morel-Rouhier M, Morin E, Pangilinan J,
592 Ram AFJ, Wösten HAB, Ruiz-Deñás FJ, Riley R, Record E, Grigoriev IV, Rosso M-N. 2020.
593 Conserved white-rot enzymatic mechanism for wood decay in the Basidiomycota genus
594 *Pycnoporus*. *DNA Res Int J Rapid Publ Rep Genes Genomes* 27.

- 595 43. Miyauchi S, Rancon A, Drula E, Hage H, Chaduli D, Favel A, Grisel S, Henrissat B, Herpoël-
596 Gimbert I, Ruiz-Dueñas FJ, Chevret D, Hainaut M, Lin J, Wang M, Pangilinan J, Lipzen A, Lesage-
597 Meessen L, Navarro D, Riley R, Grigoriev IV, Zhou S, Raouche S, Rosso M-N. 2018. Integrative
598 visual omics of the white-rot fungus *Polyporus brumalis* exposes the biotechnological potential
599 of its oxidative enzymes for delignifying raw plant biomass. *Biotechnol Biofuels* 11:201.
- 600 44. Miyauchi S, Navarro D, Grisel S, Chevret D, Berrin J-G, Rosso M-N. 2017. The integrative omics
601 of white-rot fungus *Pycnoporus coccineus* reveals co-regulated CAZymes for orchestrated
602 lignocellulose breakdown. *PLoS ONE* 12:e0175528.
- 603 45. Miyauchi S, Navarro D, Grigoriev IV, Lipzen A, Riley R, Chevret D, Grisel S, Berrin J-G, Henrissat
604 B, Rosso M-N. 2016. Visual Comparative Omics of Fungi for Plant Biomass Deconstruction. *Front*
605 *Microbiol* 7.
- 606 46. Paës G, Navarro D, Benoit Y, Blanquet S, Chabbert B, Chaussepied B, Coutinho PM, Durand S,
607 Grigoriev IV, Haon M, Heux L, Launay C, Margeot A, Nishiyama Y, Raouche S, Rosso M-N, Bonnin
608 E, Berrin J-G. 2019. Tracking of enzymatic biomass deconstruction by fungal secretomes
609 highlights markers of lignocellulose recalcitrance. *Biotechnol Biofuels* 12:76.
- 610 47. Poidevin L, Berrin J-G, Bennati-Granier C, Levasseur A, Herpoël-Gimbert I, Chevret D, Coutinho
611 PM, Henrissat B, Heiss-Blanquet S, Record E. 2014. Comparative analyses of *Podospora*
612 *anserina* secretomes reveal a large array of lignocellulose-active enzymes. *Appl Microbiol*
613 *Biotechnol* 98:7457–7469.
- 614 48. Reyre J-L, Grisel S, Haon M, Navarro D, Ropartz D, Le Gall S, Record E, Sciara G, Tranquet O,
615 Berrin J-G, Bissaro B. 2022. The Maize Pathogen *Ustilago maydis* Secretes Glycoside Hydrolases
616 and Carbohydrate Oxidases Directed toward Components of the Fungal Cell Wall. *Appl Environ*
617 *Microbiol* 88:e0158122.

- 618 49. Ribeaucourt D, Saker S, Navarro D, Bissaro B, Drula E, Correia LO, Haon M, Grisel S, Lapalu N,
619 Henrissat B, O'Connell RJ, Lambert F, Lafond M, Berrin J-G. 2021. Identification of Copper-
620 Containing Oxidoreductases in the Secretomes of Three *Colletotrichum* Species with a Focus on
621 Copper Radical Oxidases for the Biocatalytic Production of Fatty Aldehydes. *Appl Environ*
622 *Microbiol* 87:e0152621.
- 623 50. Meinken J, Asch DK, Neizer-Ashun KA, Chang G-H, R.Cooper JR C, Min XJ. 2014. FunSecKB2: a
624 fungal protein subcellular location knowledgebase. *Comput Mol Biol*
625 <https://doi.org/10.5376/CMB.2014.04.0007>.
- 626 51. Meinken J, Walker G, Cooper CR, Min XJ. 2015. MetazSecKB: the human and animal secretome
627 and subcellular proteome knowledgebase. *Database* 2015:1–14.
- 628 52. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf
629 C, Sjöstedt E, Asplund A, Olsson IM, Edlund K, Lundberg E, Navani S, Szgyarto CAK, Odeberg J,
630 Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J,
631 Nilsson P, Schwenk JM, Hamsten M, Von Feilitzen K, Forsberg M, Persson L, Johansson F,
632 Zwahlen M, Von Heijne G, Nielsen J, Pontén F. 2015. Tissue-based map of the human
633 proteome. *Science* 347.
- 634 53. Uhlén M, Karlsson MJ, Hober A, Svensson AS, Scheffel J, Kotol D, Zhong W, Tebani A, Strandberg
635 L, Edfors F, Sjöstedt E, Mulder J, Mardinoglu A, Berling A, Ekblad S, Dannemeyer M, Kanje S,
636 Rockberg J, Lundqvist M, Malm M, Volk AL, Nilsson P, Månberg A, Dodig-Crnkovic T, Pin E,
637 Zwahlen M, Oksvold P, von Feilitzen K, Häussler RS, Hong MG, Lindskog C, Ponten F, Katona B,
638 Vuu J, Lindström E, Nielsen J, Robinson J, Ayoglu B, Mahdessian D, Sullivan D, Thul P, Danielsson
639 F, Stadler C, Lundberg E, Bergström G, Gummesson A, Voldborg BG, Tegel H, Hober S,
640 Forsström B, Schwenk JM, Fagerberg L, Sivertsson Å. 2019. The human secretome. *Sci Signal* 12.

- 641 54. Lum G, Meinken J, Orr J, Frazier S, Min XJ. 2014. PlantSecKB: the Plant Secretome and
642 Subcellular Proteome KnowledgeBase. *Comput Mol Biol* 4.
- 643 55. Hooper CM, Castleden IR, Tanz SK, Aryamanesh N, Millar AH. 2017. SUBA4: the interactive data
644 analysis centre for Arabidopsis subcellular protein locations. *Nucleic Acids Res* 45:D1064–
645 D1074.
- 646 56. Zhao Z, Liu H, Wang C, Xu JR. 2014. Correction to Comparative analysis of fungal genomes
647 reveals different plant cell wall degrading capacity in fungi [BMC Genomics 14(2013) 274]. *BMC*
648 *Genomics* 15:1–15.
- 649 57. Miyauchi S, Kiss E, Kuo A, Drula E, Kohler A, Sánchez-García M, Morin E, Andreopoulos B, Barry
650 KW, Bonito G, Buée M, Carver A, Chen C, Cichocki N, Clum A, Culley D, Crous PW, Fauchery L,
651 Girlanda M, Hayes RD, Kéri Z, LaButti K, Lipzen A, Lombard V, Magnuson J, Maillard F, Murat C,
652 Nolan M, Ohm RA, Pangilinan J, Pereira M de F, Perotto S, Peter M, Pfister S, Riley R, Sitrit Y,
653 Stielow JB, Szöllősi G, Žifčáková L, Štursová M, Spatafora JW, Tedersoo L, Vaario L-M, Yamada A,
654 Yan M, Wang P, Xu J, Bruns T, Baldrian P, Vilgalys R, Dunand C, Henrissat B, Grigoriev I V.,
655 Hibbett D, Nagy LG, Martin FM. 2020. Large-scale genome sequencing of mycorrhizal fungi
656 provides insights into the early evolution of symbiotic traits. *Nat Commun* 11:5125.
- 657 58. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009.
658 BLAST+: architecture and applications. *BMC Bioinformatics* 10:421.
- 659 59. Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment,
660 interactive sequence choice and visualization. *Brief Bioinform* 20:1160–1166.
- 661 60. UniProtKB/Swiss-Prot Release 2023_04 statistics.
662 <https://web.expasy.org/docs/relnotes/relstat.html>. Retrieved 14 September 2023.

- 663 61. Hawkins CL, Davies MJ. 2019. Detection, identification, and quantification of oxidative protein
664 modifications. *J Biol Chem* 294:19683–19708.
- 665 62. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, Stroe O, Wood G,
666 Laydon A, Židek A, Green T, Tunyasuvunakool K, Petersen S, Jumper J, Clancy E, Green R, Vora
667 A, Lutfi M, Figurnov M, Cowie A, Hobbs N, Kohli P, Kleywegt G, Birney E, Hassabis D, Velankar S.
668 2022. AlphaFold Protein Structure Database: massively expanding the structural coverage of
669 protein-sequence space with high-accuracy models. *Nucleic Acids Res* 50:D439–D444.
- 670 63. Ezraty B, Grimaud R, El Hassouni M, Moinier D, Barras F. 2004. Methionine sulfoxide reductases
671 protect Ffh from oxidative damages in *Escherichia coli*. *EMBO J* 23:1868–1877.
- 672 64. Laugier E, Tarrago L, Vieira Dos Santos C, Eymery F, Havaux M, Rey P. 2010. *Arabidopsis*
673 *thaliana* plastidial methionine sulfoxide reductases B, MSRBs, account for most leaf peptide
674 MSR activity and are essential for growth under environmental constraints through a role in the
675 preservation of photosystem antennae. *Plant J Cell Mol Biol* 61:271–282.
- 676 65. Rouyère C, Serrano T, Frémont S, Echard A. 2022. Oxidation and reduction of actin: Origin,
677 impact in vitro and functional consequences in vivo. *Eur J Cell Biol* 101:151249.
- 678 66. Bender A, Hajieva P, Moosmann B. 2008. Adaptive antioxidant methionine accumulation in
679 respiratory chain complexes explains the use of a deviant genetic code in mitochondria. *Proc*
680 *Natl Acad Sci U S A* 105:16496–16501.
- 681 67. Melnyk RA, Youngblut MD, Clark IC, Carlson HK, Wetmore KM, Price MN, Iavarone AT,
682 Deutschbauer AM, Arkin AP, Coates JD. 2015. Novel mechanism for scavenging of hypochlorite
683 involving a periplasmic methionine-rich Peptide and methionine sulfoxide reductase. *mBio*
684 6:e00233-00215.

- 685 68. Luo S, Levine RL. 2009. Methionine in proteins defends against oxidative stress. *FASEB J Off*
686 *Publ Fed Am Soc Exp Biol* 23:464–472.
- 687 69. Castaño JD, Zhou M, Jonathan Schilling. 2021. Towards an Understanding of Oxidative Damage
688 in an α -L-Arabinofuranosidase of *Trichoderma reesei*: a Molecular Dynamics Approach. *Appl*
689 *Biochem Biotechnol* 193:3287–3300.
- 690 70. Peculyte A, Samuelsson L, Olsson L, McFarland KC, Frickmann J, Østergård L, Halvorsen R, Scott
691 BR, Johansen KS. 2018. Redox processes acidify and decarboxylate steam-pretreated
692 lignocellulosic biomass and are modulated by LPMO and catalase. *Biotechnol Biofuels* 11:165.
- 693 71. Scott BR, Huang HZ, Frickman J, Halvorsen R, Johansen KS. 2016. Catalase improves
694 saccharification of lignocellulose by reducing lytic polysaccharide monooxygenase-associated
695 enzyme inactivation. *Biotechnol Lett* 38:425–434.
- 696 72. Kwon S-I, Anderson AJ. 2001. Catalase Activities of *Phanerochaete chrysosporium* Are Not
697 Coordinately Produced with Ligninolytic Metabolism: Catalases from a White-Rot Fungus. *Curr*
698 *Microbiol* 42:8–11.
- 699 73. Hansberg W, Salas-Lizana R, Domínguez L. 2012. Fungal catalases: function, phylogenetic origin
700 and structure. *Arch Biochem Biophys* 525:170–180.
- 701 74. Tarrago L, Grosse S, Siponen MI, Lemaire D, Alonso B, Miotello G, Armengaud J, Arnoux P,
702 Pignol D, Sabaty M. 2018. *Rhodobacter sphaeroides* methionine sulfoxide reductase P reduces
703 R- and S-diastereomers of methionine sulfoxide from a broad-spectrum of protein substrates.
704 *Biochem J* 475:3779–3795.
- 705