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Proteome of the Wood Decay Fungus *Fomitopsis pinicola* Is Altered by Substrate

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ABSTRACT The brown rot fungus *Fomitopsis pinicola* efficiently depolymerizes wood cellulose via the combined activities of oxidative and hydrolytic enzymes. Mass spectrometric analyses of culture filtrates identified specific proteins, many of which were differentially regulated in response to substrate composition.

Two-liter flasks containing 250 mL of basal salt medium were supplemented with 1.25 g of ground and sieved (number 10 screen) quaking aspen (*Populus tremuloides*), loblolly pine (*Pinus taeda*), or white spruce (*Picea glauca*) wood as the sole carbon source, as described (1, 2). The medium was inoculated with *Fomitopsis pinicola* strain FP-58527 (= *Fomitopsis schrenkii* [3]) and placed on a rotary shaker (150 rpm). After 5 days of incubation at 22°C to 24°C, cultures were filtered through Whatman GF/C filters followed by Corning 0.22-μm polystyrene filters (catalog number 430531). Filtrate proteins were precipitated with 10% (wt/vol) trichloroacetic acid and purified (4). Three replicated cultures were harvested for each wood species.

Nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) was used to identify proteins (5–7). Equal amounts of total protein per sample were digested with trypsin/LysC and purified with OMIX C_{18} SPE cartridges (Agilent Technologies), and 2 μg was loaded for nano-LC-MS/MS analysis, using an Agilent 1100 nanoflow system (Agilent Technologies) connected to a hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap Elite; Thermo Fisher Scientific) equipped with an EASY-Spray electrospray source. Chromatography of peptides prior to MS analysis was accomplished using a capillary emitter column (PepMap C_{18} column, 3 μm, 100 Å, 150 by 0.075 mm; Thermo Fisher Scientific), onto which 2 μl of purified peptides was automatically loaded. The nano-LC system delivered solvents as described (4), and survey MS scans were acquired in the Orbitrap mass spectrometer with a resolution of 120,000, followed by MS2 fragmentation of the 20 most intense peptides detected in the MS1 scan from *m/z* 380 to 1800, with redundancy limited by dynamic exclusion. Raw MS/MS data were converted to the mgf file format using MSConvert (ProteoWizard) for downstream analysis. Resulting mgf files were used to search against forward and decoyed-reversed *F. pinicola* protein databases via the Joint Genome Institute (JGI) portal (https://mycocosm.jgi.doe.gov/Fompi3/Fompi3.info.html),

with a list of common laboratory contaminants (available at https://reprint-apms.org/?q= chooseworkflow) to establish the false discovery rate (FDR) (37,222 total entries) using an in-house Mascot search engine v2.2.07 (Matrix Science) with variable methionine oxidation and asparagine and glutamine deamidation and fixed cysteine carbamidomethylation. Scaffold v4.7.5 (Proteome Software Inc., Portland, OR) was used for spectrum-based quantification. Peptide identifications were accepted if they could be established at >80.0% probability to achieve an FDR of <1.0% by the Scaffold local FDR algorithm. Protein identifications were accepted if they could be established at >99.0% probability to achieve an FDR of <1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (8). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

A total of 200 proteins were confidently identified, of which 56 were carbohydrate-active enzymes (CAZymes) (9). Analysis of variance (ANOVA) revealed ≥2-fold accumulation of 38 proteins; of those, 30 were more abundant in aspen than in pine. These data will serve as a useful resource for studying the influence of substrate composition on protein secretion by *F. pinicola*.

Data availability. The MS proteomic data and supplemental ANOVA results have been deposited in the ProteomeXchange through PRIDE with the identifier PXD033887 (http://www.ebi.ac.uk/pride/archive/projects/PXD033887).

REFERENCES

1. Wu B, Gaskell J, Held BW, Toapanta C, Vuong TV, Ahrendt S, Lipzen A, Zhang J, Schilling JS, Master E, Grigoriev IV, Blanchette RA, Cullen D, Hibbett DS. 2021. Retracted and republished from: "Substrate-specific differential gene expression and RNA editing in the brown rot fungus *Fomitopsis pinicola*." Appl Environ Microbiol 87:e00329-21. https://doi.org/10.1128/AEM.00329-21.

2. Gaskell J, Blanchette RA, Stewart PE, BonDurant SS, Adams M, Sabat G, Kersten P, Cullen D. 2016. Transcriptome and secretome analyses of the wood decay fungus *Wolfiporia cocos* support alternative mechanisms of lignocellulose conversion. Appl Environ Microbiol 82:3979–3987. https://doi.org/10.1128/AEM.00639-16.

3. Haight JE, Nakasone KK, Laursen GA, Redhead SA, Taylor DL, Glaeser JA. 2019. *Fomitopsis mounceae* and *F. schrenkii*: two new species from North America in the *F. pinicola* complex. Mycologia 111:339–357. https://doi.org/10.1080/00275514.2018.1564449.

4. Bhatnagar JM, Sabat G, Cullen D. 2019. The foliar endophyte *Phialocephala scopiformis* DAOMC 229536 proteome when grown on wood used as the sole carbon source. Microbiol Resour Announc 8:e01280-18. https://doi.org/10.1128/MRA.01280-18.

5. Hori C, Ishida T, Igarashi K, Samejima M, Suzuki H, Master E, Ferreira P, Ruiz-Duenas FJ, Held B, Canessa P, Larrondo LF, Schmoll M, Druzhinina IS, Kubicek CP, Gaskell JA, Kersten P, St John F, Glasner J, Sabat G, Splinter BonDurant S, Syed K, Yadav J, Mgbeahuruike AC, Kovalchuk A, Asiegbu FO, Lackner G, Hoffmeister D, Rencoret J, Gutierrez A, Sun H, Lindquist E, Barry K, Riley R, Grigoriev IV, Henrissat B, Kues U, Berka RM, Martinez AT, Covert SF, Blanchette RA, Cullen D. 2014. Analysis of the *Phlebiopsis gigantea* genome,

transcriptome and secretome provides insight into its pioneer colonization strategies of wood. PLoS Genet 10:e1004759. https://doi.org/10.1371/journal.pgen.1004759.

6. Fernandez-Fueyo E, Ruiz-Duenas FJ, Ferreira P, Floudas D, Hibbett DS, Canessa P, Larrondo LF, James TY, Seelenfreund D, Lobos S, Polanco R, Tello M, Honda Y, Watanabe T, Ryu JS, Kubicek CP, Schmoll M, Gaskell J, Hammel KE, St John FJ, Vanden Wymelenberg A, Sabat G, Splinter BonDurant S, Syed K, Yadav JS, Doddapaneni H, Subramanian V, Lavin JL, Oguiza JA, Perez G, Pisabarro AG, Ramirez L, Santoyo F, Master E, Coutinho PM, Henrissat B, Lombard V, Magnuson JK, Kues U, Hori C, Igarashi K, Samejima M, Held BW, Barry KW, LaButti KM, Lapidus A, Lindquist EA, Lucas SM, Riley R, Salamov AA, et al. 2012. Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis. Proc Natl Acad Sci U S A 109:5458–5463. https://doi.org/10.1073/pnas.1119912109.

7. Hori C, Gaskell J, Cullen D, Sabat G, Stewart PE, Lail K, Peng Y, Barry K, Grigoriev IV, Kohler A, Fauchery L, Martin F, Zeiner CA, Bhatnagar JM. 2018. Multi-omic analyses of extensively decayed *Pinus contorta* reveal expression of a diverse array of lignocellulose-degrading enzymes. Appl Environ Microbiol 84:e01133-18. https://doi.org/10.1128/AEM.01133-18.

8. Nesvizhskii AI, Keller A, Kolker E, Aebersold R. 2003. A statistical model for identifying proteins by tandem mass spectrometry. Anal Chem 75:4646–4658. https://doi.org/10.1021/ac0341261.

9. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The Carbohydrate-Active Enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495. https://doi.org/10.1093/nar/gkt1178.