Screening Decay Fungi for Potential Lignin Valorization

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Abstract

Lignin is the second most abundant biopolymer and contains many aromatic and phenolic structures suitable for strong polymers, solvents, fuels, and could replace petroleum in many high-value chemicals. Lignin is also a massive component of organic waste from both cellulosic conversions and agricultural residues. The only natural means of converting lignin in any significant quantity come from decay-fungi. Despite decades of study in model organism, the means by which fungi attack whole lignin and how they handle smaller monomers remains largely unknown. Thanks to years of careful collecting and culture maintenance, the OSU Biodeterioration lab has a large array of living fungi with wood degrading abilities.

In this study 110 fungi are being grown on media containing 5 compounds that represent lignin's primary carbon intermediates (paracoumaryl alcohol, coniferyl alcohol, sinapyl alcohol, para-hydroxy benzoic acid (PHBA) and catechol). Growth rates of fungi are being monitored by total biomass as well as changes in UV absorbance of each fungal-media combination. Early results show clear differences within the abilities of fungi generally lumped together as "lignin degrading". Not only do these results indicate a complex community approach to whole lignin degradation in nature but point to many new pathways for selective lignin modification. Results from screening will inform further genomic comparisons and analysis of novel products from these fungi. Identification of lignin metabolic pathways in wood decay fungi will expand the molecular toolkit available to biologically upgrade lignin to value added chemicals. A wide screen of decay fungi is required to adequately describe the diversity of lignin-metabolism. Selective lignin modification will also drive new "green chemistry" and renewable materials beyond cellulosic biofuel, and will be essential for lignocellulosic waste valorization

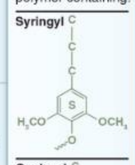
TL,DR;

- **Lignin can replace petroleum.**
- **The only common way to modify lignin is with decay-fungi, but we don't know many details.**
- **We tested 110 fungi against 5 lignin-like compounds to identify targets for further research.**

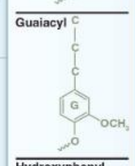
Plant resource	% Hemicellulose	% Cellulose	% Lignin*
MISCANTHUS	24-33	45-52	9-13
SWITCHGRASS	26-33	37-32	17-18
CORN STOVER	31	37	18
POPLAR	16-22	42-48	21-27
EUCALYPTUS	24-28	39-46	29-32
PINE	23	46	28

*Typical aromatic polymer containing:

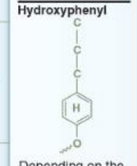
Syringyl



Guaiacyl



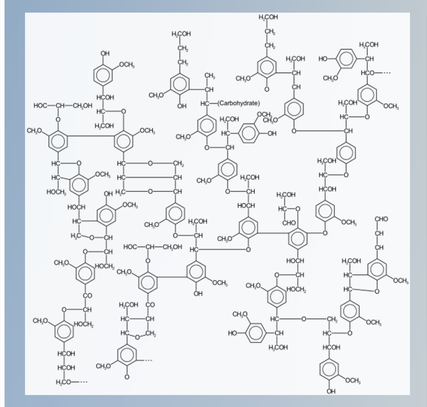
Hydroxyphenyl



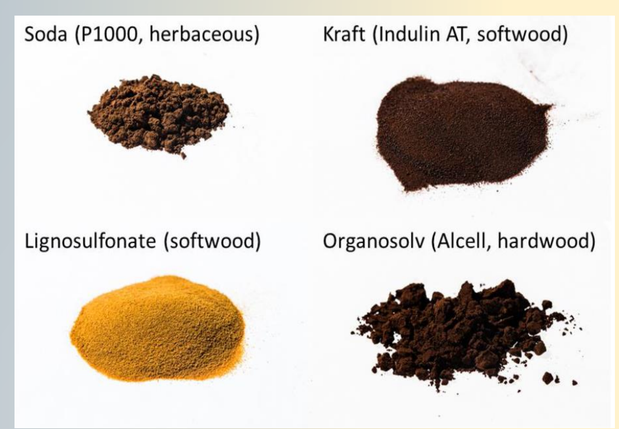
Depending on the bioresource and isolation method-ology, molecular weights for native lignin have been reported from 78,400 [in spruce (*118*)] to 8300 [in Miscanthus (*119*)] g mol^{-1}, which are derived from C9 monolignols as described in Fig. 2.

Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, Davison BH, Dixon RA, Gilna P, Keller M, et al. 2014. Lignin Valorization: Improving Lignin Processing in the Biorefinery. Science. 344(6185):1246843–1246843.

Lignin - Where it comes from and where it goes.



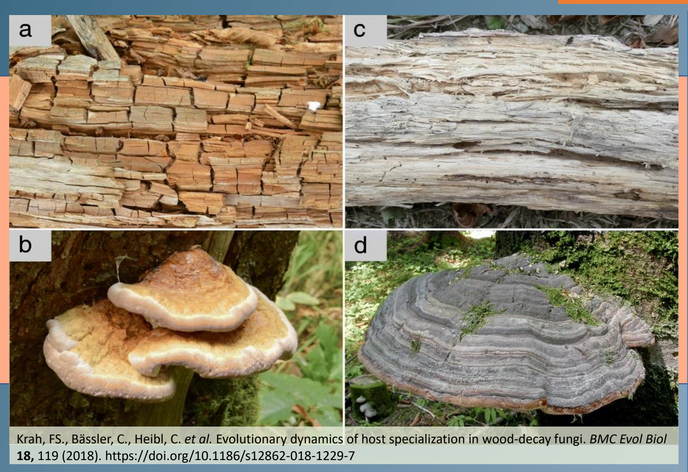
Glazer AN, Nikaido H. 2007. Microbial biotechnology: fundamentals of applied microbiology [Internet]. Cambridge; New York: Cambridge Univ Press; [accessed 2021 Jan 11]. https://doi.org/10.1017/CBO9780511811227



Bruijnincx P, et al., Agro-Paper-Chemistry P. The Importance of a Full Value Chain Approach :22.

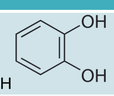
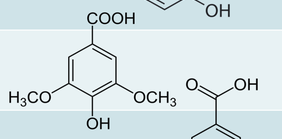
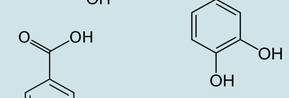
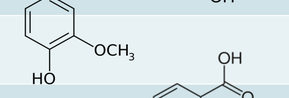
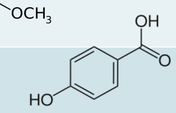
- Lignin is the second most abundant biopolymer on the planet and makes up 20-30% of most plants (right).
- Lignin is a very large, very complex polymer made up of phenolic units (center).
- Over 100 million tons of lignin are created as waste byproducts every year.
- About 1 million pounds are in the form of technical lignin (right) and have some industrial application.
- Over 98 million tons are burned as low-grade fuel if they are used at all
- Burning lignin replaces coal (value: $70/ton) but with slight modification it can replace formaldehyde in resin ($500/ton) or adipic acid in nylon ($1700/ton).

Fungi vs Lignin Monomers



Krah, FS., Bässler, C., Heibl, C. *et al.* Evolutionary dynamics of host specialization in wood-decay fungi. *BMC Evol Biol* **18,** 119 (2018). https://doi.org/10.1186/s12862-018-1229-7

- Wood is primarily decayed by "brown-rot" and "white-rot fungi.
- Brown-rot (a) forms cubicle patterned lignin-rich residue. Lignin is modified but not degraded. *Fomitopsis pinicola* (b).
- White-rot (c) causes all wood polymers to degrade and completely attacks many parts of lignin. *Fomes fomentarius* (d).
- We do not yet know how whole lignin is attacked. Currently known enzymes only act on limited fractions of lignin.
- White-rot fungi also demonstrate aromatic-ring cleavage and produce strong selective oxidases with several industrial applications.

Compound	OCM	Formula	UV peak (nm)	Primary lignin source	
Catechol	1	$C_6H_6O_2$	238	Universal	
Syringate (Gallic acid)	3	$C_9H_{10}O_5$	274	S-lignin	
Dihydrobenzoate (Protocatechuate)	9	$C_7H_6O_4$	258, 293	Universal	
Vanillate	10	$C_8H_8O_4$	259, 292	G-lignin	
Para-hydroxybenzoate	31	$C_7H_6O_3$	252	H-lignin	

- Five phenolic monomers used to represent different sources and fractions of lignin.
- Applied in 1 gram/ liter as organic carbon media (OCM) in liquid cultures of 110 fungi.
- Concentrations tracked with UV absorbance.

INTERPRETATION:

Decay fungi are usually described as "white-rot" (degrading lignin and all other wood polymers) or "brown-rot" (degrading all wood polymers except lignin). Our screening supports a much finer resolution of decay categories and a diversity of lignin attacking mechanisms. We used several methods to characterize fungal metabolism against lignin derivable monomers.

Sole Carbon Metabolism.

Different fungi can grow on agar amended with only one compound. This study was cut short when we determined that most decay fungi grow on un-amended agar even better than on glucose (creating false positives). But results indicated that different metabolic groups exist within the larger "white-rot" paradigm more commonly described. Fungi showed clear preference for various substrates, possibly explaining why specific fungi prefer certain woods.

UV analysis. Using UV-spectrometry to track substrate in liquid media revealed that fungi can either degrade a compound completely (see below) or create a new compound as revealed by new UV peaks (see bottom right.) In this example a new peak was formed at 288 nm from syringate, which absorbs at 262 nm. Only some fungi had this effect.

Color changes

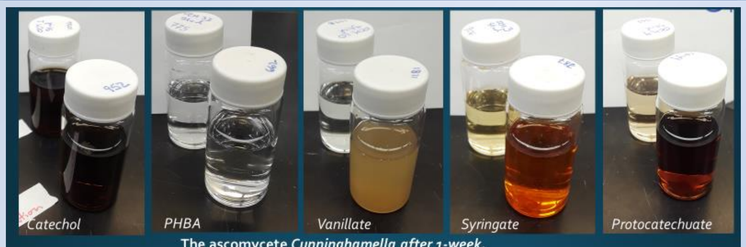
Within the sets that cause a change in UV absorbance, some fungi cause color changes, while others do not. At right is an example of OCM standards (back) and color change (fore).

These do not always appear in the 200 to 400 nm range and will be investigated further with a full spectrophotometric scan.

New peaks and chemical modifications will have to be identified by a battery of chemical analysis, including GCMS.

For many of the fungi tested this will be the first comparison of their metabolism for novel functions. In other experiments of fungi against these monomers there was no metabolism of vanillic acid, an essential portion of lignin modification and a pathway already in demand. Because of the scope of our screening this may be the first opportunity to target genes specific to vanillic acid metabolism.

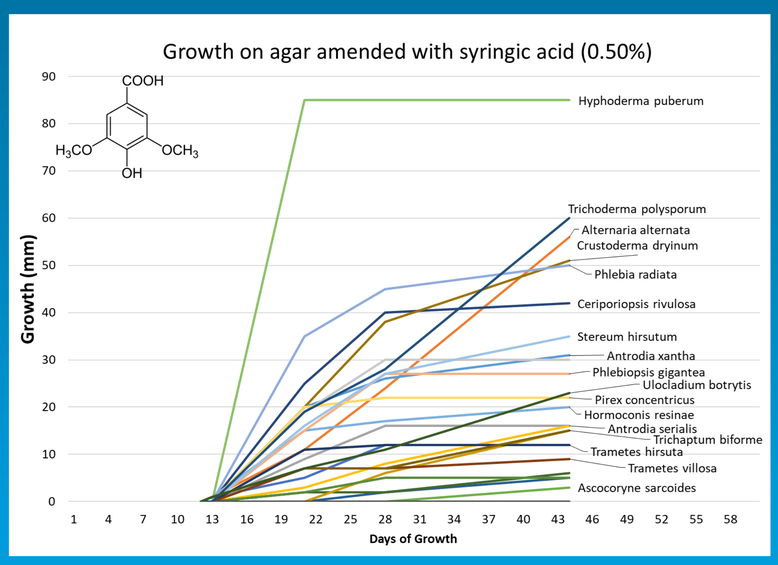
Modification observed through color change

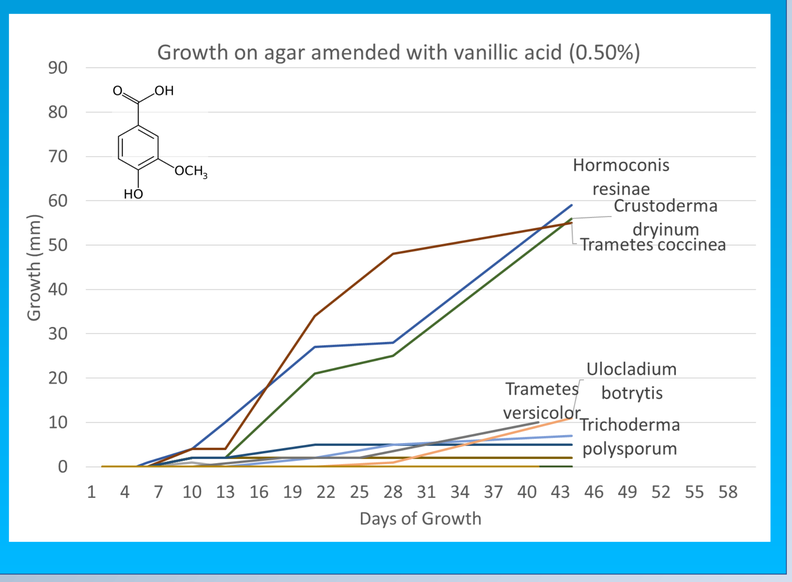


The ascomycete *Cunninghamella after 1-week.*

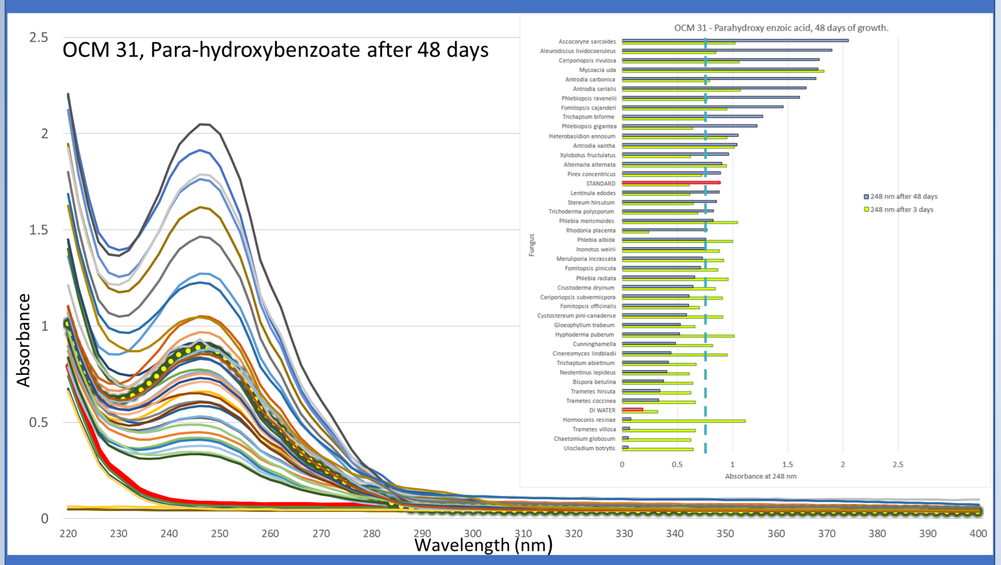
- Color changes in protocatechuate and syringate appear to be associated,
- Color changes in PHBA and vanillate have slightly less overlap
- Color change in PHA did not occur in under two weeks for any fungi

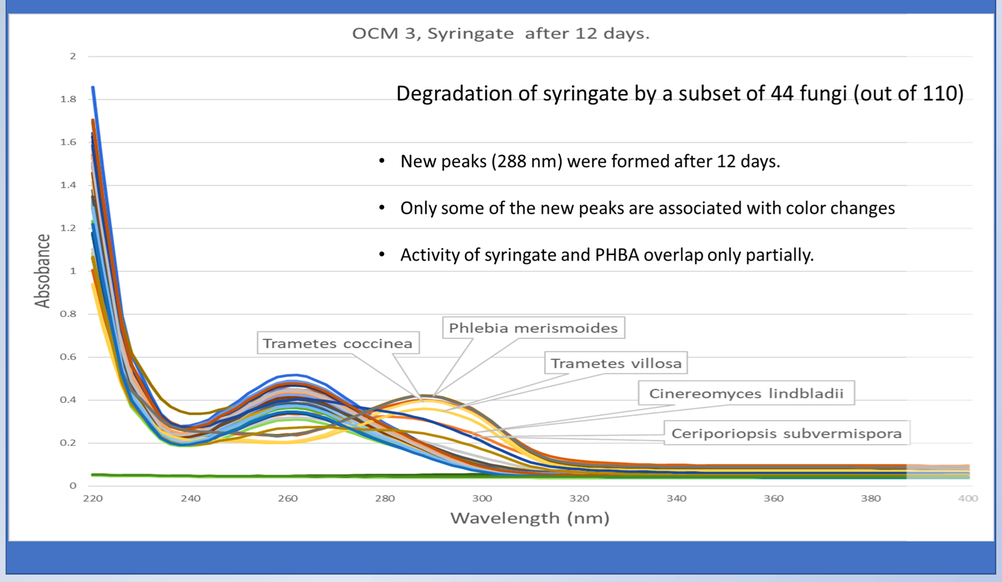
Metabolic utilization determined by growth on amended media.





Chemical modification determined by UV spectrophotometry

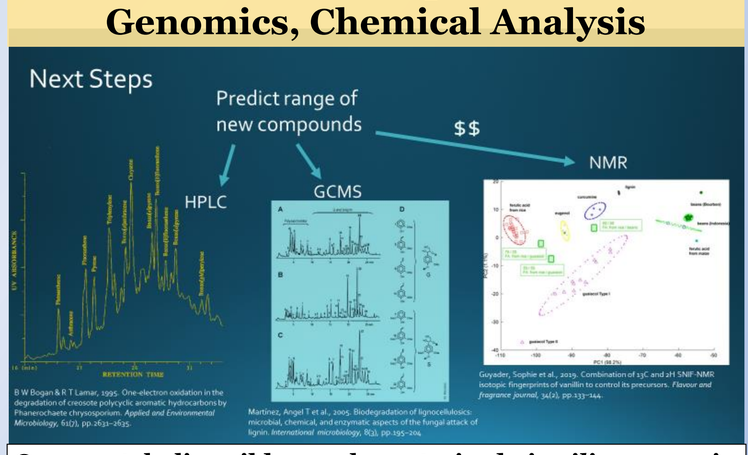




Degradation of syringate by a subset of 44 fungi (out of 110)

- New peaks (288 nm) were formed after 12 days.
- Only some of the new peaks are associated with color changes
- Activity of syringate and PHBA overlap only partially.

Next Steps; Genomics, Chemical Analysis



B W Bogan & R T Lamar, 1995. One-electron oxidation in the degradation of creosote polycyclic aromatic hydrocarbons by Phanerochaete chrysosporium. *Applied and Environmental Microbiology, 61*(7), pp.2633–2635.

Martínez, Angel T et al., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International microbiology*, 8(3), pp.195–204.

Guyader, Sophie et al., 2019. Combination of 13C and 1H SNIF-NMR Isotopic Fingerprints of vanillin to control its precursors. *Flavour and fragrance journal*, 34(2), pp.133–144.

Once metabolic guilds are characterized, *in silico* genomic comparison can be performed to identify gene-targets related to lignin decay mechanisms. These will eventually lead to the ability to modify microbes for bio-reactors, or quickly assess fungi for lignin modification potential.

By better matching fungi to substrates this could leverage technologies for bioremediation and wood preservation. One outcome of our screening will be a catalogue of fungi with novel anti-aromatic and anti-phenolic capabilities.