



Elucidating Aromatic Catabolic Pathways in White-Rot Fungi: An EMSL-NREL Collaborative Effort

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Carlos del Cerro, Erika Erickson, Tao Dong, Allison R. Wong, Elizabeth K. Eder, Samuel O. Purvine, Hugh D. Mitchell, Karl K Weitz, Lye Meng Markillie, Meagan C. Burnet, David W. Hoyt, Rosalie K. Chu, Jan-Fang Cheng, Kelsey J. Ramirez, Rui Katahira, Wei Xiong, Michael E. Himmel, Venkataramanan Subramanian, Jeffrey G. Linger, and Davinia Salvachúa. **Intracellular pathways for lignin catabolism in white-rot fungi.**

In revision in PNAS.



Outline

- **Project overview**
- **Why we chose EMSL for our project**
 - Previous collaborations
 - Capabilities, expertise, instrumentation
- **Why you should submit a proposal to EMSL**
- **Tips for a successful proposal**

Lignin and white-rot fungi

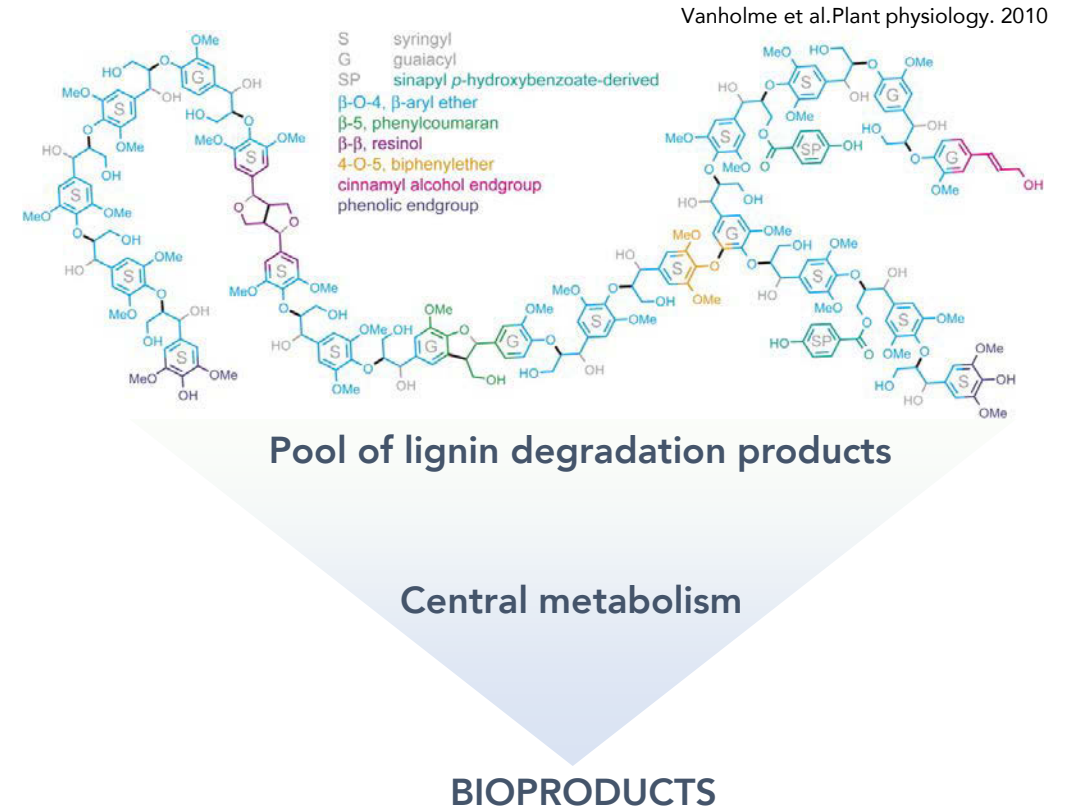
Lignocellulose

- Cellulose
- Hemicellulose
- Lignin



White-rot fungi

By Melissa Kuo



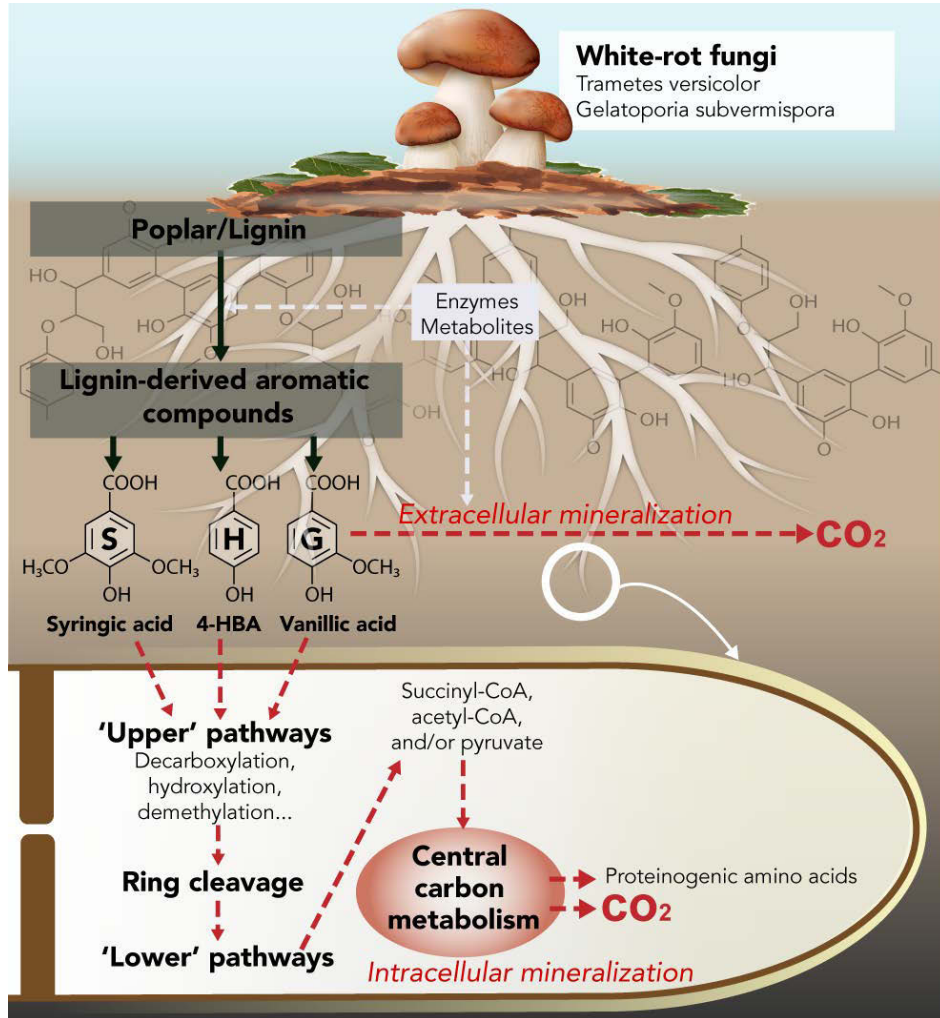
IN NATURE

- Lignin accounts for 30% of the organic carbon on Earth
- White-rot fungi are the most efficient organisms mineralizing lignin in Nature
- White-rot fungi play a pivotal role in Earth's carbon cycle

IN A BIOREFINERY

- Lignin valorization is key to enhance the feasibility of lignocellulosic biorefineries
- Lignin depolymerization is a challenge
- White-rot fungi are a promising biological tool for lignin upgrading

Our project at EMSL



del Cerro, C., Erickson, E., .., Salvachua, D. Intracellular pathways for lignin catabolism in white-rot fungi. In revision in PNAS.

We know that...

- White-rot fungi mineralize lignin to CO₂ and H₂O
- White-rot fungi secrete enzymes and metabolites to depolymerize lignin

We do not know...

- Carbon flux between lignin and CO₂ or the location (extracellular, intracellular)
- If lignin degradation products are utilized as a carbon source



Isotopic labeling experiments demonstrated that white-rot fungi utilize ¹³C-ring labeled 4-hydroxybenzoic acid as a carbon source...

... how?

Enzymes
Regulation
Pathways

Systems biology at EMSL

Proteomics
Transcriptomics
Metabolomics

Capabilities, expertise, and instrumentation

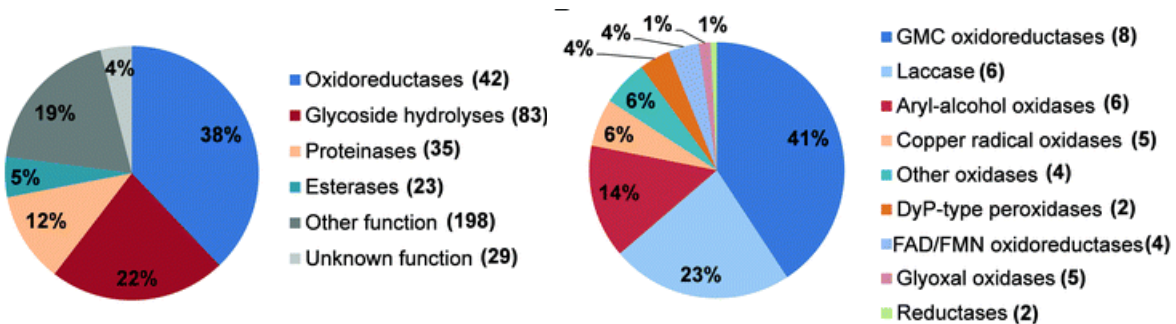
Successful previous collaborations at EMSL

How do fungi breakdown lignin?

- Proteomics in *Pleurotus eryngii*
- What enzyme cocktail is secreted by this fungus?

Functional classification

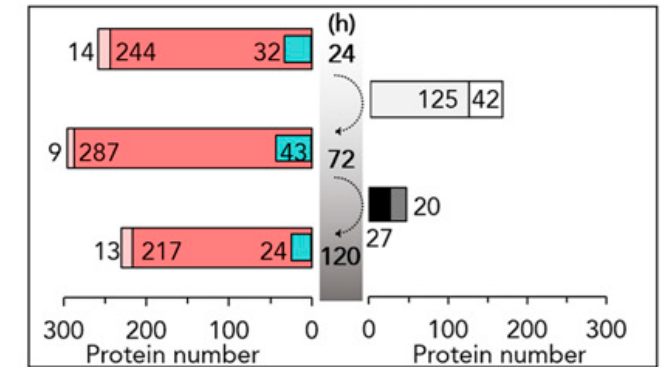
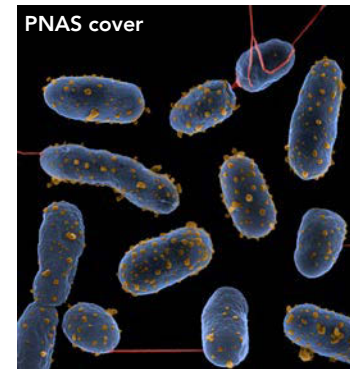
Oxidoreductases



Salvachúa, D., Katahira, R., ..., Beckham, G.T.. Lignin depolymerization by fungal secretomes and a microbial sink. **Green Chemistry**, 2016.

How do bacteria breakdown lignin?

- Proteomics in *Pseudomonas putida* and other bacteria
- Differential analyses (substrates) and spatial-temporal resolution



Salvachúa, D., Werner, A., Pardo, I., ..., Beckham, G.T.. Outer membrane vesicles catabolize lignin-derived aromatic compounds in *Pseudomonas putida* KT2440. **PNAS**. 2020

Proteomics and transcriptomics at EMSL

How do white-rot fungi catabolize lignin?



NREL

- Differential analyses (substrates) and spatial resolution in two white-rot fungi
- ~ 60 samples for proteomics and ~ 30 for transcriptomics

EMSL

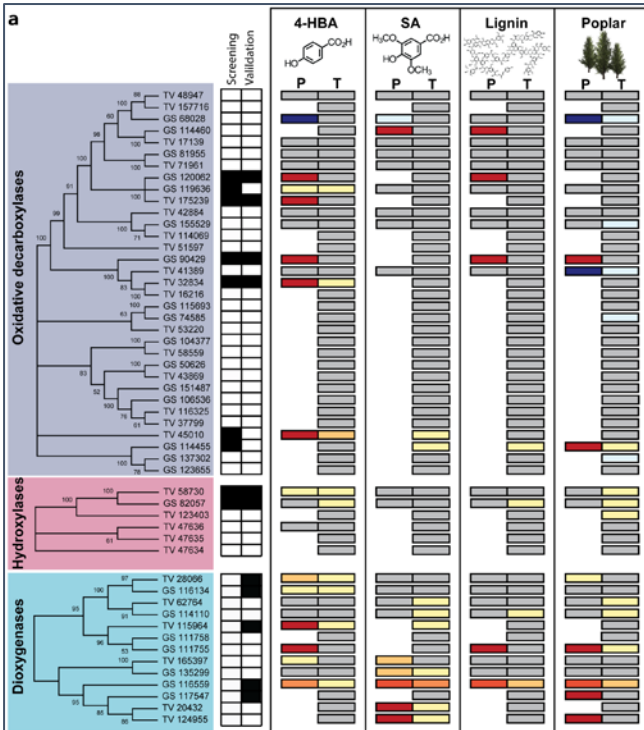
High throughput analyses, state-of-the-art capabilities

Proteomics

- Metabolite, Protein, Lipid Extraction (MPLE^{Ex}) extraction (proteomics)
- Advanced mass spectrometers (Q-Exacte Plus hybrid quadrupole/Orbitrap)
- Data analyses (statistics, bioinformatics)

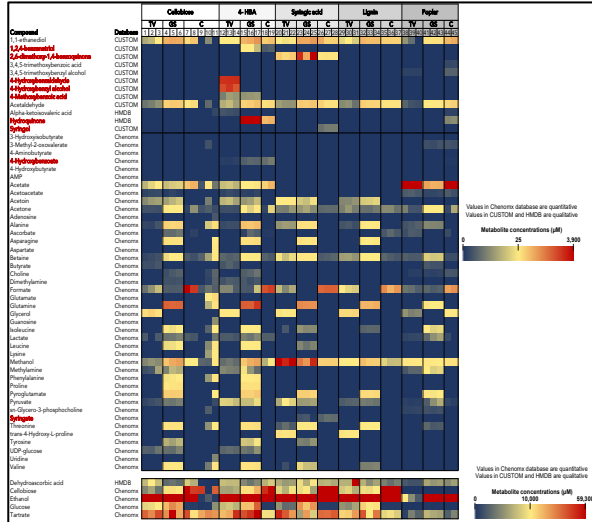
Transcriptomics

- Sample processing and RNA assessment
- Next generation (NextSeq 500) Sequencing Systems
- Data analyses (statistics, bioinformatics)



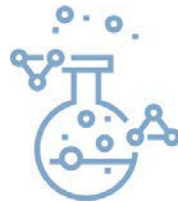
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Metabolomics at EMSL



NREL

- Differential (substrate) and spatial resolution in two white-rot fungi
- ~ 60 samples for metabolomics



EMSL

- Metabolite, Protein, Lipid Extraction (MPLEx) extraction and water extraction

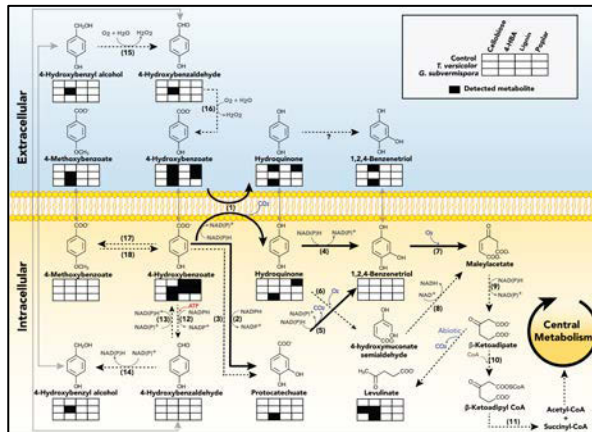


- Targeted and untargeted // quantitative and qualitative



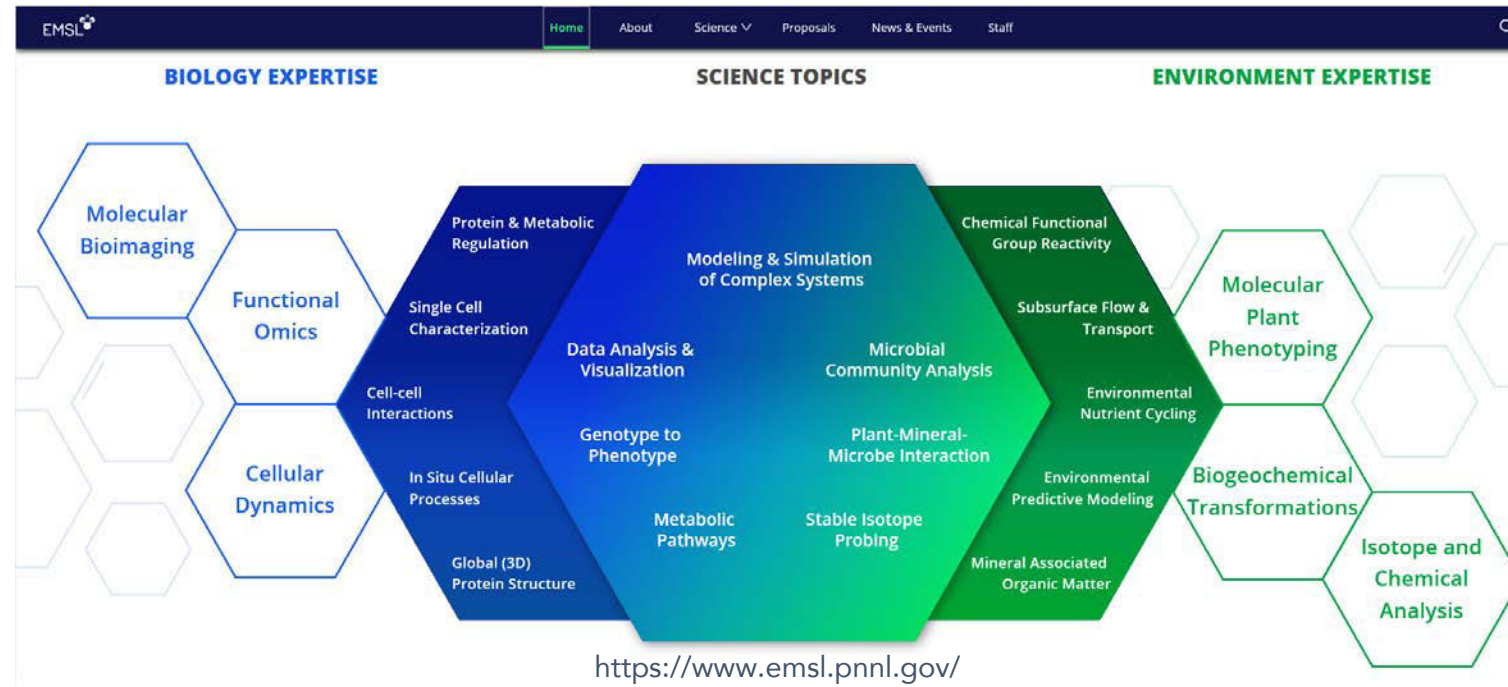
- Greater metabolome coverage: combined **NMR spectroscopy** and liquid chromatography-MS, gas chromatography-MS, and ion mobility spectrometry-MS

- NMR libraries: ChemoMx, Human Metabolome Database, and **custom in-house** (we built a library to target and cover our hypothetical catabolic pathways)



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Why you should submit a proposal to EMSL



- Very diverse capabilities, expertise, and state-of-the-art instrumentation → **high quality data**



- Study a broad spectrum of organisms and environments: fungi, bacteria, plants, microbial communities, soils...



- Constantly developing new tools: Lipidomics, MS imaging (spatial, qualitative, and quantitative molecular information)...



- **Rewarding interaction with EMSL scientists:** from proposal writing to manuscript writing

Tips for a successful proposal



Before proposal writing

- Peruse the EMSL website
- Identify the appropriate call for your proposal (EMSL website)
- Carefully read the **four review criteria** (every section has its own score!)
- **Contact EMSL researchers** once you know what you want to propose. They will recommend specific instruments and hours needed for your work and/or even different techniques that you did not have in mind to accomplish your work.

	Research focus	Call open period
Exploratory Research	This call invites proposals for projects up to 5 months. The research focus will vary year-by-year.	Jan, Feb, Mar Apr, May, Jun Jul, Aug, Sep Oct, Nov, Dec
		Jan, Feb, Mar Apr, May, Jun Jul, Aug, Sep Oct, Nov, Dec
Large-Scale EMSL Research	This call invites proposals for projects up to 24 months. The research focus will vary year-by-year.	Jan, Feb, Mar Apr, May, Jun Jul, Aug, Sep Oct, Nov, Dec
		Jan, Feb, Mar Apr, May, Jun Jul, Aug, Sep Oct, Nov, Dec
Limited Scope	This call invites proposals for a focused research sprint of no longer than 30 days.	Jan, Feb, Mar Apr, May, Jun Jul, Aug, Sep Oct, Nov, Dec

Proposal writing

- Clear hypothesis and aims in the first paragraph
- Preliminary experiments and figures that support your hypothesis/experimental design
- Clear methodology (**detailed**)
- Data organization/integration (what will you do with the data?)
- Include your expertise in the proposal (apart from the CV!)

If you don't get a project, you receive good feedback!



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Thanks for your attention! Questions?

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