



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

Davinia Salvachúa National Renewable Energy Laboratory December 1st, 2020 AGU Fall Meeting

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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

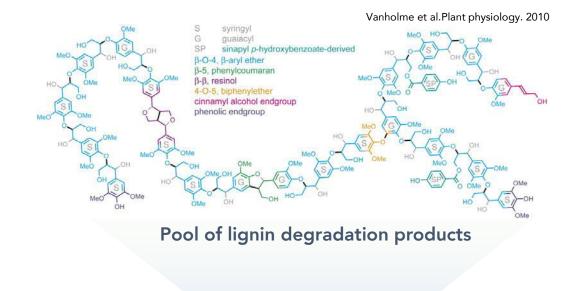
Lignin and white-rot fungi



- Cellulose
- Hemicellulose
- Lignin

White-rot fungi —





Central metabolism

BIOPRODUCTS

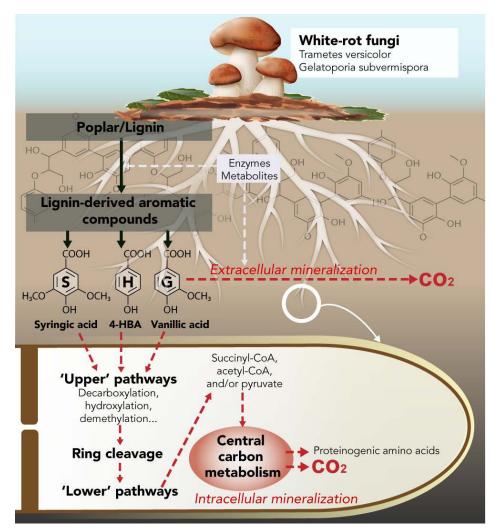
IN A BIOREFINERY

- 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

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

Our project at EMSL



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

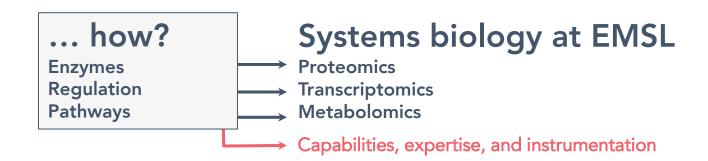
We know that...

- White-rot fungi mineralize lignin to CO_2 and H_2O
- 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...



Successful previous collaborations at EMSL

PNAS cover

How do fungi breakdown lignin?

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

How do bacteria breakdown lignin?

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

14 244

13 217

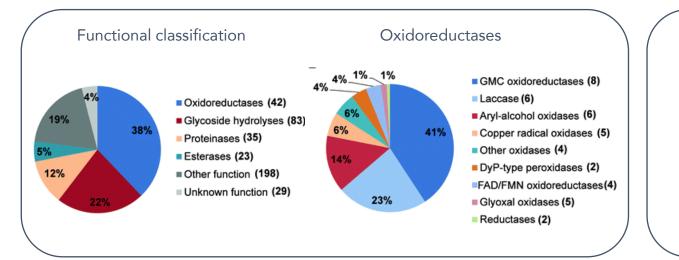
200

Protein number

100

9 287

300



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

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

32 (h) 32 24

43

24 120

0

0

125 42

100

200

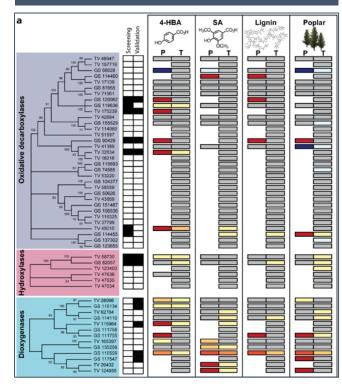
Protein number

300

20

Proteomics and transcriptomics at EMSL

How do white-rot fungi catabolize lignin?



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



- 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

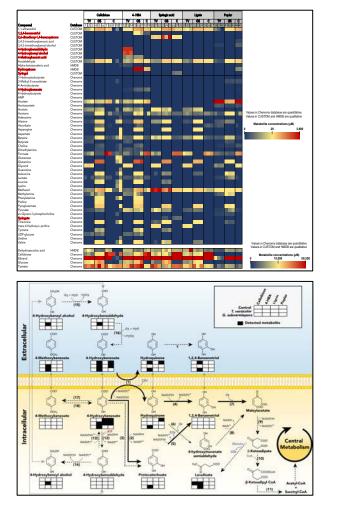
Proteomics

- Metabolite, Protein, Lipid Extraction (MPLEx) extraction (proteomics)
- Advanced mass spectrometers (Q-Exactive Plus hybrid quadrupole/Orbitrap)
- Data analyses (statistics, bioinformatics)

Transcriptomics

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

Metabolomics at EMSL



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

NREL

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

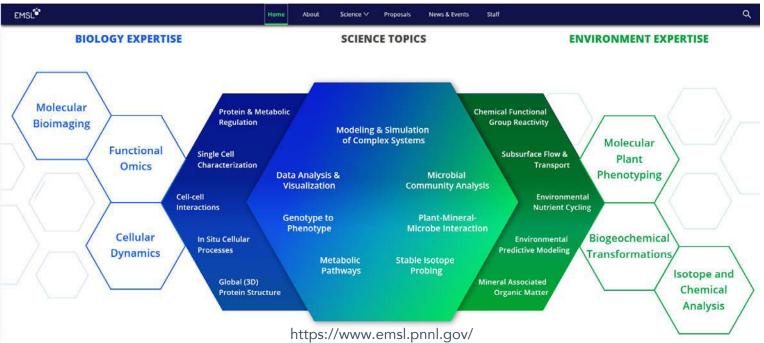
EMSL

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

Why you should submit a proposal to EMSL





- Very diverse capabilities, expertise, and state-of-the-art instrumentation \rightarrow high quality data
- Study a broad spectrum of organisms and environments: fungi, bacteria, plants, microbial communities, soils...

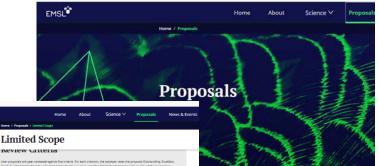


• Constantly developing new tools: Lipidomics, MS imaging (spatial, qualitative, and quantitative molecular information)...



• Rewarding interaction with EMSL scientists: from proposal writing to manuscript writing

Tips for a successful proposal



Science V

Types of Proposals Overview

The table below shows the types of proposals calls that are open throughout the year. the window to submit your proposal, and your project duration.

Research focus

up to 9 months. The research focus will

up to 24 months. The research focus

This call invites proposals for a focused

research sprint of no longer than 30

days

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Call open period

Review Criteria Details

Criterion 1: Scientific merit and quality of the proposed research

antial Considerations: How important is the proposed activity to advancing knowledge and inderstanding within its own field or across different fields? To what extent does the proposed tivity suggest and creative and original concepts? How well conceived and organized is proposed activity/

Criterion 2: Qualifications of the proposed research team to achieve proposal goals and contribute to high-impact science

ations: Does the proposal team, combined with relevant EMSL staff exp possess the breadth of skill/knowledge to successfully perform the proposed research and drive progress in this science area? If successful, would the proposed research deliver high-impact produ (for example, be publishable in high-impact journals)?

Note: impact factors are a measure of the average number of citations per published articles. ournals with higher impact factors reflect a higher average of citations per article and are conre influential within their scientific field.

Criterion 3: Relevance of the proposed research to EMSL's mission

biological and environmental processes across temporal and spatial scales. EMSL leads the scientific community toward a predictive understanding of complex biological and environmental systems to nable sustainable solutions to the nation's energy and environmental challenges.

ports the mission of the Biological and Environmental Research (BER) prog tment of Energy to achieve a predictive understanding of complex biological, earth, and mental systems for energy and infrastructure security, independence, and prosperity. BER veeks to understand the biological, biogeochemical, and physical processes that span from molecula and genomics-controlled scales to the regional and global scales that govern changes in watershed ynamics, climate, and the earth system.

Zarting with the genetic information encoded in organisms' genomes, BER research seeks to disc the principles that guide the translation of the generic code into the functional proteins and the metabolic and regulatory networks underlying the systems biology of plants and microbes as they spond to and modify their environments. This predictive understanding will enable design and ingineering of microbes and plants underpinning energy independence and a broad clean energ tribilo, including improved biofuels and bioproducts, improved carbon storage capabilities, and ntrolled biological transformation of materials such as nutrients and contaminants in the

IER research further advances the fundamental understanding of dynamic, physical, and ogeochemical processes required to systematically develop Earth system models that integra cross the atmosphere, land masses, oceans, sea ice, and subsurface. These predictive tools and ded to inform policies and plans for ensuring the security and resilience of the ical infrastructure and natural resources

with direct relevance in these areas will have the best chance for sel rolects of scientific significance also are welcomed, but the applicant should clearly outline how the piect will further a DOE mission or other areas with economic or societal impact.

tions: What is the relationship of the proposed research to FMSU's n oes the research project significantly advance the mission goals? How well does the project plan present a unique or innovative application or development of EMSL capabilities?

Criterion 4: Impact of the proposed research on one or more EMSL Science Areas

inderstanding of issues pertaining to one or more EMSL Science Areas? To what extent does the proposed research suggest and explore creative and original concepts related to one or more EMS cience Areas? How strongly does it relate to the science area's focused topics as outlined in the mos nt Call for Proposals? How well will it advance EMSL along the directions specifically outlined in

Before proposal writing

- Peruse the FMSL 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.

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!









CSP New Investigator Call



Early Career Award Research Program Biological and Environmental Research (BER)

Thanks for your attention! Questions?

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