

## Lignin research yields additional answers into bacteria's role

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A collaboration between the National Renewable Energy Laboratory (NREL) and three other national laboratories has yielded new insight into the ability of bacteria to break down the hardy part of a plant known



as lignin.

The additional understanding of the process could open a path to industrial applications.

Plants are protected by a complex cell wall made up of polymers of cellulose, hemicellulose, and lignin. The first two components can be broken down into sugars by various methods, and then turned into valuable biofuels and bioproducts. But lignin has proven recalcitrant, and its use largely limited.

"For more than a century, researchers have been trying to figure out what to do with lignin," said Gregg Beckham, a researcher at NREL and senior author of a new paper published in the *Proceedings of the National Academy of Sciences* of the United States of America. "There's a famous phrase about this polymer: You can make anything out of lignin except money."

But knowing exactly how bacteria are able to digest and use lignin is a step toward producing something of value.

The newly published research builds upon previous observations at NREL, reported in 2015, on the ability of 14 strains of bacteria to break down lignin oligomers and take advantage of those components. With that knowledge, researchers from NREL embarked on a <u>collaborative</u> <u>effort</u> to examine the three bacteria that did the best at attacking lignin.

The new paper, titled "Outer membrane vesicles catabolize ligninderived <u>aromatic compounds</u> in Pseudomonas putida KT2440," is the result of work at NREL, Argonne National Laboratory, Oak Ridge National Laboratory, and the Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory. The research was funded by the Center for Bioenergy Innovation supported by the Office



of Biological and Environmental Research in the DOE Office of Science.

"This is a team effort that required lignin chemists, molecular biologists, microscopists, microbiologists, and systems biologists," said Beckham. His NREL co-authors are Davinia Salvachúa, Allison Werner, Isabel Pardo, Brenna Black, Bryon Donohoe, Stefan Haugen, Rui Katahira, Sandra Notonier, Kelsey Ramirez, and Antonella Amore.

The new research examined the three best-performing bacteria able to convert a fraction of the lignin oligomers. Going into the project, the researchers were unaware how the bacteria were able to accomplish that feat. They hypothesized that the oligomers of lignin were not able to move through the bacteria cell membrane, so the researchers theorized something must be happening outside of the cell for them to be broken down. They tested that theory by examining what proteins were found outside the cell when the bacteria were fed lignin.

One bacterium—Pseudomonas putida KT2440—produced the most proteins by far.

A close look at the bacteria under the microscopes at NREL and Argonne National Laboratory revealed that the bacteria were secreting outer membrane vesicles (OMVs). Scientists at NREL and Oak Ridge then worked together to provide a partial answer to what's inside these vesicles: namely enzymes that are able to modify lignin-related compounds.

The research is far from finished. Beckham noted that it will be several years before it's understood exactly how the <u>bacteria</u> breaks the <u>lignin</u> down.

"We don't have the full picture yet," he said, "but we know a bit more



than we did five years ago."

**More information:** Davinia Salvachúa et al. Outer membrane vesicles catabolize lignin-derived aromatic compounds in Pseudomonas putida KT2440, *Proceedings of the National Academy of Sciences* (2020). <u>DOI:</u> 10.1073/pnas.1921073117

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