# Researchers tailor E. coli to convert plants into renewable chemicals

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Sandia National Laboratories scientists Seema Singh, left; and Fang Liu hold vials of vanillin and fermentation broth, which are critical for turning plant matter into biofuels and other valuable chemicals. Credit: Dino Vournas

What does jet fuel have in common with pantyhose and plastic soda bottles? They're all products currently derived from petroleum. Sandia National Laboratories scientists have demonstrated a new technology based on bioengineered bacteria that could make it economically feasible to produce all three from renewable plant sources.

Economically and efficiently converting tough plant matter, called lignin, has long been a stumbling block for wider use of the energy source and making it cost competitive. Piecing together mechanisms from other known lignin degraders, Sandia bioengineer Seema Singh and two postdoctoral researchers, Weihua Wu, now at Lodo Therapeutics Corp., and Fang Liu, have engineered E. coli into an efficient and productive bioconversion cell factory.

"For years, we've been researching cost-effective ways to break down lignin and convert it into valuable platform chemicals," Singh said. "We applied our understanding of natural lignin degraders to E. coli because that bacterium grows fast and can survive harsh industrial processes."

The work, "Towards Engineering *E. coli* with an Auto-Regulatory System for Lignin Valorization," was recently published in the *Proceedings of the National Academy of Sciences* and was supported by Sandia's Laboratory Directed Research and Development program.

## Engineering a costly process into profitability

Lignin is the component of plant cell walls that gives them their incredible strength. It is brimming with energy, but getting to that energy is so costly and complex that the resulting biofuel can't compete economically with other forms of transportation energy.

Once broken down, lignin has other gifts to give in the form of valuable platform chemicals that can be converted into nylon, plastics, pharmaceuticals and other valuable products. Future research may focus on demonstrating the production to these products, as they could help bring biofuel and bioproduction economics into balance. Or as Singh puts it, "they valorize lignin."

## Solving three problems: cost, toxicity and speed

Singh and her team have solved three problems with turning lignin into platform chemicals. The first was cost. *E. coli* typically do not produce the enzymes needed for the conversion process. Scientists must coax the bacteria into making the enzymes by adding something called an inducer to the fermentation broth. While effective, for activating enzyme production, inducers can be so costly that they are prohibitive for biorefineries.

The solution was to "circumvent the need for an expensive inducer by engineering the E. coli so that lignin-derived compounds such as vanillin serve as both the substrate and the inducer" Singh said.

Vanillin is not an obvious choice to replace an inducer. The compound is produced as lignin breaks down and can, at higher concentrations, inhibit the very *E. coli* working to convert it. This posed the second problem: toxicity.

"Our engineering turns the substrate toxicity problem on its head by enabling the very chemical that is toxic to the *E. coli* to initiate the complex process of lignin valorization. Once the vanillin in the fermentation broth activates the enzymes, the *E. coli* starts to convert the vanillin into catechol, our desired chemical, and the amount of vanillin never reaches a toxic level," Singh said. "It auto regulates."

The third problem was efficiency. While the vanillin in the fermentation broth moves across the membranes of the cells to be converted by the enzymes, it was a slow, passive movement. The researchers looked for effective transporters from other bacteria and microbes to fast track this process, Wu said.

"We borrowed a transporter design from another microbe and engineered it into *E. coli*, which helps pump the vanillin into the bacteria," Liu said. "It sounds pretty simple, but it took a lot of fine tuning to make everything work together."

Engineering solutions like these, which overcome toxicity and efficiency issues have the potential to make biofuel production economically viable. The external inducer-free, auto-regulating method for valorizing lignin is just one way that researchers are working to optimize the biofuel-making process.

"We have found this piece of the lignin valorization puzzle, providing a great starting point for future research into scalable, cost-effective solutions," Singh said. "Now we can work on producing greater quantities of platform chemicals, engineering pathways to new end products, and considering microbial hosts other than *E. coli*."

**More information:** Weihua Wu et al, Toward engineeringE. coliwith an autoregulatory system for lignin valorization, *Proceedings of the National Academy of Sciences* (2018). DOI: 10.1073/pnas.1720129115

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