BIOFUELS

Engineered Superbugs Boost Hopes Of Turning Seaweed Into Fuel

In the search for renewable fuels, there's no perfect solution. Biofuels can be readily made from corn starch and sugar cane, but they take land and resources away from food crops. Feedstocks such as switchgrass and wood sidestep that problem—but they are hamstrung by a molecule called lignin, which makes it harder to extract the sugars that ferment into ethanol.

Enter seaweed: It has no lignin and requires no land, fresh water, or fertilizer. Several countries have pilot programs for generating biofuels from seaweed. But there's a hitch: About a third of the sugars in seaweed take the form of alginate, a complex polymer that industrial microbes can't convert into ethanol.

On page 308, researchers led by Yasuo Yoshikuni of Bio Architecture Lab (BAL), a 4-year-old biotech company in Berkeley, California, describe a strain of *Escherichia coli* that they have genetically engineered to break down and ferment alginate and all the other major sugars in seaweed into ethanol. "This is very impressive work," says James Liao, a metabolic engineer at the University of California (UC), Los Angeles. But experts wonder whether enough seaweed can be harvested to make it a significant contributor to petroleum independence or even a lower-carbon economy. "Is this a game-changer? Probably not," says molecular biologist Stephen Mayfield of UC San Diego. "But it's a step in the right direction."

Yoshikuni's team started by giving *E. coli* a gene that enabled it to break down alginate, a polymer of uronic acids, into short fragments called oligomers. The gene—for an enzyme called alginate lyase (Aly)—came from the marine bacterium *Pseudoalteromonas sp*. To beef up the modified bug's alginate-busting ability, the researchers tacked on a so-called autotransporter molecule that forced the cells to secrete all the Aly they made.

Next, the researchers had to modify *E. coli* so that it could take up the alginate oligomers floating nearby. The plan was to install a cellular transport system for the oligomers, but first they had to find one. "It wasn't an easy process," Yoshikuni says. Their clue was pectin, another kind of polymer abundant in fruit peels and other plant materials. Because the genes behind the pectin transport system are known, the team could search for similar genes in a genomics database. Bingo; they found that the bacterium *Vibrio splendidus* 12B01 carries a stretch of DNA similar to that for pectin transport and bearing genes for alginate-degrading enzymes.

But the researchers weren't sure which genes in this stretch they needed because many of their exact functions were a mystery. Turning to natural selection for help, they created thousands of random snippets of *V. splendidus* 12B01's genome and tucked them individually into *E. coli* cells. Then they dropped cells into a thin alginate-oligomer broth to see which ones could use it as food. One 13-gene snippet allowed the *E. coli* to thrive.

Fuel line? Refiners will need a cheap supply of seaweed to make it a feasible biofuel.

The team then deleted individual genes to figure out their functions. Additionally, the team sought and introduced other *V. splendidus* genes that might function in alginate metabolism. The resulting *E. coli* strain was able to take up all the available alginate oligomers and further break them down into even simpler components. Thanks to the added DNA, the *E. coli* could also convert them into molecules, such as pyruvate, used to make chemical building blocks. Finally, the researchers added a pathway—borrowed from *Zymomonas mobilis*, a bacterium originally isolated from fermented cane juice—that turns the pyruvate into ethanol.

When the fully engineered *E. coli* was fed a slurry of the common brown seaweed called kombu (*Saccharina japonica*), the cells fermented the brew up to a concentration of 5% ethanol—comparable to the benchmark for yeast-based fermentation of woody biomass. It's also more than twice as good as existing fermentation methods for seaweed and can produce more than 80% of the theoretical maximum yield of ethanol from sugars in seaweed. "This is a big step," says Richard Sayre of Los Alamos National Laboratory in New Mexico. As with other microbial systems, BAL's *E. coli* could be modified to create other fuels and valuable chemicals.

A big question, especially for the United States, is where to get enough seaweed. "It's cultivation at the scale that we need—that is a major hurdle," says Guri Roesijadi of Pacific Northwest National Laboratory (PNNL) in Sequim, Washington. In a 2010 report on seaweed for the Department of Energy, he and other PNNL researchers calculated that replacing 1% of the U.S. gasoline supply with ethanol would require growing seaweed over nearly 11,000 square kilometers. Several other countries grow seaweed for food, animal feed, fertilizer, or polymers, but there is almost no such farming in U.S. waters.

Seaweed supply directly affects two other key factors: cost and sustainability. If refiners have to haul seaweed long distances, that will raise the cost of seaweed-based ethanol and give it a larger carbon footprint, which in turn can reduce tax breaks and other subsidies from the federal government. "This technology will be competitive only in locations where very large amounts of seaweed are readily available, such as coastal areas," predicts Michael Henson, a chemical engineer at the University of Massachusetts, Amherst. BAL's CEO, Daniel Trunfio, says the company has contracted a life-cycle analysis to show exactly how competitive and "green" its process is; results should be available later this year.

–ERIK STOKSTAD

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