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From Gasoline to Grassoline: Microbes Produce Fuels Directly from Biomass

CONTACT US

Office of Science
U.S. Department of Energy
1000 Independence Ave., SW
Washington, DC 20585
P: (202) 586-5430
More Information »

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A microbe that can produce an advanced biofuel directly from biomass was developed by researchers with the U.S. Department of Energy's Joint BioEnergy Institute.

Two-thirds of the world's petroleum resources are now used for transportation. The ever-increasing costs and shrinking — and inherently unstable — supplies of petroleum make it imperative that we, as a nation, develop alternative fuels that are home-grown and can be produced in a sustainable fashion. As an added incentive, a new domestic transportation fuel industry would also generate thousands of jobs.

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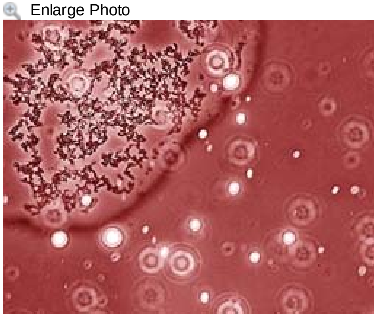

Image by Eric Steen, JBEI

Once E. coli have secreted oil, they sequester themselves from the droplets as shown by this optical image, thereby facilitating oil recovery.

Scientific studies have consistently shown that liquid fuels derived from plant biomass are one of the best alternatives to petroleum if a cost-effective means of commercial production can be found. Major research efforts to this end are focused on fatty acids — the energy-rich molecules in plant cells that have been dubbed nature's petroleum. This past year, a collaboration of researchers led by chemical engineer Jay Keasling, who heads the U.S. Department of Energy's Joint BioEnergy Institute (JBEI), developed the first microbe that can produce an advanced biofuel directly from fatty acids in biomass. Deploying the tools of synthetic biology, the JBEI researchers engineered a strain of *Escherichia coli (E. coli)* bacteria to produce biodiesel fuel and other important chemicals.

"The fact that our microbes can produce a diesel fuel directly from biomass with no additional chemical modifications is exciting and important," says Keasling, who is one of the world's foremost practitioners of synthetic biology. "Given that the costs of recovering biodiesel are nowhere near the costs required to distill ethanol, we believe our results can significantly contribute to the ultimate goal of producing scalable and cost-effective advanced biofuels and renewable chemicals."

Keasling has earned international recognition and a humanitarian award from the biotechnology industry for his use of synthetic biology techniques to develop a simple and much less expensive means of making artemisinin, the key component in artemisinin-based combination therapies (ACTs), which are the World Health Organization's recommended first-line treatment for malaria. Microbial-based artemisinin is expected to dramatically reduce the production time and cost of artemisinin for ACTs, making these powerful anti-malaria drugs far more accessible to the people who need them most. According to the World Health Organization, each year nearly 500 million people become infected with malaria, and nearly three million, mostly children, die from it. The synthetic biology technology behind this breakthrough can also be applied to the development of new and advanced biofuels.

"Artemisinin is hydrocarbon and we built a microbial platform to produce it," Keasling says. "We can remove a few of the genes to take out artemisinin and put in a different hydrocarbon to make biofuels."

To develop the biodiesel-producing strain of *E. coli* bacteria, Keasling, and a team from JBEI's Fuels Synthesis Division that included Eric Steen, Yisheng Kang, and Gregory Bokinsky, worked with a research team from LS9, a privately-held industrial biotechnology firm based in South San Francisco. The LS9 team was headed by Stephen del Cardayre and included Zhihao Hu, Andreas Schemer, and Amy McClure.

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Fuels and chemicals have been produced from the fatty acids in plant and animal oils for more than a century. These oils now serve as the raw materials not only for biodiesel fuel, but also for a wide range of important chemical products including surfactants, solvents, and lubricants.

"The increased demand and limited supply of these oils has resulted in competition with food, higher prices, questionable land-use practices and environmental concerns associated with their production," Keasling says. "A more scalable, controllable, and economic alternative route to these fuels and chemicals would be through the microbial conversion of renewable feedstocks, such as biomass-derived carbohydrates."

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E. coli is a well-studied microorganism whose natural ability to synthesize fatty acids and exceptional amenability to genetic manipulation make it an ideal target for biofuels research. The combination of *E. coli* with new biochemical reactions realized through synthetic biology enabled Keasling, Steen, and their colleagues to produce structurally tailored fatty esters (biodiesel), alcohols, and waxes directly from simple sugars.

"Biosynthesis of microbial fatty acids produces fatty acids bound to a carrier protein, the accumulation of which inhibits the making of additional fatty acids," Steen says. "Normally *E. coli* don't waste energy making excess fat, but by cleaving fatty acids from their carrier proteins, we're able to unlock the natural regulation and make an abundance of fatty acids that can be converted into a number of valuable products. Further, we engineered our *E. coli* to no longer eat fatty acids or use them for energy."

After successfully diverting fatty acid metabolism toward the production of fuels and other chemicals from glucose, the JBEI researchers engineered their new strain of *E. coli* to produce hemicellulases — enzymes that are able to ferment hemicellulose, the complex sugars that are a major constituent of cellulosic biomass and a prime repository for the energy locked within plant cell walls.

"Engineering *E. coli* to produce hemicellulases enables the microbes to produce fuels directly from the biomass of plants that are not used as food for humans or feed for animals," Steen says. "Currently, biochemical processing of cellulosic biomass requires costly enzymes for sugar liberation. By giving the *E. coli* the capacity to ferment both cellulose and hemicellulose without the addition of expensive enzymes, we can improve the economics of cellulosic biofuels."

The JBEI team is now working on maximizing the efficiency and the speed by which their engineered strain of *E. coli* can directly convert biomass into biodiesel. They are also looking into ways of maximizing the total amount of biodiesel that can be produced from a single fermentation.

"Productivity, titer, and efficient conversion of feedstock into fuel are the three most important factors for engineering microbes that can produce biofuels on an industrial scale," Steen says. "There is still much more research to do before this process becomes commercially feasible."

The DOE Joint BioEnergy Institute, led by Lawrence Berkeley National Laboratory, is one of three DOE Bioenergy Research Centers established by the U.S. Department of Energy's Office of Science in 2007. The centers support multidisciplinary, multi-institutional research teams pursuing the fundamental scientific breakthroughs needed to make production of cellulosic biofuels, or biofuels from nonfood plant fiber, cost-effective on a national scale. The centers are led, respectively, by Lawrence Berkeley National Laboratory, Oak Ridge National Laboratory, and the University of Wisconsin-Madison in partnership with Michigan State University.

—Lynn Yarris, Lawrence Berkeley National Laboratory, lcyarris@lbl.gov

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E.J. Steen et al. "Microbial production of fatty-acid-derived fuels and chemicals from plant biomass," *Nature* **463** 559 (2010).

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