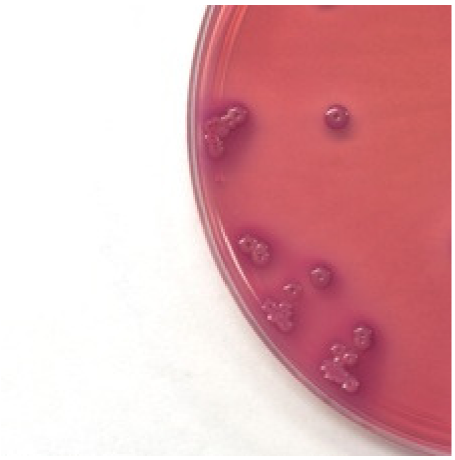
SCIENTIFIC
AMERICAN

Bacteria Transformed into Biofuel Refineries

Synthetic biology has allowed scientists to tweak *E. coli* to produce fuels from sugar and, more sustainably, cellulose. By David Biello | Wednesday, January 27, 2010



MICROBIAL FUEL: Scientists have transformed *E. coli* to produce diesel and other hydrocarbons from sugars--as well as secrete enzymes to break down cellulose. Image: © iStockphoto.com / Erik van Hannen

The bacteria responsible for most cases of food poisoning in the U.S. has been turned into an efficient biological factory to make chemicals, medicines and, now, fuels. Chemical engineer Jay Keasling of the University of California, Berkeley, and his colleagues have manipulated the genetic code of *Escherichia coli*, a common gut bacteria, so that it can chew up plant-derived sugar to produce diesel and other hydrocarbons, according to results published in the January 28 issue of *Nature*. (*Scientific American* is part of Nature Publishing Group.)

"We incorporated genes that enabled production of biodiesel—esters [organic compounds] of fatty acids and ethanol—directly," Keasling explains. "The fuel that is produced by our E. coli can be used directly as biodiesel. In contrast, fats or oils from plants must be chemically esterified before they can be used."

Perhaps more importantly, the researchers have also imported genes that allow E. coli to secrete enzymes that break down the tough material that makes up the bulk of plants—cellulose, specifically hemicellulose—and produce the sugar needed to fuel this process. "The organism can produce the fuel from a very inexpensive sugar supply, namely cellulosic biomass," Keasling adds.

The E. coli directly secretes the resulting biodiesel, which then floats to the top of a fermentation vat, so there is neither the necessity for distillation or other purification processes nor the need, as in biodiesel from algae, to break the cell to get the oil out.

This new process for transforming E. coli into a cellulosic biodiesel refinery involves the tools of synthetic biology. For example, Keasling and his team cloned genes from Clostridium stercorarium and Bacteroides ovatus—bacteria that thrive in soil and the guts of plant-eating animals, respectively—which produce enzymes that break down cellulose. The team then added an extra bit of genetic code in the form of short amino acid sequences that instruct the altered E. coli cells to secrete the bacterial enzyme, which breaks down the plant cellulose, turning it into sugar; the E. coli in turn transforms that sugar into biodiesel.

The process is perfect for making hydrocarbons with at least 12 carbon atoms in them, ranging from diesel to chemical precursors—and even jet fuel, or kerosene. But it cannot, yet, make shorter chain hydrocarbons like gasoline. "Gasoline tends to contain short-chain hydrocarbons, say C8, with more branches, whereas diesel and jet fuel contain long-chain hydrocarbons with few branches," Keasling notes. "There are other ways to make gasoline. We are working on these technologies, as well."

After all, the U.S. alone burns some 530 billion liters of gasoline a year, compared with just 7.5 billion liters of biodiesel. But Keasling has estimated in the past that a mere 40.5 million hectares of Miscanthus giganteus—a more than three-meter tall Asian grass—chewed up by specially engineered microbes, like the E. coli here, could produce enough fuel to meet all U.S. transportation needs.* That's roughly one quarter of the current amount of land devoted to raising crops in the U.S.

E. coli is the most likely candidate for such work, because it is an extremely well-studied organism as well as a hardy one. "E. coli tolerated the genetic changes quite well," Keasling says. "It was somewhat surprising. Because all organisms require fatty acids for their cell membrane to survive, if you rob them of some fatty acids, they turn up the fatty acid biosynthesis to make up for the depletion."

E. coli "grows fast, three times faster than yeast, 50 times faster than Mycoplasma, 100 times faster than most agricultural microbes," explains geneticist and technology developer George Church at Harvard Medical School, who was not involved in this research. "It can survive in detergents or gasoline that will kill lesser creatures, like us. It's fairly easily manipulated." Plus, E. coli can be turned into a microbial factory for almost anything that is presently manufactured but organic—from electrical conductors to fuel. "If it's organic, then, immediately, it becomes plausible that you can make it with biological systems."

The idea in this case is to produce a batch of biofuel from a single colony through E. coli's natural ability to proliferate and, after producing the fuel, dispose of the E. coli and start anew with a fresh colony, according to Keasling. "This minimizes the mutations that might arise if one continually subcultured the microbe," he says. The idea is also to engineer the new organism, deleting key metabolic pathways, such that it would never survive in the wild in order to prevent escapes with unintended environmental impacts, among other dangers.

But ranging outside of its natural processes, E. coli is not the most efficient producer of biofuel. "We are at about 10 percent of the theoretical maximum yield from sugar," Keasling notes. "We would like to be at 80 to 90 percent to make this commercially viable. Furthermore, we would need a large-scale production process," such as 100,000 liter tanks to allow mass production of microbial fuel.

Nevertheless, several companies, including LS9, which helped with the research, as well as Gevo and Keasling-founded Amyris Biotechnologies, are working on making fuel from microbes a reality at the pump—not just at the beer tap.

*Erratum (1/28/10): This sentence was edited after publication to correct a measurement conversion error in the number of hectares stated.