# Researchers expand yeast's sugary diet to include plant fiber

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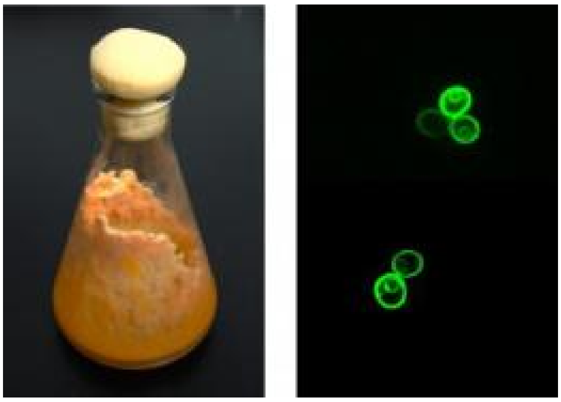

A colony of the fungus *Neurospora crassa* (left) and the yeast *Saccharomyces cerevisiae*. Sugar transporters from *Neurospora* that have been inserted into the yeast are tagged with green fluorescent protein. Credit: Jamie Cate and Susan Jenkins, UC Berkeley

(PhysOrg.com) -- University of California, Berkeley, researchers have taken genes from grass-eating fungi and stuffed them into yeast, creating strains that produce alcohol from tough plant material - cellulose - that normal yeast can't digest.

The feat could be a boon for the biofuels industry, which is struggling to make cellulosic ethanol - ethanol from plant fiber, not just cornstarch or sugar - economically feasible.

"By adding these genes to yeast, we have created strains that grow better on plant material than does wild yeast, which eats only glucose or sucrose," said Jamie Cate, UC Berkeley associate professor of molecular and cell biology and faculty scientist at Lawrence Berkeley National Laboratory (LBNL). "This improvement over the wild organism is a proof-of-principle that allows us to take the technology to the next level, with the goal of engineering yeast that can digest and ferment plant material in one pot."

The researchers hope to insert the same fungal genes into industrial yeast that now is used to turn sugar into ethanol biofuel in order to improve the efficiency of the fermentation process.

"The use of these cellodextrin transporters is not limited to yeast that makes ethanol," Cate said. "They could be used in any yeast that's been engineered to make, for example, other alcohols or jet fuel substitutes."

Cate and his UC Berkeley and LBNL colleagues, including first author Jonathan M. Galazka, a UC Berkeley graduate student, report their success this week in the journal *Science* Express. The work is funded by the Energy Biosciences Institute (EBI), a research collaboration between UC Berkeley, the University of Illinois, LBNL and the funding sponsor, BP.

Currently, the biofuel industry employs brewer's yeast, the single-celled fungus Saccharomyces cerevisiae, to convert sugar, cornstarch or other simple carbohydrates into ethanol by fermentation. But plants contain sugar polymers that yeast cannot eat - in particular, cellulose, a tough molecule composed of glucose molecules linked together in long chains. The biofuels industry is now building demonstration plants that will use "cellulosic" sources such as corn stalks, leaves and cobs, paper waste and other plant material to make ethanol.

But cellulosic processes are complex and expensive, Cate said. The plant material must first be broken down into sugars through a process called saccharification. Enzymes called cellulases are added to convert cellulose to short-chain sugars, called cellodextrins, and these must be further broken down into glucose molecules by the enzyme beta-glucosidase. Only then can yeast work its magic and turn the glucose into alcohol.

Other fungi, however, can digest cellulose, though they don't produce alcohol. One of these, *Neurospora crassa*, a common fungus whose preferred diet is fire-damaged plants, has been studied in the laboratory for more than 100 years, Cate said.

Last year, Chaoguang Tian, a former UC Berkeley post-doctoral fellow in Professor Louise Glass's laboratory who now is at the Tianjin Institute of Industrial Biotechnology in China, and William T. Beeson, a graduate student in UC Berkeley's College of Chemistry, along with Cate and other UC Berkeley researchers, conducted a genome-wide analysis of *Neurospora crassa* to locate genes that are turned on when the fungus grows on cellulose.

The genome-wide systems analysis turned up a family of genes which produces proteins that transport sugars into the *Neurospora* cell to be used as fuel. The researchers suspected that some of these transporters would allow *Neurospora* to import cellodextrins - in particular, the two-, three- and four-glucose molecules (cellobiose, cellotriose and cellotetraose, respectively). A search through the genomes of other fungi that grow on plants turned up similar genes in many of them, including the black truffle, which is symbiotic on tree roots.

Thanks to previous work funded by the National Institutes of Health, the team easily obtained *Neurospora* "knock-out" strains missing specific transporter genes and confirmed that, without all of them, the fungus could no longer eat cellodextrins as quickly.

"Most sugar-transporters let one sugar in at a time," Galazka said. "The sugar-transporters we found in *Neurospora* actually let in an entire chain of sugars. This means that four sugars can enter the fungus at once, if they are linked together.

Galazka subsequently created six strains of yeast, each with one extra gene from the *Neurospora* transporter family, along with a beta-glucosidase gene, also from *Neurospora*. The yeast strains produced *Neurospora* transporter proteins, and two of the strains were able to grow on cellodextrin as well as on glucose. One strain produced 60 percent more alcohol than normal yeast when grown on the two-glucose molecule, cellobiose.

Apparently, Galazka said, while normal yeast can't import cellodextrins or digest them once they're inside the cell, if they are given a *Neurospora* transporter and a beta-glucosidase from the fungus that stays inside the cells, it's able to do both.

"We've effectively made yeast more compatible with the enzymes used to break down woody plants," he said. "We think that the discovery of these transporters is a key step towards the efficient conversion of plant matter now considered waste into fuel."

"We now have to get these genes into industrial yeast strains - the hearty, rock 'em, sock 'em yeast used commercially - and get them to use more complicated plant material," Cate said.

He noted that a cellulosic process using yeast with transporter proteins could avoid having to add beta-glucosidases to the fermentation chamber, but enzymes would still be needed to break down cellulose into cellodextrins.

He and his colleagues are now collaborating with other EBI researchers to create improved transporter proteins and yeast strains.

Provided by University of California - Berkeley