Scientists overcome major obstacles to cellulosic biofuel production

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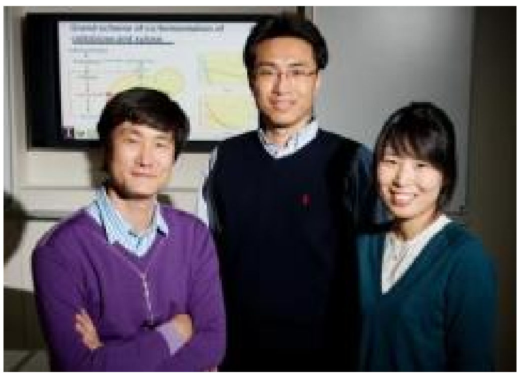

Illinois food science and human nutrition professor Yong-Su Jin (center), postdoctoral researcher Suk-Jin Ha (left), graduate student Soo Rin Kim and their colleagues engineered a yeast that outperforms the industry standard in the production of ethanol from cellulosic biomass. The effort involved researchers at Illinois, the Lawrence Berkeley National Laboratory, the University of California at Berkeley, Seoul National University and the oil company BP. Credit: L. Brian Stauffer, U. of I. News Bureau.

A newly engineered yeast strain can simultaneously consume two types of sugar from plants to produce ethanol, researchers report. The sugars are glucose, a six-carbon sugar that is relatively easy to ferment; and xylose, a five-carbon sugar that has been much more difficult to utilize in ethanol production. The new strain, made by combining, optimizing and adding to earlier advances, reduces or eliminates several major inefficiencies associated with current biofuel production methods.

The findings, from a collaborative led by researchers at the University of Illinois, the Lawrence Berkeley National Laboratory, the University of California and the energy company BP, are described in the Proceedings of the National Academy of Sciences. The Energy Biosciences Institute, a BP-funded initiative, supported the research.

Yeasts feed on sugar and produce various waste products, some of which are useful to humans. One type of yeast, *Saccharomyces cerevisiae*, has been used for centuries in baking and brewing because it efficiently ferments sugars and in the process produces ethanol and carbon dioxide. The biofuel industry uses this yeast to convert plant sugars to bioethanol. And while *S. cerevisiae* is very good at utilizing glucose, a building block of cellulose and the primary sugar in plants, it cannot use xylose, a secondary – but significant – component of the lignocellulose that makes up plant stems and leaves. Most yeast strains that are engineered to metabolize xylose do so very slowly.

"Xylose is a wood sugar, a five-carbon sugar that is very abundant in lignocellulosic biomass but not in our food," said Yong-Su Jin, a professor of food science and human nutrition at Illinois. He also is an affiliate of the U. of I. Institute for Genomic Biology and a principal investigator on the study. "Most yeast cannot ferment xylose."

A big part of the problem with yeasts altered to take up xylose is that they will suck up all the glucose in a mixture before they will touch the xylose, Jin said. A glucose transporter on the surface of the yeast prefers to bind to glucose.

"It's like giving meat and broccoli to my kids," he said. "They usually eat the meat first and the broccoli later."

The yeast's extremely slow metabolism of xylose also adds significantly to the cost of biofuels production.

Jin and his colleagues wanted to induce the yeast to quickly and efficiently consume both types of sugar at once, a process called co-fermentation. The research effort involved researchers from Illinois, the Lawrence Berkeley National Laboratory, the University of California at Berkeley, Seoul National University and BP.

In a painstaking process of adjustments to the original yeast, Jin and his colleagues converted it to one that will consume both types of sugar faster and more efficiently than any strain currently in use in the biofuel industry. In fact, the new yeast strain simultaneously converts cellobiose (a precursor of glucose) and xylose to ethanol just as quickly as it can ferment either sugar alone.

"If you do the fermentation by using only cellobiose or xylose, it takes 48 hours," said postdoctoral researcher and lead author Suk-Jin Ha. "But if you do the co-fermentation with the cellobiose and xylose, double the amount of sugar is consumed in the same amount of time and produces more than double the amount of ethanol. It's a huge synergistic effect of co-fermentation."

The new yeast strain is at least 20 percent more efficient at converting xylose to ethanol than other strains, making it "the best xylose-fermenting strain" reported in any study, Jin said.

The team achieved these outcomes by making several critical changes to the organism. First, they gave the yeast a cellobiose transporter. Cellobiose, a part of plant cell walls, consists of two glucose sugars linked together. Cellobiose is traditionally converted to glucose outside the yeast cell before entering the cell through glucose transporters for conversion to ethanol. Having a cellobiose transporter means that the engineered yeast can bring cellobiose directly into the cell. Only after the cellobiose is inside the cell is it converted to glucose.

This approach, initially developed by co-corresponding author Jamie Cate at the Lawrence Berkeley National Laboratory and the University of California at Berkeley, eliminates the costly step of adding a cellobiose-degrading enzyme to the lignocellulose mixture before the yeast consumes it.

It has the added advantage of circumventing the yeast's own preference for glucose. Because the glucose can now "sneak" into the yeast in the form of cellobiose, the glucose transporters can focus on drawing xylose into the cell instead. Cate worked with Jonathan Galazka, of UC Berkeley, to clone the transporter and enzyme used in the new strain.

The team then tackled the problems associated with xylose metabolism. The researchers inserted three genes into *S. cerevisiae* from a xylose-consuming yeast, *Picchia stipitis*.

Graduate student Soo Rin Kim at the University of Illinois identified a bottleneck in this metabolic pathway, however. By adjusting the relative production of these enzymes, the researchers eliminated the bottleneck and boosted the speed and efficiency of xylose metabolism in the new strain.

They also engineered an artificial "isoenzyme" that balanced the proportion of two important cofactors so that the accumulation of xylitol, a byproduct in the xylose assimilitary pathway, could be minimized. Finally, the team used "evolutionary engineering" to optimize the new strain's ability to utilize xylose.

The cost benefits of this advance in co-fermentation are very significant, Jin said.

"We don't have to do two separate fermentations," he said. "We can do it all in one pot. And the yield is even higher than the industry standard.

We are pretty sure that this research can be commercialized very soon."

Jin noted that the research was the result of a successful collaboration among principal investigators in the Energy Biosciences Institute and a BP scientist, Xiaomin Yang, who played a key role in developing the co-fermentation concept and coordinating the collaboration.

More information: The paper, "Engineered Saccharomyces cerevisiae capable of simultaneous cellobiose and xylose fermentation," is published in *PNAS*.

Provided by University of Illinois at Urbana-Champaign

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