

Researchers enlist sturdy yeast to help make cost-effective ethanol

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Yeasts play a key role in converting ("fermenting") sugars from plants into ethanol fuel. But not all yeasts are created equal. Some are better fermenters than others because they can tolerate the harsh conditions of

the bioreactors in which they're used.

Clavispora NRRL Y-50464 is just such a yeast.

A team of Agricultural Research Service (ARS) scientists at the agency's National Center for Agricultural Utilization Research in Peoria, Illinois, used a standard microbiology procedure called "adaptive laboratory evolution" to generate the hardy yeast strain, which in tests outperformed the industry standard, *Saccharomyces cerevisiae*.

Cornstarch has long been a leading source of simple sugars that can be fermented into [ethanol fuel](#) as a cleaner-burning alternative to gasoline. However, there are many competing uses for the versatile commodity, prompting researchers and fuel makers to focus on alternative sources—perennial grasses, forestry byproducts and crop residues like rice straw and corn stover, among them.

To get to these sugars, the "brick and mortar" of plant cells—cellulose and lignin (a.k.a., lignocellulose)—must first be broken down using enzymes or diluted acids. However, this produces furfural and other chemical byproducts that are harmful to the yeasts, hobbling their ability to ferment sugars released from lignocellulose.

Fortunately, *Clavispora* NRRL Y-50464 is no ordinary yeast.

The strain is the toughest of the tough selected by the researchers from a natural population of *Clavispora* yeasts originally found growing on sweet sorghum. This toughness includes heat tolerance, [fast growth](#) and an ability to detoxify harmful byproducts like furfural while producing [ethanol](#). The yeast strain also makes its own beta-glucosidase, an enzyme which catalyzes the breakdown of simple sugars like glucose from lignocellulose so they can be fermented into ethanol. This eliminates the need to add beta-glucosidase "and lowers the enzyme cost of cellulosic

ethanol production," explained Z. Lewis Liu, a [molecular biologist](#) (retired), formerly with the ARS center's Bioenergy Research Unit in Peoria.

That's kind of a big deal—so much so that ARS patented the yeast strain for use in lignocellulose-based ethanol production systems, whose enzyme costs are typically 10 times those of starch-based ones.

Laboratory trials conducted by Liu, ARS chemical engineer Bruce Dien and their collaborators bare out the yeast strain's super-star status as a fermenter of sugars from lignocellulose-rich sources like rice straw and corn stover (which refers to the corn plant's unharvested stalk, leaves, husks and cob).

In flask, beaker and bioreactor trials using a two-in-one step called "simultaneous saccharification and fermentation," *Clavispora* NRRL Y-50464 outperformed genetically engineered *Saccharomyces* yeast strains.

Highlights of results recently published in the *International Journal of Microbiology* include:

- an ethanol concentration (or, "titer") of 47 grams per liter (g/L) of purified cellulose within 72 hours—over the minimum required standard of 40 g/L for industrial use. What's more, *Clavispora* NRRL Y-50464 supplied its own beta-glucosidase, so none had to be added.
- an ethanol titer of 32 g/L of pre-treated corn stover and 25 g/L of rice straw within 48 and 36 hours, respectively. That's also a plus, given this represents the shortest time needed to complete cellulosic ethanol production from agricultural residues (leftover).
- better resistance to acetic acid (used in pre-treatments to liberate

sugars from lignocellulose) and tolerance to temperatures of up to 102 degrees Fahrenheit (39 degrees Celsius).

Liu said that although the *Clavispora* yeast doesn't ferment all types of simple sugars available from lignocellulose sources, it still has the potential to reduce the cost of producing ethanol. Additionally, the [genetic makeup](#) behind the [yeast](#)'s unique combination of traits could yield important clues to improving the use of other microbial strains in lignocellulose-based ethanol production systems. The key, Liu added, will be making critical process engineering improvements to the bioreactors now being used, including their ability to stir the cellulosic mix with high loads of fermentable solids.

More information: Z. Lewis Liu et al, Cellulosic Ethanol Production Using a Dual Functional Novel Yeast, *International Journal of Microbiology* (2022). [DOI: 10.1155/2022/7853935](https://doi.org/10.1155/2022/7853935)

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