

Displacing petroleum-derived butanol with plants

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As a chemical for industrial processes, butanol is used in everything from brake fluid, to paint thinners, to plastics. According to a University of Illinois researcher, butanol made from plant material could displace butanol made from petroleum, just not at the fuel pump.

"Yes you can drive your car around with 100 percent butanol, but butanol is much more valuable - about three times more valuable -- as a chemical than as a liquid fuel," said Hans Blaschek, microbiologist in the College of Agricultural, Consumer and Environmental Sciences at Illinois.

Blaschek said that butanol has all kinds of attributes that would make it a good candidate for liquid fuel—it burns cleaner, it has higher energy density than ethanol, but it's more expensive currently.

"It would displace petroleum and that's huge - clearly it could be used as a liquid fuel, but right now it's still too expensive to use that way. Right now it follows the price of propylene," Blaschek said.

He has been studying microorganisms that are used in fermentation processes for over 25 years. About 10 years ago, his lab at Illinois had a breakthrough with the development of a mutant strain of a soil bacterium called *Clostridium beijerinckii* that produces higher concentrations of butanol when added to a vat of plant byproduct.

Simply put -- what yeast is to the process used to create ethanol,

Clostridium beijerinckii is to the process that results in butanol.

Blaschek explained, "One of the beauties of *Clostridium*, is that unlike yeast that can only use six carbon sugars, this organism can use five or six carbon sugars, so you're not limited. You can use distiller's grains, biomass, pretty much anything that can be deconstructed to sugars and can be fermented. *Clostridium* eats both and it does it naturally. You don't have to engineer the organism like people have been doing for the last 20 years with yeast trying to get it to use five carbon sugars."

Because the mutant strain produces higher concentrations of butanol, it's the basis for Tetravita BioSciences, a local company that licensed the patented strain from the University of Illinois and is scaling up to use the over-productive strain on a large scale - the size of an ethanol plant.

"When we did the original study 10 years ago that resulted in the mutant strain, we didn't do it in a nice, careful way using sophisticated molecular biology. We did it using brute force and it worked. However, the problem with that approach is that you don't really know what genetic alterations caused the enhanced production."

Blaschek's most recent research on *Clostridium* was at the genetic level. "In 2004 we put a request in to the Department of Energy to sequence the parent strain," he said. "After we had access to the sequencing information, we were able to do the first global evaluation of the two strains - the one that over-produces butanol together with the parent strain -- to see what genetic alterations were responsible for this attribute."

In the lab, the two strains went through fermentation separately. Samples were taken during the course of the fermentation. The RNA was isolated and micro-array technology was used to tell how much RNA was present at a given time in the fermentation. The assumption is that if there is

more RNA, there's more protein. This was done for a series of 500 different genes. This analysis was used to look at the wild type alongside the mutant.

Blaschek found that the amount of RNA being produced for certain enzymes involved in butanol production was much greater in the mutant strain than in the wild type. There was also a difference in the ability of the mutant to make spores.

Blaschek said that the organism doesn't make any butanol until later in the fermentation process. So it has been thought that if you can prevent the organism from going into the next physiological state, which is sporulation, that you can keep it more or less producing butanol.

"The next step is to take that knowledge and produce a second generation strain by not using the brute force approach that I used earlier, but actually going in and very specifically making those genetic alterations in a targeted sort of way. You would take the wild strain and mutate the gene for the characteristic that you're interested in. And now that we have the sequence, we actually know where those genes are," he said.

The research comparing the two strains was published in the January 2009 issue of *Applied and Environmental Microbiology*.

Source: University of Illinois at Urbana-Champaign

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