Garbage bug may help lower the cost of biofuel

29 November 2012

One reason that biofuels are expensive to make is that the organisms used to ferment the biomass cannot make effective use of hemicellulose, the next most abundant cell wall component after cellulose. They convert only the glucose in the cellulose, thus using less than half of the available plant material.

"Here at the EBI and other places in the biofuel world, people are trying to engineer microbes that can use both," said University of Illinois microbiologist Isaac Cann. "Most of the time what they do is they take genes from different locations and try and stitch all of them together to create a pathway that will allow that microbe to use the other sugar."

Cann and Rod Mackie, also a U of I microbiologist, have been doing research at the Energy Biosciences Institute on an organism that they think could be used to solve this problem.

Mackie, a long-distance runner, found the microbe in the garbage dump of a canning plant while running in Hoopeston, Ill., in 1993. He noticed that the ground was literally bubbling with microbial activity and took samples. He and his son Kevin, who was in high school at the time, isolated microbes from the samples.

Among these was a bacterium that was later named *Caldanaerobius polysaccharolyticus*. "We found many exciting enzymes from this organism," said Cann, who joined the project when he came to Mackie's lab as a postdoctoral researcher.

Specifically, the bacterium contains all of the proteins and enzymes needed to break down xylan, which is the most common hemicellulose, and then to transport the fragments into the cell and metabolize them. All of the genes are located in a single cluster on the microbe's genome.

"So instead of taking a piece from here and from there and stitching them together, we can just take this part of the gene," Cann explained. "You can cut this and put it into another microbe."

On the surface of the cell, there is an enzyme that cuts the xylan into small pieces and a protein that binds to the pieces and brings them inside the cell. Enzymes within the cell metabolize the sugar.

The reason that this microbe, unlike most others used to make biofuels, is able to degrade xylan is that it has evolved an enzyme that allows it to remove the side chains, or decorations, that are part of xylan's structure. They hinder the degradation process by preventing complete accessibility of the enzymes to the sugar chain.

Once the side chains have been removed, another enzyme in the microbe breaks the sugar chain down into single sugars, or xylose. Other enzymes within the cell then metabolize the xylose.

Having the enzymes next to each other on the genome is convenient for scientists who are working on engineering microbes that can degrade both cellulose and hemicellulose. The cluster could be designed as a cassette and put into a microbe that normally degrades only cellulose.

Moreover, being next to each other allows them to work efficiently. "You have a set of enzymes that have co-evolved," Cann explained. "If they have co-evolved over millions of years, it means they have been fine-tuned to work together."

Another advantage of *Caldanaerobius polysaccharolyticus* is that it is a thermophilic bacterium, and its enzymes are resistant to temperatures as high as 70 degrees Celsius. Biofuel fermentation is usually done at 37 degrees Celsius, a temperature at which most microbes can survive. This means that the material in the fermentation vats is easily contaminated.

The next step for Cann and his collaborators is to develop techniques for transferring this gene cluster, which is quite large, into microbes.

More information: The research was recently published in the *Journal of Biological Chemistry* and is available at www.jbc.org/content/287/42/34946.full

Provided by University of Illinois at Urbana-Champaign