New microbial genetic system dissects biomass to biofuel conversion

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A research team at the DOE Great Lakes Bioenergy Research Center (GLBRC) has developed a powerful new tool that promises to unlock the secrets of biomass degradation, a critical step in the development of cost-effective cellulosic biofuels. The details of this method were published online on June 11 in the journal *Applied and Environmental Microbiology*.

Fulfilling the promise of cellulosic biofuels requires developing efficient strategies to extract sugar molecules in biomass polymers like cellulose. Microorganisms such as bacteria and fungi are capable of converting biomass to simple sugars, but historically have been difficult to study using genetic approaches.

A breakthrough by a team of University of Wisconsin-Madison researchers at the GLBRC has made it possible to perform genetic analysis on Cellvibrio japonicus, a promising bacterium that has long been known to convert biomass to sugars. Using a technique called vector integration, the team has developed a method to generate a mutation in any gene within the organism.

As a test of the technique, the team constructed a mutation that inactivated a key component of a protein complex called a Type II Secretion System, and the disruption of this system prevented the bacterium from efficiently converting biomass into sugars. This proves for the first time that Cellvibrio uses the Type II Secretion System to secrete key enzymes for breakdown of biomass polymerase, thus

providing key insight into how this bacterium obtains sugars from biomass.

"Realizing the promise of cellulosic biofuels requires identifying more efficient methods of releasing sugars from biomass", says GLBRC associate scientist David Keating, who led the team. "This new genetic method will allow us to understand how bacteria carry out this conversion, which should provide new avenues for improving the industrial process."

Plant cell wall deconstruction is a very complex process that requires a large number of enzymes, many with overlapping specificities, says Professor and Eminent Scholar in Bioenergy Harry Gilbert, of the University of Georgia's Complex Carbohydrate Research Center.

"As genetic systems for many bacteria that orchestrate this process have not been developed, the use of null mutations (inactivating specific genes) to explore the functional significance of specific enzymes has not been possible," says Gilbert. "Keating's group has provided the ability to do that — inactivate specific genes in Cellvibrio japonicus — which displays an extensive plant cell wall degrading apparatus. This enables you to ask critical biological questions about how the system is regulated and how the enzymes work together to degrade this hugely complex molecule. This is a substantial and important development in the field."

Provided by University of Wisconsin-Madison

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