Team explains the higher cellulolytic activity of a vital microorganism

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Researchers at the Energy Department's National Renewable Energy Laboratory (NREL) and the BioEnergy Science Center (BESC) say better understanding of a bacterium could lead to cheaper production of cellulosic ethanol and other advanced biofuels.

Their discovery was made during an investigation into the performance of *Clostridium thermocellum*. The scientists found the microorganism utilizes the common cellulase degradation mechanisms known today (free enzymes and scaffolded enzyme attached to the cell), and a new category of scaffolded enzymes not attached to the cell.

The discovery came as a surprise to the researchers and explains the superior performance of *C. thermocellum* on biomass. A paper reporting the potential for the bacterium, "Dramatic performance of *Clostridium Thermocellum* explained by its wide range of cellulase modalities," appears in the current issue of the journal *Science Advances*.

This anaerobic bacterium is a major candidate for the production of biofuels from biomass feedstocks because it already possesses both an external cellulase system and the internal metabolic pathways to convert biomass to ethanol. *C. thermocellum* is ubiquitous and has been isolated from soil, compost, herbivores, and hot springs.

"*C. thermocelum* can be revived from anywhere, no matter where you are, if biomass is present and the temperature is right, it will be there." said NREL scientist Yannick Bomble, who is the project leader and senior author of the paper.

C. thermocellum uses both a free-enzyme system and a tethered cellulosomal system (cellulosome) wherein carbohydrate active enzymes (CAZymes) are organized by primary and secondary scaffoldin proteins to generate large protein complexes attached to the bacterial cell wall. "These enzyme complexes are an amazing machinery," Bomble said. "They can include up to 63 biomass-degrading enzymes. One can think of a cellulosome as a nanoscale octopus wrapping and digesting cellulose microfibrils from all angles."

BESC researchers at NREL used newly published cloning strategies, enabled by a collaboration with Dartmouth College, to probe the importance of the primary and secondary scaffoldins of *C. thermocellum* using scaffoldin deletion strains. They found the scaffoldins were essential to the cell wall defibrillation mechanism used by C. thermocellum. Native cellulosomes are capable of creating or at least maintaining increased substrate surface area during deconstruction by splaying and dividing the biomass particles. This ability is completely lost with any modification of these cellulosomes, such as the removal of the primary or secondary scaffoldins.

These interesting observations were not the only discovery the researchers made. Using the same mutant strains as background, they also found a new type of enzyme assembly that is not tethered to the cell and allows the microorganism more freedom to explore for additional biomass or provides a redundancy in its cellulolytic system to assure a consistent source of sugars.

The findings have important implications for industry, and were fascinating for the scientists. "We are learning a lot about this microorganism, how it can thrive in almost any environments, and how it operates on biomass. However, we realize that there is still work to be done to bring it to its full potential. We are constantly working to improve its activity on biomass and increase renewable fuel yields," Bomble said.

"Our mission is to enable and indeed accelerate the emergence of the cellulosic biofuels enterprise through our fundamental research," said Paul Gilna, director of BESC. "*C. thermocellum* is recognized as one of the most effective cellulose-degrading bacteria in the biosphere, thus the discovery of this new mode of action represents significant progress in the scientific underpinnings of advanced approaches for biofuel production."

This discovery, enabled by the BioEnergy Science Center, will influence the strategies used to improve the cellulolytic activity of biomass degrading microbes going forward. Biomass conversion affects many areas of science, ranging from herbivore health and biofuels production to the dynamics of hot spring ecosystems.

"The multi-institutional nature of the BioEnergy Science Center allows impactful studies such as the one reported here," said Michael Himmel, one of the authors of the research paper and activity lead in the center.

More information: Dramatic performance of Clostridium thermocellum explained by its wide range of cellulase modalities, DOI: 10.1126/sciadv.1501254 , advances.sciencemag.org/content/2/2/e1501254

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