Method to customize microbes for better biofuel production

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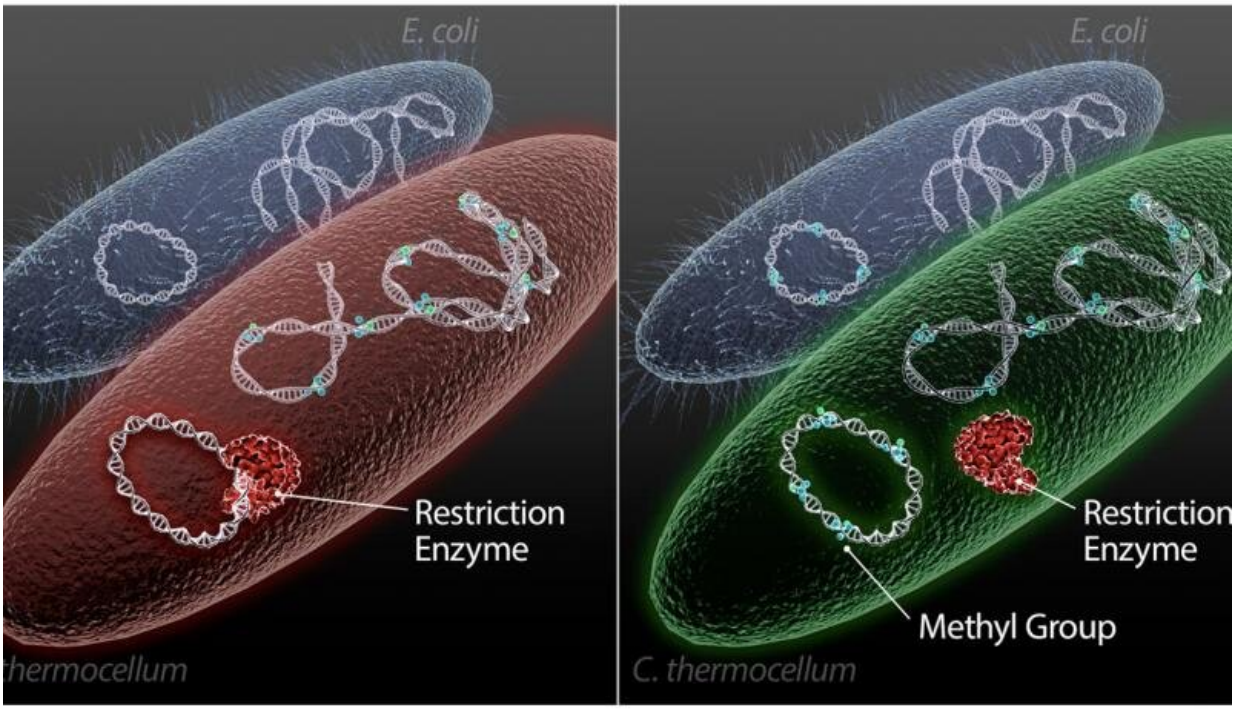

A new method uses E. coli to generate DNA with methylation patterns that target microbes recognize and accept as their own, facilitating customization of microbes for biofuels production. Credit: Oak Ridge National Laboratory

Scientists at the US Department of Energy's Oak Ridge National Laboratory have demonstrated a method to insert genes into a variety of

microorganisms that previously would not accept foreign DNA, with the goal of creating custom microbes to break down plants for bioenergy.

Microbes are the most abundant life forms on earth. They influence the growth of plants, digest food in the human gut, transform pollutants in the environment, and perform a host of other functions that affect everyday life.

Researchers at the DOE Center for Bioenergy Innovation (CBI) at ORNL are harnessing the power of microbes to turn non-food biomass like corn stalks, switchgrass and poplar into biofuels and bioproducts. To increase the efficiency of the conversion process, microbes are needed that can break down cellulose and ferment it into biofuels in a single set of reactions. Dubbed consolidated bioprocessing (CBP), this approach improves the economics of biofuels production.

Though the CBI team has demonstrated the feasibility of consolidated bioprocessing, they need better microbes to achieve greater yields of biofuels. The target: microbes that eat cellulose to produce desired fuels and thrive in high-temperature environments without oxygen.

Enhancing or introducing target traits in these more unusual microbes can be challenging. There are few tools available for engineering non-model microbes, and the organisms have developed defense mechanisms that can foil attempts to insert new genes.

Forging signatures

Meant to ward off viruses, these defense mechanisms guard microbes against unintentionally copying foreign DNA. To distinguish their own DNA from others, each microbe places a methyl group on a handful of specific DNA sequences. These methylated sequences are unique to the organism and act like a signature. Special enzymes called restriction

enzymes patrol the cell and chew up any DNA that lacks methyl groups on the signature sequences.

Guss and his team have demonstrated a way to leverage this defense system to coax microbes into accepting bioengineered DNA as their own.

Using two sequencing methods, the scientists first identified a microbe's signature sequences and the enzymes that methylate them. Then they expressed the enzymes, known as methyltransferases, in a common laboratory microbe, *E. coli*. With the right methyltransferases in place, *E. coli* are able to make copies of DNA with the expected methylation patterns, ensuring the target microbe would accept and use the new DNA.

The researchers recently published their method and the results of the experiment validating that the gene they inserted into Clostridium thermocellum ATCC27405—a CBP organism that has been challenging to transform—actually functions as anticipated. They have had similar success with ten other species and counting. These species were previously unamenable to genetic engineering.

Guss foresees many benefits of this rapid domestication method for applied and basic research, especially in identifying gene function. With this approach, scientists can remove or over express genes of interest in microbes to determine how that affects the organisms' traits. In addition to bioenergy, the method can be employed in biomedical and other fundamental research.

"What Adam and his team have done is to remove one of the major stumbling blocks to transforming these organisms," CBI Chief Scientist Brian Davison said. "This opens the door to take these microbes with really tough-to-replicate traits and be able to tune them to do more of

what we want, such as increasing biofuel yields."

More information: Lauren A. Riley et al. Rational development of transformation in Clostridium thermocellum ATCC 27405 via complete methylome analysis and evasion of native restriction–modification systems, *Journal of Industrial Microbiology & Biotechnology* (2019). DOI: 10.1007/s10295-019-02218-x

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