Engineers unveil ultra-efficient method for making high-value chemicals

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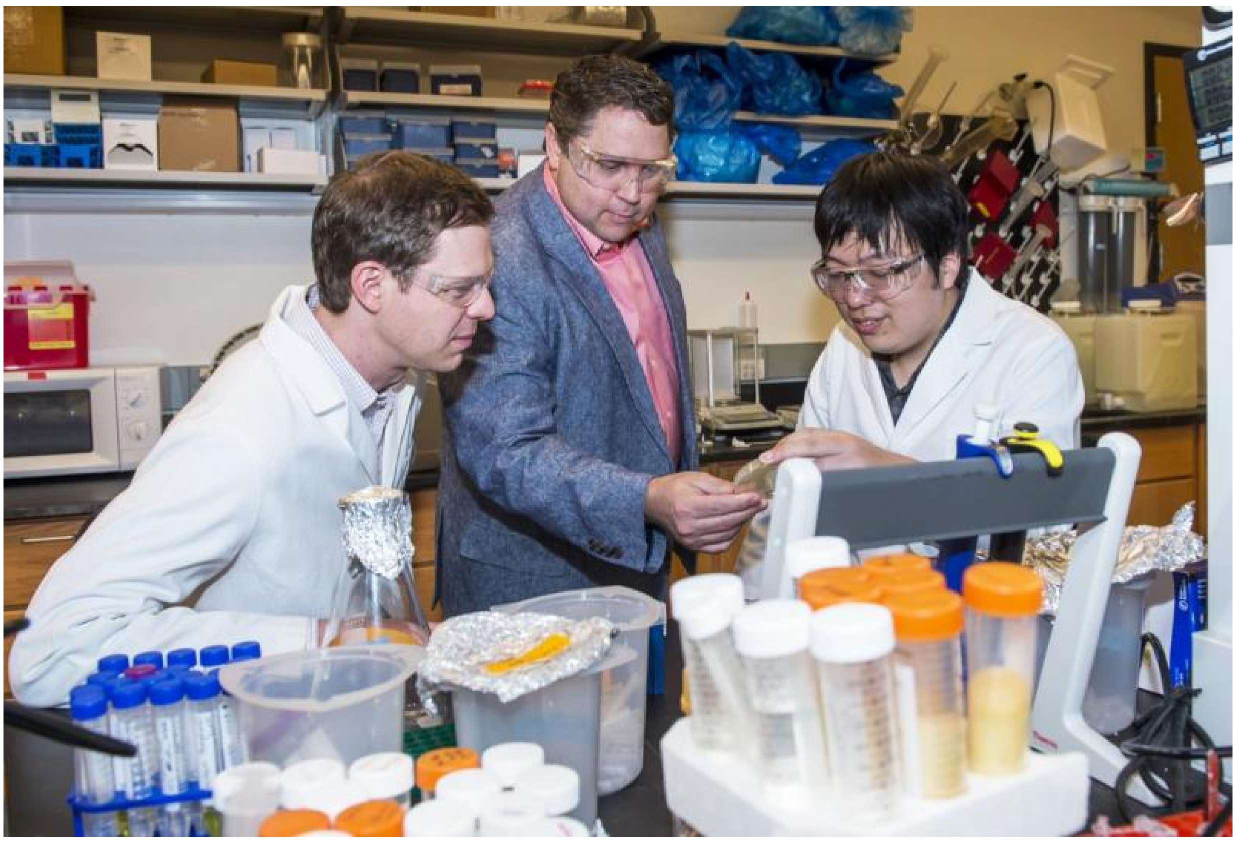

Rice researchers (from left) James Clomburg, Ramon Gonzalez and Seokjung Cheong have discovered a way to reprogram bacteria to use naturally occurring enzymes to quickly and efficiently produce high-value chemicals via new metabolic pathways unlike any found in nature. Credit: Tommy LaVergne/Rice University

Sometimes nature needs a helping hand, but the latest breakthrough from Rice University's Department of Chemical and Biomolecular Engineering might be more accurately described as a whole new arm.

The work from Rice metabolic engineer Ramon Gonzalez, who specializes in creating genetically engineered bacteria for the biotechnology industry, is described in this week's issue of *Nature Biotechnology*. Gonzalez's new bacteria use naturally occurring enzymes in ways that never appear in nature, and the result is a highly efficient system for quickly and efficiently producing high-value chemicals. Eight of the 18 chemicals described in the new study have not previously been produced via microbial fermentation.

"We have created a new metabolic pathway that doesn't exist in nature, and which doesn't interfere or have any cross talk with the organism's natural metabolism," said Gonzalez. "All of the components of our pathway—the individual steps and enzymes—do exist in nature. We are not tweaking any enzymes or creating any new enzymes for the paper. We are just creating a new pathway with existing enzymes."

A metabolic pathway is a series of chemical reactions that occur in stepwise fashion in a living cell. Cells often employ dozens of metabolic pathways simultaneously, and these pathways fall into three categories: Catabolic pathways are those that break down large molecules into small building blocks called metabolites; anabolic pathways are those that build up large molecules from metabolites; and central metabolic pathways produce both energy and precursor metabolites that cells thrive upon.

"Our goal was to design a pathway that builds complex and interesting molecules in a carbon- and energy-efficient manner, which is not something that is found in nature," Gonzalez said. "Catabolic pathways are efficient, but they break things down rather than build. Anabolic pathways build lots of interesting molecules, but they are largely inefficient, with exception of fermentative pathways, which are among the most carbon- and energy-efficient in all metabolic pathways, but they also produce some of the least interesting molecules.

"We created a hybrid pathway that combines some of the best features of these in terms of carbon- and energy-efficiency as well as in terms of the functionalized molecules that are produced," Gonzalez said. "If you look at the paper, you're going to see products with a lot of different stuff like branches, oxygen atoms, aromatic rings and other chemical functionalities. The products we've made include phenylalkanoic acids, hydroxyl acids and dicarboxylic acids of varying lengths, as well as more exotic products like tiglic acid and 2-methylpentanoic acid."

Each of the chemicals was produced by a strain of Escherichia coli that had been modified with a new metabolic pathway tailored to make that product. Though 18 such organisms and their products were featured in the study, Gonzalez said the method used to produce them could yield dozens more chemicals. He and study co-authors James Clomburg, a research scientist, and Seokjung Cheong, a graduate student, have identified hundreds of potential products that could be produced with the method.

Each of the metabolic pathways in the study encodes a four-step metabolic process. By substituting different enzymes into one or more of the steps, the process can be tailored to start with different precursor metabolites as well as to process metabolites in unique ways that result in a different final product.

"In biotechnology, people have traditionally looked to co-opt or optimize metabolic pathways that exist in nature," Clomburg said. "People almost always keep processes in the same domain, either catabolic or anabolic, where they originated. But anabolic processes in nature are rarely efficient, and there are often fixed limits to how efficient you can make an anabolic process."

Cheong said the team was able to improve the efficiency of its pathways by mixing and matching features from the anabolic and catabolic domains.

"By starting with a clean slate, we were able to incorporate some of the carbon- and energy-efficient processes from catabolic pathways and come up with unique new pathways that are extremely efficient," Cheong said.

More information: Seokjung Cheong et al. Energy- and carbon-efficient synthesis of functionalized small molecules in bacteria using non-decarboxylative Claisen condensation reactions, *Nature Biotechnology* (2016). DOI: 10.1038/nbt.3505

Provided by Rice University