To produce more food, scientists look to get more mileage out of plant enzymes

March 22 2021

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Enzymes play essential roles in the cells of every living thing, from bacteria, to plants to people. Some do their jobs a few times and fizzle out. Others can repeat a task hundreds of thousands of times before they quit.

Organisms put a lot of energy into replacing worn out enzymes, energy they could put into other processes. In plants grown for food, fuel, fiber or other purposes, longer lasting enzymes could translate into increased yields, according to Andrew Hanson, eminent scholar and professor in the UF/IFAS horticultural sciences department.

"Replacing enzymes is a huge energy cost to organisms, but no one had ever really asked, 'how long do enzymes last and what determines that?' If you want to improve enzymes' lifespans, you need to know which enzymes to target," said Hanson, lead author of a new study in which researchers present a new benchmark for evaluating the durability of any enzyme.

This benchmark, called Catalytic Cycles until Replacement, or CCR, is the first step toward improving enzyme longevity and one day producing more food, fuel and fiber for the world. The study is published in the journal *Proceedings of the National Academy of Sciences* and supported by a grant from the National Science Foundation.

To explain how CCR works, Hanson draws an analogy between the parts in a car and the enzymes in a cell.

Like parts in a car, enzymes perform a specific task over and over, which causes wear and tear. In cars, the manufacturer knows how many times a part can perform before it needs to be replaced. The CCR provides this kind of information on enzymes, telling bioengineers how many times an enzyme can do its job before it wears out.

"If you're bringing your car into the shop all the time to replace parts, that's a big investment and it's not very efficient. But plants we grow as crops, they spend a lot of energy on enzyme maintenance, which leaves less energy for growing the grain or other parts we harvest," Hanson said. "Many enzymes in plants could be improved, and with the CCR, we know where to start."

Hanson's lab has already begun working to improve one of these plant enzymes, THI4. This enzyme catalyzes a chemical reaction that makes thiamine, a B vitamin essential to many biological processes.

But THI4 is what could be called a "throw-away" enzyme, one that catalyzes the reaction once and then—poof—self-destructs.

"One possible solution is to find another enzyme that can also make thiamine but lasts longer than THI4," Hanson said.

Hanson's lab is looking to refashion processes like these using a technique called directed evolution.

An organism's genome is an instruction manual for making everything in that organism, including its enzymes. In plants, traditional breeding techniques can identify and manipulate the parts of a plant's genome that contain instructions for desirable traits. That involves cross breeding lots of individual plants and isolating those plants that have the genetic combination that results in the desired trait. However, because plants reproduce seasonally, this needle-in-a-haystack search can take years.

Directed evolution harnesses the natural processes of mutation and selection in bacteria or yeast to improve genes containing instructions for enzymes or other proteins at a much faster rate than could ever occur in a plant. The improved genes can then be transferred to a plant by using technologies like CRISPR to edit the plant's genome, and, as a result, improve the enzymes the plant makes—leading to a more productive plant.

More information: Andrew D. Hanson el al., "The number of catalytic cycles in an enzyme's lifetime and why it matters to metabolic engineering," *PNAS* (2021). www.pnas.org/cgi/doi/10.1073/pnas.2023348118

Provided by University of Florida

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