Genetic sequence allows photosynthetic organisms to stably produce ethylene

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Liquid cyanobacteria culture is grown in Jianping Yu's lab at NREL to advance sustainable ethylene production. Credit: Dennis Schroeder, NREL

For decades engineers have dreamed of programming organisms to sustainably produce ethylene, a chemical that is nicknamed "the king of petrochemicals" for its importance in plastics. Now, one hopeful pathway to this petrochemical is nearing reality, via a photosynthetic bacterium that is genetically specialized to turn sunlight and carbon dioxide (CO_2) into ethylene. But before industry can load up on tanks of living green liquid, researchers are first overcoming some metabolic barriers around ethylene production.

A cross-institution research team led by the National Renewable Energy Laboratory (NREL) made important progress toward deciphering the photosynthetic enzyme pathway. In a *Nature Communications* article titled "A guanidine-degrading enzyme controls stability of ethylene-producing cyanobacteria," the researchers report their discovery and demonstration that a certain gene can induce stability in bacteria that produce ethylene. Their discovery is a welcome breakthrough, as past attempts at employing this ethylene pathway had led to genetic instability in the bacteria.

"Until now, a major roadblock to photosynthetic ethylene production has come from the organism itself—it creates a toxic byproduct alongside the ethylene," said Jianping Yu, an NREL author on the paper. "With this work, we now know that the toxic byproduct can be dealt with using a genetic technique."

Guanidine: An unwanted guest in solar-driven ethylene production

The researchers' intended approach is straightforward: Hijack the gene to produce ethylene from a common plant pathogen (Pseudomonas Syringae, a bacterium that causes brown spots on leaves), and introduce that gene into a cyanobacteria, which use photosynthesis for energy. If everything works correctly, the cyanobacteria will then convert solar radiation and CO_2 into ethylene; indeed, more efficiently than any other biological pathway. But instead, the cyanobacteria slowly died; the researchers showed that the introduced gene pathway also produces guanidine, a toxin that creates genetic instabilities in cyanobacteria.

"Our objective is to understand the source of guanidine toxicity in this pathway and how cells can thwart it. To that end, we now have a pretty compelling approach," Yu said.

Guanidine causes a disorder of pigment metabolism in cyanobacterial cells—an obviously bad byproduct when the cells' purpose is to use its pigment to harvest light. Fortunately, a particular cyanobacteria beloved by scientists—Synechocystis 6803—can degrade guanidine. The trick, then, is to capture that genetic mechanism and reinsert it into other cyanobacteria cells. In other words, introduce another gene that stabilizes the first, and leads to unobstructed ethylene production.

Genomic stability for enduring ethylene yields

The researchers hypothesized that a specific gene in Synechocystis 6803 was at play in the guanidine degradation, based on the gene's higher expression in the ethylene-producing strain and its sequence similarity to other known guanidine-related metabolic machinery. That guess was affirmed when the researchers knocked the gene out from the cyanobacterium and observed the cells' decline when exposed to guanidine. To further validate the gene's role in guanidine degradation, the researchers then added the gene to another species.

In the other cyanobacterium—Synechococcus 7942, another favorite species that scientists engineered—the research team assessed whether the gene conferred the same ability to degrade guanidine. Sure enough, just as in the first species, the modified cyanobacterium could metabolize the guanidine, thereby preventing genetic problems and enabling persistent ethylene production. For both organisms, the gene effectively neutralized guanidine, converting the toxic chemical into harmless urea and ammonia.

Opportunity for a clean chemical alternative

Biologically produced ethylene is a double-win for clean energy—it recycles CO_2 and displaces fossil-based feedstocks that industry currently depends on. Compared to other biological pathways, which use plant biomass as a starting material, the method pursued in this work is fueled directly by the sun, making it potentially more energetically favorable.

Industrial application to decarbonize the chemical industry is tantalizing; hope persists for producing PVC pipes for clean water, and even in Mars colonization. This work shows a possibility to scale up bioethylene production by clearing certain biological barriers. Future research could create even more efficient guanidine-degrading enzymes, possibly through evolution of the same gene described in this study. For now, the team's work advances the knowledge of guanidine metabolism in nature and demonstrates a functional approach for enhancing ethylene production.

More information: Bo Wang et al, A guanidine-degrading enzyme controls genomic stability of ethylene-producing cyanobacteria, *Nature Communications* (2021). DOI: 10.1038/s41467-021-25369-x

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