Decades-old conclusion about energy-making pathway of cyanobacteria is corrected

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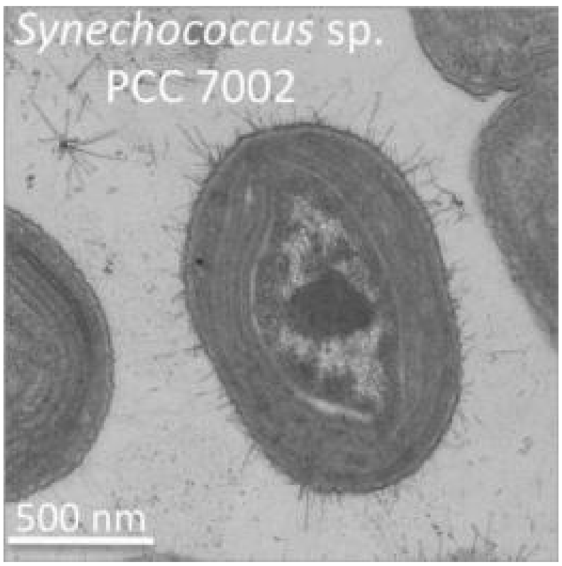


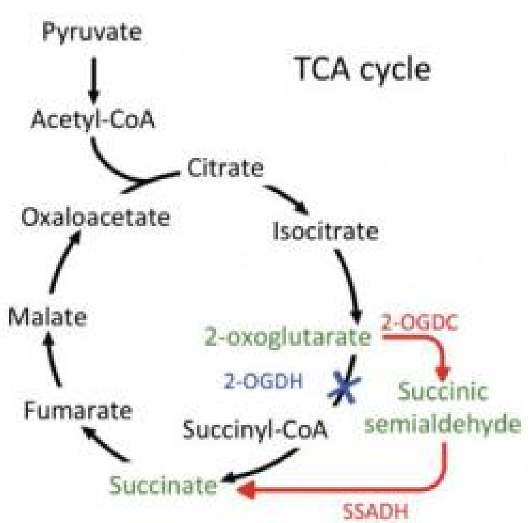
Research expected to help scientists to discover new ways of genetically engineering bacteria to manufacture biofuels overturns a generally accepted 44-year-old assumption about how certain kinds of bacteria make energy and synthesize cell materials. Donald Bryant, at Penn State University, performed biochemical and genetic analyses on a cyanobacterium called Synechococcus sp. PCC 7002, scouring its genome for genes that might be responsible for making alternative energy-cycle enzymes.With this new understaning of how cyanobacteria make energy, it might be possible to genetically engineer a cyanobacterial strain to synthesize 1,3-butanediol -- an organic compound that is the precursor for making not only biofuels but also plastics. Credit: Bryant lab, Penn State University

A generally accepted 44-year-old assumption about how certain kinds of bacteria make energy and synthesize cell materials has been shown to be incorrect by a team of scientists led by Donald Bryant, the Ernest C. Pollard Professor of Biotechnology at Penn State University and a research professor in the Department of Chemistry and Biochemistry at Montana State University.

The research, which will be published in the journal *Science* on 16 December 2011, is expected to help scientists discover new ways of genetically engineering bacteria to manufacture biofuels -- energy-rich compounds derived from biological sources. Many textbooks, which cite the 44-year-old interpretation as fact, likely will be revised as a result of the new discovery.

Bryant explained that, in 1967, two groups of researchers concluded that an important energy-making cycle was incomplete in cyanobacteria -- photosynthetic bacteria formerly known as blue-green algae. This energy-producing cycle -- known as the tricarboxylic acid (TCA) cycle or the Krebs cycle -- includes a series of chemical reactions that are used for metabolism by most forms of life, including bacteria, molds, protozoa, and animals. This series of chemical reactions eventually leads to the production of ATP -- molecules responsible for providing energy for cell metabolism. "During studies 44 years ago, researchers concluded that cyanobacteria were missing an essential enzyme of the metabolic pathway that is found in most other life forms," Bryant explained. "They concluded that cyanobacteria lacked the ability to make one enzyme, called 2-oxoglutarate dehydrogenase, and that this missing enzyme rendered the bacteria unable to produce a compound -- called succinyl-coenzyme A -- for the next step in the TCA cycle. The absence of this reaction was assumed to render the organisms unable to oxidize metabolites for energy production, although they could still use the remaining TCA-cycle reactions to produce substrates for biosynthetic reactions. As it turns out, the researchers just weren't looking hard enough, so there was more work to be done."

Bryant suspected that the decades-old finding needed to be re-evaluated with a fresh set of eyes and new scientific tools. He explained that, after researchers in the 1960s concluded that cyanobacteria had an incomplete TCA cycle, that false assumption was compounded by later researchers who used modern genomics-research methods to confirm it. "One idea we had was that the 1967 hypothesis never was corrected because modern genome-annotation methods were partly to blame," Bryant said. "Computer algorithms are used to search for strings of genetic code to identify genes. Sometimes important genes simply can be missed because of matching errors, which occur when very similar genes have very different functions. So if researchers don't use biochemical methods to validate computer-identified gene functions, they run the risk of making premature and often incorrect conclusions about what's there and what's not there."



Research expected to help scientists to discover new ways of genetically engineering bacteria to manufacture biofuels overturns a generally accepted 44-year-old assumption about how certain kinds of bacteria make energy and synthesize cell materials. Researchers in the 1960s had concluded that cyanobacteria had an incomplete tricarboxylic acid (TCA) cycle. The TCA cycle includes a series of chemical reactions that are used for metabolism by most forms of life, including bacteria, molds, protozoa, and animals. With this new understaning of how cyanobacteria make energy, it might be possible to genetically engineer a cyanobacterial strain to synthesize 1,3-butanediol -- an organic compound that is the precursor for making not only biofuels but also plastics. Credit: Bryant lab, Penn State Unviersity

To re-test the 1967 hypothesis, the team performed new biochemical and genetic analyses on a cyanobacterium called Synechococcus sp. PCC 7002, scouring its genome for genes that might be responsible for making alternative energy-cycle enzymes. The scientists discovered that Synechococcus indeed had genes that coded for one important alternative enzyme, succinic semialdehyde dehydrogenase, and that adjacent to the gene for this enzyme was a misidentified gene that subsequently was shown to encode a novel enzyme, 2-oxo-glutarate decarboxylase. "As it turns out, these two enzymes work together to complete the TCA cycle in a slightly different way," Bryant said. "That is, rather than making 2-oxoglutarate dehydrogenase, these bacteria produce both 2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase. That combination of enzymes allows these organisms to move to the next intermediate -- succinate -- and to complete the TCA cycle." Bryant also said that his team found that the genes coding for the two enzymes are present in all cyanobacterial genomes except those of a few marine species. Bryant's co-author on the Science paper is Shuyi Zhang, a graduate student in the Department of Biochemistry and Molecular Biology at Penn State.

Bryant hopes to use the findings of his research to investigate new ways of producing biofuels. "Now that we understand better how cyanobacteria make energy, it might be possible to genetically engineer a cyanobacterial strain to synthesize 1,3-butanediol -- an organic compound that is the precursor for making not just biofuels but also plastics," Bryant said.

Bryant also said that his team's discoveries about cyanobacteria show how science is an ever-evolving process, and that firm conclusions never should be drawn from studies with negative results. "Sadly, the conclusion that cyanobacteria have an incomplete TCA cycle is written into many textbooks as fact, simply because the research teams in 1967 misinterpreted their failure to find a particular enzyme," Bryant said. "But in science there is never really an end. There always is something new to discover."

Provided by Pennsylvania State University

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