Cyanobacterium demonstrates promise for biotechnology feedstock production

April 17 2012

Harvard Medical School researchers have engineered a photosynthetic cyanobacterium to boost sugar production, as a first step towards potential commercial production of biofuels and other biotechnologically and industrially useful carbon compounds. As feedstock producers, cyanobacteria have advantages over plants, particularly land plants. They need little fertilizer. They don't compete with food crops, because they can grow on marginal land. At commercial scale, the engineered cyanobacteria could potentially produce five times more sugar per acre than traditional crops, including sugarcane, says first author Daniel Ducat. The research is published in the April *Applied and Environmental Microbiology*.

Cyanobacteria were likely candidates for feedstock production because many freshwater species accumulate sucrose when subjected to salty environments, says Ducat, who is a postdoctoral researcher in Pamela Silver's laboratory at the Harvard Medical School. They do this to mitigate osmotic pressure, which otherwise would dehydrate them, he explains. "We hypothesized that this natural defense mechanism could be employed as a method to continuously produce sugar."

But to maintain continuous sugar production, it was necessary to provide a mechanism to continuously expel the sugar. Mechanisms for moving ions and chemical compounds in or out of cells, against osmotic gradients abound among bacteria. Ducat et al. chose a sucrose permease, which is used by other bacteria to scavenge sucrose from the environment. Since the chemical gradients between the cell and the environment are reversed in cyanobacteria, “we hypothesized that this same transporter might move sucrose out of the photosynthesizing bacteria,” says Ducat.

Everything worked as expected, only better. The cyanobacteria expressing the sucrose transporter expelled sucrose at a constant rate so long as the cells were illuminated to provide energy for photosynthesis. Serendipitously, the rate of photosynthesis in the sugar-exporting cyanobacteria—which belong to the freshwater species Synechococcus elongates—was actually higher than normal. “They display more activity in the enzymes involved in harvesting sunlight—specifically the water-splitting complex, photosystem II—and are capable of fixing carbon dioxide at higher rates than those cyanobacteria not exporting sucrose,” says Ducat.

Furthermore, “We found that the levels of sucrose exported in these cyanobacteria could be modulated by both the concentration of salt in the culture and the genetic background of the cyanobacteria,” says Ducat.

“Our results provide good proof-of-principle that cyanobacterial cultures could be used to produce biotechnology feedstocks with great efficiency,” says Ducat. The researchers also showed that the sugars could support the growth of yeast, organisms used to produce biofuels and other valuable compounds. “Therefore, the sugars produced by cyanobacteria could be used by other microbes without the need to extensively process them,” says Ducat—if the process can be scaled up.

That “if” is not inconsequential, says Ducat. “One of the major problems that some earlier scale-up efforts ran into when attempting to culture open raceways of algae were competing species of microbes and algae predators,” he says. An alternative is to grow cyanobacteria in a semi-enclosed reactor. Cost then becomes an issue, and “there aren’t a lot of great examples of large, inexpensive, fully enclosed photobioreactors," he says.

But if scale-up can be accomplished, the much greater efficiency of production for water-borne organisms is not all that surprising, especially to Ducat's Harvard University colleague, forestry professor Michele Holbrook. In an article in the Harvard University alumni publication, Colloquy, several years ago, Holbrook explained that land-based photosynthesis seems wildly improbable when one examines the numbers. The concentration of carbon dioxide in the atmosphere, 3.8 hundredths of a percent, is far lower than in water. A plant has to hold huge quantities of air inside its leaves in order to obtain adequate CO2, but the extensive surfaces it uses for absorbing CO2 lose water fast, she told Colloquy. Thus, roughly 500 water molecules must cycle through the plant for every carbon dioxide that gets captured. "If I turned the mass of my body into sunflower leaves, I'd have to drink two liters every 30 seconds," she said.

The results of this experiment raise unanticipated scientific questions, says Ducat. One would think that removal of the sucrose in the engineered cells would render them less fit, and thus less productive—less able to produce more sugar as well as cell biomass. The fact that they can boost overall productivity suggests that wild-type cells of this species do not naturally fix carbon as rapidly as they are able. Understanding the mechanisms behind this "may pave the way towards improving photosynthetic efficiencies generally," says Ducat. "We are following up on the mechanisms that these cyanobacteria use to sense and upregulate their photosynthetic activity.

More information: D.C. Ducat, et al., 2012. Rerouting carbon flux to enhance photosynthetic productivity. *Appl. Environ. Microbiol.* 8:2660-2668. doi:10.1128/AEM.07901-11