Champion hydrogen-producing microbe

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Research scientist Jana Stöckel, Ph.D., and postdoctoral fellow Anindita Banerjee, Ph.D., examine cultures of algae in one of the environmental chambers in Himadri Pakrasi's lab at Washington University in St. Louis. The two have recently been studying a strain of cyanobacteria (once considered algae) that produces copious amounts of hydrogen gas as a side effect of fixing nitrogen. Credit: Whitney Curtis/WUSTL

Inside a small cabinet the size of a dorm refrigerator in one of Himadri B. Pakrasi's labs, a blue-green soup percolates in thick glass bottles under the cool light of red, blue and green LEDS.

This isn't just any soup, however. It is a soup of champions.

The soup is colored by a strain of blue-green bacteria that bubble off roughly 10 times the hydrogen gas produced by their nearest competitors—in part because of their unique genetic endowment but also in part because of tricks the scientists have played on their metabolism.

Hydrogen gas can be produced by microbes that have enzymes called hydrogenases that take two hydrogen ions and bind them together. Although the soup microbes have hydrogenases, most of the hydrogen they evolve is a byproduct instead of an exceptionally efficient nitrogenase, an enzyme that converts the nitrogen in air to a nitrogen-containing molecule the microbes can use.

The microbe's gas-producing feat is described in December 14,2010 issue of the online journal *Nature Communications*.

Biohydrogen, like that bubbling up from the microbial soup, is one of the most appealing renewable energy fuels. Produced by splitting water with energy from the sun, it releases mostly water when it burns. It's hard to get any cleaner than that.

The strain growing in the Roux bottles in the cabinet, called *Cyanothece* 51142 was originally found in the Gulf of Mexico by Louis A. Sherman of Purdue University, one of the article's authors. Its genes were sequenced in 2008 at the Genome Sequencing Center at the School of Medicine.

Cyanothece 51142 may be new to science, but cyanobacteria, the group of organisms to which it belongs, have existed for at least 2.5 billion years, says Pakrasi, PhD, the George William and Irene Koechig Freiberg professor of biology in Arts & Sciences, and professor of energy in the School of Engineering. These ancient organisms have had to survive a wide variety of chemical environments and have the metabolic tricks to show for it.

All cyanobacteria have the ability to fix carbon from the atmosphere, stuffing it away in starch or glycogen, but *Cyanothece* is among the rarer strains that can also fix nitrogen, converting atmospheric nitrogen to ammonia and eventually to larger nitrogen-rich molecules.

Because it can fix both carbon and nitrogen, when conditions warrant *Cyanothece* can survive on air, water and sunlight alone. It is about as self-reliant an organism as it is possible to be.

There is one catch. Nitrogenase is very sensitive to oxygen and so carbon fixing (photosynthesis), which produces oxygen as a byproduct, has to separated from nitrogen-fixing in some way.

Himadri Pakrasi, PhD, the George William and Irene Koechig Freiberg professor of biology at Washington University in St. Louis, and research scientist Jana Stöckel, with a culture of *Chorella vulgaris*, otherwise known as green algae. This algae has talents of its own but hydrogen production is not among them. Credit: Whitney Curtis/WUSTL

Cyanothece accomplishes this by time division; it has an internal biological clock that establishes a circadian rhythm. (Cyanobacteria are the only prokaryotes (organisms without nuclei) that have a clock.)

So *Cyanothece* fixes carbon glycogen molecules during the day, producing oxygen as a byproduct, and it fixes nitrogen in ammonia during the night, producing hydrogen as a byproduct. For every nitrogen molecule that's fixed, says Pakrasi, one hydrogen molecule is produced.

Each half of the cycle powers the other. The glycogen produced in the day is consumed in the energy intensive process of fixing nitrogen at night. The fixed nitrogen produced at night is used to make nitrogen-containing proteins during the day.

Pakrasi, who is also the director of I-CARES, the International Center for Advanced Renewable Energy and Sustainability, calls the microbes biobatteries because they store daytime energy for use at night and nighttime energy for use in the day.

The separation in time prevents the two metabolic processes from competing with one another. At night the bacteria begin to metabolize the glycogen (or respire).
Quickly consuming intracellular oxygen, respiration creates the oxygen-free or anoxic conditions inside the bacteria the nitrogenase needs to do its work.

Cyanothece's clock is set by the environmental cue of changing light levels. But once entrained by the day/night cycle, the clock continues to run even in the absence of the cues. Just as a prisoner kept in solitary confinement will maintain a roughly 24-hour sleep/wake cycle, *Cyanothece* will continue to fix nitrogen even if it is incubated under continuous light.

Cyanothece 51142 may soon populate the giant remotely controlled bioreactors (the glowing containers) in the Advanced Coal and Energy Research Facility that just began operation at Washington University in St. Louis. Here Richard L. Axelbaum, professor of energy, environmental and chemical engineering, explains the facility to international visitors. Credit: Mary Butkus/WUSTL

As Pakrasi puts it, the entrained microbes are still experiencing "subjective dark" for 12 hours of the day.

More strangely, entrained *Cyanothece* incubated under continuous light evolve more hydrogen than those cycling between light and dark. This is probably because the energy in light somehow fuels the energy-intensive nitrogenase reaction, says Anindita Bandyopadhyay, PhD, a postdoctoral fellow in Pakrasi's lab. The scientists are still trying to understand exactly why this happens.

In addition to keeping the microbes awake all night, the scientists have another trick up their lab coat sleeves. *Cyanothece* can survive on the starvation diet of sunlight and air but adaptable microbe that it is, it can also live on carbon-containing molecules or on a mix of sunlight and carbon-containing molecules.

The scientists found that the microbes produced more hydrogen if they were grown in cultures that contained glycerol, a colorless, sweet-tasting molecule that is frequently used as a food additive.

The additional carbon in the glycerol revs up the nitrogenase to meet the increased demand for nitrogen in the cells, Pakrasi says. And the more active the nitrogenase, the more hydrogen is produced.

Despite journalistic hype, Pakrasi warns, hydrogen is not the fuel of tomorrow. It's hard to transport and its energy density is too low. The fuel tank for a semi-trailer powered by hydrogen would take up half the trailer, he says.

What intrigues him about the microbes is not their utility but rather their ingenuity. Their unique metabolism gives them the ability to produce hydrogen, a clean fuel, while disposing of two wasteproducts: glycerol, a copious byproduct of biodiesel production, and carbon dioxide, a waste product from coal-fired power plants. "They give you a lot of bang for your buck," he says.

Cyanothece may soon be moving house—from cramped flasks in Pakrasi's lab to the giant bioreactors in Washington University's Advanced Coal and Energy Facility. There scientists will be able to monitor their every metabolic move as they feast on carbon-dioxide-rich flue gas from the site's combuster and bubble up hydrogen.

Provided by Washington University in St. Louis

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