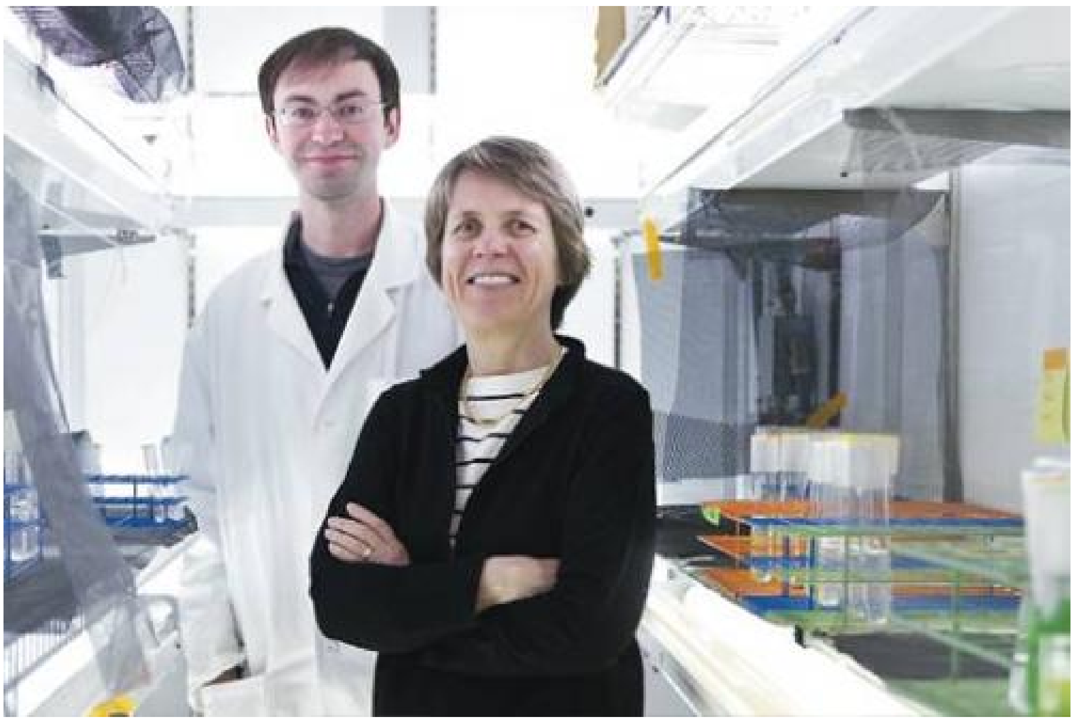
Ocean microbes display a hidden talent: Releasing countless tiny lipid-filled sacs

July 8 2014, by Nancy W. Stauffer



Steven Biller (left) and Sallie "Penny" Chisholm have discovered that abundant ocean-dwelling bacteria continually release tiny, never-before-observed spherical structures that contain lipids — a finding that could one day lead to new approaches for manufacturing biofuels. Credit: Stuart Darsch

In the search for a renewable energy source, systems using algae look like a good bet. Algae can grow quickly and in high concentrations in areas unsuitable for agriculture; and as they grow, they accumulate large quantities of lipids, carbon-containing molecules that can be extracted and converted into biodiesel and other energy-rich fuels. However, after three decades of work, commercially viable production of biofuels from algae hasn't been achieved, in part because the processes needed to break apart the algae and recover the lipids are costly and energy-intensive.

Another option is to use bacteria. For the past 25 years, Sallie (Penny) Chisholm, the Lee and Geraldine Martin Professor of Environmental Studies, has been studying Prochlorococcus, an ocean-dwelling bacterium that she calls "a pretty spectacular organism." Of all organisms that perform photosynthesis, this single-celled bacterium is both the most abundant and the smallest—less than 1 micron in diameter. It accounts for fully 10 percent of all photosynthesis on Earth and forms the base of the ocean food chain. It also has the smallest genome of any known photosynthetic cell. "Three billion years of evolution has streamlined its genome, and it now contains the least amount of information that can make biomass from solar energy and carbon dioxide," says Chisholm, who has a joint appointment in civil and environmental engineering (CEE) and biology. "It makes sense that we try to understand it—inspired by its simplicity—and see if we can use this understanding to help us design microorganisms that efficiently produce biofuels directly from sunlight."

In 2010, Chisholm's much-studied bacterium delivered a surprise: As it grows, it naturally releases small, spherical, membrane-bound vesicles containing fatty oils related to those that make algae so appealing. This was a serendipitous discovery. In 2008, Chisholm's group needed some images of Prochlorococcus for a publication. Using a scanning electron microscope, then-graduate-student Anne Thompson PhD '10 took the images—and they showed small spheres near the surfaces of the Prochlorococcus cells. The spheres remained a mystery to the ocean biologists until 2010, when Steven Biller joined Chisholm's group as a postdoctoral associate in CEE. Based on his work with soil bacteria, he proposed—and subsequently confirmed—that the spheres are lipidbound vesicles.

That finding is remarkable for two reasons. While many species are known to release vesicles, the behavior has never before been observed in a marine organism—and it could significantly change today's understanding of marine ecosystems, including their influence on the global carbon cycle. "Prochlorococcus is making organic carbon from sunlight and then packaging it up and releasing it into the seawater around it," says Chisholm. "What we need to figure out now is, Why and how? And what role do these vesicles play in ocean food webs and the ocean carbon cycle?"

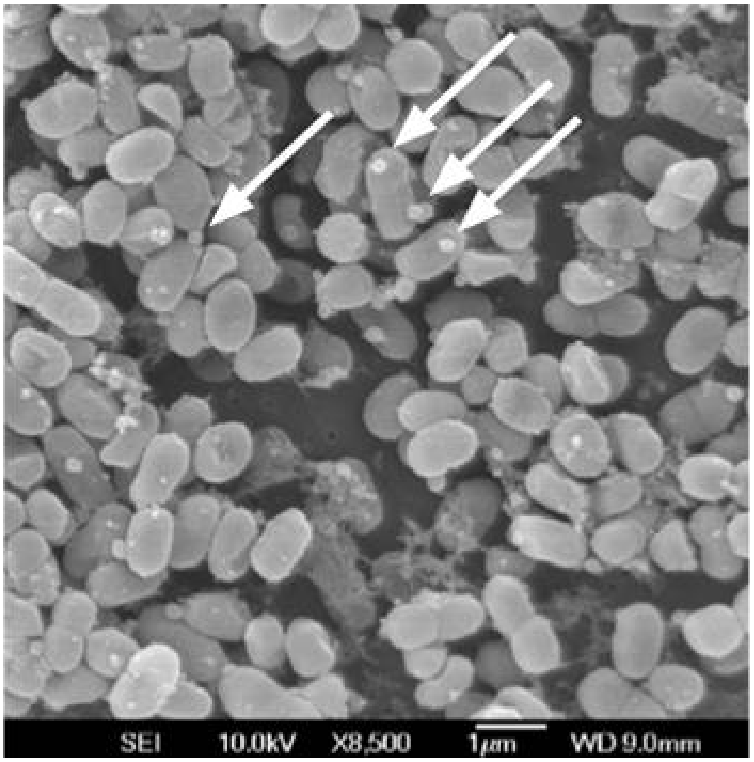
Equally surprising, this is the first observation of vesicle release in an organism that performs photosynthesis. The implications for industrial use—including biofuels production—are significant. Given just sunlight, carbon dioxide, and water, Prochlorococcus would continually release lipid-containing vesicles, which could be collected without disturbing the growing bacteria. "With algae, retrieving the lipids requires destroying one batch of cells and starting with a new batch," says Biller. "With Prochlorococcus, it could be a 'continuous culture.'"

Technical challenges, new insights

Chisholm stresses that such commercial applications are "way down the road." For now, research in her lab focuses on developing a fundamental understanding of the newly observed behavior. For example, how often does a Prochlorococcus cell release vesicles? How many does it release? And what's inside them?

To answer those questions, Biller overcame a series of technical challenges. First he developed improved methods of culturing large quantities of Prochlorococcus cells. Then he designed techniques for filtering off the vesicles and concentrating and purifying them—while keeping them intact. But his biggest problem was how to count the individual vesicles. Standard methods of counting particles don't provide sufficient resolution to look at the vesicles, which are less than 100 nm in diameter. After some trial and error, Biller was successful in adapting recent advances in nanoparticle analysis techniques to studying these tiny bacterially derived structures.

Using his new approaches, he determined that vesicles are present in large concentrations in growing cultures. Indeed, they outnumber the Prochlorococcus cells themselves—in some cases by a factor of 10. They are generated by strains of Prochlorococcus that grow in bright light (such as near the ocean surface) as well as in dimmer light (typical of the deep ocean). Vesicles appear to be produced continually during some phases of cell growth, and they are stable under laboratory conditions: Over the course of two weeks, the size and concentration of vesicles in a laboratory culture remained essentially unchanged. Finally, the vesicles contain not only lipids but also DNA, RNA, and a diverse set of proteins.



This scanning electron micrograph shows cells of a lab-cultured strain of Prochlorococcus plus small, spherical vesicles (indicated by arrows), which are released by the cells as they grow. This is the first time vesicle formation and release have been detected in a marine organism or in an organism that performs photosynthesis. The vesicles contain DNA, RNA, a variety of proteins, and lipids — molecules that potentially could be used to produce biofuels.

Unfortunately, the lipids in the vesicles from Prochlorococcus are not the optimal kind for making biofuels, notes Biller. "But because of its simple genome, it's a good model for us to use in exploring the mechanisms that control the formation and extrusion of vesicles and determine their content," he says. "Once we understand how it works, that mechanism could eventually be utilized in more robust and fast-growing organisms, and the contents of the vesicles could be manipulated."

Fieldwork expands the options

Based on their laboratory data, the researchers estimated that Prochlorococcus worldwide could release on the order of 1,027 vesicles per day—a significant contribution to the marine ecosystem. But many factors could influence vesicle production in the wild, so the team decided to take direct measurements. They collected hundreds of liters of seawater in two locations: the nutrient-rich coastal waters of Vineyard Sound in Massachusetts and the nutrient-sparse waters of the Sargasso Sea near Bermuda. They used their laboratory techniques—scaled up to handle larger volumes of water—to test the samples on board research vessels. As with their lab cultures, they found numerous vesicles in the samples from both types of ocean environments. And their analyses showed that the vesicles contained DNA from many kinds of bacteria—not just Prochlorococcus.

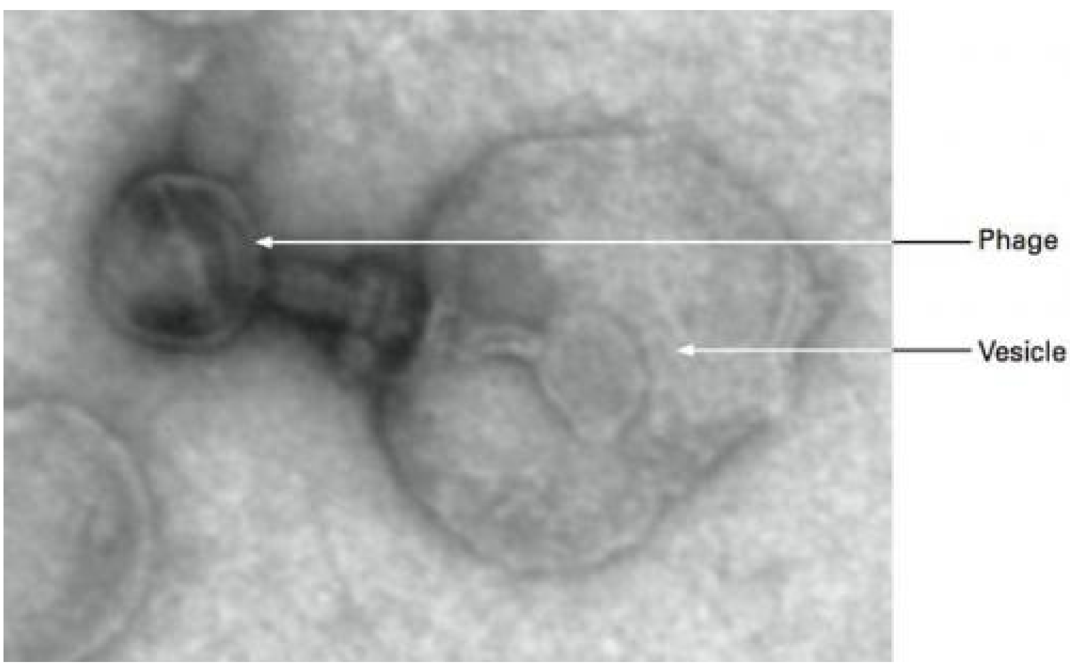
That finding potentially extends vesicle production to organisms that are ubiquitous in ocean systems extending from pole to pole. "This adds a whole new dimension to marine microbial ecosystems that we hadn't realized was there," says Biller. "And while Prochlorococcus was our entry point into this concept for biofuels production, it looks like there may be applications to many other organisms."

Vesicles as nutrients for other bacteria

These curves show the growth of a marine heterotroph—a nonphotosynthetic organism—in three laboratory cultures. One culture includes a mixture of organic carbon compounds ("+organic carbon mix"); another includes only added Prochlorococcus vesicles ("+vesicles"); and the last has no source of fixed carbon ("control"). Optical density, or OD, is a common measure used for estimating cell concentrations in liquid cultures. The data show that the vesicles alone provide enough nourishment for the cells to increase in number over 50 hours. Prochlorococcus thus appears to facilitate the growth of heterotrophs—and in return, the heterotrophs may protect Prochlorococcus by neutralizing toxic compounds that would harm it.

Wasteful behavior?

An intriguing question is why Prochlorococcus would make and release vesicles. Jettisoning their hard-earned organic carbon seems inconsistent with the need for this streamlined organism to make efficient use of scarce resources. What function could the vesicles serve? Biller and Chisholm don't have an answer to that question, but they've come up with several hypotheses—ideas with potential impacts on both understanding marine ecosystems and developing commercial-scale biofuels systems.



Electron micrograph of a phage attached to a vesicle. The shortened tail of the phage suggests that it has injected its DNA, rendering it unable to infect again. The surface of each vesicle includes protein receptors from its parent cell that serve as a target for phage. The vesicles thus act as a decoy, diverting phage away from the Prochlorococcus cells.

In working with Prochlorococcus, Chisholm and her colleagues have found that the bacterium is "happier" in the company of heterotrophs—organisms that can't synthesize their own food and need a source of organic carbon to grow. "We went through heroic efforts to separate the Prochlorococcus and their heterotrophic friends in seawater samples," says Chisholm. "Then we realized that when we grow them together, the cultures grow faster and are more stable." In a series of experiments, Biller showed that the newly identified lipid vesicles can serve as nutrients for the heterotrophs.

What does Prochlorococcus get in return? It is not fully understood, but others have shown that in the process of becoming streamlined, Prochlorococcus lost certain enzymes that other species use to neutralize toxic oxygen compounds produced during metabolism. The heterotrophs can perform that detoxification task, taking care of the problem for Prochlorococcus.

Another hypothesis is that the vesicles help protect Prochlorococcus from phage, viruses that infect bacteria. The surface of a vesicle contains material from the outer membrane of its parent cell, including protein receptors that phage use to identify their "prey." The vesicles therefore may serve as a decoy—"much as a fighter jet trying to evade an incoming missile may throw out chaff so that the missile goes after the chaff instead of the jet," says Biller. To test that idea, Biller mixed purified Prochlorococcus vesicles with a phage known to infect the Prochlorococcus source of the vesicles. Electron micrographs revealed many phage attached to vesicles. Moreover, their shortened tails suggest that they have injected their DNA into the vesicles, thereby becoming inactive.

A final hypothesis is that the vesicles assist in the exchange of genetic material between individual bacteria—a phenomenon known to occur in some bacteria as a means of developing genetic diversity and sharing useful genes. "We know that bacteria are swapping genes among themselves at surprisingly high frequencies—maybe by using phage or direct cell-to-cell contact," says Biller. "But it wasn't clear that those mechanisms alone could explain the apparent rates at which genes are moving around. This is one possibility of another way that DNA might be exchanged in these communities."

Benefits of multiple-scale study

The researchers' latest results confirm the validity of Chisholm's decades-long approach to studying Prochlorococcus. "Our studies of this bacterium have ranged in scale from genes to cells to populations and then to the community they're embedded in and up to the global scale," she says. That approach, called integrative systems biology, has obvious benefits for understanding global ecosystems and—in the longer term—for developing practical systems involving mass cultures that are fast-growing, stable, and productive. Says Chisholm, "Studying model systems such as Prochlorococcus in an expansive sense—from the phage that infect them to the other microbes that they grow with in nature—will ultimately have relevance for any kind of large-scale production of biomass for biofuels and other types of high-energy compounds."

More information: Bacterial vesicles in marine ecosystems. *Science* 10 January 2014: Vol. 343 no. 6167 pp. 183-186. DOI: 10.1126/science.1243457

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