Unusual symbiosis discovered in single-celled algae and nitrogen-fixing bacteria

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Scientists have discovered an unusual symbiosis between tiny single-celled algae and highly specialized bacteria. Their partnership plays an important role in marine ecosystems, fertilizing the oceans by taking nitrogen from the atmosphere and "fixing" it into a form that other organisms can use.

Details of the symbiosis, published in the September 21 issue of *Science*, emerged from the investigation of a mysterious nitrogen-fixing microbe with a drastically reduced genome. First detected in 1998 by Jonathan Zehr, a professor of ocean sciences at the University of California, Santa Cruz, it now appears to be the most widespread nitrogen-fixing organism in the oceans. The microbe belongs to a group of photosynthetic bacteria known as cyanobacteria, but it lacks the genes needed to carry out photosynthesis and other essential metabolic pathways. Apparently, its association with a photosynthetic host cell makes those genes unnecessary.

"The cyanobacterium is a nitrogen-fixer, so it provides nitrogen to the host cell, and the host cell provides carbon to the cyanobacterium, which is lacking the metabolic machinery to get its own carbon," said Anne Thompson, a lead author of the paper and postdoctoral researcher in Zehr's lab at UC Santa Cruz. Rachel Foster of the Max Planck Institute for Marine Microbiology is the other lead author and contributed equally to this work.

Although the partners in the symbiosis have not been grown in the laboratory, Zehr's team and their collaborators have been able to characterize both partners using cell sorting, gene sequencing, and other techniques. The host cell is a type of single-celled algae in a class known as "prymnesiophytes," which are found throughout the world's oceans. In seawater samples sorted by flow cytometry, which separates cells by size and color, the host cells were discovered among the "photosynthetic picoeukaryotes," a mixed population of tiny single-celled algae in the 1- to 3-micron size range.

"Aside from the importance of nitrogen fixation in marine ecosystems, this is such an interesting symbiosis from an evolutionary perspective, because it can be seen as analogous to an early stage in the endosymbiosis that led to chloroplasts," Zehr said.

Chloroplasts, which carry out photosynthesis in all plants, evolved from symbiotic cyanobacteria that eventually became incorporated into their host cells in a process known as endosymbiosis. In the newly discovered nitrogen-fixing partnership, the cyanobacteria are mostly seen in an indentation at one end of the host cell.

"At this point, it's unclear exactly how the cyanobacteria are associated with the host cells. It looks like there may be a little groove in the host cell where the cyanobacteria fits," said Thompson. "The association is robust enough to go through the cell sorter and other preparations, but delicate enough that they separate if they're filtered or frozen and thawed."

In previous work, Zehr's team had studied the cyanobacteria, which they called UCYN-A, in samples processed at sea and brought back to the lab for cell sorting and genetic analysis. Despite being unable to grow it in the lab, they were able to sequence the microbe's complete genome and discover that it was missing the genes for several key metabolic pathways, suggesting that it might live in association with another organism. Thompson said researchers were only able to see the symbiotic partners together when they sorted freshly collected seawater samples on board the ship.

"Our collaborators at the University of Hawaii, Dave Karl and Ken Doggett, put a cell sorter into a portable laboratory, like a lab in a box, so now we can take the machine to sea and sort cells that minutes before were in their natural environment. That's how we found the association," Thompson said.

The exchange of carbon and nitrogen between the two partners was demonstrated using powerful analytic techniques developed and carried out by collaborators from the Max Planck Institute for Marine Microbiology in Bremen, Germany. Seawater samples were incubated with stable isotopes of carbon and nitrogen, then the cells in the samples were sorted by flow cytometry. A highly specific genetic probe was used to identify the UCYN-A cells among the "photosynthetic picoeukaryotes" separated out by the cell sorting equipment. Another key technique was nanometer-scale secondary ion mass spectrometry (nanoSIMS), which helped identify the associated cells and allowed researchers to quantify and image the carbon and nitrogen isotopes within individual cells.

"This combination of techniques is a great tool for microbiology because it couples phylogenetic identification with metabolic analysis," Thompson said. "We could see that the cyanobacteria were fixing the labeled nitrogen and transferring it to the host cells."

Genetic analysis of the host cell indicates its closest relative is the species Braarudosphaera bigelowii. In many species of prymnesiophytes, including B. bigelowii, the cells form external calcified plates, suggesting that the host cell in the symbiosis may have plates that are easily dislodged during processing of seawater samples. "That would be important, because cells with plates sink faster than other organisms, so the carbon they fix could end up being transported to the deep sea or the seafloor," Zehr said.

Zehr noted that it is very difficult to estimate the contribution of this symbiosis to global carbon and nitrogen cycles. Other algae are more abundant and probably much more important in terms of oceanic carbon fixation than the algal host in this symbiosis. But the cyanobacterial partner probably makes a significant contribution to global nitrogen fixation in the oceans, he said.

"Planktonic symbioses are very understudied and difficult to study, as the associations are often fragile and difficult to keep intact," said Foster. "Here we used multiple tools and kept the relationship integrity, and also identified one of the first examples of a seemingly mutualistic partnership present in the plankton."

Zehr has named the cyanobacterium *Candidatus Atelocyanobacterium thalassa*. ("Candidatus" indicates a candidate or provisional name, since the rules of bacteriological nomenclature require that a microbe be grown in culture before the name becomes official.)

More information: "Unicellular Cyanobacterium Symbiotic with a Single-Celled Eukaryotic Alga," by A.W. Thompson et al. *Science*, 2012.

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