Unexpected source of nutrients fuels growth of toxic algae from Lake Erie

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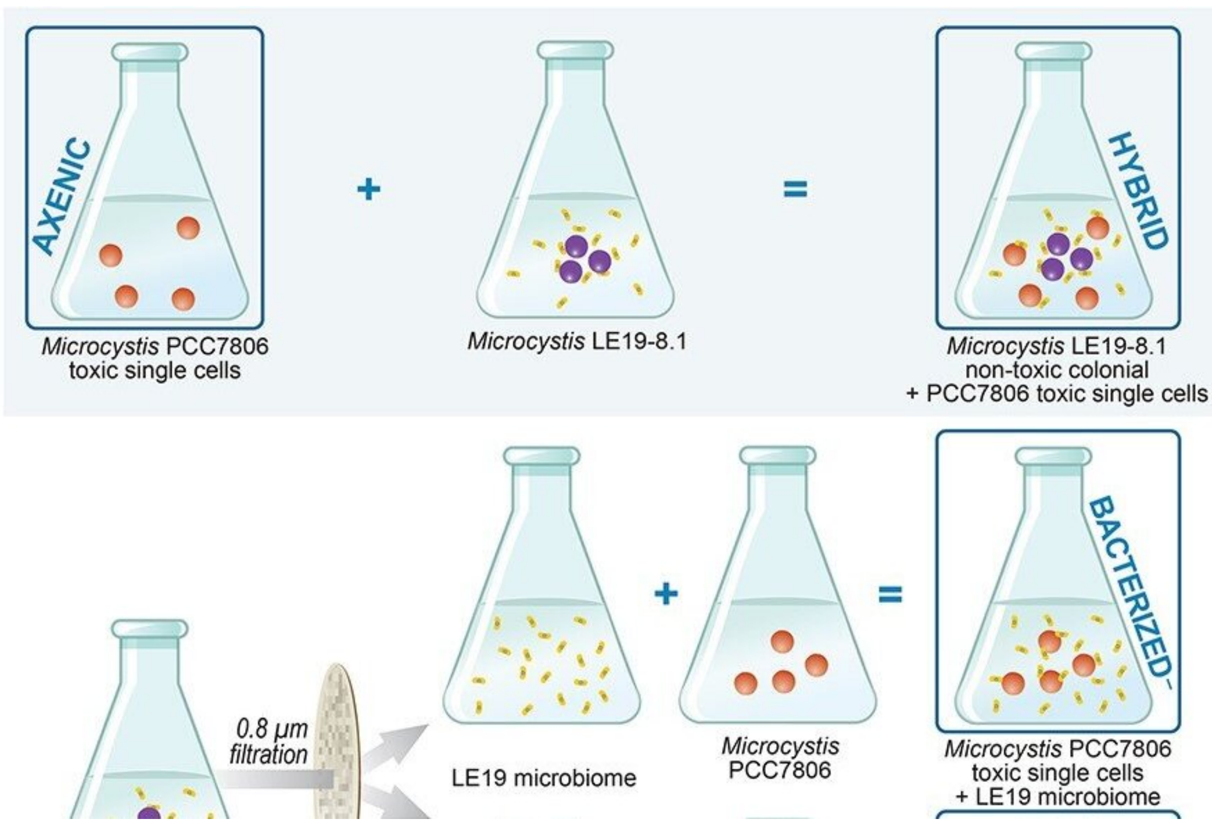

Credit: *The ISME Journal* (2024). DOI: 10.1093/ismejo/wrae082

Climate change, such as warming and changes in precipitation patterns, affects the frequency and severity of harmful algal blooms (HABs) globally, including those of toxin-producing cyanobacteria that can contaminate drinking water.

These nutrient-induced blooms cause worldwide public and ecosystem health concerns. Since the mid-1990s, Lake Erie, the shallowest and warmest of the Great Lakes and a source of drinking water for 11 million people, has experienced seasonal cyanobacterial blooms dominated by several species. Microcystis, the most abundant and most toxic, is recognized as the major producer of cyanotoxins in Lake Erie.

In an effort to better understand the factors that lead to HABs in Lake Erie, Lawrence Livermore National Laboratory (LLNL) scientists and collaborators from the University of Toledo and the University of Michigan have investigated the cyanotoxin production and microbiome community structure of several Microcystis cultures collected from algal blooms in Lake Erie.

One area that requires increasing research to better understand and ultimately predict HAB dynamics is how biological interactions in the lake ecosystems drive bloom formation and decline and how these interactions change under different nutrient conditions. That's what the team aimed to do, starting in the laboratory.

They examined the role of the cyanobacterial microbiome in impacting growth and cyanotoxin production under low inorganic nutrients to understand how microbial cycling of organic nutrients may impact HABs. Cyanobacterial HABs are usually linked to excessive inorganic phosphorus and nitrogen input (which are both found in fertilizer). Phosphorus has been widely recognized as a major contributor to phytoplankton biomass in freshwater.

"But nitrogen is now emerging as a limiting nutrient in these ecosystems, especially during algal blooms, where its availability often restricts the growth of cyanobacteria," said LLNL scientist Wei Li, lead author of the paper appearing in *The ISME Journal*.

"Most studies have focused on inorganic forms of nitrogen such as nitrate and ammonium, but the role of organic molecules in fueling HABs is not well-characterized. Organic nitrogen, which includes compounds like amino acids, proteins and urea, could be a significant source of nitrogen for algal blooms, but its dynamics and impact are less well-understood. This gap in knowledge prevents our ability to predict and manage HABs effectively, as organic nitrogen sources might play a critical role in sustaining these blooms."

In the study, scientists used microbiome transplant experiments, cyanotoxin analysis and nanometer-scale stable isotope probing to measure nitrogen incorporation and exchange at single-cell resolution. First, they found that the type of organic nitrogen available shaped the microbial community associated with Microcystis, and that external organic nitrogen input led to similar levels of cyanotoxin produced as with inorganic nitrogen.

This suggested that the microbiome could help maintain enough nitrogen levels for the cyanobacteria to make the nitrogen-rich toxin molecules. Dragan Isailovic, professor of chemistry at the University of Toledo, provided the expertise in cyanotoxin analysis.

Next, LLNL scientists conducted single-cell nitrogen incorporation analysis after doing incubations with nitrogen, 15 labeled amino acids and protein that revealed that some bacterial communities competed with Microcystis for organic nitrogen, but other communities promoted increased nitrogen uptake by Microcystis, likely through modification of the organic nitrogen to other molecules that the algae could incorporate.

Using LLNL's nanoSIMS, a complex mass spectrometer, the team was able to determine whether the toxic algae or the microbiome (or both) were able to incorporate the isotope labeled nitrogen.

"Without this instrument, it would be nearly impossible to figure this out because the microbiome and the toxic algae are all stuck together in these biofilms," said LLNL scientist Xavier Mayali, senior author and lead investigator of the study.

The nanoSIMS enabled the separation of the isotope signal from the cyanobacteria and the smaller microbiome cells from samples that had been preserved and dried. Additional microscopy of live samples in three dimensions, obtained by co-author and LLNL staff scientist Ty Samo, revealed the close associations between Microcystis and its microbiome.

Researchers at the University of Michigan contributed to experiments and genomic analysis in the collaborative project, leveraging a collection of Microcystis cultures that they isolated from the lake and maintained in the laboratory.

"We are really just beginning to understand how the microbiome affects the biology and toxicity of cyanobacterial blooms. This project allowed us to bring together nanoSIMS, microbiology, genomics and cyanotoxin analysis," said University of Michigan assistant research scientist and co-author Anders Kiledal.

The laboratory culture data showed that organic nitrogen input could potentially support Microcystis blooms and toxin production in nature, and the Microcystis-associated microbial communities likely play critical roles in this process. However, these hypotheses will require testing directly in Lake Erie, which the team hopes to do in the future.

LLNL has close ties with the University of Toledo after formalizing a collaboration agreement last fall. The agreement calls for the institutions to exchange science and technology ideas, to support student opportunities and internships and to pursue research and development in areas like solar energy and other renewable energy technologies, climate and environmental science, biomedical sciences and hydrogen.

"This project to gain a better understanding of the role of the cynanobacterial microbiome in the growth of harmful algal blooms in Lake Erie and other waterways in Northwest Ohio is one of a number of critical science and engineering challenges the University of Toledo is tackling with LLNL," said Frank Calzonetti, University of Toledo vice president for innovation and economic development. "Our scientists are benefiting greatly through our access to one of the top research facilities in the world."

Other University of Toledo contributors include graduate students Sanduni Premathilaka and Sharmila Thenuwara. Other LLNL researchers include David Baliu-Rodriguez (a former graduate student at University of Toledo), Jeffrey Kimbrel, Christina Ramon and Peter Weber.

More information: Wei Li et al, Microbiome processing of organic nitrogen input supports growth and cyanotoxin production of Microcystis aeruginosa cultures, *The ISME Journal* (2024). DOI: 10.1093/ismejo/wrae082

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