Indeed, some of these mutations are already in coronavirus-infected people, according to work by Adam Godzik, a bioinformatics expert at the University of California, Riverside. Godzik and his colleagues scoured the GISAID database, a catalogue of more than 10 million SARS-CoV-2 genomes sequenced from viruses isolated from infected individuals, searching for amino acid changes at positions in M^{pro} near where nirmatrelvir binds. In a bioRxiv preprint posted on 30 May, they reported that mutations to amino acids 166 and 167—two of the resistance mutations flagged by the Belgian group—were already in circulating viruses. Because these mutations occurred before widespread use of Paxlovid, they likely occurred randomly, Godzik says. However, he adds, they reveal the enzyme has some flexibility at these positions that could help the virus work around the drug.

And the list of potential resistance mutations keeps growing. In a paper posted last week on bioRxiv, Jun Wang, a medicinal chemist at Rutgers University, and colleagues report 66 common mutations to M^{pro} near the nirmatrelvir binding site. Like Godzik's team, they scanned the GISAID database to find altered versions of the protease, but then went a step further. Adding the gene for each of these variants of M^{pro} to *Escherichia coli* bacteria, they created supplies of the mutated enzymes for additional tests: first to determine whether each variant still carried out the essential duties of cutting viral proteins, and second to determine whether the mutations allowed M^{pro} to resist nirmatrelvir. Eleven of the 66 variants retained the protease's function (it was impaired in most of the others), and five of the 11 were resistant to nirmatrelvir, requiring at least a 10-fold increase in the drug to kill half the virus in the sample. One of those variants had a previously seen resistance mutation, at position 166, but the other four had novel workarounds at positions 144, 165, 172, and 192. The bottom line from all this work, Wang says: "It's just a matter of time before we see resistance emerge."

So, why is Paxlovid still working? One possibility is that not enough people have taken the drug yet to force the virus to mutate. Another explanation, Wang says, is that it may take multiple mutations in M^{pro} for the virus to get around Paxlovid while remaining both fully functional and easily transmissible. Thus far, adds Aditya Shah, an infectious disease specialist at the Mayo Clinic, studies show that when symptoms recur in COVID-19 patients on Paxlovid, which happens in just 2% or fewer of those who take the drug, the rebound does not seem to be due to resistance mutations. "It's reassuring," Shah says, but not proof the virus won't eventually find its way around the drug.

Pfizer says its Paxlovid regimen may forestall resistance. Patients only take the drug for a short period and typically get a dose "manyfold higher" than that required to prevent the virus from replicating in cells, thereby minimizing the opportunities for the virus to mutate, says Kit Longley, a company spokesperson.

Giving patients multiple antivirals could help prevent resistance by making it harder for the virus to evolve its way around different compounds at the same time, a strategy that has proved highly effective in treating other viruses, including HIV and hepatitis C, Ho says. Two other SARS-CoV-2 antivirals are authorized in the United States, but they have drawbacks. The other oral drug, molnupiravir, has proven considerably less effective than Paxlovid, and has raised safety concerns because it induces random genetic mutations in the virus—that typically stops it from replicating but could also spawn dangerous new variants, some scientists caution. And remdesivir, which interferes with the ability of the virus to copy its genome, is approved for use in patients with mild to moderate symptoms who are at risk of severe disease, but it must be delivered intravenously. A preprint posted on bioRxiv last week suggests combining molnupiravir and nirmatrelvir is more effective in combating SARS-CoV-2 infections than either antiviral given alone, at least in mice. But the strategy has yet to be widely embraced by doctors.

> **"When you put pressure on the virus, it escapes."**
> **David Ho**, Columbia University

Meanwhile, pharmaceutical companies are racing to complete clinical trials on additional SARS-CoV-2 antivirals, some targeting M^{pro} at different sites. But those aren't available yet. And numerous researchers, including representatives of the nonprofit Drugs for Neglected Diseases Initiative, have complained that Pfizer has not made Paxlovid easily available for trials of combination therapies. The company has said it plans to do those studies itself, although some are skeptical.

Until more antiviral drugs become available, Paxlovid will remain essentially alone, raising fears that sooner or later it will lose its punch. When pressed by a single antiviral, viruses usually find a way around the drug, Gottwein says. "If it can happen it will happen." ■

ECOLOGY

It takes a (microbial) village to make an algal bloom

More than nutrient levels may drive toxic lake growths

By **Elizabeth Pennisi**

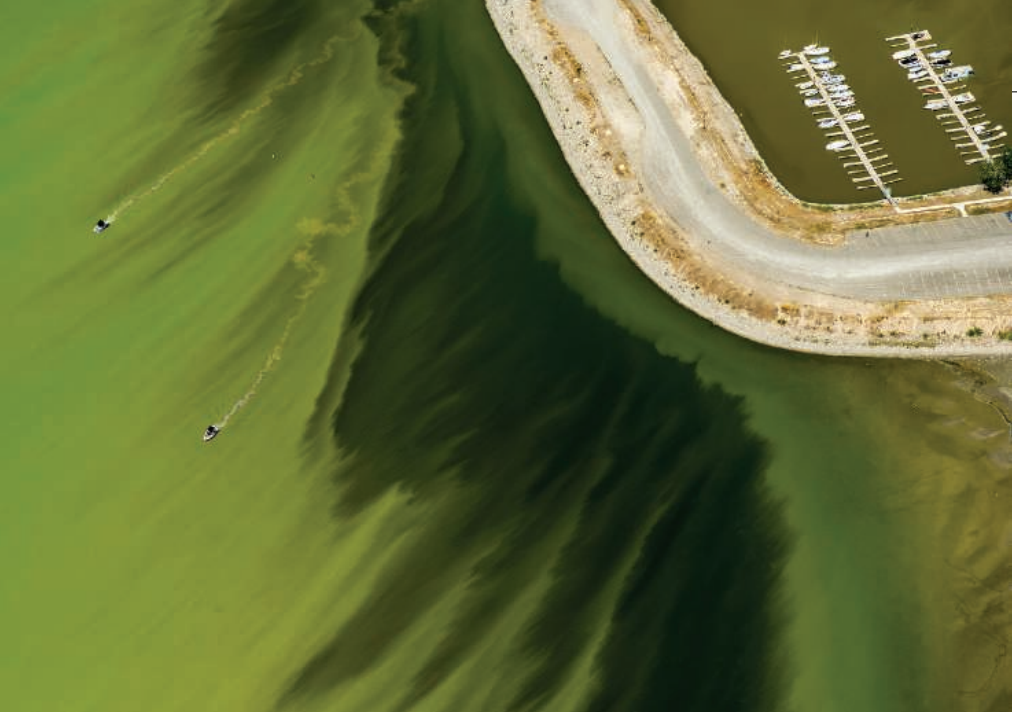
Every summer, surges of toxic green muck plague lakes worldwide, sickening hikers who fail to purify drinking water, closing favorite swimming holes, and killing fish. The most feared—and studied—cause of these freshwater "algal" blooms is a genus of cyanobacterium called *Microcystis*. Its explosive summer growth is thought to be spurred by rising levels of phosphorus, nitrogen, and other nutrients, perhaps from fertilizer run off or other pollution sources. But new research, driven by advances in DNA sequencing, suggests other types of microbes also play key roles in these massive overgrowths.

According to one study, viruses killing off a main competitor of toxic *Microcystis* may help pave the way for blooms; another indicates nitrogen fixation by other bacteria may provide the needed boost. The results suggest that reducing nutrients may not be enough to stop these slimy explosions, some scientists say. That doesn't mean curbing pollution is unimportant, they stress, but ecological factors must be considered.

"Interspecies biological interactions help determine blooms," says Kevin Johnson, a marine scientist at the Florida Institute of Technology who was not involved in the work. "The more details we understand of bloom creation, the better our knowledge of how they might be prevented or controlled."

With the warming climate and continuing inflows of pollution, harmful algal blooms are on the rise, becoming more frequent and longer lasting in ever more places across the globe. They are "a pretty wicked problem," says Ariane Peralta, a microbial ecologist at Eastern Carolina University.

In some lakes, reducing fertilizer runoff at first seemed to thwart blooms—then they came back. Similar plans for bloom-choked Lake Erie might backfire, a team of academic microbiologists and water quality experts



Toxins produced by this algal bloom in a lake in Utah sickened several people.

PHOTO: RICK EGAN/*THE SALT LAKE TRIBUNE*/AP IMAGES

funded by the National Science Foundation and other U.S. agencies reported in May. A 2014 bloom there caused such severe shortages of drinking water in the nearby city of Toledo, Ohio, that Canada and the United States have agreed to cut phosphorus going into the lake by 40%.

But a simulation of that strategy, along with an analysis of more than 100 related scientific papers, led the team to conclude that although limiting phosphorus might shrink Lake Erie blooms, they could also grow more toxic: with lower overall growth of microbes, any photosynthetic *Microcystis* left would receive more sunlight and have more nitrogen available, two conditions that favor an increase in their production of microcystin, a substance that make the blooms toxic (*Science,* 26 May, p. 1001). They suggested the lake's nitrogen should also be curtailed.

That simuation hinted that other microbes can indirectly influence the impact of *Microcystis*. But researchers studying blooms have tended to overlook lakes' many microbial inhabitants, which can include huge numbers of diatoms and other eukaryotes, as well as viruses and various types of bacteria, including smaller than average ones called picocyanobacteria. "Everyone glosses over them as not of managerial concern," says Cody Sheik, a microbial ecologist at the University of Minnesota, Duluth.

Part of the problem has been that it's been difficult to sort out which microbes are doing what in a lake. But Lauren Krausfeldt, a microbiologist at Nova Southeastern University, recently turned to metagenomics, a strategy of sequencing all the DNA in samples of water and other environments, to reconstruct the microbial ecosystem in Florida's Lake Okeechobee. The largest lake in the U.S. southeast, Okeechobee's annual summer blooms have begun to spread down rivers and spill into the Gulf of Mexico and Atlantic Ocean, forcing beaches to close. Between April and September in 2019, the bloom season, Krausfeldt and her colleagues collected multiple water samples at 21 places across the lake. From the fragments of DNA isolated from the samples and sequenced, they pieced together whole genomes belonging to specific species.

The analysis uncovered 30 kinds of cyanobacteria never before detected in the lake, and in some cases new to science, including 13 that could potentially cause blooms, she reported last month at Microbe 2022, the annual meeting of the American Society for Microbiology. "I was surprised at the diversity," Krausfeldt says.

When there was no bloom, the most common organisms were the picocyanobacteria. But as the season progressed, DNA belonging to bacterial viruses, known as phages, that infect the picocyanobacteria rose steeply. Shortly thereafter, the concentration of toxic *Microcystis* began to skyrocket. An analysis of its genome suggested why: *Microcystis* contains several antiviral defenses, such as the system that spawned the genome editor CRISPR, that picocyanobacterial lack. In addition, the bloom-forming cyanobacterium has genes that enable it to store nitrogen, a key nutrient, which may provide another competitive advantage over the many lake microbes that did not.

Krausfeldt suspects the phages lie dormant until some unknown environmental cue activates them. Then, after the viruses start slaying more and more picocyanobacteria, newly available nitrogen, phosphorus, and more light fuel a *Microcystis* bloom, Krausfeldt suggests. The phages' destruction of its hosts' cells may release even more nutrients, playing a key role in enabling algal blooms, she concludes.

Sheik, who says he had not considered phages as a factor in blooms but now wants to explore such viral dynamics, embraces Krausfeldt's ecosystem mindset. "By taking a holistic approach, we can better understand how supporting organisms can help sustain blooms," he says.

Sheik and his colleagues have also added metagenomics, as well as gene activity assessments, to his studies of several small lakes in Minnesota. Those lakes, he reported at the meeting, contain not only some *Microcystis*, but also another bloom-forming cyanobacterium called *Dolichospermum*. In 2020 and 2021, when he and colleagues tracked the microbial dynamics in one lake throughout the summer, they saw *Dolichospermum* become the most abundant microbe only to have its population crash by July. Nitrogen levels in the lake rose and fell in parallel with the microbe, suggesting it was fixing nitrogen and boosting its concentration in the water.

Nitrogen is usually quite scarce in these relatively pristine lakes, yet the nutrient is essential for the production of microcystin. That might explain why Sheik and his colleagues saw levels of *Microcystis* and its toxin rise after the bloom in nitrogen-fixing *Dolichospermum*. *Microcystis* must rely on other members of the freshwater ecosystem to fix nitrogen or to recycle it by breaking down other life forms, Sheik says.

"I'm blown away" by the metagenomic work, says Benjamin Wolfe, a microbiologist at Tufts University, because it can illuminate in great detail the lake's microbial interactions.

The case of *Dolichospermum* illustrates how complicated algal blooms can be. The good news, however, is that unlike in Europe, where this bacterium causes toxic blooms, *Dolichospermum* species in the United States lack the genes to make toxins—at least for now, says Sheik, who plans to keep watching for them in his metagenomic studies.

How the microbial dynamics that drive blooms can be interrupted is still unknown, and the picture is getting more complicated all the time. "We are grappling with understanding what parts of complex microbial communities are changing and what we can change to produce a different outcome," Peralta says. But she's optimistic that in time, "we can figure out what levers we can move." ■

It takes a (microbial) village to make an algal bloom

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