RESEARCH ARTICLE

Discovery of *Cyanophage* (A virus that kills Cyanobacteria) against *Microcystis aeruginosa* from the stagnant water of the River Ganga

Raghvendra Raman Mishra[1*], Pragya Mishra[2], Mandira Narzary[1], Abhijeet Kumar[1]
[1]Medical Laboratory Technology, RGSC, DDU KAUSHAL Kendra, Banaras Hindu University, Varanasi, Uttar Pradesh-211005, India
[2]Food Processing and Management, RGSC, DDU KAUSHAL Kendra, Banaras Hindu University, Varanasi, Uttar Pradesh-211005, India
Corresponding Author Email ID: raghvendra.mishra4@bhu.ac.in
† All authors equally contributed

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Abstract

Microcystis aeruginosa (*M. aeruginosa*) is one of the highly noxious cyanobacteria that frequently form dense blooms in eutrophic freshwaters. Its toxic nature is responsible for the death of livestock and wildlife, and is also associated with serious problems in water management.

Aim: In this study plan, our aim was to identify toxic cyanobacteria, specifically *M. aeruginosa*, in the stagnant water of the River Ganga, and also to discover lytic viruses (cyanophages) against them.

Materials and Methods: Surface water sample of a cyanobacterial bloom was collected from accumulated water near the river Ganga bank in the Mirzapur region during January 15th to March 5th, 2025. It was cultured in BG-11 medium by using a cycle consisting of 12 hours (h) of darkness and 12 h of light (ca. 40 mol photons m -2 s -1) provided by cool white fluorescent illumination at 30°C. These water samples were successively filtered through 0.8µm and 0.2µm polycarbonate membranes, and then 100µl of the filtrate was inoculated into 900µl exponentially growing cyanobacterial culture strains. The cultures were incubated for a week. Growth inhibition was observed only in the culture inoculated with the filtrate. An infectious agent was isolated from the lysed culture using three cycles of an extinction dilution procedure followed by plaque assay. 200µl of the above infectious suspension was added to 800µl exponentially growing cultures of the hosts. The cultures were incubated as described above and monitored daily for host cell lysis using optical microscopy and also analyzed host range of specific cyanophages against *M. aeruginosa.*

Results: Identified cyanobacteria were morphologically *M. aeruginosa*. Induced viruses that kill cyanobacteria are considered cyanophages. Cyanophages lysed the cultures after 8 days was considered to show the presence of cyanophage against *M. aeruginosa* in the water sample. The cyanophages were specific to corresponding host i.e. *M. aeruginosa.*

Conclusion: The presence of *M. aeruginosa* in the Ganga River water is a serious threat to the water. But the discovery of its specific cyanobacterial lytic virus i.e. Cyanophages opens new hope for the removal of such blooms.

Keywords: *Microcystis aeruginosa*, Cyanobacteria, Cyanophage, River Ganga, Bioavailability, Micronutrients.
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Introduction

Algae are broadly classified based on characteristics such as pigment composition, cellular organization, and habitat preferences. The major taxonomic groups include Green Algae (Chlorophyta), Red Algae (Rhodophyta), Brown Algae (Phaeophyta), Diatoms (Bacillariophyta), Dinoflagellates (Dinophyta), and Blue-Green Algae (Cyanobacteria).[1] While true Cyanobacteria are a class of prokaryotic organisms that share characteristics with algae, which are eukaryotic, they show the same photosynthetic capabilities. *Microcystis aeruginosa (M. aeruginosa)*, a type of cyanobacterium responsible for harmful algal blooms [2], is one of the poisonous cyanobacteria [3] that frequently form dense blooms in eutrophic freshwater. Its toxic nature causes the death of livestock and wildlife and is associated with serious water management. There are no biological procedures for removing such overgrowth.

Here are reports of some viruses that are involved in to killing Cyanobacteria are named as "Cyanophages". That works by selectively breaking down the cyanobacteria, but their effectiveness can be influenced by factors like environmental conditions and cellular stress, which may affect the lysis process and toxin release comes under cyanophage technology.[4] Cyanophages are considered a promising biological alternative to chemical treatments for controlling harmful algal blooms.[5] They target and lyse specific cyanobacteria without harming other organisms. Environmental conditions such as temperature, pH, and nutrient availability can impact the interaction between

cyanophages and *Microcystis*. During the lysis process, cyanophages can sometimes release microcystins (toxins) into the water, which needs to be considered for the practical application of phage-based control.[6]

In this project, we detect *M. aeruginosa*-associated water blooms around the stagnant water of Rivere Ganga bank. Our core aim was to find lytic viruses that are considered "cyanophages" that combat hazardous cyanobacteria, particularly against *M. aeruginosa*, in the Ganga River's water. We describe the physical and biological traits of cyanophages in this effort. The cyanophage that is particular to *M. aeruginosa* has its specific hosts. This work supports the growing interest in employing freshwater cyanophages to regulate bloom-forming cyanobacteria by providing fundamental information supporting freshwater cyanophages.

Materials & Methods

The present study was conducted at the Medical Laboratory Technology, DDU Kaushal Kendra RGSC, Barkachha, Banaras Hindu University.

Water sampling

Water samples containing full cyanobacterial *(Blue Green Algae)* blooms were collected in sterile bottles from stagnant fresh water near the Ganga River bank in Mirzapur district (Uttar Pradesh, India) from January 15th to March 5th, 2025 (Figure 1).

Figure 1: Site of collection of the water sample near Shastri Bridge, District Mirzapur, Uttar Pradesh, India

Cyanobacterial Culture under Laboratory Conditions

Cyanobacterial culture was performed in a modified Blue-Green-11 (BG-11) medium (50 mL). It was maintained at 30°C in a light-dark photoperiod cycle consisting of 12 hours (h) of darkness and 12 h of light (ca. 40 mol photons m $^{-2}$ s $^{-1}$) provided by cool white fluorescent illumination (Figure 2). Culture extracts (1 mL) were collected every 24 hours for monitoring the growth of cyanobacteria and the production of microcystin toxins. All extracts in the BG11 freshwater liquid medium for cyanobacteria were kept at –20 °C until use, and the supernatant was tested in triplicate after centrifugation at 3000 *rpm* to remove cells.

Microscopy

Microscopic observation of cultured cyanobacteria was performed under oil-immersion at 100X on a Magnus MLX-B binocular microscope allowed for detailed analysis of cellular morphology, size, arrangement, and the presence of specialized structures.

Cyanophage isolation

Collected water samples were successively filtered through 0.8-µm and 0.2-µm polycarbonate membranes. 100 µl of the filtrate was inoculated into 900-µl exponentially growing cultures of identified cyanobacteria strains. The cultures were incubated for 1 week. Observation of growth inhibition was done in culture inoculated with the filtrate. An infectious agent was isolated from the lysed culture using three cycles of an extinction dilution procedure

Cyanobacteria growth and plaque assay

To assess the growth characteristics of cyanobacteria cultures after infection (with cyanophage), the density of cyanobacterial cells was determined daily by a hemacytometer. The cyanobacterial cultures at 72 h were used for the plaque assay technique.[7] Briefly, the supernatant fraction obtained from above was filtered over 0.2-µm sterile syringe filters, and serial dilutions of the phage-containing filtrate were plated on the cyanobacterial lawns in pour-plates of 0.4% ultra-pure low-melting-point agarose in BG11 medium. Presence of cyanophage-form plaque on some susceptible cyanobacterial lawns. The titer of the cyanophage at 24-h intervals was estimated by the most-probable-number technique.

Host range analysis.

40µl of a fresh cyanophage suspension was added to 800µl exponentially growing cultures of the host. The cultures were incubated as described above and monitored daily for

host cell lysis using optical microscopy. It also dropped on the culture plate of *M. aeruginosa* to determine specificity.

Results and Discussion

Under microscopy, cell colonies are initially spherical but can lose coherence and become irregularly shaped, perforated, or sponge-like in appearance, resembling M. aeruginosa (Figure 2).

Freshwater cyanobacteria belonging to the genus *M. aeruginosa* are commonly found in environments that are meso to eutrophic. Their photosynthetic activities caused the Earth's atmosphere to oxidise [9, 10], and their phylum was formed some 3 billion years ago [8]. A modified BG 11 medium was utilised for culture of *M. aeruginosa* in the laboratory. A prominent growth were observed after 8th day in controlled conditions (Figure 3).

Through processes that have enabled them to thrive in the face of growing human impact on aquatic ecosystems, they have effectively adapted to harsh settings throughout time. [11] Today's worldwide Microcystis species can be harmful to human health when freshwaters impacted by blooms are used for recreational, drinking, irrigation, and fishing. [9, 12] Due to climate change-related factors such as rising temperatures, CO_2 levels, and eutrophication, blooms have become more widespread, frequent, and intense, [13, 14] posing greater threats to aquatic life and people. Blooms were observed in 108 nations, of which 79 verified the presence of the powerful hepatotoxin microcystin, according to a global survey of the current level of knowledge of *Microcystis* (e.g., geographic distribution, toxins, genomes, phylogeny, and ecology). [15] Cyclic heptapeptides called microcystins can kill aquatic invertebrates, [16, 17] mammals, [18] and people. [19] The toxicity of the more than 250 distinct microcystin congeners that have been identified [20] is influenced by variations in their molecular structures [21, 22] and the concentration of these congeners within cyanobacterial cells is influenced by variations in environmental factors such as temperature, [23] light intensity, [24, 25] pH [18], and nutrient concentrations. [26] Microcystin-LR, one of the most deadly forms of microcystins, is one of these congeners that is frequently found and extensively investigated. Actually, because microcystin-LR is so common, the World Health

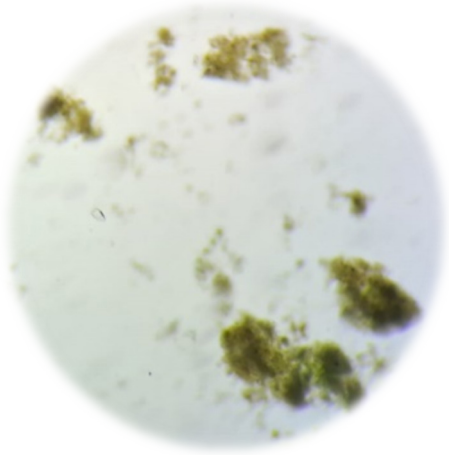

Figure 2: Spongy pale blue-green/ brown culture of *Microcystis aeruginosa*.

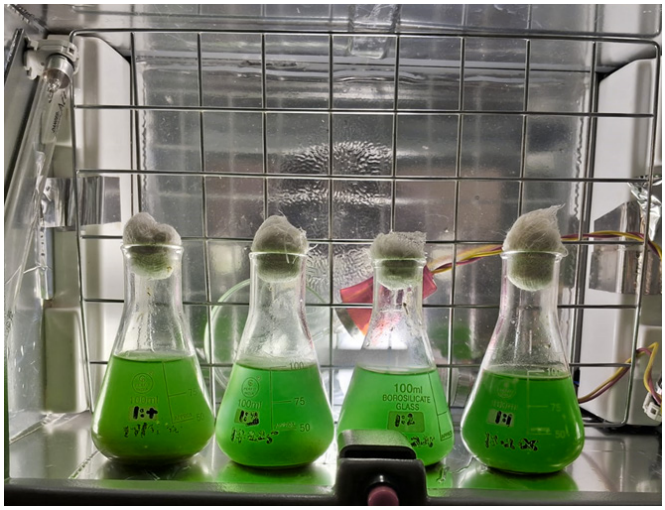

Figure 3: Culture of *Microcystis aeruginosa* in BG 11 media under laboratory conditions.

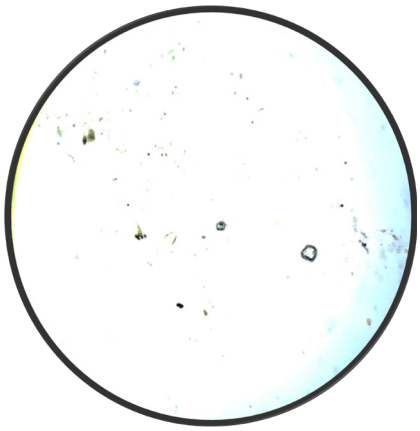

Figure 4: Microscopy of cultured *M. aeruginosa* under oil-immersion at 100X on a Magnus MLX-B

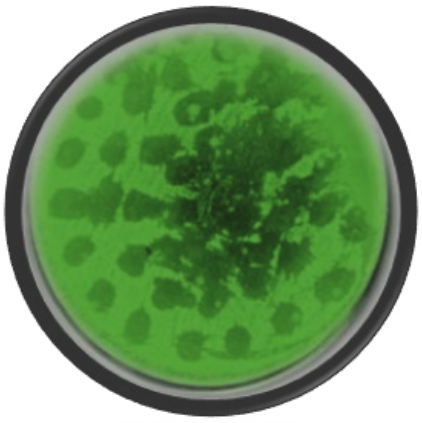

Figure 5: Application of expressed cyanophages on host *M. aeruginosa* for the host range analysis

Organization established a drinking water guideline, [27] which is followed in the majority of nations. We had properly identified M. aeruginosa in microscopy (Figure 4)

The necessity for research aimed at better understanding the generation and dynamics of algal toxins has been brought to light by growing concern over the effects of toxic algal blooms caused by cyanobacteria. In order to provide new insights into cyanophage management, we looked at the discovery of cyanophage from natural sources like the Ganga water. Cyanophages are now the most promising bloom management strategy when compared to conventional techniques. [28] Cyanophages have no effect on the geochemical cycle or the natural environment and provide many benefits in bloom management. It is vital to isolate and purify more broad-spectrum cyanophages in comparison to the ones that have been isolated thus far. Our discovered cyanophages are specific to the corresponding host, i.e. *M. aeruginosa*; the host lytic activity is prominent in the cyanophage (Figure 5)

Conclusion

Research on *M. aeruginosa* is active and promising for controlling harmful algal blooms (HABs) in aquatic systems, such as eutrophicated rivers and lakes. This is a unique discovery of a cyanophage in the River Ganga, which may be one of the reasons to protect its water. Several specific cyanophages that are effective against *M. aeruginosa* have been identified and studied, but direct application in the particular context of the River Ganga is not detailed in the research that is currently available. Furthermore, research is needed for managing blooms with different cyanobacteria in the future.

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