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ORIGINAL ARTICLE

Phylogenic diversity of bacteria associated with potentially toxic cyanobacteria *Microcystis aeruginosa*: a synthesis on its bloom dynamics

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Abstract

The occurrence of toxic bloom-forming cyanobacteria, *Microcystis aeruginosa*, has been frequently reported worldwide. These colony forming toxic cyanobacteria harbour a wide range of heterotrophic bacterial communities. The present study has attempted to understand the bloom dynamics of *M. aeruginosa* along with isolating their colony-associated culturable heterotrophic bacteria from two freshwater ponds in south India with a persisting cyanobacterial bloom. The monthly monitoring of these study areas revealed the conducive role of warm, stagnant waters with high nutrients in forming *M. aeruginosa* bloom. The peak values of temperature, nitrate, and phosphate at station 1 reached up to 30.5 °C, 4.48 mg/L, 1.64 mg/L, and at station 2, 31 °C, 3.45 mg/L, and 0.62 mg/L, respectively. Twenty-eight bacterial isolates belonging to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes were obtained during the study. Among these 28 isolates, Firmicutes was dominant with the *M. aeruginosa* bloom from both the study areas.

Keywords *Microcystis aeruginosa* · Mucilaginous colony · Heterotrophic bacteria · 16S rRNA · South India

Introduction

Recent decades have witnessed the global expansion of harmful cyanobacterial blooms, mainly in freshwater environments, in terms of frequency, intensity, and toxicity (Paerl and Barnard 2020). The toxic cyanobacterium *Microcystis* is one of the most pervasive species responsible for around 90% of the cyanobacterial bloom incidents reported in freshwater bodies (Preston et al. 1980; Chen et al. 2003; Paerl and Otten 2013). They can produce various secondary metabolites, including hepatotoxins called microcystins (Welker et al. 2006). The established toxicity of these cyanotoxins has attained much interest among researchers due to the impacts arising from buildups of such toxins, primarily in freshwater aquatic ecosystems (Schneider 2017). The presence of *Microcystis* species in aquatic systems is linked to various environmental variables, such as the abundance of nutrients and warmer temperatures with a stable water column (Reynolds and Walsby 1975). Strain level variations among *Microcystis* and its associated microbial interactions are other factors that influence their development, distribution, and toxicity (Dick et al. 2021). *Microcystis* can form colonies in response to environmental stressors like limiting nutrients, varying light, protection from grazing, etc. (Xiao et al. 2018). The colony phycosphere around the phytoplankton provides a nutrient-rich region where various specific interactions, such as mutualism, parasitism, commensalism, competition, etc., occur between phytoplankton and bacteria (Hoke et al. 2021; Serra et al. 2022). *Microcystis* can form large mucilaginous aggregates, with its phycosphere generally colonized by various associated bacteria that share a specific relationship with these *Microcystis* cells (Smith et al. 2021). These heterotrophic bacterial interactions could bring significant physiological changes in the morphology, photosynthetic pigment concentrations, electron transport rate, and extracellular polysaccharide of *Microcystis* (Shen et al. 2011). These cyanobacteria can release large amounts of organic matter into the environment (Worm and Søndergaard 1998), making their phycosphere a suitable niche for many heterotrophic bacteria (Sapp et al. 2007). The organic matter released by cyanobacterial cells attracts various bacteria, whereas the cyanobacterial cells benefit from the nutrients and

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vitamins provided by bacteria in this phycosphere region (Hoke et al. 2021). The presence of bacteria associated with *Microcystis* can promote the growth of cyanobacteria and assist in the formation of specific colony patterns (Khong et al. 2019). *Microcystis* can also modify environmental conditions by limiting oxygen concentrations, light penetration (Paerl and Otten 2016), and altering the levels of CO_2 and pH, thereby affecting the microorganisms nearby (Havens 2008). Several heterotrophic bacteria have been reported to exhibit antagonistic activity against different cyanobacteria, which could be utilised as an alternative strategy to counteract the CyanoHABs (Osman et al. 2017). The association between bloom-forming microalgae and their associated bacteria has attained much importance owing to their close interaction and significant influence on bloom development and degradation (Yang et al. 2012; Woodhouse et al. 2016; Grossart et al. 2005; Cai et al. 2013). These microbes interact with each other, forming various ecological networks and achieving different ecosystem services rather than living separately (Deng et al. 2016; Shi et al. 2016). The *Microcystis*-phycosphere-associated microbiome is considered to play a vital role in the persistence of *Microcystis* bloom through optimal and suboptimal environmental conditions (Gobler and Jankowiak 2022). Despite this, several bacterial species are also known to negatively affect the growth of *Microcytis* by parasitism, predation, or the production of various allelopathic compounds (Van Wichelen et al. 2016). They may also significantly impact microalgal growth or even cause adverse health effects on humans and animals (Eiler and Bertilsson 2004; Sigee 2005). Therefore, detailed studies and prediction of changes in their network structure are vital components of microbial ecology and help in developing different practical applications as well as biodiversity conservation and proper management of the ecosystem (Zhou et al. 2010; Yang et al. 2017). Also, studying the associated bacteria in comparison to free-living bacteria might help to understand the beneficial bacterial species and their role in the dominance of the cyanobacteria over other phytoplankton in eutrophic freshwater ecosystems (Mankiewicz-Boczek and Font-Nájera 2022). *Microcystis* blooms are becoming more common worldwide, resulting in loss of ecosystem services, hazards to human health, and fatalities of aquatic and terrestrial animals due to poisoning through the food chain or direct contact (Wilhelm et al. 2020). Thus, studying the different heterotrophic bacteria in the *Microcystis aeruginosa* colonies and their possible interaction is critical in thoroughly understanding the bloom dynamics of such toxic cyanobacteria. This study aims to look into the dynamics of *M. aeruginosa* bloom and identify the different culturable heterotrophic bacteria present in the *Microcystis* colonies isolated from two different stations in central Kerala, southwest coast of India.

Materials and methods

Study area

A preliminary survey of cyanobacterial blooms in different aquatic ecosystems along Kerala was carried out, and based on the cell abundance, the present study areas were selected for further monitoring and routine sampling. Bloom samples of potentially toxic cyanobacterial species, *Microcystis*, were collected from freshwater ponds; Station 1 (Lat. 9°58′6.7″N; Long. 76°16′56.8″E) and Station 2 (Lat. 9°57′32″N; Long. 76°17′21″E) situated at Ernakulam district of Kerala, southwest coast of India (Fig. 1). Routine monitoring of the study area was carried out from October 2021 to June 2022 representing three seasons: pre-monsoon (February, March, April, May), monsoon (June), and post-monsoon (October, November, December, January). The selected study areas were frequently used for different domestic purposes like bathing and washing clothes.

Sample collection and hydrographic parameters

The surface water with microalgal bloom was filtered (~50 L) through a sieve of mesh size 63 µm to a sterile plastic container for storage and analysis. Hydrographic parameters like

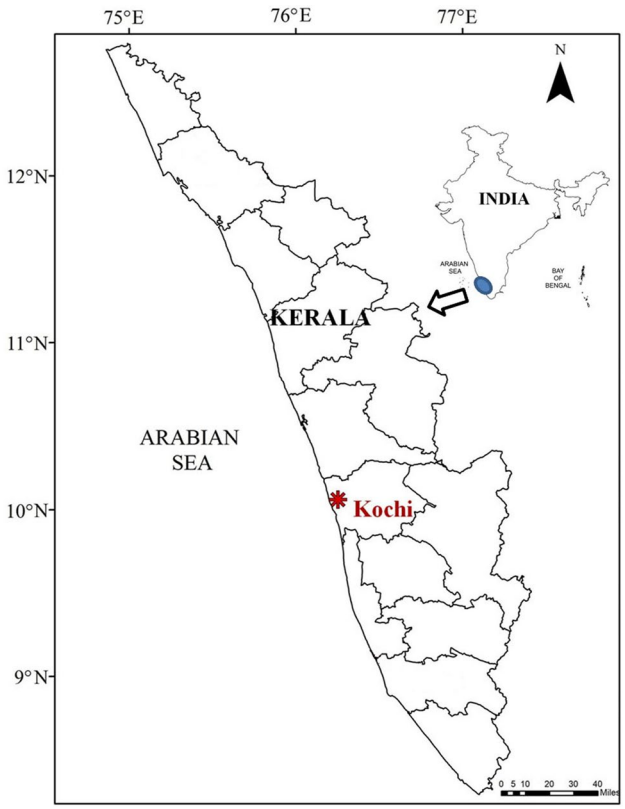

Fig. 1 Study area located at central Kerala along the southwest coast of India

temperature and pH were measured in situ using a calibrated digital thermometer and pH meter (Eutech, PCTestr 35). All the other parameters, such as dissolved oxygen, nutrient concentration, and chlorophyll *a* concentration, were determined in the laboratory. The dissolved oxygen concentrations in the surface waters from both study stations were obtained through Winkler's iodometric titration method (Winkler 1888). The levels of major nutrients like nitrate, phosphate, and silicate in the surface waters were determined with the help of a Hitachi UV-VIS spectrophotometer, USA, at their respective wavelengths according to the standard methods (Grasshoff et al. 1983). The photosynthetic pigment chlorophyll *a* concentration in the study areas was obtained spectrophotometrically using the 90% acetone extraction method (Parsons et al. 1984). The samples were fixed in Lugol's iodine before the qualitative analysis was performed using a Leica DM 2000 Phase contrast microscope with standard identification keys (Desikachary 1959). Enumeration of the phytoplankton cells using Sedgewick-Rafter counting cell was carried out, and the total number of phytoplankton cells present in the sample were expressed as cells per L. Correlation among cell density and environmental parameters was analysed through Primer (V6) using Principal Component Analysis (PCA) plot (Clarke and Gorley 2006).

SEM analysis

Scanning electron microscopic observation was done to confirm the presence of various bacteria attached to the colonies and cells of *M. aeruginosa*. The *Microcystis* samples obtained from the study area were centrifuged at 11,000 rpm for 10 min, followed by a gentle wash with PBS twice. The pellets were fixed in 2.5% glutaraldehyde for an incubation period of 24 h and dehydrated using increasing ethanol concentrations of 30, 50, 75, 90, and 100% for 10 min each at room temperature. The samples were finally gold-coated prior to the SEM imaging (Zhou et al. 2016; Czerwińska-Główka et al. 2021).

Isolation of bloom-associated bacteria

The water samples collected in sterile vials during the pre-monsoon season from the study stations were filtered through Whatman GF/F filter paper to fractionate the sample into two, targeting the bacteria attached to the colonies (B) and free-living bacterial fraction (W). Serial dilution of both fractions was performed and spread plated in nutrient agar medium with an incubation period of 24 h at 37 °C to isolate the bacteria from both samples (B and W). Pure colonies were isolated through quadrant streaking technique and differentiated based on distinct morphology, gram staining, catalase test, and motility test following the standard procedures to exclude the repeating isolates.

Molecular identification of isolated bacteria

DNA isolation of the bacterial isolates was performed following the boiling method (Dashti et al. 2009), and the template DNA obtained was amplified using PCR with universal bacterial 16S primer pair 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Chen et al. 2015). The amplification of the bacterial gene was performed under the following conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 30 s of denaturation at 95 °C, annealing for 30 s at 55 °C, elongation for 1 min 30 s at 72 °C, final extension for 5 min at 72 °C and 4 °C final hold. The bloom samples were centrifuged at 11,000 g for 10 min, and the DNA isolation of *Microcystis* from the pelleted cells was carried out with the help of Qiagen DNeasy blood and tissue kit as per the protocol with minor modifications. Specific primer pairs 16S_F683(5′-CGAAAGCGTGCTACTGGGCTGTAT-3′) and 16S_R1217(5′-TCGCTGGCTCTCGCGAGTTC-3′) were used for the identification of *Microcystis*. The PCR conditions were set to initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s of denaturation at 95 °C, annealing for 30 s at 55 °C, elongation for 30 s at 72 °C, final extension for 5 min at 72 °C and 4 °C final hold thereafter (Yuan et al. 2020). The PCR products were analysed by agarose gel electrophoresis with 1% agarose gel prepared in TAE buffer. Ethidium bromide was used in gel preparation for visualizing the amplified products. All the resultant PCR products were subjected to sequencing via the Sanger sequencing method. The sequences thus obtained were examined using NCBI BLAST. These sequences were then aligned using clustalW, and a phylogenetic tree with the 28 isolates was constructed based on the neighbour-joining method (Saitou and Nei 1987) using MEGA-X (Kumar et al. 2018).

Results

Hydro-biological characteristics of the study area

The colour of both the ponds remained green throughout the study period, with a continuous bloom of *M. aeruginosa* (Fig. 2), which appeared as green scum floating on the water surface with an unpleasant odour. Physicochemical parameters analysed from the study area are shown in Figs. 3, 4, 5, 6, 7, and 8. The surface water temperature variation was measured from the two stations in the range of 27 to 31 °C. The temperature at both stations was maximum with values of 30.5 °C (February) at station 1 and 31 °C (February) at station 2 during the pre-monsoon period, followed by a decline towards the monsoon season. The pH values obtained from the surface waters ranged from 8.4 to 9.5 at station 1 and 8.8 to 9.5 at station 2. The pH values at

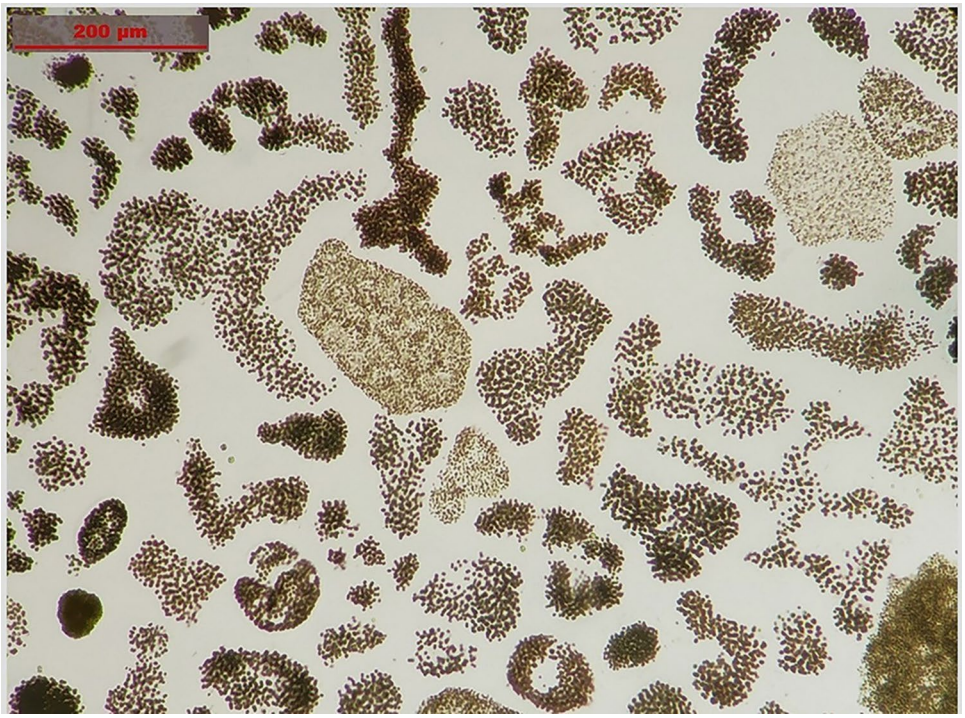

Fig. 2 Microphotograph of cyanobacterium *Microcystis aeruginosa* obtained from the study area

both stations were found to be alkaline throughout the study period. The dissolved oxygen concentrations analysed from the study area differed from 6.88 to 14 mg/L. Maximum dissolved oxygen concentrations were recorded during pre-monsoon, with values of 12.8 mg/L (May) at station 1 and 14 mg/L (April) at station 2. Lower values obtained were 7.31 mg/L from station 1 and 6.88 mg/L from station 2 during the post-monsoon period.

The nitrate levels in the study area ranged from 0.4 to 4.48 mg/L at station 1 and 0.35 to 3.45 mg/L at station 2. At station 1, nitrate levels were moderate during the post-monsoon season and elevated during the pre-monsoon season. The post-monsoon season showed an increased rate of nitrate, which declined with the progress of the pre-monsoon season at station 2. In the present study, phosphate concentration was observed to be greater during monsoon season at station 1 with a value of 1.64 mg/L, while station 2 reported the maximum phosphate level during the post-monsoon season (December) with a value of 0.62 mg/L. Both the study areas have the lowest concentration of phosphate during the

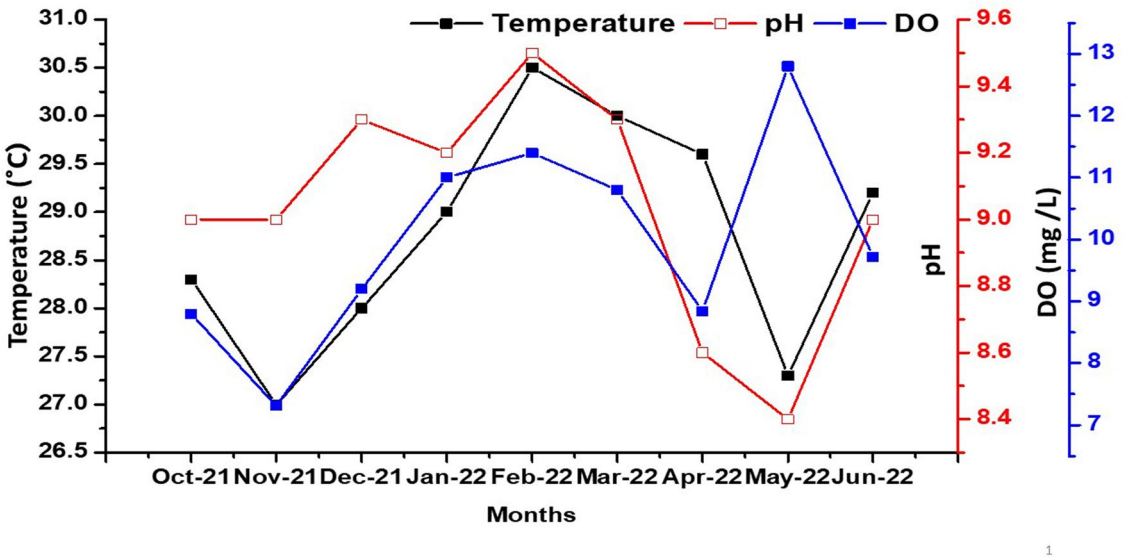

Fig. 3 Monthly variations in hydrographic parameters: temperature, pH, and dissolved oxygen recorded from station 1

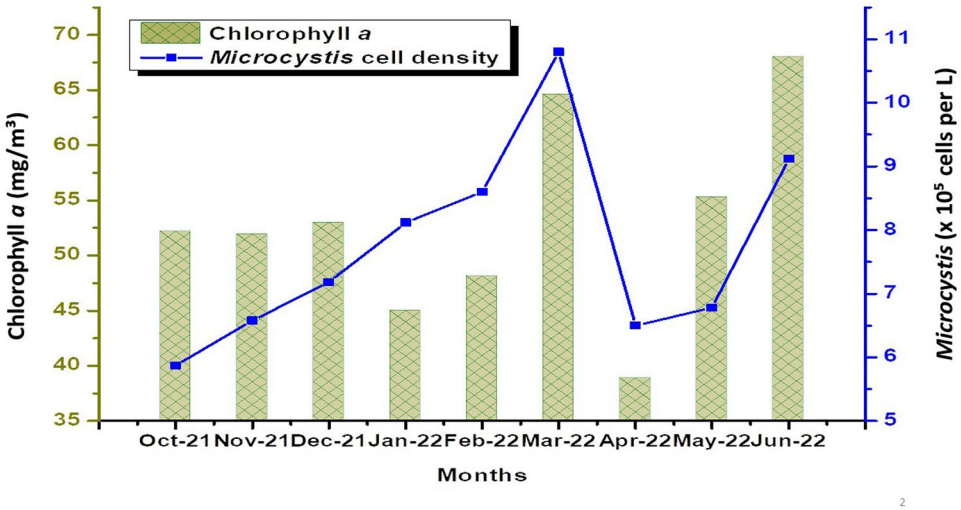

Fig. 4 Monthly variations in chlorophyll *a* concentration and *Microcystis aeruginosa* cell density recorded from station 1

post-monsoon season (October at station 1 and January at station 2). The phosphate levels gradually increased during the pre-monsoon and monsoon periods. There was a more significant fluctuation in silicate concentration at both stations. The highest and lowest silicate concentrations were recorded in station 1 during the pre-monsoon period, with values of 18.25 mg/L in April and 1.85 mg/L in February, respectively. At station 2, the maximum concentration was recorded during the post-monsoon (January: 14 mg/L) and the lowest during the pre-monsoon period (April: 6 mg/L). The concentration of photosynthetic pigment chlorophyll *a* ranged between 38.9 and 68 mg/m^3 at station 1, of which the maximum value was recorded during monsoon (June) and lowest during pre-monsoon (April). A higher concentration of chlorophyll *a* was observed from station 2 with 167 mg/m^3 (February), which was obtained during pre-monsoon, and the lowest, 56.08 mg/m^3 (October), measured during post-monsoon season. *M. aeruginosa* cell concentrations varied between 5.87×10^5 and 10.8×10^5 cells per L at station 1, while station 2 exhibited a denser bloom with cell concentration varying between 9.8×10^5 and 2.1×10^8 cells per L. Higher abundance in both the study stations was recorded during the beginning of the pre-monsoon period (station 1: March and station 2: February), while the lower cell concentration was noticed during the post-monsoon period (station 1: October and station 2: January). The results obtained from principal component analysis (Fig. 9) showed 59.3% of the cumulative variation for the first two principal components (PC 1 and PC 2) at station 1 (Table 1). A total variance of 77.8% was obtained with the addition of PC 3. In station 2, PC 1 and PC 2 together accounted for a total variance of 73.2%, and the addition of principal component 3 marked a

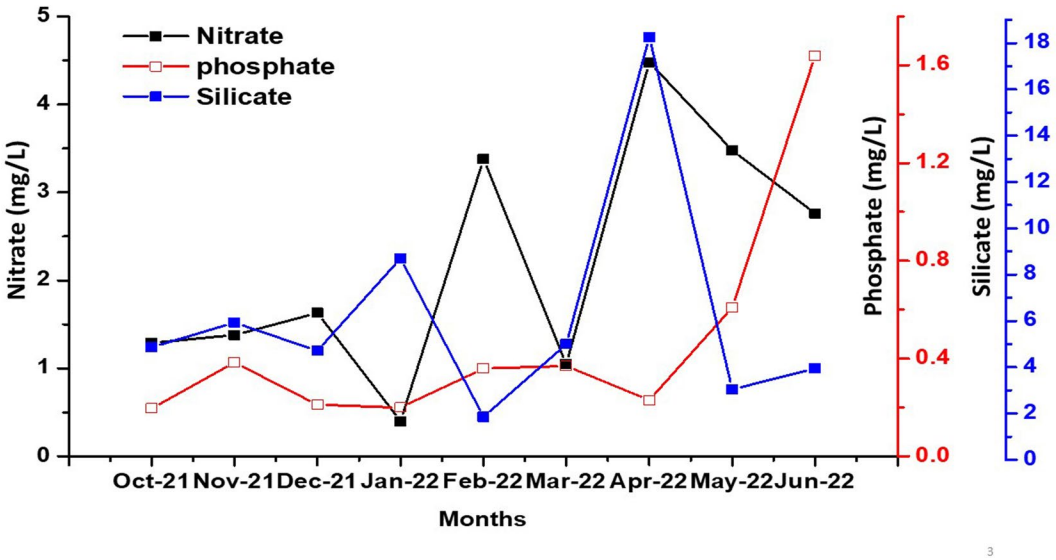

Fig. 5 Monthly variations in concentration of major nutrients such as nitrate, phosphate, and silicate recorded from station 1

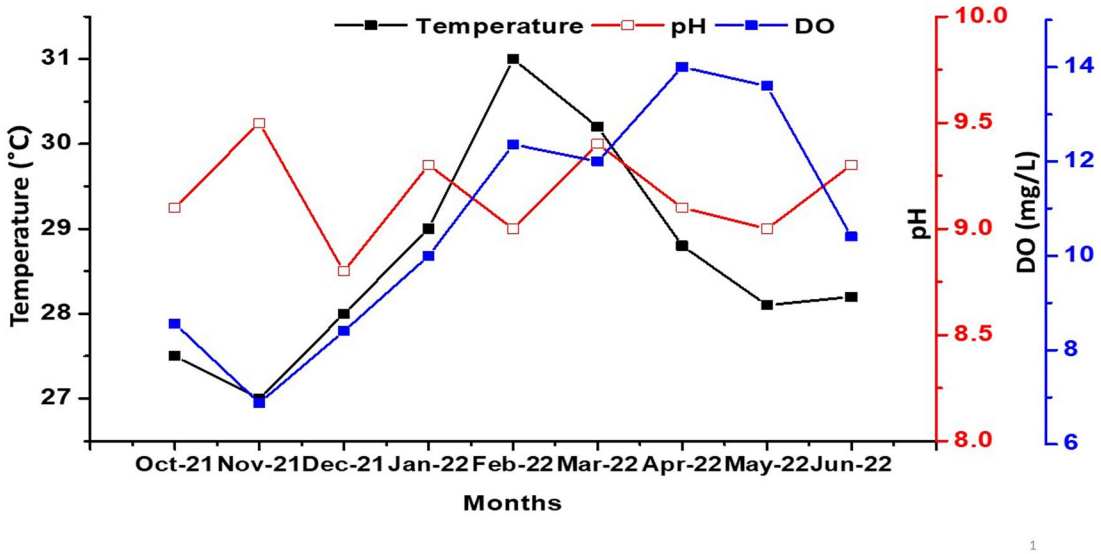

Fig. 6 Monthly variations in hydrographic parameters: temperature, pH, and dissolved oxygen recorded from station 2

total cumulative variance of 90.1% (Table 2). In both study areas, the *M. aeruginosa* abundance was correlated well with chlorophyll *a* concentration and temperature. A positive correlation was also shown by the chemical parameters like pH and nutrients, except for silicate, which negatively correlated with *M. aeruginosa* cell density.

Culturable heterotrophic bacteria associated with bloom species

M. aeruginosa-associated bacterial interaction was examined with the help of scanning electron microscopic images. The SEM micrographs are given in Fig. 10. The SEM images revealed the rod and cocci-shaped bacterial flora attached to the surface of colonies as well as cells of *M. aeruginosa.* For the isolation of bloom-associated bacteria, filtration of bloom samples and their serial dilutions were prepared to isolate pure colonies. The dilutions plated from the filtrate fraction W contained only one distinct colony of bacteria at both stations compared to that of the bloom concentrate fraction B, indicating the abundance of bloom-associated bacteria than free-living ones. Twenty-eight isolates of culturable bacteria were isolated from both stations, of which seventeen gram-positive and eleven gram-negative species were recorded. The motility test revealed that nineteen of the isolates were found to be motile, and nine were non-motile bacteria. Most of the isolated bacteria were shown to be catalase-positive except one isolate.

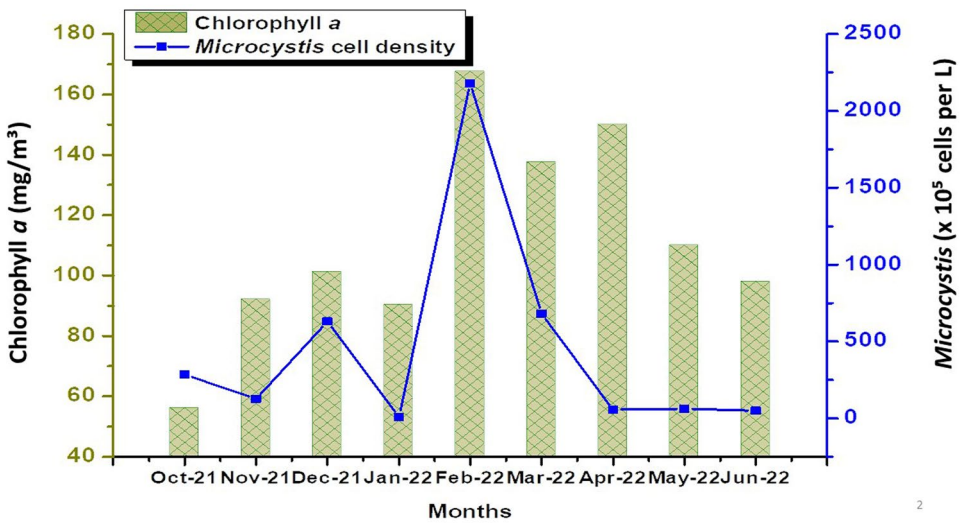

Fig. 7 Monthly variations in chlorophyll *a* concentration and *Microcystis aeruginosa* cell density recorded from station 2

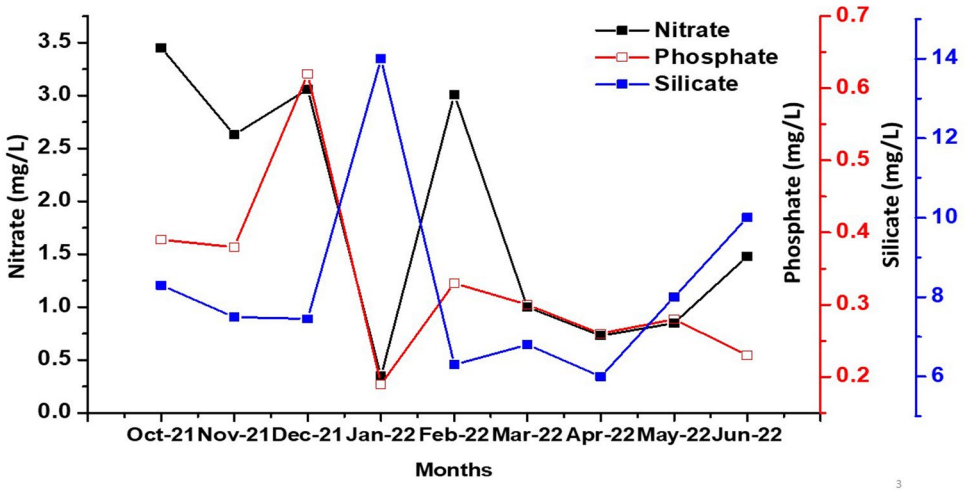

Fig. 8 Monthly variations in concentration of major nutrients such as nitrate, phosphate, and silicate recorded from station 2

Molecular identification of isolated bacteria by 16S rRNA gene sequencing

The 16S rRNA gene sequence confirmed that the bloom samples were dominated by cyanobacteria *M. aeruginosa*, and the phylogenetic analysis of cultured bacterial isolates showed that they belong to 5 different clades: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes (Table 3). The neighbour joining tree of all the 28 bacterial isolates obtained during the study was computed based on its 16S rRNA sequences (Fig. 11). The numbers at the branches depict the bootstrap values (1000 replications). Alphaproteobacteria included species such as *Brevundimonas* sp. and *Brevundimonas diminuta*. Species like *Ralstonia pickettii*, *Comamonas* sp., and *Hydrogenophaga* sp. obtained represented the Betaproteobacteria. Thirteen species, including *Bacillus cereus*, *B. altitudinis*, *B. pumilus*, *B. xiamenensis*, *B. safensis*, *B. albus*, *Rossellomorea vietnamensis* (*Bacillus vietnamensis*), *Fictibacillus* sp., *Metabacillus indicus*, *Staphylococcus epidermidis*, *S. pasteuri*, *and S. warneri*, identified were Firmicutes. Two species, including *Microbacterium* sp. and *Kocuria marina*, represented the Actinobacteria. Gammaproteobacteria were represented by species such as *Acinetobacter* sp., *Aeromonas* sp., *Pseudomonas* sp., and *Klebsiella variicola*. *Staphylococcus epidermidis* and *Rosellomorea vietnamensis* were the isolates obtained from sample fraction W which were the free-living bacteria not

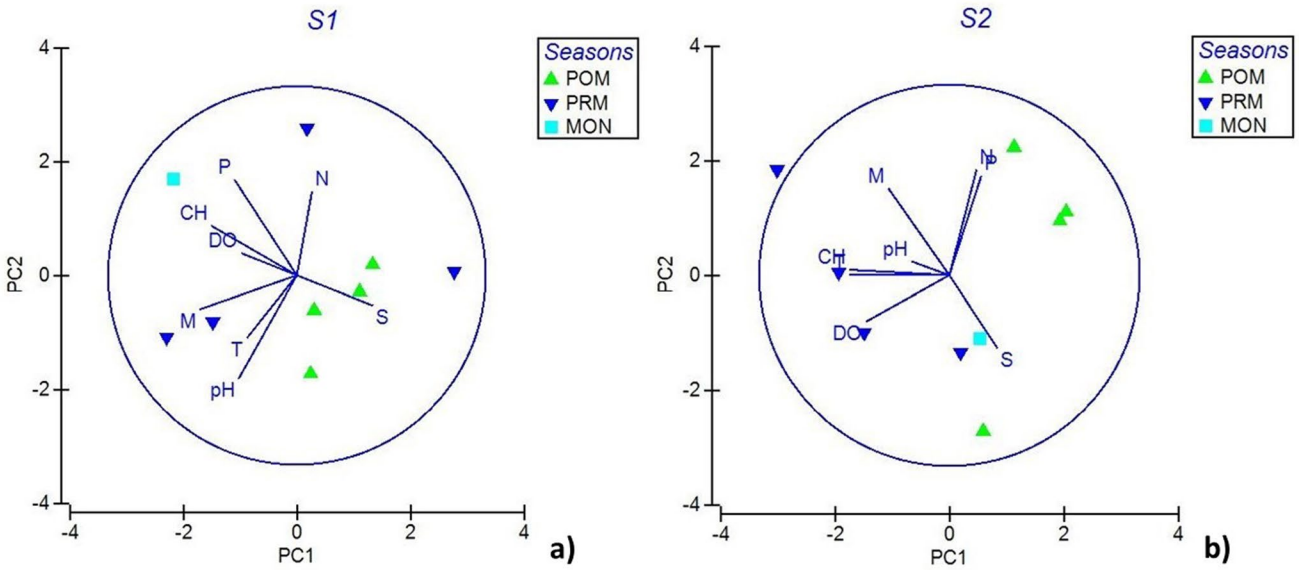

Fig. 9 PCA plot of variations in hydrographic parameters at **a** station 1 (S1) and **b** station 2 (S2). PRM-Pre-monsoon, MON-Monsoon, POM-Post-monsoon, T-Temperature, N-Nitrate, P-Phosphate, S-Silicate, CH- Chlorophyll *a*, M-*Microcystis aeruginosa* cell density, DO-Dissolved oxygen

Table 1 Eigenvalues and cumulative variations of principal component analysis (PCA) station 1

PC	Eigenvalues	% Variation	Cum. % variation
1	2.89	36.1	36.1
2	1.86	23.2	59.3
3	1.48	18.5	77.8

Table 2 Eigenvalues and cumulative variations of principal component analysis (PCA) station 2

PC	Eigenvalues	% Variation	Cum. % variation
1	3.12	38.9	38.9
2	2.74	34.3	73.2
3	1.35	16.9	90.1

associated with the colonies. *Pseudomonas* sp., *Bacillus pumilus*, and *Brevundimonas* sp. were the isolates found commonly from both stations.

Discussion

Microcystis is a well-known toxic bloom-forming cyanobacterium in various aquatic ecosystems with a worldwide distribution (Harke et al. 2016). Eutrophic water conditions with warm temperature enhance *Microcystis* blooms in aquatic systems (Davis et al. 2009). Comprehensive studies on *M. aeruginosa* blooms from south India, predominantly freshwater system along Kerala, are scanty. This study attempts to characterize the bloom dynamics of *M. aeruginosa* and the associated bacterial community along freshwater aquatic environments in south India. Hydrographic conditions like warmer temperature and pH values, low turbulence, buoyancy regulation in the water column, and high nutrient inputs (particularly phosphorus and nitrogen) are the primary factors that enhance the development of cyanobacteria in various aquatic ecosystems, resulting in surface blooms and its accumulation as scum (Oliver and Ganf 2000). The temperature influences bloom dynamics and directly affects the species distribution, activity, and density of bacterial assemblages (Caron et al. 1986). Peak temperature values (above 30 °C) were obtained during the pre-monsoon period in both study stations. *Microcystis* species often predominate during the summer seasons, favoured with higher surface water temperatures, influencing the bloom formation (Imai et al. 2009). The bloom formation and high temperature enhance the growth and metabolism of microorganisms.

pH values recorded from both stations remained alkaline in the range of 8.4 to 9.5 throughout the study period, which was ambient for the succession of *M. aeruginosa* over other phytoplankton. Increased pH levels often favour the growth of *Microcystis*, with the advantage of their dominance over other microalgal species (Yang et al. 2018). An increase in alkalinity facilitates the conversion of aqueous

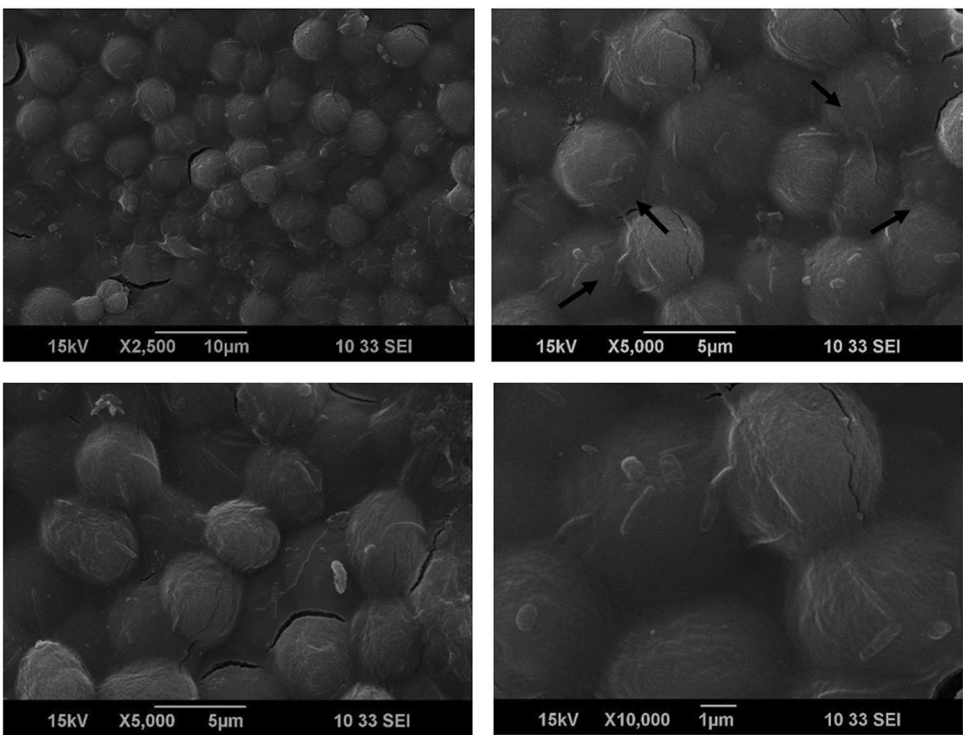

Fig. 10 Scanning electron microscopic images of bacteria attached to the surface of *M. aeruginosa* cells

Table 3 List of the bacterial isolate samples obtained from the study area with their closest species matches from NCBI

Station	Samples	Accession no:	Closest bacteria from NCBI with accession no:	Similarity %	Taxon
S1	S1B12	OQ202324	*Bacillus safensis* WB-12 OP941656	100	Firmicutes
	S1B13	OQ154970	*Pseudomonas* sp. voya 40th634, MT626363	98.79	Gammaproteobacteria
	S1B19	OQ34982	*Acinetobacter* sp. PUCARPATA_27F, MK264767	99.30	Gammaproteobacteria
	S1B22	OQ154971	*Aeromonas* sp. OS6, KC182747	100	Gammaproteobacteria
	S1B25	OQ308977	*Staphylococcus pasteuri* A2-37c-19, JX517276	100	Firmicutes
	S1B31	OQ154982	*Microbacterium* sp. K2NRBAY012, MK110494	100	Actinobacteria
	S1B32	OQ380533	*Kocuria marina* PS01, MK615922	99.76	Actinobacteria
	S1B35	OQ244513	*Ralstonia pickettii* CHP10, MT341804	100	Betaproteobacteria
	S1B41	OQ309004	*Brevundimonas diminuta* JCM13047, LC507944	98.90	Alphaproteobacteria
	S1B44	OQ345992	*Microbacterium* sp. S2-AOM-10, MN179707	99.87	Actinobacteria
	S1B51	OQ202236	*Bacillus pumilus* AS_S2 (sw) OQ1957704	100	Firmicutes
	S1B61	OQ309002	*Bacillus cereus* MI 15, MF360044	99.62	Firmicutes
	S1W51	OQ202253	*Staphylococcus epidermidis* K28-2, OQ225701	99.87	Firmicutes
	S1M	OQ355833	*Microcystis aeruginosa* NIES-90, LC557463	100	Cyanophyceae
S2	S2B14	OQ195902	*Metabacillus indicus* ROA028, MT510170	99.57	Firmicutes
	S2B15	OQ202114	*Klebsiella variicola* M34, MF289160	99.40	Gammaproteobacteria
	S2B16	OQ202117	*Comamonas* sp. EB172, EU847238	99.28	Betaproteobacteria
	S2B17	OQ284062	*Fictibacillus* sp. ZS12W13, MH255990	99.75	Firmicutes
	S2B18	OQ326845	*Bacillus xiamenensis* J8BS8, MT415984	99.74	Firmicutes
	S2B21	OQ326859	*Bacillus albus* BPB24, MK748262	99.87	Firmicutes
	S2B24	OP964604	*Bacillus altitudinis* ASI7, MT318661	100	Firmicutes
	S2B26	OQ2021225	*Bacillus pumilus* AS_S2 (sw) OQ1957704	99.86	Firmicutes
	S2B27	OQ326860	*Hydrogenophaga* sp. 784, MT568553	99.13	Betaproteobacteria
	S2B34	OQ380534	Betaproteobacteria BIWA 25, LC217412	99.65	Betaproteobacteria
	S2B36	OQ284057	*Brevundimonas* sp. C5-8, MT255149	99.87	Alphaproteobacteria
	S2B38	OQ326840	*Pseudomonas* sp. GMCo37, KM370370	99.01	Gammaproteobacteria
	S2B42	OQ345981	*Bacillus* sp. BC2-10, KY625565	99.48	Firmicutes
	S2B43	OQ195931	*Staphylococcus warneri* EMB8, MH267807	100	Firmicutes
	S2W13	OQ202134	*Bacillus vietnamensis* 0028, KP236172	96.55	Firmicutes
	S2M	OQ355832	*Microcystis aeruginosa* NIES-90, LC557463	100	Cyanophyceae

CO_2 to HCO_3^-, which can be absorbed and directly utilized by *Microcystis.* Acidification of water causes a decline in *Microcystis* growth and negatively affects its competitive domination over other green algae (Talling 1976). The excessive and active proliferation of microalgal cells during *Microcystis* bloom could have contributed to the increased amount of dissolved oxygen observed in the surface waters of the study areas. The values, including both stations, ranged from 6.88 to 14 mg/L. Dissolved oxygen slightly declines with the post-bloom period due to the decaying of algal cells (Huang and Chen 2013).

The decreased levels of macronutrients such as phosphorus and nitrogen are considered the limiting factors for the growth of cyanobacteria in freshwater ecosystems. Hence, elevated concentration of these nutrients favours the intense surface bloom of cyanobacteria, resulting in subsequent deterioration of water quality (Paerl et al. 2011). High nutrient load, limited water exchange, and temperature rise in a eutrophic environment encourage the mass proliferation of *Microcystis* (Rejmánková et al. 2004). Increased phosphorus (P) concentrations are thought to be a significant component in eutrophication and cyanobacterial blooms. The frequent usage of soaps as well as detergents in these study areas for domestic purposes, along with natural causes, might have increased the phosphate content that enhanced the development and persistence of *M. aeruginosa* bloom in these freshwater ecosystems, as previously reported (Mohan et al. 2020).

The photosynthetic pigment chlorophyll *a* is widely used to estimate phytoplankton biomass (Felip and Catalan 2000). Chlorophyll *a* is a frequently monitored parameter in water quality assessments, which exhibits a strong positive correlation with microcystin-LR concentrations (Yuan et al. 2014). The chlorophyll *a* content observed was almost positively correlated with the abundance of *M. aeruginosa* at station 1. Even though the chlorophyll value increased with maximum cell density at station 2 during February 2022

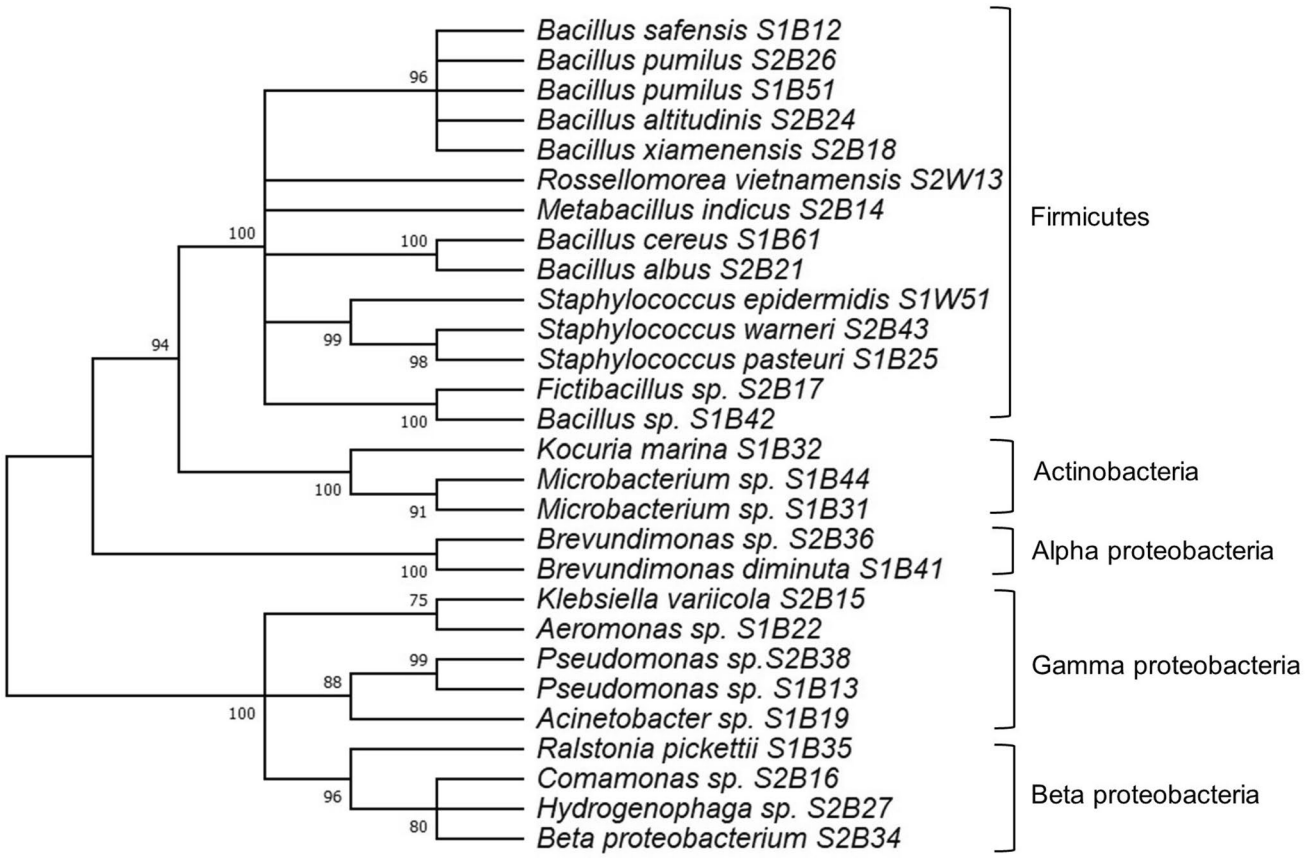

Fig. 11 Neighbour joining tree constructed with all 28 bacterial isolates obtained during the study with bootstrap values greater than 70% from 1000 replicates

(pre-monsoon), an increase in chlorophyll *a* concentration with minimum cell density was also observed during April 2022 (pre-monsoon). This might be due to the presence of other microalgae with *Microcystis*, where they all contribute to the chlorophyll *a* pigment.

The polysaccharide mucilage surrounding the *Microcystis* cells usually harbours several bacterial species (Brunberg 1999). Such a nutrient-rich environment often consists of bacterial communities that are distinct from those present in the free-living community and those in association with other microalgae (Smith et al. 2021; Dick et al. 2021). Both positive and negative associations are observed between heterotrophic bacteria and *Microcystis* colonies. The associated bacteria can absorb the excess organic carbon contributed by *Microcystis* with the exchange of inorganic compounds required by microalgae through the mineralization of various organic substances (Shi et al. 2010). In contrast, some bacterial species possess specific genes which code for enzymes that can significantly degrade various cyanotoxins in aquatic systems (Kormas and Lymperopoulou 2013). The microbiome composition is distinct for each *Microcystis* genotype, and closely related genotypes might have more similar bacterial diversity in their phycosphere (Pérez-Carrascal et al. 2021). This associated bacterial community varies continuously with the development of algal blooms and depends on other environmental factors (Mankiewicz-Boczek and Font-Nájera 2022). Sequencing of the 16S rRNA gene in the present study identified different bacterial isolates belonging to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes, some of which are previously observed in association with *M. aeruginosa* (Shi et al. 2009). Firmicutes dominated the *Microcystis* bloom in both the study areas, followed by Actinobacteria and Gammaproteobacteria at station 1 and Betaproteobacteria at station 2. Species belonging to Firmicutes, Alphaproteobacteria, and Gammaproteobacteria have a significant role in the bloom formation and its development through their influence on the size of the colonies, zooplankton grazing, and vertical migration of *Microcystis* (Wu et al. 2019). Some Alphaproteobacteria can degrade the hepatotoxin microcystin (Dziallas and Grossart 2011). Several bacteria belonging to Alphaproteobacteria can actively uptake organic monomers like amino acids (Cottrell and Kirchman 2000).

The isolate *Brevundimonas diminuta* (closely related to bacterial isolate S1B41) has been reported to have an algicidal effect against *Microcystis*. However, large colony

sizes of *Microcystis* in the upper layers were found to be less prone to algicidal bacteria. Even with high algicidal bacteria, dramatic elevations in *Microcystis* cell numbers have been observed. Although these bacteria could remove the dissolved nitrogen in the culture system, they facilitate the release of dissolved phosphorous (Zhou et al. 2022). Some species of *Brevundimonas* were observed to promote the growth of *M. aeruginosa* (Kim et al. 2020). *Acinetobacter* sp., *Aeromonas* sp., *Pseudomonas* sp., and *Klebsiella variicola* were the Gammaproteobacteria which are the closest genus of the bacterial isolates S1B19, S1B22, S1B13, S2B38, and S2B15, respectively. Among them, *Aeromonas* and *Pseudomonas* were earlier observed within the *Microcystis* colony (Cai et al. 2014; Shi et al. 2010). Many bacterial isolates within Gammaproteobacteria can fix nitrogen (Liba et al. 2006).

Pseudomonas sp. can provide the phosphorous required by *M. aeruginosa* and eventually influence their growth and bloom formation by facilitating phosphorous exchange with *Microcystis* (Jiang et al. 2007). The growth of unicellular *Microcystis* has been reported to be enhanced by strains of *Aeromonas* attached to cyanobacterial bloom (Berg et al. 2009). Species of *Pseudomonas* and *Acinetobacter* release amino acids and 3-indoleacetic acid, which enhances cyanobacterial growth, and *Acinetobacter* also exhibits phosphate-solubilizing activity (Liba et al. 2006). *Pseudomonas* and a few other species of Alphaproteobacteria were observed in the cultures of colony-forming *Microcystis*, which reportedly disappeared upon the disintegration of colonies into single cells, indicating their possible role in maintaining the colony form (Wang et al. 2015). Hydrogen-oxidizing species of *Hydrogenophaga* were observed with *Microcystis* in a minor proportion while studying the bacterial community in the phycosphere of *M. aeruginosa*. The hydrogen gas production by *M. aeruginosa* as a result of fermentation possibly acted as a suitable substrate for *Hydrogenophaga* species (Kim et al. 2020). *Bacillus safensis*, *Bacillus altitudinis*, *Fictibacillus* sp., *Metabacillus indicus*, and *Staphylococcus warneri* (closest genus of the bacterial isolates S1B12, S2B24, S2B17, S2B14, and S2B43, respectively) are the firmicutes identified in association with *Microcystis* bloom. Several *Bacillus* species which can produce 3-methyl-1-butanol resulting in cyanobacterial cell lysis can inhibit the growth of *Microcystis* (Wright and Thompson 1985; Wright et al. 1991). They can decrease the colony size of *Microcystis* probably by degrading the extracellular polysaccharide (Wu et al. 2019) and temperature-dependant degradation of microcystins (Kansole and Lin 2016).

Staphylococcus epidermidis and *Bacillus vietnamensis* were the two isolates among Firmicutes isolated from the filtered fraction of the sample representing the free-living microbes. The *Bacillus* sp. and *Aeromonas* sp. have been observed to be associated with *Microcystis* colonies obtained from different lakes in China (Wang et al. 2016). *Acinetobacter* sp. (Li et al. 2016) and *Bacillus altitudinis* were observed to have algicidal activity against colony-forming *M. aeruginosa* (Hou et al. 2023). *Aeromonas veronii* and *Bacillus cereus* isolated from *Microcystis* mucilage possibly were able to induce colony formation and EPS (extracellular polysaccharides) production in unicellular *M. aeruginosa* (Wang et al. 2016). *Acinetobacter*, *Ralstonia*, and a few other species were reported along with *Microcystis* bloom (Chentaizi River, China), which was suggested to have a possible positive role in forming colonies in *Microcystis* (Dai et al. 2022). *Comamonas* sp. with microcystin-degrading properties was isolated from a bacterial assemblage in eutrophic aquatic systems (Bukowska et al. 2018). The nitrogen-fixing genera, including *Pseudomonas* and *Acinetobacter*, can release amino acids and solubilize phosphates that benefit their host (Shi et al. 2009). Gammaproteobacteria have also been identified in association with declining *Microcystis*. Such bacteria could play a crucial role in influencing the dynamics of microalgal populations and contribute to the rapid decline of harmful algal blooms (Meng-ke et al. 2019).

Actinobacteria have been known to degrade microcystin. *Microbacterium* sp. and *Kocuria marina* (closely related genus to the isolates S1B31, S1B44, and S1B32) come under Actinobacteria, which has been isolated from station 1. Strains of *Microbacterium* sp. capable of degrading the hepatotoxin microcystin were isolated from Daechung reservoir, Korea (Ko et al. 2013). Strains of Actinobacterium belonging to *Microbacterium* genus were isolated from Lake Dianchi, China, which could dissolve insoluble and inorganic phosphate, which in turn could facilitate the growth of *Microcystis* (Zhang et al. 2017). Different bacteria strains that can degrade cyanotoxins are also included within Alphaproteobacteria and Betaproteobacteria (Jones et al. 1994; Park et al. 2001; Saito et al. 2003). Such bacterial strains in aquatic systems can have a crucial part in naturally removing the much more stable cyanobacterial hepatotoxins. Numerous bacteria, including haemolytic strains, have been previously reported from samples containing cyanobacteria. Some of them include known pathogens from Gammaproteobacteria (*Aeromonas*, *Pseudomonas*, *Acinetobacter*), Firmicutes (*Staphylococcus*, *Bacillus*), etc., which are opportunistic with potential harmful health-related risks in humans and animals (Berg et al. 2009). Detailed studies on the identification of *Microcystis*-associated bacterial diversity, their complex interactions, and the hydrographical parameters could provide insight into the mechanisms of bloom formation and its development in the aquatic system.

Conclusion

Microcystis bloom dynamics and colony-associated culturable heterotrophic bacterial communities from two freshwater ponds of central Kerala, south India, were explored during the study. The hydrographic parameters such as warm temperature, increased nutrients (nitrate and phosphate), and alkaline pH in the stagnant waters have elevated the density of *M. aeruginosa* and favoured maintaining the continuous bloom. Changing environmental conditions and anthropogenic effects lead to eutrophication that promotes bloom formation. Cyanobacterial mass occurrences and their toxin can cause ecological, economic, and health problems worldwide. Twenty-eight distinct bacterial isolates belonging to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes were obtained from the colonies of *M. aeruginosa* from the study area, with Firmicutes being the dominant ones. Some of the isolated bacteria were reported to have possible positive interaction with *Microcystis* in previous studies, while few others were reported to have microcystin degrading capability, growth inhibition, etc. Several bacteria isolated from *M. aeruginosa* bloom are potential opportunistic pathogens, which can negatively affect humans exposed to such waterbodies containing cyanobacterial bloom harbouring these microorganisms. However, the bacterial diversity and its role keep changing with each *Microcystis* species responsible for the bloom formation. Understanding such interactions is necessary to provide a basis for unravelling the mechanisms influencing the outbreak of *Microcystis* blooms.

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