Identifying the Microbial Community in the Phycosphere of *Microcystis aeruginosa*

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Abstract

Microcystis aeruginosa is a representative freshwater cyanobacterium and is known to form a phycosphere with other microorganisms to create a mutualism or commensalism relationship. These satellite microorganisms may play an important role for *M. aeruginosa* in the surface bloom formation. We aim to identify the microbial community in the phycosphere of several *Microcystis aeruginosa* cultures. To identify the heterotrophic bacteria living in the phycosphere of different *M. aeruginosa* host strains, heterotrophic bacteria were first isolated with Luria broth agar plates and partial 16S rRNA gene sequence were determined. The 16S rRNA gene sequences were analyzed using MEGA software. The isolated bacteria were members of different classes such as Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Actinobacteria. Majority of the isolates are Plant Growth Promoting Bacteria (PGPB) such as *Rhizobium*, *Sphingomonas*, and *Microbacterium*. Other satellites belonged to lineages known as an inhibitor, such as *Delftia*, or as an indicator for *M. aeruginosa* being in a terminal state, such as *Stenotrophomonas* sp. Microbial diversity within the phycosphere will vary depending on the conditions and state that *M. aeruginosa* is found in. This will influence the *Microcystis*-associated bacteria and could amplify different relationship. Further investigation is required to have an in depth understanding of the different relationships M. *aeruginosa* has with its satellites.

Introduction

- Members of the genus *Microcystis* commonly form a solidified group of cells (colony) ranging from 50-200 µm and are known to form massive toxic blooms (Kim et al., 2019).
- Around solidified cells exists a niche for various bacteria known as a phycosphere.
- *M. aeruginosa* blooms are known to be genetically diverse, and that different bacteria live inside the phychosphere depending on the state of *M. aeruginosa.*
- Phycosphere microbial communities can exhibit diversity across or within species and strains.
- Learning what bacteria are present in the phycosphere allows for increased understanding of their relationship with *M. aeruginosa* and a reason as to why they interact.
- The objective of this research is to identify the phycosphere community of several *M. aeruginosa* strains.

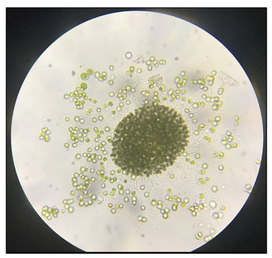

Fig.1. *Microcystis aeruginosa*, *Pseudoanabaena*, green algae, and heterotrophic bacteria aggregate together to form a phychosphere. Sample collected from the Caloosahatchee River.

Table 1. *Microcystis aeruginosa* strains used in this study

Strains	Location	Country	Year	Toxin production
FL10	Caloosahatchee River	United States	2020	toxic
FL11	Caloosahatchee River	United States	2020	toxic
FL12	Caloosahatchee River	United States	2020	toxic
FL13	Caloosahatchee River	United States	2020	toxic
FL14	Caloosahatchee River	United States	2020	toxic
FL16	Caloosahatchee River	United States	2020	toxic
FL17	Caloosahatchee River	United States	2020	toxic
FL19	Caloosahatchee River	United States	2020	toxic
FL26	Caloosahatchee River	United States	2020	N.d
FD2	Caloosahatchee River	United States	2018	toxic
FD4	Caloosahatchee River	United States	2018	nontoxic
AL2	Alva Bridge	United States	2019	toxic
RP1	Rosen Park	United States	2019	toxic
LO8	Lake Okechobee	United States	2018	nontoxic
NIES-87	Lake Kasumigaura	Japan	2018	nontoxic
NIES-88	Lake Kawaguchi	Japan	1981	toxic
NIES-90	Lake Kawaguchi	Japan	2018	toxic
NIES-102	Lake Kasumigaura	Japan	2018	toxic
NIES-103	Lake Kasumigaura	Japan	1978	toxic
NIES-933	Little Rideau Lake	Canada	1954	toxic
NIES-1022	Mitsu Bay	Japan	2001	nontoxic
NIES-1123	Lake Ohnuma	Japan	2018	nontoxic

Methods

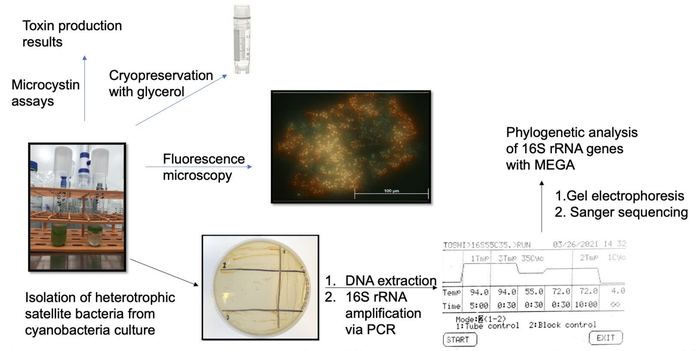

Fig. 2. Heterotrophic bacteria were isolated from *Microcystis* strains to identify species via 16S rRNA gene sequencing. Isolated bacteria were observed under fluorescence microscopy to view host and satellite relationships. Microcystin assay was performed to test the toxin production capability.

Results

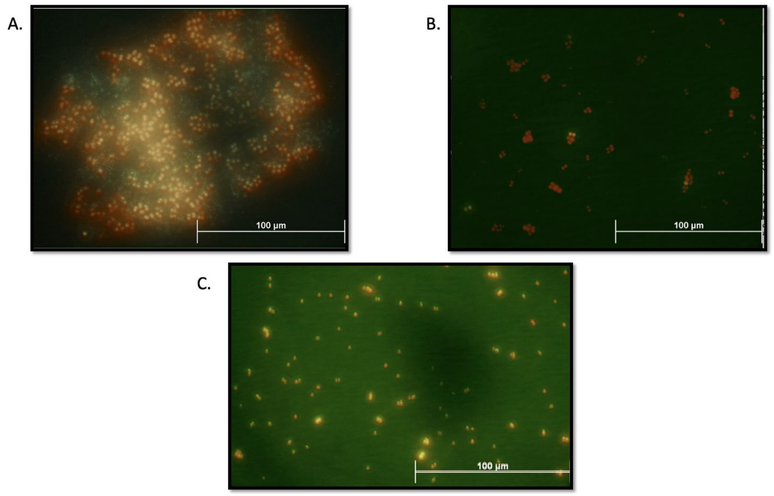

Fig. 3. *M. aeruginosa* and satellite heterotrophic bacteria overlapped fluorescence microscopy photos for samples AL2 (A), FD4 (B), and RP1 (C). Cyanobacteria (red) layer superimposed on surrounding satellite (green) layer. Satellites, including heterotrophic bacteria, are found in higher quantities around AL2 *M. aeruginosa*. FD4 had no significant number of satellites found around the host species. RP1 has a less notable number of satellites in comparison to AL2.

Table 2. YSI water quality measurements. Hydrogen peroxide, microcystin, and chlorophyll *a* were analyzed according to the methods reported by Ndungu et al. (2019)

Location	Coordinates	Date (m/d/y)	Time	Hydrogen peroxide (uM)	Microcystin (ug/L)	Chl a (ug/L)	Iron (mg/L)	Temp (°C)	DO (mg/L)	pH
Fort Denaud	26°44'41"N 81°30'37"W	6/27/18	10:00am	0.27	450.51	72.5	1.32	32.4	3	7.7
Rosen Park	26°62'46"N 81°92'37"W	7/24/18	2:00pm	5.07	24	65.4	4.56	30.4	5.5	7.9
Alva Bridge	26°42'50"N 81°36'36"W	6/27/18	2:00pm	0.83	308.07	100.7	2.43	31	1.6	7.1

Discussion

- Many satellites found were Plant Growth Promoting Bacteria (PGPB), including *Rhizobium* sp. and *Sphingomonas* sp. (Ramanan et al., 2015).
- *Rhizobium* sp. have been shown to reduce oxidative stress in *M. aeruginosa*, in exchange *Rhizobium* sp. consumes the host species carbon source.
- *Sphingomonas* sp. have been found to be a microcystin degrader. *Sphingomonas* numbers will increase to take advantage of host toxin production as a source of nutrients.
- A few Gammaproteobacterial species were found, such as *Stenotrophomonas*, and could be an indicator for the host species being in its terminal stage. *Stenotrophomonas* can provide insight to the host species state and indicate a potential bloom event.
- *Pseudomonas* sp. was expected since it is believed to have an important factor in cyanobacteria blooms but none were found (Jiang et al., 2007); this may be an artefact of laboratory isolation and culturing.
- The relationship between *M. aeruginosa* and the bacterium *Cupriavidus metalliduran* identified is unclear. However, it is possible to infer where there is an abundance of heavy metal, *Cupriavidus metalliduran* may have a presence in the phycosphere, or when *M. aeruginosa* cells lyse during terminal stage and bacteria uptake released nutrients.
- Interestingly an algae inhibitory bacterium, *Delftia* sp., was found which has been demonstrated to reduce *M. aeruginosa* colony size by 36%-51% (Wu et al., 2019). *Delftia* sp. has the protentional to change phycosphere microbial composition by promoting the growth of *M. aeruginosa* from a steady to a terminal state.
- The only Actinobacterial species found was *Microbacterium* sp., which was shown to promote the growth of *M. aeruginosa* during its exponential phase. It was only associated with two toxic strains, NIES-933 and NIES-103.

Conclusion

- Taxonomically diverse *Microcystis*-associated bacteria were identified. They may have different roles within the phycosphere of *M. aeruginosa*.
- Taxonomic positions of satellite bacteria differ depending on host strains.
- This research provides a firmer basis for further investigations to obtain a deeper understanding of this complex network of microorganisms and their interactions and dependence upon *M. aeruginosa*.

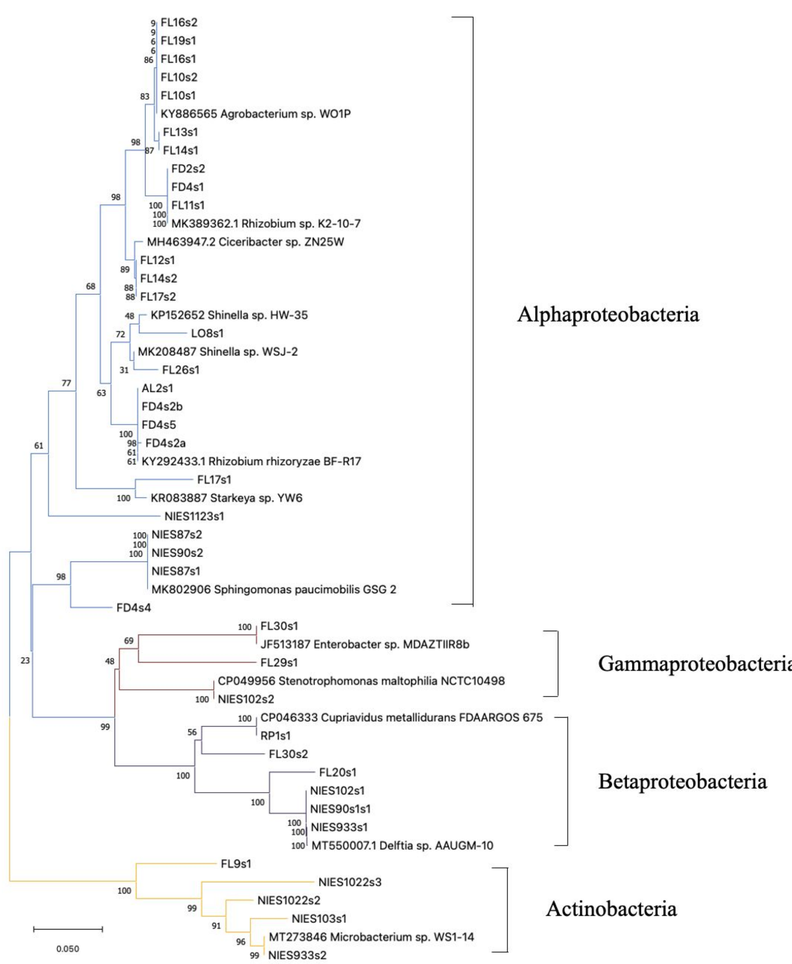

Fig. 4. Neighbor Joining phylogenetic tree based on 16S rRNA genes. Satellites isolated in this study and model reference organisms are shown. Three different classes of Proteobacteria were identified: Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria. Actinobacteria were also found. Numbers near the nodes show bootstrap values.

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