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ORIGINAL ARTICLE

Morphological changes of *Microcystis aeruginosa* colonies in culture

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ABSTRACT

We investigated the changes in the morphology, genetics and photosynthetic characteristics of Microcystis aeruginosa *colonies during two months of cultivation. The colonies were collected in Lake Taihu, kept under 25°C on a 12h:12h light/dark cycle at a light density of about 45 μmol* m^{-2} s^{-1}. M. novacekii*-type colonies were detected on the* 10^{th} *day and their percentage of the population tended to increase until the* 60^{th} *day. With* M. novacekii*-like colonies increased, the proportion of* M. aeruginosa *colonies decreased and reached almost zero by the end of the experiment.* M. novacekii*-like colony size (*D_{50}*; this value indicates that particles below* D_{50} *accounts for 50%) was greater than 500 μm when these colonies first appeared and was similar to that of* M. aeruginosa *in the experiment. No differences in cell size were found between these two* Microcystis *morphotypes either collected from Lake Taihu or cultured in the laboratory. Through molecular tools (16S rDNA, 16S-23S ITS and* cpc*BA-IGS), there were strong evidences to claim that the original* M. aeruginosa *colonies isolated from Lake Taihu and the later* M. novacekii*-like colonies in our cultures were the same species. We suggest that* M. aeruginosa *colonies consistently changed their colonial morphology to that of a typical* M. novacekii.

Key words: Microcystis aeruginosa, Microcystis novacekii, morphospecies, morphological changes, seasonal succession.

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INTRODUCTION

Frequent cyanobacterial blooms cause serious ecological and environmental problems (Paerl *et al.,* 2001; Otten and Paerl, 2011). *Microcystis* spp. is one of the most widely distributed and harmful bloom-forming cyanobacteria (Cao *et al.,* 2005; Sivonen and Jones, 1999). *Microcystis* form large irregular or quasi-spherical colonies in freshwater ecosystems and several species (*e.g.*, *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Microcystis ichthyoblabe* and *Microcystis novacekii, etc*.) have been identified based on their morphological characteristics (Desikachary, 1959, Komárek, 1991; Komárek and Komárková, 2002).

Seasonal succession of *Microcystis* morphospecies has been reported in varying inland waters. These inland waters included Lake Suwa (Park, 1993), Lake Biwa (Ozawa *et al.,* 2005), an eutrophic pond in Japan (Yamamoto *et al.,* 2009), Lake Chaohu (Jia *et al.,* 2011), and Lake Taihu (Li *et al.,* 2013b). The dynamics of the seasonal succession of *Microcystis* morphospecies are important in understanding the occurrence of *Microcystis* blooms and in evaluating the risk of toxic microcystins, because growth and toxin production vary among morphospecies (Watanabe *et al.,* 1988, 1989; Imai *et al.,* 2009).

Our previous study (Li *et al.,* 2014a) argued that the seasonal succession of *Microcystis* morphospecies could be explained by colonial morphological changes induced by mucilage solubilization. Patterns of colonial morphological changes were summarized as *M. ichthyoblabe*, *M. wesenbergii* and *M. aeruginosa* successively, which was similar to the succession patterns of *Microcystis* noted in field studies. Merely, the morphological variation after *M. aeruginosa* needs further investigation. Moreover, the results of our previous study were obtained after *Microcystis* colonies were soaked in deionized water in darkness at 4°C (Li *et al.,* 2014a), which differed from common culture conditions and the data were not conclusive. Thus, it was necessary to investigate the morphological changes of *Microcystis* in culture. Otsuka *et al.* (2000) reported that the morphology of *M. wesenbergii* changed to that of *M. aeruginosa* under culture condition. Nevertheless, the knowledge of morphological changes of *Microcystis* colonies was still insufficient.

Phenotypic plasticity of *Microcystis* has been well reported (Cao and Yang, 2010; Yang *et al.,* 2008). It is well known that *Microcystis* colonies usually lose their characteristic features, disaggregate to single cells after long-term culture (Zhang *et al.,* 2007). Conversely, it has been found that specific genotypes of freshwater single-cell *Synechococcus* can form microcolonies, transitional forms towards colonial cyanobacteria, as a fast response to UV radiation (Callieri *et al.,* 2011, 2012). Several re-

searchers (Yang *et al.,* 2005; Shen *et al.,* 2011) tried to induce colony formation of unicellular *Microcystis* by changing various factors under culture conditions. Many biotic and abiotic factors (*e.g.*, zooplankton, bacteria, light, temperature, nutrient level) were reported effective in inducing colony formation of *Microcystis* (Yang *et al.,* 2008; Shen *et al.,* 2011; Yang and Kong, 2013; Li *et al.,* 2013c). However, the morphology of induced colonies was still different from that of *Microcystis* colonies in field. The investigation of the morphological changes of *Microcystis* under culture conditions would be helpful to understand the difference in colonial morphology between colonies induced under culture conditions and in the field.

Combined with the descriptions above, it is necessary to investigate the morphological changes of *M. aeruginosa* under culture condition. In the current study, *M. aeruginosa* colonies collected in Lake Taihu were cultured. Both the morphology and photosynthetic characteristics of *Microcystis* were investigated. The photosynthetic characteristics were analyzed to check whether cells were in normal activity. If so, the morphological changes occurred in the cultures would be not induced by stress.

METHODS

Colony collection and culture experiment

Microcystis colonies were collected in Meiliang Bay (31°30.06′ N; 120°10.97′ E) in Lake Taihu, southeast China, on 20th October 2013 (water temperature was 18°C, wind speed was less than 3 m s^{-1}) when *M. aeruginosa* was the dominant *Microcystis* morphospecies (Li *et al.,* 2013b). Water at the depth of 30 cm below lake surface was collected by a sampling barrel and then a phytoplankton net (63 μm mesh size) was used to entrap the colonies. The collected colonies were put into 500-mL plastic bottles and taken back to the laboratory immediately.

Colonies were dispersed by BG-11 culture medium. Each colony was observed under a CX31 optical microscope. Only colonies with the morphological characteristics of *M. aeruginosa* were collected for the culture experiment. The selected colonies were cultured in triplicate in 75 mL BG-11 medium in 100-mL conical flasks at 25°C under a 12h:12h L/D cycle with a photon flux density of about 45 μmol m^{-2} s^{-1} provided by daylight fluorescent lamps. Cell density was counted after incubation following the methods described by Li *et al.,* (2013c) and the initial cell density was $1.3\pm0.2\times10^5$ cells mL^{-1}.

Analysis of colonial morphology and colony size

The morphology of *Microcystis* including colonial morphology, colony size, and cell size was analyzed every 10 days. Each sample was shaken gently and thoroughly and a 5-mL sample was drawn out to analyze colonial morphology. Microphotographs were taken using a CX31 optical microscope (100×) equipped with an Olympus C-5050 digital camera to determine the morphological composition and the colony size. Cell size was also analysed. Colonies were divided into *M. aeruginosa* and other morphotypes based on the colonial morphological characteristics listed in Tab. 1 referring to Watanabe (1996) and Komarek and Anagnostidis (1999).

The photomicrographs were analyzed using UTHSCSA Image Tool Version 3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA) to measure colony size. About 100 colonies of each sample were analyzed each time. As colonies of different morphospecies were irregular, the longest axis (the longest length of each colony) and the shortest axis (the longest length that is perpendicular to the longest axis) of each colony was measured. The colony diameter (μm) was estimated as:

$$R = \sqrt{R_1 \times R_2} \qquad \text{(eq. 1)}$$

where R_1 is the longest axis, and R_2 is the shortest axis.

Biovolumes of measured colonies were calculated from colony size using a sphere volume calculation formula. The percentages of different *Microcystis* morphotypes and D_{50} (the colony size at which 50% of the total mass of the particles was smaller than this size; Li *et al.,* 2014b) were calculated using biovolume.

Tab. 1. Colony characteristics of *M. aeruginosa* and other morphotypes.

Morphospecies	Colony characteristics
M. aeruginosa	Colonies are more or less firm, more or less spherical, elongate, lobate, or reticulated. Margin of colony is indistinct. Cells are somewhat densely and irregularly agglomerated
M. novacekii	Colonies are microscopic, composed of subcolonies, almost spherical, no lobate, composed of tightly aggregated cells and a thick surrounding gelatinous substance
M. ichthyoblabe	The distribution of cells in colonies is homogeneous, irregular, or sponge-like, and the margin of the colony is regular and invisible without treatment

According to Watanabe (1996) and Komarek and Anagnostidis (1999).

Cell size analysis

After microphotographs of colonies were taken, about 10 *Microcystis* colonies (with the same colonial morphological characteristics) from the 5-mL sample were randomly isolated. Colonies with the same morphological characteristics were put together and disaggregated using the alkaline hydrolysis process described by Joung *et al.,* (2006). After that, the microphotographs of unicellular cells were taken using a CX31 optical microscope (1000×) equipped with an Olympus C-5050 digital camera. UTHSCSA Image Tool Version 3.00 software was used to analyze the cell diameter. The diameters of 200 cells were measured and the maximum, minimum, and average diameters of different morphotypes were calculated.

Photosynthetic parameters

The photosynthetic characteristics of *Microcystis* were analyzed by Phyto-PAM (Walz, Effeltrich, Germany) to determine the activity of *Microcystis* colonies at the beginning and at the end of the experiment. Accurate 10 mL medium from each sample was taken and mixed, then a 3-mL mixed sample was taken for analysis. The sample was kept in the dark for 10 min, then the F_v/F_m (maximum quantum yield of PSII) and the rapid light curve (RLC) was determined. The three characteristic parameters of the RLC were analyzed: the slope of the curve in the light-limiting region (a) that is proportional to the efficiency of PSII (Schreiber, 2004), the maximum electronic transport rate (ETR_{max}), which determines the maximum photosynthetic capacity of phytoplankton (Schreiber, 2004), and the saturating irradiance (E_k) that represents the capacity of phytoplankton to tolerate short-term changes in light (Ralph and Gademann, 2005).

PCR amplification and sequencing

DNA for polymerase chain reaction (PCR) templates was extracted from *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies at the end of the experiment by using the modified procedure (Zhou *et al.,* 1996). *Microcystis* pellets were dispersed in 0.8 mL extraction buffer (1.5 M NaCl, 1% CTAB, 100 mM Tris-HCl, 100 mM Na_2EDTA, 100 mM Na_3PO_3, pH 0.8) and 20 μL proteinase K (30 mg mL^{-1}), incubated at 37°C for 30 min. Thereafter, 480 μL 20% SDS was added to each sample, which was then incubated at 65°C for 1 h. Samples were extracted using phenol-choroform-isoamyl (25:24:1) and choroform-isoamyl (24:1) successively. Centrifuged at 8000×g for 5 min, the supernatant was moved to new tubes. Afterwards, 0.6 mL 100% isopropyl alcohol was added to purify the DNA sample. Centrifuged at 16,000×g for 20 min, the DNA sample was then rinsed with 70% ethanol. Each DNA sample was dried and dissolved in 100 μL TE. The quantity and quality of DNA were determined by Nanodrop-2000.

Six pairs of primers were used for amplification and sequencing of all samples. The reactions were performed in a volume of 50 μL, containing 25 μL 2× mixture buffer (Bioteke, Beijing, China), 2 μL DNA (20 ng μL^{-1}), 1.2 μL Forward primer (10 μM), 1.2 μL Reverse primer (10 μM) and 20.6 μL ddH_2O. The amplification program was set at 94°C for 5 min, followed by 34 cycles of 94°C for 50 s, 50°C for 50 se (MSR1, MSR2, MSR3 and ITS) or 52°C for 50 s (*cpc*BA-IGS), and 72°C for 60 s followed by a final extension at 72°C for 10 min. The amplified products were purified by a Gel Recovery Purification Kit (AxyPrep™, Union City, CA, USA) and sequenced in Shanghai Sunny Biotechnology Co., Ltd.

Tab. 2 shows the primers applied in the current study which were cited by Otsuka *et al.,* (1998, 1999a) and Neilan *et al.,* (1995). MSR1, MSR2, MSR3 and *cpc*BA-IGS were used for amplification and sequencing, ITS (A) for 16S-23S ITS amplification, ITS (S) for 16S-23S ITS sequencing.

Alignment and phylogenetic analyses

Using CLUSTAL X 2.0, we aligned 16S rDNA sequences isolated in this study with those of 9 reference strains for which morphospecies classification appears reliable (Otsuka *et al.,* 1998). We similarly aligned 16S-23S ITS isolated in this study with those of 8 reference strains from Otsuka *et al.* (1999a) and *cpc*BA-IGS sequences with those of 4 reference strains from Wu *et al.* (2007) and 3 from the NCBI GenBank database. Maximum like-

Tab. 2. List of six pairs of primers for amplification and sequencing in *Microcystis*.

Primer	For sequence (5'-3')	Rev sequence (5'-3')	Reference
MSR1	TTGATCCTGGCTCAGGATGA	GTCGTTAAGCAACCTGATTTG	Otsuka *et al.,* 1998
MSR2	CGGTAATACGGGGGAGGCAA	CCAACATCTCACGACACGAG	Otsuka *et al.*, 1998
MSR3	CTGGTGAAAGCTGGGGGTGC	GGTTACCTTGTTACGACTTC	Otsuka *et al.,* 1998
ITS(A)	TCAGGTTGCTTAACGACCTA	(G/T)TTCGCTCGCC(A/G)CTAC	Otsuka *et al.*, 1999
ITS(S)	CCAGTGAAGTCGTAACAAGG	GGGTT(T/G/C)CCCCATTCGG	Otsuka *et al.,* 1999
*cpc*BA-IGS	GGCTGCTTGTTTACGCGACA	CCAGTACCACCAGCAACTAA	Neilan *et al.*, 1995

lihood tree of phylogeny analysis was constructed by software MEGA 5.0 (Tamura *et al.*, 2011), with the parameters being bootstrapped for 1000 replications.

RESULTS

Morphological variability of colonies in culture

Most of the colonies maintained the morphological characteristics of *M. aeruginosa* (Fig. 1a) for 10 days. Then, some colonies showed morphological variations (Fig. 1b), which gradually dispersed into small subcolonies with cells aggregated tightly. Thirty days later, the colonies were discontinuously elongated and resembled the morphotype of *M. novacekii* (Fig. 1c). After the first 60 days, the colonies became *M. novacekii*-like, which composed of small and firm subcolonies (Fig. 1d). In addition, some of these colonies were surrounded by thick, refractive, gelatinous substance that is characteristic of *M. novacekii*. After 65 days, the aggregation of cells in subcolonies became soft and the colonies gradually began to disperse to single cells (Fig. 1e). Nevertheless, some *M. aeruginosa* colonies had became homogeneous or sponge-like that is the characteristic of *M. ichthyoblabe* (Fig. 1f).

The composition of *Microcystis* morphotype during the experiment changed greatly over time (Fig. 2). Samples were dominated by *M. aeruginosa* in the first 30 days. The percentage of *M. aeruginosa* decreased throughout the culture, and became <50% after 40 days. Meanwhile, the proportion of the intermediate morph between *M. aeruginosa* and *M. novacekii* also changed, increasing from 0 to 48% in the first 40 days. Subsequently, *M. novacekii*-like colonies dominated from the 50th to 60th day evidently. And some *M. ichthyoblabe*-like colonies that were irregular or sponge-like existed throughout the experiment, but even the highest proportion of these colonies was much smaller than that of *M. novacekii*-like colonies on the 60th day.

Colony size of *Microcystis*

When *M. novacekii*-like colonies appeared on the 10th day, the D_{50} value of them was more than 500 μm, which was similar to that of *M. aeruginosa* colonies (Fig. 3). From then on, the size of *M. novacekii*-like colonies was always larger than that of *M. aeruginosa* along with the increase of proportion of *M. novacekii*-like colonies.

Photosynthetic activity of *Microcystis*

To characterize the photosynthetic characteristics of the algae, F_v/F_m and rapid light curve of these samples was tested at the beginning and the final phase (after culturing for 70 days). The F_v/F_m of the initial *Microcystis* colonies

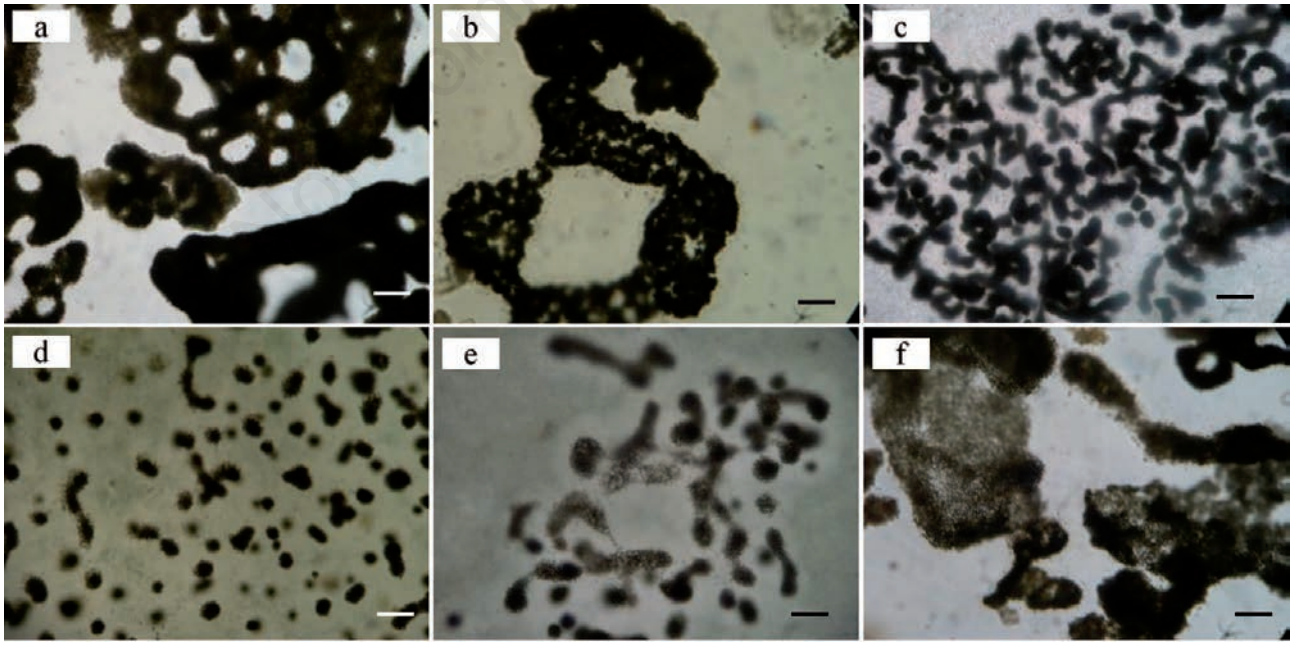

Fig. 1. Morphological variability of *M. aeruginosa* colonies in culture. a) A colony of *M. aeruginosa* which was picked out for culture experiment. b) A colony, which was cultured for 10 days, gradually dispersed into small subcolonies. c) A colony, which was cultured for 30 days, had elongated and discontinuous subcolonies. d) A colony, which was cultured for 60 days, was composed of small and firm subcolonies. e) A colony, which was cultured for 65 days, was less tightly arranged. F) A soft colony of *M. aeruginosa* on day 10 had sponge-like subcolonies. Scale bars: 100 μm.

was 0.51, and the value on the 70th day was 0.24. The slope of the linear section (α) was changed from 0.232 to 0.115. The saturating irradiance (E_k) varied from 352.6 to 773.5 μmol·m^{-2}·s^{-1}, and the maximum electron transport rate (ETR_{max}) was from 81.6 to 88.8 μmol·m^{-2}·s^{-1} (Fig. 4).

Comparison of morphological characteristics

According to the *Microcystis* colony characteristics described in Tab. 1, morphological characteristics (colony form, mucilage structure, cell distribution, colony size, and cell size) of different morphotypes were compared in detail (Tab. 3). All cells of these *Microcystis* morphospecies were spherical, and their diameters were very similar. Additionally, the mucilage of most colonies (except for some *M. novacekii*-like colonies) was invisible without treatment. In culture, the size of *M. aeruginosa*, *M. novacekii*-like colony as well as that of the intermediate morph was similar.

16S rDNA and 16S-23S ITS sequences

M. aeruginosa colonies at the beginning and *M. novacekii*-like colonies on the 60th day of our experiments were sequenced. The length of 16S rDNA and 16S-23S ITS were 1426 bp and 360 bp, respectively. The phylogenetic tree based on the analysis of sequences of the 16S rDNA and 16S-23S ITS showed that *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies at the end of our experiments formed a defined cluster (Figs. 5 and 6), which located within the major *Microcystis* cluster.

*cpc*BA-IGS sequences

A length of 663 bp of *cpc*BA-IGS sequences was identified. Tab. 4 showed that the similarity of *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies on the 60th day of our experiments was 99.2, which was higher than that of *M. aeruginosa* FACHB 936 and *M. aeruginosa* FACHB 912 (99.0%) and that of *M. novacekii* UAM259 and *M. novacekii* UAM258 (96.0%). This result suggested that *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies at the end of our experiments be the same species.

DISCUSSION

Our experiment showed that *M. novacekii*-like colonies appeared after the 10th day. The percentage of these colonies tended to increase until the 60th day. Along with the increase of the percentage of these colonies, the percentage of *M. aeruginosa* decreased and reduced to almost zero by the end of the experiment (Figs. 1 and 2). Colony size (D_{50}) of *M. novacekii*-like colonies was >500 μm when they firstly appeared on the 10th day which was

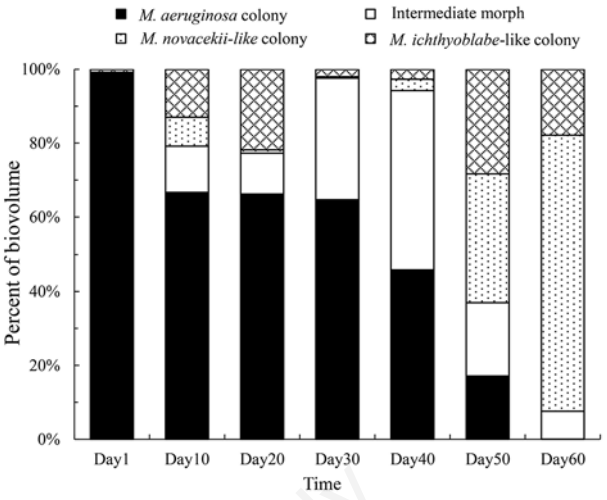

Fig. 2. Composition of *Microcystis* morphotypes in culture at different times.

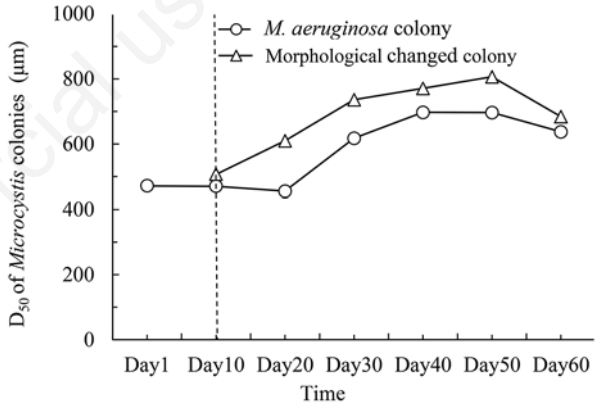

Fig. 3. The variation of D_{50} of *M. aeruginosa* and *M. novacekii*-like colonies in culture.

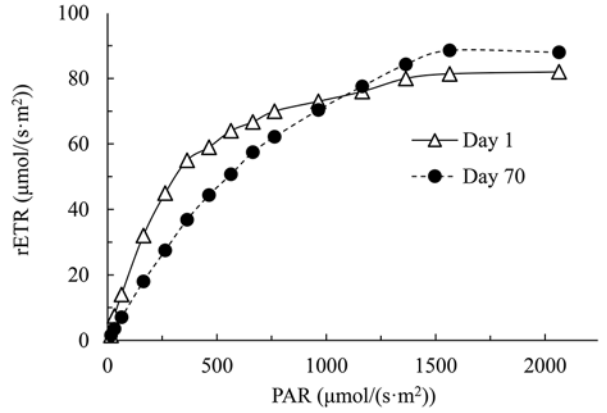

Fig. 4. Rapid light curve of *Microcystis*. The relative electron transport rate is plotted against the PAR irradiance (μmol m^{-2} s^{-1}).

similar to that of *M. aeruginosa* in culture. Our previous work showed that colonies with a maximum size of 100 μm formed after culturing *Microcystis* for 14 days under standard conditions (Li *et al.,* 2013c). Yang and Kong (2012) also reported that the diameter of some colonies reached over 180 μm during 50 days of culture. Even though the interval of morphological investigation in the current study was long and the first time *M. novacekii*-type colonies appeared was not precisely determined, it can still be inferred that unicellular *Microcystis* could not have formed such large colonies (500 μm) in 10 days. Thus, the appearance of *M. novacekii*-like colonies was not generated by cell growth, but from morphological changes of the existing *M. aeruginosa* colonies.

It was proposed that algal cell size is useful for the discrimination of *Microcystis* morphospecies (Desikachary, 1959; Komárek, 1991). We also analysed cell size of both *M. aeruginosa* and *M. novacekii*-like colonies in our experiment (Tab. 3), but the result showed that the cell size of them was similar. This result also supported the conclusion that the appearance of *M. novacekii*-like colonies could be due to morphological changes in the existing *M. aeruginosa* colonies.

By using molecular tools (16S rDNA, 16S-23S ITS and *cpc*BA-IGS), there were stronger evidences to claim that the original *M aeruginosa* colonies isolated from Lake Taihu and the later *M. novacekii*-like colonies in our cultures were the same genospecies. Even though, the similarity of 16S rDNA sequences cannot be used to prove that the two *Microcystis* were actually the same species

Tab. 3. Colony characteristics of cultured *Microcystis* morphotypes.

Morphotypes	Colony characteristics
M. aeruginosa	Colonies are irregularly branched or reticulated, varying greatly in size (500-1200 μm). In Lake Taihu, can be as small as 70 μm. Mucilage is invisible without treatment. Cells are somewhat densely and spherical. The cell diameter is 1.7 5.9 μm, and the average diameter is 3.3 μm. (Fig. 1a)
Intermediate morph	Colonies (160-1400 μm) keep the framework of *M. aeruginosa*, contain some subcolonies that connected to each other unobtrusively. The cell diameter is 1.8-5.3 μm, and the average diameter is 3.6 μm. (Fig. 1b)
M. novacekii-like morph	Colonies are composed of several or even hundreds of subcolonies, varying greatly in size (60-1600 μm). Cells are tightly aggregated, later becoming soft. The cell diameter is 1.9-5.6 μm, and the average diameter is 3.5 μm. Margin of colony is indistinct (Fig. 1c)

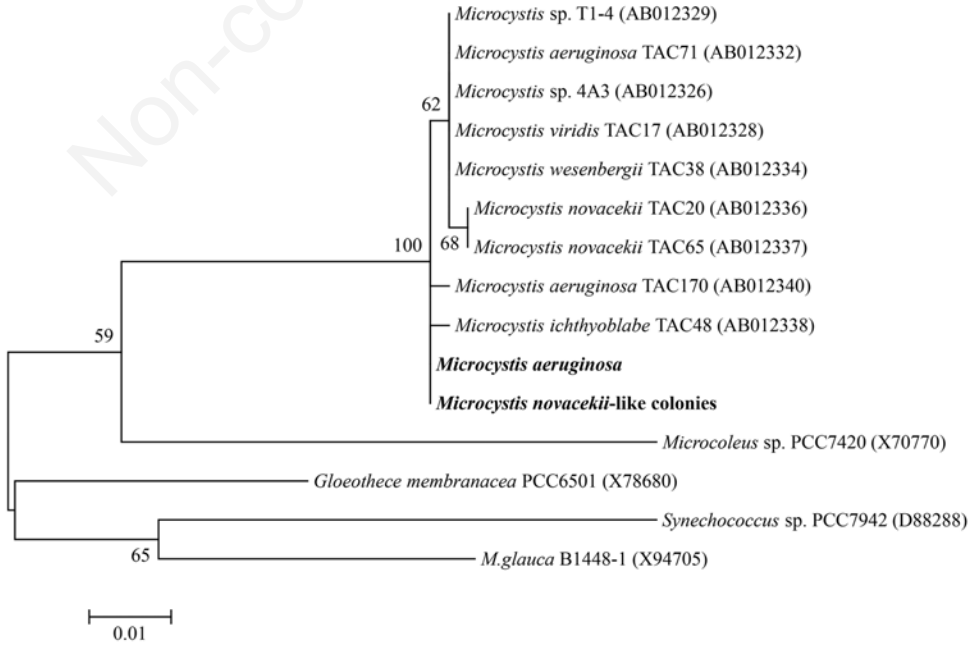

Fig. 5. Phylogenetic tree based on analysis of sequences of the 16S rDNA. The bold characters indicated sequences of *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies on the 60th day in the current study.

(Otsuka *et al.,* 1998; Xu *et al.,* 2014), the use of 16S-23S ITS and *cpc*BA-IGS (Otten and Pearl, 2011; Tan *et al.,* 2010) gave our results more robustness.

Both culture temperature (25°C) and nutrient concentration in the current study was higher than that in Lake Taihu during sampling. These differences will affect colonial morphology of *Microcystis* (Li *et al.,* 2013c: Yang and Kong, 2013). However, this will not affect the results of morphological changes of *M. aeruginosa* under culture condition because *Microcystis* grew well under this condition. Li *et al.* (2013a) measured the photosynthetic activity of *Microcystis* in Lake Taihu in the middle of autumn, and found that the α values were 0.085-0.27, and the ETR_{max} and E_k values were 22.3-202.7 μmol m^{-2} s^{-1} and 168.6-651.7 μmol m^{-2} s^{-1}, respectively. The three characteristic parameters of the RLCs obtained in our study were similar to those in the above field investigation. The F_v/F_m of *Microcystis* changed from 0.51 to 0.24, which means the photosynthetic efficiency of the algae on the 70th day was lower. However, F_v/F_m of *Microcystis* colonies in Lake Chaohu was as low as 0.25 (Wu *et al.,* 2007). Therefore, even if F_v/F_m of *Microcystis* colonies during the experiment decreased eventually was more similar to the colonies in the lake. With our study we proposed that *M. aeruginosa* colonies can change their morphological characteristics and turned into *M. novacekii*-like colonies, if cultivated under favourable growing conditions.

During the experiment, some soft and sponge-like colonies were also found. These colonies did not show the morphological characteristics of *M. novacekii* but had similar morphological characteristic of *M. ichthyoblabe*. However, the proportion of these colonies were much lower than that of *M. novacekii*-like colonies. It was reported that *Microcystis* colonies lost their characteristic features easily and disaggregated to single cells after long-term culture (Zhang *et al.,* 2007). These *M. ichthyoblabe*-like colonies

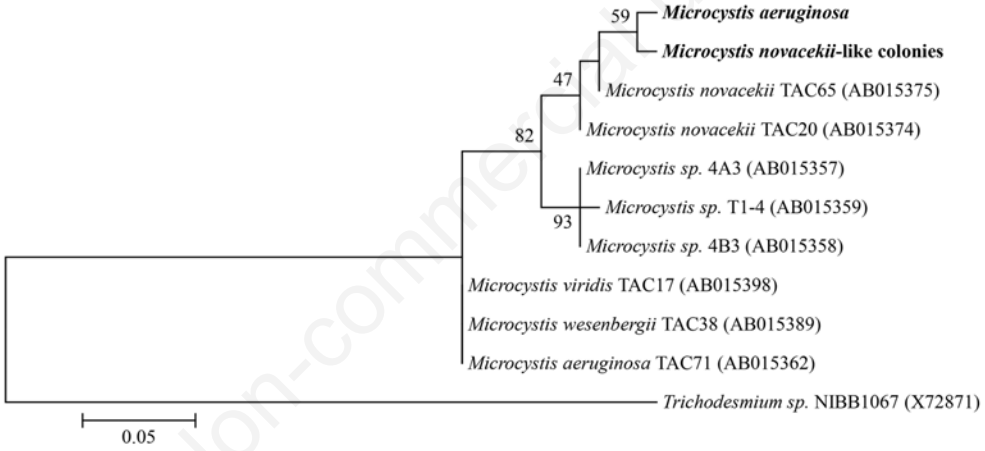

Fig. 6. Phylogenetic tree based on analysis of sequences of the 16S-23S ITS. The bold characters indicated sequences of *M. aeruginosa* colonies at the beginning and *M. novacekii*-like colonies on the 60th day in the current study.

Tab. 4. Similarities between each *Microcystis* strain based on *cpc*BA-IGS sequences.

	1	2	3	4	5	6	7	8	
									1-*M. aeruginosa*
2	99.2								2-*M. novacekii*-like colonies
3	98.6	99.0							3-*M. aeruginosa* FACHB-936
4	99.0	99.8	99.0						4-*M. aeruginosa* FACHB-912
5	95.0	94.9	94.6	94.8					5-*M. novacekii* UAM259
6	97.6	98.0	98.5	97.9	93.5				6-*M. virids* NIES-102
7	99.0	99.8	99.0	100.0	94.6	98.4			7-*M. sp.* FACHB-574
8	98.4	99.2	99.0	99.5	94.2	98.0	99.5		8-*M. aeruginosa* UWOCC001
9	98.1	97.4	96.9	97.2	96.3	96.0	97.2	96.5	9-*M. novacekii* UAM258

The underlined characters are referred to the sequences of this study as M. aeruginosa *colonies at the beginning and* M. novacekii-*like colonies on the 60th day in the current study.*

would be due to disaggregation of *M. aeruginosa* colonies, and are the result of a well known phenomenon. Conversely, the *M. novacekii*-like colonies were formed by morphological changes of *M. aeruginosa* colonies, and this is a new interesting finding. We also analysed the proportion and the morphological characteristics of *M. novacekii* in Lake Taihu during our routine monitoring (Li *et al.,* 2013b). *M. novacekii* appeared mainly from September to November when *M. aeruginosa* was the dominant *Microcystis* in Lake Taihu. The percentage of *M. novacekii* was very small (0.4-1.1%) and the colony size was 50-400 μm, both were much smaller than those of *M. aeruginosa. Microcystis* declined with biomass decrease and colony disaggregation during this time (Wang *et al.,* 2012). The increase of space between subcolonies in *M. novacekii* made colonies more susceptible to disperse from wind shear under natural conditions. As a result, the composition of *M. novacekii* in Lake Taihu was fairly low and its colony size was much smaller than that under culture conditions without turbulence. A mass of small colonies found during this period (Li *et al.,* 2013b) would be a circumstantial evidence for the disaggregation of *Microcystis* colonies. Moreover, *M. novacekii* and *M. aeruginosa* colonies appeared in the field at the same time when masses of small colonies were also found. Thus, we suggested that *M. novacekii* colonies were derived from the morphological changes of *M. aeruginosa*.

Otsuka *et al.,* (2000) described three other morphotypes of *M. novacekii* in his study: i) colonies were discontinuously elongated; ii) colonies were lobated, elongated, or reticulated and resembled the morphotype of *M. aeruginosa*; and iii) colonies were less tight and nonhomogeneous or soft and homogeneous. Since *M. novacekii* can show several colony-forms corresponding to several morphospecies, such as *M. aeruginosa*-type or *M. ichthyoblabe*-type colonies, it is difficult to distinguish the *M. novacekii* morphospecies from others. In our study, we also found some colonies that were soft and homogeneous, but classified them as *M. ichthyoblabe*-like colonies based on morphospecies level taxonomy.

It was challenging to evaluate the seasonal succession of *Microcystis* morphospecies in the literature because the morphological criteria for *Microcystis* classification was confused. Colonies of different morphospecies may have similar morphological characteristics (Otsuka *et al.,* 2000; Komárek and Komárková, 2002). Culture experiments also showed that *Microcystis* colonies sometimes varied their characteristics to resemble those of other morphospecies (Otsuka *et al.,* 2000; Nguyen *et al.,* 2012). Besides, taxonomy based on the morphological characteristics of *Microcystis* was not supported by phylogenetic analysis based on 16S rRNA, 16S-23S ITS sequences and *cpc*BA-IGS (Otsuka *et al.,* 1999b; Nguyen *et al.,* 2012). There is a limit to the botanical taxonomy of *Microcystis*, and the different morphospecies might be morphological variations of the same *Microcystis* genotype.

CONCLUSIONS

M. aeruginosa colonies collected in Lake Taihu were cultured under culture conditions. *M. novacekii*-like colonies were detected on the tenth day and its percentage of the population tended to increase until the 60th day. As *M. novacekii*-like colonies increased, the percentage of *M. aeruginosa* decreased and almost disappeared by the end of the experiment. By using molecular tools (16S rDNA, 16S-23S ITS and *cpc*BA-IGS), there were stronger evidences to claim that the original *M. aeruginosa* colonies isolated from Lake Taihu and the later *M. novacekii*-like colonies in our cultures were the same species. We suggested that *M. aeruginosa* colonies consistently changed their colonial morphology to that of a typical *M. novacekii*.

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