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Molecular diversity of *Microcystis* strains (Cyanophyceae, Chroococcales) based on 16S rDNA sequences

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Abstract. *– Partial 16S rDNA sequences were determined for six* Microcystis *strains from lakes in the region of Plön (Northern Germany) assigned to four different species on the basis of their morphology. These sequences appear very similar to each other and to those of 71* Microcystis *strains from four continents available in the databases. This great genotypic homogeneity, as measured by 16S rDNA sequence similarity (334 characters), is contrasting with the conspicuous morphological differences observed for the studied strains.*

Keywords: Microcystis, *Cyanophyceae, Cyanobacteria, 16S rDNA*

Abbreviations: *PCR, Polymerase Chain Reaction; rDNA, ribosomal DNA; ITS, Internally Transcribed Spacer; BSA, Bovine Serum Albumin*

Introduction

The genus *Microcystis* contains unicellular planktonic Cyanophyceae forming microscopic or macroscopic colonies of various sizes and forms (Komárek & Anagnostidis 1998). They are held together by mucilage of various consistencies. Gas vesicles are present in the cells that are spherical and divide by binary fission successively in three planes. Members of this genus may proliferate in certain waterbodies and can form toxic blooms. The toxins produced include microcystins (*M. aeruginosa, M. viridis* and *Microcystis* spp.) and anatoxin-a (*M. aeruginosa*) (Sivonen 1996; Park & al. 1993). Based on morphological differences in colony type, mucilage characteristics and cell sizes, different species have been described. It is interesting to test their molecular relatedness because many phenotypic characters exhibit a quite considerable variability or may even be lost in culture.

Six *Microcystis* strains, belonging to four morphospecies, were isolated from five lakes of the region of Plön (Schleswig-Holstein, North Germany), and were used for a molecular study based on 16S rDNA sequences. These sequences are the first ones obtained from strains isolated in Germany. A more detailed analysis of the diversity of this genus, including a critical appraisal of the morphology and the use of other molecular methods, is currently prepared.

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Table 1. Identification, origin and date of collection of the strains sequenced in this study.

Strain	Identification	Origin	Date of collection
BM Mi 1	*M. viridis*	Dobersdorfer See Germany	01/09/1994
BM Mi 9	*M. aeruginosa*	Edebergsee Germany	30/08/1994
BM Mi 14	*M. flos-aguae*	Passader See Germany	01/09/1994
BM Mi 16	*M. wesenbergii* 1	Holzsee	

Material and methods

Strains

The studied strains are listed in table 1, together with their identification, origin and date of collection. Original strains were established from single colonies taken from net plankton samples using the micropipette method and grown in 2.5 cm diameter polysterol petri-dishes in liquid WC medium under a 12:12 L/D cycle and illuminated with cool fluorescent bulbs adjusted to ca. 20 µmol m^{-2} sec^{-2}.

Culture conditions

The strains were cultivated in medium WC (Stein 1973) at 23 °C under a light intensity of 10-40 µE m^{-2} s^{-1}.

DNA extraction and PCR amplification of a part of the rDNA operon

A crude cell lysis was performed according to Scheldeman & al. (1999). In summary, the cells are incubated with proteinase K for 1 hour at 37 °C, followed by a boiling step for 5 min. For PCR, the lysis was subjected to different dilutions (2 to 1000 times) with sterile water, and multiple reactions were performed to find the best conditions. In a total volume of 50 µl, 0.5 µl of the (diluted or not) cell lysis was added to 0.2 mM dNTPs, 0.4 µM of primer 1 (Wilmotte & al. 1993) and of primer B23S 5'R (CTTCGCCTCTGTGTGCCTAGGT), 1 mg ml^{-1} BSA, 1X Super Taq Plus buffer and 0.8 U Super Taq Plus polymerase (HT Biotechnology Ltd, UK). The PCR cycles included 1 cycle of 5 min at 94 °C, 10 cycles of 45 sec. at 94 °C, 45 sec at 57 °C, 120 sec at 68 °C followed by 25 cycles of 45 sec. at 92 °C, 45 sec at 54 °C, 120 sec at 68 °C. A final incubation of 7 min at 68 °C was carried out.

Sequence analysis and construction of a distance tree

The PCR products, containing the entire 16S rDNA (except the eight first bases at the 5' end) plus the ITS, were concentrated by ethanol precipitation. The sequence determination was performed by the company Genome Express (Grenoble, France) using primer 1. Depending on the strain, between 369 and 580 bases were determined. Using the complementary sequence of primer 359F (Nübel et al. 1997) as a sequencing primer, 270 bases were determined on the second DNA strand for strains BMMi14, BMMi16, and BMMi20.

The sequences were submitted to EBI and received the following accession numbers BMMi1: AJ271677; BMMi9: AJ271676; BMMi14: AJ271675; BMMi16: AJ271679; BMMi17: AJ271680; BMMi20: AJ271678. The sequences were aligned with all available *Microcystis* sequences (August 1999) which had sequence data for the first 500 bases at the 5'end of the 16S rDNA (Table 2) by the software Clustal 1.8 (http://www2.ebi.ac.uk/clustalw/). 334 positions, corresponding to positions 135 to 498 of *Escherichia coli* (Neefs & al. 1990), were common to all the 81 sequences and used for the phylogenetic analysis. This restricted alignment was used to construct a distance tree by the Neighbor-joining method (Saitou & Nei 1987) with the software package TREECON 1.2. (Van De Peer & De Wachter 1994). A distance matrix was calculated after correction of the dissimilarity values for multiple mutations by the formula of Jukes & Cantor (1969). The indels were not taken into consideration. The outgroup was *Synechocystis* PCC6803, which has about 85% 16S rDNA sequence similarity to all *Microcystis* strains tested (Neilan et al. 1997). A bootstrap analysis was performed, involving the construction of 500 resampled trees.

Table 1. Identification, origin and date of collection of the strains sequenced in this study.

Strain	Identification	Origin	Date of collection
BM Mi 1	*M. viridis*	Dobersdorfer See Germany	01/09/1994
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BM Mi 14	*M. flos-aquae*	Passader See Germany	01/09/1994
BM Mi 16	*M. wesenbergii I*	Holzsee Germany	28/08/1994
BM Mi 17	*M. wesenbergii I*	Groβer Plöner See Germany	30/08/1994
BM Mi 20	*M. wesenbergii II*	Passader See Germany	01/09/1994

Table 2. Genbank data of the used *Microcystis* sequences.
Strain designation, accession number, geographical origin (when available), and authors of submission to Genbank of the sequences used in fig. 1.
Authors of submission (right column): [1] : Kondo, R & Watanabe, M; [2] : Kondo, R; [3] : Neilan, BA; [4] : Otsuka, S, Oyaizu, H & Watanabe, MM; [5] : Seung, CS, Lee, SW, Hahn, YS, Chung, JH, Lee, JA & Park, YS.

Strain designation	Accession number	Geographical origin	
4B3 *Microcystis* sp.	AB012327	Hubei, P.R. China	[4]
4A3 *Microcystis* sp.	AB012326	Hubei, P.R. China	[4]
AWT139 *M. aeruginosa*	U40331	Australia	[3]
KND9506 *Microcystis* sp.	U66194	Sonakdong river, Corea	[5]
LMK9508-3 *M. aeruginosa*	AB008318	Lake Mikata, Japan	[2]
LMK9508-4 *M. wesenbergii*	AB008319	Lake Mikata, Japan	[2]
LMK9508-5 *M. flos-aquae*	AB008320	Lake Mikata, Japan	[2]
LMK9508-7 *Microcystis* sp.	AB008321	Lake Mikata, Japan	[2]
LMK9508-8 *M. aeruginosa*	AB008322	Lake Mikata, Japan	[2]
LMK9508-10 *M. aeruginosa*	AB008323	Lake Mikata, Japan	[2]
LMK9508-12 *Microcystis* sp.	AB008324	Lake Mikata, Japan	[2]
LMM9508-4 *Microcystis* sp.	AB008325	Lake Mikata, Japan	[2]
NIES42 *M. elabens*	AB001724 D89035	Japan	[2]
NIES42 *M. elabens*	U40335	Japan	[3]
NIES43 *M. holsatica*	D89036	Japan	[2]
NIES43 *M. holsatica*	U40336	Japan	[3]
NIES87 *M. aeruginosa*	D89031	Japan	[2]
NIES88 *M. aeruginosa*	AB023255	Japan	[1]
NIES89 *M. aeruginosa*	U03403	Japan	[3]
NIES90 *M. aeruginosa*	AB023256	Japan	[1]
NIES91 *M. aeruginosa*	AB023257	Japan	[1]
NIES98 *M. aeruginosa*	D89032	Japan	[2]
NIES98 *M. aeruginosa*	U40337	Japan	[3]
NIES99 *M. aeruginosa*	AB023258	Japan	[1]
NIES100 *M. aeruginosa*	AB023259	Japan	[1]
NIES101 *M. aeruginosa*	AB023260	Japan	[1]
NIES102 *M. viridis*	D89033	Japan	[2]
NIES102 *M. viridis*	U40332	Japan	[3]
NIES104 *M. wesenbergii*	AB023266	Tokyo, Japan	[1]
NIES105 *M. wesenbergii*	AB023267	Japan	[1]
NIES106 *M. wesenbergii*	AB023268	Japan	[1]
NIES107 *M. wesenbergii*	U40333	Japan	[3]
NIES108 *M. wesenbergii*	AB023269	Japan	[1]
NIES109 *M. wesenbergii*	AB023270	Japan	[1]
NIES110 *M. wesenbergii*	AB023271	Japan	[1]
NIES111 *M. wesenbergii*	D89034	Ibaraki, Japan	[2]
NIES112 *M. wesenbergii*	U40334	Japan	[3]
NIES112 *M. wesenbergii*	AB023272	Japan	[1]
NIES298 *M. aeruginosa*	AB023261	Japan	[1]
NIES299 *M. aeruginosa*	AB023262	Japan	[1]
NIES604 *M. wesenbergii*	AB023273	Japan	[1]
PCC7005 *M. aeruginosa*	U40338	Lake Mendota, Wisconsin, USA	[3]
PCC7806 *M. aeruginosa*	U03402	Braakman Reservoir, The Netherlands	[3]
PCC7820 *M. aeruginosa*	U40339	Loch Balgavies, Scotland	[3]
PCC7941 *M. aeruginosa*	U40340	Little Rideau Lake, Ontario, Canada	[3]
T17-1 *Microcystis* sp.	AB012330	Chon Buri, Thailand	[4]
T1-4 *Microcystis* sp.	AB012329	Bangkok, Thailand	[4]

TAC17 *M. viridis*	AB012328	Ibaraki, Japan	[4]
TAC20 *M. novacekii*	AB012336	Ibaraki, Japan	[4]
TAC38 *M. wesenbergii*	AB023274	Ibaraki, Japan	[1]
TAC38 *M. wesenbergii*	AB012334	Ibaraki, Japan	[4]
TAC48-1 *M. ichthyoblabe*	AB023280	Nagano, Japan	[1]
TAC48 *M. ichthyoblabe*	AB012338	Nagano, Japan	[4]
TAC51 *M. ichthyoblabe*	AB023281	Nagano, Japan	[1]
TAC52-1 *M. wesenbergii*	AB023275	Nagano, Japan	[1]
TAC52 *M. wesenbergii*	AB012335	Nagano, Japan	[4]
TAC57-1 *M. wesenbergii*	AB023276	Nagano, Japan	[1]
TAC65-2 *M. novacekii*	AB023285	Nagano, Japan	[1]
TAC65 *M. novacekii*	AB012337	Nagano, Japan	[4]
TAC66 *M. novacekii*	AB023286	Nagano, Japan	[1]
TAC71 *M. aeruginosa*	AB012332	Ishikawa, Japan	[4]
TAC75 *M. novacekii*	AB023287	Shiga, Japan	[1]
TAC78 *M. viridis*	AB023277	Fukui, Japan	[1]
TAC78 *M. viridis*	AB012331	Fukui, Japan	[4]
TAC86 *M. aeruginosa*	AB012333	Shimane, Japan	[4]
TAC91 *M. ichthyoblabe*	AB023282	Hokkaido, Japan	[1]
TAC91 *M. ichthyoblabe*	AB012339	Japan	[4]
TAC92 *M. viridis*	AB023278	Hokkaido, Japan	[1]
TAC125 *M. ichthyoblabe*	AB023283	Hokkaido, Japan	[1]
TAC140 *M. viridis*	AB023279	Fukuoka, Japan	[1]
TAC146 *M. ichthyoblabe*	AB023286	Hokkaido, Japan	[1]
TAC157-2 *M. aeruginosa*	AB023263	Chiba, Japan	[1]
TAC169 *M. aeruginosa*	AB023264	Tokyo, Japan	[1]
TAC170 *M. aeruginosa*	AB012340	Tokyo, Japan	[4]
TAC192 *M. aeruginosa*	AB023265	Tokyo, Japan	[1]

Results and discussion

Fig. 1 shows the Neighbor-joining tree, based on 334 positions of the 16S rDNA, containing the six studied strains and 75 available *Microcystis* sequences. A clear dichotomy separates a major cluster containing 77 *Microcystis* strains and a minor cluster of four sequences assigned to *M. elabens* NIES42 and *M. holsatica* NIES43 that were determined by two different authors (Neilan & al. 1997; Kondo & al. 1998). Surprisingly, the sequences from what should be the same strains are different and they have no particular relatedness to the strains in the major *Microcystis* cluster.

Two problems are revealed by this observation. The first one concerns the correctness of the identification of the strains called NIES 42 and 43. The second one concerns the belonging of the two species *M. elabens* and *M. holsatica* to the genus *Microcystis*. Since Geitler's work (1932), both taxa

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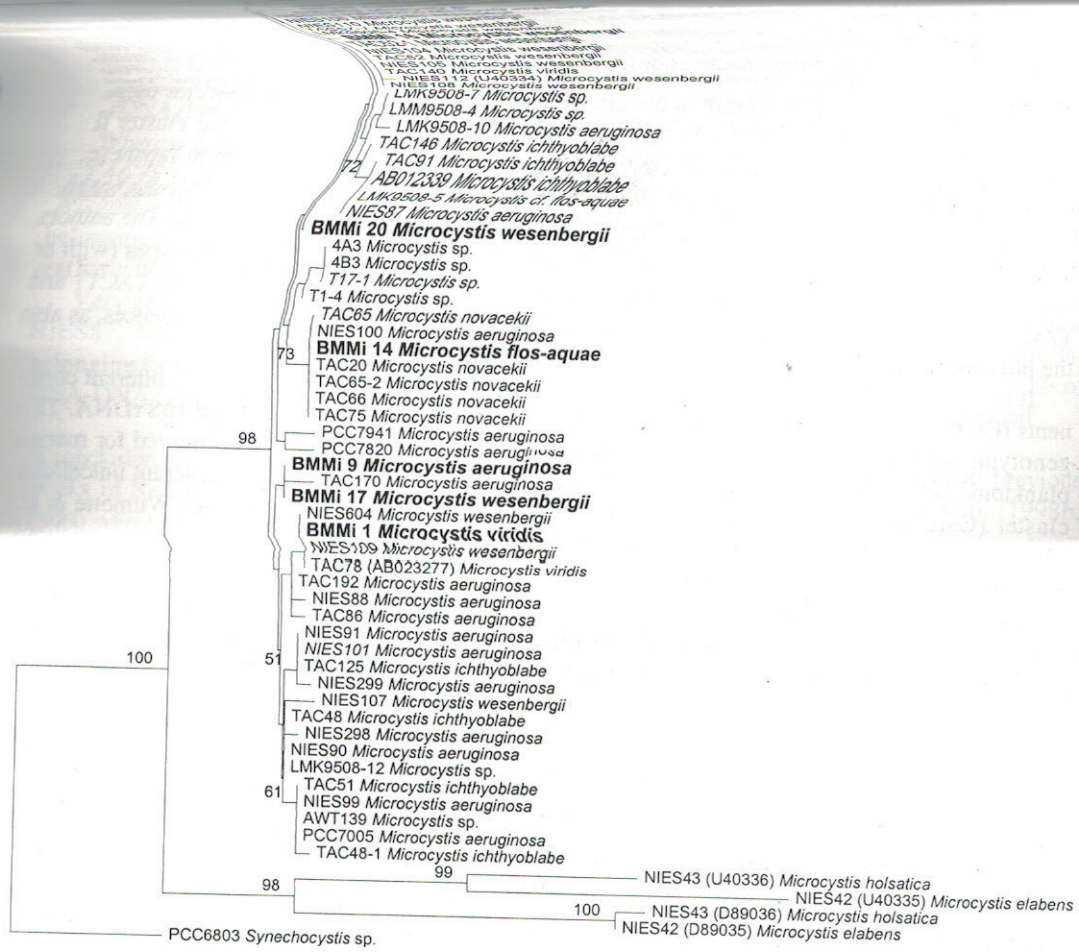
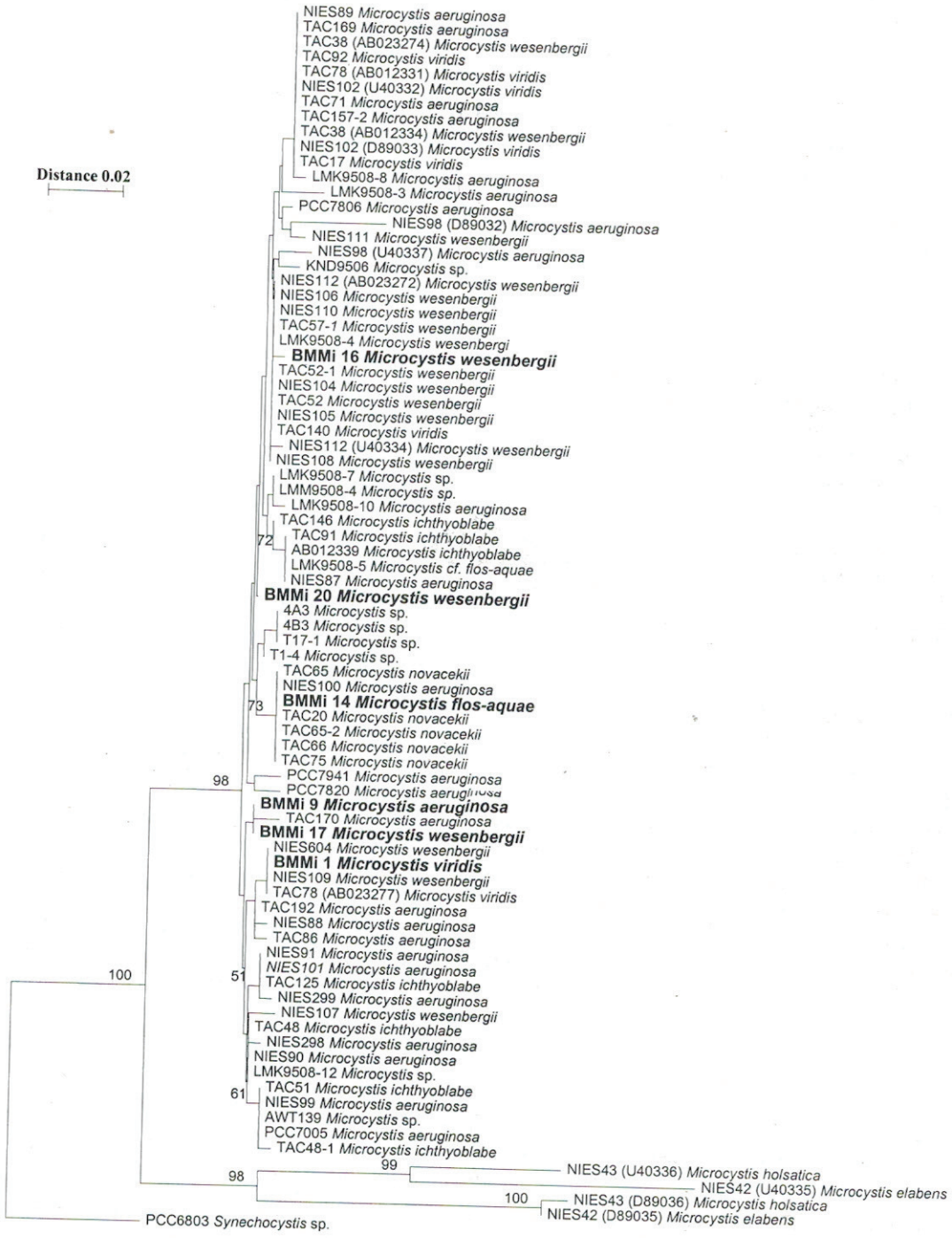
Figure 1.
Distance tree based on 334 positions of the 16S rDNA sequences of 81 Microcystis *strains and the outgroup* Synechocystis *PCC6803.*
Distance values were calculated by the formula of Jukes & Cantor (1969) as implemented in the software TREECON 1.2 (Van De Peer & De Wachter, 1994) and the distance matrix was used to construct a tree by the Neighbor-joining method of Saitou & Nei (1987).
Distances between two strains, expressed in numbers of substitutions per nucleotide, are obtained by summing the length of the horizontal branches connecting them, taking into account the scale on top. Indels were not taken into account.
The bootstrap values higher than 50% are indicated besides the concerned nodes.
The six strains studied in this paper are indicated in bold.


LMK9508-7 Microcystis sp.
LMM9508-4 Microcystis sp.
LMK9508-10 Microcystis aeruginosa
TAC146 Microcystis ichthyoblabe
TAC91 Microcystis ichthyoblabe
AB012339 Microcystis ichthyoblabe
LMK9508-5 Microcystis cf. flos-aquae
NIES87 Microcystis aeruginosa
BMMi 20 Microcystis wesenbergii
4A3 Microcystis sp.
4B3 Microcystis sp.
T17-1 Microcystis sp.
T1-4 Microcystis sp.
TAC65 Microcystis novacekii
NIES100 Microcystis aeruginosa
BMMi 14 Microcystis flos-aquae
TAC20 Microcystis novacekii
TAC65-2 Microcystis novacekii
TAC66 Microcystis novacekii
TAC75 Microcystis novacekii
PCC7941 Microcystis aeruginosa
PCC7820 Microcystis aeruginosa
BMMi 9 Microcystis aeruginosa
TAC170 Microcystis aeruginosa
BMMi 17 Microcystis wesenbergii
NIES604 Microcystis wesenbergii
BMMi 1 Microcystis viridis
NIES109 Microcystis wesenbergii
TAC78 (AB023277) Microcystis viridis
TAC192 Microcystis aeruginosa
NIES88 Microcystis aeruginosa
TAC86 Microcystis aeruginosa
NIES91 Microcystis aeruginosa
NIES101 Microcystis aeruginosa
TAC125 Microcystis ichthyoblabe
NIES299 Microcystis aeruginosa
NIES107 Microcystis wesenbergii
TAC48 Microcystis ichthyoblabe
NIES298 Microcystis aeruginosa
NIES90 Microcystis aeruginosa
LMK9508-12 Microcystis sp.
TAC51 Microcystis ichthyoblabe
NIES99 Microcystis aeruginosa
AWT139 Microcystis sp.
PCC7005 Microcystis aeruginosa
TAC48-1 Microcystis ichthyoblabe
NIES43 (U40336) Microcystis holsatica
NIES42 (U40335) Microcystis elabens
NIES43 (D89036) Microcystis holsatica
NIES42 (D89035) Microcystis elabens
PCC6803 Synechocystis sp.
72
73
98
100
51
61
99
98
100


Distance 0.02
NIES89 Microcystis aeruginosa
TAC169 Microcystis aeruginosa
TAC38 (AB023274) Microcystis wesenbergii
TAC92 Microcystis viridis
TAC78 (AB012331) Microcystis viridis
NIES102 (U40332) Microcystis viridis
TAC71 Microcystis aeruginosa
TAC157-2 Microcystis aeruginosa
TAC38 (AB012334) Microcystis wesenbergii
NIES102 (D89033) Microcystis viridis
TAC17 Microcystis viridis
LMK9508-8 Microcystis aeruginosa
LMK9508-3 Microcystis aeruginosa
PCC7806 Microcystis aeruginosa
NIES98 (D89032) Microcystis aeruginosa
NIES111 Microcystis wesenbergii
NIES98 (U40337) Microcystis aeruginosa
KND9506 Microcystis sp.
NIES112 (AB023272) Microcystis wesenbergii
NIES106 Microcystis wesenbergii
NIES110 Microcystis wesenbergii
TAC57-1 Microcystis wesenbergii
LMK9508-4 Microcystis wesenbergi
BMMi 16 Microcystis wesenbergii
TAC52-1 Microcystis wesenbergii
NIES104 Microcystis wesenbergii
TAC52 Microcystis wesenbergii
NIES105 Microcystis wesenbergii
TAC140 Microcystis viridis
NIES112 (U40334) Microcystis wesenbergii
NIES108 Microcystis wesenbergii
LMK9508-7 Microcystis sp.
LMM9508-4 Microcystis sp.
LMK9508-10 Microcystis aeruginosa
TAC146 Microcystis ichthyoblabe
72
TAC91 Microcystis ichthyoblabe
AB012339 Microcystis ichthyoblabe
LMK9508-5 Microcystis cf. flos-aquae
NIES87 Microcystis aeruginosa
BMMi 20 Microcystis wesenbergii
4A3 Microcystis sp.
4B3 Microcystis sp.
T17-1 Microcystis sp.
T1-4 Microcystis sp.
TAC65 Microcystis novacekii
NIES100 Microcystis aeruginosa
78
BMMi 14 Microcystis flos-aquae
TAC20 Microcystis novacekii
TAC65-2 Microcystis novacekii
TAC66 Microcystis novacekii
TAC75 Microcystis novacekii
98
PCC7941 Microcystis aeruginosa
PCC7820 Microcystis aeruginosa
BMMi 9 Microcystis aeruginosa
TAC170 Microcystis aeruginosa
BMMi 17 Microcystis wesenbergii
NIES604 Microcystis wesenbergii
BMMi 1 Microcystis viridis
NIES109 Microcystis wesenbergii
TAC78 (AB023277) Microcystis viridis
TAC192 Microcystis aeruginosa
NIES88 Microcystis aeruginosa
TAC86 Microcystis aeruginosa
NIES91 Microcystis aeruginosa
100
51
NIES101 Microcystis aeruginosa
TAC125 Microcystis ichthyoblabe
NIES299 Microcystis aeruginosa
NIES107 Microcystis wesenbergii
TAC48 Microcystis ichthyoblabe
NIES298 Microcystis aeruginosa
NIES90 Microcystis aeruginosa
LMK9508-12 Microcystis sp.
61
TAC51 Microcystis ichthyoblabe
NIES99 Microcystis aeruginosa
AWT139 Microcystis sp.
PCC7005 Microcystis aeruginosa
TAC48-1 Microcystis ichthyoblabe
99
NIES43 (U40336) Microcystis holsatica
98
NIES42 (U40335) Microcystis elabens
100
NIES43 (D89036) Microcystis holsatica
NIES42 (D89035) Microcystis elabens
PCC6803 Synechocystis sp.

have been removed from the genus *Microcystis*. *M. elabens* was placed in *Aphanothece* by Elenkin (1938) and *M. holsatica* in *Aphanocapsa* by Cronberg & Komàrek (1994). These assignements were accepted by Komàrek and Anagnostidis (1998). The three genera exhibit, besides diagnostic morphological features, different modes of division: three perpendicular directions in *Microcystis*, one in *Aphanothece* and two in *Aphanocapsa*.

The first six sequences from Northern Germany, determined in this study, belong to the major *Microcystis* cluster which forms a tightly related group supported by 98% of bootstrap trees. Inside this cluster, no well-supported subcluster (with a bootstrap value of at least 95%) can be observed. The most divergent strain in the cluster is *M. aeruginosa* NIES98 (Accession D89032) which shows a maximum of 3.7 % sequence divergence with the other strains. Another sequence from the same strain has been determined by other authors, with a slightly different outcome: NIES 98 (Accession U40337) appears more related to KND9506. Differences between sequences of the same strains which were determined by different authors are observed in several other cases (NIES98, NIES 112, TAC48), and further research would be necessary to search for the origin of this discrepancy.

The existence of a genotypically homogeneous *Microcystis* cluster has already been observed by other authors. Kondo & al. (1998) have determined 457 positions of the 16S rDNA for 41 *Microcystis* strains and they distinguished six subclusters based on tiny sequence differences. Their 457 positions overlap completely with the 334 positions used for the tree of fig. 1 which contains twice as many strains. Their clusters I, III, IV, V and VI are also present in the tree of fig. 1 though the bootstrap support for these clusters is not significant (less than 95%). In contrast, strain TAC146 is removed from their cluster II and appears slightly more related to cluster I in fig.1. Unfortunately, this paper is written in Japanese, what precludes a more detailed comparison of data. Another study by Otsuka & al. (1998b) was based on 15 complete 16S rDNA sequences from Japan, Thailand and People's Republic of China. The authors have shown that *Microcystis* strains belonging to five morphospecies and two pigment types (with or without phycoerythrin) were grouped in a tight cluster. They also observed that *M. viridis* TAC17 and TAC78, *M. wesenbergii* TAC38 and *M. aeruginosa* TAC71 had identical 16S rDNA sequences, as also the phycoerythrin-containing *Microcystis* sp. 4A3 and 4B3.

It is quite remarkable that the major *Microcystis* cluster contains strains from four different continents (Europe, Asia, Australia, and America, see tab. 2) which have almost identical 16S rDNA. This genotypic homogeneity combined with a cosmopolitan distribution was already observed for marine planktonic taxa (Mullins & al. 1995), for *Microcoleus chthonoplastes* and a halotolerant unicellular cluster (Garcia-Pichel & al. 1996, 1998), for the genera *Phormidium/Leptolyngbya* (Wilmotte & al. 1997), and *Arthrospira* (Scheldeman & al. 1999). In the latter genus, it is associated with a conspicuous morphological variability, like for *Microcystis*.

It is also surprising that it is not possible to find a genotypic support for the different species designation in the tree of fig. 1. Though the four strains (of which TAC65 is duplicated) identified as *M. novacekii* appear to have identical sequences, the other strains assigned to *M. aeruginosa*, *M. wesenbergii*, *M. viridis* and *M. ichthyoblabe* are located in a seemingly random manner throughout the tree. We can put forward two hypotheses. First, a number of strains might have been misidentified. This is a concern raised by Komárek (1994). Secondly, these differences in sheath structure, cell characteristics, and colony pattern which are used to distinguish the species might be coded by a part of the genome which varies more rapidly than genes like the 16S rDNA. Thus it would be possible that these genetic differences are not reflected at the level of the 16S rDNA which is a quite conserved region, but could be detected by other methods based on more sensitive molecular markers.

In order to solve this problem, several steps should be followed. First, it would be useful to perform a taxonomic reassessment of all the strains with a detailed morphological description and a test of the morphological variability in culture. Indeed, Krüger et al. (1981) have observed a pronounced effect of

the age of the culture and the light conditions on the variability of the cell sizes of two *Microcystis* strains. Morphological information is available for a few strains only. Otsuka et al. (1998a) have described the cell and colony morphology of four phycoerythrin-containing *Microcystis* sp. strains. In addition, Otsuka et al. (1999) have shown that 24 other strains belonging to four morphospecies (*M. wesenbergii*, *M. aeruginosa*, *M. novacekii*, *M. viridis*) had overlapping size ranges, whereas all six strains of *M. ichthyoblabe* had smaller cell sizes. Secondly, more variable molecular markers should be tested to observe whether the major *Microcystis* cluster could be divided into different subclusters. Then, if subclusters can be obtained by the use of molecular tools with a higher resolution power (like fingerprints or spacer sequences), one should look whether there are phenotypic characteristics consistent with the genotypic groupings

The use of the ITS sequence between the 16S and 23S rDNA was recently tested by Otsuka & al. (1999) with the 15 strains studied in their paper of 1998 and 32 additional ones. They found that the similarities of ITS sequences were still surprisingly high (93.3 to 100 % without taking indels into consideration). They could distinguish three clusters but only *M. viridis*, represented by six strains, showed a correspondence between morphology and ITS clustering. The other morphospecies appeared heterogeneous in terms of genotypic relations and were mixed in the three clusters. The authors concluded by speculating that all the studied strains would belong to only one genotypic species.

After an analysis of four allozymes in 28 strains, Kato & al. (1991) had observed groupings of strains assigned to *M. viridis* and *M. wesenbergii*, but not to *M. aeruginosa*. For a number of these strains, 16S rDNA data is now available and shows that there is no complete agreement between the two approaches. Whereas for *M. wesenbergii*, strains TAC52 and 57 appear identical by both methods, *M. wesenbergii* TAC38 is more similar to *M. viridis* strains TAC78 and 92 on the basis of the 16S rDNA sequence. Nishihara et al. (1997) have tested the use of a genetic fingerprint technique, RAPD, with 18 strains belonging to five morphospecies. They observed a quite good agreement between groupings obtained earlier with allozymes and the new RAPD data.

Very recently, Kondo et al. (2000) have performed DNA-DNA reassociation between strains NIES87, NIES89, NIES98, NIES298, NIES102, NIES104, NIES111, NIES112 and NIES604, belonging to *M. aeruginosa*, *M. viridis*, and *M. wesenbergii*. All these strains present at least 70% of DNA-DNA hybridisation and thus they belong to the same species if bacterial criteria are applied.

In conclusion, the analysis of partial 16S rDNA of *Microcystis* strains from Germany confirms previous works of Neilan & al. (1997), Rudi & al. (1997, 1998), Kondo & al. (1998), Otsuka & al. (1998b, 1999) who have shown that there is a genetically homogeneous cluster of typical *Microcystis* strains, based on 16S rDNA sequences. This is in contrast with the morphological variability inside this genus and with the different pigment characteristics. The use of the morphological differences in colony morphology, cell size, characteristics of the mucilage to distinguish different species should be carefully reconsidered, but this paper is only a first step in this direction.

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