J. Phycol. **37,** 810–818 (2001)

GENETIC VARIABILITY OF BRAZILIAN STRAINS OF THE *MICROCYSTIS AERUGINOSA* COMPLEX (CYANOBACTERIA/CYANOPHYCEAE) USING THE PHYCOCYANIN INTERGENIC SPACER AND FLANKING REGIONS (*cpc*BA)[1]

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The genetic and morphological variability among 15 Brazilian strains of *Microcystis aeruginosa* (Kütz.) Kütz. collected from four locations was examined and compared with several reference strains of *M. aeruginosa*, *M. viridis* (A. Br.) Lemm. and *M. wesenbergii* (Kom.) Kom. in Kondr. Brazilian strains were classified by morphological features and by comparison of the nucleotide sequences of the *cpc*BA intergenic spacer and flanking regions. Our results indicate that Brazilian strains classified as *M. aeruginosa* are phylogenetically diverse compared with reference strains of *M. aeruginosa* and that the current taxonomy underestimates genetic diversity within *M. aeruginosa*. The data also demonstrate that morphological criteria alone are inadequate to characterize *Microcystis* species. Although colonial characters were shown to vary considerably in culture, some genetic lineages demonstrated consistent cellular diameter ranges, indicating that cell size has value as a taxonomic character. The detection of six *M. aeruginosa* genotypes in a single water body indicates that morphological approaches can also seriously underestimate the diversity of *Microcystis* bloom populations.

Key index words: ***cpc*BA-IGS; Cyanobacteria; Cyanophyceae; DNA; genetic diversity; intergenic spacer; *Microcystis*; *Microcystis aeruginosa*; phycocyanin; phylogeny; systematics**

Cyanobacterial blooms of the genus *Microcystis* are a serious ecological and public health concern because of their ability to dominate the planktonic environment and produce toxins, which affect aquatic organisms or animals and humans that consume the water. On a worldwide basis, *Microcystis aeruginosa* is the most commonly reported species causing hepatotoxicity and odor problems in lakes and water supplies (Sivonen et al. 1990, Azevedo et al. 1994, Carmichael 1996, Codd et al. 1999, Dinga et al. 1999).

Existing taxonomy of the genus is based primarily on the morphological characteristics: cell size, shape, and distribution; colony shape and structure; and thickness and character of the mucilage around the cells (Komárek and Anagnostidis 1986, 1999). However, in the laboratory many of these characters show morphological changes when subjected to different culture conditions. Effects such as change or loss of colony structure and gas vesicles and the reduction of cell size are well documented (Krüger et al. 1981, Doers and Parker 1988, Kato et al. 1991, Komárek 1991, Roberts and Zohary 1992, Bolch and Blackburn 1996, Otsuka et al. 2000). As a result, the taxonomic criteria used for distinguishing *Microcystis* species have been questioned (e.g. Otsuka et al. 1999, 2000) or thought to be completely artificial (e.g. Nishihara et al. 1997).

Most field studies continue to use morphological criteria to assign morphotypes to particular *Microcystis* species, necessitating the correlation of phenotype with genotype if possible. However, developmental variation in colonial morphology means it is often impossible to assess colonial characters evident only in mature colonies (Bittencourt-Oliveira 2000). Many blooms also show considerable morphological polymorphism or are composed of more than one morphological species (e.g. Krüger et al. 1981, Baker 1992), making it difficult to assign morphotypes to any one species. It is also not clear whether the level of variation observed represents phenotypic variation of a single genotype or represents mixtures of different genotypes.

The sequences of the ribosomal RNA genes (rDNA) have been successfully used to aid morphological taxonomy in cyanobacteria (Wilmotte and Golubic 1991, Wilmotte et al. 1992, Nelissen et al. 1996). However, at the intrageneric level the ribosomal RNA (16S rRNA) gene sequences have demonstrated little clearly interpretable variation among *Microcystis* strains. Average sequence divergence has been generally less

[1]Received 31 July 2000. Accepted 11 July 2001.

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than 3% (Neilan et al. 1997a, Kondo et al. 1998) and often much less than 1% (Rudi et al. 1997, Otsuka et al. 1998). Comparison of 16S rDNA sequences from the same strain obtained by different workers also often differ by more than that found between different strains (Bolch, unpublished data), indicating that minor sequencing errors may account for much of the variation and that 16S sequences are potentially uninformative below genus level.

An alternative to the conserved 16S rDNA is to examine intergenic spacer regions, which usually show considerably more variation. For example, restriction site polymorphism (RFLP) and DNA sequencing studies of the 16–23S rDNA internal transcribed spacer have indicated up to 7% sequence variation (Neilan et al. 1997b, Otsuka et al. 1999). The c-phycocyanin genes *cpc*B and *cpc*A and the intervening intergenic spacer (*cpc*BA-IGS) display similar levels of sequence variation and are capable of resolving distinct genotypes within a range of genera (Neilan et al. 1995, Bolch et al. 1996, 1999a,b, Hayes and Barker 1997). The sequences are also somewhat easier to align due to limited insertions and deletions and easier to interpret due to the codon structure within the coding regions.

In this work we use *cpc*BA-IGS sequence data to examine genetic variation of Brazilian *M. aeruginosa* strains and their relationship to reference *M. aeruginosa*, *M. viridis*, and *M. wesenbergii* strains from European, North American, and Japanese regions. We also sought to compare the morphological data with the sequencing data in the *Microcystis* strains examined.

MATERIALS AND METHODS

Strains and growth conditions. Water samples were collected on a monthly basis from a eutrophic artificial reservoir, Garças reservoir, São Paulo, SP, Brazil (by C. Bicudo, D. Bicudo, and C. Sant'Anna) using a Kemmerer bottle at three depths in relation to the euphotic zone: surface, middle (1% light penetration, around 1.5 m), and bottom (4.0 m; permanently at the aphotic zone). Additional water samples were also collected from Americana (Americana, SP, Brazil) and Cantareira reservoirs (Mairiporã, SP, Brazil) by P. A. Senna and C. Sant'Anna. A single 25-μm plankton net sample was also collected from Usina Santa Rita reservoir (Maracaí, SP, Brazil). The unialgal strain NPLS-1, isolated from Lagoa Santa reservoir (Lagoa Santa, MG, Brazil), was obtained from the Laboratory for Culture and Ecophysiology of Cyanobacteria (NPPN-UFRJ). The 16 clonal and nonaxenic strains of *Microcystis* were isolated by growth on solid medium prepared by surface spreads containing cycloheximide (50 $mg \cdot L^{-1}$, Sigma-Aldrich, Dorset, UK). The strains were grown at 21° C ± 1° C and 130 μmol $photons \cdot m^{-2} \cdot s^{-1}$ of cool-white fluorescent light (40 W, General Electric, Fairfield, CT) under a 14:10-h light:dark photoperiod in tubes containing 10 mL of modified liquid BG-11 (Rippka et al. 1979) culture medium (Bittencourt-Oliveira 2000).

Morphological analysis. All strains were cultured under the same conditions and observed in mid-logarithmic phase. Strains were classified into morphological groups based on the diacritical characteristics used for identification of *Microcystis* species (Komárek and Anagnostidis 1986, 1999): form and development of the colony, cellular aggregation pattern, cellular diameter, and nature and thickness of mucilage. Mean cell diameter was calculated ($n = 50$ cells) for each strain and compared statistically using the Duncan test with 95% confidence level (Duncan 1955). Photomicrographs of colonies and cells were taken with a Leica DMLS microscope (Leica Microsystems, Wetzlar, Germany) equipped with a video camera system (Image Pro Plus version 4.0, Media Cybernetics, Silver Spring, MD). Colonies were examined after staining with ink to show the outline of colonial mucilage. It was not possible to obtain morphological data on colony structure for some strains because they did not form colonies in culture; cellular diameter only was measured in these cases.

DNA sequencing. DNA for PCR was prepared using the InstaGene™ Matrix (BioRad Laboratories, Hercules, CA) method of Neilan et al. (1995) modified as described by Bolch et al. (1996). PCR amplifications were performed using modified protocols and *cpc*BA-IGS oligonucleotide primers described by Bolch et al. (1996). PCβ-F: 5′-GGCTGCTTGTTTACGCGACA-3′; PCα-R: 5′-CCAGTACCACCAGCAACTAA-3′. Amplifications were carried out in 100-μL volumes in a GeneAmp 2400 thermocycler (Perkin-Elmer, Foster City, CA). Reactions contained 2 μL of cell lysate, 5 pmol of each primer, 2.5 U of *Taq* polymerase (Amersham Pharmacia Biotech, Piscataway, NJ) with buffer containing 1.5 mM $MgCl_2$ and 200 μM of each dNTP (Boehringer-Mannheim, Mannheim, Germany). The following cycling parameters were used: 92° C for 2 min, followed by 40 cycles of 92° C for 40 s; 55° C for 50 s; and 72° C for 2 min, followed by a final extension at 72° C for 8 min. Unreacted PCR components, primers, and buffers were removed by purification through QiaQuick columns (Qiagen, Valencia, CA), and the PCR products were inspected for size and amplification artifacts by horizontal gel electrophoresis (Sambrook et al. 1989).

Both strands of PCR products were sequenced using ABI Prism® Big Dye™ Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer) using 70 ng of PCR product template and 3.2 pmol of PCR primers PCβ-F and PCα-R using standard cycle-sequencing protocols and an ABI 310 capillary sequencer (Perkin-Elmer). Automated base calls for both strands were checked by manual inspection and ambiguous calls and conflicts resolved by alignment and comparison using Sequencher version 3.0 (Gene Codes, Ann Arbor, MI) to establish a consensus sequence for each strain.

Phylogenetic analysis. Completed *M. aeruginosa* sequences were aligned with each other and those of eight reference strains of *M. aeruginosa* (PCC7941, PCC7806, PCC7820, NIES98, NIES99), *M. viridis* (NIES102), and *M. wesenbergii* (NIES111 and NIES112). Alignments were carried out using the Clustal aligment option of Sequence Navigator version 1.0.1 (Applied Biosystems, Foster City, CA), using the default settings for gap inclusion and extension. All sequences were submitted to the GenBank (accession numbers AF385368 to AF385391).

Phylogenetic analysis of the data was carried out with the computer program PAUP* 4.0 (Swofford 1998). Hierarchical structure in the data set was evaluated by the random tree method and probability tables of Hillis and Huelsenbeck (1992). The phylogenetic approach used was a maximum likelihood analysis. Assumed nucleotide frequences were determined empirically from the data set and, to account from substitution rate heterogeneity, the analysis assumed 4 rate categories and a γ distribution of 0.0032 determined from preliminary analyses. The *M. wesenbergii* strains were designated as outgroups for rooting the resulting trees. Support for tree branch points was estimated by the bootstrap approach (Felsenstein 1985) using 1000 replicates of the heuristic search algorithm.

RESULTS

All the *Microcystis* strains presented spherical cells with gas vesicles, with or without mucilage present around the solitary cells (Figs. 1–3). The colonial characteristics of the morphotypes are shown in Table 1, and the measurements of the cellular diameter, SD, and variance of the different strains are shown in Table 2. By morphological criteria the strains could be

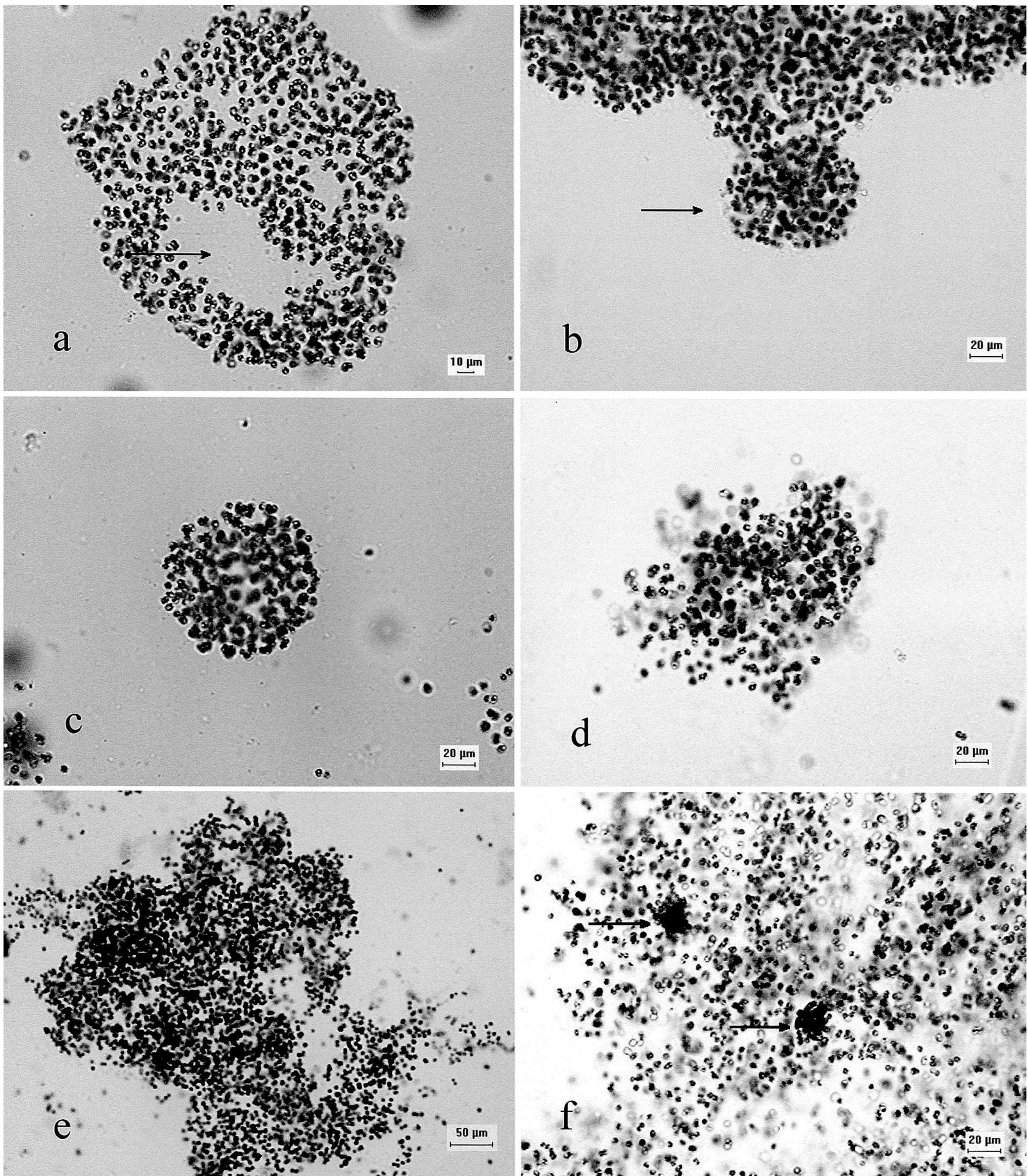

FIG. 1. Morphological characteristics of *Microcystis aeruginosa.* (a) Morphotype M1. Mature colony from strain FCLA-199 with irregular outline and distinct holes (arrow). (b) Morphotype M1. Mature colony from strain FCLA-158 releasing a "daughter" colony (arrow) via projection from the "mother" colony. (c) Morphotype M1. Juvenile colony morphology from strain FCLA-199 after released of mother colony. (d) Morphotype M2. Strain FCLA-003 showing irregular young colony. (e) Morphotype M2. Mature colony from strains FCLA-003. (f) Morphotype M2. Strain FCLA-003 with two daughter colonies (arrows) inside the mother colony and densely aggregated irregularly distributed cells in the mature colony.

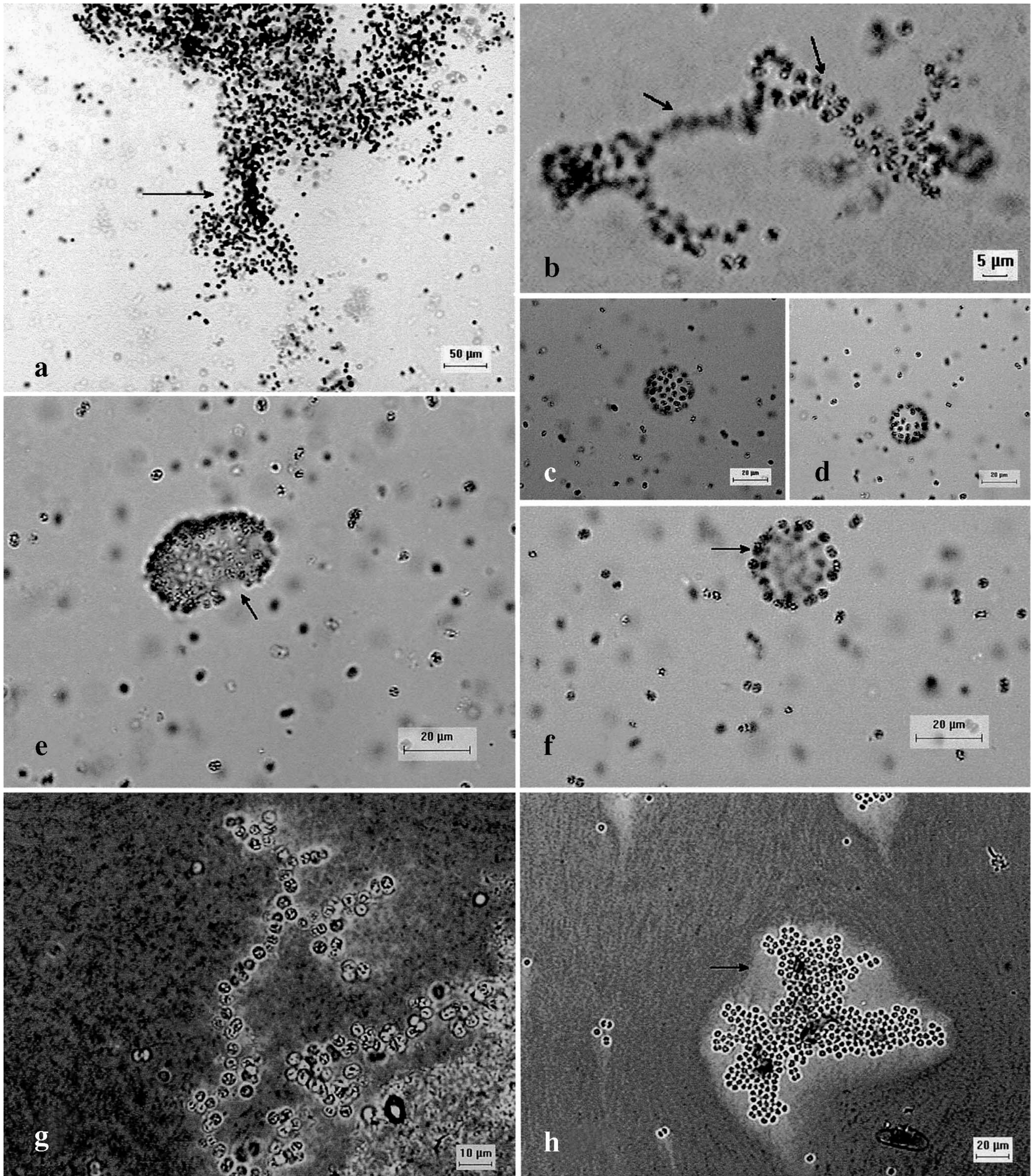

FIG. 2. Morphological characteristics of *Microcystis aeruginosa*. (a) Morphotype M2. Strain FCLA-003 showing irregular young colony released through fragmentation (arrow). (b–f) Morphotype M3 from strain FCLA-030. (b) Mature colony with lateral buddings (arrows). (c and d) Surface focus detail of a spherical juvenile colony showing distribution of cells. (e) Colony breaking in the middle (arrow). (f) Juvenile colony with a single cell layer (arrow). (g) Morphotype M4. Ribbon-like mature colony from strain FCLA-200. (h) Morphotype M4. Mature colony with densely packed cells arranged in a single plane with narrow diffluent mucilage (arrow).

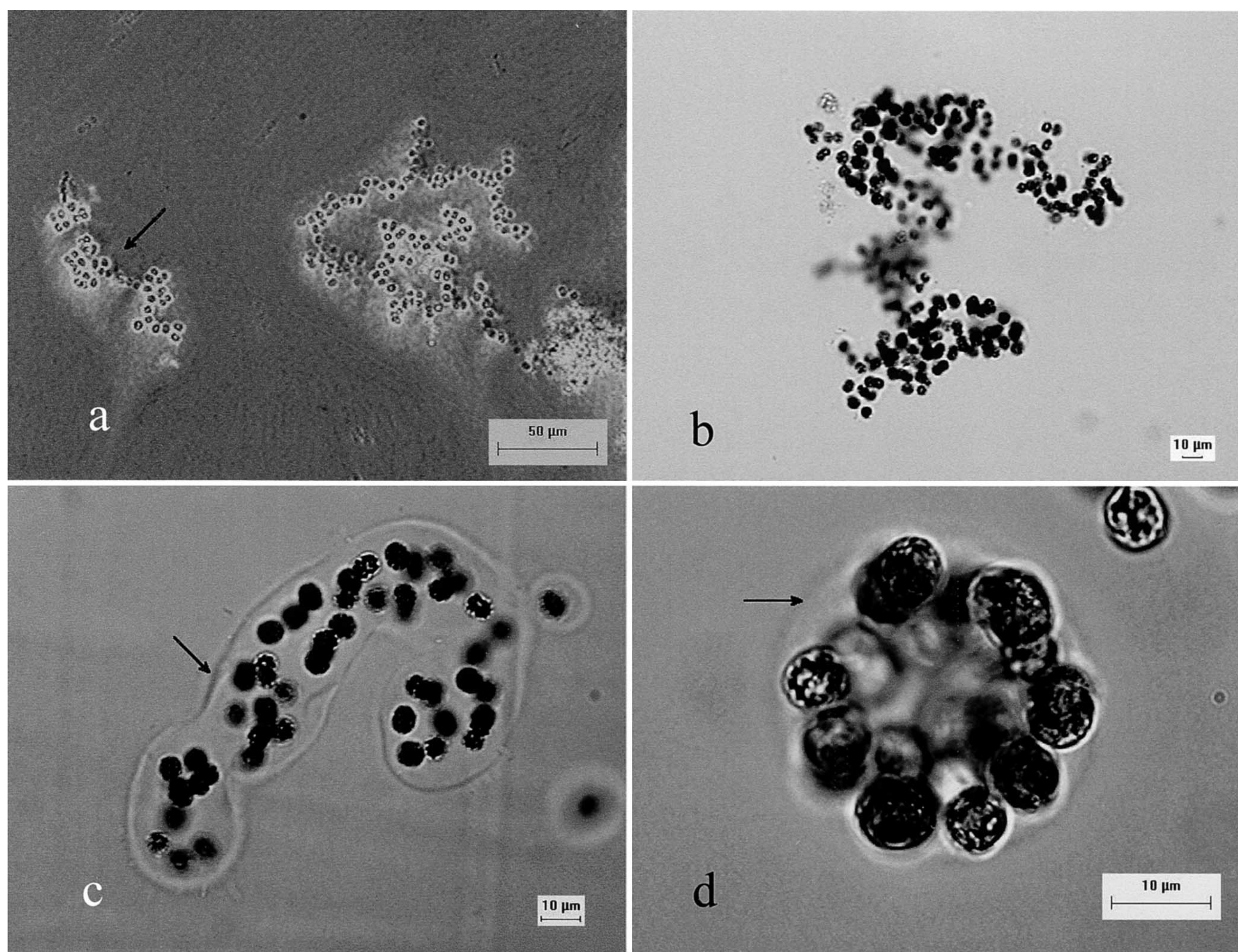

FIG. 3. Morphological characteristics of *Microcystis aeruginosa* and *M. wesenbergii.* (a) Morphotype M4. Juvenile ribbon-like colony (arrow) (b). Morphotype M5. Low magnification view of mature colony from strain FCLA-Ninf. (c) Morphotype M5. Mature colony of *M. wesenbergii* from nature with a refractive edge (arrow). (d) Morphotype M5. Detail of a young colony from strain FCLA-Ninf with peripherally distributed cells and wide mucilage (arrow).

separated into five morphotypes (M1–M5), with a sixth morphotype for those that no longer formed colonies. The population from Usina de Santa Rita reservoir, Maracaí, SP (morphotype M5) was morphologically identified as *M. wesenbergii* (Fig. 3, b–d), and this identification is supported by *cpc*BA sequence comparison with *M. wesenbergii* NIES111 and 112. All other strains analyzed were identified as *M. aeruginosa, M. panniformis* Kom. et al., or *Microcystis* sp. (Sant'Anna et al. 1978, 1989, Azevedo et al. 1996, Bittencourt-Oliveira 2000, Komárek et al. 2001) but here are considered to belong within the unresolved "*M. aeruginosa* complex."

Consensus PCR sequencing of the *cpc*BA-IGS region did not show evidence of sequence polymorphism (evident as dual base calls). Of the approximately 660 base pairs (bp), fragment 574 bp could be clearly resolved and unambiguously aligned for phylogenetic analysis.

No insertions or deletions were noted in the coding or IGS regions among the 24 strains examined. Nucleotide substitutions were heterogeneously distributed between the gene coding regions and the IGS. The sequenced region contained a total of 59 (6.9%) unambiguously variable sites, 16 (6.9%) in the *cpc*B gene, 5 (7.6%) in the IGS, and 38 (13.7%) in the *cpc*A gene. The bulk of the variable sites in *cpc*B (15 sites, 6.5%) and the IGS (5 sites, 7.6%) could be attributed to within the *M. aeruginosa* complex. In contrast, the 38 variable sites in the *cpc*A gene varied both within *M. aeruginosa* complex (26 sites, 9.4%) and between *M. wesenbergii* and *M. aeruginosa* (12 sites, 4.3%).

Among the Brazilian *Microcystis* strains, nine distinct *cpc*BA-IGS sequence genotypes could be resolved, eight of which were identified as *M. aeruginosa* complex, designated as genotypes G1 to G8 (Table 2). Pairwise sequence divergence between genotypes ranged from 1 bp (0.17%) between G2 and G4, up to 37 bp (6.43%) between G2 and *M. wesenbergii* FCLA-Ninf. Identical *cpc*BA genotypes were recovered from different reservoirs (G6 at Garças and Cantareira reservoirs). At the

TABLE 1. Description of *Microcystis* morphotype groups.

Morphotype	Juvenile colony: Shape	Juvenile colony: Mucilage	Juvenile colony: Arrangement of cells in colony	Mature colony: Shape	Mature colony: Mucilage	Mature colony: Arrangement of cells in colony	Daughter-colony release
M1	Spherical or subspherical	Diffluent, narrow	Arranged and homogeneously distributed	Irregular, lobate; clathrate	Diffluent, narrow, or broad	Irregular distributed with daughter colonies in their interior	Projections of the margins of the mother colony
M2	Spherical, subspherical, or irregular	Diffluent, broad	Densely packet in the center	Irregular, lobate; clathrate	Diffluent, broad	Densely aggregated; irregular	Irregular fragmentation from the larger colony; disintegration into small clusters
M3	Spherical, single cell layer	Diffluent, narrow	Homogeneously distributed in periphery of colony; sometimes in a single layer inside	Irregular, ribbon-like, branched with connections between the daughter colonies	Diffluent, narrow	Homogeneously distributed in periphery of colony in a single layer inside	Colony divides and globose buddings emerge from the laterals; buddings may or may not be liberated
M4	Plate-like, ribbon-like; flat	Diffluent, narrow	Cells close to one another; more or less plane	Irregular, elongate, plate-like; clathrate	Diffluent, narrow	Cells close to one another; more or less planar	Fragmentation
M5	Spherical, subspherical	Firm, refractive, narrow	Densely packed cells in the center or in periphery of colony	Irregular, subspherical, lobate, composite colonies; clathrate	Firm, refractive	Irregularly spaced; cells peripherally distributed	Budding from surface of mother colony
M6	No colonies						

main sampling site, Garças reservoir, six different genotypes of *M. aeruginosa* complex were recovered, including two coexisting genotypes in March and April 1997.

Tests for phylogenetic signal in the data set by examining the distribution of randomly generated trees showed that distributions were significantly skewed than expected from random data ($g_1 = -0.62$, $P < 0.01$), indicating that the data contained phylogenetic structure. The maximum likelihood analysis of the Brazilian *Microcystis cpc*BA data separated the genotypes into five distinct lineages (L1–L5, Fig. 4) with moderate to high bootstrap support. Strain FCLA-Ninf (*M. wesenbergii*, morphotype M5, Fig. 3, b–d) clustered clearly with the designated outgroup taxa NIES111 and NIES112, consistent with the distinct nature (refractile edge to the colonial mucilage) of this species compared with *M. aeruginosa*.

The *M. aeruginosa* complex strains formed a monophyletic group with four distinct lineages, which did not correlate clearly with morphological groupings. All genotypes with two or more strains (G2, G6, and G8) contained strains classified into two or three morphotype categories. The Duncan size–class categories

TABLE 2. Genotype and morphotype (see Table 1) of the Brazilian *Microcystis* spp. strains examined.

Strain	Sample location	Sample date	Depth	Genotype	Morphotype	Cell diameter (μm): Mean	SD	Min-max	Duncan test
NPLS-1	L	Unknown	S	G1	M6	5.02	0.49	3.4-6.4	I
FCLA-262	G	Mar 1997	M	G2	M2	5.73	0.55	4.4-6.9	II
FCLA-298	G	May 1997	B	G2	M4	4.39	0.40	3.5-5.4	III
FCLA-299	G	Feb 1997	M	G2	M4	4.68	0.52	3.7-5.9	IV
FCLA-232	G	Mar 1997	S	G3	M6	4.31	0.44	3.4-4.9	III
FCLA-235	G	Apr 1997	S	G4	M6	4.11	0.47	3.0-4.0	V
FCLA-200	A	Unknown	S	G5	M4	3.81	0.49	3.0-4.9	VI
FCLA-009	G	May 1996	S	G6	M4	3.08	0.40	2.0-4.0	VII
FCLA-030	G	Nov 1996	S	G6	M3	3.00	0.37	2.5-4.0	VII
FCLA-225	C	Mar 1997	S	G6	M6	3.14	0.38	2.5-4.4	VII
FCLA-310	G	Apr 1997	M	G6	M6	3.20	0.39	2.9-4.0	VII
FCLA-450	G	Jul 1997	S	G7	M6	6.67	0.66	4.9-7.9	VIII
FCLA-003	G	Dec 1996	B	G8	M2	4.96	0.32	4.0-5.9	I
FCLA-158	G	Dec 1996	S	G8	M1	5.10	0.64	3.9-6.1	I
FCLA-199	G	Dec 1996	M	G8	M1	5.52	0.43	4.9-6.4	IX
FCLA-Ninf	U	Feb 1998	S	G9	M5	7.01	0.14	4.9-9.9	X

Sample locations are Americana Reservoir (A), Cantareira Reservoir (C), Garças Reservoir (G), Lagoa Santa Reservoir (L), and Usina Santa Rita Reservoir (U). S, surface; M, middle; B, bottom. NPLS, Núcleo de Pesquisas Lagoa Santa; FCLA, Faculdade de Ciências e Letras de Assis. See text and legend to Figure 4 for explanation of codes used for genotypes and morphotypes.

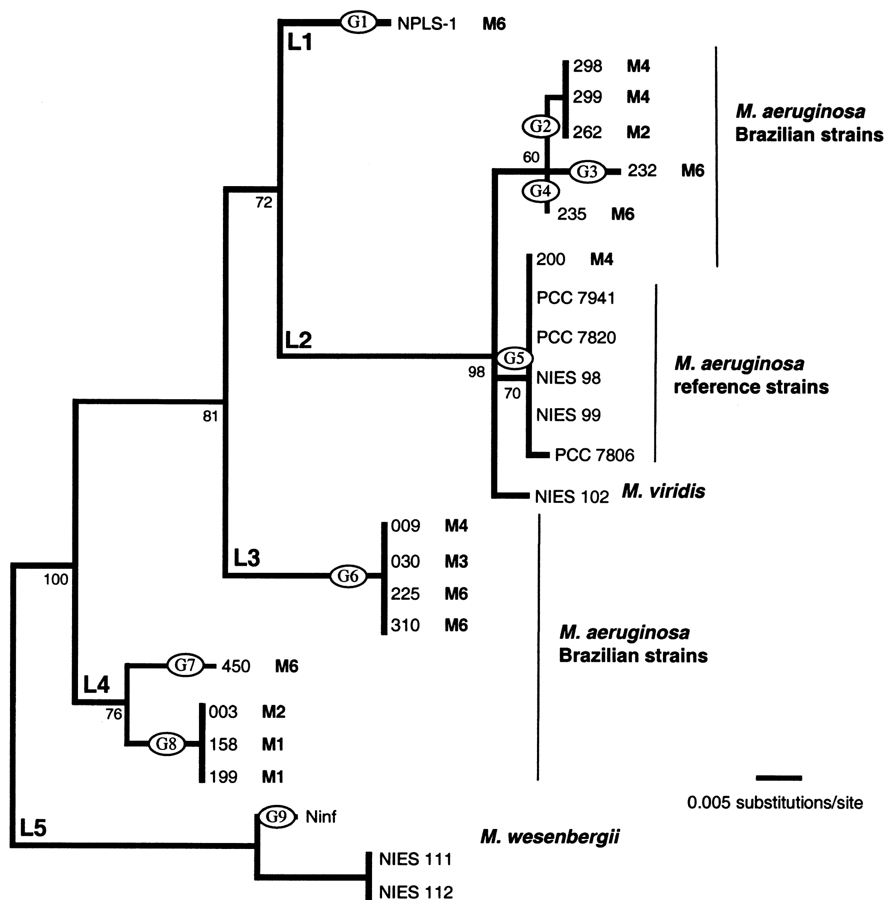

FIG. 4. Maximum likelihood analysis of Brazilian *Microcystis aeruginosa* and reference *M. aeruginosa, Microcystis viridis,* and *Microcystis wesenbergii* cpcBA-IGS genotypes; NIES111 and NIES112 included as outgroup taxa ($g_1 = -0.62$, $P < 0.01$). *Microcystis aeruginosa* lineages are marked (L1–L4) at the base of each branch; distinct genotypes (G1–G9) are shown in white ovals at terminal branches; morphological groupings (M1–M6, see Table 1) are indicated to the right of each strain designation. Figures under each branch point are bootstrap support values obtained from 1000 replications using the heuristic search algorithm.

(Table 2) generally correlated poorly with the lineages; however, some consistent features were apparent. The four strains in lineage L3 correlated completely with Duncan group VII and all displayed small mean cell diameters close to 3.0 μm (Table 2, Fig. 4). Lineage L4 contained two related genotypes and three Duncan groupings, yet comparison of mean diameters indicates that all strains possessed larger mean diameters close to or exceeding 5.0 μm. Lineage L2, which contained four related genotypes, showed considerable variation in cell size, consistent with the observed genetic variation among the genotypes (Table 2, Fig. 4).

When compared with PCC and NIES collection *M. aeruginosa* reference strains, the *cpc*BA sequences of Brazilian strains showed considerable genetic diversity. All reference *M. aeruginosa* strains and *M. viridis* NIES102 clustered within lineage L2. The *cpc*BA-IGS sequence *M. aeruginosa* strains NIES98, NIES99, PCC7820, and PCC7941 were identical to that of strain FCLA-200; *M. aeruginosa* PCC7806 differed by only a single base pair. The reference *M. viridis* (NIES102) clustered closely to the reference *M. aeruginosa* genotype, along with genotypes G2, G3, and G4 (Fig. 4).

DISCUSSION

The present work confirms the utility of *cpc*BA-IGS sequences to resolve distinct genotypes within the genus *Microcystis*. The maximum within-genus *cpc*BA variation found here (up to 6.4%) compares closely with the variation noted over the shorter 360-bp 16–23S rRNA spacer region (Otsuka et al. 1999). The variation is also similar to the 6.7% *cpc*BA-IGS variation within *Nodularia* (Hayes and Barker 1997, Bolch et al. 1999a), although *Microcystis* showed comparatively higher variation in the *cpc*A gene (13.7%) than *Nodularia* (8.4%). Much of this additional *cpc*A variation was between *M. wesenbergii* and *M. aeruginosa* genotypes in *wesenbergii*-specific region 145–160 bp from the start of the *cpc*A gene, a potentially useful target for development of group-specific probes or PCR primers to discriminate *Microcystis* lineages.

Early studies of morphological and genetic variation of *Microcystis* morphospecies have shown some correlation between morphological and genetic groupings. Allozyme studies indicated that *M. wesenbergii,* and *M. viridis* were distinct but that *M. aeruginosa* could be separated into two groups on the basis of cells size: (1) *M. aeruginosa* L type with larger cells (4.58–5.08 μm diameter), a consistent colony morphology (Kato et al. 1991), considered to correspond to *M. aeruginosa* (Komárek 1991) and (2) a morphologically and genetically variable group with smaller cells (3.25–4.19 μm diameter) referred to as *M. aeruginosa* S type. This latter group was considered to contain a number of morpho-

types transitional between *M. aeruginosa* and *M. ichthyoblabe* (Komárek 1991). These findings were generally supported by recent randomly amplified polymorphic-PCR DNA studies that also noted a high level of genetic polymorphism among strains classified as *M. ichthyoblabe* and *M. aeruginosa* (Nishihara et al. 1997). In contrast, more recent studies have failed to support current morphological groupings. Comparison of 16–23S rRNA sequences of five species of *Microcystis* resolved 25 genotypes into three broad clusters (Otsuka et al. 1999). Two clusters contained primarily *M. viridis* and *M. wesenbergii*, respectively, but also *M. aeruginosa* strains. The third and largest cluster was genetically highly variable and contained a mix of strains classified as *M. aeruginosa*, *M. novacekii*, and *M. ichthyoblabe*.

These latter genetic studies concluded that that morphology does not correlate with genotype in *Microcystis aeruginosa*, or *Microcystis* generally, and that one genotype could present more than one morphotype, a conclusion generally supported by the current work. This poor overall correlation of genotype and morphospecies results from culture-induced variation (Krüger et al. 1981, Doers and Parker 1988, Otsuka et al. 2000), effects of changing environmental conditions on morphology (Krüger et al. 1981), overlapping morphological traits (Kato et al. 1991), and transitional colonial morphology (Komárek 1991, Bittencourt-Oliveira 2000). All these factors complicate the placement of strains into particular morphological species, and it is difficult to envisage a morphological scheme that can adequately account for the morphological and genetic variability in the genus. However, close examination of previous works (e.g. Kato et al. 1991, Nishihara et al. 1997, Otsuka et al. 1999) and the data presented here indicate that some genotypes or lineages display consistent morphological characteristics. Some have consistently smaller or larger cell sizes (e.g. L3 and L4), and some morphospecies appear to correspond largely with a particular genotype or cluster of closely related genotypes (e.g. *M. viridis* and *M. wesenbergii*) (Kato et al. 1991, Nishihara et al. 1997, Otsuka et al. 1999, this study). This argues strongly for a polyphasic approach, combining the development and routine use of genetic detection with existing morphotaxonomic criteria, to discriminate and characterize the complexity of *Microcystis* populations.

The high diversity of the Brazilian *M. aeruginosa* lineages compared with the PCC and NIES reference strains from Europe, North America, and Japan demonstrates that strains or populations routinely classified as *M. aeruginosa* in one region (Brazil in this case) can be genetically distinct entities to similarly classified *Microcystis* from other regions. Without reference to more widely collected material, studies of *Microcystis* from restricted geographic or environmental ranges could therefore under-represent diversity and produce misleading taxonomic or phylogenetic conclusions. Similarly, global generalizations of the physiological or environmental tolerance, toxicity, or bloom dynamics of individual *Microcystis* species are also potentially invalid.

In this study identical *cpc*BA-IGS genotype could be recovered from widely separated water bodies. The most extreme example is genotype G5, which is known from Brazil (strain FCLA-200), Japan (NIES98, 99), Scotland (PCC7820), and North America (PCC7941). It is possible that this genotype may represent "cosmopolitan species" as suggested for a proportion of temperate cyanobacterial species (Komárek 1985). However, identical *cpc*BA-IGS genotypes of *Nodularia spumigena* can be resolved into distinct genotypes using genetic fingerprinting approaches (Bolch et al. 1999a); therefore, it is unlikely that *Microcystis cpc*BA genotypes represent one genetic clone throughout their distribution but rather a series of related clonal populations sharing a common phylogenetic history.

Although some previous data sets have indicated the presence of two or three *M. aeruginosa* genotypes in a single water body (Kato et al. 1991, Bolch et al. 1997), this study recovered six *M. aeruginosa* genotypes from Garças reservoir during the 14-month sampling period. This high level of genetic diversity of *Microcystis* strains demonstrates the potential for major shifts in dominant genotypes and accompanying bloom toxicity. Such shifts in genotype may further explain the often poor correlations of *Microcystis* cell numbers with microcystin content in many field studies (e.g. Kotak et al. 1995, Jungmann et al. 1996), a phenomenon usually attributed to environmental effects on cellular toxicity.

Supported by FAPESP (The State of São Paulo Research Foundation, 97/10741-3 and 95/9182-4), FUNDUNESP (082/97), and CNPQ (301052/96-4 and 300163/94-0). We thank Dr. Sandra M. O. Azevedo from Universidade Federal do Rio de Janeiro for providing NPLS-1 strain and Drs. C. Bicudo, D. Bicudo, Célia Sant'Anna, Maria Tereza P. Azevedo, and Pedro A. Senna for bloom samples. We thank Maria Cristina F. Costa for helping with the DNA sequencing.

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