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Identification and Phylogenetic Analysis of Toxigenic Cyanobacteria by Multiplex Randomly Amplified Polymorphic DNA PCR

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Randomly amplified polymorphic DNA PCR was used to generate unique and identifying DNA profiles for members of the cyanobacterial genera *Anabaena* and *Microcystis*, which are responsible for much of the production of nuisance blooms in various freshwater systems, including recreational and drinking water supplies. A method based on the combination of two 10-mer oligonucleotides in a single PCR was developed to provide specific and repeatable DNA fingerprints for cyanobacterial isolates. The strain-specific randomly amplified polymorphic DNA profiles made it possible to discriminate among all toxigenic cyanobacteria studied to the three taxonomic levels of genus, species, and strain. Analysis of DNA typing results obtained by the described method clearly distinguishes between the genera *Anabaena* and *Microcystis*. The markers produced for each strain were also applied to a phylogenetic analysis to infer genetic relatedness in this group of prokaryotes.

Members of the cyanobacterial genera *Microcystis* and *Anabaena* are commonly present as a mixed population during blue-green algal blooms. Responding to various ecological stimuli, including eutrophication of their freshwater habitat, these oxygenic phototrophs rapidly form blooms, causing extensive physical and chemical damage to affected aquatic environments (19, 21). The frequency and severity of bloom events continue to rise, most probably as a direct result of increased nutrient loading of water systems worldwide. Specific detrimental effects of cyanobacterial blooms on drinking water quality include the production of taste- and odor-causing compounds and several toxic molecules. Specifically, the neurotoxins and hepatotoxins associated with cyanobacterial blooms are responsible for deaths in wild and domesticated animal populations and have various acute and chronic pathogenic effects on humans (5, 9).

Current cyanobacterial taxonomy is based primarily on observed morphological characteristics which do not identify potentially bloom-forming or toxigenic strains. More importantly, the morphology of cyanobacteria in laboratory cultures is often considerably altered from the original morphology of environmental isolates, and the diversity of strains within a culture may be reduced because of selective culturing conditions (6, 26). Analyses of photosynthetic pigment content, isozyme variation, or differentiated cell structures may also be misleading because of the variable expression of cyanobacterial gene products in culture (11, 20). In addition, plasmid profiles have not indicated any relationship to strain toxicity or bloom potential (12).

The randomly amplified polymorphic DNA (RAPD) technique, in conjunction with PCR, has been employed to identify many organisms to the strain level of classification (27, 29). This technique is sensitive and specific because the entire genome of an organism is used as the basis for generating a DNA profile. In this study RAPD-PCR technology was used for the detection of genetic heterogeneity among axenic cultures of cyanobacteria. The methods reported are a modification of standard RAPD protocols, which use a single 10-mer primer, and, because of the increased stringency of the method for amplification reaction, provide a highly reproducible RAPD pattern for each strain analyzed. The increased reproducibility provided by the presented method is primarily a result of the multiplexing of random 10-mer oligonucleotide primers. The cyanobacteria used in this study are commonly encountered bloom-forming and toxic species, and the results presented indicate specific genetic profiles which may be used to rapidly identify them in axenic cultures. The inferred phylogeny of this group, based on the RAPD data, is also presented.

MATERIALS AND METHODS

Cyanobacterial cultures. Strains were chosen from the culture collections of the Pasteur Culture Collection, France (PCC), the National Institute of Environmental Studies, Japan (NIES), and Australian Water Technologies, Australia (AWT). Only those cultures reported to be axenic and unicyanobacterial were used in this study. The strains used are species of the filamentous genus *Anabaena* and the unicellular genus *Microcystis*, which are commonly found in cyanobacterial blooms and which produce neurotoxins and hepatotoxins, respectively. The strains used in the present study are listed in Table 1.

DNA extraction and PCR template preparation. Samples of type cultures were harvested immediately upon receipt, and PCR templates were prepared according to two methods. In the first method, total genomic DNA was extracted by a modification of a method for purification of DNA from gram-negative bacteria, including cyanobacteria (24). A 1-ml aliquot of mid- to late-log-phase culture was pelleted by centrifugation, the medium was decanted, and the pellet was resuspended in 500 μl of 50 mM Tris-HCl (pH 8.0)–5 mM EDTA (pH 8.0)–50 mM NaCl. Lysozyme was added to obtain a final concentration of 1 mg/ml, and the solution was incubated at 55°C for 30 min. After the addition of 10 μl of proteinase K (10 mg/ml) and 20 μl of 10% sodium dodecyl sulfate, the mixture was incubated at 55°C for 10 min or until the solution cleared (complete cell lysis). The solution was chilled on ice and extracted with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1). The organic extraction was repeated, and the supernatant was added to an equal volume of 4 M ammonium acetate. Total genomic DNA was precipitated by the addition of 2 volumes of isopropanol followed by centrifugation for 10 min at room temperature.

Alternatively, the PCR template was prepared by the rapid lysis of cyanobacterial cells and the liberation of DNA into the supernatant. In this procedure approximately 10^5 to 10^7 cells were centrifuged to a pellet and the culture

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TABLE 1. Cyanobacterial strains used in this study

Strain	Culture collection[a]	Source
Microcystis aeruginosa PCC7806	1	Braakman Reservoir, The Netherlands
Microcystis sp. strain AWT102	2	Penrith Lake, Australia
Microcystis sp. strain AWT104	2	Lake Oberon, Australia
Microcystis sp. strain AWT107	2	Newcastle, Australia
Microcystis sp. strain AWT114	2	Botany Pond 5, Australia
Microcystis sp. strain AWT139	2	Manly Dam, Australia
Microcystis aeruginosa NIES89	3	Lake Kawaguchi, Japan
Microcystis wesenbergii NIES107	3	Lake Kawaguchi, Japan
Microcystis holsatica NIES43	3	Lake Kasumigaura, Japan
Microcystis elabens NIES42	3	Lake Kasumigaura, Japan
Microcystis viridis NIES102	3	Lake Kasumigaura, Japan
Anabaena circinalis NIES41	3	Lake Kasumigaura, Japan
Anabaena spiroides NIES78	3	Lake Kasumigaura, Japan

[a] 1, PCC; 2, AWT; 3, NIES.

medium was removed. The cells were resuspended in 200 μl of Instagene matrix (Bio-Rad, Hercules, Calif.) supplemented with 20 μl of 1% Nonidet P-40 or 1% Triton X-100. Cells were incubated at 55°C for 30 min, vortexed for 1 min, and heated to 95°C for 10 min. The solution was again vortexed for 1 min, and the cell debris was pelleted by brief centrifugation. Between 1 and 5 μl of the supernatant contained sufficient genetic material for a successful PCR amplification. The supernatant of this cell lysate had been stored at 4°C for over 2 years and was found to retain sufficient DNA for a PCR template.

RAPD-PCR and electrophoretic analysis. The standard, optimized PCR was performed in a total volume of 50 μl containing 3 mM $MgCl_2$, 200 μM each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 10 pmol of each PCR primer, 1 U of *Taq* DNA polymerase (Biotech International, Perth, Australia), and 10 ng of genomic DNA or 2 μl of cyanobacterial cell lysate supernatant. The reaction product was overlaid with mineral oil, and thermal cycling was achieved in an FTS-1 thermal sequencer (Corbett Research, Sydney, Australia) according to the following program: initial denaturation at 94°C for 4 min; 30 cycles of 94°C for 20 s, 45°C for 30 s, 72°C for 60 s; and a final extension at 72°C for 5 min. Conditions for the small-volume (10-μl) capillary PCR have been previously described (17) and were applied to the RAPD-PCR of cyanobacteria in this study to highlight the robustness of the proposed method. The random 10-mer oligonucleotide primers used are presented in Table 2. Primers were obtained from commercial sources (Operon Technologies, Alameda, Calif.) or synthesized on an Oligo 1000 DNA synthesizer (Beckman, Fullerton, Calif.) by using previously published RAPD experiment data (1, 14). The multiplexing of RAPD primers was performed after single primers provided adequate DNA fingerprints at an annealing temperature of 40°C. Primers were combined in an equimolar ratio for the multiplex reactions, and the cycling annealing temperature was raised.

PCR products were resolved on either a 2% low-melting-point–1% agarose gel in Tris-acetate-EDTA buffer or on a 4 to 20% gradient, native polyacrylamide gel (Novex, San Diego, Calif.) in Tris-borate-EDTA buffer. Agarose and polyacrylamide gels were stained in an ethidium bromide solution (0.5 mg/ml) to visualize RAPD bands. The polyacrylamide gels were also stained with silver nitrate (18).

Calculation of genetic distances with RAPD markers. Each band visualized on a gel was considered a RAPD marker and part of the total RAPD fingerprint generated for a strain of cyanobacteria. Bands bisected by similar perpendicular lines drawn across the gel were considered homologous characteristics. With this approach to RAPD marker identification, the comigration of bands between cyanobacterial accessions was used as an indication of genetic relatedness. The assumption that these bands contained homologous primer recognition sequences and identical intervening sequence lengths was made. Therefore, the presence or absence of a band at any position on the gel was used to construct a binary matrix for cyanobacterial RAPD markers from the described multiplex reaction. Genetic distances between strains were calculated by using the algorithm of Nei and Li (15) as provided in the RAPDistance software package developed by Armstrong et al. (3). A pairwise comparison of genetic distances for all cyanobacterial patterns was used to create a phenogram based on the neighbor-joining method and the program NJTREE (22), which was also supplied with the RAPDistance package. Another 17 metrics supplied by the RAPDistance package were applied to the data to support the tree inferred by the Nei and Li algorithm distances, including metrics previously used for RAPD marker analysis (2).

TABLE 2. Decamer primers used for RAPD analysis of cyanobacteria

Primer	Sequence	% G+C	Reference or source
CRA22	CCGCAGCCAA	70	14
CRA23	GCGATCCCCA	70	14
CRA25	AACGCGCAAC	60	14
CRA26	GTGGATGCGA	60	14
OPA-08	GTGACGTAGG	60	Operon Technologies
OPA-11	CAATCGCCGT	60	Operon Technologies
OPA-13	CAGCACCCAC	70	Operon Technologies
OPA-18	AGGTGACCGT	60	Operon Technologies

RESULTS

Optimization and selection of a multiplex RAPD-PCR. A total of 48 primers were initially chosen to generate RAPD patterns for 27 strains of cyanobacteria. The criteria for choosing these primers were their availability and the generally accepted bias towards oligonucleotides of high G+C content (30). This number of 10-mer oligonucleotides was reduced to eight, these being the primers which produced informative and reproducible genetic markers for the cyanobacteria. Primers which produced consistently even product intensities throughout a pattern were favored because of the high reproducibility of markers in these reactions. Of the primers selected, three had a G+C content of 70%. Primers with a G+C content of 60% which did not produce RAPD patterns with 10 pmol of primer were successful with 100 pmol per reaction. Higher concentrations of primers with 70% G+C resulted in complicated and unrepeatable PCR patterns. Single primers which gave similar patterns across a range of PCR annealing temperatures up to 42°C were chosen for further optimization of the reaction. Two primers, CRA22 and CRA23, were combined in equimolar ratios and used at 10 pmol per primer per 50-μl reaction mixture. RAPD patterns generated from this multiplex reaction were identical over a range of PCR annealing temperatures between 35 and 45°C and template DNA levels from 100 pg to 100 ng (Fig. 1). The annealing temperature of 45°C provided the most stringent thermal cycling conditions that provided reproducible PCR products. Increasing the annealing temperature to 50°C led to no amplification in the multiplex reaction but produced an altered pattern in the single-primer PCR. RAPD patterns in the dual-primer PCR were also highly reproducible with neat and diluted cyanobacterial cell lysate as the PCR template (Fig. 1).

Reproducibility was also achieved for this multiplex RAPD in a number of other areas previously considered drawbacks of RAPD analyses. First, no change in pattern was seen among reactions from different regions of the thermal cycling heating blocks used. Similarly, patterns among various thermal cycling instruments were identical, although cycling times were extended for machines which do not employ an in-tube thermocouple to monitor the actual reaction temperature (such as Perkin-Elmer Cetus model 480). The transfer of the technology to other laboratories with different thermal cyclers and reagents did not alter the RAPD profile obtained for strains PCC7806 and PCC7005 (data not shown). Second, identical patterns were obtained with thermostable DNA polymerases from different suppliers, e.g., AmpliTaq (Perkin-Elmer, Norwalk, Conn.). Assuming similar unit activity values for different enzymes, reproducible results should be achieved when this parameter is altered. The concentration of $MgCl_2$ in the PCR was held constant at 3 mM for all experiments, as was the thermostable polymerase at 1 U per 50-μl reaction mixture, on the basis of previously reported RAPD reaction optimizations (7).

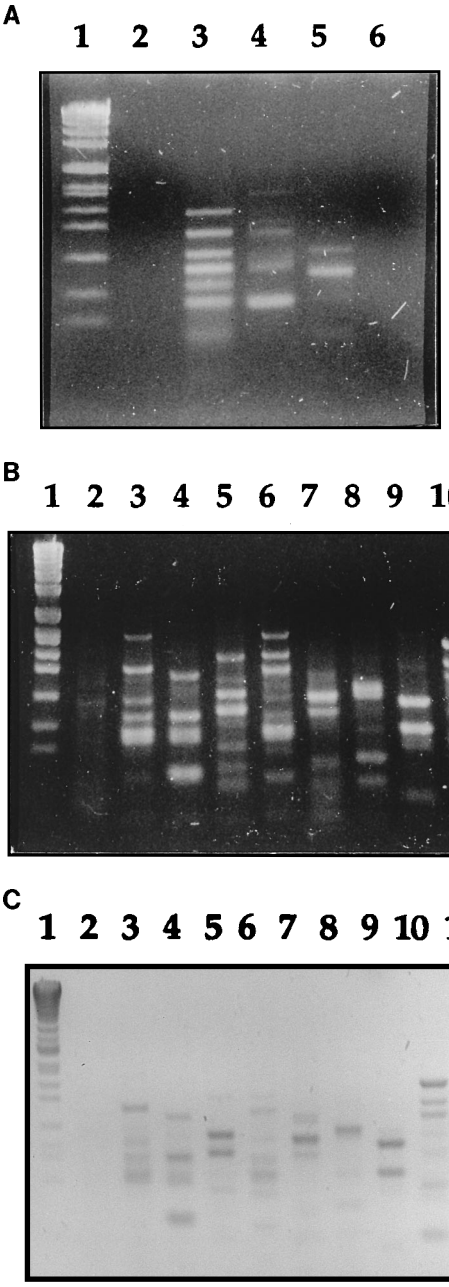

FIG. 1. Effects of altered PCR annealing temperatures on single- and dual-primer RAPD. (A) RAPD profiles for *M. aeruginosa* PCC7806. Lane 1, SPP-1 and *Eco*RI; lane 2, no DNA control (primers CRA22 and CRA23); lanes 3 and 6, primers CRA22 and CRA23 with PCR annealing at 35 and 50°C, respectively; lanes 4 and 5, primer CRA23 with PCR annealing at 35 and 50°C, respectively. (B) Multiplex RAPD (with primers CRA22 and CRA23) with PCR annealing at 35°C. Lane 1, SPP-1 and *Eco*RI; lane 2, no DNA control; lanes 3 to 11, cell lysates from *Microcystis* sp. strains NIES107, NIES102, NIES42, NIES89, NIES43, AWT114, and AWT104 and *Anabaena* sp. strains NIES41 and NIES78, respectively (strains described in Table 1). (C) Multiplex RAPD as in panel B but with the PCR annealing temperature increased to 42°C (refer to Fig. 4 for the result of PCR with annealing at 45°C). Samples were loaded on 2% low-melting-point–1% agarose gels in Tris-acetate-EDTA buffer. All gels were stained with ethidium bromide; the positive photographic images are presented in panels A and B, while the negative image is presented in panel C.

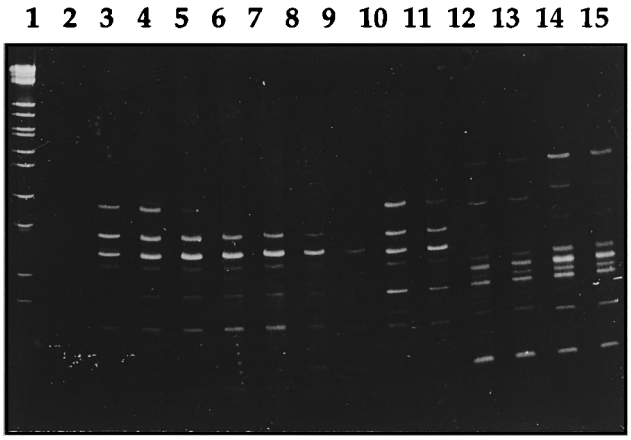

FIG. 2. Effects of template DNA concentration and use of crude cell lysates for multiplex RAPD-PCR. Primers used are CRA22 and CRA23 in a 50-μl PCR mixture, as described in the text. Lane 1, SPP-1 and *Eco*RI; lane 2, no DNA control; lanes 3 to 9, 1,000 ng, 100 ng, 10 ng, 1 ng, 100 pg, 10 pg, and 1 pg, respectively, of extracted DNA from *M. aeruginosa* PCC7806; lanes 10 and 11, 3 and 0.3 μl, respectively, of cell lysate from PCC7806; lanes 12 and 13, 10 and 1 ng, respectively, of genomic DNA from *M. aeruginosa* NIES89; lanes 14 and 15, 3 and 0.3 μl, respectively, of cell lysate from NIES89. The gel used was 4 to 20% polyacrylamide in Tris-borate-EDTA buffer.

Generation of DNA profiles and measurement of genetic relatedness. The electrophoretic patterns for 11 strains of bloom-forming and toxigenic cyanobacteria derived from eight single-primer reactions and one double-primer reaction were analyzed to provide diagnostic fingerprints for each culture and genetic distances between strains based on RAPD markers. The multiplex RAPD-PCR was chosen, because of its greater degree of reproducibility and its stringency, for the accurate differentiation of cyanobacteria to the strain level and the inference of cyanobacterial phylogeny. The DNA fingerprint produced consists of bands between ca. 1,400 and 100 bp and clearly distinguishes among all strains tested. Successive analyses of cultures have shown that these RAPD profiles are consistent over periods of time up to 18 months and after several PCR template extraction protocols (Fig. 2). Several type cultures were reordered from axenic culture collections over the course of this study, and all batches of the same strain gave identical profiles. The results of a single multiplex RAPD-PCR, like the results shown for the two-primer RAPD-PCR, were also able to distinguish among strains of *Microcystis* spp. isolated from different geographical locations and exhibiting various levels of microcystin production (Fig. 3). The results also show the ability of the RAPD method to identify *Microcystis* species isolated and purified from a common freshwater habitat (Fig. 4).

Genetic diversity among strains tested was determined with banding patterns from the multiplex RAPD reaction. Each band was considered a genetic marker for the strain from which it was amplified. Comigrating bands were recorded as similar markers but were not sequenced or probed for further identification. The multiplexing of primers CRA22 and CRA23 generated a total of 33 RAPD markers and 29 RAPD markers when analyzed on polyacrylamide and agarose gels, respectively (Fig. 3). The data produced, comprising 29 markers from 10 strains of *Anabaena* and *Microcystis* spp., were used to calculate genetic distances, and by pairwise comparisons (data not shown) the phenogram in Fig. 5 was constructed. As expected, the tree illustrates the similarity of RAPD patterns seen on the gels. The phenogram clearly supports the delinea-

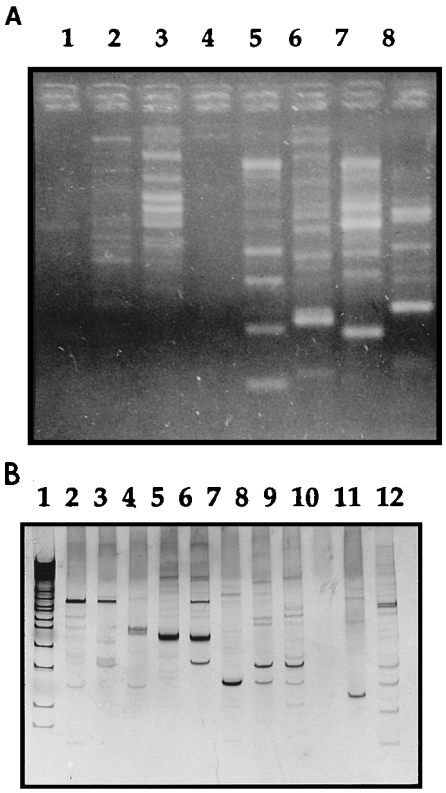

FIG. 3. (A) RAPD profiles for *M. aeruginosa* PCC7806 with 10-mers OPA-08, OPA-11, OPA-13, OPA-18, CRA22, CRA23, CRA25, and CRA26 on agarose gel lanes 1 to 8, respectively. (B) Silver-stained 4 to 20% polyacrylamide and Tris-borate-EDTA gel showing RAPD profiles derived from a single-primer (CRA22) PCR. The reaction annealing temperature used was 42°C. Lane 1, SPP-1 and *Eco*RI; lanes 2 to 12, AWT102, NIES298, NIES102, NIES107, AWT139, AWT104, AWT107, NIES41, a *Pseudoanabaena* sp., PCC7005, and PCC7806, respectively, as outlined in Table 1.

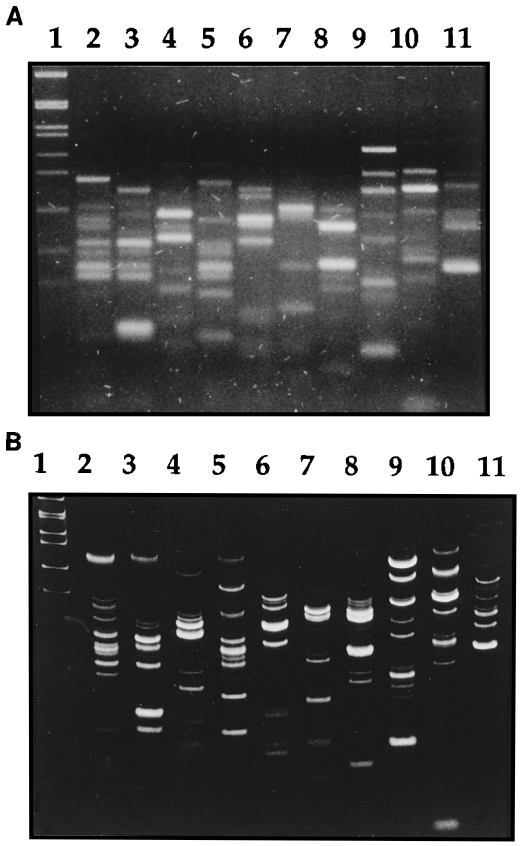

FIG. 4. Multiplex (with primers CRA22 and CRA23) RAPD-PCR of cyanobacterial strains and electrophoresis for successive phylogenetic analysis. PCR was performed at an annealing temperature of 45°C. Lane 1, SPP-1 and *Eco*RI; lanes 2 to 11, strains NIES107, NIES102, NIES42, NIES89, NIES43, AWT114, AWT104, NIES41, NIES78, and PCC7806, respectively (Table 1). (A) PCR products separated on 2% low-melting-point–1% agarose gels in Tris-acetate-EDTA buffer. (B) Electrophoresis of RAPD markers on a 4 to 20% polyacrylamide gel in Tris-borate-EDTA buffer.

tion of the genera *Anabaena* and *Microcystis*, as shown by the primary bifurcation of the tree. This clustering of morphologically distinct groups is supported by previous work describing the phylogeny of freshwater cyanobacteria obtained with 16S rRNA gene sequences (10, 16). However, the RAPD-PCR-derived phylogeny does not clearly discriminate among the species of *Microcystis* in this study, with traditionally classified taxa dispersed throughout the *Microcystis* domain. The relatively large genetic distances, seen in both RAPD and 16S rDNA (DNA encoding RNA) data, among strains of the species *Microcystis wesenbergii* and those of *Microcystis aeruginosa* are indicative of the problems associated with the current taxonomy based on largely undifferentiated morphologies (13).

DISCUSSION

The combination of PCR and random oligonucleotide primers, 10 nucleotides long, has provided a method for the rapid and sensitive delineation of animal, plant, fungal, algal, and bacterial strains (1, 14, 23, 27, 29). RAPD profiling of these organisms' genomes has been widely accepted as a valid taxonomic and phylogenetic tool. Current RAPD technology has been optimized for the identification of cyanobacterial cultures to the strain level. The combination of two primers in a single reaction has proven to be useful for optimizing RAPD reactions, as has the correct selection of primers and other PCR components. The choice of primers with high G+C contents provided good DNA profiles at a low concentration, but oligonucleotide base composition does not appear to be linked to the target molecule base content, as cyanobacterial G+C content is in the range of 40 to 70%, with the average for *Microcystis* species genomes being 40 to 43% G+C (8). The primers synthesized in this study were chosen because of their previous usefulness when applied to both plant and bacterial genomes (1, 14). Multiplexing 10-mer primers has resulted in increased reproducibility, efficiency, and sensitivity of the RAPD-PCR. Lowering primer and DNA concentrations as well as raising the PCR annealing temperature compared with previously described concentrations and temperatures has resulted in reaction conditions with increased stringency. The products of the multiplex RAPD method under more stringent conditions were reproducible across a range of varied parameters such as enzyme preparation, type of thermal cycler, and quantity and quality of template DNA. Multiplexing also had the advantage of providing an increased number of informative genetic markers from a single reaction compared with the results produced with only one primer per reaction. Comparison of single- and multiple-primer reactions showed that most bands derived from the single priming were retained, while some were lost and several new products were synthesized as a result of multiplexing.

The strains used in this study were axenic laboratory cul-

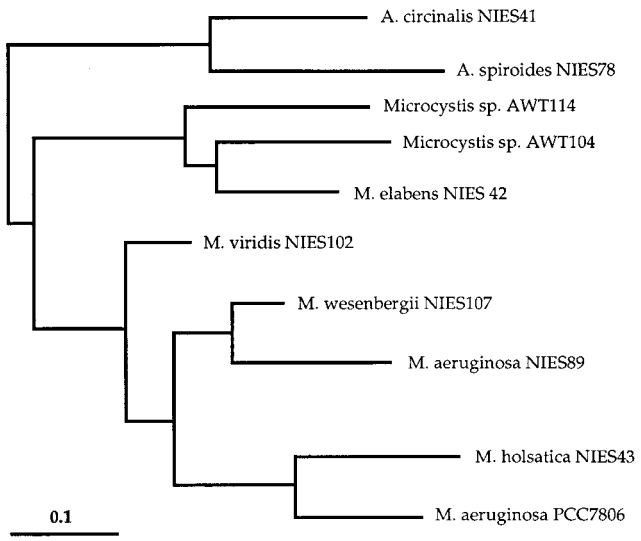

FIG. 5. Phenogram based on RAPD markers amplified from 10 strains of bloom-forming and toxigenic cyanobacteria. Markers were amplified by the described two-primer RAPD-PCR and electrophoresed on an agarose gel (Fig. 4). Genetic distances were calculated and a neighbor-joining tree was constructed with the RAPDistance software package. The scale indicates genetic distance measurements as calculated by the algorithm of Nei and Li (15).

tures, as the described RAPD-PCR would be altered by the presence of any contaminating microorganisms. For the described technique to be applicable to environmental isolates, the sample must be made axenic by selective culturing or by using specific cyanobacterial cells segregated by micromanipulation or laser capture methods. We have previously reported taxonomic and phylogenetic relationships for the genus *Microcystis* based on 16S rRNA gene sequences (16). The specificity of the RAPD-PCR was highlighted by the distinguishing DNA fingerprints for *M. aeruginosa* PCC7806 and NIES89; these fingerprints resulted in the placement of the strains in separate lineages in the presented phenogram (Fig. 5). Morphological data support the discrimination of these two strains of *M. aeruginosa*, with PCC7806 being unicellular and NIES89 displaying a unicellular, colonial habit in culture. The results of 16S rRNA gene sequencing, on the other hand, provided data indicating relatively little genetic divergence, with a sequence similarity of 99.6% for these two strains. DNA sequence heterogeneity detected in the phycocyanin operon and 16S rDNA of cyanobacteria revealed multiple evolutionary origins for strains of the *Microcystis* species as they are currently defined (data not shown). The lack of consistent clustering of strains from morphologically determined *Microcystis* species delineations was also indicated by the inferred RAPD phylogeny of this cyanobacterial radiation (Fig. 5). For the accessions studied, the current species definitions for the genus *Microcystis* were not supported by the DNA profiles generated.

It has been shown that as few as three primers used separately provide enough polymorphic information to identify species of the symbiotic genus *Anabaena* and to create a phylogenetic tree with a topology similar to that derived with 22 primers (25). Similarly, a single 10-mer proved sufficient for distinguishing among 64 strains of *Helicobacter pylori* (1). The data from the present study suggest that this level of polymorphic content can be analyzed by a single reaction with multiple primers. In this system the multiplex RAPD-inferred cyanobacterial diversity is closely supported by the evolutionarily significant 16S rRNA gene sequences (10, 16).

In addition, the RAPD technique does not require previous knowledge of an organism's gene sequences and requires only 1/1,000 the amount of DNA required for traditional restriction fragment length polymorphism-Southern hybridization experiments. The combination of 10-mer primers in a single reaction has not been previously reported, although the use of multiple 5-mers (4) or dual arbitrary priming with longer primers (at least 18-mers) has been suggested (28). The technique used in this study was also performed without the need for radioactive labelling of the RAPD-PCR products or for hybridization. The use of a single, multiple-primer RAPD-PCR may not be suitable for inferring an accurate phylogeny for all taxonomic groups. The results show that the genetic relatedness among the genera of bloom-forming cyanobacteria is supported by both the described multiplex RAPD markers and 16S rRNA gene sequences. Thus, the sensitivity of RAPD, which is derived by using the entire genome as the PCR template, provides a taxonomy and phylogeny for the cyanobacteria which are comparable to those achieved by more traditional methods. The novel RAPD-PCR-based classification of toxic and bloom-forming cyanobacteria is an alternative and complementary approach to the traditional methods for studying cyanobacterial systematics. It should be noted that RAPD profiles may be altered by the presence of transposable elements and plasmids in prokaryotes, as well as by contaminating bacteria in a sample. The multiplex RAPD-PCR method presented here has also been applied to the identification of species of lactic acid bacteria and archival Australian native plant embryos (unpublished data). The DNA profiles produced for the cyanobacteria provide a rapid and reliable basis for the genetic typing of clonal and axenic cultures.

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REFERENCES

1. **Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg.** 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res. **20:**5137–5142.
2. **Apostol, B. L.** 1993. Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*. Theor. Appl. Genet. **86:**991–1000.
3. **Armstrong, J., A. Gibbs, R. Peakall, and G. Weiller.** 1994. The RAPDistance package. ftp://life.anu.edu.au (150.203.38.74) pub/molecular_biology/rapd-pack.exe.
4. **Caetano-Anolles, G., B. J. Bassam, and P. M. Gresshoff.** 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology **9:**553–557.
5. **Carmichael, W. W.** 1992. Cyanobacterial secondary metabolites—the cyanotoxins. J. Appl. Bacteriol. **72:**445–459.
6. **Doers, M. P., and D. L. Parker.** 1988. Properties of *Microcystis aeruginosa* and *M. flos-aquae* (Cyanophyta) in culture: taxonomic implications. J. Phycol. **24:**502–508.
7. **Ellsworth, D. L., K. D. Rittenhouse, and R. L. Honeycutt.** 1993. Artifactual variation in randomly amplified polymorphic DNA banding patterns. BioTechniques **14:**214–215.
8. **Fahrenkrug, P. M., M. B. Bett, and D. L. Parker.** 1992. Base composition of DNA from selected strains of the cyanobacterial genus *Microcystis*. Int. J. Syst. Bacteriol. **42:**182–184.
9. **Falconer, I. R., and T. H. Buckley.** 1989. Tumour promotion by *Microcystis* sp., a blue-green alga occurring in water supplies. Med. J. Aust. **150:**351.
10. **Giovannoni, S. J., S. Turner, G. J. Olsen, S. Barns, D. J. Lane, and N. R. Pace.** 1988. Evolutionary relationships among cyanobacteria and green chloroplasts. J. Bacteriol. **170:**3584–3592.
11. **Kato, T., M. F. Watanabe, and M. Watanabe.** 1991. Allozyme divergence in microcystis (Cyanophyceae) and its taxonomic inference. Arch. Hydrobiol. Suppl. Bd. **64:**129–140.

12. **Kohl, J. G., T. Borner, M. Henning, W. Schwabe, and A. Weihe.** 1988. Plasmid content and differences in ecologically important strains of *Microcystis aeruginosa*. Arch. Hydrobiol. Suppl. Bd. **53:**195–201.
13. **Komarek, J.** 1991. A review of water-bloom forming *Microcystis* species, with regard to populations in Japan. Arch. Hydrobiol. Suppl. Bd. **64:**115–127.
14. **Kresovich, S., J. G. K. Williams, J. R. McFerson, E. J. Routman, and B. A. Schaal.** 1992. Characterisation of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor. Appl. Genet. **85:**190–196.
15. **Nei, M., and W. H. Li.** 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA **76:**5269–5273.
16. **Neilan, B. A., P. T. Cox, P. R. Hawkins, and A. E. Goodman.** 1994. 16S ribosomal RNA gene sequence and phylogeny of toxic *Microcystis* sp. (cyanobacteria). DNA Sequence **4:**333–337.
17. **Neilan, B. A., A. Gurvitz, D. Leigh, L. Y. C. Lai, and B. McDonald.** 1993. Rapid preparation of limited biological samples for capillary PCR. PCR Methods Applic. **2:**261–262.
18. **Neilan, B. A., D. A. Leigh, E. Rapley, and B. L. McDonald.** 1994. Microsatellite genome screening: rapid non-denaturing, non-isotopic dinucleotide repeat analysis. BioTechniques **17:**708–712.
19. **Reynolds, C. S., and A. E. Walsby.** 1975. Water-blooms. Biol. Rev. **50:**437–481.
20. **Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier.** 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. **111:**1–61.
21. **Rosenberg, D. B., and S. M. Freeman.** 1991. Succession theory, eutrophication, and water quality, p. 239–251. *In* J. Rose (ed.), Water and the environment. Gordon and Breach Science Publishers, Philadelphia.
22. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
23. **Scott, M. P., K. M. Haymes, and S. M. Williams.** 1992. Parentage analysis using RAPD PCR. Nucleic Acids Res. **20:**5493.
24. **Smoker, J. A., and S. R. Barnum.** 1988. Rapid small-scale DNA isolation from filamentous cyanobacteria. FEMS Microbiol. Lett. **56:**119–122.
25. **Van Coppenhole, B., I. Watanabe, C. Van Hove, G. Second, N. Huang, and S. R. McCouch.** 1993. Genetic diversity and phylogeny analysis of *Azolla* based on DNA amplification by arbitrary primers. Genome **36:**686–693.
26. **Ward, D. M., R. Weller, and M. M. Bateson.** 1990. 16S rRNA sequences reveal uncultured microorganisms in a natural community. Nature (London) **345:**63–65.
27. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
28. **Welsh, J., and M. McClelland.** 1991. Genomic fingerprinting using arbitrarily primed PCR and a matrix of pairwise combinations of primers. Nucleic Acids Res. **19:**5275–5279.
29. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.
30. **Yu, K., and K. P. Pauls.** 1992. Optimisation of the PCR program for RAPD analysis. Nucleic Acids Res. **20:**2606.