NOTES

Base Composition of DNA from Selected Strains of the Cyanobacterial Genus *Microcystis*

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Average DNA base compositions, as determined from 50% thermal denaturation temperatures and from buoyant densities, were 38, 41, 43, and 40 to 41 mol% G+C for *Microcystis* sp. strains P1-15, C5-34, AK1-6, and C3-40, respectively. These phenotypically similar organisms, which are representatives of the *Microcystis* cluster within the *Synechocystis* group of chroococcacean cyanobacteria, have a smaller range of DNA base compositions (38 to 43 mol% G+C) than the *Synechocystis* group as a whole does (30 to 48 mol% G+C). Because the DNA base ratios of the *Microcystis* cultures adjoin or overlap those of the low- and high-G+C-content clusters of the *Synechocystis* group, DNA base composition data do not differentiate the *Microcystis* cluster from these two *Synechocystis* clusters. By bridging the gap between the low- and high-G+C-content clusters of the *Synechocystis* group, the data for *Microcystis* create a continuum of DNA base ratios within the *Synechocystis* group and diminish the usefulness of G+C values for discerning clusters within the phenotypically heterogeneous *Synechocystis* group.

Unicellular cyanobacteria that divide in two or three successive planes at right angles to one another have been provisionally assigned to an assemblage designated the *Synechocystis* group (8–10, 12, 19–21, 23), which consists of the following four strain clusters: the marine cluster, the low-G+C-content cluster, the high-G+C-content cluster, and the *Microcystis* cluster (24). The four clusters are “equivalent to genera but have not been formalized because their characterization is based on an incomplete data set, with a relatively limited number of phenotypic properties used” (24). For brevity and for consistency with previous publications, organisms belonging to the *Microcystis* cluster are referred to below as *Microcystis* strains, without implying more than a provisional taxonomic conclusion.

Previously, the DNA base composition of only one *Microcystis* culture, strain ATCC 27153 (= PCC 7005), has been determined (23). Below we describe a method for extracting DNAs from additional axenic *Microcystis* sp. cultures and report their DNA base compositions as estimated from 50% thermal denaturation temperatures (T_m) and from buoyant densities in CsCl gradients.

Cultures. The purification, origins, morphologies, and certain properties of *Microcystis* sp. strain P1-15, C5-34, AK1-6, and C3-40 (a subclone of strain C3-9) have been described previously (7, 16). All other cultures were obtained from the American Type Culture Collection.

DNA extraction. Calf thymus DNA (3, 17) and *Micrococcus luteus* DNA (2) were obtained from Sigma Chemical Co. DNAs from *Escherichia coli* ATCC E25404 (1, 6) and *Salmonella typhimurium* ATCC 15277 (1) were extracted by using the Marmur method (14) and cells grown on brain heart infusion agar (Difco). Cyanobacterial cultures in Jansen medium (4) were grown at 26 to 30°C without shaking and with continuous irradiance of 15 to 30 microeinsteins $m^{-2} s^{-1}$ (1,076 to 2,152 lx) from cool-white fluorescent lights to a

density of 10^7 to 10^8 cells per ml. Cells from each culture were washed three times by suspension and centrifugation in double-distilled water. Each cell pellet (2 to 3 g, wet weight) was resuspended in 15 ml of saline EDTA (0.15 M NaCl in 0.01 M sodium EDTA, pH 8). The cell suspension was sequentially treated by adding 15 mg of lysozyme (Sigma), incubating the preparation for 45 min at 37°C, adding 1 ml of 20% (wt/vol) sodium dodecyl sulfate, incubating the preparation for an additional 45 min at 37°C, adding 2.5 ml of 5 M sodium perchlorate, and extracting the preparation with an equal volume of chloroform-isoamyl alcohol (23:1, vol/vol) at 20°C for 30 min. The DNA was precipitated with ethanol and resuspended in 2 ml of $0.1\times$ SSC buffer, which was then adjusted to $1\times$ SSC (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0). This solution was incubated with 50 μ g of pancreatic RNase at 37°C for 30 min and then extracted at least twice with phenol-chloroform (1:1, vol/vol) (5). For T_m analysis, the DNA was further purified by hydroxylapatite column chromatography (18). The yields of cyanobacterial DNA averaged 164 μ g of DNA per g (wet weight) of cells. The UV absorbance spectra between 190 and 300 nm were as predicted for good-purity nucleic acid (11); the ratios of A_{260} to A_{280} were ≥ 1.80 (range, 1.80 to 2.14), and the ratios of A_{260} to A_{230} were ≥ 2.01 (range, 2.01 to 5.00).

Thermal denaturation. Thermal denaturation of DNA was examined by using the method of Mandel and Marmur (13). A 10- μ g portion of DNA in 1 ml of $0.1\times$ SSC was heated at a rate of 1.0°C per min in a Perkin-Elmer model 552 spectrophotometer equipped with a 10-cell heatable chamber that allowed parallel examination of control DNA. Table 1 shows the T_m data.

Buoyant densities. Buoyant densities were measured by using the method of Meselson et al. (15), modified as described below. Tris-HCl buffer (0.02 M 2-amino-2 hydroxymethyl-1,3-propanediol, pH 8.5) containing 54 to 320 μ g of test DNA per ml and 5.8 to 17.4 μ g of internal standard *Micrococcus luteus* DNA per ml was adjusted to a final density of 1.710 g/ml by adding optical grade CsCl (U.S.

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TABLE 1. Mean DNA base compositions as determined by T_m and buoyant density measurements

Source of DNA	T_m (°C) ^a	Buoyant density (g/ml) ^b	G+C content (mol%)		
			This study		Other studies
			T_m method	Buoyant density method	
Calf thymus	75.4 ± 0.8	1.6991 ± 0.0000	42	40	42, ^c 39 ^d
<i>E. coli</i> E25404	78.9 ± 1.9	1.7086 ± 0.0013	50	50	50, ^e 51 ^f
<i>Micrococcus luteus</i>	ND ^g	1.7306 ± 0.0000	ND	72	72 ^h
<i>S. typhimurium</i> 15277	79.4 ± 0.8	ND	51	ND	50 ^e
<i>Synechocystis</i> sp. strain 27150 ^T	72.3 ± 2.5	1.6942 ± 0.0006	36	35	35, ⁱ 35 ^j
<i>Synechocystis</i> sp. strain 27178 ^T	ND	1.7057 ± 0.0009	ND	47	47, ⁱ 48 ^k
<i>Microcystis</i> sp. strain P1-15	73.1 ± 0.9	ND	38	ND	ND
<i>Microcystis</i> sp. strain C3-40	74.7 ± 0.6	1.6996 ± 0.0006	41	40	ND
<i>Microcystis</i> sp. strain C5-35	74.8 ± 1.8	ND	41	ND	ND
<i>Microcystis</i> sp. strain AK1-6	75.9 ± 0.5	ND	43	ND	ND

^a For most organisms the data are means ± standard deviations for three replicates from individually grown cultures; duplicate cultures were used for the *Synechocystis* sp. strain 27150^T and *Microcystis* sp. strain C5-34 determinations.

^b For most organisms the data are means ± standard deviations for four replicates from individually grown cultures; duplicate cultures were used for calf thymus determinations, and six replicates were used for *Microcystis* sp. strain C3-40 determinations.

^c Data from reference 3.

^d Data from reference 17.

^e Data from reference 1.

^f Data from reference 6.

^g ND, not determined.

^h Data from reference 2.

ⁱ Data from reference 23.

^j Data from reference 8.

^k Data from reference 10.

Biochemical Corp.). This solution was centrifuged for 48 to 70 h at 35,000 rpm (85,000 × *g*) in a type 50Ti rotor in a Beckman model L5-50 ultracentrifuge at 25°C. Fractions (5 drops) were collected from the bottom of the centrifuge tube and stored in tightly sealed vials. The densities of fractions were determined from refractive indices measured with a Valentine refractometer. To each fraction we added 0.2 ml of 0.1 M Tris-HCl buffer (pH 8.4), and the A_{260} values were determined with a Beckman model 35 spectrophotometer. Table 1 shows the buoyant density of each DNA sample and the corresponding G+C content, which was calculated as described by Schildkraut et al. (22).

DNA base composition. For all of the DNA samples which we analyzed, the G+C compositions calculated from the T_m data were in agreement with the G+C compositions calculated from buoyant densities (Table 1). Both methods yielded data for controls that were consistent with the following previously published G+C values: 39 to 42 mol% for calf thymus DNA (3, 17), 50 to 51 mol% for *E. coli* DNA (1, 6), 50 mol% for *S. typhimurium* DNA (1), 72 mol% for *Micrococcus luteus* DNA (2), 35 mol% for *Synechocystis* sp. strain 27150^T (T = type strain) DNA (8, 23), and 47 to 48 mol% for *Synechocystis* sp. strain 27178^T DNA (10, 23). The latter two organisms are the type strains of the low- and high-G+C-content clusters of the *Synechocystis* group, respectively (9, 10, 23).

The various *Microcystis* cultures had fairly similar base compositions (38 to 43 mol% G+C) (Table 1). When the value of 45 mol% G+C that has been reported previously for *Microcystis* sp. strain ATCC 27153 (24) is included, the range of G+C contents for all currently characterized *Microcystis* cultures is 38 to 45 mol%. These G+C values are greater than the values published previously (24) for the marine *Synechocystis* cluster (30 to 32 mol% G+C), are contiguous with the values for the low-G+C-content *Synechocystis* cluster (35 to 37 mol% G+C), and overlap the

values for the high-G+C-content *Synechocystis* cluster (42 to 48 mol% G+C). Because of insufficient differences, DNA base composition cannot be used to distinguish the latter two clusters from the *Microcystis* cluster. In fact, the values for *Microcystis* cultures bridge the gap between the values for the low- and high-G+C-content clusters, creating a continuum of DNA base ratios within the *Synechocystis* group and potentially eliminating one of the primary criteria for separating the low- and high-G+C-content clusters of the *Synechocystis* group.

It should be noted, however, that the two G+C clusters of the *Synechocystis* group differ in phenotypic properties, including the occurrence of facultative heterotrophy, the presence of phycoerythrin, the polyunsaturated fatty acid content, and cell size (24). Likewise, the *Microcystis* cultures share several characteristics that are generally absent in *Synechocystis* cultures, as discussed below. Although the *Synechocystis* group is a phenotypically heterogeneous assemblage with a wide range of DNA base ratios (24), the *Microcystis* cultures have similar phenotypes (4, 7, 16) and have a limited range of base compositions (Table 1). Therefore, we favor the continued provisional recognition of the various clusters within the *Synechocystis* group until data concerning levels of DNA homology, levels of rRNA similarity, and additional phenotypic properties are available.

The *Microcystis* cultures in Table 1 are organisms that have similar ecology and morphology (4, 7, 16). All were isolated from planktonic organisms in eutrophic, alkaline, freshwater lakes or rivers in the midwestern United States. All produce spherical cells with prominent gas vesicles. At the time of culture isolation, all occurred in large multicellular aggregates within copious capsules or slime layers, which are still formed by strains P1-15, C3-40, and AK1-6 although not by strain C5-34 (4, 7, 16). The cell diameters under standard growth conditions are between 3.5 and 8 µm (3.6 ± 0.5 µm for strain P1-15 [7], 4 to 5 µm for strain C5-34

[16], and 6 to 8 μm for strain C3-9 and its subclone, C3-40 [16]). Several of these properties are characteristic of the genus *Microcystis* but not of the *Synechocystis* clusters. In particular, gas vesicles are present in six of the seven organisms assigned by Waterbury and Rippka to the *Microcystis* cluster, but in none of the cultures of the *Synechocystis* clusters (24). The diameters of the *Microcystis* cells are larger than the values of 2 to 3 μm observed for members of the high-G+C-content *Synechocystis* cluster (24). Several *Microcystis* cultures differ from *Synechocystis* cultures in the production of toxin(s), β -cyclocitral, or cell aggregates involving a capsule (7, 16, 24). Because *Microcystis* isolates share several phenotypic properties that are often absent from other members of the *Synechocystis* group, it is not too surprising that these isolates have a relatively narrow range of DNA base compositions compared with the *Synechocystis* group as a whole.

As originally isolated, strain C5-34 exhibited the morphology of *Microcystis aeruginosa* (16), whereas the other *Microcystis* strains in Table 1 corresponded to *Microcystis flos-aquae* (7, 16). The G+C composition of strain C5-34 is in the middle of the range of G+C values for the three *Microcystis flos-aquae* cultures and thus does not differ from the G+C composition of *Microcystis flos-aquae*.

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