Genetic Relatedness of Heterocystous Cyanobacteria by Deoxyribonucleic Acid-Deoxyribonucleic Acid Reassociation

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I assessed the genetic relatedness of 45 strains of heterocyst-forming cyanobacteria assigned to eight genera by Rippka et al. and of 19 undescribed strains of the same group by in vitro reassociation of radioiodinated deoxyribonucleic acids. The members of the genera *Nodularia*, *Cylindrospermum*, *Chlorogloeopsis*, and *Fischerella* formed discrete clusters (intrageneric values of relative binding, more than 55%) and showed intergeneric relatedness of less than 40%, results consistent with the classification proposed by Rippka et al. The genus *Nostoc* was heterogeneous; four strains previously assigned to *Anabaena* appeared to belong to *Nostoc*. The genus *Calothrix* comprised four clusters with various degrees of internal homogeneity and two strains which showed low relatedness to any others. The general relatedness (i.e., relative binding) of heterocyst formers to various non-heterocystous cyanobacteria belonging to groups II and III (both unicellular and filamentous) was on the order of 10 to 20%.

Traditionally, the classification of cyanobacteria has been based on the morphology of specimens in their natural habitats. When these organisms are studied in pure culture, several differentiating features (for example, colony formation) disappear and thus cannot be used as taxonomic criteria (9). An examination of nutritional patterns does not constitute a useful alternative, since cyanobacteria are essentially obligate photoautotrophs. Consequently, the criteria used in classifying axenic cultures of cyanobacteria are confined almost exclusively to the structure of the cells and the macromolecules present. Although cyanobacteria are quite diverse in deoxyribonucleic acid (DNA) base composition (3), those cyanobacteria which form heterocysts display a narrow range (38.3 to 46.7 mol%) of guanine-plus-cytosine (G+C) contents, supporting the classification of these taxa in a single major taxonomic group (9).

In this paper, I describe the genetic relatedness among heterocyst-forming cyanobacteria, as assessed by DNA-DNA reassociation.

MATERIALS AND METHODS

Microorganisms. Axenic cultures of cyanobacteria were obtained from the Pasteur Culture Collection (PCC; Institut Pasteur, Paris, France) and were grown as described previously (9). Centrifuged cells were stored frozen until needed. The strains listed in Table 1 have been described and classified by Rippka et al. (9). The strains listed in Table 2 have not been de-

scribed yet, and they await deposition in the American Type Culture Collection. Provisional generic assignments (R. Rippka and G. Guglielmi, personal communications) are shown in Table 2.

Determination of base composition. The thermal denaturation of native DNA samples was monitored spectrophotometrically (6). A solution of DNA (absorbance at 260 nm, 0.33) in 0.028 M sodium phosphate buffer (pH 6.8) was placed into an electrically heated cuvette linked to a thermoprogrammer (Gilford Instrument Laboratories Inc., Oberlin, Ohio) that was set to raise the temperature at a rate of 0.5°C/min, and the absorbance at 260 nm was recorded.

The DNA extracted from *Anabaena* strain PCC 7120 (42.5 mol% G+C [3]) was used as an internal standard. The equation of Marmur and Doty (7) appropriate for these conditions was used to calculate the G+C contents from three separate determinations.

DNA reassociation. DNA was extracted, purified, radioiodinated, and reassociated as described previously (5). Each reassociation experiment included a number of heterologous crosses (radioactive reference DNAs with unlabeled DNAs from other strains) and at least one homologous test (unlabeled reference DNA with tracer DNA) to provide an internal control. The relative binding (RB) value was calculated as described by Brenner et al. (1). Duplicate experiments were conducted separately at 60°C. When possible, pairs of DNAs exhibiting significant reassociation (usually 30% or more) were reannealed once more and eluted thermally from hydroxyapatite, thus allowing the determination of the thermal denaturation point [$T_{m(e)}$] of heteroduplex DNA. Several confirmatory experiments were also performed at 70°C, a more restrictive temperature. Lower degrees of reassociation were obtained, but no inconsistencies with the reassociations at the more permissive temperature were found. Consequently, they contributed no additional information.

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TABLE 1. *Strains described by Rippka et al. that were used in this study*^a

Classification	PCC strain no.	ATCC no. ^b	G+C content (mol%) ^c	Mol wt of genome (×10 ⁹) ^d
Heterocystous strains of section IV				
<i>Anabaena</i>	6309	29211	38.3	3.6
	6411	27898	42.9	3.9
	7108	29898	42.3 ^e	3.7
	7118	27892	43.4	3.9
	7119	29151	42.4	3.7
	7120	27893	42.5	3.2
	7122	27899	43.9	5.7
<i>Cylindrospermum</i>	73101	29335	44.7 ^e	5.7
	7417	29204	44.0 ^e	
	7604	33001	42.9	
<i>Nodularia</i>	73104	29167	40.5	3.3
<i>Nostoc</i>	6302	27897	45.1	5.7
	6310	27896	44.2	4.5
	6314	27904	43.9	5.1
	6705	29131	43.4	5.6
	6719	29105	43.9	4.0
	6720	27895	41.8	
	7107	29150	41.8	
	73102	29133	45.5	4.9
	7413	29106	42.7	4.5
	7416	29107	42.4	4.8
	7422	29132	41.6	6.4
	7423	29168	42.2	4.9
	7524	29411	39.0	5.6
<i>Scytonema</i>	7110	29171	44.4	7.4
<i>Calothrix</i>	6303	29156	41.8	5.1
	7101	27914	41.2	7.8
	7102	27901	39.8	8.6
	7103	27905	39.8	5.2
	7111	29199	44.4	5.4
	7116	29111	40.8	5.4
	7204	29190	40.5	5.3
	7415	29157	41.8	5.4
	7426	29343	40.9	8.2
	7504	29158	41.5	
	7507	29112	42.8	5.5
Heterocystous strains of section V				
<i>Chlorogloeopsis</i>	6718	27181	42.1	6.2
	6912	27193	42.9	5.7
<i>Fischerella</i>	7115	27929	44.4	4.6
	73103	29114	42.4	4.0
	7414	29161	43.5 ^e	4.8
	7520	29537	42.5	
	7521	29538	42.3	3.6
	7522	29539	41.9	
	7523	29540	41.9	
Unicellular strains				
<i>Gloeotheca</i>	6501	27151	41.7	
	73108	29164	42.7	5.0
<i>Synechocystis</i>	6702	27171	47.4	
	7005	27153	45.4	2.1
<i>Chroococcidiopsis</i>	6712	27176	40.2	3.3
	7436	29383	46.4	
Non-heterocystous, filamentous strains				
<i>Oscillatoria</i>	6412	29205	42.5	3.2
LPP group	73110	29407	47.4	5.2
	7409	29541	43.6	4.6

^a See reference 9.^b ATCC, American Type Culture Collection, Rockville, Md.^c Values from Herdman et al. (3), except as noted below.^d Values from Herdman et al. (2).^e Values determined as described in the text.

TABLE 2. *Strains used in this study which were not described by Rippka et al.*

Generic assignment ^a	PCC strain no.	G+C content (mol%) ^b
<i>Nodularia</i>	7804	44.7
	7121	44.5
<i>Nostoc</i>	7706	44.5
	7803	42.7
<i>Calothrix</i>	7807	47.2
	6305	44.8
	6601	44.3
	7601	42.7
	7708	
	7709	41.8
	7710	46.1
	7711	
	7712	43.1
	7713	
	7714	41.6
	7715	41.4
<i>Fischerella</i>	7716	41.3
	7810	
Unidentified	7603	42.9
	7126	
LPP group	7705	58.9

^a Generic assignments by R. Rippka or G. Guglielmi (personal communications).

^b Values were determined as described in the text.

RESULTS AND DISCUSSION

Use of radioiodinated DNA. The DNAs of 24 strains were radioiodinated. Determinations of $C_{0t_{1/2}}$ for a representative number of DNAs showed a range of from 3 to 7 mol·s/liter in this group. Table 3 shows the specific activities of the DNA samples, their values for actual binding to hydroxyapatite (after reaching a C_0t value of 25 mol·s/liter) in both the presence and the absence of unlabeled homologous DNA, and the thermal elution points of homoduplexes. Measurements of actual binding immediately after denaturation showed that undenatured DNA accounted for almost all of the background values reported as self-renaturation. Since these values were not excessive, no correction factor was applied at any level.

Orosz and Wetmur (8) have shown that excessive iodination of nucleic acids can affect their renaturation properties. The differences (D values) between the $T_{m(e)}$ values measured by thermal elution of DNA from hydroxyapatite and the values obtained spectrophotometrically (Tables 1 and 2) are listed in Table 3. In a few cases (e.g., strains PCC 6303 and PCC 7111), these D values appeared to be excessively high, and the actual binding values were correspondingly low. However, there was no correlation between poor reassociation characteristics (high D value, low actual binding) and high specific

activity. The reason for this is not clear. Although most of the DNA samples were labeled in the presence of 5 μ M potassium iodide, as recommended for the labeling of RNA (10), I later found that increasing the concentration of carrier iodide to 15 μ M resulted in a definite improvement in reassociation characteristics, independent of specific radioactivity (Table 3). This improvement may have been due to a more stable incorporation of isotope into the DNA. The results obtained with reference strains PCC 6303 and PCC 7111 were considered unreliable and are not reported.

General patterns of DNA relatedness. The RB and $\Delta T_{m(e)}$ values of the cyanobacteria surveyed are shown in Tables 4 through 9.

Reciprocal tests generally agreed within the limits of experimental error, as most (83%) deviations were less than 10% RB. In some cases, larger differences were observed. The reciprocal crosses involving strain PCC 7102 on the one hand (Table 5) and PCC 7103 and PCC 7709 on the other hand (Table 8) were remarkable in this respect, with RB deviations of 13 and 21%, respectively. The high degree of genetic complexity in strain PCC 7102 (8.6×10^9 daltons [3]) could account for this, since in parts of its genome this strain should have sequences not present in strain PCC 7103 and possibly not present in strain PCC 7709 (genome size was not determined in the latter strain). Thermal elution points were subject to much less deviation in reciprocal experiments.

Table 4 shows the results of attempts to find significant reassociation between DNAs from non-heterocystous strains and labeled DNAs from heterocyst formers. No significant reactions were observed. The highest RB value was found between *Cylindrospermum* strain PCC 73101 and *Oscillatoria* strain PCC 6412. An RB value of 21% with this reference strain was considered below the level of significance. Little is known about the genetic relatedness among non-heterocystous cyanobacteria. However, the results obtained by Kelly and Cowie (4) and by Stam and Venema (11) suggest that members of the genera *Lyngbya*, *Plectonema*, and *Phormidium* (the LPP group proposed by Rippka et al. [9]) are rather homogeneous. The absence of reassociation between three LPP strains and the heterocystous reference strains used in this study indicates that heterocystous cyanobacteria are quite distinct from these filamentous types. Unicellular strains of *Gloeotheca* able to incorporate atmospheric nitrogen aerobically (9) also failed to show significant relatedness with heterocystous reference strains.

Within the heterocyst-forming cyanobacteria

TABLE 3. *Characteristics of radioiodinated DNA preparations from reference strains*

PCC strain no.	Sp act (Ci/g)	G+C content (mol%)	$T_{m(e)}$ (°C) ^a	D (°C) ^b	% Self-renaturation ^c	% Actual binding
6302 ^d	1.2	45.1	87.9	0.8	4	86
6303	3.4	41.8	76.7	10.7	4	54
6705	0.7	43.4	84.4	3.6	2	90
6718 ^d	3.5	42.1	88.8	-1.3	4	93
6720	0.7	41.8	83.5	3.9	4	85
7101	2.5	41.2	81.5	5.6	3	62
7102	5.9	39.8	83.1	3.5	3	74
7103 ^d	2.8	39.8	87.3	-0.7	4	92
7107	1.7	41.8	82.6	4.8	7	69
7111	4.0	44.4	78.5	9.9	4	54
7116	0.7	40.8	87.0	0.0	4	90
7120	6.5	42.5	83.6	4.1	5	84
7122	0.6	43.9	81.2	7.0	2	86
73101	0.6	44.7	87.6	1.0	5	87
73102	0.1	45.5	86.0	2.9	6	79
73104 ^d	3.9	40.5	88.3	-1.5	2	95
7413	8.6	42.7	82.5	5.2	2	78
7415	3.0	41.8	85.1	2.3	3	69
7504	1.6	41.5	82.8	4.5	4	75
7520 ^d	1.4	42.5	87.9	-0.2	3	93
7523 ^d	5.0	41.9	87.5	-0.1	2	94
7524	12.3	39.0	81.7	4.5	3	81
7709 ^d	0.3	41.8	87.3	0.1	2	89
7810 ^d	1.8		87.4		2	91

^a Thermal elution midpoint of reassociated DNA adsorbed onto hydroxyapatite.^b Depression of homologous $T_{m(e)}$ due to iodination (see text).^c Percent RB of labeled DNA reassociated in the absence of unlabeled DNA.^d Labeling was in the presence of 15 µM KI.TABLE 4. *DNA reassociation between strains of non-heterocystous cyanobacteria and reference strains with which maximum RB values were obtained*

Test strain	Maximum RB value (%)	Reference strain	No. of reference strains tested
<i>Oscillatoria</i> PCC 6412	21	<i>Cylindrospermum</i> PCC 73101	1
<i>Chroococcidiopsis</i>			
PCC 7436	19	<i>Chlorogloeopsis</i> PCC 6718	1
PCC 6712	13	<i>Chlorogloeopsis</i> PCC 6718	1
<i>Synechocystis</i>			
PCC 6702	13	<i>Cylindrospermum</i> PCC 73101	1
PCC 7005	10	<i>Cylindrospermum</i> PCC 73101	1
LPP group			
PCC 73110	13	<i>Nostoc</i> PCC 6302	6
PCC 7409	11	<i>Cylindrospermum</i> PCC 73101	16
PCC 7705	6	<i>Nostoc</i> PCC 7413	5
<i>Gloeotheca</i>			
PCC 6501	10	<i>Calothrix</i> PCC 7415	4
PCC 73108	5	<i>Anabaena</i> PCC 7122	1

included in this survey intergroup RB values generally ranged from ca. 10 to 30%. There was a definite gap in the RB values obtained with each reference strain between closely related and distantly related strains. This gap might be regarded as an adequate basis for establishing clustering limits. However, in practice it would be desirable to set a more stringent limit within which strains may be treated as members of a

same operational taxonomic unit. In the discussion below, I consider RB values of 80% and $T_{m(e)}$ values of 2°C to be criteria for defining the boundaries of nomenclatures.

Groups of interrelated heterocystous cyanobacteria. With a few exceptions, the heterocystous cyanobacteria formed DNA reassociation clusters consistent with the assignment of these organisms by Rippka et al. (9) to genera

or sections on the basis of morphology and physiology.

Table 5 shows the results for those strains of section IV which do not form hormogonia. *Anabaena* isolates which normally form motile trichomes, conical end cells, and akinetes but which are neither sensitive to phage N-1 nor able to grow as even suspensions (9) comprise two strains which show high relatedness and one which reassciated only poorly with the reference strain. The high RB value found between strains PCC 7122 and PCC 6309 was unexpected, as these strains differed by 5.6 mol% in their G+C contents. This difference (3) was confirmed with DNA extracted from newly grown cells. The significance of this is not clear, but an examination of these strains revealed that PCC 6309 had lost its motility, suggesting some changes in its genotype during its many years in axenic culture. Loss or acquisition of DNA by cells in culture or the repeated presence of a contaminant in the DNA extract might account

for this irregularity. Rippka et al. (9) have presented evidence for considering strains PCC 7122 and PCC 6309 conspecific. The above-mentioned results fully support this conclusion.

Although PCC 7108 showed only 32% RB with the reference strain (Table 5), it is regarded as having significant (albeit low) relatedness to PCC 7122, since all other RB values obtained with this reference strain were 23% or lower. PCC 7108 can be regarded as a member of a separate species of *Anabaena*.

The three strains assigned to *Cylindrospermum* on the basis of the formation of elongate akinetes adjacent to a terminal heterocyst exhibited results somewhat analogous to those reported for *Anabaena* (Table 5). The G+C content difference of 4.6 mol% between PCC 73101 and PCC 7417 reported by Herdman et al. (3) could not be confirmed. Newly purified DNAs showed a difference of 0.7 mol% (Table 1). Strain PCC 7604 appeared moderately related to the reference strain, and thus it probably represents

TABLE 5. DNA reassociation among strains of heterocystous cyanobacteria with no developmental cycles^a

Test strain	Reference strains					
	<i>Anabaena</i> PCC 7122		<i>Cylindrospermum</i> PCC 73101		<i>Nodularia</i> PCC 73104	
	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)
<i>Anabaena</i>						
PCC 7122	(100)	(0)			33	
PCC 6309	93	0	31			
PCC 7108	32					
<i>Cylindrospermum</i>						
PCC 73101			(100)	(0)	41	
PCC 7417	23		100	0		
PCC 7604			59	8	41	18
<i>Nodularia</i>						
PCC 7804	19		32		65	6
PCC 73104			31		(100)	(0)

^a See reference 9. $\Delta T_{m(e)} = T_{m(e)} \text{ homologous} - T_{m(e)} \text{ heterologous}$. Parentheses show homologous reactions.

TABLE 6. DNA reassociation among interrelated strains of *Anabaena* and *Nostoc*^a

Test strain	Reference strains							
	<i>Anabaena</i> PCC 7120		<i>Nostoc</i> PCC 6705		<i>Nostoc</i> PCC 7413		<i>Noctoc</i> PCC 7524	
	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)
<i>Anabaena</i>								
PCC 6411	96	0	80	1	65		30	
PCC 7118	96	0	86	0	68	6	34	
PCC 7120	(100)	(0)	86	0	69	6	36	14
PCC 7119	102	0						
<i>Nostoc</i>								
PCC 6719	96	0	88	0	67	6	33	14
PCC 6705	93	1	(100)	(0)	67		36	
PCC 7413	55	6	62	6	(100)	(0)	37	13
PCC 7423	41	11	50	12	48		32	
PCC 7524	29		40	13	43	14	(100)	(0)

^a $\Delta T_{m(e)} = T_{m(e)} \text{ homologous} - T_{m(e)} \text{ heterologous}$. Parentheses show homologous reactions.

TABLE 7. DNA reassociation among *Nostoc* strains not included in Table 6^a

Test strain	Reference strains							
	PCC 73102		PCC 6302		PCC 7107		PCC 6720	
	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)	% RB	$\Delta T_{m(e)}$ (°C)
PCC 73102	(100)	(0)	56	12	23		35	
PCC 6310	59	9	56	11			33	
PCC 6302	53	9	(100)	(0)			28	
PCC 7422	56	11	64	7			38	14
PCC 7121	52	16	64	8			38	
PCC 7807	49		63	9				
PCC 7706			57	11				
PCC 7803			54	12				
PCC 6314	36	18	36		23			
PCC 7107	26				(100)	(0)	67	5
PCC 7416	34				93	0	75	6
PCC 6720	31				69	4	(100)	(0)

^a See footnote a, Table 6.

a separate species of *Cylindrospermum*. Strain PCC 7417 differs from the other two *Cylindrospermum* strains by its ability to assimilate fructose and sucrose (9). Its apparent conspecificity with PCC 73101 (RB value, 100%) is an indication that the few nutritional tests available to cyanobacterial taxonomists may not be of much significance in species delimitation.

The two strains that were assigned to *Nodularia* principally on the basis of their disk-shaped cells revealed moderate relatedness (Table 5), and they can be considered separate species of the same genus. Their DNA G+C contents differed by 4.2 mol%.

Some isolates classified as either *Anabaena* or *Nostoc* (9) showed significant interrelatedness (Table 6). Strains PCC 6411, PCC 7118, PCC 7119, and PCC 7120, which were considered almost identical by Rippka et al. (9), had significant phenotypic differences compared with other *Anabaena* isolates. They formed nonmotile trichomes, produced neither conical end cells nor akinetes, were susceptible to lysis by phage N-1, and grew as even suspensions. Until recently, they had not been observed to form hormogonia, a diagnostic criterion of the genus *Nostoc*. However, Rippka (personal communication) has demonstrated hormogonium production in PCC 7119. The three other strains may or may not be induced to undergo the same development, but they should be regarded as members of the genus *Nostoc* since they clustered with strains PCC 6719 and PCC 6705 at a level high enough to allow treating all six as members of a single nomenspecies.

The above-mentioned strains also showed a moderate degree of relatedness with PCC 7413 (Table 6), suggesting that the latter belongs to a separate species. All had DNA G+C contents

of between 42.4 and 43.9 mol%. Strains PCC 7423 and PCC 7524 showed RB values with this group well above background levels, and these strains may be considered distantly related, separate species.

The remaining *Nostoc* strains and their DNA relatedness values are shown in Table 7. Strains PCC 73102 through PCC 7803 exhibited a moderate degree of interrelatedness. Preliminary experiments (Guglielmi, personal communication) using labeled DNA from PCC 7803 gave RB values of 56, 50, 51, 49, and 48% for strains PCC 7706, PCC 7807, PCC 7121, PCC 7422, and PCC 6302, respectively, which confirms the structure of this cluster. These strains stand out by their slightly higher average base compositions (44.4 ± 1.7 mol% G+C) compared with the other *Nostoc* strains (42.3 ± 1.5 mol% G+C). These values do overlap and thus cannot provide a sharp discriminatory criterion. These *Nostoc* strains may also be distinguished by the formation of C-phycoerythrin, a pigment not present in other strains of this genus; the latter form phycoerythrocyanin instead (D. Bryant, personal communication).

Strain PCC 7121, which appeared to be related to strain PCC 6302 (Table 7), has lost the ability to grow as a multicellular organism (Rippka, personal communication). Strain PCC 6314 did not cluster at a significant level with any reference strain, but it appeared to be distantly related to other *Nostoc* strains. Reference strains PCC 6720 (received as *Anabaenopsis circularis*) and PCC 7107 clustered together with PCC 7416. The latter two strains appear to be conspecific, a result which had been anticipated by Rippka et al. (9) on the basis of structural and nutritional properties.

The results for strains assigned to the genus

TABLE 8. DNA reassociation among strains of *Calothrix*^a

Test strain	Reference strains											
	PCC 7101			PCC 7415			PCC 7103			PCC 7102		
	% RB	$T_{m(e)}$ (°C)		% RB	$T_{m(e)}$ (°C)		% RB	$T_{m(e)}$ (°C)		% RB	$T_{m(e)}$ (°C)	
Cluster 1												
PCC 7101	(100)	(0)		67	3	37				7		17
PCC 7601	70	4		90	0	35						
PCC 7710	54	5		72	1	33						
PCC 7708	56	6		57	5	37						
Cluster 2												
PCC 6305	24			31		61						
PCC 6601	29			31		61						
PCC 7415	26			30		(100)				16		14
PCC 7507	18			22		27				8		14
Cluster 3												
PCC 7103	16			9		10						
PCC 7713				10		15				52	5	19
PCC 7102	13			12		19				54	6	5
PCC 7715						16				(100)	(0)	15
PCC 7716										5	6	4
PCC 7709										53	6	1
PCC 7714				15		13				53	5	1
Cluster 4										(100)	(0)	(0)
PCC 7116	9			10		10				53	5	10
PCC 7426	8			6						37	11	53
PCC 7810										7		(0)
PCC 7204				11						6		18
PCC 7711												13
PCC 7111	10			8		10						60
PCC 7815						13				49	14	60
PCC 6303	11			12		10				45	15	(0)
										45	15	14
										40	14	53
										36	9	34
										9		12

^a See footnote a, Table 6.

TABLE 9. DNA reassociation among strains of section V^a

Test strain	Reference strains					
	<i>Chlorogloeopsis</i> PCC 6718		<i>Fischerella</i> PCC 7520		<i>Fischerella</i> PCC 7523	
	% RB	<i>T</i> _{m(e)} (°C)	% RB	<i>T</i> _{m(e)} (°C)	% RB	<i>T</i> _{m(e)} (°C)
<i>Chlorogloeopsis</i>						
PCC 6718	(100)	(0)	40		42	
PCC 6912	93	0	40		45	
<i>Fischerella</i>						
PCC 7115	42		98	0	84	5
PCC 7520	42		(100)	(0)	84	5
PCC 7414	41		100	0	84	5
PCC 7603	41		100	0	87	5
PCC 73103	40		99	0	85	5
PCC 7523	37		74	6	(100)	(0)
PCC 7521	37		73	6	91	0
PCC 7522	34		72	5	87	2

^a See reference 9. See also footnote a, Table 6.

Calothrix by Rippka et al. (9) are shown in Table 8. The first cluster included strains PCC 7101 and PCC 7601, which were received as *Tolypothrix tenuis* and *Fremyella* sp., respectively. The high relatedness among PCC 7504, PCC 7601, and PCC 7712 justifies placing them in the same nomenclature. The second cluster included two strains (PCC 6305 and PCC 6601) which were received as *Microchaete* sp. Strain PCC 7507, the only *Calothrix* strain reported to form akinetes (9), stood alone. Its highest RB value was obtained with reference strain PCC 7415. It also differs from the above-mentioned strains in its accessory pigment composition. Whereas strains of the two clusters just described form adaptable levels of phycoerythrin, PCC 7507 forms a constitutive phycoerythrocyanin (Bryant, personal communication).

A third cluster included strains PCC 7102 and PCC 7103 (Table 8), which were received as *Calothrix desertica* and *Nodularia sphaerocarpa*, respectively. This cluster is relatively homogeneous in base composition (39.8 to 41.8 mol% G+C) but shows appreciable heterogeneity in phycoerythrinoid pigment composition (Bryant, personal communication) and in genome complexity (2). A recent isolate, PCC 7713, appears to be conspecific with PCC 7103. Strains PCC 7709, PCC 7715, and PCC 7716 can also be considered members of the same nomenclature.

The last cluster (Table 8, cluster 4) contained all isolates recognized as marine organisms; the growth of these isolates in a laboratory requires the use of a special medium (9). This cluster also included strains identified by Guglielmi (personal communication) as *Isactis* sp. (PCC 7426), *Calothrix pulvinata* (PCC 7810), and *Calothrix crustacea* (PCC 7815). The RB values in this group were relatively low but were distinctly

above background level compared with the reference strains employed. Thus, the separation of these isolates into different genera cannot be justified.

The DNA extracted from strain PCC 6303 (received as *Calothrix parietina*) did not reassociate significantly with any other DNA tested. As mentioned above, the quality of this DNA preparation was doubtful, and the results reported here should be considered provisional.

Section IV (9) also includes the genus *Scytonema*. Preliminary reassociation experiments performed with the DNA of *Scytonema* strain PCC 7110 failed to show significant relatedness between this strain and any of the reference strains.

An unidentified isolate, PCC 7126, gave a RB value of 35% with *Nodularia* strain PCC 73104. Its taxonomic position remains uncertain.

Strains of section V (9) differ from all other heterocyst formers in that cell division in these strains is in more than one plane. These strains clustered in perfect conformity with their generic classification by Rippka et al. (9). The two *Chlorogloeopsis* isolates appeared to be conspecific (Table 9) and showed a low but significant degree of relatedness to the *Fischerella* strains. The latter formed two highly related clusters; each of these clusters had a high enough internal homogeneity to regard it as a separate nomenclature. No correlation was found between the DNA relatedness among these strains and the previous assignment of some of them to the genus *Mastigocladus* (9).

DNA relatedness and the taxonomy of heterocystous cyanobacteria. The molecular taxonomic results presented above indicate clearly that the application of the bacteriological approach to cyanobacterial systematics (9) is a

major step toward the classification of these organisms. The genera *Anabaena*, *Nodularia*, *Cylindrospermum*, *Chlorogloeopsis*, and *Fischerella* now appear to be well defined. The heterogeneity elucidated in *Nostoc* and *Calothrix* suggests future directions for investigation, which should probably include the search for larger numbers of phenotypic characters to use in the classification of these organisms.

The addition of discriminatory criteria other than hormogonium formation to delimit the genera *Nostoc* and *Anabaena* would be desirable in view of the difficulty with which this developmental cycle is detected in some strains of *Nostoc*. The formation of conical end cells in *Anabaena* is potentially one such useful trait.

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LITERATURE CITED

1. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for the thermal elution of DNA from hydroxyapatite. *Anal. Biochem.* **28**:447-459.
2. Herdman, M., M. Janvier, R. Rippka, and R. Y. Stanier. 1979. Genome size of cyanobacteria. *J. Gen. Microbiol.* **111**:73-85.
3. Herdman, M., M. Janvier, J. B. Waterbury, R. Rippka, R. Y. Stanier, and M. Mandel. 1979. Deoxyribonucleic acid base composition of cyanobacteria. *J. Gen. Microbiol.* **111**:63-71.
4. Kelly, M. L., and D. B. Cowie. 1972. DNA-DNA hybridization studies of blue-green algae. *Carnegie Inst. Washington Yearb.* **71**:276-281.
5. Lachance, M. A. 1980. Simple method for determination of deoxyribonucleic acid relatedness by thermal elution on hydroxyapatite microcolumns. *Int. J. Syst. Bacteriol.* **30**:433-436.
6. Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol.* **12B**:195-206.
7. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
8. Orosz, J. M., and J. G. Wetmur. 1974. *In vitro* iodination of DNA. Maximizing iodination while minimizing degradation; use of buoyant density shifts for DNA-DNA hybrid isolation. *Biochemistry* **13**:5467-5473.
9. 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.* **iii**:1-61.
10. Scherberg, N. H., and S. Refetoff. 1974. The radioiodination of ribopolymers for use in hybridizational and molecular analyses. *J. Biol. Chem.* **249**:2143-2150.
11. Stam, W. T., and G. Venema. 1977. The use of DNA-DNA hybridization for determination of the relationship between some blue-green algae (cyanophyceae). *Acta Bot. Neerl.* **26**:327-342.