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Deoxyribonucleic Acid Base Composition of Cyanobacteria

By MICHAEL HERDMAN,* MONIQUE JANVIER, JOHN B. WATERBURY,† ROSMARIE RIPPKA AND ROGER Y. STANIER

Unité de Physiologie Microbienne, Département de Biochimie et Génétique Microbienne, Institut Pasteur, 28 rue du Docteur Roux, Paris 75015, France

AND MANLEY MANDEL

Department of Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Texas 77030, U.S.A.

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The DNA base compositions of 176 strains of cyanobacteria were determined by thermal denaturation or by CsCl density gradient centrifugation. A summary of all data now available for this prokaryotic group is presented and the taxonomic and evolutionary implications are discussed.

INTRODUCTION

Edelman *et al.* (1967) reported mean DNA base compositions for a limited number of strains of cyanobacteria, representative of many different sub-groups. More extensive data were published by Stanier *et al.* (1971) for one sub-group, the unicellular cyanobacteria. In this paper we present data for a large number of additional strains, broadly representative of this major prokaryotic taxon.

METHODS

Strains. The strains examined are all maintained in the Pasteur Culture Collection (PCC) and have now been deposited in the American Type Culture Collection (ATCC). They are identified here by both PCC and ATCC strain numbers. Full strain histories, media employed for cultivation and the explanation of generic terminology are given by Rippka *et al.* (1979). An additional strain (*Oscillatoria agardhii*) was isolated by Dr F. I. Kappers from the Veluwemeer, The Netherlands, and is now in pure culture as strain PCC 7805.

Extraction of DNA. DNA for use in thermal denaturation experiments was purified by a method modified from that of Britten *et al.* (1968). Harvested cells (approximately 5 g wet wt) were suspended in 20 ml lysis mixture containing 8 M-urea, 1 M-$NaClO_4$, 0·01 M-EDTA (disodium salt) and 1 % (w/v) sodium dodecyl sulphate (SDS) in 0·24 M-phosphate buffer (pH 7·0), disrupted by passage through a French press at 76 MPa, and partially deproteinized by shaking with an equal volume of chloroform/3-methylbutan-1-ol (24:1, v/v) at room temperature for 30 min. Following centrifugation at 3000 *g* for 15 min the upper (aqueous) phase, containing DNA, was retained. DNA-grade hydroxyapatite (HAP; 2 g; Bio-Rad) was suspended in 20 ml 0·24 M-phosphate buffer (pH 7·0), boiled for 30 min, centrifuged at low speed, and washed twice with 15 ml MUP (Britten *et al.*, 1968) containing 8 M-urea in 0·24 M-phosphate buffer (pH 7·0), being sedimented after each wash by brief low-speed centrifugation. The deproteinized nucleic acid solution was mixed with the washed HAP and stirred (5 min) to permit binding of DNA. The bound DNA was purified by washing the mixture (by centrifugation) seven times with 15 ml MUP, to remove RNA and residual protein, and then four times with 15 ml 0·014 M-phosphate buffer (pH 7·0), to remove urea. The purified DNA was eluted

* Present address: Department of Biological Sciences, The University, Dundee DD1 4HN.

† Present address: Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, U.S.A.

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from the HAP in two washes (7 ml) of 0·4 M-phosphate buffer (pH 7·0), transferred to cleaned dialysis sacs and concentrated to at least 250 μg ml^{-1} in Aquacide III (polyethyleneglycol, B grade, Calbiochem). The preparation was finally dialysed exhaustively against several changes of 0·1 × SSC buffer (SSC buffer, pH 7·0, is 0·15 M-NaCl/0·015 M-trisodium citrate), centrifuged to remove any remaining traces of HAP, and stored at 4 °C over chloroform.

This method permitted the rapid, simultaneous purification of up to eight DNA samples in a 8 × 50 ml rotor. DNA solutions were routinely checked spectrophotometrically, by a modification (Taggart, 1967) of the orcinol reaction (Schneider, 1957) and by measurement of hyperchromicity following thermal denaturation, in order to confirm their purity.

DNA for CsCl ultracentrifugation studies was extracted after disruption in the Hughes Press at −18 °C. The paste was thawed in saline/EDTA containing 2% SDS and deproteinized by shaking with an equal volume of freshly redistilled neutralized phenol saturated with the saline/EDTA buffer and a 24:1 (v/v) mixture of chloroform and 3-methylbutan-1-ol. Following centrifugation at 8000 *g* for 10 min the aqueous phase was removed and impure nucleic acids were precipitated with 2 vol. 95% (v/v) ethanol. The spooled fibres were washed twice in 70% ethanol, air-dried and dissolved in a small volume of SSC buffer.

Estimation of DNA base composition. Thermal denaturation of DNA in 0·1 × SSC buffer was performed in sealed cuvettes, in triplicate, in a Gilford 240 spectrophotometer fitted with automatic cuvette programmer, thermosensor, and analogue multiplexer. The fourth cuvette contained a solution of guanine to enable correction to be made for solvent expansion. The temperature increment was 0·1 °C min^{-1}. The thermosensor and temperature recording equipment were calibrated with DNA samples of known base composition. The DNA base composition was calculated from the temperature midpoint of thermal denaturation (T_m) using the equation of Marmur & Doty (1962), and expressed as percentage guanine plus cytosine to total base content (mol% GC). Buoyant densities in neutral CsCl were measured as previously described (Stanier *et al.*, 1971) using the same reference DNA samples as in that study.

RESULTS

The data on DNA base composition in Tables 1 to 5 include all values now available for cyanobacteria, derived both from the present study and from preceding publications (Edelman *et al.*, 1967; Craig *et al.*, 1969; Kaye *et al.*, 1967; Stanier *et al.*, 1971; Rippka *et al.*, 1974). A careful comparison of cyanobacterial strain histories (Rippka *et al.*, 1979) has shown that a few of the strains, previously assumed to be of independent origin, are probably identical isolates; in such cases, a value for only one strain is included in the tabulations.

The assignment of strains to sections and genera follows the taxonomic proposals of Rippka *et al.* (1979). The failure to assign specific names to most strains is deliberate; the reasons for this policy are discussed by Rippka *et al.* (1979). Within each genus, the strains are listed in order of the increasing GC content of their DNA. Strains of independent origin which appear to be closely related in terms of their phenotypic properties (Rippka *et al.*, 1979) are enclosed within braces in the Tables.

Section I includes all unicellular cyanobacteria that reproduce either by binary fission (most genera) or by budding (genus *Chamaesiphon*). The two largest assemblages of strains in this section are representatives of the genera *Synechococcus* and *Synechocystis*, defined, respectively, as rod-shaped and spherical unicellular organisms, not enclosed by sheaths, and multiplying by binary fission. Both genera are characterized by wide DNA base compositional spans and by clear-cut internal discontinuities in base composition (Table 1), as had been recognized previously by Stanier *et al.* (1971). The analysis of additional strains has revealed the existence of one further discrete base compositional cluster in the genus *Synechococcus*. The members of this genus fall into three distinct groups with mean DNA base compositions in the ranges 39 to 43, 47 to 56 and 66 to 71 mol% GC. Phenotypic analyses suggest that each base compositional cluster includes representatives of two or more species (Stanier *et al.*, 1971; unpublished observations). Two strains in the low base compositional cluster, PCC 7418 and 7424, are readily distinguishable by their large cells, which resemble in size and shape those of the ensheathed cyanobacteria of the genus *Gloeothece*. All other strains of *Synechococcus* have much smaller cells and differ little from

Table 1. *Mean DNA base compositions of strains assigned to Section I*

Genus	Strain number PCC	Strain number ATCC	Mol % GC from: Buoyant density	Mol % GC from: Thermal denaturation
Synechococcus	7202	29140		39·0
	7502	29172		40·5
	7424	29155		41·2
	7418	29534		42·4
	7511	29154		43·2
	7335	29403		47·4
	6910	27191	48·0[a]	47·5
	7117	29139		48·5
	7425	29141		48·6
	7002	27264	49·0[a]	49·1
	73109	29404	48·5	49·0
	7003	27265	49·5[a]	49·3
	6312	27167	50·2[a]	
	6717	27180	52·0[a]	
	6715	27149	53·6[a]	
	6716	27179	53·6[a]	
	6301	27144	55·1[a], 56[b]	55·1
	6311	27145	55·1[a], 56[b]	54·8
	6908	27146	55·6[a]	56·0
	7009	29203		66·6
	6603	27168	65·7[a], 63[b]	
	6911	27192	66·3[a]	
	6904	29138		67·8
	6710	27174	68·4[a]	
	6713	27177	69·4[a]	
	6307	27147	69·7[a], 71[b]	
	6907	27148	71·4[a]	
	7001	27194	69·4[a]	69·5
Synechocystis	6308	27150	34·7[a], 35[b]	
	6701	27170	35·7[a]	35·8
	6808	27189	35·7[a]	36·0
	6804	27185	36·2[a]	36·9
	6711	27175	36·7[a]	37·0
	6902	29108		42·1
	7509	29235		42·5
	7008	29110		44·9
	7005	27153	45·4[a]	
	6906	27266		46·9
	6806	27187	46·4[a]	46·6
	7201	29152		46·1
	6905	29109		47·1
	6714	27178	47·4[a]	47·9
	6702	27171	47·4[a]	
	6803	27184	47·4[a]	47·5
	6805	27186	48·0[a]	
Gloeobacter	7421	29082		64·0[c]
Gloeocapsa	7512	29115		39·8
	7501	29113		40·9
	73106	27928		41·7
	7428	29159		46·0
Gloeothece	73107	29116		40·4
	6501	27151	40·8[a]	41·7
	6909	27152	41·3[a]	41·5
	7109	29163		42·0
	73108	29164		42·7
Chamaesiphon	7430	29397		46·7
	6605	27169	46·9[a]	46·9

[a] From Stanier *et al.* (1971); [b] from Edelman *et al.* (1967); [c] from Rippka *et al.* (1974).

Table 2. *Mean DNA base compositions of strains assigned to Section II*

Genus	Strain number PCC	Strain number ATCC	Mol % GC from: Buoyant density	Mol % GC from: Thermal denaturation
Dermocarpa	7438	29372		38·3
	7437	29371		40·7
	7302	29368		42·9
	7303	29369		43·6
	7301	29367	44[a]	44·0
	7304	29370		44·0
Xenococcus	7307	29375		43·6
	7305	29373		44·2
Dermocarpella	7326	29376		45·1
Myxosarcina	7325	29378		42·7
	7312	29377		44·0
Chroococcidiopsis	6712	27176	40·8[b]	40·2
	7434	29382		45·8
	7203	27900		45·8
	7431	29379		45·9
	7432	29380		46·0
	7439	29384		46·3
	7433	29381		46·4
	7436	29383		46·4
Pleurocapsa group	7440	29394		39·3
	7516	29396		41·0
	7310	29385		42·8
	7320	29389		43·0
	7322	29391		43·0
	7321	29390		43·0
	7319	29388		43·2
	7314	29386		43·3
	7324	29392		43·3
	7317	29387		45·4
	7327	29393		46·5

[a] From Edelman *et al.* (1967); [b] from Stanier *et al.* (1971).

one another in gross structural respects. Thus members of different base compositional clusters may be virtually indistinguishable by microscopic examination.

Base compositional data on six newly isolated *Synechocystis* strains have not significantly extended the overall range for this genus (Stanier *et al.*, 1971). *Synechocystis* contains two distinct strain clusters with mean DNA base compositions in the ranges of 35 to 37 and 42 to 48 mol% GC. In the latter (high GC) group, seven independent isolates appear, in terms of their phenotypic properties, to be members of a single species; they are also closely similar in base composition.

The four other genera assigned to Section I are each represented by a small number of strains. The spans of DNA base composition within these genera may therefore be wider than they now appear (Table 1). The four strains assigned to the genus *Gloeocapsa* differ structurally but have a relatively narrow base compositional span of 40 to 46 mol% GC. The five members of the genus *Gloeothece*, which may represent at least two different species, are closely similar in DNA base composition (40 to 43 mol% GC). *Chamaesiphon* is represented by two strains, of identical base composition (47 mol% GC), which are readily distinguishable by their phenotypic properties (Waterbury & Stanier, 1977). *Gloeobacter* is represented by a single strain of mean DNA base composition 64 mol% GC.

The cyanobacteria of Section II, the pleurocapsalean group, are distinguished by a special mode of reproduction: the release of small, spherical daughter cells (baeocytes) which arise

Table 3. *Mean DNA base compositions of strains assigned to Section III*

Genus	Strain number PCC	Strain number ATCC	Mol % GC from: Buoyant density	Mol % GC from: Thermal denaturation
Oscillatoria	7515	29209		40·1
	7805	—		40·9
	6412	29205		42·5
	6506	29081	44·6	
	6407	27906	45·4	
	6602	27935	45·4	
	6401	29215	46·9, 45[a]	
	7412	29135		47·3
	6304	27930	49·0, 48[a]	
	7112	29134	49·5	
Pseudanabaena	7429	29536		43·8
	6903	27190	44·9[b]	
	6901	27263	45·9[b]	45·9
	6802	27183	45·9[b]	
	7403	29210		46·3
	7402	29207		46·6
	7367	29137		47·7
	6406	29118		52·2
Spirulina	7345	29408		44·3
	6313	29542	53·6	53·4
LPP group	7408	29344		41·7
	7419	29346		43·3
	7409	29541		43·6
	7420	29128		45·5
	7505	29170		45·9
	7410	29136		47·1
	6402	27902	47·4	
	6306	27894	48[a]	48·8
	73110	29407	47·4, 47[c]	49·9, 48[c]
	7376	29410		46·0
	6703	27907	46·6	
	7113	29206		46·7
	7375	29409		47·8
	6409	29119	48·0	
	7427	29129		48·7
	7406	29125		49·2
	7404	29165		50·7
	7004	27913	50·7	50·9
	7114	29121	52·0	
	7105	29120		53·0
	7124	29123	53·6	
	7407	29126		56·2
	7104	29117	57·7	
	7123	29122	59·2	
	7411	29127		67·3

[a] From Edelman *et al.* (1967); [b] from Stanier *et al.* (1971); [c] from Kaye *et al.* (1967).

by multiple fission within the outer wall layer of a much larger vegetative cell. The genera included are diverse in developmental respects (Waterbury & Stanier, 1978) and are, in fact, assigned to two separate orders, the Dermocarpales and the Pleurocapsales, in the traditional phycological classification of the cyanobacteria (e.g. Bourrelly, 1970). However, the section is remarkably uniform in DNA base composition (Table 2), the total span for the 30 strains examined being 38 to 47 mol% GC. Within the genus *Chroococcidiopsis*, six of the eight strains appear to be members of one species in terms of their phenotypic pro-

Table 4. *Mean DNA base compositions of strains assigned to Section IV*

Genus	Strain number PCC	Strain number ATCC	Mol % GC from: Buoyant density	Mol % GC from: Thermal denaturation
Anabaena	6309	29211		38·3
	7122	27899		43·9
	7119	29151		42·4
	7120	27893		42·5
	6411	27898	42·9	
	7118	27892	43·4, 44[a]	41–43[b]
Nostoc	7524	29411		39·0
	7422	29132		41·6
	6720	27895	41·8	
	7107	29150	41·8	
	7416	29107		42·4
	7423	29168		42·2
	7413	29106		42·7
	6705	29131	43·4	
	6314	27904	43·9	
	6719	29105	43·9	
	6310	27896	44·2, 46[a]	
	6302	27897		45·1
	73102	29133		45·2
Cylindrospermum	7417	29204		42·1
	7604	33001		42·9
	73101	29535		46·7
Nodularia	73104	29167		40·5
Scytonema	7110	29171		44·4
Calothrix	7103	27905	39·8	
	7102	27901	39·8	
	7204	29190		40·5
	7116	29111	40·8	
	7426	29345		40·9
	7101	27914	43[a]	41·2
	7504	29158		41·5
	7415	29157		41·8
	6303	29156	41·8, 43[a]	
	7507	29112		42·8
	7111	29199	44·4	

[a] From Edelman *et al.* (1967); [b] from Craig *et al.* (1969).

perties; they are also closely similar in DNA base composition, and significantly different in this respect from the one other strain of this genus so far examined.

The generic sub-division of the filamentous, non-heterocystous cyanobacteria assigned to Section III still presents many unsolved problems, as discussed by Rippka *et al.* (1979). Three genera – *Oscillatoria*, *Pseudanabaena* and *Spirulina* – can be recognized and distinguished on structural grounds. However, a clear-cut generic assignment for many of the strains cannot yet be made and, since these strains share, in various combinations, the properties which have been ascribed to the genera *Lyngbya*, *Plectonema* and *Phormidium*, they have been placed in a provisional category, termed the LPP group.

Oscillatoria and *Pseudanabaena*, represented by 10 and 8 strains respectively, have relatively narrow and similar base compositional spans of 40 to 50 and 44 to 52 mol% GC (Table 3). The two strains of *Spirulina* analysed contained DNA with 44 and 54 mol% GC. These two strains differ greatly in phenotypic respects: strain PCC 7345 contains gas vacuoles and forms very thick filaments whereas strain 6313 does not form gas vacuoles and has much thinner filaments.

Table 5. *Mean DNA base compositions of strains assigned to Section V*

Genus	Strain number PCC	Strain number ATCC	Mol % GC from: Buoyant density	Mol % GC from: Thermal denaturation
Chlorogloeopsis	6718	27181		42·1
	6912	27193	42·9[a]	41–42[b]
Fischerella	7522	29539		41·9
	7523	29540		41·9
	7521	29538		42·3
	73103	29114		42·4
	7520	29537		42·5
	7115	27929	44·4	
	7414	29161		46·3

[a] From Stanier *et al.* (1971); [b] from Craig *et al.* (1969).

The base compositional span of DNA for the 25 strains of the LPP group is wide: 42 to 67 mol% GC (Table 3). Although the group is phenotypically diverse, there appears to be little correlation between phenotypic characters and DNA base composition. The bracketed cluster of five strains are the only representatives of the LPP group which appear closely similar to one another in phenotypic respects; they are also similar in base composition (46 to 50 mol% GC). This strain cluster includes the organism that has been widely used for physiological studies (Stewart & Lex, 1970; Padan *et al.*, 1971) and as a host for LPP phages (Padan *et al.*, 1970) under the name *Plectonema boryanum*.

Section IV comprises heterocystous cyanobacteria which form unbranched filaments. Only four base compositional values for cyanobacteria of this section have been previously published. The 35 strains included cover a considerable range of structural diversity; they include representatives both of the nostocacean (*Anabaena*, *Nostoc*, *Cylindrospermum*, *Nodularia*) and rivularian (*Calothrix*) types. The overall base compositional span is narrow: 38 to 47 mol% GC (Table 4). For genera in which numerous strains have been analysed (e.g. *Nostoc* and *Calothrix*), the intrageneric spans almost correspond to that for Section IV in its entirety.

The strains assigned to Section V are the heterocystous cyanobacteria which form true branches (genus *Fischerella*) and *Chlorogloeopsis*, a virtually unicellular heterocyst-former which, like the true branchers, divides in more than one plane. The base compositional range for the nine strains analysed is narrow (42 to 46 mol% GC, Table 5) and not significantly different from that of the heterocyst-formers of Section IV.

DISCUSSION

The wide span of mean DNA base composition among cyanobacteria – only slightly less than that for all prokaryotes – was established by Edelman *et al.* (1967) and Stanier *et al.* (1971). These workers analysed a relatively limited number of strains, heavily biased in favour of unicellular forms. The present study, conducted on a much larger and more representative strain collection, has revealed that major DNA base compositional divergences are confined to two cyanobacterial sub-groups: unicellular organisms that reproduce by binary fission (35 to 71 mol% GC) and filamentous, non-heterocystous cyanobacteria (40 to 67 mol% GC). For all other cyanobacteria, the overall range is only 38 to 47 mol% GC. These include the heterocystous and the pleurocapsalean cyanobacteria, two sub-groups which are large, internally diverse and very different with respect to structure and development. For both sub-groups, the DNA base compositional range is the same. This stands in striking contrast to the situation among unicellular cyanobacteria, where the range

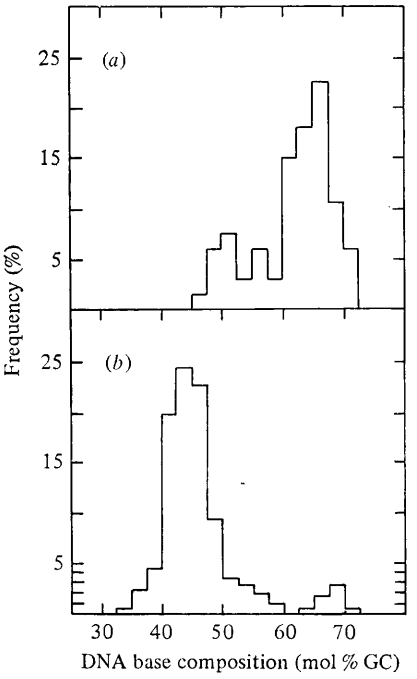

Fig. 1. Distribution of mean DNA base composition of (*a*) photosynthetic bacteria (after Mandel *et al.*, 1971) and (*b*) cyanobacteria.

for a single form genus, *Synechococcus*, extends from 39 to 71 mol% GC. There is, accordingly, little or no correlation among cyanobacteria between divergences in mean DNA base composition on the one hand and diversity in structural and developmental respects on the other. This is paradoxical, since it is precisely in structural and developmental respects that the group appears most highly diverse; the gross metabolic properties of cyanobacteria are remarkably uniform (Stanier & Cohen-Bazire, 1977).

Except possibly at the specific level, DNA base composition is not a useful taxonomic character among pleurocapsalean and heterocystous cyanobacteria. Among unicellular cyanobacteria, on the other hand, it emerges as a most important differential property. Both major form genera, *Synechococcus* and *Synechocystis*, can be sub-divided by virtue of significant base compositional discontinuities. The three ranges for *Synechococcus* are: 39 to 43, 47 to 56 and 66 to 71 mol% GC. The two ranges for *Synechocystis* are: 35 to 37 and 42 to 48 mol% GC. In bacteriological taxonomic practice, discontinuities as marked as these could be used to justify generic separations.

Singer & Ames (1970) hypothesized that the wide divergences of bacterial mean DNA base compositions, which range from 29 to 74 mol% GC, are attributable at least in part to environmental selection by solar irradiance. They suggested that DNA with a high GC content might have been selected as a means of avoiding thymine-specific damage from ultraviolet irradiation; in support of this proposal, they showed that bacterial genera of high GC content are, in general, composed of organisms subject to solar irradiance in the natural habitat, whereas those of low GC content tend to be composed of organisms from habitats not subject to solar irradiance. Bak *et al.* (1972) listed a number of exceptions to this rule and concluded that (apart from counter-selection of extreme values) the distribution of bacterial GC contents is largely random. Obviously, all phototrophic prokaryotes are subject to solar irradiance; and, as predicted by Singer & Ames (1970), nearly all purple and green bacteria possess DNAs which fall in the upper half of the GC scale. This is shown in

Fig. 1(*a*), which summarizes the extensive data of Mandel *et al.* (1971). However, the cyanobacteria show a markedly different mean DNA base distribution (Fig. 1*b*), very few representatives possessing DNAs that fall into the upper range of the GC scale. The DNAs of the majority cluster slightly below the middle of the GC scale, and a few lie near its lower limit.

In many natural habitats, the intrinsic oxygen tolerance of cyanobacteria permits them to develop in direct contact with the atmosphere. This makes them considerably more liable to ultraviolet exposure than the purple and green bacteria, which develop in oxygen-free aqueous habitats, where they are covered by an ultraviolet-absorbing water overlayer. The DNA base compositional range of cyanobacteria therefore appears to be incompatible with the Singer–Ames hypothesis. We conclude that the nature of the selective pressures which have led to prokaryotic DNA base composition divergence remain largely unexplained.

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