Deoxyribonucleic Acid Base Composition of Flexibacteria

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SUMMARY

The base compositions of the deoxyribonucleic acids extracted from 93 cultures of flexibacteria classified in the genera *Cytophaga*, *Flexibacter*, *Microscilla*, *Saprospira*, *Herpetosiphon*, *Vitreoscilla* and *Beggiatoa* have been determined. The data demonstrate that the guanine plus cytosine contents (GC values) of the representative cultures ranged from 30 to 53 %. A number of the recently proposed nomen species are shown to contain representatives with different GC values.

INTRODUCTION

Marmur, Falkow & Mandel (1963) reviewed the arguments for considering comparative analyses of DNA base compositions as a cardinal criterion in microbial taxonomy. In brief, they concluded that a necessary, but not sufficient, condition for substantial genetic homology between pairs of organisms is an overall similarity in DNA base composition. As the difference in composition increases, so does the likelihood of less homology, and hence greater evolutionary divergence. A critical parameter in this regard is the 'GC value', the proportion of guanine + cytosine in the deoxyribonucleic acid, expressed as a molar percentage of total bases.

In this paper we present GC values obtained by density-gradient determinations for DNA of 90 of the strains of flexibacteria isolated and characterized by Lewin & Lounsbery (1969), and for three other organisms. These data were used, together with other biochemical and physiological information, in a computer analysis designed to associate these microbes in groups which could serve as a basis for their classification (Fager, 1969). Largely on the basis of Fager's analysis Lewin (1969) assigned these strains to a number of named genera and species. In the present paper, we also discuss the status of Lewin's proposed taxa as viewed solely from the standpoint of their GC values.

METHODS

Organisms were harvested by centrifugation of cultures in liquid media when they had attained maximum growth as described by Lewin & Lounsbery (1969). The organisms were suspended in a small volume of 0.15 M-NaCl containing 0.1 M-Na₂ ethylenediamine tetra-acetate (EDTA) at pH 8.0 (saline EDTA) and were lysed by the addition of sodium dodecyl (lauryl) sulphate to a final concentration of 2 % (w/v). The lysate was immediately deproteinized with fresh phenol saturated with saline-EDTA at neutral pH, the aqueous phase separated by centrifugation, and the DNA precipitated from it by the

addition of two volumes of 99% (v/v) ethanol was 'spooled' on a clean glass rod. Spools prepared in California were shipped in ethanol to Texas for analysis. The spool was freed of phenol by successive immersions in two changes of 70% (v/v) ethanol in water, drained, and then dissolved in 0.15 M-NaCl + 0.015 M-Na₃ citrate at pH 7.0 (solution SSC). When samples were to be analysed by thermal denaturation, highly purified DNA was prepared from the lysates by the procedure of Marmur (1961). All samples were stored over CHCl₃ at 5° until they were analysed.

Table 1. *Buoyant density and GC content of DNA of flexibacteria*

Values given for mean density are averages of at least two determinations of the buoyant density in CsCl at 44,770 and 42,040 rev./min. Specific epithets and strain numbers correspond to the designations of Lewin (1969).

Name	Strain	Mean density (g./cm. ³)	GC (mole %)
<i>Cytophaga latercula</i>	SIO-1	1.6935	34.2
<i>C. lytica</i>	B-9	1.6925	33.2
	WFB-21	1.6935	34.2
	BON	1.693 (1.7165*)	33.7 (57.7*)
	ENS	1.6925	33.2
	LIM-21	1.693	33.2
<i>C. diffluens</i> (?)	DD-1	1.694	34.7
<i>C. diffluens</i> var. <i>aprica</i>	DUB-4	1.696	36.7
	ST-1	1.6965	37.2
	JL-4	1.6945	35.2
<i>C. diffluens</i> var. <i>carnea</i>	Q-1	1.6965	37.2
<i>C. diffluens</i>	Y-1	1.700	40.8
	GOL-12	1.700	40.8
	N-3	1.6995	40.3
	NN-3	1.6995	40.3
	B-1	1.7015	42.3
	LIM-1	1.7015	42.3
	O-2	1.7007	41.5
<i>Flexibacter litoralis</i>	SIO-4	1.6897	30.3
<i>F. aurantiacus</i>	DWO	1.6905	31.1
	PSY	1.6910	31.6
<i>F. aurantiacus</i> var. <i>copepodarus</i>	COP	1.691	31.6
<i>F. aurantiacus</i> var. <i>excathedrus</i>	CR-134	1.6935	34.2
<i>F. giganteus</i>	CR-103	1.6925	33.2
	CR-104	1.691	31.6
	CR-124	1.6906	31.2
<i>F. roseolus</i>	CR-141	1.6935	34.2
	CR-155	1.698	38.8
<i>F. ruber</i>	GEY	1.696	36.7
<i>F. flexilis</i>	CR-63	1.700	40.8
	CR-81	1.702	42.9
	A-52	1.7015	42.3
	WAR-5	1.6995	40.3
<i>F. flexilis</i> var. <i>pelliculosus</i>	FLE	1.6985	39.3
<i>F. flexilis</i> var. <i>iolanthae</i>	BA-24	1.7005	41.3
<i>F. sancti</i>	BA-3	1.705	45.9
	BA-23	1.706	46.9
	MIC	1.705	45.9
<i>F. elegans</i>	NZ-1	1.7065	47.4

(Table 1 cont.)

Name	Strain	Mean density (g./cm. ³)	GC (mole %)
<i>Microscilla arenaria</i>	HJ-1	1·6915	32·1
<i>M. aggregans</i>	Q-3	1·7015	42·3
	QQ-1	1·6985	39·3
	QQ-11	1·6993	40·1
	JL-13	1·695	35·7
	NN-13	1·696	36·7
<i>M. aggregans</i> var. <i>calatatica</i> (<i>Flexithrix dorotheae</i>)	HI-3	1·694	34·7
	QQ-3	1·6965	37·2
<i>Microscilla tractuosa</i>	EE-13	1·697	37·8
	EG-13	1·6965	37·2
	GH-1	1·6935	34·2
	GH-2	1·6945	35·2
	HI-15	1·695	35·7
	H-43	1·6955	36·2
	JK-11	1·696	36·7
	T-13	1·6965	37·2
<i>M. sericea</i>	SIO-7	1·698	38·8
	SIO-9	1·698	38·8
<i>M. marina</i>	SIO-8	1·7015	42·3
<i>M. furvescens</i>	TV-2	1·704	44·9
<i>Saprospira thermalis</i>	BEG	1·696	36·7
	CR-123	1·6947	35·4
	CR-125	1·692	32·7
<i>S. toiviformis</i>	A-1	1·6976	38·4
<i>S. albida</i>	CAR	1·699	39·8
	HH	1·7015	42·3
	MUR	1·702	42·9
<i>S. grandis</i>	WH	1·7068	47·8
	PA-1	1·7064	47·3
	PA-2	1·7055	46·4
	PA-3	1·7055	46·4
	LJA	1·7058	46·7
	E-1	1·7055	46·4
	E-2	1·707	48·0
<i>S. grandis</i>	M-1	1·7065	47·4
	M-2	1·7065	47·4
	ROB	1·7065	47·4
	SG-1	1·706	46·9
	SG-2	1·707	48·0
	DAW-1	1·707	48·0
	DAW-2	1·706	46·9
	DUB-2	1·706	46·9
	DUB-3	1·705	45·9
	A-2	1·705	45·9
	A-3	1·705	45·9
	JL-1	1·7065	47·4
<i>S. flammula</i>	SNZ	1·7073	48·3
<i>Herpetosiphon cohaerens</i>	II-2	1·704	44·9
<i>H. persicus</i>	T-3	1·7115	52·6
<i>H. nigricans</i>	SS-2	1·712	53·1
<i>H. geysericolus</i>	GC-42	1·7075	48·5

* Second component present in trace amount.

The buoyant density in CsCl was determined as described by Schildkraut, Marmur & Doty (1962). All samples were analysed at least once in each of two different analytical ultracentrifuges, one operating at 44,770 rev./min. and recording photographically, the other operating at 42,040 rev./min. and using a photoelectric multiplier scanning system. (For details of operation, see Mandel, Schildkraut & Marmur, 1968.) Samples of 1 to 2 μ g. of the DNA of unknown density were brought to isopycnic equilibrium in CsCl gradients containing as a reference sample 0.5 μ g. DNA of bacteriophage SP 8 of a density of 1.742 g./cm.³ (calibrated against DNA of *Escherichia coli*, assumed to be of a density of 1.710 g./cm.³). The guanine + cytosine (GC) content of the extracted DNA was estimated from the mean buoyant density as described in the above references. This is the GC value as discussed throughout this paper.

Thermal denaturation was done in solution SSC at a DNA concentration of 20 μ g./ml. using a Beckman DU spectrophotometer, as described by Marmur & Doty (1962).

RESULTS

The results are summarized in Tables 1 (all strains examined) and Table 2 (combined data for species).

Cytophaga

The 18 strains assigned to the genus *Cytophaga* had GC values of 33 to 42%. The strain BON, assigned to *Cytophaga lytica*, yielded DNA which displayed also a second minor component at equilibrium in the CsCl gradient. Upon heat denaturation both major and minor bands increased in density by about 15 and 12 mg./cm.³ respectively; hence we concluded that each was a double stranded DNA molecule. No obvious contaminating organism has been isolated from this culture, but the possibility of contamination has not been excluded beyond question.

The strains assigned to *C. lytica* were homogeneous with respect to GC values. The standard deviation of the twelve analyses (0.0008 g./cm.³) was not appreciably different from the standard deviation usually encountered when a like number of analyses is performed on a single DNA sample. The single strain assigned to *C. latercula* had a GC value similar to that of *C. lytica*.

The remaining strains assigned to the genus *Cytophaga* can be conveniently divided into two groups (Table 2). The first group, with a mean GC value of about 36%, included the strain DD-1, tentatively assigned to *C. diffluens*, and the varieties *C. diffluens* var. *aprica* and *C. diffluens* var. *carnea*. The standard deviation of the analyses is somewhat large and may indicate a lack of homogeneity in this group. The second group, also assigned to the species *C. diffluens*, had a GC value of about 41% and appears homogeneous.

Flexibacter

Lewin (1969) assigned to the genus *Flexibacter* 21 strains ranging in GC value from 30 to 47% (Table 1). The monotypic *F. litoralis* represented the low extreme, with not very different values observed for the groups designated as *F. aurantiacus* and *F. giganteus*. The combination of *F. aurantiacus* with its varieties *copepodarus* and *excathe-drus* yielded a standard deviation that appeared uncomfortably large (see Table 2). If strain GR-134 were to be excluded from this group, the S.D. would be appreciably decreased. A similar reservation has to be made for the association of the three strains

included under *F. giganteus*. *F. roseolus* is represented by two isolates with significantly different GC values, of the order of 4 to 5% apart, indicating that the organisms may be related but are not likely to be members of the same genospecies in the sense of Ravin (1963). The strain designated as *F. ruber* had a GC value intermediate between the two values for the strains of *F. roseolus*. Six isolates assigned to *F. flexilis* and its varieties *pelliculosus* and *iolanthae* have GC values ranging from 39 to 43%, with a mean of 41.6% and a standard deviation of 1.6%. We conclude that these groups, too, display inhomogeneity in this regard, although there is no obvious way by which an effective division may be made with the data at hand. The three strains assigned to *F. sancti* comprised a uniform assemblage with GC values of $46.2 \pm 0.9\%$. The single strain of *F. elegans* was only slightly but not significantly richer in GC content, its value being 47.4%.

Table 2. Buoyant density and GC content of DNA calculated for groups of strains of flexibacteria

Species or group	No. of analyses	Mean value and standard deviation	
		Density (g./cm. ³)	GC (moles %)
<i>Cytophaga latercula</i>	(2)	1.6935	34.2
<i>C. lytica</i>	(12)	1.6929 ± 0.0008	33.6 ± 0.8
<i>C. diffluens</i> group I	(10)	1.6955 ± 0.0014	36.2 ± 1.4
<i>C. diffluens</i> group II	(16)	1.7000 ± 0.0010	40.8 ± 1.0
<i>Flexibacter litoralis</i>	(4)	1.6897 ± 0.0005	30.3 ± 0.5
<i>F. aurantiacus</i> group	(8)	1.6915 ± 0.0014	32.1 ± 1.4
<i>F. giganteus</i>	(9)	1.6911 ± 0.0013	31.7 ± 1.3
<i>F. roseolus</i>	(4)	1.6958 ± 0.0026	36.5 ± 2.6
<i>F. ruber</i>	(2)	1.696	36.7
<i>F. flexilis</i> group	(14)	1.7004 ± 0.0016	41.6 ± 1.6
<i>F. sancti</i>	(6)	1.7053 ± 0.0009	46.2 ± 0.9
<i>F. elegans</i>	(2)	1.7065	47.4
<i>Microscilla arenaria</i>	(2)	1.6915	32.1
<i>M. aggregans</i> group	(15)	1.6974 ± 0.0025	38.2 ± 2.5
(<i>Flexithrix dorotheae</i>)	(2)	1.6965	37.2
<i>M. tractuosa</i>	(16)	1.6956 ± 0.0013	36.3 ± 1.3
<i>M. sericea</i>	(4)	1.698 ± 0.0008	38.8 ± 0.8
<i>M. marina</i>	(2)	1.7015	42.3
<i>M. furvescens</i>	(2)	1.704	44.9
<i>Saprospira thermalis</i>	(10)	1.6947 ± 0.0022	35.4 ± 2.2
<i>S. toiviformis</i>	(5)	1.6976 ± 0.0009	38.4 ± 0.9
<i>S. albida</i>	(6)	1.7005 ± 0.0015	41.3 ± 1.5
<i>S. grandis</i>	(49)	1.7061 ± 0.0009	47.0 ± 0.9
<i>S. flammula</i>	(4)	1.7073 ± 0.0010	48.3 ± 1.0
<i>Herpetosiphon cohaerens</i>	(2)	1.704	44.9
<i>H. persicus</i>	(2)	1.7115	52.6
<i>H. nigricans</i>	(2)	1.712	53.1
<i>H. geysericolus</i>	(2)	1.7075	48.5

Microscilla and *Flexithrix*

In this group of 20 strains, *Microscilla arenaria* was at the lower limit with a GC value of 32%, and *M. furvescens* was at the other extreme with a value of 45%. The *M. aggregans* group appeared to be heterogeneous in this respect, and might be better subdivided into a group of 3 strains, Q-3, QQ-1 and QQ-11, with a mean GC value of 41% and a second group, JL-13, and NN-13, which, together with the variety *catalatica* (HI-3), would have

a mean value of 36%. The strain QQ-3, bearing the provisional epithet *Flexithrix dorotheae*, might have an affinity with this latter group or with members of the *M. tractuosa* group. Members of this group, with an average GC value of $36.3 \pm 1.3\%$, may eventually prove to be divisible into at least two species. The two strains assigned to *M. sericea* had indistinguishable GC values. The remaining strain, designated as *M. marina*, had a GC value of 42%, it was thereby readily distinguished from all other strains of the *Microscilla* group except the higher GC subgroup of *M. aggregans*.

Saprospira

Of the 27 strains assigned to this genus, 19 are considered to be representative of *Saprospira grandis*; the similarity of their GC values (Table 1) is in agreement with this conclusion. The mean value for this species was $47.0 \pm 0.9\%$. Strain A-1 was originally considered to be a member of this species, but the lower density of its DNA indicated a GC value of about 38%. (Melting curves confirmed this difference: the DNA of A-1 melted at a temperature 4 to 5° below that of the DNA from DAW-3). Further phenotypic characterization led to our decision to distinguish this strain as a separate monotypic species, *Saprospira toviformis* (Lewin & Mandel, 1969).

The representatives of *S. thermalis* had DNA base compositions quite distinct from those of *S. grandis*. The GC value for strain CR-125 (33%) was lower than that obtained for BEG and CR-123 (35 to 37%), which it otherwise closely resembled. (In fact, CR-123 and CR-125 were isolated from the same mud sample).

The strain SNZ, designated as *S. flammula* (Lewin, 1965), did not differ significantly in base composition from *S. grandis*, which it closely resembled also in phenotypic appearance, pigmentation and nutrition. Although originally isolated from a non-saline hot spring, it grew well in marine media, so we cannot regard it as specifically distinct from *Saprospira grandis*.

The three strains designated as *S. albida* had GC values very close to 41%.

Herpetosiphon

Holt & Lewin (1968) described a new genus of sheathed gliding bacteria with the type species *Herpetosiphon aurantiacus*. Three strains of this species were examined and found to have GC values of $48.1 \pm 1.2\%$. Four other isolates of morphologically similar filamentous organisms have been assigned to this genus (Lewin 1969). The strain designated as *H. geysericolus* had a GC value indistinguishable from that of the type species *H. aurantiacus*. The representative of *H. cohaerans* was significantly lower in GC content (45%), while *H. persicus* and *H. nigricans* each had GC values about 53%. The genus is therefore clearly divisible into at least three species on the basis of GC values.

Other apochlorotic gliding microbes

We have determined the DNA base compositions of a few similar organisms other than those included in the study of Lewin & Lounsbery (1969). A *Vitreoscilla* strain provided by Dr B. J. Bachmann (originally isolated by Professor E. G. Pringsheim) had a DNA of a buoyant density indicating a GC value of 43.6%: it was thus indistinguishable from strain V-1 obtained from Dr C. Hageage. Professor R. Y. Morita provided a cell harvest of *Beggiatoa leptomitiformis*, grown as described by Burton & Morita (1964); we obtained for this sample a GC value of 37%.

DISCUSSION

The GC values obtained for 90 of the cultures of flexibacteria studied by Lewin & Lounsbery (1969) ranged from 31 to 53%.

The 18 cultures assigned to the genus *Cytophaga* all had GC values between 33 and 42%, within the range previously reported for that genus by Mandel & Leadbetter (1965). Among the strains assigned by Lewin (1969) to *C. diffluens*, the range of GC values may be too wide for a single species, and it is to be expected that further studies of this group will lead to its division into at least two, possibly three, species.

The 21 cultures assigned to the genus *Flexibacter* ranged in GC values from 30 to 48%. Since most of the species were represented by no more than a few strains, judgments as to homogeneity of GC values can be only tentative. The GC values obtained for two strains assigned to *F. roseolus* differed by at least 4%, and perhaps strain CR-141 should be set apart as a separate species.

The 20 cultures of the genus *Microscilla* had GC values ranging from 32 to 45% GC. *Microscilla aggregans* and *M. tractuosa* may eventually prove to embrace more than one species.

The genus *Saprospira*, represented by 27 isolates, had members with GC values of 53 to 48%. We have reservations as to the uniformity of the members of *S. thermalis* and *S. albida*. *S. toviiformis* and *S. flammula* are monotypic; the latter cannot be separated from *S. grandis* on the basis of base composition.

The genus *Herpetosiphon* had representatives whose GC values ranged from 45 to 53%. Three species are monotypic at present, while in the type species, *H. auranticiacus*, the three strains are nearly identical in DNA base composition, with GC values averaging 48%.

The GC values of the *Vitreoscilla* cultures and of *Beggiatoa leptomitiformis*, as reported in this paper, were within the range found for the filamentous flexibacteria. *Leucothrix* strains have been reported with GC values, 47 to 49% in the same range (Brock & Mandel, 1966).

The gliding bacteria may be regarded as apochlorotic relatives of the filamentous Cyanophyta, notably the Oscillatoriales (Pringsheim, 1949; Harold & Stanier, 1955). Edelman *et al.* (1967) found GC values for 29 representatives of the blue-green algae to range from 35 to 71%, and commented upon the overlap of GC values for the filamentous gliding bacteria and the Oscillatoriales. In their survey, the 22 representatives of the order Oscillatoriales had GC values which ranged from 39 to 51%. Zimmerman (1966) reported that a strain of *Anacystis nidulans* had a GC value of 49% (*cf.* the value 56% reported by Edelman *et al.* 1967). This strain, when obtained originally from Kratz *via* Myers, has apparently been incorrectly identified, and should more correctly be called *Phormidium mucicola* (Silva, 1962). Its GC value falls neatly in the range of the Oscillatoriales. The additional data now available for filamentous flexibacteria extend the range of their GC values below those for blue-green algae. The argument for relatedness is strengthened by the coincidence of GC values for the sheathed representatives, *Lyngbya* (47 and 51%) and *Herpetosiphon* (44 to 53%).

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