The Base Composition of Deoxyribonucleic Acids of *Streptomyces*

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SUMMARY

The base composition of DNA preparations from 12 strains of *Streptomyces* and 1 strain of *Nocardia* were determined from their denaturation temperature (T_m) and buoyant density.

The % GC (guanine + cytosine) contents ranged from 74.4 to 78.5 (from T_m) and from 69.4 to 73.4 (from buoyant density). The correlation between the two sets of data and the found differences are discussed. The range of pH values which did not affect T_m and the degree of hyperchromic effect have been related to the ionic strength of the solvent. With 0.01 M-phosphate as solvent the pH indifference range was narrower and the hyperchromic effect smaller than with 0.2 M- Na^+ solvent.

Though the difference between the maximum and minimum % GC in *Streptomyces* is sufficient to distinguish the respective strains, within the extreme values there is a continuous progression of base composition which does not permit taxonomic divisions to be made on the basis of overall % GC alone.

INTRODUCTION

The base composition of bacterial DNA, expressed as % GC (guanine + cytosine), varies widely from one genus to another, but, generally, very little from one species to another in the same genus (Lee, Wahl & Barbu, 1956; Belozersky & Spirin, 1960; Marmur, Falkow & Mandel, 1963). The DNA from any one strain is, however, very homogeneous in base composition, as shown by its narrow banding in caesium chloride density gradient ultracentrifugation, the narrow range of temperature over which it denatures, and its readiness to 'renature' under suitable cooling conditions. Close genetic relationship can be indicated by the formation *in vitro* of hybrid DNA molecules in which the two strands originate from different DNAs. To enable the formation of such hybrids, the two parent DNAs should have, as a first requirement, the same overall base composition and, as a second requirement, similar base sequences, which, in turn, imply similar genetic information, since the latter is held to be coded in the DNA precisely in the sequences of the four bases, guanine (G), cytosine (C), adenine (A), thymine (T). Therefore, we thought it opportune as a first step to determine the base composition of DNAs from strains of *Streptomyces* and *Nocardia* that had been included in a previous phenetic classification (Silvestri, Turri, Hill & Gilardi, 1962). For this purpose, two techniques were used: (a) to determine for each DNA sample the 'melting temperature' (T_m), which, according

to Marmur & Doty (1962), is linearly related to % GC; (b) to determine the buoyant density of the samples in CsCl gradient centrifugation, which according to Schildkraut, Marmur & Doty (1962) is again linearly related to % GC.

METHODS

Organisms. The strains of *Streptomyces* and *Nocardia* used were selected from those comprised in the study by Silvestri *et al.* (1962) according to three criteria: (a) that they were located in different spheres (or phenetic taxa) in the earlier phenetic classification; (b) that they were as near as possible to the respective geometrical centres of their spheres; (c) that they were nomenclatural type cultures or, at least, reference strains deposited in recognized collections. The strains chosen as satisfying these criteria are listed in Table 1.

Table 1. *Organisms used in this work*

PSA* no.	Name	Other reference nos.	Sphere	Group
<i>Streptomyces</i>				
99	<i>S. kentuckensis</i>	ATCC* 12691	II	A
107	<i>S. parvulus</i>	ATCC 12434	XIV	D
45	<i>S. griseus</i>	ATCC 10137	VII	C
145	<i>S. parvus</i>	NRRL B 1455	IV	C
101	<i>S. griseolus</i>	ATCC 3325	XII	C
108	<i>S. flaveolus</i>	ATCC 3319	X	C
148	<i>S. diastaticus</i>	NRRL B 1270	III	B
22	<i>S. bobilliae</i>	ATCC 3310	VI	B
156	<i>S. fradiae</i>	ATCC 10745	IX	B
96	<i>S. intermedius</i>	ATCC 3329	XVII	B
60	<i>S. albus</i>	ATCC 618	XV	E
61	<i>S. fradiae</i>	ATCC 10745	XIII	F
<i>Nocardia</i>				
165	<i>N. asteroides</i>	IMRU 727	XI	E

* ATCC = American Type Culture Collection. NRRL = North Regional Research Laboratory, Peoria, Ill., U.S.A. IMRU = Institute of Microbiology, Rutgers University, N.J., U.S.A. PSA = Progetto Sistematica Actinomiceti, Università Statale, Milano, Italia.

See the catalogue: *The Culture Collection of the Progetto Sistematica Actinomiceti*, 1962, and Silvestri *et al.* 1962.

Cultivation of organisms. Strains were inoculated with a wire-loop from slope cultures into 250 ml. flasks with an anti-vortex baffle containing 70 ml. of a liquid medium composed of: Bacto-Peptone (Difco) 5 g.; Bacto-Yeast Extract (Difco) 8 g.; glucose, 10 g.; Bacto-Casitone (Difco) 1 g.; Bacto-Beef Extract 2 g.; tap water 1 l. (For the *Nocardia* strain, better growth resulted with Bacto-Peptone and Bacto-Casitone replaced by Peptone and Caseina, 'Costantino', respectively.) Flasks were incubated at 30° on an agitator for 48–60 hr. Subcultures were then made into the same medium by transferring 0.5 ml. samples of fully grown stationary phase culture into several flasks which were re-incubated for 24–30 hr. By this procedure growth as pellets was generally avoided and the cultures were in the logarithmic phase when the mycelia were collected by Buchner filtration or centrifugation. From 5 to 10 g. (wet weight) of mycelia were used for each DNA preparation.

Preparation of DNA specimens. The procedure of Marmur (1961) was followed.

Particular attention had to be paid to render optimal the lysis by lysozyme and sodium lauryl sulphate. Incubation with lysozyme was continued for 2–3 hr. but suspensions, though becoming viscous, did not become transparent until the detergent was added. *Nocardia asteroides* was normally resistant to lysozyme, but partial lysis was induced by pretreating 25 ml. suspension with 0.5 ml. ethane thiol ($\text{C}_2\text{H}_5\text{SH}$), overnight in the cold with agitation. According to Dr M. Mandel (personal communication) 2-mercapto-ethanol, $\text{CH}_2(\text{OH})\text{CH}_2\text{SH}$, is probably more efficient in uncovering lysozyme-sensitive sites. Final isopropanol precipitates were fibrous in most cases and were stored in the dry state.

Table 2. *Solutions used to determine the effect of ionic strength and pH value on T_m value with DNA from Streptomyces bobilliae*

Soln.	KH_2PO_4	Na_2HPO_4	EDTA	Diluted	Molarity	pH value	Specific conductance	T_m (°)
	0.2 M (ml.)	0.2 M (ml.)	0.1 M (ml.)	to (ml.)	PO_4^{3-} (M)		$\times 10^{-3}$ mho	
1	2.5	2.5	10	200	0.005	6.7	0.881	75.6
2	2.5	2.5	10	100	0.01	6.8	1.570	80.5
3	2.5	2.5	10	50	0.02	6.7	2.979	85.8
4	2.5	2.5	10	25	0.04	6.7	5.590	90.8
5	4.5	0.5	10	100	0.01	5.85	1.250	76.5
6	3.5	1.5	10	100	0.01	6.40	1.440	79.25
7	1.5	3.5	10	100	0.01	7.10	1.720	81.6
8	0.5	4.5	10	100	0.01	7.50	1.923	81.8

Determination of T_m values. Solutions containing 10–20 μg . DNA/ml. were heated in a Beckman DU spectrophotometer fitted with thermal spacers (Marmur & Doty, 1962) and the increase in extinction on denaturation of the DNA (hyperchromic effect) observed. An initial reading was made at 25° and then, from 3–4° before the onset of denaturation to 2–3° after it had terminated, extinction readings were made at 1° intervals, the temperature being raised at the rate of about 1°/10 min. T_m value is defined as the mid-point (50%) of the hyperchromic effect.

Solvents. The % GC content of a *Streptomyces* DNA has been reported to be in the order of 70–74% (Belozersky & Spirin, 1960; Marmur & Doty, 1962) and therefore the T_m determination in the usual solvent SSC (standard saline citrate; 0.15 M-NaCl + 0.015 M- Na_3 citrate, pH 7.0) requires temperatures higher than 100° (Marmur & Doty, 1962). Use has therefore to be made of more dilute solvents. It is reported in the literature (e.g. Ts'o, Helmkamp & Sander, 1962), that the T_m value is proportional to the logarithm of the ionic strength of the solvent and that, with SSC solvent, the pH value does not influence the T_m value within the range pH 5.5–8.5. Two sets of solutions were prepared (Table 2) to determine the effect on T_m value of changing ionic strength (pH constant) and of changing pH value in a region of an ionic strength lower than that of SSC solvent (molarity being constant). Specific conductance of solutions was determined with a Philips Conductivity Bridge, model GM 4249. By plotting T_m values against the logarithm of specific conductance (Fig. 1) it is seen that the first four solutions, changing ionic strength, pH constant, fall on a straight line. With 0.01 M- PO_4^{3-} , pH changing, the indifference range of pH values was narrower than that reported for SSC solvent, since solutions at pH 6.4 and 7.5 already departed from the straight line. During these experiments

it was noticed that the hyperchromic effect is also influenced by ionic strength. A quadratic relationship was found. The more dilute the solvent the less was the hyperchromic effect (e.g. in solution 1 the hyperchromic effect in duplicate samples was 23.1 and 21.9%, whereas in solution 4 it was 27.2 and 27.9%) (Fig. 2). The degree of hyperchromicity is, however, less reproducible than T_m in replicate experiments. The hyperchromicity varies with conductance according to the following parabolic function:

$$\% \text{ hyperchromicity} = 22.40 - 0.28 \log x + 9.74 (\log x)^2,$$

where x is the specific conductance of the solution.

Fig. 1

Fig. 1. The relationship between T_m values and ionic strength (DNA from *Streptomyces bobilliae*). Solvents 1–8, see Table 2. SSC = standard saline citrate solvent. A = phosphate solvent used for T_m determinations.

Fig. 2

Fig. 2. The relationship between the hyperchromic effect and ionic strength. DNA from *Streptomyces bobilliae*, duplicate samples.

The solvent finally chosen, as giving T_m values 20° less than the SSC solvent, was: Na_2HPO_4 , 0.2M, 2.5 ml. + NaH_2PO_4 , 0.2M, 2.5 ml. + EDTA, 0.01M, 10 ml.; diluted to 100 ml.; pH 6.8; specific conductance 1.345×10^{-3} mho. Under our conditions, the specific conductance of SSC solvent was 16×10^{-3} mho and, from Fig. 1, the above phosphate solution (indicated by A in Fig. 1) should yield T_m values 20.1° less than in SSC solvent.

Buoyant density. The technique of Meselson, Stahl & Vinograd (1957) was used with *Escherichia coli* B DNA as reference (1710 g./cm.³). CsCl was obtained from Merck (Darmstadt; ELH grade) and used with no further purification. Runs were made in a Spinco model E analytical ultracentrifuge and lasted 24 hr at 44,770 rev./min.

RESULTS

Thermal denaturation. We previously reported on the reproducibility of T_m values (Hill & Silvestri, 1963); in the present work the largest difference obtained with duplicate samples was 0.25° with the same batch of solvent. T_m values for the various

Fig. 8. The correlation between T_m and buoyant density. Numbers in the figure refer to the organisms in Table 1. $r = 0.502$, $n = 11$, $P = 0.07$.

Table 3. % GC from T_m and buoyant density (ρ) values of various DNA preparations

Strain	(1) T_m (°)	(2) % GC from T_m	(3) in g./cm. ³	(4) % GC from ρ	(5) Difference 2-4 (%)
<i>Streptomyces</i>					
<i>S. kentuckensis</i> 99	80.8	76.8	1.728	69.4	+7.4
<i>S. parvulus</i> 107	80.9	77.2	1.730	71.4	+5.8
<i>S. griseus</i> 45	80.8	76.8	1.730	71.4	+5.4
<i>S. parvus</i> 145	80.25	75.5	1.729	70.4	+5.1
<i>S. griseolus</i> 101	80.3	75.6	1.729	70.4	+5.2
<i>S. flaveolus</i> 108	80.0	74.8	1.729	70.4	+4.4
<i>S. diastaticus</i> 148	81.2	77.8	1.729	70.4	+7.4
<i>S. bobilliae</i> 22	79.8	74.4	1.729	70.4	+4.0
<i>S. fradiae</i> 156	81.5	78.5	1.732	73.4	+5.1
<i>S. intermedius</i> 96	79.5	73.6	1.728	69.4	+4.2
<i>S. albus</i> 60	79.75	74.3	1.730	71.4	+2.8
<i>S. fradiae</i> 61	81.5	78.5	1.731	72.4	+6.1
<i>Nocardia</i>					
<i>N. asteroides</i> 165	81.0	77.3	1.728	69.4	+7.9

Notes. Col. 1 in 0.01M- PO_4 +0.001M-EDTA (pH 6.8). Col. 2 $T_m = 49.3 + 0.41(\text{GC})$. Col. 3 relative to *Escherichia coli* B NDA = 1.710 g./cm.³ Col. 4 $\rho = 1.660 + 0.098 (\text{GC})$.

samples are listed in column 1, Table 3, together with % GC calculated (column 2) by means of the linear relationship (Marmur & Doty, 1962): $T_m = 49.3 + 0.41$ (GC).

Buoyant density, ρ . Values are listed in column 3, Table 3, together with % GC calculated (column 4) by means of the linear relationship (Schildkraut *et al.* 1962): $\rho = 1.660 + 0.098$ (GC).

Correlation between T_m and buoyant density values. The correlation coefficient between the two sets of values was calculated, $r = 0.502$, $n = 11$, $P = 0.07$ (Fig. 3). The correlation coefficient between T_m values (Hill & Silvestri, 1963) in an Na_2HPO_4 solvent, adjusted to pH 7.0 with NaOH, gave the following results: $r = 0.6275$, $n = 10$, $P = 0.03$.

The better correlation of the previously published data can be attributed to the fact that then determinations were conducted with the same batch of solvent. Changing batches with such dilute solvents introduces another source of variance.

Difference in % GC according to T_m and buoyant density values. In Table 3, column 5, the differences in % GC calculated from T_m and from buoyant density are recorded. Those calculated from T_m were always higher than those calculated from buoyant density, the differences ranging from a minimum of 2.8% to a maximum of 7.9%; the average difference was 5.46% GC.

In the studies of Marmur & Doty (1962) and Schildkraut *et al.* (1962), only one streptomycete DNA (from *Streptomyces viridochromogenes*) was examined for T_m and buoyant density. These authors too found a difference between % GC calculated from T_m (74%) and from buoyant density (70%). Chromatographic analysis of the bases yielded a % GC of 73.8% for this species (Belozersky & Spirin, 1960). Schildkraut *et al.* (1962) discussed several explanations for this discrepancy, perhaps the most important of which was the possible existence of bases other than the usual four.

With respect to our data, the range of % GC calculated from T_m values using the equation of Marmur & Doty (1962) is from a minimum of 73.6% to a maximum of 78.5%.

Range of % GC within *Streptomyces*. The 2° difference between the minimum T_m value (strain 96 *Streptomyces intermedius*) and the maximum T_m (strains 61 and 156, both *S. fradiae*) corresponds to a % GC range of 4.9%. Again, the % GC from the buoyant density data is 4% (0.004 g./cm.³) difference between the minimum buoyant density (strains 99, 96, 165) and the maximum (strain 156).

DISCUSSION

The range of % GC in *Streptomyces* seems to be very restricted. Jones & Bradley (1963), with fewer strains, found a yet narrower range. Though the difference between maximum and minimum % GC is large enough to allow a clear distinction between the extreme strains, the fact that there is a continuous progression of base composition precludes an attempt to make taxonomic divisions on the basis of overall % GC composition alone. Only one strain of *Nocardia* was included in this study, but apparently distinction between the two genera on this basis alone seems impossible. This continuity of DNA composition within a genus was also observed by De Ley & Schell (1963) in acetic acid bacteria. Given that the maximum difference in % GC was about 4%, then even the strains most distant from one

another could have in common many DNA molecules (Sueoka, 1961). Broad-spectrum actinophages are known such as 1091 (Silvestri *et al.* 1962) which are active against strains which differ by as much as 2% GC. Furthermore, reports exist in the literature of transformation between *Streptomyces aureofaciens* and *S. griseus* (Jarai, 1961), of heterokaryosis between *S. cyaneus* and *S. griseus* (Bradley & Lederberg, 1956) and of recombination between *S. rimosus*, *S. coelicolor* and *S. aureofaciens* (Alacevic, 1963). On the basis of the present data concerning DNA base composition, phage sensitivity and genetic exchange, it seems legitimate to doubt of the existence of isolated species within the *Streptomyces* taxon, which is presently considered to merit a generic rank. It is probable that the process of 'splitting' has been carried too far. By phenetic taxometric studies it is easier to resolve the genus *Streptomyces* into distinguishable groups. However, in view of the available facts, we are doubtful whether the phenetic groups (spheres) individuated by electronic computer could be properly considered species deserving distinct binomials. On the other hand, the relative scarcity of molecular and genetic data within the genus does not permit a more definite conclusion at present, neither an evaluation of the taxonomic rank of the phenetic taxa.

It is interesting to note that strain 99 *Streptomyces kentuckensis*, which is in the middle of the % GC range found here within the genus, occupied also a central position in the phenetic hyperspace of our previous taxometric study (Silvestri *et al.* 1962). This organism is near the geometric centre of the second sphere, which is itself located in a very central position in respect to the entire system.

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