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**

<table>
<thead>
<tr>
<th>PSA* no.</th>
<th>Name</th>
<th>Other reference nos.</th>
<th>Sphere</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>S. kentuckensis</td>
<td>ATCC 12091</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>107</td>
<td>S. parvulus</td>
<td>ATCC 12434</td>
<td>XIV</td>
<td>D</td>
</tr>
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<td>45</td>
<td>S. griseus</td>
<td>ATCC 10137</td>
<td>VII</td>
<td>C</td>
</tr>
<tr>
<td>145</td>
<td>S. parvus</td>
<td>NRRL B 1455</td>
<td>IV</td>
<td>C</td>
</tr>
<tr>
<td>101</td>
<td>S. griseolus</td>
<td>ATCC 3225</td>
<td>XII</td>
<td>C</td>
</tr>
<tr>
<td>108</td>
<td>S. flavescens</td>
<td>ATCC 3219</td>
<td>X</td>
<td>C</td>
</tr>
<tr>
<td>148</td>
<td>S. diastaticus</td>
<td>NRRL B 1270</td>
<td>III</td>
<td>B</td>
</tr>
<tr>
<td>22</td>
<td>S. bobiliae</td>
<td>ATCC 3210</td>
<td>VI</td>
<td>B</td>
</tr>
<tr>
<td>156</td>
<td>S. fradiae</td>
<td>ATCC 10745</td>
<td>IX</td>
<td>B</td>
</tr>
<tr>
<td>96</td>
<td>S. intermedius</td>
<td>ATCC 3229</td>
<td>XVII</td>
<td>B</td>
</tr>
<tr>
<td>60</td>
<td>S. albus</td>
<td>ATCC 618</td>
<td>XV</td>
<td>E</td>
</tr>
<tr>
<td>61</td>
<td>S. fradiae</td>
<td>ATCC 10745</td>
<td>XIII</td>
<td>F</td>
</tr>
<tr>
<td>165</td>
<td>N. asteroides</td>
<td>IMRU 727</td>
<td>XI</td>
<td>E</td>
</tr>
</tbody>
</table>

* 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.


*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, overnight in the cold with agitation. According to Dr M. Mandel (personal communication) 2-mercapto-ethanol, CH,\((OH)CH,SH\), 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 Tₘ value with DNA from Streptomyces bobiliae

<table>
<thead>
<tr>
<th>Soln.</th>
<th>(KH₄PO₄) (mol/l)</th>
<th>(Na₄HPO₄) (mol/l)</th>
<th>EDTA (mol/l)</th>
<th>Diluted to (mol/l)</th>
<th>Molarity (PO₄^{3-}) (mol)</th>
<th>pH value of (PO₄^{3-}) (mol)</th>
<th>Specific conductance (\times 10^{-3}) mho</th>
<th>(Tₘ) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>10</td>
<td>200</td>
<td>0.005</td>
<td>6.7</td>
<td>0.881</td>
<td>75.6</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>10</td>
<td>200</td>
<td>0.01</td>
<td>6.8</td>
<td>1.570</td>
<td>80.5</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>2.5</td>
<td>10</td>
<td>50</td>
<td>0.02</td>
<td>6.7</td>
<td>2.970</td>
<td>85.6</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>2.5</td>
<td>25</td>
<td></td>
<td>0.04</td>
<td>6.7</td>
<td>5.590</td>
<td>90.8</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>0.5</td>
<td>10</td>
<td>100</td>
<td>0.01</td>
<td>5.85</td>
<td>1.250</td>
<td>76.5</td>
</tr>
<tr>
<td>6</td>
<td>3.5</td>
<td>1.5</td>
<td>10</td>
<td>100</td>
<td>0.01</td>
<td>6.40</td>
<td>1.440</td>
<td>79.25</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
<td>3.5</td>
<td>10</td>
<td>100</td>
<td>0.01</td>
<td>7.10</td>
<td>1.720</td>
<td>81.6</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>4.5</td>
<td>10</td>
<td>100</td>
<td>0.01</td>
<td>7.50</td>
<td>1.923</td>
<td>81.8</td>
</tr>
</tbody>
</table>

**Determination of \(Tₘ\) 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. \(Tₘ\) 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 \(Tₘ\) determination in the usual solvent SSC (standard saline citrate; \(0.15\%\) NaCl + \(0.015\%\) Na₂ 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 \(Tₘ\) 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 \(Tₘ\) value within the range pH 5.5–8.5. Two sets of solutions were prepared (Table 2) to determine the effect on \(Tₘ\) 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 \(Tₘ\) 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 \(0.01\%\) \(PO₄^{3-}\), 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:

$$\% \text{ hyperchromicity} = 22.40 - 0.23 \log x + 9.74 (\log x)^2,$$

where $x$ is the specific conductance of the solution.

The solvent finally chosen, as giving $T_m$ values 20° less than the SSC solvent, was: Na$_2$HPO$_4$, 0.2 M, 2.5 ml. + NaH$_2$PO$_4$, 0.2 M, 2.5 ml. + EDTA, 0.01 M, 10 ml.; diluted to 100 ml.; pH 6.8; specific conductance 1.345 x 10$^{-4}$ mho. Under our conditions, the specific conductance of SSC solvent was 16 x 10$^{-4}$ 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, 1968); 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
**DNA base composition of streptomyces**

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>
<thead>
<tr>
<th>Strain</th>
<th>$T_m$ ($^\circ$C)</th>
<th>% GC</th>
<th>% GC from $T_m$</th>
<th>% GC from $\rho$</th>
<th>Difference $2-4$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. kentuckensis</td>
<td>99</td>
<td>80.8</td>
<td>76.8</td>
<td>1.728</td>
<td>69.4</td>
</tr>
<tr>
<td>S. parvulus</td>
<td>107</td>
<td>80.9</td>
<td>77.2</td>
<td>1.730</td>
<td>71.4</td>
</tr>
<tr>
<td>S. griseus</td>
<td>45</td>
<td>80.8</td>
<td>76.8</td>
<td>1.728</td>
<td>71.4</td>
</tr>
<tr>
<td>S. parvus</td>
<td>145</td>
<td>80.25</td>
<td>75.5</td>
<td>1.729</td>
<td>70.4</td>
</tr>
<tr>
<td>S. griseolus</td>
<td>101</td>
<td>80.3</td>
<td>75.6</td>
<td>1.729</td>
<td>70.4</td>
</tr>
<tr>
<td>S. flavescens</td>
<td>108</td>
<td>80.0</td>
<td>74.8</td>
<td>1.729</td>
<td>70.4</td>
</tr>
<tr>
<td>S. diastaticus</td>
<td>148</td>
<td>81.2</td>
<td>77.8</td>
<td>1.729</td>
<td>70.4</td>
</tr>
<tr>
<td>S. bobiline</td>
<td>22</td>
<td>79.8</td>
<td>74.4</td>
<td>1.728</td>
<td>70.4</td>
</tr>
<tr>
<td>S. fradiae</td>
<td>156</td>
<td>81.5</td>
<td>78.5</td>
<td>1.735</td>
<td>73.4</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>96</td>
<td>79.5</td>
<td>73.6</td>
<td>1.728</td>
<td>69.4</td>
</tr>
<tr>
<td>S. albicus</td>
<td>60</td>
<td>79.75</td>
<td>74.3</td>
<td>1.730</td>
<td>71.4</td>
</tr>
<tr>
<td>S. fradiae</td>
<td>61</td>
<td>81.5</td>
<td>78.5</td>
<td>1.731</td>
<td>72.4</td>
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</table>

<table>
<thead>
<tr>
<th>Nocardia</th>
<th></th>
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<tbody>
<tr>
<td>N. asteroides</td>
<td>165</td>
<td>81.0</td>
<td>77.3</td>
<td>1.728</td>
<td>69.4</td>
</tr>
</tbody>
</table>

**Notes.** Col. 1 in 0.01M-PO$_4$ + 0.001M-EDTA (pH 6.8). Col. 2 $T_m = 49.3 + 0.41$(GC). Col. 3 relative to *Escherichia coli* NDA = 1.710 g./cm.$^3$ Col. 4 $\rho = 1.660 + 0.098$ (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, \( \rho \).** 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 \( \text{Na}_2\text{HPO}_4 \) 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, 163) 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.

We are indebted to Professors P. Caldirola and R. Fieschi for allowing us to utilize a spectrophotometer of the solid state laboratory of the Institute of Physics of the University of Milan. The authors are also indebted to Professors P. Polli and L. Di Mayore. Ultracentrifuge runs were carried out by Mr G. Conti.

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REFERENCES