Base Composition of Deoxyribonucleic Acid from *Spirillum volutans*, *S. serpens* and *S. itersonii*

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INTRODUCTION

Spirillum volutans, *S. serpens* and *S. itersonii* have been placed in the same genus largely on the basis of cell physiology (Williams & Rittenberg, 1957). Similarities other than their characteristic shape include the accumulation of poly β -hydroxybutyrate in cytoplasmic granules (Martinez, 1963), the presence of a cytochrome-linked electron-transfer chain, and the absence of *a*-type cytochromes (Cole & Rittenberg, 1971). However, *S. itersonii* differs from the other species in its ability to grow anaerobically with nitrate, and *S. volutans* is uniquely sensitive to oxygen (Williams & Rittenberg, 1957; Wells & Krieger, 1965). Although cell shape has traditionally been a highly weighted character in classification, its importance in relation to DNA base composition was recently challenged by Bøvre & Holten (1970) who suggested that the rod-shaped *Neisseria elongata* should be included in a genus of cocci. Because of the biochemical differences between the three spirilla, their DNA base compositions have been determined. The results suggest that *S. volutans*, *S. itersonii* and *S. serpens* are not closely related.

METHODS

Spirillum volutans (Pringsheim), American Type Culture Collection number (ATCC) 19553, *S. serpens* var. *serpens*, ATCC12683 and *S. itersonii* var. *itersonii*, ATCC12639 were obtained and cultured as described by Cole & Rittenberg (1971). *Escherichia coli* K12 W1485 was obtained from J. W. T. Wimpenny, and *Proteus mirabilis* DNA was a gift from F. Priest.

The DNA isolation procedure was based on the methods of Marmur (1961) and Saito & Muiira (1963). Pellets of frozen bacteria (3 g wet wt) were thawed, suspended in 25 ml 0.5 M-NaCl and 0.1 M-ethylenediamine tetraacetate, pH 8.0, and incubated for 20 min at 37 °C with 2 mg/ml lysozyme. Sodium dodecyl sulphate (0.5 %, w/v) and pronase (50 mg/ml) were added with gentle mixing, and the solution incubated at 37 °C for 16 h. Two volumes of freshly distilled phenol – saturated with 0.015 M-trisodium citrate and 0.15 M-NaCl (SSC) – were added and the flask shaken for 20 min at 0 °C. After centrifuging, the nucleic acids were precipitated from the aqueous layer by adding two volumes of ethanol at 0 °C. The fibrous precipitate was dissolved in SSC and incubated with deoxyribonuclease-free ribonuclease (50 μ g/ml for 30 min at 37 °C). The extraction with phenol was repeated, and the clarified aqueous layer added to 10 vol ethanol at 0 °C. Strands of DNA were spooled on a glass rod, washed successively in 70 %, 80 % and 90 % (v/v) ethanol, redissolved in 0.1 SSC and dialysed exhaustively against 0.1 SSC. The ratio of the extinctions at 257 nm and 280 nm was usually in the range 1.9 to 2.1. Samples with ratios below 1.8 were further purified by precipitating them with 0.7 vol cold isopropanol, and redissolving them in 0.1 SSC.

Table 1. *Thermal denaturation, buoyant density and GC content of DNA from Spirillum volutans, S. serpens and S. itersonii*

Organism	T_m *	% GC calculated from mean T_m	Buoyant density†	% GC calculated from buoyant density‡
<i>Spirillum volutans</i>	67.2; 66.9	32.2	1.697	36.3
<i>S. serpens</i>	73.2; 73.4	47.4	1.709	48.8
<i>S. itersonii</i>	78.1; 78.2	59.3	1.721	61.2

* DNA dissolved in 0.1 SSC at a concentration of 20 µg/ml was contained in 4 ml stoppered quartz cuvettes. The optical density at 260 nm, measured in a Gilford 2000 attachment to a Beckman DU spectrophotometer, and the temperature in the cuvette chamber were recorded at 15 s intervals. The temperature of the circulating water was increased continuously by 0.2 °C/min.

† Duplicates differed only in the fourth decimal figure.

‡ Calculated from the relationship: % GC = 1038.47 ($p - 1.6616$) (De Ley, 1970).

The guanine plus cytosine mole fraction (GC content) was determined from the thermal denaturation profile of DNA dissolved in 0.1 SCC (Marmur & Doty, 1962). The mid-point of the profile (T_m) was estimated and the GC content derived from the equation:

$$\% \text{ GC} = 2.44 (T_m - 53.9) \quad (\text{Mandel \& Marmur, 1968}).$$

Escherichia coli K12 DNA with a T_m of 74.5 °C in 0.1 SSC was included as an internal standard with each batch of determinations.

GC contents derived from T_m measurements were confirmed by determining the buoyant densities of the DNA in CsCl with a Beckman Model E Analytical Ultracentrifuge (Mandel, Schildkraut & Marmur, 1968). *Escherichia coli* K12 DNA (buoyant density 1.710) or *Proteus mirabilis* DNA (buoyant density 1.700) was included in each run as an internal standard.

RESULTS AND DISCUSSION

The GC contents of DNA from three spirilla were calculated from T_m and buoyant density measurements (Table 1). *Spirillum volutans* DNA melted at a much lower temperature than *S. itersonii* DNA, and an intermediate T_m was obtained for *S. serpens* DNA. The more precise buoyant density measurements gave slightly higher GC contents than the T_m measurements, but confirmed the large differences between them (36 %, 49 % and 61 % GC for *S. volutans*, *S. serpens* and *S. itersonii* respectively; Table 1).

Although similarity in GC content does not necessarily indicate genomic similarity, differences are more significant. De Ley (1969) has calculated that DNA samples which differ in GC content by more than 20 % have practically no identical nucleotide sequences. *Spirillum volutans* and *S. itersonii* are therefore probably unrelated. A similar conclusion can be drawn from the GC contents of *S. anum* DNA (28 %; Marmur, Falkow & Mandel, 1963) and *S. serpens* DNA. On the basis of these GC content measurements, it appears that the taxonomy of the genus *Spirillum* requires revision.

I am grateful to Professor S. C. Rittenberg for drawing my attention to the Ph.D. thesis of J. S. Wells (Virginia Polytechnic Institute, Blacksburg, Virginia, U.S.A.) in which T_m determinations for various spirilla are reported. The GC contents calculated from these data are slightly higher than those derived from buoyant density determinations, but confirm the wide range within the genus.

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