DEOXYRIBONUCLEIC ACID BASE COMPOSITION OF
PROTEUS AND PROVIDENCE ORGANISMS

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ABSTRACT

Falkow, Stanley (Walter Reed Army Institute of Research, Washington D.C.), I. R. Ryman, and O. Washington. Deoxyribonucleic acid base composition of Proteus and Providence organisms. J. Bacteriol. 83:1318-1321. 1962.—Deoxyribonucleic acids (DNA) from various species of Proteus and of Providence bacteria have been examined for their guanine + cytosine (GC) content. P. vulgaris, P. mirabilis, and P. rettgeri possess essentially identical mean GC contents of 39%, and Providence DNA has a GC content of 41.5%. In marked contrast, P. morganii DNA was found to contain 50% GC. The base composition of P. morganii is only slightly lower than those observed for representatives of the Escherichia, Shigella, and Salmonella groups. Aerobacter and Serratia differ significantly from the other members of the family by their relatively high GC content. Since a minimal requirement for genetic compatibility among different species appears to be similarity of their DNA base composition, it is suggested that P. morganii is distinct genetically from the other species of Proteus as well as Providence strains. The determination of the DNA base composition of microorganisms is important for its predictive information. This information should prove of considerable value in investigating genetic and taxonomic relationships among bacteria.

Evidence has now been accumulated to support the hypothesis that genetic homology occurs only among organisms which have similar deoxyribonucleic acid (DNA) base compositions (Lanni, 1960; Marmur, Schildkraut, and Doty, 1961). As a corollary to this hypothesis, one should expect that organisms which show extensive phenotypic similarity (such as those employed in bacterial classification) should resemble one another in DNA base composition. The studies of Lee, Wahl, and Barbu (1956), as well as those of Belozersky and Spirin (1960), largely uphold this idea, although several notable exceptions occur. One exception, noted by Belozersky and Spirin (1960), is the wide divergence between the DNA base compositions of Proteus vulgaris and P. morganii. The base composition for the other species of Proteus and for the closely related Providence bacteria (Stuart, Wheeler, and McGann, 1946; Shaw and Clarke, 1955), however, were not determined.

In the present study, we have examined the DNA base composition of members of the genus Proteus and of Providence bacteria. The results are discussed from the viewpoint of the significance of base composition with regard to systematics, phylogeny, and possible genetic relationships.

MATERIALS AND METHODS

Cultures. Ten strains each of P. vulgaris, P. mirabilis, P. rettgeri, P. morganii, and Providence (29911) organisms were obtained from C. A. Stuart, Department of Biology, Brown University, Providence, R.I. Other cultures of Enterobacteriaceae, which will be mentioned, came from the culture collection of this institution.

Isolation of DNA. DNA was isolated from each of the bacterial strains by the method of Marmur (1961), and dissolved in standard saline citrate (SSC; 0.15 m NaCl + 0.015 m Na citrate, pH 7.0). These samples were stored at 5 C over a few drops of chloroform, and were stable.

Determination of DNA base composition. Approximately 20 µg per ml of DNA in glass-stoppered quartz cuvettes were heated in the chamber of a Beckman DU spectrophotometer equipped with thermospacers and the absorbance (corrected for thermal expansion) recorded at 260 µm as a function of temperature (Marmur and Doty, 1959). The mid-point of the absorbance rise, melting temperature (Tm), occurs
within a narrow temperature range and is dependent on the guanine + cytosine (GC) content of the DNA sample (Marmur and Doty, 1959; Marmur et al., 1961). The over-all GC composition of bacterial DNA samples may be simply determined, therefore, by measuring the Tm value. Figure 1 shows the dependence of the Tm on the GC content of various DNA samples. The GC values used in this figure were obtained from the published chemical determinations of Lee et al. (1956) and Belozersky and Spirin (1960).

The Tm value for each of the DNA preparations was determined at least twice and the results averaged. In every case, the Tm could be reproduced for a given sample within 0.4 C. Moreover, the Tm for different DNA preparations of the same culture was similarly reproducible.

RESULTS

The mean Tm values for the Proteus and Providence cultures examined are shown in Table 1. P. vulgaris, P. mirabilis, and P. rettgeri exhibited essentially identical mean Tm values of 85.3 to 85.4 C, corresponding to a GC content of 39%. The standard deviation within these three groups of organisms was on the order of ±0.6 C or ±1.2% GC.

The DNA from Providence strains melted at a temperature of 86.3 C or 41.5% GC. These strains formed a fairly homogenous group, the Tm values all falling within a 0.4 C range. This increased mean GC content is significantly higher (p = 0.001) than that of P. vulgaris, P. mirabilis, and P. rettgeri.

The strains of P. morganii differed markedly in base composition from the other Proteus species examined. The DNA isolated from this organism displayed a mean Tm value of 89.8 C.

**TABLE 1. Deoxyribonucleic acid base composition of Proteus and Providence organisms**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. strains examined</th>
<th>Mean (Tm, C)</th>
<th>Guanine + cytosine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus vulgaris</td>
<td>10</td>
<td>85.4 ± 0.5*</td>
<td>39.3 ± 1.2</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>10</td>
<td>85.4 ± 0.6</td>
<td>39.3 ± 1.4</td>
</tr>
<tr>
<td>P. rettgeri</td>
<td>10</td>
<td>85.2 ± 0.6</td>
<td>39 ± 1.5</td>
</tr>
<tr>
<td>P. morganii</td>
<td>10</td>
<td>89.8 ± 0.3</td>
<td>50 ± 0.7</td>
</tr>
<tr>
<td>Providence</td>
<td>10</td>
<td>86.3 ± 0.2</td>
<td>41.5 ± 0.6</td>
</tr>
</tbody>
</table>

* Standard deviation.

**FIG. 2. Absorbance-temperature denaturation profiles of representative Proteus and Providence deoxyribonucleic acid (DNA) samples.** The DNA, in 0.15 M NaCl + 0.015 M Na citrate, is heated in the chamber of a Beckman DU spectrophotometer, and the absorbance (corrected for thermal expansion) relative to 25 C is plotted against temperature.
TABLE 2. Deoxyribonucleic acid base composition of representative members of the Enterobacteriaceae

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains examined</th>
<th>Mean (Tm, °C)</th>
<th>Guanine + cytosine %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>5</td>
<td>90.0</td>
<td>50.5</td>
</tr>
<tr>
<td>Salmonella</td>
<td>10</td>
<td>90.2</td>
<td>51</td>
</tr>
<tr>
<td>Shigella</td>
<td>4</td>
<td>90.0</td>
<td>50.5</td>
</tr>
<tr>
<td>Bethesda-Ballerup</td>
<td>3</td>
<td>90.1</td>
<td>50.7</td>
</tr>
<tr>
<td>Arizona</td>
<td>2</td>
<td>90.2</td>
<td>51</td>
</tr>
<tr>
<td>Aerobacter aerogenes</td>
<td>3</td>
<td>93.5</td>
<td>57.8</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>3</td>
<td>93.6</td>
<td>58</td>
</tr>
</tbody>
</table>

which corresponds to 50% GC. Absorbance-temperature profiles of representative _Proteus_ and Providence strains are shown in Fig. 2.

A comparison with the Tm values of representative members of the _Enterobacteriaceae_ shows that _P. morganii_ has a base composition only slightly lower than those observed for the _Escherichia-Shigella-Salmonella_ groups (Table 2). _Aerobacter aerogenes_, _Serratia marcescens_, and related organisms differed significantly from the other members of the family by their relatively high (55%) GC content. At the other extreme, the other species of _Proteus_, as well as Providence, with their low GC content, appeared as the exceptions to a family of organisms otherwise characterized by equimolar (50%) or expressed GC-type DNA.

**DISCUSSION**

The proposition that genetic homology is related to equality of DNA base composition does not imply that all organisms with similar base compositions will exhibit genetic compatibility. Similarity of composition is, however, a minimal requirement. In this respect, _P. vulgaris_, _P. mirabilis_, _P. rettgeri_, and possibly Providence organisms fulfill this minimal requirement; _P. morganii_ does not. Density gradient ultracentrifugation studies (Sueoka, Marmur, and Doty, 1959) have demonstrated that the distribution of molecular composition about the mean base composition values is relatively small compared with the range of mean values found in bacteria (30 to 78%). Thus, for pairs of bacteria having substantially different mean DNA base compositions, it can be said that they will have few DNA molecules in common. On this basis, _P. morganii_ would exhibit essentially no DNA molecules of similar composition in common with the other _Proteus_ species and Providence. Providence DNA would show considerable overlapping with that of _P. vulgaris_ and _P. rettgeri_, but should contain some DNA molecules which do not overlap.

Unfortunately, there is little information available on the genetics of the _Proteus_ group to test our data. Coetzee and Sacks (1960) demonstrated that many bacteriophages lyse _P. mirabilis_ and _P. vulgaris_ with equal efficiency, although bacteriophage-mediated genetic exchange has been successful only among strains of _P. mirabilis_. Along similar lines, Vieu (1956) has reported that phages have been isolated which lyse _P. vulgaris_, _P. mirabilis_, and an occasional strain of _P. rettgeri_, but not _P. morganii_ or Providence. Although genetic transfer mechanisms are, thus far, unavailable in _Proteus_, the technique of molecular hybridization (Marmur et al., 1961; Schildkraut, Marmur, and Doty, 1961) should offer a rational approach to the study of homologies between the DNA of _Proteus_ species as well as Providence.

There is certainly sufficient diversity among bacteria to permit a fairly complex system of classification in which definite orders and families may be established. At lower taxonomic levels, especially the species level, distinct diversity falls off and overlapping appears. It is at these lower taxonomic levels that the determination of DNA base composition would seem to be of most value. It should be clear that base composition has considerable utility, not only as an indicator of genetic homology, but also in helping to extend the area over which correlations might be tested and perhaps in estimating the degree of evolutionary diversity within a defined taxonomic group. Therefore, the determination of DNA base composition is important for its predictive information.

The similarity of base composition between _P. morganii_ and members of other genera could indicate previously unrecognized homologies. On the other hand, it is possible that this equality of composition is merely a coincidence of evolution. It should be noted that compositional heterogeneity within a genus is not restricted to _Proteus_. Marmur et al. (1961) have reported that the GC content of members of the genus _Bacillus_ range from 33 to 50.5% GC. Moreover, Catlin and Cunningham (1961) have demonstrated that _Neisseria catarrhalis_ (40% GC)
differs markedly in base composition from the other species of Neisseria (50% GC). Although there has been a report (Belozersky and Spirin, 1960) of several compositional deviations via mutation from organisms initially possessing equimolar DNA to lower (42% GC) and higher (65 % GC) values, the distribution of GC content of the DNA of an organism is unimodal, narrow in range, and apparently a stable characteristic (Sueoka, 1961). Since there is no general agreement concerning the scope of a bacterial genus, the phylogenetic significance of compositional diversity within Proteus (and other genera) is not clear.

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LITERATURE CITED


