Deoxyribonucleic Acid Base Composition of *Rothia dentocariosa* as Determined by Thermal Denaturation

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The moles per cent guanine plus cytosine (GC) of 10 filamentous strains of *Rothia dentocariosa* ranged from 65.4 to 69.7. Major differences were not observed in the base composition of a filamentous form (69.7 moles% GC) and its coccal variant (68.0 moles% GC).

The taxonomic position of an actinomycete previously classified as *Actinomyces dentocariosus*, *Nocardia dentocariosa*, and *N. salivae* was recently clarified when Georg and Brown (5) reviewed their biochemical characteristics, placed these organisms in synonymy, and created the new genus, *Rothia*. *R. dentocariosa*, the single species in the genus, has recently been the focus of considerable attention because of its possible role in oral disease (10). This organism appears to be unique among the actinomycetes because of its bizarre morphological variations (1) and distinctive serological properties (6). However, no information is available on the deoxyribonucleic acid (DNA) base composition of this organism and how its DNA compares with those of other actinomycetes. It was of interest to determine whether differences in DNA base composition accompany the morphological transformation of a single strain from the filamentous phase to the coccal phase. This report describes the DNA base composition of several filamentous strains and one coccal form of *R. dentocariosa*.

Cultures of *R. dentocariosa* were obtained from the American Type Culture Collection; L. K. Georg, Center for Disease Control; H. V. Jordan, Forsyth Dental Center; and from the stock culture collection of the Department of Microbiology, University of Pennsylvania School of Dental Medicine. The coccal variant of *R. dentocariosa* ATCC 17931 was obtained by subculturing a fecal sample of a gnotobiotic Sprague-Dawley 87-day-old rat monocontaminated for 60 days with the typical filamentous form. The subculture was provided by S. S. Socransky of the Forsyth Dental Center. All strains were grown for 48 hr in Trypticase Soy Broth (BBL), and the cells were harvested by centrifugation, washed in saline ethylenediaminetetraacetate, and extracted by using the procedures of Marmur (8). The most reproducible results were obtained when the washed cells were pretreated for 1 hr with 0.5 M 2-mercaptoethanol before exposure to lysozyme and sodium lauryl sulfate. Melting temperature (*T*<sub>m</sub>) values of the purified DNA extracts were determined with a Gilford (model 2400) automatic spectrophotometer by using the procedures of Marmur and Doty (9). All DNA samples were dissolved in the phosphate (0.01 M)-ethylenediaminetetraaceta (0.01 M) buffer as recommended by Frontali, Hill, and Silvestri (4) in cases where denaturation temperatures exceed 100 C. The *T*<sub>m</sub> values obtained in phosphate buffer were corrected by adding 20.1 to give a comparable *T*<sub>m</sub> value in standard saline-citrate buffer. Moles per cent guanine plus cytosine (GC) was calculated by using the equation of Marmur and Doty (9).

The *T*<sub>m</sub> values in Table 1 represent an average of at least two different determinations and were reproducible for a given sample within 0.2 C. All DNA samples gave monophasic absorbance-temperature denaturation profiles. A comparison of the GC values of the 11 strains of *R. dentocariosa* listed in Table 1 reveals a reasonably homogeneous group. All of the strains, including the coccal variant, had a GC content between 65.4 and 69.7, with a mean of 66.5 moles % GC and a standard deviation of 0.77 %. Duplicate *T*<sub>m</sub> determinations made on different DNA samples from the same strains showed some variation, but in no instance was the difference in *T*<sub>m</sub> greater than 0.5 C (less than 1% change in GC content). It therefore seemed likely that the differences in GC ratios listed in Table 1 are probably not ascribable to experi-
dentocariosa strains.
form chemically composition. All of homogeneity come (filamentous); 4, It
tained rabbit C, coccal (Georg); (2) H. V.
R. dentocariosa RC44 (filamentous); 4, (1) Communicable Disease Center Collection
Rothia R. dentocariosa ATCC 17931
R. dentocariosa X-303 (filamentous). (1)..
R. dentocariosa X-346 (filamentous). (1).
R. dentocariosa RC27 (filamentous). (1).
R. dentocariosa RC29 (filamentous). (2).
R. dentocariosa RC30 (filamentous). (2).
R. dentocariosa RC37 (filamentous). (2).
R. dentocariosa RC44 (filamentous). (2).
R. dentocariosa H69 (filamentous). (2).

* (1) Communicable Disease Center Collection (Georg); (2) H. V. Jordan cultures; (3) University of Pennsylvania.

Table 1. Moles per cent guanine plus cytosine content (mole% GC) and thermal denaturation values of R. dentocariosa strains

<table>
<thead>
<tr>
<th>Organism and origin*</th>
<th>Tm</th>
<th>Moles% GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rothia dentocariosa ATCC 17931 (filamentous)</td>
<td>77.8 ± 0.5</td>
<td>69.7</td>
</tr>
<tr>
<td>R. dentocariosa 17931 (coccal)</td>
<td>77.1 ± 0.3</td>
<td>68.0</td>
</tr>
<tr>
<td>R. dentocariosa X-614a (1)</td>
<td>76.1 ± 0.2</td>
<td>65.6</td>
</tr>
<tr>
<td>R. dentocariosa X-303 (1)</td>
<td>76.2 ± 0.3</td>
<td>65.7</td>
</tr>
<tr>
<td>R. dentocariosa X-346 (1)</td>
<td>76.1 ± 0.2</td>
<td>65.6</td>
</tr>
<tr>
<td>R. dentocariosa RC27 (2)</td>
<td>77.1 ± 0.4</td>
<td>68.1</td>
</tr>
<tr>
<td>R. dentocariosa RC29 (2)</td>
<td>76.0 ± 0.2</td>
<td>65.4</td>
</tr>
<tr>
<td>R. dentocariosa RC30 (2)</td>
<td>76.3 ± 0.2</td>
<td>66.1</td>
</tr>
<tr>
<td>R. dentocariosa RC37 (2)</td>
<td>76.1 ± 0.4</td>
<td>65.6</td>
</tr>
<tr>
<td>R. dentocariosa RC44 (2)</td>
<td>76.1 ± 0.2</td>
<td>65.6</td>
</tr>
<tr>
<td>R. dentocariosa H69 (3)</td>
<td>76.2 ± 0.3</td>
<td>65.7</td>
</tr>
</tbody>
</table>

It is interesting to note that these strains had come from diverse origins, having been maintained on different media, in different laboratories, and probably under other slightly different conditions and yet, with the exception of the coccal form, they all showed a reasonable degree of uniformity in DNA base composition and other properties.

A repeatable difference of less than 2% in GC content is not considered of taxonomic significance among bacteria (7); hence, it is concluded that the coccal and filamentous forms are probably in the same taxonomic group. It will be of interest to see whether the similarity in the DNA base composition of the coccal and filamentous forms is reflected in their respective biochemical and serological profiles. Preliminary data on cell wall analysis revealed minor quantitative differences in amino acid and amino sugar content of these two forms. However, the coccal and filamentous forms were serologically related as determined by Ouchterlony immunodiffusion techniques (6); a reaction of identity was observed for at least one soluble antigen detected in sonic extracts of both forms (Fig. 1). Similarly, the fructose-containing antigen unique to R. dentocariosa (filamentous) cell wall was also detected in the coccal form by fluorescent antibody techniques as described previously (6).

The similarity of the Rothia GC values to the GC values of other actinomycetes is also noteworthy and give further support to the inclusion of Rothia in the Actinomycetaceae. Most of the other genera that have been tested, including Actinoplanes, Micromonospora, Streptomyces, Nocardia, and Actinomyces, all cluster in this same general vicinity of 65 to 70 mole % GC (2, 3). Of course, the similarity in GC values is only indirect evidence of genetic similarity and less meaningful than the similarities observed in hybridization studies measuring DNA homology.

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LITERATURE CITED


