Guanine-Plus-Cytosine Content of *Rothia dentocariosa*

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The guanine-plus-cytosine contents of *Rothia dentocariosa* strains ATCC 17931^T (T = type strain) and ATCC 19426 were determined by thermal denaturation and found to be 54 and 57 mol%, respectively. These values are lower than the values given in many publications, but are consistent with data cited in some other references.

The guanine-plus-cytosine (G+C) content of *Rothia dentocariosa* was reported by Hammond (4) to be 65.4 to 69.7 mol%, and these values are included in the description of the species in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (1). These base contents are given in later publications which cite Hammond directly (8) or indirectly (2, 3). However, Slack and Gerencser (10) published lower figures, 47 to 53 mol% G+C, for five strains and cited unpublished data from Hammond. These data were cited by Schaal and Pulverer (9).

To resolve this discrepancy, we determined the G+C contents of two American Type Culture Collection strains of *R. dentocariosa* and one clinical isolate. Strains ATCC 17931^T (T = type strain) and ATCC 19426 were obtained from the American Type Culture Collection and were grown on casein soy agar supplemented with 10% sheep blood and in brain heart infusion broth. Small broth cultures were used to inoculate 100-ml portions of brain heart infusion broth, which were then used to start 1-liter cultures. All cultures were tested for purity by streaking onto blood agar plates and by Gram staining. The characteristic large white colonies and pleomorphic cells were observed.

The growth of 1-liter cultures was monitored turbidimetrically for about 26 h. Then 10,000 U of penicillin G (10 µg/ml) was added, and the cultures were incubated for an additional 1 h and harvested. The cells were suspended in 15 ml of 0.015 M NaCl and incubated with 10 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.) for 1 h at 37°C and then with 200 U of mutanolysin (Sigma) at 55°C for 1 h. The suspension was made 0.15 M NaCl-0.0015 M sodium citrate and heated to 60°C, and the cells were lysed by adding 1 ml of 25% (wt/vol) sodium dodecyl sulfate. The deoxyribonucleic acid (DNA) was purified by phenol and chloroform deproteinizations, treatment with ribonuclease, and precipitation with ethanol (6). The yields of DNA from these slowly growing, lysis-resistant bacteria were small. About 0.5 mg was isolated from each 1-liter culture. The values for absorbance of ultraviolet light by this DNA at 260, 230, and 280 nm had a ratio of 1:0.56:0.57, indicating that there was some residual carbohydrate but very little protein (6).

The G+C contents were determined by thermal denaturation in 0.15 M NaCl-0.015 M sodium citrate, using the method and formula of Marmur and Doty (7). The temperature was raised 0.5°C/min by an automatic cuvette heater (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) while the absorbance was continually recorded. The system was calibrated by simultaneous denaturation of DNA from *Escherichia coli* strain K-12 (51.5 mol% G+C [5]). The tempera-

ture of the cuvette chamber was also calibrated by using a thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) whose probe was inserted into the chamber. These measurements showed that the *E. coli* DNA denatured at 90.4°C (51.5 mol% G+C) when the cuvette heater registered about 91.5°C (54.1 mol% G+C). Repeated measurements with *E. coli* DNA indicated an error of -1.07°C, and all results were corrected by -2.6 mol% G+C.

The G+C content of *R. dentocariosa* ATCC 17931^T was 54.1 (mol% range for three determinations, 53.7 to 54.7 mol%; standard deviation, ±0.43 mol%), and the value for *R. dentocariosa* ATCC 19426 was 56.6 mol% (range for three determinations, 55.8 to 57.11 mol%; standard deviation, ±0.57 mol%). These values are only about 3 mol% higher than those published by Slack and Gerencser (10) (51 mol% G+C for strain ATCC 17931^T) and are far below the values in an earlier publication (4) (69.7 mol% G+C for the filamentous form of strain ATCC 17931^T). The value for the clinical isolate was 52.9 mol% (range for four determinations, 52.0 to 53.3 mol%; standard deviation, ±0.31 mol%).

Our results show that *R. dentocariosa* G+C contents are lower than the values found in many publications (2-4) and that the lower values that have been recorded previously (9, 10) are credible. We believe that the data presented here should be included in future descriptions of this species.

(Details concerning the clinical isolate will be published elsewhere [M. A. Gerber et al., *Lancet*, in press]. The G+C contents in that report do not reflect the correction factor reported here.)

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