Guanine-plus-Cytosine Content and Production of Phenylacetic Acid by *Bacteroides putredinis* (Weinberg et al., 1937) Kelly (1957) and *Bacteroides splanchnicus* Werner et al. (1975)

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The guanine-plus-cytosine contents of *Bacteroides putredinis* and *Bacteroides splanchnicus* deoxyribonucleic acids were determined after the deoxyribonucleic acids were purified by gel chromatography. In addition, both species were tested for the production of phenylacetic acid. The guanine-plus-cytosine content of *B. putredinis* deoxyribonucleic acid was 50 to 52 mol%, and that of *B. splanchnicus* deoxyribonucleic acid was 38 to 40 mol%. Both species produced phenylacetic acid.

Bacteroides putredinis and *Bacteroides splanchnicus* are butyrate-producing *Bacteroides* species that are on the Approved Lists of Bacterial Names (10). However, the guanine-plus-cytosine (G+C) content of neither *B. putredinis* deoxyribonucleic acid (DNA) nor *B. splanchnicus* DNA has been reported previously.

Several workers have shown that phenylacetic acid is produced from tyrosine in certain *Bacteroides* species (9, 11). For example, phenylacetic acid is formed by *Bacteroides gingivalis* (5), but the phenotypically similar species *Bacteroides asaccharolyticus* is negative for phenylacetic acid production. Other butyrate-producing species, including *B. putredinis* and *B. splanchnicus*, have not been investigated previously for phenylacetic acid production.

MATERIALS AND METHODS

The type strain (ATCC 29800^T) and strains S27-35 and 47-79 (both clinical isolates) of *B. putredinis* and the type strain (NCTC 10825^T), strain K1-2 (from feces), and strain S2-34 (a clinical isolate) of *B. splanchnicus* were included in this study.

Strains were cultured and identified by the methods of Werner and Rintelen (14) and Holdeman et al. (4). The acid end products from PYG and PY fermentation broths were determined by gas chromatography. Volatile acids were extracted in diethyl ether after acidification, and nonvolatile acids were detected after methylation (4). The gas chromatographic methods which we used have been described elsewhere (3).

DNA was extracted and purified as follows. A 2- or 3-g (wet weight) sample of cells from a 48-h culture in PYG was washed in saline ethylenediaminetetraacetate (8), lysed, and purified to the first deproteinization step of the procedure of Marmur (8) with chloroform-

isoamyl alcohol. The DNA was separated by a brief centrifugation (5 min, 5,000 × g), and then precipitated with 2 volumes of ethanol and removed by gently spooling on a glass rod. The precipitated DNA was dissolved in 0.1 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Further purification was accomplished by adding 50 µg of ribonuclease (Sigma, Munich, Federal Republic of Germany) per ml, followed by incubation for 30 min at 37°C. Pronase (10 µg/ml; Serva, Heidelberg, Federal Republic of Germany) was added, and the mixture was incubated for 2 h at 37°C. A 2-ml portion of the resulting DNA solution was applied directly to a column (0.9 by 60 cm) of Sepharose 4B (Pharmacia, Uppsala, Sweden). The components were eluted with 0.1 × SSC at a flow rate of about 0.15 ml/min; 50-drop fractions were collected, and the first two fractions of the first elution peak (measured at 260 nm) were collected and used for further G+C determinations. The principle of this purification method has been discussed by Gibson and Ogden (2) and Zdražil et al. (16).

G+C contents were estimated by the thermal melting point method (7) in 0.1 × SSC. Measurements were performed with a Zeiss model DMR 11 double-beam spectrophotometer equipped with thermostatable cuvette holders at 260 nm. Cuvettes were heated with a Thermomix model 1460 constant-temperature circulator (Braun, Melsungen, Federal Republic of Germany). Temperatures were checked with an equilibrated thermometer. Measurements were done twice with different preparations, and several *Bacteroides* strains whose G+C contents were known were included for reference purposes. The G+C content was calculated from the following formula: $G+C = (T_m - 53.9) \times 2.44$, where T_m was the thermal melting point.

RESULTS

The morphological and physiological properties of all six strains studied were in accordance

FIG. 1. Gas chromatograms of chloroform extracts of methylated PYG broth cultures of *B. putredinis* 47-79 (A) and *B. splanchnicus* NCTC 10825^T (B), showing the nonvolatile acids produced. S, succinic acid; Pha, phenylacetic acid. Traces of lactic acid (L) also were found. t, Time.

with the results described for the type strains (1, 13, 15). In addition to acetic, propionic, isobutyric, butyric, isovaleric, and succinic acids, all strains of *B. splanchnicus* and *B. putredinis* produced considerable amounts of phenylacetic acid (Fig. 1).

The G+C contents of *B. splanchnicus* strains NCTC 10825^T, K1-2, and S2-34 were 38.3, 39.8, and 39.0 mol%, respectively. The G+C contents of *B. putredinis* strains ATCC 29800^T, S27-35, and 47-79 were 50.5, 51.5 and 51.0 mol%, respectively.

DISCUSSION

The DNA purification procedure described above seems to be a useful tool for quick and reliable DNA purification, especially for bacteria with a low DNA yield. With this method we were able to obtain reproducible results in determinations of the DNA base contents of many strains of bacteria, including *B. putredinis* and *B. splanchnicus*.

Strains of *B. asaccharolyticus* and *B. putredinis* can be differentiated by phenylacetic acid production. Although these two species have similar G+C contents, only *B. putredinis* produces phenylacetic acid.

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