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Production of Phenylacetic Acid by Strains of *Bacteroides asaccharolyticus* and *Bacteroides gingivalis* (sp. nov.)

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Strains of *Bacteroides asaccharolyticus* and *Bacteroides melaninogenicus* subspecies isolated from human and animal sources were examined for the production of phenylacetic acid. *B. asaccharolyticus* strains isolated from oral sites in humans and monkeys always produced phenylacetic acid. *B. asaccharolyticus* strains isolated from human nonoral sites consistently failed to produce this product. This metabolic difference correlates with the genetic dichotomy recently found to exist between oral and nonoral *B. asaccharolyticus* strains.

Recent studies have implicated *Bacteroides asaccharolyticus* and *Bacteroides melaninogenicus* in the periodontal disease process (9, 10). Of particular interest is *B. asaccharolyticus*, which is usually found in deep periodontal pockets and may comprise, when present, as much as 67% of the total flora at this site (10).

We have found that oral *B. asaccharolyticus* strains are distinctly different from nonoral isolates of this species. Oral strains have much lower deoxyribonucleic acid (DNA) base contents (46.5 to 48.5 mol% versus 51 to 52 mol% guanine plus cytosine), and their DNAs do not form hybrid duplexes with DNA of nonoral *B. asaccharolyticus*. We consider the oral strains to represent a separate species called *Bacteroides gingivalis* (A. L. Coykendall, F. S. Kaczmarek, and J. Slots, Int. J. Syst. Bacteriol., in press). Because these two species are phenotypically so similar, we and others have sought simple methods to differentiate them. Slots and Genco found that the oral isolates agglutinate sheep erythrocytes whereas nonoral isolates do not (7). Reed et al. (M. J. Reed, J. Slots, C. Mouton, and R. J. Genco, manuscript in preparation) have found that the oral strains do not react with a commercial *B. asaccharolyticus* fluorescent-antibody preparation (Fluoretec-M, Pfizer Diagnostics Division, New York). Mayrand (5) has reported that all the strains of *B. asaccharolyticus* from humans that were tested produced an unusual nonvolatile organic acid, phenylacetic acid, whereas *B. melaninogenicus* subsp. *intermedius* and *B. melaninogenicus* subsp. *melaninogenicus* did not. We have found that oral *B. asaccharolyticus* (*B. gingivalis*) produces phenylacetic acid, but nonoral strains ("true" *B. asaccharolyticus*) do not.

The strains used in this study were isolated from human and other animal (monkey and dog) sources (see Table 1). Although they are genetically diverse, they all have the phenotypic characteristics of *B. asaccharolyticus* (1). Some monkey strains were catalase positive and were among those described by Slots and Genco (8) and called *Bacteroides melaninogenicus* subsp. *macacae*. They are molecularly distinct from both *B. asaccharolyticus* and *B. gingivalis* as determined by DNA analysis. Most canine isolates are also unrelated to these two species. All the isolates tested were grown in a peptone-yeast-glucose broth as outlined by Shah et al. (6) but without the horse serum. Tubes containing the broth were inoculated with cells grown on peptone-yeast-glucose agar supplemented with 5% defribinated sheep erythrocytes. The cultures were then incubated for 48 h in a plastic anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.) under an atmosphere of 5% CO_2, 10% H_2, and 85% N_2.

Methyl derivatives of nonvolatile acid standards, including phenylacetic acid (Sigma Chemical Co., St. Louis, Mo.), and of the culture material were prepared by the methods of Holdeman and Moore (2). The derivatives were analyzed with a Capco 700 gas chromatograph (Clinical Analysis Products Co., Sunnyvale, Calif.). The column was 6 ft by ¼ in. (ca. 1.82 m by 6.3 mm), packed with SP-1000 and 1% phosphoric acid, and supported by Chromosorb 100/120 mesh. Injector, column, and detection blocks were held at 139°C. The carrier gas, helium, had a flow rate of 120 ml/min. The detector current was set at −95 mA and the attenuator was at 1. The analysis time was 12 min. The retention of phenylacetic acid was about 9.75 min.

The amount of phenylacetic acid produced in cultures was estimated by comparing the area under the appropriate peak to the area under the peak produced by the phenylacetic acid stan-

TABLE 1. *Strains of black-pigmenting Bacteroides species tested for production of phenylacetic acid*

Species	No. of strains	Source	Catalase	mol% G+C[a]	Phenylacetic acid
B. gingivalis	6	Human mouth		46.5–48.5	+
	1	Dog gingival sulcus		48.2	+
	1	Human nonoral infection		48.0	+
	1	Monkey gingival sulcus		47.5	+
B. asaccharolyticus	13	Human infections		50–53	–
	1	Dog mouth		51.6	–
B. melaninogenicus subsp. *macacae*	3	Monkey gingival sulcus	+	44–45	+
Resembling *B. asaccharolyticus*[b]	3	Dog mouths	–	41–43	–
	2	Dog gingival sulcus	+[c]		+
B. melaninogenicus subsp. *intermedius*	15	Various sources			–
B. melaninogenicus subsp. *melaninogenicus*	1				–

[a] Moles percent guanine plus cytosine.
[b] Strains that resemble *B. asaccharolyticus* but are catalase positive or have unusual base contents.
[c] Not related to *B. gingivalis* or *B. asaccharolyticus* by DNA hybridization.

dard, the concentration of which was 1 mg/ml.

We determined the base composition of the DNA (moles percent guanine plus cytosine) by observing its thermal denaturation of 0.15 M NaCl–0.015 M sodium citrate (4) in a Gilford model 2527 programmable cuvette heater; absorbance was recorded by a Gilford model 6051 recorder. DNA was extracted and purified according to the procedure developed by Marmur (3). A minimum of three determinations were made for each DNA.

The production of phenylacetic acid by the various strains of *B. asaccharolyticus* tested is shown in Table 1. All human oral isolates (*B. gingivalis*) tested produced phenylacetic acid, as found by Mayrand (5). The amounts ranged from 150 to 450 μg/ml and varied among strains and over time. The nonoral strains consistently failed to produce phenylacetic acid, even upon prolonged incubation of 7 days. Addition of up to 1 mg of phenylalanine per ml to the medium neither enhanced phenylacetic acid production by oral strains (*B. gingivalis*) nor elicited phenylacetic acid production by the nonoral (*B. asaccharolyticus*) strains. (It is possible that these organisms do not take up the free amino acids [11]). The presence or absence of glucose in the medium had no effect. Oral *B. asaccharolyticus* strains from monkey subgingival plaque (*B. melaninogenicus* subsp. *macacae*) also produced phenylacetic acid. Among the canine oral strains, only the catalase-positive strains produced phenylacetic acid; the catalase-negative strains, with the exception noted below, did not. Analysis of DNA indicated that these canine strains are not related to either *B. gingivalis* or true *B. asaccharolyticus*. All *B. melaninogenicus* subsp. *intermedius* strains, and the one strain of *B. melaninogenicus* subsp. *melaninogenicus* tested, failed to produce phenylacetic acid regardless of the source of the isolate.

Initially three strains did not fit the overall pattern of phenylacetic acid production. Strain 754.1, isolated from canine subgingival plaque, was catalase negative but produced phenylacetic acid. However, by DNA hybridization, this strain proved to be a strain of *B. gingivalis*. Strain 612 (derived from *B. asaccharolyticus* NCTC 9337) was originally isolated from a nonoral source (infected hemorrhoids) but unexpectedly produced phenylacetic acid. Determination of the base content of this strain's DNA indicated that it was a strain of *B. gingivalis* with 48.0 mol% guanine plus cytosine. A catalase-negative strain isolated from a monkey also proved to be *B. gingivalis* (47.7 mol% guanine plus cytosine) and produced phenylacetic acid.

Thus the only strains that produced phenylacetic acid were *B. gingivalis* and the catalase-positive strains. The latter have not been found in humans and, of course, can be distinguished by the catalase test.

B. gingivalis appears to prefer the mouth for its habitat and therefore should be suspected when a black-pigmenting *Bacteroides* species is isolated from infections involving the face or mouth, and in cases of bite wounds. Our results indicate that this species can be distinguished

easily from *B. asaccharolyticus* by its production of phenylacetic acid.

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