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Differentiation of *Bacteroides fragilis* Species by Gas Chromatographic Detection of Phenylacetic Acid

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Of 382 strains tested, 261 strains of the species *Bacteroides fragilis, B. thetaiotaomicron, B. ovatus,* and *B. distasonis* produced phenylacetic acid; the remaining strains, belonging exclusively to the species *B. vulgatus,* failed to do so. This differentiation characteristic may be useful in routine clinical bacteriology.

Analysis of fermentation products in culture media is very useful in the taxonomy of certain anaerobic bacteria (5). On the other hand, species differentiation within a genus is based mostly on biochemical reactions (6) because the same short-chain organic acids may be produced by several strains within a genus.

During the course of studying the fermentation patterns of hundreds of saccharolytic bacteroides strains, a nonidentified metabolic product appeared useful for the separation of *Bacteroides vulgatus* from *B. fragilis, B. thetaiotaomicron, B. distasonis,* and *B. ovatus.* Identification of this unknown eluant was performed by gas-liquid chromatography-mass spectrometry. The apparatus consisted of a Pye Unicam gas chromatograph 104 equipped with an SE 30 column (1.5% SE 30; 1.5 m; carrier gas, helium) and connected to an AEI MS 20 mass spectrometer. Mass spectral data of the metabolic product indicated that it was phenylacetic acid. The mass spectrum (electronic mass with relative abundance in parentheses) was: 150(25), 91(100), 65(12), 59(10), 51(5), 40(12), 39(10), 38(5), 36(17). As far as we know, this characteristic product has never been reported or identified in chemotaxonomic studies.

Representative strains from the established species of the *Bacteroides fragilis* group (2) were used in this study. These strains included the type or neotype strains of each of the species studied: *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29184, *B. distasonis* ATCC 8503, *B. ovatus* ATCC 8483, *B. vulgatus* ATCC 8482, 40 strains donated by the collections of other investigators, and 337 strains isolated from fecal material of piglets.

Confirmation of the identities of the bacteroides species was performed in our laboratory by using the procedures of Holdeman and Moore (5) as a guide. Tests were performed in peptone-yeast extract PY, PY-glucose, and Rosenow (1) culture media.

Methyl derivates of nonvolatile short-chain organic acids and phenylacetic acid (Sigma Chemical Co.) were analyzed by methods described in the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (5) on a Hewlett-Packard model 5700A dual-column gas chromatograph equipped with flame ionization detectors. Samples were separated on a coiled stainless-steel column (1.8 m by 0.3-cm OD) packed with 10% Carbowax 20 M-terephtalic acid on 80- to 100-mesh Chromosorb W (AW-DMCS; Applied Science Laboratories, State College, Pa.). Nitrogen was used as carrier gas at a flow rate of 30 ml/min. Temperature conditions were: injection and detector blocks, 200°C; oven isothermal, 135°C. Detector sensitivity was set at 10 × 4. Chromatograms were drawn by a Hewlett-Packard 3380 A integrator (5.0 mm/min).

Table 1 shows that, of 382 bacteroides strains tested, all except the 121 *B. vulgatus* strains produced phenylacetic acid. The presence of this taxonomically useful acid in culture media has not been reported previously. Although there was a larger amount of the aromatic acid in cultures incubated for 7 days, it was possible to detect it chromatographically in 24-h cultures

TABLE 1. *Phenylacetic acid production of saccharolytic Bacteroides species*

Species	No. of strains tested	No. of strains producing phenylacetic acid
B. fragilis	198	198
B. thetaiotaomicron	41	41
B. distasonis	14	14
B. ovatus	8	8
B. vulgatus	121	0

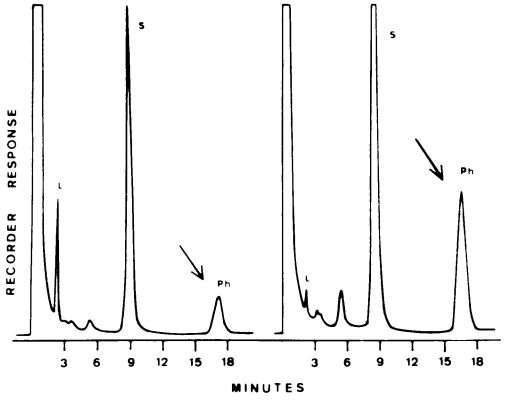

FIG. 1. *Chromatograms of chloroform extracts of methylated PY broth cultures of B. thetaiotaomicron; 24-h culture (left) and 1-week culture (right). L, Lactic acid; S, succinic acid; Ph, Phenylacetic acid.*

(Fig. 1). Analysis of phenylacetic acid in culture media with and without glucose (PY and PY-glucose) gave similar results; however, larger quantities were produced at a faster rate in the absence of this monosaccharide. On the other hand, no trace of phenylacetic acid could be detected in cultures of *B. vulgatus* (Fig. 2).

Because *B. fragilis* and *B. thetaiotaomicron*, which are two of the most commonly gram-negative bacilli involved in infection (3, 4), produce phenylacetic acid and *B. vulgatus*, a predominant isolate from feces (7), does not, the detection of phenylacetic acid may be useful in clinical bacteriology. Possibly, this sensitive gas chromatographic analysis will also provide a helpful tool for differentiation of other bacteria.

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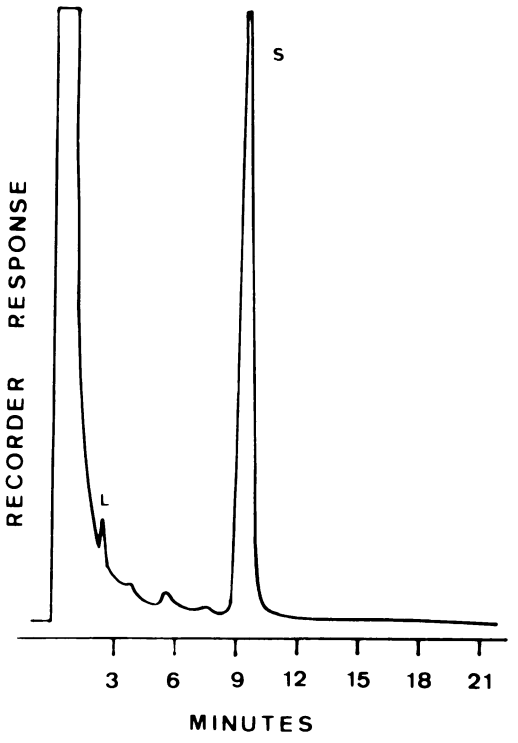

FIG. 2. *Chromatogram of chloroform extract of methylated PY broth culture of B. vulgatus incubated for 1 week. L, Lactic acid; S, succinic acid.*