Identification of *Bacteroides* Species by Cellular Fatty Acid Profiles

WILLIAM R. MAYBERRY, DWIGHT W. LAMBE, JR., AND KAETHE P. FERGUSON

Department of Microbiology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, Tennessee 37614

The total acid hydrolysates of 160 strains representing 12 species and subspecies of *Bacteroides* were analyzed for cellular fatty acids by capillary gas-liquid chromatography. Fatty acid profiles were analyzed mathematically to generate ratios among selected components. Certain of these ratios appeared to be characteristic, permitting separation of the species and subspecies tested.

The identification of bacteroides (vernacular plural of *Bacteroides*; pronounced bacteroides) has been based on biochemical methods involving analysis of end products of glucose utilization, carbohydrate fermentation patterns, and various hydrolytic capabilities (7-9). Further taxonomic characterization generally requires expensive, often time-consuming techniques such as deoxyribonucleic acid homology and serological testing (1, 10, 15-18).

Bacteroides contain sphingolipids as major constituents of their cellular lipids (4, 5, 12-14, 31, 33, 34). This property was used by Fritzche and Thelen (5) to separate *Bacteroides* and *Sphaerophorus*. The *Bacteroides* species contain a characteristic pattern of branched-chain, non-hydroxylated, predominantly 15-carbon fatty acids (12, 14, 30, 31, 33, 34). This property was used by Prefontaine and Jackson (30) to generate a visual "fingerprinting" method to distinguish *Bacteroides* from *Eikenella*, *Pasteurella*, *Yersinia*, and *Haemophilus*.

Cellular fatty acid profiles, as derived from total cell hydrolysates, are useful indicators of species in the order *Spirochaetales* (2, 21), in some genera of *Enterobacteriaceae* (22), and in *Neisseria* (19, 20, 27), *Clostridium* (28), *Bacillus* (11), *Pseudomonas* (29), and *Propionibacterium* (26).

Recently, Miyagawa et al. (25) analyzed 21 strains encompassing 14 species and subspecies of the genus *Bacteroides* as well as one strain each of six species of *Fusobacterium*. Using a similarity coefficient calculation, these authors constructed a dendrogram of these gram-negative, obligately anaerobic rods based on the fatty acid composition of not more than 3 strains in any given taxon.

To our knowledge, there has been no concerted effort to determine whether the fatty acid profiles of *Bacteroides* species and subspecies vary in a consistent or predictable manner. We

report here a study of nine or more strains of each of 12 species and subspecies of *Bacteroides*. This report indicates the utility of determining characteristic ratios among the fatty acids in the cellular profile, including both the non-hydroxy and the recently demonstrated (4, 23-25) hydroxy acid components.

(Portions of this work were presented at the 80th Annual Meeting of the American Society for Microbiology, Miami, Fla., 11 to 16 May 1980.)

MATERIALS AND METHODS

Bacterial strains. A total of 160 strains of *Bacteroides* were analyzed in this study. Strains of the following *Bacteroides* species were isolated from clinical specimens at Emory University, Atlanta, Ga., or New England Deaconess Hospital, Boston, Mass.: *B. melaninogenicus* subsp. *melaninogenicus*, 7 strains; *B. melaninogenicus* subsp. *intermedius*, 6 strains; *B. asaccharolyticus*, 9 strains; "*B. melaninogenicus* subsp. *levii*" (names in quotation marks are not on the Approved Lists of Bacterial Names [32] and have not been validly published since 1 January 1980), 5 strains; *B. oralis*, 9 strains; *B. bivia*, 14 strains; *B. disiens*, 9 strains; *B. fragilis*, 16 strains; *B. distasonis*, 8 strains; *B. thetaiotaomicron*, 6 strains; and *B. vulgatus*, 4 strains.

Sources of other strains included: American Type Culture Collection (ATCC); L. V. Holdeman and J. Johnson, Anaerobe Laboratory, Virginia Polytechnic Institute and State University (VPI), Blacksburg; M. Sebald, Service des Anaerobies, Institut Pasteur, Paris, France; P. E. Riely, Marion Laboratories, Inc., Kansas City, Mo.; R. J. Genco and J. Slots, Department of Oral Biology, State University of New York at Buffalo; B. J. Mansheim, Department of Medicine, University of Florida, Gainesville; S. A. Syed, Department of Oral Biology, School of Dentistry, The University of Michigan, Ann Arbor; S. S. Socransky, Forsyth Dental Institute, Boston, Mass.; D. Danielsson, Central County Hospital, Orebro, Sweden; M. Lev, Southern Illinois University, Carbondale; and D. J. Blazevic, University of Minnesota, Minneapolis.

The strains obtained from the above-mentioned

sources follow. The first strain listed for each species is the type or other reference strain. *B. melaninogenicus* subsp. *melaninogenicus* strains were 10-80 (← ATCC 25845) and 209-74 (← VPI 4196 ← Hardie) (ATCC 25847). *B. melaninogenicus* subsp. *intermedius* strains were 22-81 (← VPI 4197) (ATCC 25611), 161-79 (← VPI 6018), 731-74 (← VPI 9145), 732-74 (← VPI 9146), 168-79 (← VPI 9854), 157-79 (← VPI 11329), 160-79 (← VPI 11335), 12-80 (← ATCC 15032), 13-80 (← ATCC 15033), 11-80 (← ATCC 25261), 509-78 (← Genco 20-3), and 508-78 (← Genco RP1-1). *B. asaccharolyticus* strains were 8-80 (← ATCC 25260), 9-80 (← ATCC 27067), 207-79 (← Sebal 362/76), 202-79 (← Sebal 363/76), 204-79 (← Sebal 336/78), 206-79 (← Sebal 361/78), and 122-80 (← Genco IN4). *B. gingivalis* strains were 121-80 (← Slots 2561) (ATCC 33277), 1754-75 (← Hardie ← Werner W50), 504-78 (← Genco 1021), 502-78 (← Genco 1112), 86-80 (← Mansheim ← Socransky 381), 87-80 (← Mansheim ← Socransky 382), 127-80 (← Mansheim ← Socransky 2018), 128-80 (← Mansheim ← Socransky 2020), 88-80 (← Mansheim ← Socransky 2022), 90-80 (← Mansheim ← Socransky BMD-3), 106-80 (← Riely ← Syed C2), and 107-80 (← Riely ← Syed BAN-2). '*B. melaninogenicus* subsp. *levii*' strains were 189-75 (← VPI 3300 ← Lev), 31-76 (← VPI 10176), 33-76 (← VPI 10360), 14-80 (← VPI 10449 ← Lev), 121-77 (← VPI 11330 ← Williams JP-2), and 15-80 (← VPI 10451). *B. oralis* strains were 23-81 (← VPI D27B-24), 590-76 (← VPI 8906D), and 591-76 (← VPI 10688 ← Blazevic 087-G). *B. disiens* strains were 19-81 (← VPI 8057) (ATCC 29426) and 2166-77 (← VPI 10944S ← Maryland). *B. bivia* strains were 2177-77 (← VPI 6822 ← U. Minn. 653C) (ATCC 29303), 163-79 (← VPI 6685), 164-79 (← VPI 6690), and 2172-77 (← VPI 9498 ← Medical College of Ohio 6444). *B. thetaiotaomicron* strains were 463-74 (← VPI 5482) (ATCC 29148), 464-74 (← VPI 2302), 1173-73 (← VPI C22-15), 121-79 (← Sebal 28/78), 130-79 (← Sebal Du 96), 179-79 (← Sebal BD 541/79), 186-79 (← Sebal BF 561/79), 189-79 (← Sebal BD 528/79), and 196-79 (← Sebal BD 628/79). *B. vulgatus* strains were 20-81 (← VPI 4245) (ATCC 8482), 119-79 (← Sebal 384/78), 123-79 (← Sebal 9N3), 124-79 (← Sebal R232), 140-79 (← Sebal Ty50), 187-79 (← Sebal BV 614/79), and 194-79 (← Sebal BF 601/79). *B. distasonis* strains were 21-81 (← VPI 4243) (ATCC 8503), 1169-73 (← VPI B6-11), and 546-71 (← Danielsson ON71-3/1). The *B. fragilis* strain was 192-76 (← VPI 2553 ← Sonnenwirth EN-2 ← NCTC 9343) (ATCC 25285).

Strains were identified by biochemical tests as described by Cato et al. (7) and by fluorescent-antibody serogrouping (15-17) when possible.

Cultures were grown in an anaerobic chamber in an atmosphere of 80% N₂, 10% H₂, and 10% CO₂ in 5 ml of brain heart infusion broth (Difco Laboratories) supplemented with yeast extract, hemin, and menadione (18). After 48 h at 35°C, 5 to 20 ml (one to four tubes) of each culture was harvested and washed free from medium components by centrifugation once at 4°C with 30 ml of distilled water.

Hydrolysis, extraction, and derivatization. Cell pellets were suspended in 2.5 ml of 2 M HCl and hydrolyzed overnight at 100°C. The hydrolysates were extracted once with 5 ml of chloroform. The organic layers were esterified with 2.5 ml of 1 M HCl in methanol at 100°C for 30 min. The reaction mixtures

were washed twice by partition against 5-ml portions of water. The organic phases were evaporated to dryness under N₂. The methyl esters were analyzed by gas-liquid chromatography, first directly and then, after derivatization, with 0.2 ml of trifluoroacetic anhydride-dichloromethane (1:2, vol/vol) for 15 min at room temperature.

Chromatography. Gas-liquid chromatography was carried out with a Hewlett-Packard 5840A gas chromatograph equipped with hydrogen flame ionization detectors. Analysis was done on a 10-m capillary column coated with the dimethylsiloxane liquid phase SP-2100 (J. and W. Scientific Co., purchased from Supelco, Inc., Bellefonte, Pa.). The instrument was operated in the splitless mode under temperature-programmed conditions such that retention times of the fatty acid methyl esters were linear with respect to chain length (23). The esters were identified by their 'equivalent chain length' values, as characterized previously (23). Molar response factors for quantitative analysis were determined empirically.

Calculations. The fatty acid profile of each strain was calculated as moles percent of each fatty acid. Ratios among various peaks or groups of peaks were calculated to determine which, if any, were consistent within a species or were discriminatory between species.

Chemicals and standards. Chloroform and methanol were redistilled before use. Dichloromethane (Fischer Scientific Co., Fairlawn, N.J.) and trifluoroacetic anhydride (Eastman Kodak Co., Rochester, N.Y.) were used as supplied. Saturated straight-chain fatty acids were purchased individually (Sigma Chemical Co., St. Louis, Mo.) and were weighed to provide a standard mixture. Branched-chain fatty acids were purchased as BC-Mix L and BC-Mix 1 (Applied Science Laboratories, Inc., State College, Pa.). Straight chain saturated 3-hydroxy acids of 12 to 20 carbons were prepared from hydrolysates of strains of *Escherichia coli* and *Acholeplasma axanthum*.

RESULTS

The predominant cellular fatty acids of all strains tested were the branched 15-carbon non-hydroxy acids, followed, in general, by the branched 17-carbon 3-hydroxy acids. Together, these two classes of fatty acids comprised 75 to 80% of the total fatty acid profile of each strain. Typical fatty acid profiles are shown, as histograms, in Fig. 1.

The species tested could be readily divided, by inspection of the fatty acid profile, into three major groups: (i) species with a significant amount (5 mol% or greater) of normal 15-carbon non-hydroxy acid (n15), characteristic of *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. distasonis*; (ii) species in which n15 was negligible and the non-hydroxylated anteiso-branched 15-carbon acid (a15) was greater than the iso-branched 15-carbon acid (i15), for example, in *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, *B. bivia*, *B. disiens*, and *B. oralis*; and (iii) species in which i15 was equal to or greater than a15, such

TYPICAL FATTY ACID PROFILES

FIG. 1. Cellular fatty acid profiles of typical bacteroides. Elution pattern is that for a nonpolar capillary column. Open bars, non-hydroxy methyl esters; solid bars, D-(-)-3-hydroxy methyl esters, trifluoroacetate derivatives. Abbreviations: a, anteiso-branched; i, iso-branched; n, normal (straight chain); h, hydroxy. Equivalent chain length is a measure of retention relative to a series of straight-chain saturated fatty acid methyl esters.

TABLE 1. Species with significant n15:0^a

Fatty acids	Ratio, mean (SD)											
	<i>B. distasonis</i> (11 strains)			<i>B. vulgatus</i> (11 strains)			<i>B. thetaiotaomicron</i> (15 strains)			<i>B. fragilis</i> (17 strains)		
br15:n15	5.1	(2.2)	5.8	4.9	(1.1)	5.0	7.0	(2.1)	5.2	6.6	(2.2)	6.3
a15:i15	4.1	(1.4)	4.8	4.8	(1.4)	4.1	4.0	(0.6)	3.5	1.8	(0.3)	1.8
n15:i15	1.2	(0.6)	1.0	1.2	(0.4)	1.0	0.7	(0.2)	0.8	0.4	(0.2)	0.4
Σ15:n16	28.4	(11.7)	38.7	22.2	(12.2)	39.9	16.5	(5.4)	10.3	13.7	(3.6)	17.0
Non-hydroxy:	2.2	(0.7)	2.3	2.0	(1.0)	1.9	1.4	(0.3)	1.3	1.8	(0.7)	1.3
hydroxy												
br17h:n16h	15.5	(9.5)	15.7	5.6	(1.4)	4.3	6.6	(1.4)	5.0	3.8	(1.6)	2.9
i17h:a17h	16.2	(7.3)	20.2	6.5	(2.9)	4.1	5.0	(1.2)	5.1	11.1	(3.5)	8.1

^a Abbreviations: a, anteiso; i, iso; br, branched (iso + anteiso); n, normal; h, hydroxy; Σ, total. Values in italics are those determined for type strains.

as *B. asaccharolyticus*, *B. gingivalis*, and "*B. melaninogenicus* subsp. *levii*."

Relative concentration ratios (moles percent/moles percent) were calculated for various combinations of individual peaks and groups of peaks for each strain. Those ratios which were of value in distinguishing among the species in each of the three groups are shown in Tables 1, 2, and 3, respectively. The values are the mean for each ratio, with the standard deviation included in parentheses to provide some indication of the variability among strains in a given species. The values for the type strain are shown in italics. The ratios are set up so most values are greater than 1.

DISCUSSION

In Table 1, comparison of the ratios of straight-chain C₁₅ to iso-branched C₁₅ (n15:i15) showed that *B. vulgatus* and *B. distasonis* (n15:i15 greater than 1) could be separated from *B. fragilis* and *B. thetaiotaomicron* (n15:i15 less than 1). In *B. distasonis*, the iso-branched C₁₇ hydroxy acid (i17h) was nearly the sole hydroxy

acid, as shown by the ratios br17h:n16h and i17h:a17h greater than 10. This permitted separation of *B. distasonis* from *B. vulgatus*, in which there were sufficient quantities of straight-chain C₁₆ hydroxy and anteiso-branched C₁₇ hydroxy acids to yield br17h:n16h and i17h:a17h ratios of less than 10. The ratio i17h:a17h also could be used to separate *B. thetaiotaomicron* (i17h:a17h less than 6) from *B. fragilis* (i17h:a17h greater than 8). The other ratios in Table 1, although informative, did not contribute to the separation of these four species.

Strains of *B. oralis* and *B. disiens* (Table 2) contained low but significant quantities of the branched hydroxylated C₁₅ acid (br15h), showing br17h:br15h ratios of 10 to 20, as contrasted to *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, and *B. bivius*, in which no br15h could be detected. *B. disiens* strains contained high concentrations of the non-hydroxylated branched C₁₃ acids (br13), showing br15:br13 ratios of less than 7, which permitted separation from *B. oralis*, which showed br15:br13 ratios much greater than 10.

TABLE 2. Bacteroideis with negligible n15:0; a15:0 greater than i15:0^a

Fatty acids	Ratio, mean (SD)														
	<i>B. oralis</i> (12 strains)			<i>B. disiens</i> (11 strains)		<i>B. bivius</i> (18 strains)		<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i> (9 strains)		<i>B. melaninogenicus</i> subsp. <i>intermedius</i> (18 strains)					
a15:i15	1.9	(0.4)	2.1	1.9	(0.6)	1.9	3.4	(1.6)	1.2	2.4	(0.9)	1.9	1.7	(0.5)	2.1
br15:n16	29.4	(19.4)	8.7	17.7	(8.9)	11.9	7.7	(3.0)	13.3	6.8	(1.8)	7.8	27.2	(17.4)	34.6
br15:br13	25.3	(15.5)	>100	3.6	(1.5)	5.6	>30 ^b		32.0	42.8	(45.5)	>100	16.6	(14.4)	22.0
br17h:br15h	10.6	(8.3)	18.2	10.8	(5.8)	15.0	— ^c		—	—		—	—		—
Non-hydroxy:	3.9	(2.8)	1.4	3.8	(2.5)	4.9	2.3	(1.1)	1.5	2.8	(0.8)	2.0	2.5	(1.5)	1.6
hydroxy															
br17h:n16h	4.8	(2.5)	1.5	6.0	(2.3)	10.6	12.4	(5.7)	15.3	3.3	(3.6)	4.4	7.5	(4.3)	11.7
i17h:a17h	2.5	(0.6)	3.5	10.5	(4.8)	6.1	>15		>100	>100		>100	8.4	(4.1)	6.7

^a See footnote to Table 1.

^b Fewer than half of the strains tested contained a detectable quantity of the minor component. The value indicated is the lowest ratio calculated for those strains.

^c No br15h detected.

As can be seen in Table 2, the distinctions among the remaining taxa—*B. bivius* and the two subspecies of *B. melaninogenicus*—become more tenuous.

Strains of *B. bivius* and *B. melaninogenicus* subsp. *melaninogenicus* tend to contain higher levels of palmitic acid (n16), showing br15:n16 ratios in the range of 7 to 15, whereas the corresponding ratios for *B. melaninogenicus* subsp. *intermedius* tend to center near 30, although the range overlaps somewhat. All *B. melaninogenicus* subsp. *intermedius* strains tested contained considerable anteiso-branched hydroxylated C₁₇ fatty acid (a17h), showing i17h:a17h ratios ranging from approximately 4 to 12. Only 5 of the 18 strains of *B. bivius* showed any significant a17h, but the i17h:a17h ratios ranged from approximately 15 to 30. *B. melaninogenicus* subsp. *melaninogenicus* strains had fairly high concentrations of the straight-chain hydroxylated C₁₆ fatty acid (n16h), showing br17h:n16h ratios near 4, in contrast to *B. bivius* strains, for which br17h:n16h ratios centered near 12.

The rather large standard deviations seen for several of the ratios in Table 2 indicate the wide variability among strains of these species. Heterogeneity is found in other properties as well (8). Lambe and co-workers demonstrated two distinct serogroups in *B. melaninogenicus* subsp. *intermedius* (15, 17). Heterogeneity in phenotypic tests, protein electrophoretic mobility, and deoxyribonucleic acid homology studies led Holdeman et al. to propose that *B. melaninogenicus* subsp. *melaninogenicus* be divided into three species, "*B. loescheii*," "*B. socranskii*," and *B. melaninogenicus*, and that *B. melaninogenicus* subsp. *intermedius* be divided into at least two species, "*Bacteroides corporis*" and *B. intermedius*, deoxyribonucleic acid homology groups I and II (L. V. Holdeman, J. L. Johnson, and W. E. C. Moore. J. Dent Res. Spec. Issue A 60:414, abstr. 415, 1981).

In Table 3, *B. asaccharolyticus* was readily separated from *B. gingivalis* and "*B. melanino-*

genicus subsp. *levii*" solely on the ratio i15:a15. In all the strains tested, the iso-branched C₁₅ non-hydroxylated compound was the predominant fatty acid in the profile (approximately 65 to 80 mol%). "*B. melaninogenicus* subsp. *levii*" contained considerable anteiso-branched C₁₇ hydroxy acid (i17h:a17h less than 10); this distinguished it from *B. gingivalis*, which contained negligible amounts of the anteiso compound (i17h:a17h greater than 100). In addition, "*B. melaninogenicus* subsp. *levii*" strains contained quite high levels of branched hydroxylated C₁₅ fatty acid (br15h), such that the total br15h was nearly equal to the total br17h, giving br17h:br15h ratios ranging from 1 to 2, as contrasted to ratios generally higher than 5 in *B. gingivalis* strains.

The separations listed above strongly indicate the potential usefulness of considering the cellular fatty acid profiles as part of the identification, and perhaps as part of the taxonomy, of the genus *Bacteroides*. The information in Table 3 is particularly striking, especially in terms of the taxonomic history of those members of the genus which produce a black pigment on media containing laked blood.

In *Bergey's Manual for Determinative Bacteriology*, 8th ed. (9), *B. melaninogenicus* is divided into three subspecies: *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, and "*B. melaninogenicus* subsp. *asaccharolyticus*," separable largely on the basis of carbohydrate fermentation or lack thereof. A fourth subspecies, "*B. melaninogenicus* subsp. *levii*," was described by Holdeman, Cato, and Moore (7). In 1977, "*B. melaninogenicus* subsp. *asaccharolyticus*" was elevated to species rank as *B. asaccharolyticus* (6). However, there appeared to be considerable difference between "intestinal" and "oral" strains of *B. asaccharolyticus*. This anomaly has been resolved by the recent creation of a new species, *B. gingivalis*, which includes the asaccharolytic pigmented *Bacteroides* strains of oral origin (3).

The ratios in Table 3 clearly support the

TABLE 3. *Bacteroides* with negligible n15:0; i15:0 greater than a15:0^a

Fatty acids	Ratio, mean (SD)					
	<i>B. asaccharolyticus</i> (16 strains)		<i>B. gingivalis</i> (12 strains)		" <i>B. melaninogenicus</i> subsp. <i>levii</i> " (11 strains)	
i15:a15	31.6	(4.3) 27.7	2.2	(0.4) 2.8	1.7	(0.3) 1.5
br15:n16	35.5	(12.8) 32.6	5.2	(1.5) 3.3	21.9	(17.0) 21.2
br15:br13	32.2	(26.8) 24.7	20.6	(13.9) 36.2	9.0	(2.6) 7.9
br17h:br15h	2.2	(0.8) 2.4	14.3	(9.7) 8.0	1.1	(0.5) 1.7
Non-hydroxy:hydroxy	5.8	(2.1) 4.1	2.0	(1.1) 1.8	4.6	(1.9) 5.8
br17h:n16h	4.9	(p3.0) 7.9	4.7	(1.8) 2.2	2.4	(1.4) 3.7
i17h:a17h	>100	>100	>100	>100	5.5	(1.2) 8.0

^a See footnote to Table 1.

TABLE 4. Key for the identification of *Bacteroides* taxa by characteristic ratios of cellular fatty acids

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- I. Negligible normal C₁₅, br15:n15 > 100:1
- A. Iso-C₁₅ greater than anteiso-C₁₅, i15:a15 > 1:1
1. i15:a15 > 25:1; *B. asaccharolyticus*
 2. i15:a15 < 5:1
 - a. Negligible anteiso hydroxy C₁₇, i17h:a17h > 100:1; *B. gingivalis*
 - b. Significant anteiso hydroxy C₁₇, i17h:a17h < 10:1; "*B. melaninogenicus* subsp. *levii*"
- B. Iso-C₁₅ less than anteiso-C₁₅, i15:a15 < 1:1
1. Significant branched hydroxy C₁₅, br17h:br15h < 20:1
 - a. High concentrations of branched C₁₃, br15:br13 < 7:1; *B. disiens*
 - b. Low branched C₁₃, br15:br13 > 10:1; *B. oralis*
 2. Negligible branched hydroxy C₁₅, br17h:br15h > 100:1
 - a. Relatively low normal C₁₆, br15:n16 > 20:1, high a17h, i17h:a17h < 10:1; *B. melaninogenicus* subsp. *intermedius*
 - b. Relatively high normal C₁₆, br15:n16 < 15:1
 - (1) Relatively high normal hydroxy C₁₆, br17h:n16h < 7:1; *B. melaninogenicus* subsp. *melaninogenicus*
 - (2) Relatively low normal hydroxy C₁₆, br17h:n16h > 8:1; *B. bivius*
- II. Significant normal C₁₅, br15:n15 < 10:1
- A. Normal C₁₅ greater than iso-C₁₅, n15:i15 > 1:1
1. Low anteiso hydroxy C₁₇, i17h:a17h > 10:1; *B. distasonis*
 2. Significant anteiso hydroxy C₁₇, i17h:a17h < 10:1; *B. vulgatus*
- B. Normal C₁₅ less than iso-C₁₅, n15:i15 < 1:1
1. Relatively low anteiso hydroxy C₁₇, i17h:a17h > 8:1; *B. fragilis*
 2. Relatively high anteiso hydroxy C₁₇, i17h:a17h < 6:1; *B. thetaiotaomicron*
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recognition of *B. asaccharolyticus* and *B. gingivalis* as separate species and suggest that "*B. melaninogenicus* subsp. *levii*" should be elevated to species status as well.

The determination of characteristic peak ratios from the cellular fatty acid profiles of *Bacteroides* species can provide useful information leading to rapid presumptive identification. A practical key for an identification scheme based on the ratios given in Tables 1–3 is shown in Table 4.

In general, fatty acid profiles of the various species of *Bacteroides* considered in this report were quite similar to those reported for corresponding species by Miyagawa et al. (25), although the medium and growth conditions used by these authors and by us were dissimilar.

The correlation coefficient calculation used by Miyagawa et al. separates most of the *Bacteroides* species from the *Fusobacterium* species but tends to group the strains of *B. fragilis* and *B. melaninogenicus* subspecies (similarity index greater than 0.9). *B. asaccharolyticus* and two strains listed as *B. splanchnicus* and *B. oralis*, however, show a high similarity to one another but a low similarity to the other species of the genus.

By contrast, the use of characteristic ratios, as described in this report, permitted clear separation of *B. fragilis* and similar organisms from the subspecies of *B. melaninogenicus* as well as what appeared to be reasonable separation among the other species studied.

The work of Miyagawa et al., as well as that reported here, indicates that cellular fatty acid

profiles serve as a useful taxonomic tool; the results obtained thereby agree with those obtained by other techniques, such as biochemical and serological tests and deoxyribonucleic acid homology determinations. The fatty acid profiles confirm both the current identification of *Bacteroides* species based on biochemical characterization (7) and the serological classification, by fluorescent antibody and agglutination techniques, of *B. fragilis*, *B. melaninogenicus* subspecies, and *B. asaccharolyticus* (15–18).

Since the fatty acid profiles and serological classification schemes are specific when applied to *Bacteroides* species, the combination of these two methods may suffice for identification.

ACKNOWLEDGMENTS

We acknowledge, with thanks, those investigators who provided us with reference strains. We thank L. V. Holdeman for valuable discussions and information regarding the current taxonomy of the pigmented strains.

This research was supported in part by Biomedical Research Development Awards 1-SO8-RR-09171-01 and -02 from the National Institutes of Health.

REPRINT REQUESTS

Address reprint requests to: Dr. W. R. Mayberry, Department of Microbiology, College of Medicine, East Tennessee State University, Johnson City, TN 37614.

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