BACTEROIDES ORALIS, PROPOSED NEW SPECIES ISOLATED FROM THE ORAL CAVITY OF MAN

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ABSTRACT

LOESCHE, W. J. (Forsyth Dental Center and Harvard School of Dental Medicine, Boston, Mass.), S. S. SOCRANSKY, AND R. J. GIBBONS. *Bacteroides oralis*, proposed new species isolated from the oral cavity of man. J. Bacteriol. **88**:1329-1337. 1964.—Twenty-seven strains of oral bacteroides were characterized and compared with a National Collection of Type Cultures strain of *Bacteroides fragilis* as well as strains of intestinal bacteroides. A combination of acid end products, gas production, pentose fermentation, and 10% bile tolerance afforded a basis for separation of oral strains from intestinal strains. The oral strains produced succinic, lactic, acetic, and formic acids but no gas. They were inhibited by 10% bile, and did not ferment xylose and arabinose. Intestinal strains consistent with the description for *B. fragilis* produced succinic, formic, acetic, and propionic acids and gas, with a few strains forming lactic acid. They were stimulated by 10% beef bile, and fermented xylose and arabinose. The oral strains are sufficiently different from intestinal strains of the *B. fragilis* type, so that they appear to constitute a separate species. It is proposed that they be recognized as *B. oralis*, sp. n.

The mucous membranes of mammals support large numbers of strictly anaerobic nonsporulating, gram-negative rods. Cultural and microscopic studies of the intestinal flora of man established their numerical importance in this site (Eggerth and Gagnon, 1933; Zubrycki and Spaulding, 1962; Rosebury, 1962). Recent quantitative investigations of the human gingival crevice microbiota demonstrated that gram-negative anaerobic rods constitute 16% of the total cultivable organisms (Gibbons et al., 1963). *Bacteroides melaninogenicus* accounted for one-third of these organisms, whereas the remaining strains were reported as fusobacteria and fermentative and nonfermentative "bacteroides-like" organisms which were not further characterized. It was subsequently found (Loesche, unpublished

data) that the nonfermentative group was similar to *Vibrio sputorum* as described by Macdonald (1953). The present investigation was initiated to identify the remaining nonpigmented bacteroides-like organisms. These fermentative organisms appeared similar in some respects to intestinal bacteroides; consequently, 19 intestinal strains were included in this study. Strains of *B. melaninogenicus* were omitted from this investigation, because this organism was previously characterized (Sawyer, Macdonald, and Gibbons, 1962).

MATERIALS AND METHODS

Isolation of oral strains. Fifteen strains were obtained from the study of the predominant cultivable flora of the gingival crevice area of man (Gibbons et al., 1963). Strain K92 was obtained from J. B. Macdonald and was a member of an experimental mixed anaerobic infection (Macdonald et al., 1956). The remaining 11 strains were isolated as follows. Debris from the gingival crevice area of man was collected in 0.067 M phosphate buffer (pH 7) and was dispersed for 30 sec in a Raytheon 9-kc sonic oscillator according to the method of Williams and Eickenberg (1952). Samples (0.1 ml) of tenfold serial dilutions were spread with a glass rod on Heart Infusion Agar (Difco) containing 10% defibrinated horse blood, and were incubated anaerobically for 4 to 7 days at 35 C in Brewer jars filled with 95% H₂ and 5% CO₂. The resulting colonies were streaked aerobically and anaerobically on the above blood medium. Colonies which grew only anaerobically were retained, and facultative colonies were discarded. All colonies were Gram-stained, and gram-negative obligately anaerobic organisms were restreaked to assure culture purity.

Isolation of intestinal strains. A fecal sample was dispersed in 0.067 M phosphate buffer (pH 7), and samples of high dilutions were plated as described above. The majority of colonies re-

sulting from high dilutions (i.e., 10^{-8} , 10^{-9}) were found to be gram-negative anaerobic rods. *B. fragilis* strain 9343 was obtained from the National Collection of Type Cultures, London, England. The American Type Culture Collection was not able to furnish cultures of *B. fragilis*.

Maintenance of cultures. All strains grew, though some poorly, in several commercially available media, i.e., Trypticase Soy Broth (BBL), PPLO broth with and without crystal violet (BBL), Spirolate broth (BBL), and Brewer Thioglycollate Medium (Difco). Hemin ($5\text{ }\mu\text{g/ml}$) enhanced the growth of the poorer-growing strains, and was added routinely to all media. Thioglycollate Medium without Dextrose (BBL), supplemented with 0.2% yeast extract (Difco) and hemin, was used as a basal medium and will be referred to as such throughout. Stock cultures were maintained on blood-agar plates as well as in the basal medium supplemented with 0.5% glucose. All cultures were incubated at 35 C in Brewer jars containing an atmosphere of 95% H_2 and 5% CO_2 .

Fermentation studies. Stock solutions (20%) of carbohydrates and carbohydrate derivatives were autoclaved separately, and were added aseptically to the basal medium to give a final concentration of 1%. A 2% starch solution was sterilized by ethylene oxide (Judge and Pelczar, 1955) and was added to the test medium in a 0.2% concentration. The pH after 6 to 8 days of incubation was measured with a glass electrode. A pH drop of 0.4 to 0.6 units below control cultures grown without added sugar was interpreted as slight fermentation, and greater acidities were considered positive. Starch hydrolysis was determined by adding 0.5 ml of a solution of 5% iodine in 10% potassium iodide to the fermentation tube and observing the presence or absence of purplish-black coloration. All fermentation tests were duplicated at a later time.

Acid end-product analysis. All strains were tested for the production of lactic, succinic, and volatile acids by the Celite column chromatographic method of Wiseman and Irvin (1957) as modified by R. D. McCarthy (*personal communication*). The basal medium was modified to contain 1% glucose and the sodium thioglycollate was omitted. The uninoculated medium was found to contain small amounts of acetic and lactic acids; consequently, the fermentation values were corrected for these amounts. These contaminating acids could be attributed to the

Trypticase in the medium. It was found that lactic and succinic acids overlapped on the column; consequently, colorimetric lactic acid determinations (Barker and Summerson, 1941) were performed on all cultures. The chemically determined lactate values were subtracted from the acid recovered in the column lactate-succinate fraction, and the difference was considered to represent succinic acid. The presence of both succinic and lactic acids in the column fractions was verified by paper chromatography with the use of an ethanol- NH_4OH -water (80:5:15) solvent after converting the acids to ammonium salts (Block, Durrum, and Zweig, 1956). Strains GA33 and B₁4 of *B. ruminicola* subsp. *brevis* (Bryant et al., 1958), known to produce succinic acid, were used as succinate controls. Carbon dioxide was determined by use of the Conway (1963) microdiffusion technique.

Differential biochemical tests. Nitrate reduction was determined by use of the basal medium supplemented with 0.1% KNO_3 , according to procedures described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957). The presence of residual nitrate was determined by the addition of zinc dust to acidified samples of the culture. Acidogenic strains were tested for nitrate reduction at pH 5.0 and 6.7 according to the suggestion of Rogosa (1961). Indole production was determined in the basal medium supplemented with 0.02% L-tryptophan. Gelatin liquefaction was determined in the basal medium to which 4% gelatin was added. Cultures were examined at 4 C after 3 to 15 days of incubation. Hydrogen sulfide production was tested by use of Peptone Iron Agar (Difco) and also lead acetate paper strips over Trypticase Soy Broth. The nitrate, indole, gelatin, and H_2S tests were performed in both the presence and absence of 0.5% glucose. Lysine, arginine, and ornithine decarboxylases were determined by the method of Ewing (1960). Tolerance to 10% bile (Oxgall, Difco) and 6.7% NaCl was determined in the basal medium supplemented with 0.5% glucose. Production of iodophilic polysaccharides was tested by the method of Gibbons and Socransky (1962). Lipolysis was determined in Spirit Blue Agar medium (BBL) supplemented with Wesson oil (Starr, 1941). Urease, catalase, and acetyl-methylcarbinol were sought by procedures described in the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957).

L-Threonine utilization (Béerens, Guillaume, and Petit, 1959) was determined in the basal medium to which 0.5% L-threonine was added. Acid production from L-threonine was sought by the column chromatography procedure described previously. Gas production was determined in the basal medium supplemented with 0.5% glucose by the appearance of bubbles in Durham tubes.

Morphology. Morphology was ascertained from Gram-stained and dark-field preparations of both agar and broth cultures. Motility was sought by dark-field microscopy of 1-, 3-, and 4-day-old cultures growing in broth.

RESULTS

Characteristics of oral isolates. Twenty-seven strictly anaerobic, saccharolytic, catalase-negative, nonsporulating rods with rounded ends were isolated from the mouths of 12 individuals. The majority of cultures contained cells 1 to 2 μ in length, with occasional longer forms. One group of six strains (recorded as *B. oralis* var. *elongatus* in Tables 1, 2, and 3) consisted of cells 3 to 8 μ in length, with occasional longer filamentous threads. This group was further distinguished by being stimulated by the addition of hemin (5 μ g/ml) to the medium. Strain 19A consisted of long, thick rods approximately 0.8 to 1 μ by 6 to 10 μ . Marked pleomorphism was observed only with strain K92. The cultures stained gram-negative, although on occasion some variability was noted.

All oral isolates were unable to reduce nitrate

at pH 5.0 and 6.7. Hydrogen sulfide was not produced in Peptone Iron Agar. However, all strains were slightly H₂S-positive when tested in Trypticase Soy Broth with lead acetate paper strips (Table 1). The oral strains did not possess decarboxylases for L-lysine, L-ornithine, and L-arginine; nor did they exhibit ureolytic activity. No strain produced acetylmethylcarbinol. They were inhibited by 6.7% NaCl and did not grow on Spirit Blue Lipid medium. All oral strains produced iodophilic polysaccharide from glucose.

A group of 22 strains (recorded as *B. oralis* in Tables 1, 2, and 3) had similar biochemical characteristics. They were uniformly indole- and gelatin-negative, did not produce gas from glucose, and were inhibited by 10% beef bile (Table 1). Of the 22 strains, 7 representative ones were tested for their ability to utilize L-threonine. L-Threonine did not enhance growth over that observed in the basal medium, and no propionic acid was detected in the spent broth. The five remaining strains, recorded as unclassified strains in Tables 1, 2, and 3, exhibited variability with regard to indole and gas production, gelatin liquefaction, and bile inhibition (Table 1). Only one oral strain (K92) was not inhibited by 10% beef bile.

The group of 22 strains uniformly fermented glucose, galactose, fructose, mannose, maltose, sucrose, lactose, cellobiose, raffinose, and starch (Table 2). The five unclassified strains were variable in their fermentation pattern. Only one oral strain (19A) fermented the pentoses, xylose and arabinose.

TABLE 1. Biochemical characteristics of *Bacteroides* strains

Determination	Oral strains							Intestinal strains		
	<i>B. oralis</i> var. <i>oralis</i>	<i>B. oralis</i> var. <i>elongatus</i>	Unclassified strains					<i>B. fragilis</i> NCTC 9343	New isolates	
			19A	K92	14A	13B	U81		Typical strains	Strain 9
No. of strains	16	6	1	1	1	1	1	1	17	1
Nitrate reduction	—	—	—	—	—	—	—	—	—	—
Indole	—	—	+	+	—	—	—	—	— (14/17)*	—
Gelatin liquefaction	—	—	—	—	—	+	—	—	—	—
H ₂ S†	—	—	—	—	—	—	—	—	—	—
Gas from glucose	—	—	+	—	—	—	—	+	—	—
Growth in 10% beef bile	—	—	—	+	—	—	—	+	+	+

* Number of indole-negative strains per total number of *B. fragilis* strains.
† Results obtained in Peptone Iron Agar. All strains were weakly positive with lead acetate paper strips in Trypticase Soy Broth.
‡ Number of gas-producing strains per total number of *B. fragilis* strains.

TABLE 2. Carbohydrate fermentation of *Bacteroides* strains

Determination	Oral strains							Intestinal strains		
	<i>B. oralis</i> var. <i>oralis</i>	<i>B. oralis</i> var. <i>elongatus</i>	Unclassified strains					<i>B. fragilis</i> NCTC 9343	New isolates	
			19A	K92	14A	13B	U81		Typical strains	Strain 9
No. of strains	16	6	1	1	1	1	1	1	17	1
Acid from										
Glucose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	—	+	—	+	+	+
Fructose	+(15/16)*	+	+	+	+	—	—	+	+	+
Mannose	+	+	+	—	—	—	+	+	+	+
Rhamnose	—(15/16)†	—	—	—	—	—	—	—	+(12/17)*	—
Maltose	+	+	+	+	—	+	+	+	+(16/17)*	+
Sucrose	+	+	+	—	+	—	+	+	+	+
Lactose	+	+(5/6)*	—	—	—	—	—	+	+	+
Cellobiose	+	+	—	—	—	—	—	+	+(11/17)*	+
Trehalose	—	—	—	—	—	—	—	—	—(11/17)†	+
Raffinose	+	+	+	—	—	—	—	+	+	—
Salicin	—	—	—	—	—	—	—	—	—(12/17)†	+
Xylose	—	—(5/6)†	+	—	—	—	—	+	+	—
Arabinose	—	—(5/6)†	+	—	—	—	—	+	+(16/17)*	—
Starch	+	+	+	—	—	+	—	+	+(16/17)*	—
Mannitol	—	—	—	—	—	—	—	—	—	—
Sorbitol	—	—	—	—	—	—	—	—	—	—

* Total number of positive strains over total number studied.
† Total number of negative strains over total number studied.

TABLE 3. Acid end products detected in 1% glucose broth

Determination	Oral strains							Intestinal strains		
	<i>B. oralis</i> var. <i>oralis</i>	<i>B. oralis</i> var. <i>elongatus</i>	Unclassified strains					<i>B. fragilis</i> NCTC 9343	New isolates	
			19A	K92	14A	13B	U81		Typical strains	Strain 9
No. of strains	16	6	1	1	1	1	1		17	1
Acid end products (μmoles/ml)										
Butyric	0	0	2	16	0	0	0	0	0 ^a	0
Propionic	0	0	0	0	11.2	29.6	0.8	3.6	1 to 4	0
Acetic	5 to 20	4 to 33	0	0	9.0	28.0	8.4	12.0	4 to 20	7.0
Formic	2 to 5	0	0	3.75	0	0	0	0	0 to 5 ^b	8.6
Lactic	0 to 4 ^c	0	11	2.75	7.8	20.4	0	1.5	0 to trace ^d	23.4
Succinic	6 to 24	11 to 14	0	2.5	0	0	17.8	18	7 to 20	0

^a One *B. fragilis* strain produced a trace amount of butyric acid.
^b Two *B. fragilis* strains did not produce formic acid.
^c Six *B. oralis* var. *oralis* strains did not produce lactic acid.
^d Four *B. fragilis* strains produced trace amounts of lactic acid.

Acid end products detected in 1% glucose broth also established the similarity of the 22 strains (Table 3). All produced acetic and succinic acids, and most strains produced formic and lactic acids. Six strains within this group (re-

corded as *B. oralis* var. *elongatus* in Tables 1, 2, and 3), distinguished by the presence of filamentous forms, failed to produce either formic or lactic acids. The five unclassified oral strains exhibited a wide variety of acid end products, with

two strains (K92, 19A) producing butyric acid and three strains (U81, 13B, and 14A) producing propionic acid.

The characteristics of the oral strains are consistent with the description given by Kelly (Breed, Murray, and Smith, 1957) for the genus *Bacteroides*. However, the oral bacteroides did not resemble exactly any of the recognized species. The group of 22 strains most resembled *B. fragilis* in being gelatin-, H₂S- and indole-negative. The oral bacteroides were compared with a known strain of *B. fragilis* (NCTC 9343) and with 18 newly isolated intestinal bacteroides.

Characteristics of intestinal strains. Morphology in broth cultures of NCTC strain 9343 and the intestinal isolates revealed cells ranging from coccobacilli approximately 1 to 2 μ in length up to and including long rods 5 to 7 μ in length. Most cells in a culture were approximately 0.5 to 1.0 μ wide and 1 to 4 μ long, with rounded ends. Chains were occasionally present. The more bizarre pleomorphic forms usually associated with *Sphaerophorus necrophorus* (*B. funduliformis*) were not observed. Single cell morphology of agar-grown organisms tended to be more uniform, consisting of rods 1 to 4 μ in length. Surface colonies on blood-agar were round with an entire edge, convex, shiny, smooth, gray to white in color, and 1 to 3 mm in diameter. Hemin (5 μ g/ml) enhanced the growth of two strains.

The NCTC strain of *B. fragilis* and the 18 intestinal isolates failed to produce H₂S in Peptone Iron Agar, but were capable of slightly blackening lead acetate paper strips in Trypticase Soy Broth (Table 1). All strains were nitrate- and gelatin-negative. Three strains produced indole demonstrable only in glucose-free medium. Fifteen strains, including the known culture of *B. fragilis*, produced in glucose broth a small visible amount of gas which was not CO₂. Gas production was slow, and did not appear until after 3 to 4 days of incubation. Beef bile (10%) stimulated the growth of most strains, including the known culture. A few strains were indifferent to bile, but no strain was inhibited. The increased growth in bile broth did not appear to be accompanied by an increase in acid end products. A wide variety of carbohydrates, including xylose and arabinose, were fermented (Table 2). The fermentation of rhamnose, cellobiose, trehalose, and salicin was variable; no strain utilized mannitol and sorbitol. Strain 9

was the only intestinal strain unable to ferment the pentoses. All strains produced acetic acid and, with one exception (strain 9), produced succinic and propionic acids (Table 3). Formic acid production was variable, and lactate was produced in trace amounts by five strains.

Eleven intestinal strains, including the known culture, gave slight to moderate growth in 0.5% L-threonine broth. Ten of these strains, including the *B. fragilis* culture, produced small amounts of propionate (i.e., from 2 to 7 μ moles/ml), comparable to that which was produced in the basal medium with and without glucose. One strain (strain D), however, produced copious amounts of propionate (i.e., 52 μ moles/ml).

DISCUSSION

The oral nonpigmented bacteroides have not been characterized previously. Although 30 species of *Bacteroides* are recognized by Kelly (Breed et al., 1957), only *B. melaninogenicus* has been isolated from the oral cavity of man. Eggerth and Gagnon (1933) fragmented the intestinal bacteroides into a variety of species, basing, in most cases, the species description on the carbohydrate fermentation pattern of five or fewer isolates. Weiss and Rettger (1937) found carbohydrate fermentation of no value in their characterization of intestinal strains (which included strains of Eggerth and Gagnon, 1933), and relied on morphological and serological criteria to establish three species of intestinal bacteroides. Lewis and Rettger (1940) published fermentation data for 46 intestinal bacteroides, but found no clear-cut subdivisions, and designated all strains as the single species, *B. vulgatus*. Prévot (1957) divided gram-negative anaerobic, nonsporulating, nonmotile rods into four genera: *Ristella* and *Capsularis* on the basis of capsule formation; *Pasteurella* to include ovate forms; *Dialister* to include short, filterable forms; and *Zuberella* to include motile forms. Béerens, Castel, and Fievez (1962), in schematic form, attempted to classify the family Bacteroidaceae according to the ability to utilize L-threonine, the volatile acids produced in glucose broth, and bile tolerance. They proposed a new genus, *Eggerthella*, to include strains which do not utilize threonine and which do not produce butyric acid from glucose.

Many recognized species are not available for comparison purposes; the tendency has been to place all relatively nonpleomorphic intestinal

bacteroides into a large group, with many biochemical determinants regarded as variable (Lewis and Rettger, 1940; Rosebury, 1962). Rosebury (1962) considered all nonpleomorphic, nonmotile, saccharolytic, and comparatively acidogenic organisms as *B. fragilis*, after the type species described by Veillon and Zuber (1898). This grouping of bacteroides species, at least with regard to the intestinal bacteroides, was supported by our data. The description of the known culture of *B. fragilis* and the fresh intestinal isolates given here is compatible with and extends the description provided by Kelly (Breed et al., 1957) for *B. fragilis*. It is also compatible with the volatile acid end products found by Guillaume, Bérens, and Osteux (1956) in *Ristella* (*Bacteroides*) species; with the fermentation data given by Lewis and Rettger (1940) for *B. vulgatus*; and with the bile stimulation of *Ristella* (*Bacteroides*) species reported by Bérens and Castel (1960). These intestinal strains would appear to be similar to *E. convexa* (Bérens et al., 1962).

Lewis and Rettger (1940) and Eggerth and Gagnon (1933) noted variation in H₂S and indole production and gelatin liquefaction in their intestinal strains, which was not observed in this study. The variation in H₂S production may be explained by differences in the medium employed. Both Lewis and Rettger (1940) and Eggerth and Gagnon (1933) incorporated lead acetate into their basal medium. In the present study, H₂S was sought in Peptone Iron Agar in both the presence and absence of 0.5% glucose and with lead acetate paper strips. The weak response observed with the lead acetate paper strips was considered as negative. In the present investigation, gelatin determinations were not carried beyond 15 days. The majority of species reported as gelatin-positive by Eggerth and Gagnon (1933) liquefied gelatin after 12 to 50 days of incubation. This delayed liquefaction would indicate lack of a constitutive gelatinase, and may represent nonspecific gelatinase activity of protoplasmic enzymes which have leaked into the medium. The differences in indole production are unexplained, except to note that the majority of the strains of Eggerth and Gagnon (1933) were indole-negative.

Carbohydrate fermentation per se has not clarified the taxonomy of the *Bacteroides*. However, pentose fermentation appears to be a consistent characteristic of intestinal bacteroides. The intestinal isolates and the culture of *B.*

fragilis fermented xylose and arabinose. In an additional study of intestinal bacteroides (van Houte, unpublished data), over 200 isolates from seven individuals uniformly fermented xylose and arabinose. Eggerth and Gagnon (1933) found 104 of 118 strains to ferment xylose and 78 of 118 strains to ferment arabinose. Lewis and Rettger (1940) reported 45 of 46 strains to ferment xylose and 34 of 46 strains to ferment arabinose.

Of 11 intestinal strains, 10, including the culture of *B. fragilis* and 7 oral strains selected from the biochemically homogeneous group of 22 strains, failed to utilize L-threonine. Because the intestinal strains were stimulated by 10% beef bile and produced acetic and propionic acids among other acids in glucose broth, they probably would be considered by Bérens et al. (1962) to be *E. convexa*. The oral strains according to this scheme might be members of the species *E. clostridiiformis*. However, Goldberg, Barnes, and Charles (1964) published data on *E. clostridiiformis* showing that the type culture of Bérens failed to lower the pH below 5.6 and produced copious amounts of gas. These findings argue against the inclusion of the homogeneous group of 22 oral strains in the species *E. clostridiiformis*.

Apparently, the relatively nonpleomorphic intestinal bacteroides of the *B. fragilis* type possess a basic pattern of biochemical activity. This would include saccharolytic activity against pentoses and other carbohydrates; the production of acetic, succinic, and propionic acids and gas other than CO₂ in glucose broth; and either stimulation or indifference to 10% beef bile.

The oral strains differed from this biochemical pattern of intestinal isolates. A biochemically homogeneous group of 22 oral bacteroides did not form propionic acid and gas, did not ferment xylose and arabinose, and were inhibited by 10% beef bile. Attempts to adapt these oral strains to bile were unsuccessful. These differences seem to warrant the recognition of this group of oral bacteroides as a new species, *B. oralis*. Two distinct morphological types were observed within the *B. oralis* species. A group of 16 strains were mostly short rods 1 to 3 μ long, whereas a group of 6 strains consisted of rods 3 to 8 μ long with frequent filamentous forms. It is proposed that these morphological types be considered as *B. oralis* var. *oralis* and as *B. oralis* var. *elongatus*, respectively.

Bacteroides oralis var. *oralis*. Sixteen oral strains of which the type strain is 7CM are placed

in this subspecies. This organism is a gram-negative nonmotile rod approximately 0.5 to 1.0 μ wide and 1 to 4 μ long with rounded ends. A few filamentous cells are usually observed in broth cultures. Surface colonies on blood-agar are round with an entire edge, convex, shiny, smooth, nonhemolytic, gray to white in color, semitransparent, and 1 to 2 mm in diameter. Often, a delicate internal granularity is observed.

Growth in liquid medium is evenly dispersed.

Strict anaerobe.

Final pH in glucose broth is 5.0 to 5.4.

Acid fermentation products detected in glucose broth include succinic, acetic, and formic acids in the following average percentages: 44, 41, and 15%. The majority of strains produce lactic acid in trace amounts.

No gas is produced.

Glucose, galactose, fructose, mannose, maltose, sucrose, lactose, cellobiose, and raffinose are fermented. Rhamnose, mannitol, sorbitol, xylose, arabinose, trehalose, and salicin are not fermented.

Iodophilic polysaccharide is produced from glucose.

Starch is hydrolyzed.

Gelatin is not liquefied.

Nitrate is not reduced. Acetylmethylcarbinol, catalase, and indole are not produced.

Hydrogen sulfide is not produced in Peptone Iron Agar. Lead acetate paper strips are slightly blackened by cultures in Trypticase Soy Broth.

Urea is not hydrolyzed.

Beef bile (10%) inhibits growth in Trypticase broth.

Source: the oral cavity of man.

B. oralis var. *elongatus*. Six oral strains of which R42 is the type strain are placed in this subspecies. Cell morphology reveals gram-negative rods approximately 3 to 8 μ long by 0.5 to 1 μ wide with rounded ends. No internal granules are detectable. Surface colonies on blood-agar are usually gray to white in color, nonhemolytic, low, convex, occasionally with an irregular outline; and exhibit an internal speckling or veining. Occasionally, long forms will dominate in a culture broth. When streaked on blood plates, these filamentous forms give rise to a rhizoid colony with growth penetrating into the agar. With subculture, the more regular colonial type returns. Growth of both colonial types in broth tends to be granular.

B. oralis var. *elongatus* strains form acetic

and succinic acids in glucose broth. Hemin (5 μ g/ml) enhances growth of most strains.

The cell morphology of *B. oralis* var. *elongatus* was suggestive of *Fusobacterium*. Some oral strains resembling *B. oralis* var. *elongatus* probably have been characterized as *Fusobacterium*. The strains of saccharolytic fusobacteria described by Morris (1954), i.e., groups A5 to A9 and the six strongly acidogenic strains of *F. nucleatum* reported by Hine and Berry (1937), might fall into this category. The carbohydrate fermentation pattern and acid end products formed by *B. oralis* var. *elongatus* clearly distinguish them from the weakly saccharolytic, butyric acid-forming *Fusobacterium* species.

Hemin (5 μ g/ml) was found to stimulate growth of five of six *B. oralis* var. *elongatus* strains and two intestinal *B. fragilis* strains. Hemin was reported by Quinto (1962) to enhance growth of certain intestinal bacteroides of man, and by White, Bryant, and Caldwell (1963) for certain *B. ruminicola* strains. A hemin requirement for *B. melaninogenicus* has been well documented (Evans, 1951; Gibbons and Macdonald, 1960). The role of hemin in these organisms, with the exception of *B. ruminicola*, needs to be elucidated because they are generally thought to lack cytochromes and are catalase-negative.

The majority of intestinal and oral strains studied formed acetic and succinic acids as the major acid end products. The acid end products of the rumen *Bacteroides* thus far classified included succinic and acetic acids (Bryant, 1959). Recently, it was found that some carbohydrate-fermenting strains of *B. melaninogenicus* also formed significant amounts of acetate and succinate (Loesche, unpublished data). *Eggerthella* strains (Béerens et al., 1962) produced acetic acid, but succinate values were not determined. These data suggest that a common pathway of carbohydrate catabolism may exist in these organisms.

High dilution plates were employed in the isolation of strains from both gingival debris and feces. Only the most numerous cultivable types were isolated. One cannot conclude from this study that organisms of the *B. oralis* type are not present in low numbers in feces, and that organisms of the *B. fragilis* type are not present in the oral cavity. The presence of several unclassified oral strains which produced propionic acid, and of one intestinal strain which did not

form propionic acid, suggests that atypical types exist in both sites.

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