Fusobacterium prausnitzii (Hauduroy et al.) Moore and Holdeman: Emended Description and Designation of Neotype Strain

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Forty strains that conform closely to the original description of *Bacteroides prausnitzii* Hauduroy et al. have been studied, and previously undescribed characteristics of this organism are presented. Because of their metabolic activity, these organisms are members of the genus *Fusobacterium* as presently defined. Strain ATCC 27768 (= VPI C13-51) is designated as the neotype strain of *Fusobacterium prausnitzii* (Hauduroy et al.) Moore and Holdeman.

In 1922, Prausnitz isolated from pus from a case of pleural empyema (8) an organism which he named *Bacillus mucosus anaerobius*. Weinberg et al. in 1937 (9) proposed that the organism be named *Bacterium zoogeleiformans* (i) because it did not belong in the genus *Bacillus*, (ii) because the rules of nomenclature required that binary combinations be used, and (iii) because the name *Coccobacterium mucosum anaerobicum* had been given by Klinger in 1912 to a completely different organism (4). However, Hauduroy et al. (3), also in 1937, proposed that the organism be placed in the genus *Bacteroides* as *Bacteroides prausnitzii* (sic) because it was an anaerobic, gram-negative, nonsporeforming rod. Their name for this organism was accepted as validly published and legitimate (1).

In 1973, following the recommendations of the International Subcommittee on Taxonomy of the Anaerobic, Gram-Negative Rods, those anaerobic, gram-negative, nonsporeforming rods whose major metabolic product is butyric acid without isobutyric and isovaleric acids were placed in the genus *Fusobacterium* (7). Since all strains of organisms that fit the original description of *Bacteroides prausnitzii* are anaerobic, gram-negative, nonsporeforming rods that produce large amounts of butyric acid and no isobutyric or isovaleric acid, this species has been reclassified as *Fusobacterium prausnitzii* (7). (The spelling of the specific epithet was corrected according to Rule 27. Since the organism was named for Prausnitz, the original spelling was a typographical error.)

Neither the strain isolated by Prausnitz nor any of the strains studied by Hauduroy et al.

(3) is extant. The original description of the species is emended on the basis of a study of 40 strains of this organism recently isolated from the normal fecal flora of man.

MATERIALS AND METHODS

Bacterial strains. The 40 strains included in this study were isolated in our laboratory from feces of clinically normal humans.

Methods. The methods used in the isolation and identification of these organisms have been previously described (6). The guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acid (DNA) preparations were determined by their thermal melting points by J. L. Johnson as previously described (2, 5).

RESULTS AND DISCUSSION

Morphologically (Fig. 1), the cells of *Fusobacterium prausnitzii* are exactly as originally described: gram-negative, slender, nonsporeforming, nonmotile rods, 0.5 to 0.9 μm $\times$ 2.4 to 14.0 μm , somewhat smaller in pure culture. Short forms are straight with tapered or rounded ends, and long cells are definitely curved. In carbohydrate media supplemented with rumen fluid, some cells may be as long as 35.0 μm . Cells occur singly, in pairs, or in short chains. They may stain irregularly with colorless vacuoles. A thick, mucous capsule can be demonstrated with a fuchsin stain.

Colonies on blood agar streak tubes are pinpoint to 1 mm in diameter, circular, entire to slightly erose, convex, and translucent to transparent; they usually have no visible inter-

nal structure but occasionally have a granular or mottled appearance. They are colorless to milky white. Of 10 strains tested, none grew on McClung-Toabe egg yolk agar plates or on anaerobic blood agar plates. These observations are in agreement with those of Hauduroy et al. (3), who reported that the organism was difficult to isolate and could only be cultured under strictly anaerobic conditions. Unheated serum was reported to be required for growth (9). We have found that prereduction of media

replaces the reported requirement of many anaerobes for fresh serum.

Growth of most strains is stimulated by the addition of sterile rumen fluid to a concentration of 5%. The temperature for optimal growth was 37 C, although most strains grew as well at 45 C. Hauduroy et al. (3) stated that broth cultures were turbid and after 6 to 7 days produced a viscous sediment. Our cultures in PRAS glucose medium (6) supplemented with rumen fluid were turbid with a viscous or ropy sediment in 1 to 3 days.

The biochemical reactions of the strains studied and reactions given in the original description of this organism are listed in Table 1 and in the following text. In addition to these results, deoxyribonuclease was formed by some strains. Hydrogen gas was not produced by cultures in fructose broth. Of 10 representative strains tested (including the neotype), a small amount of CO₂ was detected in cultures of six strains, and a small amount of H₂S was produced by five strains. Neutral red was

FIG. 1. *F. prausnitzii* ATCC 27768; 24-h-old culture in E medium (6). Bar represents 10 μ m.

TABLE 1. Biochemical reactions of *F. prausnitzii*^a

Substrate or reaction	Hauduroy et al.	<i>F. prausnitzii</i>			Neotype strain
		No. of strains tested	Test result	Positive (%)	
Amygdalin		40	—w	10	—
Cellobiose		40	—w	37.5	w
Esculin pH		40	—w	20	—
hyd.		40	+	100	+
Fructose	w	40	w—	55	w
Galactose	w	22	—w	45.5	w
Glucose	A	40	w—	70	w
Lactose	w	40	w—	52.5	w
Maltose	A	40	w—	62.5	w
Mannitol	—	39	—	0	—
Mannose		40	—	7.5	—
Raffinose		40	—w	10	—
Rhamnose		40	—w	20	w
Salicin		30	—w	40	—
Starch pH		40	—w	25	—
hyd.		40	—	0	—
Sucrose	w	40	—w	20	w
Trehalose		40	—w	22.5	—
Indole produced		40	—	0	—
Nitrate reduced		40	—	0	—
Motility	—	37	—	0	—
Gas produced	—	40	—	0	—

^a No strain fermented dulcitol, erythritol, glycerol, inositol, sorbitol, arabinose, ribose, xylose, melibiose, melezitose, glycogen, or inulin. Little if any gas produced in glucose agar deeps. Catalase was not present. Neither gelatin, chopped meat, nor milk was digested; milk was not curdled. Symbols: A, strong acid; w, final pH 5.6 to 6.0; +, reaction positive; —, reaction negative or final pH > 6.0. Where a single result is listed, the indicated reaction is produced by over 90% of the cultures. When two reactions are listed, the first is the result observed with most cultures, and the second reaction is less frequently observed.

reduced by most strains; reduction of resazurin was variable. Hippurate was not hydrolyzed.

As originally described, *B. prausnitzii* formed strong acid from glucose and maltose, weak acid from fructose, lactose, sucrose, and galactose, and no acid from mannitol. Under our test conditions, carbohydrates were not fermented strongly; Hauduroy et al. (3) reported strong fermentation, but the organism reduces its environment and probably affected the litmus used at that time to measure pH. The carbohydrates mentioned above are the ones most frequently attacked. The average final pH of peptone-yeast extract-glucose-rumen fluid broth (PYG-RF) after 5 days of incubation was 5.75 as compared with an average of 6.1 in basal medium (peptone-yeast extract-rumen fluid).

The products of fermentation of PYG-RF were butyric acid (0.5 to 3.3 meq/100 ml) and usually formic acid (0 to 1.8 meq/100 ml) and D(-)-lactic acid (0 to 2.0 meq/100 ml). Traces of succinic and of propionic acids were formed by some strains. Neither threonine nor lactate was converted to other products, but pyruvate was converted to acetate and butyrate, sometimes with small amounts of formate and lactate. Conversion of gluconate was variable,

but galacturonate was converted by 15 of 17 strains tested to acetate and butyrate, usually with formate.

The G+C content of DNA preparations from six representative strains ranged from 52 to 57 mol%. The range indicates that there may be heterogeneity within this group of organisms. However, some of the variation may have been due to technical reasons because of the uniformly low amount of growth and the poor yield of DNA that was obtained from the cells. Differing reactions of the six strains are listed in Table 2. Other reactions are as described.

ATCC 27768 (= VPI C13-51) is here designated the neotype strain of *Fusobacterium prausnitzii* (Hauduroy et al.) Moore and Holdeman. Characteristics of this strain closely conform to Prausnitz' original description as well as to the description by Hauduroy et al. (3). The G+C content of the neotype strain is 53 mol%.

The original strain of this organism was isolated twice by Prausnitz from purulent pleurisy, first in association with a pneumococcus and then in pure culture. It was not pathogenic for the guinea pig, rabbit, or mouse. Serum of the patient fixed complement in the presence of a culture of the organism, but sera

TABLE 2. Biochemical reactions of representative strains of *F. prausnitzii*

Substrate or reaction	Strains tested					
	C10-60	C13-51 ^a	C3-19	C11-27	C13-20A	C10-47
G+C (mol%)	50	53	55	55	56	57
PY	6.2 ^b	6.15	6.0	6.0	6.1	6.05
Amygdalin	6.1	6.0	5.85	6.1	6.05	6.0
Cellobiose	5.65	5.8	5.85	6.1	6.1	6.2
Fructose	5.75	5.8	5.8	6.1	6.1	5.85
Galactose	5.7	5.85	5.7	6.05	6.1	5.82
Glucose	5.5	5.9	5.75	6.0	6.0	5.75
Lactose	5.85	5.85	5.8	6.05	6.05	6.02
Maltose	5.6	5.9	5.63	6.1	6.2	5.88
Rhamnose	5.8	5.9	6.1	6.2		6.2
Salicin	5.85	6.0	5.7	6.2	6.15	5.85
Starch	6.1	5.95	5.75	6.15	6.15	6.02
Sucrose	6.15	5.8	5.9	6.25	6.35	5.85
Trehalose	5.6	6.2	6.1	6.2	6.2	6.05
CO ₂ formed	+	+	+	—	+	+
H ₂ S formed	+	+	—	—	—	—
Products from PYG	BF ^c	BL ^f	B ^f	B	BL ^f	BF

^a C13-51 = ATCC 27768; C11-27 = ATCC 27765; C13-20A = ATCC 27766.

^b Final pH after 5 days incubation.

^c B, at least 1 meq butyric acid/100 ml; F, at least 1 meq formic acid/100 ml; f, less than 1 meq formic acid/100 ml; L, at least 1 meq lactic acid/100 ml; l, less than 1 meq lactic acid/100 ml.

TABLE 3. Characteristics that differentiate *F. prausnitzii* from other species in the genus *Fusobacterium*^a

Organism	Esculin hydrolyzed	Indol produced	Threonine→ propionate	Glucose	Mannose	Products (PYG)
<i>F. prausnitzii</i>	+	—	—	w—	—	B(FLsp)
<i>F. mortiferum</i>	+	—	+	Aw	wA	BAp(LFivs4)
<i>F. necrogenes</i>	+	—	+	Aw	wA	Ba(lfpsv4)
<i>F. aquatile</i>	+	+	+—	Aw	A	Ba
<i>F. varium</i>	—	+—	+	wA	w	BLA(Sp)
<i>F. nucleatum</i>	—	+	+	—w	—	Baps(Lf)
<i>F. naviforme</i>	—	+	—	—A	—	BLap(fs)
<i>F. necrophorum</i>	—	+	+	—w	—	Bap(Lsf)
<i>F. glutinosum</i>	—	+	+	A	—	Bap(Lsf)
<i>F. gonidiaformans</i>	—	+	+	w—	—	Bap(lsf)
<i>F. plauti</i>	—	—	—	wA	—w	LBas
<i>F. bullosum</i>	—	—	—	—	—	Abp
<i>F. symbiosum</i>	—	—	—	Aw	A—	BA(Lf)
<i>F. russii</i>	—	—	—	—w	—	B(LFas)

^a Adapted from Holdeman and Moore (6). Symbols: +, reaction positive for >90% of strains; —, reaction negative for >90% of strains or final pH of >6.0; A, final pH 5.5 or lower; w, final pH 5.6 to 6.0; w—, wA, etc., first reaction most common, 10 to 40% of strains may have second reaction. Products: A, acetic acid; B, butyric acid; F, formic acid; iv, isovaleric acid; L, lactic acid; P, propionic acid; S, succinic acid; V, valeric acid; 4, butanol. Capital letters, >1 meq/100 ml; lower case, <1 meq/100 ml; (), may be present.

from two normal individuals did not. We have not isolated any strain from clinical material, but the organism appears to be a numerically significant member of the normal intestinal flora of man.

Characteristics which qualify *F. prausnitzii* as a member of the genus *Fusobacterium* Knorr. *F. prausnitzii* is a member of the genus *Fusobacterium* because it is an obligately anaerobic, gram-negative, nonsporeforming rod which produces butyric acid as a major product of fermentation without isobutyric or isovaleric acids.

The characteristics most useful in distinguishing *F. prausnitzii* from other members of the genus *Fusobacterium* are listed in Table 3. It hydrolyzes esculin, does not produce indole, and does not convert threonine to propionate. The major product of fermentation by *F. prausnitzii* is butyric acid, usually with formic, lactic, and small amounts of succinic acids. Traces of propionic acid are sometimes formed.

Some strains of *Eubacterium cylindroides* that decolorize readily are morphologically similar to *F. prausnitzii*. However, *E. cylindroides* is distinguished biochemically by its strong acid production (pH below 5.5) from fructose, glucose, and mannose and by the proportion of acids it forms from glucose. The major fermentation product of *E. cylindroides* is lactic acid with smaller amounts of butyric and sometimes formic and succinic acids.

Gram-positive cells can usually be demonstrated in young (2- to 4-h-old) *E. cylindroides* cultures.

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REPRINT REQUESTS

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