Fusobacterium perfoetens (Tissier) Moore and Holdeman 1973: Description and Proposed Neotype Strain

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In 1900, Tissier described a new bacterium, *Coccobacillus anaerobius perfoetens*, that he isolated from an infant with diarrhea and, in 1905, from nursing infants. This organism was placed by Hauduroy et al. (1937) in the genus *Bacteroides*, by Sébald (1962) in *Sphaerophorus*, and then by Moore and Holdeman (1973) in the genus *Fusobacterium*. *Sphaerophorus perfoetens* CC1, isolated in 1947 by Prévot from the cecum of a horse and studied by Sébald in 1962, has been lost. Since then, no strains conforming to the description of Tissier's organism have been known to be extant. However, recently we obtained from a normal piglet six isolates that conform to the original and subsequent descriptions of this organism. One of these isolates, PVA B₃/63, is designated as the neotype strain of *Fusobacterium perfoetens* and is available from the American Type Culture Collection as ATCC 29250.

In 1900, Tissier (11) isolated a gram-negative, anaerobic, nonsporeforming organism from an infant with diarrhea and named the organism *Coccobacillus anaerobius perfoetens*. In 1905, he isolated the same organism from nursing infants.

In 1947, Prévot isolated an identical strain from the cecum of a horse. This strain, CC1, was studied by Sébald in 1962 (10) and was transferred by her to the genus *Sphaerophorus* as *S. perfoetens*. Subsequently, the cultures of this strain were lost.

On the basis of the published descriptions of this organism, Moore and Holdeman (7) transferred the organism to the genus *Fusobacterium* as *F. perfoetens*.

Recently, we isolated from the feces of a piglet a strain whose characters agree not only with those in the original description of this organism by Tissier but also with those in subsequent descriptions (5, 8, 11).

The purpose of this paper is to update the description of *Fusobacterium perfoetens* (Tissier) Moore and Holdeman and to designate the neotype strain for it.

MATERIALS AND METHODS

Bacterial strains. We recently obtained six isolates from the feces of a 2-week-old normal piglet. The isolates were designated PVA B₃/5, PVA B₃/6, PVA B₃/31, PVA B₃/31b, PVA B₃/34, and PVA B₃/63. One of the isolates, PVA B₃/63, was deposited in the American Type Culture Collection (ATCC), Rockville, Md., under the number 29250.

Media. Initial isolation of organisms from fecal material was made by enrichment in regenerated modified Rosenow medium (1). Colonies were picked

from solid VL blood medium without selection and were incubated at 37°C in GasPak (BBL) jars using a GasPak H₂-CO₂ generator for the gaseous phase.

Cultures of the isolates were maintained in modified Rosenow medium (1), covered with paraffin, and stored deep-frozen at -20°C. The composition of the medium is as follows: proteose peptone no. 3 (Difco), 10 g; beef extract (Difco), 3 g; NaCl, 5 g; glucose, 2 g; cysteine hydrochloride, 0.3 g; Andrade solution (5% fuchsin), 10 ml; and distilled water, 1,000 ml; the pH was adjusted to 7.2. The medium was distributed in tubes, each containing 0.5 g of frozen beef brain and a piece of marble, and was sterilized at 120°C for 20 min.

Most of the biochemical tests were performed in VL base medium (1) to which supplements were added. The medium contained: tryptone (Oxoid), 10 g; NaCl, 5 g; beef extract (Difco), 2 g; yeast extract (Difco), 5 g; cysteine hydrochloride, 0.3 g; agar, 0.5 g; and distilled water, 1,000 ml. For the solid VL blood medium, 0.2% glucose, 2% agar, and 10% defibrinated horse blood were added to the VL base medium.

Methods. Cellular morphology was determined by examination of Gram-stained smears (Kopeloff modification) as described by Holdeman and Moore (4). The purity of each culture was checked by aerobic and anaerobic subculture on VL blood agar. Catalase production was determined by adding 1 drop of a 3% H₂O₂ solution to a colony on VL agar without blood, since false-positive results may occur due to the catalase in blood. These tests were performed both immediately after removing cultures from an anaerobic environment (anaerobic jar) and after at least a 1-h exposure to the air. The fermentation reactions were studied in VL base supplemented with a 1% carbon source. All carbohydrates and polyalcohols were membrane-filter sterilized as 10% (wt/vol) solutions and added to the basal medium to give a final concentration of 1%. The results were

recorded after 7 days. Nitrate reduction and H_2S production tests were performed in VL base supplemented with 5 g of $NaNO_3$ per liter and 2 g of glucose per liter, respectively, and with 0.2 g of $FeSO_4$ per liter and 0.3 g of $Na_2S_2O_3$ per liter. The presence of nitrite was detected by adding 5 drops of 0.8% sulfanilic acid in 5 N acetic acid and 5 drops of 0.6% α -naphthylamine in 5 N acetic acid to cultures grown in the above-mentioned medium. A red color indicated the presence of nitrite. H_2S production was demonstrated by the blackening of the medium. Indole and NH_3 production was determined on VL base by adding Kovac reagent or Nessler solution. Threonine deamination was determined by the method described by Buttiaux et al. (1).

The metabolic end products from peptone-yeast extract-glucose (PYG) medium and the production of propionic acid from threonine were detected by gas-liquid chromatography by the methods of Holdeman and Moore (4). Chromatography was performed on a Hewlett Packard 5700 A gas chromatograph with hydrogen flame detector and with helium as the carrier gas.

Peak areas were recorded with a digital integrator (model 3370 A). Stainless-steel columns (length, 1.8 m [6 feet]; outer diameter, ca. 0.3 cm [$1\frac{1}{8}$ inch]) containing 10% Carbowax on Diatoport W were used.

Other operating conditions were: sample size, 3 μ l; carrier make-up flow, 60 ml/min; inlet and detector temperature, 250°C; and column temperature isothermal, 120°C. Sample components were tentatively identified by comparison with standard volatile fatty acids and fatty acid methyl esters. For determination of deoxyribonucleic acid (DNA) base composition, DNA was isolated from strain PVA B₃/63 by the method of Marmur (6). The percentages of guanine plus cytosine (G+C) in the DNA samples were determined from absorbancy ratios of the DNAs by using a quick method described by De Ley (2).

To demonstrate the antibiotic susceptibility of the strains, 6-mm paper disks containing the indicated amounts of antibiotic (susceptibility disks for antibiotics, BBL) were placed on VL blood agar plates streaked with the indicated organism, and these were incubated for 2 days in a GasPak anaerobic jar (BBL).

RESULTS AND DISCUSSION

Cellular morphology. The morphology of the six isolates was found to be exactly the same as that of the strains described by Weinberg et al. (12) and Prévot (8). The isolates were nonspore-forming, gram-negative, nonmotile, short, ovoid rods. The cells measured 0.6 to 0.8 by 0.8 to 1.0 μ m; they were oval, never elongated, and occurred singly, in pairs, in chains of no more than three cells, or in irregular masses (Fig. 1) where each cell maintained its own shape. They did not elongate in old cultures but remained oval. The cell measurements were always greater in PYG than in Rosenow broth

cultures (Fig. 2). No flagella or capsules were present.

Colonial morphology. Tissier (11) described 48-h-old deep-agar colonies as lenticular and 1 mm in diameter. We also found lenticular colonies which enlarged markedly on continued incubation in VL deep agar (6 g of agar per liter). Surface colonies of isolate PVA B₃/63 on horse blood agar were 1 to 2 mm in diameter, circular with an entire edge, raised, and grayish-white to gray. Surface colonies of the other isolates were similar to those of PVA B₃/63. However, colonies of some of the isolates were slightly umbonate and had diffuse edges and a slightly mottled or finely granular appearance.

Biochemical characteristics originally and subsequently described. As described by Weinberg et al. (12), the earlier isolated strains were obligately anaerobic, did not survive 60°C for 15 min, and lived for at least 1 month at room temperature in different media. Growth in glucose broth cultures was rapid, and a fine sediment, gas, and a fetid odor were produced. Glucose and sucrose were the only carbohydrates fermented; lactose was not fermented. Milk was not coagulated, and indole was not produced. H_2S , NH_3 , and CO_2 were produced. Lactic acid and only small amounts of butyric and valeric acids were produced from glucose.

Some additional biochemical information was provided in the classification manuals of anaerobic bacteria by Prévot (8) and Prévot et al. (9). These characteristics are included in Table 1, in which also are listed the characteristics of the six isolates.

Prévot (8) reported the production of acetic, butyric, valeric, and lactic acids from glucose. However, the metabolic products from glucose as summarized by Moore and Holdeman (5), and based upon literature descriptions by Weinberg et al. (12) and by Prévot et al. (9), are acetic, butyric, and lactic acids. In our laboratory, gas chromatographic analyses revealed, as major products, butyric acid (1 to 2 meq/100 ml), lactic acid (0.6 to 1.3 meq/100 ml), acetic acid (0.1 to 0.6 meq/100 ml), and traces of propionic acid (0.0 to 0.1 meq/100 ml). The amount of acetic acid always was greater in PY (0.5 to 1.5 meq/100 ml) than in PY glucose. We believe that the chromatographic procedures available to us are more reliable than were the earlier distillation procedures used by Tissier and Prévot for the identification of the products.

The temperature for optimum growth is about 37°C; strain PVA B₃/63 grows well in Rosenow medium at 45°C, but it does not grow at 50°C and grows only sparsely at 25 to 30°C. No growth was observed after aerobic incuba-

FIG. 1. Cellular morphology of *Fusobacterium perfoetens* PVA B₃/63. Gram stain of 24-h-old Rosenow broth culture. Magnification, $\times 1,000$.

FIG. 2. Cellular morphology of *Fusobacterium perfoetens* PVA B₃/63. Gram stain of 24-h-old PYG-broth culture. Magnification, $\times 1,000$.

tion, although cultures on surface-inoculated blood agar plates exposed to the air for 24 h and later incubated in an anaerobic GasPak jar grew very well.

The other biochemical characteristics of the six isolates are in good agreement with the characteristics cited in the aforementioned descriptions of this organism. Tissier stated that *B. perfoetens* fermented only glucose and su-

crose. We obtained the same results, although weakly positive reactions were noted with fructose, galactose, trehalose, and mannose.

The G+C content of the DNA of *S. perfoetens* described by Sebald (1962) (10) (chromatographic separation) was 30 mol%. Using a method based upon absorbancy ratios (2), we found a G+C content of 28.2 mol% for strain PVA B₃/63.

TABLE 1. *Characteristics of Fusobacterium perfoetens*

Characteristic	Isolate PVA B ₃ /63 ^a	Five other iso- lates	Weinberg et al. (12)	Prévot et al. (9)
Gas from glucose	+	+	+	+
Hemolysis	-	-	.	.
Catalase produc- tion	-	-	.	.
Motility	-	-	-	-
Production of:				
Indole	-	-	-	-
H ₂ S, CO ₂ , NH ₃	+	+	+	+
Growth in:				
20% Bile	-	-	.	.
Brilliant green (0.001%)	+	+	.	.
Polymyxin (0.001%)	-	-	.	.
Nitrate reduction	-	-	.	.
Esculin hydrolysis	-	-	.	.
Milk clotting	-	-	-	-
Propionate ← threonine	+	+	.	.
Threonin deami- nation	+	+	.	.
Gelatin	-	-	.	.
Acid from:				
Amygdaline	-	-	.	.
Arabinose	-	-	.	.
Cellobiose	-	-	.	.
Dextran	-	-	.	.
Fructose	w	w	.	.
Galactose	w	w	.	.
Glucose	A	A	+	+
Glycerol	-	.	.	.
Lactose	-	-	-	-
Maltose	-	-	.	-
Mannitol	-	-	.	.
Mannose	w	w	.	.
Melibiose	-	-	.	.
Raffinose	-	-	.	.
Rhamnose	-	-	.	.
Ribose	-	.	.	.
Salicin	-	-	.	.
Sorbitol	-	.	.	.
Sucrose	A	A(w)	+	+
Trehalose	w	w	.	.
Xylose	-	.	.	.
Acids from PY- glucose broth cultures (meq/100 ml)				
Acetic				
Average	0.3	0.4	-	+
Range	0.1-0.6	0.1-0.9		
Butyric				
Average	1.4	1.5	+	+
Range	1.1-1.9	1-2.5		
Lactic				
Average	1.0	1.2	+	+
Range	0.8-1.3	0.6-1.8		
Valeric	-	-	+	-

^a Results from five replicate test sets. Symbols: +, positive reaction (100% of the strains or of the test sets positive); -, negative reaction; A, pH below 5.7; w, pH 5.7 to 6.0; ., not tested or reported.

TABLE 2. *Susceptibility of Fusobacterium perfoetens strains to antibiotics*

Antibiotic	Antibiotic conc (μg/ disk) ^a	Zone diam (mm) of inhibition of:	
		PVA B ₃ /63 ^b	Five other isolates
Aureomycin	30	25	25-27
Ampicillin	10	6	6
Bacitracin	10 ^c	6	. ^d
Carbenicillin	50	6	6
Clindamycin	2	30	29-34 ^e
Chloramphenicol	30	24	23-28
Colistin	10	17	16-18
Erythromycin	15	6	.
Kanamycin	30	6	.
Neomycin	30	6	6-12
Oleandomycin	15	6	.
Penicillin	10 ^c	6	.
Polymyxin	300 ^c	17	17-19
Rifampin	30	17	18-22
Terramycin	30	27	25-33
Tetracycline	30	24	23-29
Vancomycin	30	6	6

^a Filter paper disks (6 mm in diameter; BBL susceptibility disks).

^b Results are mean values of three replicates.

^c Antibiotic concentration expressed in units.

^d Not tested.

^e Range of diameter of zone.

The susceptibilities of the six isolates to a number of antibiotics is given in Table 2. All of the isolates had the same antibiotic susceptibility. They were resistant to penicillins, erythromycin, oleandomycin, bacitracin, vancomycin, kanamycin, and neomycin. They were highly susceptible to tetracyclines, chloramphenicol, and clindamycin and moderately susceptible to colistin, polymyxin, and rifampin.

Proposed neotype strain of *F. perfoetens*. From the data presented in Table 1, it is obvious that the characteristics of PVA B₃/63 (ATCC strain 29250) agree not only with those recorded in Tissier's original description of *Cocobacillus anaerobius perfoetens* (syn.: *F. perfoetens*) but also with those subsequently described for this species by Weinberg et al. (12), Prévot (8), and Séblad (10). Therefore, we propose strain PVA B₃/63 as the neotype strain of *Fusobacterium perfoetens* (Tissier) Moore and Holdeman.

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REPRINT REQUESTS

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