Fusobacterium simiae, a New Species from Monkey Dental Plaque

JØRGEN SLOTS AND THOMAS V. POTTS

Department of Oral Biology and Periodontal Disease Clinical Research Center, State University of New York at Buffalo, Buffalo, New York 14226

We describe the morphological, genetic, and biochemical features of *Fusobacterium simiae*, a new species which accommodates isolates from monkey dental plaque. *F. simiae* can be distinguished from currently recognized *Fusobacterium* species by its ability to produce indole, hydrolyze hippurate, grow in 2% oxgall, and ferment fructose and glucose but not 27 other carbohydrates. The type strain of this new species is 7511 R2-13 (= ATCC 33568).

There have been numerous reports on the importance of obligately anaerobic bacteria in clinical infections in humans. In contrast, relatively little is known about the role of anaerobes in clinical veterinary medicine. Furthermore, there are very few data on the range of hosts which can be colonized by a given bacterial species.

In this paper we describe a new species of *Fusobacterium* which resides in the mouth of the stump-tailed macaque (*Macaca arctoides*). The oral cavity of this monkey resembles that of humans in various aspects, including microflora, salivary composition, and gingivitis histopathology (9), and therefore this monkey has been used as an animal model in dental disease research.

MATERIALS AND METHODS

A total of 10 isolates (7511 R2-13, 7511 R3-10, 7511 L3-25, 7511 R1-11, 7511 R6-23, 7511 R4-19, 7728 R1-5, 7728 L4-15, 7728 R4-1, and 7728 R4-26) of previously undescribed fusobacteria were obtained at different sampling times from four diseased periodontal sites of two *M. arctoides* females. These organisms were isolated and characterized by procedures routinely used in our laboratory (8; Slots, Arch. Microbiol., in press). The reference strains used in the deoxyribonucleic acid (DNA) studies (Table 1) were obtained from the American Type Culture Collection. DNA was purified by the technique of Marmur (4). The guanine-plus-cytosine content of the DNA was determined by the thermal melting point method (5). DNA-DNA hybridization was carried out at 56°C and was assessed by the S1 nuclease assay (1).

RESULTS

The strains which we isolated were gram-negative, nonmotile, anaerobic organisms. The cells were 0.7 to 1.0 by 5.0 to 8.0 μm and were cigar shaped with pointed ends, a morphology typical of fusobacteria (Fig. 1). After anaerobic incubation for 5 days on brain heart infusion blood agar plates, the colonies were 1.5 to 2.0

mm in diameter, circular, convex, entire, grayish, translucent, and slightly glistening.

The DNA study revealed that the three monkey strains examined contained 27 to 28.5 mol% guanine plus cytosine (Table 1). In addition, the hybridization data obtained with a ^3H -labeled strain 7511 R2-13 DNA probe showed that these three strains were genetically similar (Table 1). Also, the DNA-DNA homologies with type strains of other *Fusobacterium* species were less than 50% (Table 1), indicating that the monkey strains possessed a unique DNA composition.

Table 2 shows the biochemical test results for the 10 isolates. There was a close overall similarity among the strains studied. These strains fermented glucose and fructose but not the 27 other carbohydrates studied. They were indole

TABLE 1. DNA characteristics of three strains of *F. simiae* and six reference strains

Source of unlabeled DNA	Guanine-plus-cytosine content (mol%) ^a	% hybridization with strain 7511 R2-13 [^3H]DNA probe
<i>F. simiae</i> 7511 R2-13	28	100
<i>F. simiae</i> 7511 R3-10	28.5	86
<i>F. simiae</i> 7511 L3-25	27	107
<i>F. necrophorum</i> ATCC 25286	34	9
<i>F. varium</i> ATCC 8501	31	8
<i>F. mortiferum</i> ATCC 25557	30	6
" <i>Fusobacterium fusiforme</i> " ATCC 23726 ^b	28	48
<i>F. nucleatum</i> ATCC 25586	27	48
<i>Escherichia coli</i> B	52	4

^a Each guanine-plus-cytosine value is the average of two or more determinations.

^b Name not on the Approved Lists of Bacterial Names (7) and not validly published as of 1 January 1980.

FIG. 1. *F. simiae* cells from brain heart infusion blood agar examined in a wet-mount preparation by phase-contrast microscopy. Bar = 5 μ m.

positive, produced hydrogen sulfide (as determined by lead acetate test paper), reduced litmus milk, hydrolyzed hippurate, and decarboxylated glutamic acid. Otherwise, the organisms were basically inert in commonly used biochemical tests.

The acid end products formed in glucose broth cultures included large amounts of butyric acid and smaller quantities of acetic and propionic acids (Table 3). An analysis of the metabolic products also showed that pyruvate was utilized and propionate was produced from threonine. Lactate was not utilized.

Table 4 shows the results of susceptibility tests. The strains were resistant to most dyes. They were also resistant to erythromycin but susceptible to chloramphenicol, clindamycin, metronidazole, penicillin, and tetracycline. In addition, the organisms grew in the presence of 2% oxgall but did not grow on MacConkey agar, Simmons citrate agar, or Levine eosin methylene blue agar.

TABLE 2. Biochemical characteristics of the 10 *F. simiae* strains, including the type strain

Test or substrate	Results	Test or substrate	Results
Hemolysis (sheep blood)	—	Glutamic acid	+
Catalase	—	Lysine	—
Oxidase	—	Ornithine	—
Benzidine	—	API-ZYM system	
Hydrogen sulfide		Alkaline phosphatase	—
Lead acetate paper	+	Acid phosphatase	—
SIM agar ^a	—	Esterase (butyrate)	+
Indole	+	Esterase-lipase (caprylate)	+
Acetylmethylcarbinol	—	Lipase (myristate)	—
Nitrate to nitrite	—	Leucine aminopeptidase	—
Gas from glucose	—	Valine aminopeptidase	—
Malonate utilization	—	Cystine aminopeptidase	—
Phenylalanine deaminase	—	Trypsin	—
Glucuronate oxidation	—	Chymotrypsin	—
Ammonia from arginine	—	Phosphoamidase	—
Litmus milk (reduced)	+	α -Galactosidase	—
Hydrolysis of:		β -Galactosidase	—
Casein	—	β -Glucuronidase	—
DNA	—	α -Glucosidase	—
Esculin	—	β -Glucosidase	—
Gelatin	—	<i>N</i> -Acetyl- β -glucosaminidase	—
Hippurate	—	α -Mannosidase	—
Lecithin	—	α -Fucosidase	—
Lipid	+	Carbohydrate fermentation ^b	
	(weak)	Glucose	+
Starch	—		(pH decrease of 0.8 U)
Tween 80	—		
Tyrosine	—	Fructose	+
Urea	—		(pH decrease of 1.5 U)
Decarboxylation of:			
Arginine	—		

^a SIM, Sulfide indole motility.

^b No strain produced acid (no pH decrease of more than 0.3 U compared with peptone-yeast extract [PY] broth [pH 7.2]) from adonitol, amygdalin, arabinose, cellobiose, dextrin, dulcitol, esculin, galactose, glycerol, glycogen, inositol, inulin, lactose, maltose, mannose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or xylose.

TABLE 3. Metabolic acid end products produced by *F. simiae*^a

Medium	Amt (meq/100 ml of medium) of the following end products:						
	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Oxalacetic acid	Succinic acid	Phenylacetic acid
PY-glucose	1.5	0.3	4.7	0.5	0.3	0.1	0
PY-pyruvate	NT ^b	NT	NT	0.7	1.6	0.1	0.3
PY-threonine	2.7	0.7	2.1	NT	NT	NT	NT

^a Pyruvate was utilized (disappearance of pyruvic peak in PY-pyruvate broth cultures), and threonine was converted to propionate. Lactate was not utilized.

^b NT, Not tested.

TABLE 4. Susceptibilities of 10 *F. simiae* strains, including the type strain, to inhibitory agents

Test agent or substrate ^a	Growth
MacConkey agar	—
Simmons citrate agar	—
Levine eosin methylene blue agar	—
Tellurite (300 µg/ml) agar	—
Sodium chloride (1.0%) agar	—
Sodium fluoride (10 µg/ml) agar	+
Sodium fluoride (50 µg/ml) agar	—
Oxgall (2.0%) agar or broth	+
Chlorhexidine (0.1 µg/ml) agar	—
Alcian blue (1,000 µg/ml) agar	+
Azocarmine B (1,000 µg/ml) agar	+
Basic fuchsin (1,000 µg/ml) agar	+
Bromocresol green (1,000 µg/ml) agar	+
Crystal violet (100 µg/ml) agar	+
Crystal violet (1,000 µg/ml) agar	—
Diazoblue B (10 µg/ml) agar	+
Diazoblue B (50 µg/ml) agar	—
Evans blue (1,000 µg/ml) agar	+
Lissamine green (1,000 µg/ml) agar	+
Malachite green (100 µg/ml) agar	+
Malachite green (1,000 µg/ml) agar	—
Methylene blue (50 µg/ml) agar	+
Methylene blue (100 µg/ml) agar	—
Naphthol blue black (1,000 µg/ml) agar	+
Neutral red (100 µg/ml) agar	+
Neutral red (1,000 µg/ml) agar	—
Phenol red (1,000 µg/ml) agar	+
Safranin (1,000 µg/ml) agar	+
Superchrome blue B (100 µg/ml) agar	+
Superchrome blue B (1,000 µg/ml) agar	—
Bacitracin (10 µg/ml) agar	+
Chloramphenicol (1 µg/ml) agar	—
ClinadAMYcin (1 µg/ml) agar	—
Erythromycin (25 µg/ml) agar	+
Kanamycin (10 µg/ml) agar	+
Metronidazole (1 µg/ml) agar	—
Neomycin (10 µg/ml) agar	+
Penicillin G (5 µg/ml) agar	—
Polymyxin B (5 µg/ml) agar	—
Streptomycin (10 µg/ml) agar	+
Tetracycline (1 µg/ml) agar	—
Vancomycin (10 µg/ml) agar	+

^a Wilkins-Chalgren agar was the basal medium used for testing susceptibility.

DISCUSSION

In humans and other animals, fusobacteria have been associated with clinical syndromes

ranging from asymptomatic infections to fatal disseminated diseases (2, 6). Although this group of bacteria obviously contains potentially pathogenic members, some confusion concerning its taxonomic status still remains.

The organism described here has the characteristics typical of the genus *Fusobacterium* (6). It is a pointed, nonsporeforming, gram-negative, anaerobic, rod-shaped bacterium which produces butyric acid as a major metabolic product. It does not produce isobutyric or isovaleric acids, products which are detected in the few butyric acid-producing *Bacteroides* species that have been described.

This organism is genetically distinct from the type strains (7) of *Fusobacterium nucleatum*, *Fusobacterium necrophorum*, *Fusobacterium varium*, and *Fusobacterium mortiferum*. Its biochemical reaction profile is quite similar to that of *F. varium* (6), but it fails to ferment mannose, produce hydrogen sulfide in sulfide indole motility medium, or form gas in an agar stab. Unnamed *Fusobacterium* strains which appear to possess biochemical characteristics similar to the characteristics of this organism have been described by Holdeman et al. (3). However, the DNA relatedness between these strains and our strains has not been determined, and therefore no definitive conclusion with respect to taxonomic relationships can be made.

In summary, the monkey strains described in this study differ genetically and physiologically from the currently recognized species of *Fusobacterium*. For these strains we propose the name *Fusobacterium simiae* sp. nov. (sim'-i-ae-L. fem. noun *simia* monkey; L. gen. noun *simiae* of the monkey). The type strain of this species is strain 7511 R2-13 (= ATCC 33568).

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grant DE04898 from the National Institute of Dental Research. The assistance of Homer S. Reynolds is greatly appreciated.

REPRINT REQUESTS

Address reprint requests to: Dr. Jørgen Slots, Department of Oral Biology and Periodontal Disease Clinical Research Center, State University of New York at Buffalo, Buffalo, NY 14226.

LITERATURE CITED

1. Crosa, J. H., D. J. Brenner, and S. Falkow. 1973. Use of a single strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J. Bacteriol.* 115:904-911.
2. Finegold, S. M. 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.
3. Holdeman, L. V., E. P. Cato, and W. E. C. Moore. 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
4. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3:208-217.
5. Marmur, J., and C. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109-118.
6. Moore, W. E. C., and L. V. Holdeman. 1975. *Fusobacterium* Knorr 1922, p. 404-416. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
7. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* 30:225-420.
8. Slots, J., and E. Hausmann. 1979. Longitudinal study of experimentally induced periodontal disease in *Macaca arctoides*: relationship between microflora and alveolar bone loss. *Infect. Immun.* 23:260-269.
9. Slots, J., E. Hausmann, C. Mouton, L. F. Ortman, P. G. Hammond, and R. J. Genco. 1980. The relationship between the periodontal microflora and alveolar bone loss in *Macaca arctoides*, p. 109-121. In D. W. Lambe, Jr., R. J. Genco, and K. J. Mayberry-Carson (ed.), *Anaerobic bacteria: selected topics*. Plenum Publishing Corp., New York.