Fusobacterium alocis sp. nov. and *Fusobacterium sulci* sp. nov. from the Human Gingival Sulcus

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Two new species, *Fusobacterium alocis* and *Fusobacterium sulci*, are described. They were isolated principally from subgingival areas associated with gingivitis or periodontitis. Strains of these species were nonreactive in the biochemical tests usually used and were differentiated most readily from each other and from other *Fusobacterium* species by patterns of soluble cellular proteins determined by polyacrylamide gel electrophoresis. The type strains of the species are *F. alocis* ATCC 35896 and *F. sulci* ATCC 35585.

During studies of the bacterial flora of gingival sulci of humans with gingivitis or periodontitis, we found that 170 isolates from 77 samples from 44 different persons had characteristics distinct from those of any species previously described. Cells of these isolates were nonsporeforming, gram-negative, obligately anaerobic rods that produced butyrate but not isobutyrate or isovalerate from peptone. They were, therefore, members of the genus *Fusobacterium* (7). By differential criteria presently accepted (1, 7), the isolates phenotypically resembled *Fusobacterium russii*: neither carbohydrates nor proteins were attacked, esculin was not hydrolyzed, propionate was not formed from threonine, and indole was not produced. However, electrophoretic patterns of soluble cellular proteins were quite distinct from those of not only *F. russii* strains but also all the described *Fusobacterium* species.

In previous papers (5, 6, 8-11), we had separated these strains into three species, *F. russii* D (dental *F. russii*), *Fusobacterium* D2, and *Fusobacterium* D9, on the basis of differences in growth characteristics (*Fusobacterium* D2 and D9 grew more slowly and more poorly than did *F. russii* D), motility (*Fusobacterium* strain D9 often showed a twitching or end-over-end type of motility), and hydrogen production (*F. russii* D usually produced abundant hydrogen, and the others produced little or none). However, since these strains grew poorly, identification by these criteria could not be made with confidence. When strains of the three groups were subjected to polyacrylamide gel electrophoresis, two distinct patterns of soluble cellular proteins emerged.

Because only 3 of the 170 isolates were from healthy gingival crevices and because the isolates were found in more subgingival than supragingival samples (67 subgingival of 77 total positive samples), these strains may be significant in the development of human periodontitis. For these strains we propose the following new species and type strains: *Fusobacterium alocis* VPI D40B-5 (=ATCC 35896) and *Fusobacterium sulci* VPI D45A-29A (=ATCC 35585).

MATERIALS AND METHODS

All isolates were from supragingival or subgingival samples taken as described previously (9, 10).

Reactions were determined in prereduced, anaerobically sterilized media by anaerobic methods described in the *Anaerobe Laboratory Manual* (1). Patterns of soluble cellular proteins were determined by polyacrylamide gel electrophoresis without sodium dodecyl sulfate (4).

John L. Johnson determined the guanine-plus-cytosine content of DNA from each type strain by the thermal melting point method (2, 3).

Antibiotic susceptibilities were tested by the broth-disk method of Wilkins and Thiel (12).

RESULTS AND DISCUSSION

Fusobacterium alocis sp. nov. (a'lo.kis. Gr. n. *alox* a furrow; N.L. gen. n. *alocis* of a furrow, referring to its isolation from a crevice of the gums). Cells are gram-negative, nonsporeforming, obligately anaerobic rods, 0.4 to 0.7 by 1.5 to 7.0 μm , with rounded to tapered ends. They occur singly, in pairs, or occasionally in short chains. Of 117 strains, 6 have slow twitching or end-over-end type of motility, but flagella are not detected.

Surface colonies on anaerobic blood agar plates are pinpoint to 1.0 mm, circular, entire, flat to low convex, translucent to transparent, shiny, smooth, and nonhemolytic. No internal colony structure is visible.

The optimum temperature for growth is 37°C, at which growth is only moderate at best. Slight growth occurs at 30 and 45°C; there is little or no growth at 25°C. Growth can be stimulated by the addition of rabbit serum (final concentration, 10 to 15% [vol/vol]) and cocarboxylase (final concentration, 5 $\mu\text{g/ml}$). Most strains grow better in an atmosphere of 10% CO_2 -90% N_2 than of 100% CO_2 .

Cultures in peptone-yeast extract-turkey (PYG) broth have a fine granular sediment and no turbidity after incubation for 5 to 7 days. The final pH of cultures in PYG broth and in basal medium incubated in a CO_2 - N_2 atmosphere is 6.8 to 7.1; under 100% CO_2 , the final pH of each is 5.9 to 6.1.

Although little or no gas is formed in PYG deep agar cultures, abundant H_2 is detected in the headspaces of broth cultures when there is sufficient growth. No H_2S is detected in sulfide-indole-motility medium (BBL Microbiology Systems). No acid is produced from adonitol, amygdalin, L-arabinose, cellobiose, dextrin, dulcitol, *meso*-erythritol, esculin, D-fructose, D-galactose, D-glucose, glycerol, glycogen, inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, pectin, raffinose, rhamnose, D-(-)-ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, trehalose, or D-xylose. Indole, acetylmethylcarbinol, catalase, lecithinase, lipase, urease, oxidase, and deoxyribonuclease are not produced. Esculin, starch, and hippurate are not hydrolyzed. Nitrate is not reduced; neither resazurin nor neutral red is reduced. There is no reaction in milk and no digestion of gelatin or chopped meat.

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FIG. 1. Polyacrylamide gel electrophoresis protein patterns of the type strains of species in the genus *Fusobacterium*. Lanes 1 and 2, *Streptococcus faecalis* U4-20, a reference strain; lanes 3 through 6, *F. nucleatum* ATCC 25586^T (lane 3), VPI 4355 (lane 4), VPI 14D4 (lane 5), and VPI E1D1 (lane 6) (these strains have the phenotypic characteristics of *F. nucleatum* but are distinct genetic subgroups [Y. Selin and J. L. Johnson, J. Dent. Res. {Special Issue A} 60:415, abstr. no. 420, 1981]); lane 7, *F. gonidiaformans* ATCC 25563^T; lane 8, *F. mortiferum* ATCC 25557^T; lane 9, *F. naviforme* ATCC 25832^T; lane 10, *F. necrogenes* ATCC 25556^T; lane 11, *F. necrophorum* ATCC 25286^T; lane 12, *F. perfoetens* ATCC 29250^T; lane 13, *F. periodonticum* ATCC 33693^T; lane 14, *F. prausnitzii* ATCC 27768^T; lane 15, *F. russii* ATCC 25533^T; lane 16, *F. simiae* ATCC 33568^T; lane 17, *F. sulci* ATCC 35588^T; lane 18, *F. varium* ATCC 8501^T.

The products of fermentation in PYG broth are small to moderate amounts of butyrate and acetate. Pyruvate, DL-lactate, D-gluconate, and DL-threonine are not utilized.

All tested strains are susceptible to chloramphenicol (12 µg/ml), clindamycin (1.6 µg/ml), erythromycin (3 µg/ml), and tetracycline (6 µg/ml); 1 of 20 strains tested is resistant to penicillin (2 U/ml).

The principal habitat of *F. alocis* is the human gingival sulcus. It has been isolated from a submandibular abscess and from a mouth ulcer.

The type strain of *F. alocis* is VPI D40B-5 (=ATCC 35896). The guanine-plus-cytosine content of the DNA of this strain is 34 mol%.

Fusobacterium sulci sp. nov. (sul'ki. L. gen. n. *sulci* of a furrow, referring to its habitat, the human gingival sulcus).

Cells are gram-negative, nonmotile, nonsporeforming, obligately anaerobic, straight rods, 0.4 to 0.7 by 1.0 to 7.0 µm, with rounded ends. They occur singly, in pairs, or in short chains, often with cells of different sizes in the same chain.

Surface colonies on blood agar plates are pinpoint to 1.0 mm, circular, entire, flat to low convex, translucent, colorless, shiny, smooth, and nonhemolytic. No internal colony structure is visible.

The optimum temperature for growth is 37°C, at which growth is only moderate at best. No growth occurs at 25 or 45°C. Growth can be stimulated by the addition of rabbit serum (10 to 15% [vol/vol]) and cocarboxylase (5 µg/ml) and in an atmosphere of 10% CO₂-90% N₂.

Cultures in PYG broth have a stringy or granular sediment and may be slightly turbid. After 5 to 7 days of incubation under CO₂-N₂, the pH of PYG and basal medium broth cultures is 6.9 to 7.1; under 100% CO₂, the pH of each is 6.0 to 6.2.

Gas is not detected in PYG deep agar cultures; trace to moderate amounts of H₂ may be produced. H₂S is not detected in sulfide-indole-motility medium.

No acid is produced from adonitol, amygdalin, L-arabinose, cellobiose, dextrin, dulcitol, meso-erythritol, esculin, D-fructose, D-galactose, D-glucose, glycerol, glycogen, inositol, inulin, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, pectin, raffinose, rhamnose, D-(-)ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, trehalose, or D-xylose. Indole, acetylmethylcarbinol, catalase, lecithinase, lipase, urease, oxidase, and deoxyribonuclease are not produced. Esculin, starch, and hippurate were not hydrolyzed. Nitrate is not reduced; neither resazurin nor neutral red is reduced. There is no reaction in milk and no digestion of gelatin or chopped meat.

The products of fermentation in PYG broth are moderate to large amounts of butyrate and trace to small amounts of acetate; traces of succinate are occasionally detected. Pyruvate, DL-lactate, D-gluconate, and L-threonine are utilized.

All 15 strains tested are susceptible to chloramphenicol (12 µg/ml), clindamycin (1.6 µg/ml), erythromycin (3 µg/ml), penicillin (2 U/ml), and tetracycline (6 µg/ml).

The principal habitat of *F. sulci* is the human gingival sulcus.

The type strain of *F. sulci* is VPI D45A-29A (=ATCC 35585). The guanine-plus-cytosine content of the DNA of this strain is 39 mol%.

Strains of *F. alocis*, *F. sulci*, and *F. russii* can be distinguished most clearly by the electrophoretic patterns of their

FIG. 2. Polyacrylamide gel electrophoresis protein patterns of *Fusobacterium* strains. Lane 1, *S. faecalis* U4-20, a reference strain; lane 2, *F. russii* ATCC 25533^T; lanes 3 through 12, *F. alocis* ATCC 35896^T (lane 3), D3A16 (lane 4), D6A6 (lane 5), D6B19 (lane 6), D40C8 (lane 7), D45B4 (lane 8), D61D29 (lane 9), D62D20 (lane 10), D73D11 (lane 11), and D84D7 (lane 12); lanes 13 through 17, *F. sulci* ATCC 35585^T (lane 13), E4Y17 (lane 14), D25A5 (lane 15), D26C13 (lane 16), and D83A25 (lane 17).

TABLE 1. Distribution of 117 isolates of *F. alocis* and 53 isolates of *F. sulci* in periodontal diseases^a

Source ^b	<i>F. alocis</i>		<i>F. sulci</i>		Total no. of isolates	No. of people
	% of isolates ^c	% of 30 tested samples	% of isolates ^d	% of 30 tested samples		
MP sub	1.30	19.75	0.70	9.62	5,146	27
MP supra	0.14	4.55	0.14	4.55	694	15
JP sub	1.97	37.50	0	0	1,064	21
JP supra	0.13	3.85	0.25	7.69	797	17
SP sub	1.17	15.22	0.29	8.70	1,711	21
SP supra	0.18	2.70	0.35	5.71	1,130	18
H sub	0	0	0	0	956	19
H supra	0.20	3.12	0.10	3.12	1,000	20
Adult						
EG10	0	0	0	0	562	4
NG10	0	0	0	0	548	11
EG11	0	0	0	0	1,332	4
NG11	0	0	0.79	8.33	380	7
EG12	0	0	0.09	2.78	1,141	4
NG12	0.79	16.67	0	0	379	8
Child						
EG10	0	0	0	0	1,449	4
NG10	0	0	0	0	318	7
EG11	0	0	0	0	1,363	4
NG11	0	0	0	0	220	4
EG12	0	0	0	0	405	4
NG12	0	0	0	0	122	3

^a Data in part are from references 5, 6, and 8 through 10.^b MP, Moderate periodontitis; JP, juvenile periodontitis; SP, severe periodontitis; H, gingiva of healthy persons; sub, subgingival samples taken from the depth of the sulcus; supra, supragingival samples taken from sites coronal to the subgingival sites sampled; EG10, EG11, and EG12, subgingival sites with gingival index scores of 0 (healthy), 1 (inflamed), and 2 (bleeding), respectively, from subjects in experimental gingivitis trials; NG10, NG11, and NG12, samples from subjects with naturally occurring gingivitis (gingival index scores are as for EG10, EG11, and EG12, respectively).^c Incidence of *F. alocis* in 20,717 isolates from 115 persons is 0.56%.^d Incidence of *F. sulci* in 20,717 isolates from 115 persons is 0.26%.

soluble cellular proteins (Fig. 1 and 2). The slow and sparse growth of *F. alocis* and *F. sulci* as compared with that of *F. russii* is a helpful differential characteristic. The production of significant amounts of H₂ by *F. alocis* and little or none by *F. sulci* and *F. russii* also is useful.

F. alocis was more common in the gingival sulcus than was *F. sulci* (Table 1). Both species were more prevalent in the subgingival flora than in residual supragingival flora and in diseased than in healthy sites. No strains were isolated from experimental gingivitis in children; in developing gingivitis in young adults, both species were detected only when the gingiva were inflamed or bleeding. They may, therefore, contribute to the etiology of both gingivitis and periodontitis.

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