Wolinella gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., and Description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from Humans with Periodontal Disease

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We compared 46 strains of gram-negative, asaccharolytic, rod-shaped bacteria which were isolated from humans with gingivitis, periodontal pockets, and lesions in alveolar bone with 10 reference strains of *Eikenella corrodens*, *Vibrio succinogenes*, *Bacteroides ureolyticus*, and species of *Campylobacter*. We divided these 56 strains into seven groups based on the guanine-plus-cytosine contents of their deoxyribonucleic acids, their deoxyribonucleic acid homologies, and cluster analyses of their phenotypic features. A total of 23 of the fresh isolates showed more than 90% similarity (Jaccard coefficient) with *E. corrodens*. Growth of the remaining 23 isolates was enhanced in broth cultures by formate and fumarate. These isolates were not members of *B. ureolyticus*, *V. succinogenes*, or previously described species of *Campylobacter*; they constituted three distinct new species. We propose *Bacteroides gracilis* sp. nov. (type strain, ATCC 33236) as the name for seven isolates of slender, gram-negative, nonmotile, anaerobic, rod-shaped bacteria that corroded agar and had deoxyribonucleic acid guanine-plus-cytosine contents of 44 to 46 mol%. All of the remaining isolates were motile by means of a single polar flagellum. Ten anaerobic strains were similar to *V. succinogenes* in phenotypic characteristics and guanine-plus-cytosine contents. However, these strains were distinct from *V. succinogenes* on the basis of deoxyribonucleic acid homology results. We propose *Wolinella* as the name of a new genus to include anaerobic, asaccharolytic, rod-shaped bacteria with single polar flagella and deoxyribonucleic acid guanine-plus-cytosine contents of 42 to 49 mol%. *Wolinella succinogenes* (Wolin et al.) comb. nov. is designated the type species of the genus, and ATCC 29543 is the type strain of *W. succinogenes*. We propose *Wolinella recta* sp. nov. (type strain, ATCC 33238) as the name for nine of the strains that formed a related but distinct group. We propose *Campylobacter concisus* sp. nov. (type strain, ATCC 33237) as the name for the six isolates of noncorroding, microaerophilic, gram-negative, rod-shaped bacteria that have predominantly curved cells and deoxyribonucleic acid guanine-plus-cytosine contents of 34 to 38 mol%. The description of the genus *Campylobacter* is amended to include species with deoxyribonucleic acid guanine-plus-cytosine contents of 30 to 38 mol%.

We isolated gram-negative, asaccharolytic, rod-shaped bacteria from humans with lesions of advanced destructive periodontal disease; the majority of these bacteria pitted or corroded agar media. These organisms were nonmotile, or they exhibited either cell "twitching" or active darting motility when they were examined by dark-field microscopy. These isolates were numerically dominant in some active disease sites (31). They were difficult to separate into distinct groups on the basis of routine characterization studies, and some of them were different from

isolates of known species. In this investigation we compared strains isolated from humans with advanced periodontal diseases and strains from other oral sites (20) with reference strains of *Eikenella corrodens* (10), *Bacteroides ureolyticus* (11), *Vibrio succinogenes* (35), and *Campylobacter sputorum* (15, 25).

We characterized the strains extensively and used their phenotypic features for a cluster analysis. The guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of the strains and DNA hybridizations were used to

clarify the relationships of phenotypically similar organisms. The feature frequencies of the homology groups were examined to determine which biochemical characteristics distinguished similar organisms. The same strains were examined serologically (1) and by using electron microscopy of thin sections (14). All of the isolates from lesions in alveolar bones and 17 strains from periodontal pockets were identified as members of *E. corrodens*. We discuss the taxonomic status of the remaining periodontal isolates, which included strains that corroded agar and strains whose growth was stimulated by formate and fumarate. We propose a new genus for the organism previously known as *Vibrio succinogenes* and three new species to include the remaining periodontal isolates.

MATERIALS AND METHODS

Bacterial strains. The origins and strain designations of the alveolar and periodontal strains used in this investigation are shown in Table 1. Using an anaerobic gas-flushed sampling device (18), we obtained fresh isolates from samples from the apical regions of periodontal pockets. Gingivitis was defined as gingival inflammation without loss of alveolar bone and periodontal pocket depths of less than 5 mm. Periodontitis samples were from adults who had gingivitis, advanced alveolar bone loss, and pocket depths greater than 6 mm. Periodontosis (juvenile periodontitis) samples were from adolescent patients with a molar-incisor pattern of advanced bone loss and minimal clinical gingival inflammation. We also used the gas-flushed sampling device to obtain samples from alveolar bone cavities associated with facial neuralgia (20). The methods used for sample dispersion, dilution, and plating have been described previously (31). We chose the gram-negative, asaccharolytic, rod-shaped bacteria that corroded agar for further study. Morphologically and physiologically similar organisms that did not corrode agar plates also were included. Reference strains (Table 2) were kindly provided by M. J. Wolin (Department of Health, Albany, N.Y.), R. M. Smibert (Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg), W. J. Loesche (University of Michigan, Ann Arbor), and the American Type Culture Collection, Rockville, Md.

Media and maintenance of strains. The organisms were maintained in the laboratory by weekly transfer on commercially prepared Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood. The broth medium used to grow organisms as sources of inocula or for mass cultures was Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) supplemented with 5 mg of hemin per liter and either 2 g of potassium nitrate per liter (for the capnophilic strains [*E. corrodens*]) or 2 g of sodium formate per liter and 3 g of sodium fumarate per liter (for all motile and all anaerobic, nonmotile isolates). Anaerobes were incubated at 35°C in a 80% N₂-10% H₂-10% CO₂ atmosphere. Reference *Campylobacter* strains were maintained in a microaerophilic atmosphere containing 80% N₂, 7.5% H₂, 7.5% CO₂, and 5% O₂. Young colonies from blood agar

TABLE 1. *Alveolar and periodontal strains isolated at the Forsyth Dental Center*

Origin	FDC strain(s) ^a
Nonmotile strains	
Gingivitis	384, 557, 1075
Periodontitis	373, 374, 401, 402, 404, 406, 464, 467, 468, 469, 470, 479, 568, 1073, 1074, 1083, 1084
Periodontosis	1078, 1079, 1080, 1081
Alveolar abscess	1006, 1007, 1008, 1010, 1011, 1012
Motile strains	
Gingivitis	483, 484, 569, 1086, 1087
Periodontitis	288, 521, 522, 267, 285, 286, 302, 303, 371, 372
Periodontosis	1219

^a FDC, Forsyth Dental Center, Boston, Mass.

plates were placed in broth supplemented with 5% dimethyl sulfoxide for slow freezing and storage in liquid nitrogen.

Characterization. Cell morphology and either active motility or cell twitching were determined by dark-field microscopy of young broth cultures. Todd-Hewitt broth partially solidified with 0.3% agar (Difco) also was used to test for motility. Carbol fuchsin was used as the counterstain in the Gram stain technique. Colonial morphology was determined by examining surface growth on agar plates with a ×10 dissecting microscope, using direct and oblique light.

The basal medium for biochemical characterization tests in broth contained (per liter) 3.1 g of beef extract (Difco), 11.2 g of yeast extract (Difco), 8.8 g of Trypticase (BBL), 2 g of NaCl, 0.4 g of Na₂HPO₄, 0.25 g of Na₂CO₃, and 5 mg of hemin; this medium was supplemented with either 2 g of potassium nitrate per liter or 2 g of sodium formate per liter and 3 g of sodium fumarate per liter, as described above. Each inoculum was added to freshly prepared basal medium, and 2-ml volumes of the mixture were pumped aseptically into tubes containing 0.2-ml portions of 10-fold concentrations of the test reagents (29). Broth tests included fermentation of carbohydrates, reduction of nitrate, nitrite, neutral red, and benzyl viologen, decarboxylation of lysine, ornithine, and arginine, production of urease, indole, and ammonia, evolution of gas, survival at 80°C for 10 min, and production of hydrogen sulfide by blackening of lead acetate strips. Biochemical characterization tests were repeated on three separate occasions.

We used an agar replicating technique with a Steers replicator (30) to determine the ability of isolates to grow at different temperatures and in different atmospheres on Trypticase soy agar containing 5% sheep blood (29). The atmospheres tested were anaerobic (80% N₂, 10% CO₂, 10% H₂), microaerophilic (5% O₂, 7.5% CO₂, 7.5% H₂, 80% N₂), and air supplemented with 10% CO₂. Minimal inhibitory concentrations of antibiotics and hydrolysis of starch, gelatin, esculin, and casein were determined on a basal medium containing (per liter) 15 g of Trypticase (BBL), 5 g of yeast extract (Difco), 2 g of sodium pyruvate, 1 g of sodium formate, 1.5 g of sodium fumarate, 100 mg of

TABLE 2. *Reference strains*^a

Species or subspecies	Strain	Source	Origin
<i>Eikenella corrodens</i>	ATCC 23834	ATCC	Sputum
	FDC 598	VPI	Human clinical
<i>Bacteroides ureolyticus</i>	VPI 7814	VPI	Anal fistula (D. W. Lambe 404-72E)
	VPI 7815	VPI	Human clinical (Jackson B912)
<i>Campylobacter sputorum</i> subsp. <i>bubulus</i>	FDC 616	Loesche	Lamb
<i>Campylobacter sputorum</i> subsp. <i>sputorum</i>	VPI S17	VPI	Oral cavity
" <i>Campylobacter fetus</i> subsp. <i>jejuni</i> " ^b	VPI H641	VPI	Human clinical
<i>Campylobacter fetus</i>	VPI 1176	VPI	Human clinical
<i>Vibrio succinogenes</i>	FDC 602 (=ATCC 29543) ^c	Wolin	Bovine rumen
	VPI 9584	VPI	Alveolar abscess (D. W. Lambe)

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; FDC, Forsyth Dental Center, Boston, Mass.; VPI, Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg.

^b Not on the Approved Lists of Bacterial Names (24).

^c This strain was deposited with the American Type Culture Collection.

sodium succinate, 5 g of sodium chloride, 5 mg of hemin, and 20 g of agar. This medium is a modification of medium V (34), which was devised for minimal inhibitory concentration testing of antibiotics. Growth in the presence of selected dyes and inhibitors was tested on Trypticase soy agar supplemented with 5% sheep blood, 1 g of sodium formate per liter, and 1.5 g of sodium fumarate per liter. The dye and inhibitor concentrations used are indicated below (see Tables 4 and 5). Growth equal to the growth in the basal medium without the added inhibitor was considered a positive reaction.

Acid end products were determined by gas-liquid chromatography of the butyl esters in the culture supernatant of the basal medium used for biochemical characterization tests in broth (22). Since complex media contain small amounts of organic acids, uninoculated media were run as controls, and differences between test results and control results greater than 4 mM were considered positive. Gaseous end products of metabolism were determined by growing the organisms for 7 days in Todd-Hewitt broth supplemented with 5 mg of hemin per liter and either 2 g of potassium nitrate per liter or 2 g of sodium formate per liter plus 3 g of sodium fumarate per liter in septum-stoppered tubes (catalog no. 2047; Bellco Glass, Inc., Vineland, N.J.). The atmosphere over the tubes was replaced by 100% argon at the time of inoculation. After growth, 0.5 ml of the headspace gas was injected into a model 1200 gas partitioner (Fisher Scientific Co., Pittsburgh, Pa.) equipped with a column (6.5 feet [1.98 m] by 0.125 inch [3.2 mm]) packed with 80- to 100-mesh Column-pak PQ and a column (11 feet [3.35 m] by 0.1875 inch [4.8 mm]) packed with 60- to 80-mesh Molecular Sieve 13X in series. The oven temperature was 45°C, and argon (flow rate, 25 ml/min) was the carrier gas. Chromatography of the culture headspace gas with authentic gas standards was used to verify retention times. The gas standards used were carbon dioxide, carbon monoxide, hydrogen, nitrogen, oxygen, nitrous oxide, and methane.

Computer analysis. A total of 155 features were recorded for each strain. The simple coefficient and the Jaccard coefficient (28) were determined. The simple matching coefficient determined the percentage of both positive and negative matches between two strains. The Jaccard coefficient, which determined the percentage of positive matches compared with the total of positive matches and mismatches, was used for the cluster analyses because it excluded negative matches and was considered more appropriate for this investigation, in which many tests were negative. A cluster analysis was performed by using an unweighted average linkage sort (28). The mean similarity and standard deviation were determined for organisms within each cluster analysis phenon and between pairs of phenons. The feature frequencies of the DNA homology groups and cluster analysis phenons were determined. In addition to the characteristics listed below and in Table 4, cell width, length, and shape, colonial morphology (convex or spreading and corroding), and growth in the presence of antibiotics at selected concentrations also were included in the data base used for the cluster analysis.

Extraction of DNA and determination of the G+C content. Broth cultures (2 to 3 liters) were grown to log phase in appropriately supplemented Todd-Hewitt broth and were harvested by centrifugation. DNA was isolated by a modification (29) of the Marmur method (16).

A recording spectrophotometer equipped with a thermoprogrammer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) was used to determine both the G+C contents and the DNA-DNA reassociation values.

The DNA solutions were dissolved in a phosphate buffer containing 9.47 g of Na₂HPO₄ per liter and 9.2 g of NaH₂PO₄ per liter. G+C contents were determined by thermal denaturation of purified unsheread DNAs (17). DNA solutions were used at concentrations of approximately 45 µg/ml, and the DNA of *Escherichia coli* strain B or strain K12 was included in each run as

a standard. Each value reported represents the mean of three determinations.

DNA-DNA hybridization. The relatedness between different DNA pairs was measured by comparing the renaturation rates of DNA solutions from pure cultures with the renaturation rates of mixtures (5). Each renaturation experiment was run in duplicate. We determined the similarities of a representative strain from each phenotypic group to the remaining strains in that group and, where possible, to representatives of other groups. When phenotypic groups contained strains showing low DNA homology, additional strains were selected for further hybridizations. All fresh isolates were tested against one or more reference strains.

RESULTS AND DISCUSSION

Historically, the classification of gram-negative, asaccharolytic organisms that corrode agar surfaces has been ambiguous. Four different organisms have been described as "*Bacteroides corrodens*" (7, 11, 12, 21). One aerobic to capnophilic group included lysine decarboxylase-positive, ornithine decarboxylase-positive, oxidase-positive strains with G+C contents of 58 to 60 mol%. This species was renamed *Eikenella corrodens* (10), and a urease-positive strict anaerobe with a G+C content of 27 to 28 mol% was considered to be *Bacteroides corrodens*; Jackson and Goodman (11) later renamed this latter organism *Bacteroides ureolyticus*.

Jackson and Goodman (11) mentioned two additional agar-corroding organisms that were motile and had DNA G+C contents of 43 to 45 mol%. Apparently motile, gram-negative, asaccharolytic, rod-shaped bacteria with similar physiological characteristics have been isolated rather frequently from a number of ecological sites. *V. succinogenes*, which was isolated by Wolin et al. (35) from a bovine rumen, is an agar-corroding, motile organism with a G+C content of 46 mol% and a metabolism similar to that of *B. ureolyticus* (27). Phenotypically similar strains identified as *V. succinogenes* and *B. ureolyticus* were isolated from humans. These microorganisms grew optimally in broth media containing formate and fumarate (27). An organism that was tentatively identified as *V. succinogenes* and had a G+C content of 48 mol% was isolated from a gingival sulcus by van Palenstein Helderman and Winkler (32). *Campylobacter* species share many characteristics with members of the above-mentioned taxa but have lower G+C contents (30 to 35 mol%). For example, *Campylobacter sputorum* subsp. *sputorum* is microaerophilic, has curved and helical cells, and, in common with *B. ureolyticus* and *V. succinogenes*, is stimulated in its growth in broth media by formate and fumarate (25, 26). For

these reasons, we included reference *E. corrodens*, *B. ureolyticus*, *V. succinogenes*, and *Campylobacter* strains to compare them with our fresh oral isolates.

The 56 strains used in this taxonomic study of gram-negative, asaccharolytic, rod-shaped bacteria had a number of characteristics in common: (i) gram-negative rods; no branching or endospore formation detected; production of gray, translucent or transparent colonies (except for reference *Campylobacter* strains); (ii) growth in a microaerophilic atmosphere; reduction of nitrate; production of carbon dioxide; (iii) failure to hydrolyze starch, dextran, esculin, casein, DNA, and gelatin; (iv) failure to produce hydrogen peroxide, lecithinase, lipase, indole, ammonia, acetylmethylcarbinol, and gas in glucose broth; (v) failure to produce acid from adonitol, amygdalin, arabinose, cellobiose, dextran, dulcitol, fructose, esculin, galactose, glucose, glycerol, inositol, inulin, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or xylose; (vi) growth in the presence of 0.00002 g of malachite green per liter, 0.0125 g of brilliant green per liter, or 0.05 g of Evans blue per liter; and (vii) no growth in the presence of 40 g of sodium chloride per liter or 0.5 g of indulin scarlet per liter. A total of 78 characteristics varied, and a cluster analysis of the phenotypic features separated the organisms into seven groups, which are illustrated in a trellis diagram in Fig. 1 and a dendrogram in Fig. 2. Figures 1 and 2 and Table 3 illustrate the high degree of phenetic similarity among the organisms which we studied. With the exception of *E. corrodens*, all of the periodontal strains were more than 60% similar to each other on the basis of this analysis. The degree of similarity among the strains within each of the phenons was more than 84%, with the exception of the *B. ureolyticus* strains (phenon 6). DNA base ratios and DNA-DNA hybridization experiments confirmed and clarified the groups.

The majority of the nonmotile, agar-corroding organisms used in this investigation clustered in phenon 7 with the reference strains of *E. corrodens* and were considered to be members of this species. Six isolates from humans with alveolar lesions, 17 strains from humans with periodontitis, periodontosis, and gingivitis pockets, and the reference strains of *E. corrodens* had small cells (approximately 1 by 4 μm) with blunt or rounded ends and parallel sides. Although these organisms did not exhibit active motility, cells from log-phase broth cultures demonstrated twitching. The minimal inhibitory concentrations of antibiotics and the biochemical charac-

FIG. 1. Trellis diagram of a cluster analysis of 56 strains of gram-negative, asaccharolytic, rod-shaped bacteria. The Jaccard coefficient was used to determine percent similarities between strains, and the sort was by average unweighted linkage. The numbers in the key indicate percent similarities. Phenons 1 through 7 are indicated by brackets. The lack of overlap between the *E. corrodens* strains and strains of the remaining species is readily apparent; there is some overlap between strains in each of the other phenons.

teristics of the *E. corrodens* strains in phenon 7 are listed above and in Tables 4 and 5. Only the 25 strains of *E. corrodens* exhibited a negative benzidine reaction and decarboxylated lysine and ornithine. Ammonia formation by *E. corrodens* strains was not detected in complex media but was demonstrated when the organisms were grown in a synthetic medium (S. S. Socransky, C. Smith, and A. D. Manganiello, J. Dent. Res. 52[Special Issue]:88, abstr. 120, 1973). The 25 *E. corrodens* strains were relatively resistant to potassium cyanide, metronidazole, clindamycin, gentamicin, neomycin, and streptomycin but relatively susceptible to nalidixic acid and rifampin. The strains which we examined had G+C contents of 50 to 58 mol%. The mean phenetic similarity of the isolates in this group was 92% (Table 3).

The remaining isolates shared the following characteristics: growth was stimulated by formate and fumarate in broth media; formate could be replaced by hydrogen when the strains were grown on agar plates; fumarate was reduced to succinate; benzyl viologen was reduced; the benzidine test was positive; hydrogen sulfide was produced; and L-arginine, L-lysine, and L-ornithine were not decarboxylated. However, these isolates did not cluster or exhibit significant DNA homology with any of the reference strains and thus could not be identified as members of *B. ureolyticus*, *V. succinogenes*, *C. sputorum*, or *Campylobacter fetus*. The lack of identity of the oral isolates with known species necessitated the creation of three new species for the strains in phenons 4, 5, and 6.

Phenon 2 strains had G+C contents of 46 and

FIG. 2. Dendrogram of the cluster analysis shown in Fig. 1. The numbers on the horizontal axis indicate percent similarities, as determined by the Jaccard coefficient. Phenons 1 through 7 are indicated by brackets. Subgroups within phenons are apparent, particularly within phenon 7 (*E. corrodens*).

TABLE 3. Mean intra- and interphenon percent similarities as determined by the Jaccard coefficient

Species	% Similarity to:						
	Phenon 1 (reference <i>Campylo-</i> <i>bacter</i> sp.)	Phenon 2 (reference <i>Wolinella</i> sp.) ^a	Phenon 3 (<i>C. concisus</i>)	Phenon 4 (<i>B. gracilis</i>)	Phenon 5 (<i>W. recta</i>) ^b	Phenon 6 (<i>B. ureolyticus</i>)	Phenon 7 (<i>E. corrodens</i>)
Reference <i>Campylobacter</i> sp.	89.2 ± 4.3 ^c						
Reference <i>Wolinella</i> sp.	72.7 ± 3.2	84					
<i>C. concisus</i>	71.2 ± 3.8	80.9 ± 2.5	92.3 ± 4.2				
<i>B. gracilis</i>	64.5 ± 3.5	66.4 ± 2.2	69.6 ± 2.7	88.9 ± 4.6			
<i>W. recta</i>	58.1 ± 2.8	58.4 ± 2.7	61.8 ± 2.6	65.2 ± 3.3	93.2 ± 5.2		
<i>B. ureolyticus</i>	56.6 ± 3.3	53.1 ± 3.3	57.1 ± 3.5	67.6 ± 3.7	69.5 ± 3.8	72.3	
<i>E. corrodens</i>	39.1 ± 1.7	33.9 ± 2.5	38.2 ± 1.7	39.4 ± 2.5	35.8 ± 2.1	41.0 ± 3.2	92.0 ± 4.3

^a *Wolinella* strains ATCC 29543 and VPI 9584.

^b *W. recta* strains and *Wolinella* strain 286.

^c Mean ± standard deviation.

47 mol% and included *V. succinogenes* strain 602 (ATCC 29543). Strains described as *V. succinogenes* did not fit descriptions of existing

genera (Table 6). The genus *Vibrio*, which includes saccharolytic, aerobic to facultatively anaerobic species (23), appeared to be inappro-

TABLE 4. Features useful in separating some gram-negative, asaccharolytic, oral organisms^a

Test	Phenon 1		Phenon 2		Phenon 3		Phenon 4		Phenon 5		No. of phenon 6 (<i>B. ureolyticus</i>) strains positive (n = 2)	No. of phenon 7 (<i>E. corrodens</i>) strains positive (n = 25)
	No. of reference <i>Campylobacter</i> strains positive (n = 4)	Reaction of <i>C. sputorum</i> subsp. <i>sputorum</i> S17	No. of reference <i>Wollnella</i> strains positive (n = 2)	Reaction of <i>W. succinogenes</i> ATCC 29543	No. of <i>C. concisus</i> strains positive (n = 6)	Reaction of strain 484	No. of <i>B. gracilis</i> strains positive (n = 7)	Reaction of strain 1084	No. of <i>W. recta</i> strains positive (n = 10)	Reaction of strain 371		
Active motility	4	+	2	+	6	+	0	-	10	+	0	0
Helical cells	4	+	2	+	0	-	0	-	0	-	0	0
Growth in air + CO ₂	4	+	0	-	0	-	0	-	0	-	0	25
Growth stimulated by nitrate	4	+	2	+	0	-	3	-	0	-	1	25
Growth stimulated by formate and fumarate	4	+	2	+	6	+	7	+	10	+	2	0
Lysine and ornithine decarboxylase	0	-	0	-	0	-	0	-	0	-	0	25
Urease	0	-	0	-	0	-	0	-	0	-	2	0
Benzidine reaction	4	+	2	+	6	+	7	+	10	+	2	0
H ₂ O ₂ decomposition	2	-	0	-	0	-	0	-	0	-	0	0
Oxidase test	4	+	0	-	6	+	0	-	0	-	1	25
Reduction of nitrite	4	+	2	+	6	+	7	+	10	+	0	0
Reduction of benzyl viologen	4	+	2	+	6	+	7	+	10	+	1	0
Reduction of neutral red	4	+	2	+	6	+	7	+	10	+	2	0
End products												
Hydrogen sulfide	3	+	2	+	6	+	7	+	10	+	2	0
Formate	0	-	0	-	0	-	1	-	0	-	0	20 ^b
Acetate	0	-	0	-	3	+	1	-	1	-	0	25
Lactate	0	-	0	-	0	-	1	-	0	-	0	0
Succinate	4	+	2	+	6	+	7	+	10	+	2	0
Pyruvate	0	-	0	-	0	-	0	-	0	-	1	0
Hydrogen	0 ^c	+	1	-	6	+	7	+	9	+	1	0
Nitrous oxide	0	-	0	-	0	-	0	-	0	-	0	25
Growth in the presence of:												
Sodium fluoride (0.5 g/liter)	4	+	1	+	6	+	7	+	0	-	2	19
Oxgall (10 g/liter)	4	+	1	-	6	+	3	+	0	-	1	6
Sodium deoxycholate (1 g/liter)	3	+	2	+	6	+	7	+	0	-	1	5
Phenol (1 g/liter)	3	+	0	-	0	-	0	-	0	-	0	0
Janus green (0.1 g/liter)	4	+	2	+	6	+	7	+	5	-	2	25
Basic fuchsin (0.05 g/liter)	4	+	2	+	6	+	7	+	1	-	0	25
Crystal violet (0.005 g/liter)	0	-	2	+	6	+	4	+	0	-	0	3
Safranine (0.1 g/liter)	0	-	1	+	6	+	5	+	0	-	0	0

Alizarine red S (0.32 g/liter)	4	+	2	+	6	+	7	+	1	-	2	17
Azure II (0.05 g/liter)	2	+	2	+	6	+	7	+	1	-	1	0
Potassium cyanide (1 g/liter)	4	+	0	-	5	+	0	-	0	-	2	25
Methyl orange (0.25 g/liter)	4	+	2	+	6	+	7	+	0	-	0	21

^a In addition, the DNA G+C contents of these organisms were as follows (in moles percent): reference *Campylobacter* strains, 29 to 35; *C. sputorum* subsp. *sputorum* S17, 30; reference *Wolinella* strains, 46; *C. concisus* strains, 34 to 38; strain 484, 36; *B. gracilis* strains, 44 to 46; strain 1084, 45; *W. recta* strains, 42 to 46; strain 371, 43; *B. ureolyticus* strains, 28; *E. corrodens* strains, 50 to 58.

^b Only 20 strains were tested.

^c Only two strains were tested.

priate. *V. succinogenes* had a G+C content of 46 mol% and thus could not be considered a member of the genus *Campylobacter* (G+C content, 30 to 35 mol%). Organisms belonging to the genera *Succinivibrio*, *Succinomonas*, and *Butyrivibrio* are saccharolytic (2-4), whereas *Desulfovibrio* strains (19) reduce sulfate but not nitrate. *Anaerovibrio lipolytica* (9) includes saccharolytic strains that form acetate and propionate. Since there were no clear indications to amend these genera to include *V. succinogenes*, we propose the formation of a new genus, *Wolinella*. The characteristics which distinguish this proposed new genus from other genera are summarized in Table 6. ATCC 29543, the type strain of the type species, *Wolinella succinogenes* (Wolin et al.) comb. nov., is a rumen strain that was isolated by Wolin et al. (35).

***Wolinella* gen. nov.** (Wol in el'la. M.L. dim. ending -ella; M. L. fem. n. *Wolinella* named for M. J. Wolin, American bacteriologist who first isolated and named the organism designated here as the type species of the genus).

Helical, curved, or straight, unbranched cells, 0.5 to 1 by 2 to 6 μ m, having rounded or tapered ends. Gram negative. Rapid, darting motility is by means of a single, polar flagellum. Endospores are not produced. Anaerobic; some strains can grow in the presence of 5% oxygen but not in air enriched with 10% CO₂. Carbohydrates are not fermented and do not support growth. Growth is stimulated in broth cultures by formate and fumarate. Hydrogen and formate are used as energy sources (35). Formate is oxidized to produce hydrogen and carbon dioxide. Fumarate is reduced to succinate. Nitrate is reduced, and hydrogen sulfide is produced. Oxidase and catalase negative. The G+C content of the DNA is 42 to 49 mol% (thermal denaturation method).

The type species is *Wolinella succinogenes* (Wolin et al.) comb. nov.

***Wolinella succinogenes* (Wolin et al.) comb. nov.** (basonym, *Vibrio succinogenes* Wolin et al. 1961).

Helical, curved, or straight, unbranched cells 0.5 to 1 by 2 to 6 μ m, having rounded or tapered ends. Gram negative. Rapid, darting motility is by means of a single, polar flagellum. Endospores are not produced.

Translucent colonies are produced on blood agar plates. There are the following three colony types: a convex variant with a diameter of 1 mm; an agar-pitting or corroding variant which is up to 5 mm in diameter; and a variant which spreads on agar up to a diameter of 5 mm.

Anaerobic; some strains can grow in the presence of 5% oxygen but not in air enriched with

TABLE 5. *Minimal inhibitory concentrations of antibiotics for the organisms studied*

Antibiotic	Minimal inhibitory concn ($\mu\text{g/ml}$) for:						
	Phenon 1 (reference <i>Campylobacter</i> sp.)	Phenon 2 (reference <i>Wolinella</i> sp.)	Phenon 3 (<i>C. concisus</i>)	Phenon 4 (<i>B. gracilis</i>)	Phenon 5 (<i>W. recta</i>)	Phenon 6 (<i>B. ureolyticus</i>)	Phenon 7 (<i>E. corrodens</i>)
Bacitracin	64->128	128	>128	>128	64->128	≥ 128	32->128
Chloramphenicol	0.5-1	2-8	4	2-8	1-2	0.5-1	0.5-1
Clindamycin	0.5-2	1-2	2-4	0.5-2	≤ 0.25 -0.5	≤ 0.25	16-64
Colistin	<0.25 -4	2-4	0.5-1	0.5-1	≤ 0.25 -1	≤ 0.25 -0.5	≤ 0.25 -1
Erythromycin	0.5-2	2-8	4	1-2	0.5-2	≤ 0.25 -0.5	2-8
Gentamicin	2-4	2-8	2-4	2-4	1-2	2	8-64
Kanamycin	2-4	8	1-2	0.5-2	≤ 0.25 -1	0.5-1	4-32
Metronidazole	≤ 0.25 -8	1-2	0.5-2	0.5-1	0.5-2	1-2	16->128
Minocycline	≤ 0.25 -4	0.05-4	2	0.5-2	≤ 0.25 -2	≤ 0.25	8
Nalidixic acid	8-64	32-128	64-128	64->128	16-128	32	1-4
Neomycin	16-32	16-32	16-32	16-32	16	16	64->128
Penicillin	≤ 0.25 -8	8->128	0.5-4	1-32	≤ 0.25 -4	≤ 0.25 -0.5	≤ 0.25 -1
Polymixin B	≤ 0.25 -16	1-8	≤ 0.25 -1	≤ 0.25 -1	≤ 0.25 -1	≤ 0.25	≤ 0.25 -1
Rifampin	64	≥ 128	16-64	4-32	2	4	0.5-1
Streptomycin	2-4	2-8	1-2	1-2	0.5-4	0.5	8-32
Tetracycline	0.25-1	0.5-2	1-2	1-8	≤ 0.25 -0.5	≤ 0.25	0.5-4
Vancomycin	>128	>128	>128	>128	>128	≥ 128	16-128

TABLE 6. *Characteristics that distinguish Wolinella from closely related genera^a*

Genus	G+C content (mol%)	Distinguishing characteristics
<i>Wolinella</i>	42-49	Anaerobic; motile, polar flagella; growth stimulated by formate and fumarate; no acid or gas from carbohydrates; oxidase and urease negative; nitrate but not sulfate reduced; hydrogen peroxide not decomposed; hydrogen sulfide produced
<i>Vibrio</i>	40-50	Facultatively anaerobic; fermentative; oxidase positive; growth on simple nutrient media
<i>Succinivibrio</i> and <i>Succinimonas</i>		Fermentative; succinate formed from glucose; nitrate not reduced; hydrogen sulfide not produced
<i>Butyrivibrio</i>		Fermentative; butyrate formed from glucose
<i>Desulfovibrio</i>	46-61	Sulfate reduced; grows in media containing sulfate with lactate or pyruvate
<i>Anaerovibrio</i>		Fermentative; propionate from fructose; nitrate not reduced
<i>Campylobacter</i>	30-35	Microaerophilic to anaerobic; oxidase positive

^a Data were derived from references 2 through 4, 9, 19, 23, and 26 and from this study.

10% CO₂.

Carbohydrates are not fermented and do not support growth. Growth is stimulated in broth cultures by formate and fumarate. Hydrogen and formate are used as energy sources. Formate is oxidized to produce hydrogen and carbon dioxide. Fumarate is reduced to succinate. Nitrates, nitrites, neutral red, and benzyl viologen are reduced. The benzidine test is positive; hydrogen sulfide is produced. Oxidase and catalase negative. Lysine, ornithine, and arginine are not decarboxylated. Starch, dextran, esculin, casein, DNA, and gelatin are not hydrolyzed. Hydrogen peroxide, urease, lecithanase, lipase, indole, ammonia, and acetylmethylcarbinol are not pro-

duced. Gas is not formed under an agar layer that is over a broth culture.

The G+C content of the DNA is 46 to 49 mol% (thermal denaturation method).

Found in bovine rumens.

The type strain is ATCC 29543.

The identification of the remaining phenon 2 strain (VPI 9584) is not clear at the present time. This strain was received from L. V. Holdeman (Virginia Polytechnic Institute and State University) as a representative strain of *V. succinogenes*, but its DNA showed no homology with the DNA of reference *V. succinogenes* strain ATCC 29543 isolated by Wolin (Table 7).

The strains of phenon 5 were distinct from the

other motile organisms (Fig. 1 and 2) and consisted of straight, actively motile rods which were approximately 0.5 by 4 μ m and had G+C contents of 42 to 46 mol%. We propose an additional species, *Wolinella recta* sp. nov. to include the straight rod-shaped bacteria which clustered with strain 371, the proposed type strain of this species.

Wolinella recta sp. nov. (rec 'ta. L. adj. *rectus* straight, direct, in a straight line).

Small, unbranched, straight cells 0.5 by 2 to 4 μ m, having rounded ends. Gram negative. Rapid, darting motility occurs in young broth cultures by means of a single, polar flagellum. Endospores are not produced.

The inner and outer cell membranes are generally straight (or smooth) and parallel and are about 20 nm apart. There is no distinct peptidoglycan cell wall layer. The outer surface of the outer membrane is covered with a distinctive array of hexagonal, packed, macromolecular subunits, each about 17 nm in diameter (14).

Translucent colonies are produced on blood agar plates. There are the following three colony types: a convex variant with a diameter of 1 mm; an agar-pitting or corroding variant which is up to 5 mm in diameter; and a variant which spreads on agar up to a diameter of 5 mm.

Anaerobic; some strains can grow in the presence of 5% oxygen but not in air enriched with 10% CO₂.

Carbohydrates are not fermented and do not support growth. In broth cultures, growth is stimulated by formate and fumarate. Hydrogen and formate are used as energy sources. Formate is oxidized to produce hydrogen and carbon dioxide. Fumarate is reduced to succinate. Nitrates,

nitrites, neutral red, and benzyl viologen are reduced. The benzidine test is positive, and hydrogen sulfide is produced. Oxidase and catalase negative. Lysine, ornithine, and arginine are not decarboxylated. Starch, dextran, esculin, casein, DNA, and gelatin are not hydrolyzed. Hydrogen peroxide, urease, lecithinase, lipase, indole, ammonia, and acetylmethylcarbinol are not produced. No gas is formed under an agar layer that is over a broth culture.

The G+C content of the DNA is 42 to 46 mol% (thermal denaturation method).

Found in gingival crevices of humans. Pathogenicity is unknown.

The type strain is FDC 371 (= ATCC 33238).

W. recta strains differ from *W. succinogenes* strains in cellular and ultrastructural morphology (14), in their serological reactions (1), and in their greater susceptibility to dyes and antibiotics. The *W. recta* strains which we tested were quite susceptible to sodium fluoride, basic fuchsin, clindamycin, colistin, minocycline, penicillin, polymyxin B, and tetracycline (Tables 4 and 5).

The results of DNA-DNA hybridizations between *W. recta* strains and helical strains of *W. succinogenes* are summarized in Table 7. Nine strains of *W. recta* were clearly closely related to each other on the basis of DNA homology. None of the *W. recta* strains was closely related to strain VPI 9584. DNA from *W. succinogenes* ATCC 29543 showed only a low level of homology with either DNA from strain VPI 9584 or DNAs from the *W. recta* strains. Strain 286 was not sufficiently similar by DNA homology to the *W. recta* isolates to be included in this species, although it was phenotypically similar to them.

The *B. ureolyticus* reference strains of phenon 6 had G+C contents of 28 mol% and were the only urease-positive isolates in this study. The cells of these strains were nonmotile, measured 0.5 by 2 to 4 μ m, and had rounded ends. One strain (VPI 7815) formed ammonia. The *B. ureolyticus* reference strains examined had a DNA-DNA homology value of 51% with each other and a 72% phenotypic similarity. This relatively low level of similarity was consistent with the observations of Jackson and Goodman (11), who found that strains VPI 7814 and VPI 7815 are atypical strains of this species.

Phenon 4 strains were clearly different from both *E. corrodens* strains and *B. ureolyticus* reference strains with respect to the G+C contents of their DNAs. Phenon 4 strains could be differentiated from *E. corrodens* strains which were oxidase, lysine decarboxylase, ornithine decarboxylase positive and grew in air containing 10% CO₂. Unlike *E. corrodens*, phenon 4 strains were benzidine positive, produced H₂S, and in

TABLE 7. Percent DNA homology values for *W. recta*, *W. succinogenes*, and strain VPI 9584

Strain	% Homology with DNA from:		
	<i>W. recta</i> 285	<i>W. recta</i> 371	Strain VPI 9584
<i>W. recta</i>			
285	100	75	30
267	100	80	30
1219	100	68	21
1087	92	78	28
1086	86		13
302	86	79	23
372	85	95	17
371	75	100	24
303	73	90	24
<i>Wolinella</i> sp. 286	44	25	43
<i>W. succinogenes</i> ATCC 29543	16	1	20

broth had their growth stimulated by formate and fumarate, with the formation of succinate as an end product. These strains were urease negative and in this way differed from the isolates of *B. ureolyticus*. In addition, the cells of phenon 4 isolates were slender and often had tapered ends. We propose *Bacteroides gracilis* sp. nov. as the name of these nonmotile, anaerobic periodontal isolates.

Bacteroides gracilis sp. nov. (gra'cil is. L. adj. *gracilis* slim, slender, thin, skinny, slight).

Small, straight, unbranched cells approximately 0.4 by 4 to 6 μ m, often having both tapered and rounded ends. Gram negative. The isolates demonstrate twitching movement during dark-field examinations of young broth cultures; no flagella have been observed. Endospores are not produced.

Inner and outer cell membranes are wavy or undulatory. The cell wall peptidoglycan layer is poorly resolved. Intracytoplasmic, electron-dense inclusions approximately 40 nm in diameter, some membrane bound, have been observed (14).

Translucent colonies are produced on blood agar plates. The following three colony types occur: a convex variant with a diameter of 1 mm; an agar-pitting or corroding variant which is up to 5 mm in diameter; and a variant which spreads on agar up to a diameter of 5 mm.

Anaerobic, but some strains can grow in the presence of 5% oxygen but not in air enriched with 10% CO₂.

Carbohydrates are not fermented and do not support growth. Growth is stimulated in broth cultures by formate and fumarate. Hydrogen and formate are used as energy sources. Formate is oxidized to produce hydrogen and carbon dioxide. Fumarate is reduced to succinate. Nitrates, nitrites, neutral red, and benzyl viologen are reduced. The benzidine test is positive, and hydrogen sulfide is produced. Oxidase and catalase negative. Lysine, ornithine, and arginine are not decarboxylated. Starch, dextran, esculin, casein, DNA, and gelatin are not hydrolyzed. Hydrogen peroxide, urease, lecithinase, lipase, indole, ammonia, and acetylmethylcarbinol are not produced. No gas is formed under an agar layer that is over a broth culture.

The G+C content of the DNA is 44 to 46 mol% (thermal denaturation method).

Found in gingival crevices of humans. Pathogenicity in unknown.

The type strain is FDC 1084 (= ATCC 33236).

The *B. gracilis* isolates were resistant to azure II, chloramphenicol, and nalidixic acid. *B. gracilis* strains showed more than 50% homology with each other, and five of the seven strains

showed more than 90% homology (Table 8). Since there was considerable overlap between *B. gracilis* and *W. recta* strains in biochemical characteristics and antibiotic susceptibilities (see above and Tables 4 and 5) and in G+C contents of the DNAs, we examined the relatedness of these strains by performing DNA-DNA homology tests. Table 8 shows that the DNAs from the nonmotile *B. gracilis* strains were not homologous with the DNA from strain 285, a representative motile strain of *W. recta*.

Phenon 1 consisted of the *Campylobacter* reference strains whose G+C contents ranged from 29 to 35 mol%. The isolates of this group were more aerotolerant than the other motile isolates. The characteristics of these organisms are listed above and in Tables 4 and 5. The *C. fetus* strains were the only strains which decomposed hydrogen peroxide (33).

The six microaerophilic strains in phenon 3 formed a fairly homogeneous group. Cells of these strains were usually curved and formed only raised, convex colonies. The G+C contents of these isolates ranged from 34 to 38 mol% and were higher than the values of 30 to 35 mol% reported for the genus *Campylobacter* (25) but lower than those found for the genus *Wolinella*. In all other respects these isolates fit the genus *Campylobacter* (26). The utilization of formate and fumarate by *C. sputorum* has been reported previously (26), as has the darting motility of isolates of this species. The sole amendment to the genus description is to extend the G+C contents from 30 to 35 mol% to 30 to 38 mol%. Thus, *Campylobacter concisus* sp. nov. is the name which we propose for the phenon 3 isolates, and the genus description should be amended as indicated above.

Campylobacter concisus sp. nov. (con'cis'us. L. part. adj. *concisus* brief, concise).

Predominantly small, curved, unbranched cells, occasionally straight or helical, 0.5 to 1 by 4 μ m, having rounded ends. Gram negative.

TABLE 8. Percent DNA homology between *B. gracilis* and *W. recta* 285

Strain	% Homology with DNA from:				
	<i>B. gracilis</i> 1083	<i>B. gracilis</i> 1084	<i>B. gracilis</i> 406	<i>B. gracilis</i> 568	<i>W. recta</i> 285
1083	100	100	69	69	23
1084	100	100	67	63	20
401	93	97	55	67	28
402	100	100	54	53	20
404	100	100	77		0
406	69	67	100	58	9
568	68	63	58	100	17

Rapid, darting motility in young broth cultures is by means of a single, polar flagellum. Endospores are not produced.

Inner and outer cell membranes follow a twisted, undulating course. There is no distinct cell wall peptidoglycan layer. Caplike cytoplasmic condensations occur at the flagellated pole.

Colonies are convex, translucent, 1 mm in diameter, and entire.

Microaerophilic to anaerobic; grows anaerobically and in the presence of 5% oxygen but not in air containing 10% carbon dioxide.

Carbohydrates are not fermented and do not support growth. Growth is stimulated in broth cultures by formate and fumarate. Hydrogen and formate are used as energy sources. Formate is oxidized to produce hydrogen and carbon dioxide. Fumarate is reduced to succinate. Nitrates, nitrites, neutral red, and benzyl viologen are reduced. Hydrogen sulfide is produced. Oxidase is produced but catalase is not. The benzidine test is positive. Lysine, ornithine, and arginine are not decarboxylated. Starch, dextran, esculin, casein, DNA, and gelatin are not hydrolyzed. Hydrogen peroxide, urease, lecithinase, lipase, indole, ammonia, and acetylmethylcarbinol are not produced. Gas is not formed under an agar layer that is over a broth culture.

The G+C content of the DNA is 34 to 38 mol% (thermal denaturation method).

Found in gingival crevices of humans. Pathogenicity is unknown.

The type strain is FDC 484 (= ATCC 33237).

C. concisus can be differentiated from *Wolliella* species on the basis of its G+C content. *C. concisus* strains are relatively resistant to sodium fluoride, crystal violet, safranin, azure II, chloramphenicol, nalidixic acid, and rifampin (Tables 4 and 5). There was no significant DNA homology between the strains of *C. concisus* and the reference strains of *Campylobacter sputorum* subsp. *sputorum* (Table 9). Four of the strains of *C. concisus* demonstrated 100% homology with each other. Strains 521 and 288 were related to the main cluster at a lower level. Values for DNA homologies between strains of *C. concisus* and strains of *W. recta* were consistently below 50%.

None of the *Campylobacter* strains produced agar-pitting colonies. The *C. concisus* strains formed small, convex, translucent colonies with entire edges. The reference *Campylobacter* strains produced raised, flat colonies. *E. corrodens*, *B. ureolyticus*, *B. gracilis*, *W. succinogenes*, and *W. recta* exhibited a range of colony types similar to the range described for *E. corrodens* (6, 8, 10-13, 27). One colonial variant corroded or pitted agar, a second had a spread-

TABLE 9. Percent DNA homology between *C. concisus* and reference *Campylobacter* strains

Strain	% Homology with DNA from:				
	<i>C. concisus</i> 483	<i>C. concisus</i> 484	<i>C. concisus</i> 521	<i>Campylobacter</i> sp. strain 288	<i>C. sputorum</i> subsp. <i>sputorum</i> S17
483	100	100	82		23
484	100	100	61	67	18
522	100	100	74		14
569	100	100	76		21
521	82	61	100	72	8
288		67	68	100	10
S17 ^a	23	18	8	10	100
616 ^b	26	15	22	18	93
1176 ^c		21	11	20	21

^a *C. sputorum* subsp. *sputorum*.

^b *C. sputorum* subsp. *bubulus*.

^c *C. fetus*.

ing, transparent, matte appearance, and a third consisted of small, transparent, convex colonies. In agreement with Khairat (13), we found that the ability of colonies to corrode and spread was medium dependent. Corroding colonies commonly appeared on media made in our laboratories but the organisms produced surface-spreading colonies when they were transferred to commercially prepared Trypticase soy agar containing 5% sheep blood. Small convex colonies appeared with either of the other types or alone. Each colonial variant could be maintained in pure culture on agar plates, but serial transfer in broth or revival from the freeze-dried state frequently led to reversion to multiple colony types.

At the beginning of this investigation, it was not clear whether the three colonial types were variations in pure cultures or represented mixtures of two or more organisms. DNAs were isolated from variants which produced consistently convex or spreading colonies on Trypticase soy agar containing 5% sheep blood. High DNA-DNA homology values between colonial variants of single strains indicated that these isolates were pure cultures, as suggested by their identical biochemical characteristics (8; see above), although we did find differences in susceptibilities to certain inhibitory agents. In general, the cells from spreading colonies were more susceptible to inhibitors than the cells from nonspreading colonies. Similar cell wall ultrastructures were observed in cells derived from different colonial types of a pure culture. However, slight antigenic differences between the two forms have been found (1). On occasion, truly mixed cultures of gram-negative, assacharolytic,

TABLE 10. *Origins of periodontal isolates*

Species	No. of strains isolated from humans with:		
	Gingivitis	Periodontitis	Periodontosis
<i>E. corrodens</i>	3	10	4
<i>B. gracilis</i>	0	7	0
<i>C. concisus</i>	3	3	0
<i>W. recta</i>	2	7	1

rod-shaped bacteria were encountered. These mixtures were difficult to separate or distinguish because the colonial morphologies of the mixtures were within the ranges observed for pure cultures.

The results of physiological characterizations and DNA base content and DNA-DNA hybridization experiments were consistent with the differences observed in serological properties and ultrastructural characteristics (1, 14).

Sources and incidence of new periodontal isolates. Table 10 shows the widespread distribution of *E. corrodens* strains, but the apparent increased frequencies of *B. gracilis* and *W. recta* in diseased sites will need further investigation.

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REPRINT REQUESTS

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LITERATURE CITED

- Badger, S. J., and A. C. R. Tanner. 1981. Serological studies of *Bacteroides gracilis*, *Campylobacter concisus*, *Wolinella recta*, and *Eikenella corrodens*, all from humans with periodontal disease. *Int. J. Syst. Bacteriol.* 31:446-451.
- Bryant, M. P. 1974. Genus *Butyrivibrio*, p. 420-421. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Bryant, M. P. 1974. Genus *Succinivibrio*, p. 422. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Bryant, M. P. 1974. Genus *Succinomonas*, p. 422. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- DeLey, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* 12:133-142.
- Eiken, M. 1958. Studies on an anaerobic rod-shaped gram-negative microorganism: *Bacteroides corrodens* n. sp. *Acta Pathol. Microbiol. Scand.* 43:404-416.
- Henriksen, S. D. 1969. Designation of the type strain of *Bacteroides corrodens*. *Int. J. Syst. Bacteriol.* 19:165-166.
- Hill, L. R., J. J. S. Snell, and S. P. Lapage. 1970. Identification and characterisation of *Bacteroides corrodens*. *J. Med. Microbiol.* 3:483-491.
- Hungate, R. E. 1966. The rumen and its microbes, p. 80-86. Academic Press, Inc., New York.
- Jackson, F. L., and Y. E. Goodman. 1972. Transfer of the facultative anaerobic organism *Bacteroides corrodens* Eiken to a new genus, *Eikenella*. *Int. J. Syst. Bacteriol.* 22:73-77.
- Jackson, F. L., and Y. E. Goodman. 1978. *Bacteroides ureolyticus*, a new species to accommodate strains previously identified as "*Bacteroides corrodens*, anaerobic." *Int. J. Syst. Bacteriol.* 28:197-200.
- Jackson, F. L., Y. E. Goodman, F. R. Bell, P. C. Wong, and R. L. S. Whitehouse. 1971. Taxonomic status of facultative strictly anaerobic "corroding bacilli" that have been classified as *Bacteroides corrodens*. *J. Med. Microbiol.* 4:171-184.
- Khairat, O. 1967. *Bacteroides corrodens* isolated from bacteraemias. *J. Pathol. Bacteriol.* 94:29-40.
- Lai, C.-H., M. A. Listgarten, A. C. R. Tanner, and S. S. Socransky. 1981. Ultrastructures of *Bacteroides gracilis*, *Campylobacter concisus*, *Wolinella recta*, and *Eikenella corrodens*, all from humans with periodontal disease. *Int. J. Syst. Bacteriol.* 31:465-475.
- Loesche, W. J., R. J. Gibbons, and S. S. Socransky. 1965. Biochemical characteristics of *Vibrio sputorum* and relationship to *Vibrio bubulus* and *Vibrio fetus*. *J. Bacteriol.* 89:1109-1116.
- Marmur, M. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* 3:208-218.
- Marmur, M., and P. Doty. 1962. Determination of the base composition of DNA from its thermal denaturation temperature. *J. Mol. Biol.* 5:109-118.
- Newman, M. G., and S. S. Socransky. 1977. Predominant cultivable microbiota in periodontosis. *J. Periodontal Res.* 12:120-128.
- Postgate, J. P. 1974. Genus *Desulfovibrio*, p. 418-420. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Ratner, E. J., P. Person, D. J. Kleinman, G. Shklar, and S. S. Socransky. 1979. Jaw bone cavities and trigeminal and atypical facial neuralgias. *Oral Surg. Oral Med. Oral Pathol.* 48:3-20.
- Robinson, J. V. A., and A. L. James. 1973. Some serological studies on *Bacteroides corrodens*. *J. Gen. Microbiol.* 78:193-197.
- Salanitro, J. P., and P. A. Muirhead. 1975. Quantitative method for gas chromatographic analysis of short-chain monocarboxylic and dicarboxylic acids in fermentation media. *Appl. Microbiol.* 29:374-381.
- Shewan, J. M., and M. Veron. 1974. Genus I. *Vibrio*, p. 340-345. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- 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.
- Smibert, R. M. 1974. Genus II. *Campylobacter*, p. 207-212. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Smibert, R. M. 1978. The genus *Campylobacter*. *Annu. Rev. Microbiol.* 32:673-709.

27. **Smibert, R. M., and L. V. Holdeman.** 1976. Clinical isolates of anaerobic gram-negative rods with a formate-fumarate energy metabolism: *Bacteroides corrodens*, *Vibrio succinogenes*, and unidentified strains. *J. Clin. Microbiol.* **3**:432-437.
28. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman and Co., San Francisco.
29. **Socransky, S. S., S. C. Holt, E. R. Leadbetter, A. C. R. Tanner, E. Savitt, and B. F. Hammond.** 1979. *Capnocytophaga*: new genus of gram-negative gliding bacteria. III. Physiologic characterisation. *Arch. Microbiol.* **122**:29-33.
30. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother.* **9**:307-311.
31. **Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodontitis in man. *J. Clin. Periodontol.* **6**:278-307.
32. **van Palenstein Helderman, W. H., and K. C. Winkler.** 1975. Elective medium for the direct count of vibrio (campylobacter), fusobacteria, bacteroides, selenomonas and veillonella in the gingival crevice flora. *J. Periodontal Res.* **10**:230-241.
33. **Véron, M., and R. Chatelain.** 1973. Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. *Int. J. Syst. Bacteriol.* **23**:122-134.
34. **Walker, C. B., T. A. Niebloom, and S. S. Socransky.** 1979. Medium V: an agar medium for use in susceptibility testing of bacteria from human periodontal lesions. *Antimicrob. Agents Chemother.* **16**:452-457.
35. **Wolin, M. J., E. A. Wolin, and N. J. Jacobs.** 1961. Cytochrome-producing anaerobic vibrio, *Vibrio succinogenes* sp. n. *J. Bacteriol.* **81**:911-917.