Wolinella curva sp. nov.: “*Vibrio succinogenes*” of Human Origin

A. C. R. TANNER,^{1*} M. A. LISTGARTEN,² AND J. L. EBERSOLE¹

Forsyth Dental Center, Boston, Massachusetts 02115¹ and University of Pennsylvania, Philadelphia, Pennsylvania 19104²

Asaccharolytic, anaerobic vibrios which require formate and fumarate for growth in broth culture have been isolated from humans. Some of these strains resemble *Wolinella succinogenes* phenotypically, but show no deoxyribonucleic acid (DNA)-DNA homology with either *W. succinogenes* or *Wolinella recta*. In this investigation, six unidentified strains were compared with reference and type strains of *W. recta* and *W. succinogenes* by using cluster analysis of phenotypic characteristics, enzyme-linked immunosorbent assays, and cell wall ultrastructure. One unidentified strain, a sewage isolate, was *W. succinogenes*. The remaining unidentified strains showed no DNA-DNA homology with either *W. succinogenes* or *W. recta*. *Wolinella curva* sp. nov. is proposed for four isolates which were distinct, as determined by serological and DNA-DNA homology experiments, from the previously described species. *W. curva* strains could also be differentiated from *W. recta* by morphology and by the growth of *W. curva* in the presence of Janus green (0.1 g/liter), basic fuchsin (0.032 g/liter), sodium deoxycholate (1.0 g/liter), indulin scarlet (0.5 g/liter), oxgall (10 g/liter), safranin (0.5 g/liter), azure II (0.025 g/liter), penicillin (16 µg/ml), and polymixin B (4 µg/ml). *W. curva* and *W. succinogenes* are similar in cell ultrastructure and in other phenotypic features.

Wolinella succinogenes (*Vibrio succinogenes*) was isolated from a bovine rumen by Wolin et al. (24). Members of this species use formate or hydrogen as an electron donor. Fumarate was found to be the most efficient electron acceptor of several organic and inorganic compounds tested (1, 24). Two other motile, metabolically similar species from humans have been described; these are *Wolinella recta*, with a guanine-plus-cytosine (G+C) content of 44 to 46 mol%, and *Campylobacter concisus*, with a C+G content of 34 to 38 mol% (20).

Several investigators have isolated from human sources anaerobic, gram-negative, asaccharolytic, motile vibrio-shaped rods with metabolism similar to that of *W. succinogenes* (17, 20, 21, 23). Deoxyribonucleic acid (DNA)-DNA homology techniques have shown that these additional strains are distinct from previously described species (21). In this investigation we determined the taxonomic status of additional formate- and fumarate-requiring strains isolated from humans.

MATERIALS AND METHODS

Characterization. The origins of the oral and reference strains which we used are shown in Table 1. The methods used to maintain and characterize these strains were similar to those described previously (20). Cell morphology and motility were determined by dark-field microscopy of young broth cultures. Biochemical tests were performed in broth cultures and on agar plates. The basal medium used for broth tests (PPLO-FF medium) contained (per liter) 21 g of Mycoplasma broth base (BBL Microbiology Systems, Cockeysville, Md.), 5 mg of hemin, 2 g of sodium formate, and 3 g of sodium fumarate and was adjusted to pH 7.4. A 30-ml portion of a log phase inoculum was added to 100 ml of freshly prepared basal medium, and 2 ml of this mixture was aseptically pumped into tubes containing 0.2 ml of 10-fold concentrations of the test reagents (19). The cultures were incubated anaerobically at 35°C in an atmosphere containing 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Acid end products were determined by gas-liquid chromatography of the butyl esters (16) of the culture supernatant in PPLO-

FF basal medium. Gaseous end products were determined from the headspace gas of cultures grown in PPLO-FF medium in a 100% argon atmosphere (20).

Atmosphere and temperature requirements were determined on Trypticase soy agar supplemented with 5% sheep blood (BBL). Starch hydrolysis; dextran, gelatin, esculin, DNA, and casein degradation; oxidase; and growth in the presence of dyes and chemical inhibitors were tested on a basal medium containing (per liter) 15 g of Trypticase, 3 g of Phytone, 1 g of sodium formate, 1.5 g of sodium fumarate, 1 g of potassium nitrate, 5 mg of hemin, and 20 g of agar at pH 7.2 to 7.4. The oxidase test was performed with 1% (wt/vol) tetramethyl-*p*-phenylenediamine (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in dimethyl sulfoxide (22). The dye and chemical inhibitor concentrations used are shown in Table 2. Growth equal to that in the basal medium without the added chemical inhibitor was considered a positive reaction.

Extraction of DNA, determination of the G+C content, and DNA-DNA hybridization experiments. Two- to three-liter broth cultures were grown to log phase in PPLO-FF medium and harvested by centrifugation. DNA was isolated by a modification (19) of the Marmur method (11). 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 the purified unsharpened DNAs (12). The results reported below are the means of triplicate determinations.

A Gilford model 260 recording spectrophotometer with a thermoprogrammer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) was used to determine the G+C contents and DNA-DNA reassociation. The G+C contents of DNAs were estimated from the melting temperatures. The midpoint of inflection was determined by the following formula: $Y = K_1 + 1/(K_2 + K_3e^{-K_4x})$, where x is time and Y is optical density. The constants K_1 , K_2 , K_3 , and K_4 were fitted by using the Marquardt curve-fitting algorithm and were computed for each curve. After the function had been fit, the midpoint of inflection was calculated. The G+C content was computed by using the formula of De Ley (4).

DNA similarities were measured by comparing the renaturation rates of DNA solutions from pure cultures with the renaturation rates of mixtures in triplicate experiments (5).

* Corresponding author.

TABLE 1. *Wolinella* strains examined and their sources

Species	Strain	Source ^a	Origin
<i>W. succinogenes</i>	ATCC 29543 ^T	M. J. Wolin ^b	Bovine rumen (= FDC 602W)
<i>W. recta</i>	ATCC 33238 ^T	FDC 371	Human periodontitis
	FDC 285	FDC	Human periodontitis
	FDC 303	FDC	Human periodontitis
Unidentified	VPI 10279 ^c	VPI	Human dental root canal ^d
	ATCC 35224 ^e	VPI 9584 ^f	Human alveolar abscess
	VPI 10296 ^e	VPI	Human dental root canal ^d
	VPI 10659 ^e	ATCC (as a previous ATCC 29543)	Human clinical isolate
	FDC 640	M. Coyle ^g	Human septicemia
	Y-IB4	T. Yoshinari	Sewage

^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 Department of Health, Albany, N.Y.

^c *Wolinella* group II described by Tanner et al. (21).

^d Isolated by G. Sundqvist, Goteborg, Sweden (17).

^e *Wolinella* group III described by Tanner et al. (21).

^f Isolated by D. W. Lambe.

^g Sent by C. A. Spiegel, Veterans Administration Medical Center, Wood, Wis.

TABLE 2. Biochemical characteristics of *Wolinella* strains used in the cluster analysis^a

Characteristic	<i>W. curva</i>		<i>W. succinogenes</i> (two strains)	<i>W. recta</i> (three strains)	<i>Wolinella</i> sp. strain VPI 10279
	Strain ATCC 35224 ^T	Three strains			
G+C content (mol %)	43	43-47	43-46	44-46	42-46
Morphology as determined by dark-field microscopy					
Ends round	+ ^b	3 ^c	1	3	+
Ends tapered	+	3	2	0	+
Sides parallel	-	3	0	3	+
Sides curved and helical	+	3	2	0	-
Length < 4 µm	+	3	1	3	+
Length 4-6 µm	+	2	1	0	-
Width 0.5-1 µm	-	0	1	3	-
Production of:					
Formate	-	1	0	2	-
Acetate	-	2	0	1	-
Growth on agar containing:					
NaF (0.5 g/liter)	-	0	2	0	-
Glycine (10 g/liter)	+	3	0	3	+
Oxgall (10 g/liter)	+	3	2	0	-
Deoxycholate (1 g/liter)	+	3	2	0	-
Janus green (0.1 g/liter)	+	3	2	0	-
Basic fuchsin (0.032 g/liter)	+	3	2	0	-
Crystal violet (0.005 g/liter)	-	0	2	0	-
Safranin (0.5 g/liter)	+	3	1	0	-
Alizarine red S (0.32 g/liter)	+	3	2	2	+
Induline scarlet (0.5 g/liter)	+	3	0	0	-
Azure II (0.025 g/liter)	+	3	1	0	+
TTC (0.1 g/liter) ^d	+	3	2	0	+
Methyl orange (0.32 g/liter)	+	3	2	1	+
Penicillin (4 µg/ml)	+	3	2	0	+
Penicillin (8 or 16 µg/ml)	+	3	2	0	-
Penicillin (32 µg/ml)	-	0	1	0	-
Polymixin B (1 µg/ml)	+	3	2	1	+
Polymixin B (2 µg/ml)	+	3	2	0	+
Polymixin B (4 µg/ml)	+	3	2	0	-
Polymixin B (8 µg/ml)	+	3	1	0	-
Kanamycin (8 µg/ml)	+	3	2	3	-

^a All strains shared the following positive characteristics: gram-negative rods, actively motile, <1 µm in diameter; growth in PPLO-FF broth; growth on agar surfaces in anaerobic (80% N₂-10% H₂-10% CO₂) and microaerophilic (5% O₂-76% N₂-9.5% H₂-9.5% CO₂) atmospheres; reduction of nitrate, nitrite, neutral red, and benzyl viologen; oxidase and benzidine positive; production of hydrogen sulfide, hydrogen, carbon dioxide, and succinate; growth in the presence of Evans blue (0.05 g/liter), malachite green (0.00002 g/liter), brilliant green (0.0125 g/liter), and kanamycin (1, 2, or 4 µg/ml). All strains shared the following negative characteristics: no growth in unsupplemented broth media or in broth media supplemented only with 0.2% NO₃; no growth on agar surfaces in air or in air containing 10% CO₂; no reduction in pH when incubated in PPLO-FF medium supplemented with 1% adonitol, 1% amygdalin, 1% arabinose, 1% cellobiose, 1% dextran, 1% dulcitol, 1% fructose, 1% galactose, 1% glucose, 1% glycerol, 1% glycogen, 1% inositol, 1% inulin, 1% lactose, 1% maltose, 1% mannitol, 1% mannose, 1% melizitol, 1% melibiose, 1% raffinose, 1% rhamnose, 1% ribose, 1% salicin, 1% sorbitol, 1% sucrose, 1% trehalose, 1% xylose, 0.25% esculin, or 0.5% starch; catalase negative; lysine, ornithine, and arginine not decarboxylated; starch, dextran, esculin, casein, DNA, and gelatin not hydrolyzed; hydrogen peroxide, urease, lecithinase, lipase, indole, ammonia, and acetylmethylcarbinol not produced; nitrous oxide, methane, formate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, caproate, lactate, and pyruvate not detected; gas not formed under an agar layer over a broth culture; no growth in the presence of NaCl (20 or 40 g/liter), or penicillin (64 µg/ml).

^b +, Positive reaction; -, negative reaction.

^c Number of strains positive.

^d TTC, Triphenyl tetrazolium chloride.

Decreases in optical density were fitted to regression lines by the method of least squares. The slopes were used to determine the DNA similarity by formula 10 of De Ley et al. (5).

Serological methods. *W. recta* strain ATCC 33238^T (= FDC 371^T; T = type strain) and *Wolinella* sp. strain ATCC 35224 (= VPI 9584) were harvested from 2 to 6 liters of PPLO-FF medium and used for preparation of antisera. The cells were washed in phosphate-buffered saline (0.02 M phosphate) containing 1 mM ethylenediaminetetraacetate and were killed with 0.5% buffered formal saline as described previously (6). Rabbit antisera were produced by two subcutaneous injections, 14 days apart, of 10⁹ Formalin-fixed cells. The globulin fraction was separated from the serum by precipitation with 40% (NH₄)₂SO₄, conjugated with horseradish peroxidase (13), and stored at -20°C until use. The conjugate was tested for antibody activity by an enzyme-linked immunosorbent assay (ELISA) (6a). After preliminary tests with 30 oral and nonoral species, dilutions lacking cross-reactions were chosen for species comparisons.

The ELISA was performed on cultures which were scraped from the surfaces of blood agar plates and suspended in phosphate-buffered saline. Cell suspensions were standardized to an optical density at 580 nm of approximately 0.300, and 50 µl-samples were distributed into wells of microtiter plates. The plates were centrifuged, and the cells were fixed to the plates with 0.25% glutaraldehyde. Exposed binding sites on the plates were blocked with 1% bovine serum albumin in 100 mM glycine buffer, and the plates were washed three times with phosphate-buffered saline supplemented with 0.05% Tween 20 (Fisher Scientific Co., Fairhaven, N.J.). Specific horseradish peroxidase-globulin was then incubated for 30 min with *Wolinella* strains, and the plates were washed three times with phosphate-buffered saline supplemented with 0.05% Tween 20. The system was developed by adding 0.03% H₂O₂ and *N,N,N',N'*-tetramethylbenzidine. (The *N,N,N',N'*-tetramethylbenzidine was dissolved in 15 mg of glacial acetic acid per ml and diluted 1:50 in 0.05 M sodium citrate buffer, pH 4.2; 0.1 ml of this solution was added to each well.) After 15 to 20 min at 25°C, NaSCN was added to a final concentration of 0.75 M. Formation of a blue precipitate indicated a positive reaction.

Electron microscopy. Two-day-old colonies from a Trypticase soy blood agar surface were fixed in 5% glutaraldehyde

TABLE 3. Levels of DNA homology between *W. curva*, *W. succinogenes*, and *W. recta* strains^a

Strain	% Homology with DNA from strain:				
	<i>W. curva</i> ATCC 35224 ^T	<i>W. curva</i> VPI 10296	<i>W. succino-</i> <i>genes</i> ATCC 29543 ^T	<i>W. recta</i> ATCC 33238 ^T	<i>W. recta</i> FDC 285
<i>W. curva</i>					
ATCC 35224 ^T	100	80	14	24	20
VPI 10296	82	100	9	34	13
VPI 10659	89	98	18	18	
FDC 640	88		13	10	25
<i>W. succinogenes</i>					
ATCC 29543 ^T	20	4	100	1	16
Y-IB4	13		100	3	13
<i>W. recta</i>					
ATCC 33238 ^T	24	34	2	100	78
FDC 303	24	25	9	90	73
FDC 285	30	24	16	78	100
<i>Wolinella</i> species strain VPI 10279	21	17	27	40	34

^a Data derived by using the initial renaturation rate method of De Ley et al. (5). Values of ≤25% are not considered significant DNA-DNA homology.

and 4% paraformaldehyde buffered to pH 7.3 with 0.185 N aqueous sodium cacodylate (8). After postfixation in 2% s-collidine-buffered osmic acid, the cells were washed, dehydrated in graded ethanol solutions, and embedded in Epon (10). Sections (0.1 µm) were cut, mounted on bare grids, stained with 1% uranyl acetate and 1% lead citrate (14), and examined and photomicrographed with a Philips model EM-300 transmission electron microscope.

Negatively stained preparations of whole cells were obtained by applying 1 drop of cellular material suspended in 2% phosphotungstic acid to a carbon-reinforced, Formvar-coated grid. Excess liquid was removed by touching the edge of the grid with filter paper and air drying. Measurements were made from electron micrographs at magnifications of ×32,800 for cell diameter and ×7,600 for cell length. Measurements of cell diameter were made from cells that were sectioned perpendicular to their surfaces, as determined from the micrographic clarity of the peripheral layers. Cell length was determined from micrographs of negatively stained whole cells. Average measurements were derived from 30 cells of each strain picked at random.

TABLE 4. Cellular dimensions of *W. curva* and *W. succinogenes*

Strain	Cell length (µm)		Cell diam (µm)	
	Range	Mean ± standard deviation	Range	Mean ± standard deviation
<i>W. curva</i>				
ATCC 35224 ^T	1.05–4.75 ^a	1.85 ± 0.75	0.30–0.40	0.35 ± 0.50
VPI 10296	0.80–4.50 ^b	1.40 ± 0.40	0.30–0.40	0.35 ± 0.00
FDC 640	0.80–4.60 ^b	1.20 ± 0.25	0.35–0.45	0.40 ± 0.05
VPI 10659	1.20–28.30 ^b	3.75 ± 2.20	0.25–0.45	0.35 ± 0.05
<i>W. succinogenes</i>				
ATCC 29543 ^T	0.90–1.85	1.40 ± 0.30	0.30–0.45	0.35 ± 0.05
Y-IB4	NA ^c	NA	0.30–0.45	0.35 ± 0.05
<i>Wolinella</i> species strain VPI 10279	0.65–1.45	0.90 ± 0.20	0.25–0.35	0.35 ± 0.05

^a All dimensions are rounded off to the nearest 0.05 µm.

^b Measurements at the high extreme of the range are not included in the mean measurement.

^c NA, Not available.

FIG. 1. Two-day-old agar culture of *W. curva* strain VPI 10296. Most cells appear as short, curved rods, some with polar flagella (arrow). $\times 7,600$.

FIG. 2. Strain FDC 640. Note polar flagellation (arrows). $\times 7,600$.

FIG. 3. Strain VPI 10659. The average cell length of this strain was greater than that of other strains. $\times 7,600$.

FIG. 4. Strain VPI 10659. Note the convoluted cell surface contour. $\times 12,600$.

FIG. 5. Typical cross-sectional appearance of strain ATCC 35224^T. Note the distinct nucleoid area (N) and the small, electron-dense cytoplasmic inclusions (arrows). $\times 32,800$.

FIG. 6. Strain Y-IB4. Typical peripheral structures include an outer membrane (OM) and an inner (cytoplasmic) membrane (IM). $\times 68,000$.

FIG. 7. Strain ATCC 35224^T. Note the polar cap (P) with a regular substructure located near the cell end. $\times 68,000$.

FIG. 8. Strain FDC 640. Note the polar cap (P) located near the cell end, in close proximity to the flagellar insertion (F). $\times 68,000$.

Cluster analysis of biochemical and serological characteristics. The Jaccard coefficient was used to compare strains. An unweighted average linkage sort was used for cluster analysis (18).

RESULTS AND DISCUSSION

The cell and colonial morphologies and biochemical characteristics of all isolates were consistent with the genus *Wolinella* (Table 2). All of the strains were gram-negative, motile, anaerobic, asaccharolytic rods that required formate and fumarate for broth growth. The G+C contents of the strains examined ranged from 42 to 47 mol%, which indicated that these strains belong to the genus *Wolinella* rather than *Campylobacter* (G+C content, 29 to 38 mol%).

Table 3 shows the results of DNA-DNA homology experiments, which indicated that five unidentified strains were not *W. succinogenes* or *W. recta* strains. The sewage isolate, strain Y-IB4, showed 100% homology with the type strain of *W. succinogenes*. Strain VPI 10279 showed no significant DNA homology with *W. succinogenes* or with the other strains. *Wolinella curva* sp. nov. is proposed for the four strains which resemble *W. succinogenes* but are of human origin.

***Wolinella curva* sp. nov.** *Wolinella curva* (curv'a. L. adj. *curva* curved, bent, arched) cells are curved rods 0.5 to 1 μ m wide by 2 to 6 μ m long and have rounded or tapered ends. Helical or straight cells may also exist. Gram negative. Rapid, darting motility. Endospores are not produced.

Cell morphology as determined by electron microscopy. In 2-day-old agar or broth cultures, cells of most strains appear as short, slightly curved rods with single polar flagella. Cells with single flagella are observed less frequently among cells of the type strain than among cells of other strains of this species. Cell length ranges from 0.65 to 4.75 μ m, with mean lengths of 1.20 to 1.85 μ m. Strain VPI 10659 exhibits greater variability in cell length, with a range of 1.20 to 28.30 μ m and a mean of 3.75 μ m (Table 4 and Fig. 1 through 8). Cell diameters of all strains range from 0.25 to 0.45 μ m, with means of 0.35 to 0.40 μ m.

In negatively stained preparations and in ultrathin sections, the cell surface has a convoluted contour that is typical of most gram-negative bacteria (Fig. 5 through 8). In

sections, a distinct nucleoid region is present in all cells. Polar caps similar to the structures described by Ritchie et al. (15) and Lai et al. (9) are present in all of the strains which we examined (Fig. 7 and 8). Cytoplasmic inclusions are rare; they appear as circular electron-dense bodies that are generally 0.05 μ m or less in diameter (Fig. 5). The outer layers of the cells consist of a highly convoluted outer membrane with a less distinct inner or cytoplasmic membrane. These layers are separated by an electron-lucent space of irregular width. A distinct, electron-dense peptidoglycan layer between the inner and outer membranes cannot readily be identified (Fig. 6).

The description given above applies equally to all strains of *W. curva*, to *W. succinogenes* (strains ATCC 29543^T and Y-IB4), and to unrelated strain VPI 10279. Morphological characteristics are of limited value in distinguishing among these species. In contrast, *W. recta* exhibits a distinctive cell wall structure in which the outer membrane is covered with a distinctive array of hexagonally packed macromolecular subunits (9).

Colonial morphology. Translucent colonies are produced on blood agar plates. The following three colony types occur: 1-mm-diameter, convex colonies, which are found with agar-pitting or -corroding colonies up to 5 mm in diameter or with spreading colonies up to 5 mm in diameter. Agar-corroding or spreading colonies appear to be medium dependent, as only one of these variants occurs at a time.

Growth requirements. Strains grow on agar surfaces in anaerobic and microaerophilic (5% oxygen) atmospheres containing H₂. There is no growth on agar surfaces in air enriched with 10% CO₂, but strains grow when they are incubated in air in the freshly prepared broth medium described by Kafkewitz (7), which contains thioglycolate as a reducing agent. All cultures require formate and fumarate for broth culture. Strains grow less well in broth media supplemented with formate and nitrate (0.2%), but do not grow in broth media supplemented only with nitrate. Hydrogen and formate are used as energy sources. Formate is oxidized. Hydrogen and carbon dioxide are produced in headspace gas over broth cultures (PPLO-FF medium). Hydrogen is also detected in the headspace gas of *W. succinogenes* cultures. This contrasts with a previous report (20), in which no hydrogen was detected with this species.

TABLE 5. Characteristics useful in differentiating *W. curva*, *W. succinogenes*, and *W. recta*

Characteristic	<i>W. curva</i>	<i>W. succinogenes</i>	<i>W. recta</i>
Source	Human clinical	Bovine rumen	Human oral
Morphology			
Helical or curved cells dominant	+	+	-
Straight cells dominant	-	-	+
Cells with tapered ends	+	+	-
Hexagonal units in cell wall	-	-	+
Growth in the presence of:			
Janus green (0.1 g/liter), Basic fuchsin (0.032 g/liter), oxgall (10 g/liter), sodium deoxycholate (1 g/liter), methyl orange (0.32 g/liter), penicillin (16 μ g/ml), or polymyxin B (4 μ g/ml)	+	+	-
Glycine (10 g/liter)	+	-	+
Crystal violet (0.005 g/liter) or sodium fluoride (0.5 g/liter)	-	+	-
Indulin scarlet (0.5 g/liter)	+	-	-
Safranine (0.5 g/liter) or azure II (0.025 g/liter)	+	V	-
Antiserum reactions			
<i>W. curva</i> ATCC 35224 ^T	+	-	-
<i>W. recta</i> ATCC 33238 ^T	-	-	+

^a +, Positive reaction; -, negative reaction; V, variable reaction.

Fumarate is reduced to succinate. Carbohydrates are not fermented and are not required for growth.

Biochemical characteristics and sensitivities to inhibitory agents. The biochemical characteristics are shown in Tables 2 and 5. *Wolinella* strains are oxidase positive, as determined with the reagent described by Tarrand and Groschel (22). This finding contrasts with the results of the test performed with aqueous *N,N,N',N'*-tetramethyl-*p*-phenylene diamine dihydrochloride (3), but is consistent with the description of oxidase-positive oral *Vibrio succinogenes*-like organisms (23) and the presence of cytochromes in *W. succinogenes* and its ability to use low levels of oxygen (24).

Antibiotic susceptibilities. Minimal inhibitory concentrations of antibiotics for *W. curva* (in micrograms per milliliter): bacitracin, >128; chloramphenicol, 2 to 4; clindamycin, 0.5 to 1; colistin, 4; erythromycin, 2; gentamicin, 2; kanamycin, 4 to 8; metronidazole, 1 to 2; minocycline, 2 to 4; nalidixic acid, 64 to 128; neomycin, 4 to 8; penicillin, 32; polymyxin B, 8; rifampin, 128 to >128; streptomycin, 2; tetracycline, 1; vancomycin, >128 (21; this study).

DNA-DNA homologies. *W. curva* isolates have G+C contents of 43 to 47 mol% (Table 2). Strains of *W. curva* form a distinct group with an intergroup homology of 80 to 100% (Table 3). The *W. curva* isolates show no significant DNA homology with the type strain of *W. succinogenes*, the type strain of *W. recta*, or strain VPI 10279.

TABLE 6. Bacterial species showing no ELISA cross-reactivity with anti-*Wolinella* antisera

Species	Strain(s) tested
Gram-negative species	
<i>Actinobacillus actinomy-</i>	FDC Y4, FDC 511, FDC 652,
<i>cetemcomitans</i>	ATCC 29523 ^a
<i>Bacteroides coagulans</i>	FDC A635
<i>Bacteroides corporis</i>	VPI 9342 ^a
<i>Bacteroides fragilis</i>	ATCC 23745
<i>Bacteroides gingivalis</i>	FDC 381 ^a
<i>Bacteroides gracilis</i>	ATCC 33236 ^T (= FDC
	1084 ^T) ^a
<i>Bacteroides intermedius</i>	FDC 581, VPI 8944 ^a
<i>Bacteroides melaninogenicus</i>	FDC 287 ^a
<i>Bacteroides oralis</i>	ATCC 33321 ^a
<i>Bacteroides ureolyticus</i>	VPI 7814, VPI 7815
<i>Campylobacter concisus</i>	ATCC 33237 ^T (= FDC 484 ^T) ^a
<i>Campylobacter fetus</i>	VPI 1176, VPI H641
<i>Campylobacter sputorum</i>	VPI S17 ^a
<i>Capnocytophaga gingivalis</i>	ATCC 33624 ^T (= FDC 27 ^T) ^a
<i>Capnocytophaga ochracea</i>	FDC 25 ^a
<i>Capnocytophaga sputigena</i>	ATCC 33612 ^T (= FDC 4 ^T) ^a
<i>Eikenella corrodens</i>	FDC 1073, FDC 373 ^a
<i>Fusobacterium nucleatum</i>	FDC 364 ^a
<i>Haemophilus aphrophilus</i>	FDC 626 ^a
<i>Haemophilus aegyptus</i>	H102 ^b
<i>Haemophilus paraphrophilus</i>	H76, H78
<i>Haemophilus canis</i>	H50
<i>Haemophilus gallinarum</i>	H100
<i>Haemophilus influenzae</i>	H9, H66, H104
<i>Haemophilus parainfluenzae</i>	H15, H20, H28, H37, H54
<i>Haemophilus suis</i>	H101
<i>Selenomonas sputigena</i>	FDC 1304 ^a
Gram-positive species	
<i>Actinomyces israelii</i>	FDC B13 ^a
<i>Actinomyces naeslundii</i>	FDC 1 ^a
<i>Actinomyces viscosus</i>	FDC T14 ^a
<i>Streptococcus sanguis</i>	FDC 254

^a Antisera to these strains showed no activity against *Wolinella* strains.

^b H strains were obtained from W. Sims, University of London, London, England.

TABLE 7. Serological ELISA reactions among *Wolinella* species

Strain	Reaction with antiserum to:	
	<i>W. curva</i> ATCC 35224 ^T	<i>W. recta</i> ATCC 33238 ^T
<i>W. curva</i>		
ATCC 35224 ^T	+	—
VPI 10296	+	—
VPI 10659	+	—
FDC 640	+	—
<i>W. recta</i>		
ATCC 33238 ^T	—	+
FDC 285	—	+
FDC 303	—	+
<i>W. succinogenes</i> ^a		
ATCC 29543 ^T	—	—
Y-IB4	—	—
<i>Wolinella</i> species strain	+	—
VPI 10279	—	—

^a No antisera were made (or tested) against *W. succinogenes*.

Serology. None of the antisera against *Wolinella* strains react with the cells of other species of oral and nonoral microorganisms (Table 6).

The serological relationships among *Wolinella* species are shown in Table 7. These reactions are consistent with the findings of Badger and Tanner (2), who used an agglutination assay. However, the ELISA has the advantage of a distinct end result with the formation of a blue color. After the specific antiglobulin has been made, prepared, and characterized, this assay can be performed faster than multiple biochemical tests. As used in these studies, the ELISA does not require broth culture of test strains.

The G+C content of the DNA is 43 to 47 mol% (melting temperature method).

Strains were isolated from lesions in human oral cavities and from a blood culture.

Pathogenicity unknown.

The type strain is strain ATCC 35224 (= VPI 9584).

Differentiation of *W. curva* from other species of *Wolinella*.

Table 8 indicates the level of similarity within and between cluster analysis groups. The data are consistent with the previous difficulty in separating these species solely on the basis of their biochemical characteristics. Figure 9 is a dendrogram of the phenotypic characteristics and serological reactions of the strains examined. The *Wolinella* strains formed two clusters. *W. curva* and *W. succinogenes* formed

TABLE 8. Mean inter- and intragroup similarities of three *Wolinella* species, as determined with the Jaccard coefficient

Taxon	Level of similarity (%)			
	<i>W. curva</i>	<i>W. succinogenes</i>	<i>W. recta</i>	VPI strain 10279
<i>W. curva</i> (four strains)	95.3 ± 1.9 ^a			
<i>W. succinogenes</i> (two strains)	82.6 ± 3.8	86.2		
<i>W. recta</i> (three strains)	53.7 ± 2.8	50.2 ± 3.5	88.7 ± 7.1	
<i>Wolinella</i> sp. strain VPI 10279	65.1 ± 1.5	66.6 ± 4.5	65.1 ± 1.5	100

^a Mean ± standard deviation.

FIG. 9. Dendrogram of cluster analysis of *Wolinella* strains. Two clusters were formed. Cluster 1 contained the *W. recta* strains. Cluster 2 contained two subgroups of *W. curva* and *W. succinogenes*. *Wolinella* sp. strain VPI 10279 joined this cluster at a lower level.

subgroups within the first cluster, with strain VPI 10279 joining this cluster at a lower level. The *W. recta* isolates clustered together in the second cluster.

Characteristics useful in differentiating *Wolinella* species are summarized in Table 5. *W. curva* and *W. succinogenes* are difficult to separate solely on the basis of biochemical tests. In practice, the origin of an isolate may be useful in determining the species to which it belongs. Strains of *W. recta* can be differentiated because the cells are predominantly straight, have a different cell wall ultrastructure, and are more sensitive to inhibitory agents than *W. curva* and *W. succinogenes*. The three described species are serologically distinct.

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