Taxonomy of the Genus *Veillonella* Prévot

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Results of deoxyribonucleic acid homology studies of 116 strains of *Veillonella* Prévot, representing the two species and seven subspecies currently recognized in this genus, showed seven deoxyribonucleic acid homology groups distinct at the species level. Because the type strains of *V. parvula* subsp. *parvula* and *V. alcalescens* subsp. *alcalescens* had high homology, we regard *V. alcalescens* Prévot 1933 as a later subjective synonym of *V. parvula* (Veillon and Zuber, 1896) Prévot 1933. The species *V. parvula*, *V. dispar* (Rogosa) comb. nov., *V. atypica* (Rogosa) comb. nov., *V. rodentium* (Rogosa) comb. nov., *V. ratti* (Rogosa) comb. nov., *V. criceti* (Rogosa) comb. nov., and *V. caviae* sp. nov. (type strain, ATCC 33540) are recognized. Because most strains of *V. criceti* produced acid in peptone-yeast extract-fructose media, the genus description is emended to include strains that ferment fructose.

Organisms in the genus *Veillonella* Prévot are anaerobic, nonmotile, nonsporulating, small, gram-negative cocci arranged as diplococci, masses, and short chains. By electron microscopy, the basic arrangement appears to be diplococcal. Members of the genus generally do not attack carbohydrates but produce propionate and acetate from lactate, pyruvate, and peptones. The type species was first described by Veillon and Zuber (25) in 1898 and was designated *Staphylococcus parvulus*. The genus *Veillonella*, proposed in 1933 by Prévot (17), included small, anaerobic, gram-negative cocci occurring in irregular masses. Two species, *V. parvula* (Veillon and Zuber) Prévot and *V. alcalescens* (Prévot), were included.

The current taxonomy of *Veillonella*, most recently reviewed by Rogosa in 1964 (18), is based on the results of extensive cultural, nutritional, biochemical, and serological studies of a large number of isolates (4, 18, 19, 21–23). *V. parvula*, with three subspecies (*V. parvula* subsp. *parvula*, *V. parvula* subsp. *rodentium*, and *V. parvula* subsp. *atypica*), and *V. alcalescens*, with four subspecies (*V. alcalescens* subsp. *alcalescens*, *V. alcalescens* subsp. *ratti*, *V. alcalescens* subsp. *criceti*, and *V. alcalescens* subsp. *dispar*), are currently recognized. The species and subspecies are differentiated serologically by whole-cell tube-agglutination reactions and on the basis of an absolute requirement for putrescine or cadaverine or of the decomposition of hydrogen peroxide by *V. alcalescens*, or both of the latter.

Preliminary deoxyribonucleic acid (DNA) homology results (T. D. Mays and J. L. Johnson,

Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, 1147, p. 179) showed that the type strains of the two species, *V. parvula* and *V. alcalescens*, had relatively high homology with each other and that some of the type strains of the various subspecies had little or no homology with each other or with the type strains of the species.

The purpose of this investigation was to ascertain the genetic groups of veillonellae by means of their DNA homologies. Many of the extant organisms investigated by Rogosa, strains from other culture collections, and some clinical isolates from the collection of the Department of Anaerobic Microbiology at Virginia Polytechnic Institute and State University (VPI) were included in the study.

MATERIALS AND METHODS

Bacterial strains. The *Veillonella* strains studied and their derivations and sources are listed in Table 1. Reference DNA for homology studies was prepared from the first strain listed in each group and from strain VPI 12134. Other strains are listed in order of decreasing homology values with the first strain of each group.

Biochemical reactions and growth requirements. Reduction of nitrate to nitrite, gas production, the pH of cultures in carbohydrate media, and volatile fatty acids produced in glucose, fructose, pyruvate, and lactate media were determined as described in the *Anaerobe Laboratory Manual* (5).

Requirements for putrescine, hypoxanthine, and vitamins were determined by serially transferring strains three or more times (or until there was no growth in fewer than three transfers) in 3 ml of prereduced Casamino Acids-lactate (CL) broth and in CL broth supplemented with combinations of putrescine, hypoxanthine, and vitamins. CL broth contained

TABLE 1. Strains used in this study

VPI No.	Derivation of VPI strains, other designations ^a
11221	*ATCC 10790, Prevot Te3, type strain of <i>V. parvula</i> subsp. <i>parvula</i>
0557	Prevot Te3, pyorrhea-gingivitis
12093	Ro-Rat 17, rat mouth
5452	Prevot 1305B, cystitis
3822	Prevot 4056A, inflammation of tongue
1724	CDC 1496B
5259	Human, pus
12173	Ro-N4282, La-N4282, E. G. D. Murray, N4282 (<i>V. gazogenes</i>)
12169	Ro-VHII
12109	Ro-Rv3, rat mouth
12161	Ro-Rv10x, rat mouth
12107	Ro-Rv7x, rat mouth
12119	Ro-Rv19, rat mouth
12092	Ro-RA26, rabbit mouth
9265	Human, mouth ulcer
12126	Ro-Rat 22, rat mouth
8463	Human, incisor wound
12156	Ro-Rv25x, rat mouth
12125	Ro-Rv2, rat mouth
12120	Ro-Rv24x, rat mouth
11224	*ATCC 17745, Ro-259, Prevot 259, human mouth, type strain of <i>V. alcalescens</i> subsp. <i>alcalescens</i>
12103	Ro-Rv11x, rat mouth
12076	Ro-RA27, rabbit mouth
8923	Human
3244A	Human
5788	Human, chest fluid
12154	Ro-Rv18, rat mouth
12168	Ro-RA15, rabbit mouth
12099	Ro-Rv24, rat mouth
12176	Ro-RA23, rabbit mouth
12145	Ro-RA11, rabbit mouth
12078	Ro-RA38, rabbit mouth
12113	Ro-RA31x, rabbit mouth
12164	Ro-RA37, rabbit mouth
10860A	Human
3420	Normal human mouth
8524	Human
12123	Ro-RA31, rabbit mouth
12097	Ro-RA3, rabbit mouth
12149	Ro-RA36, rabbit mouth
12112	Ro-RA33, rabbit mouth
12091	Ro-RA17, rabbit mouth
12111	Ro-RA28, rabbit mouth
12117	Ro-RA19, rabbit mouth
11223	*ATCC 17748, Ro-ERN, La-ERN, human mouth, type strain of <i>V. alcalescens</i> subsp. <i>dispar</i>
12114	Ro-ERN, La-ERN
6878	Human, urine
10460	Human, jaw bone
8551	Human, vaginal smear
6950	Human, oral lesion
5454	Prevot 2470, bronchitis
11506	Human, stomach aspirate
11220	*ATCC 17744, La-KON, human saliva, type strain of <i>V. parvula</i> subsp. <i>atypica</i>
5877	Prevot 3529
12121	Ro-SHE, La-SHE, human saliva
12106	*ATCC 12641, Ro-BYR2, La-BYR2, <i>V. alcalescens</i> subsp. <i>alcalescens</i> (<i>V. gazogenes</i>)
12170	Ro-11F, La-11F, human saliva

TABLE 1. Continued

VPI No.	Derivation of VPI strains, other designations ^a
12148	Ro-KON, La-KON, human saliva
12118	Ro-DOE, La-DOE, human saliva
12172	Ro-COR1, La-COR1, human saliva
12157	Ro-MIN1, La-MIN1, human saliva
12077	Ro-WAR, La-WAR, human saliva
12166	Ro-NEM, La-NEM, human saliva
12132	Ro-ECK, La-ECK, human saliva
12108	Ro-RAK, La-RAK, human saliva
12162	Ro-5, <i>V. parvula</i>
5456	Prevot 2402, bronchitis
12917	*ATCC 14894, H. C. Douglas, UW221; Foubert and Douglas, T-5; (<i>Micrococcus lactilyticus</i>)
12177	Ro-LIT3, La-LIT3, human saliva
11560	Human, lung
5418	Prevot 3227
12165	Ro-DAY, La-DAY, human saliva
3821	Prevot 2842, bronchial sample
3885	Normal mouth
12105	Ro-Z24B, La-Z24B, E. G. D. Murray, Z24B
12916	*ATCC 12642, La-CLE, M. J. Pelczar, human saliva
12138	Ro-CAM1, La-CAM1, human saliva
12088	Ro-PRI, La-PRI, human saliva
5451	Prevot 1582, tonsillitis
12159	Human saliva
3240	Ro-FOS, La-FOS, human saliva
12102	Ro-L3, La-L3, human saliva
12134	Ro-PV4, guinea pig mouth
11222	*ATCC 17743, Ro-HV19, hamster mouth, type strain of <i>V. parvula</i> subsp. <i>rodentium</i>
12146	Ro-HV18, hamster mouth
12089	Ro-HV23, hamster mouth
12155	Ro-HV12, hamster mouth
11225	*ATCC 17746, Ro-Rv12x, rat mouth, type strain of <i>V. alcalescens</i> subsp. <i>ratti</i>
12147	Ro-Rv12x, rat mouth
11226	*ATCC 17747, Ro-HV1, hamster mouth, type strain of <i>V. alcalescens</i> subsp. <i>criceti</i>
12096	Ro-HV16, hamster mouth
12175	Ro-HV25, hamster mouth
12094	Ro-HV1, hamster mouth
12141	Ro-HV13, hamster mouth
12144	Ro-HV7, hamster mouth
12158	Ro-HV2, hamster mouth
12130	Ro-HV9, hamster mouth
12127	Ro-HV24, hamster mouth
12135	Ro-HV3x, hamster mouth
12101	Ro-HV5, hamster mouth
12083	Ro-HV8, hamster mouth
12085	Ro-HV20, hamster mouth
12131	Ro-HV6, hamster mouth
12174	Ro-HV10, hamster mouth
3312A	Human, abdomen
6788D	Human, blood
12140	Ro-PV1, guinea pig mouth
12104	Ro-PV2, guinea pig mouth
12086	Ro-PV6, guinea pig mouth
12151	Ro-PV4x, guinea pig mouth
12136	Ro-PV8, guinea pig mouth
12153	Ro-PV3, guinea pig mouth
12100	Ro-PV7, guinea pig mouth

TABLE 1. *Continued*

VPI No.	Derivation of VPI strains, other designations ^a
8638	Human, vagina
12095	Ro-MV3, mouse mouth
7944	Human, urine
1148B	Normal human feces

^aThe VPI strain tested was from the first derivation cited. Ro- = Rogosa collection number; La- = Langford et al. (13) strain number. ATCC strains are designated with an asterisk (*) for convenience of location in the table.

(per 100 ml): 0.5 g of Casamino Acids (Difco), 0.7 ml of 85% lactic acid, 4 ml of salts solution (5), 0.4 ml of resazurin solution (5), and 0.05 g of cysteine HCl; pH was adjusted to 7.0. Supplements were added to CL broth from filter-sterilized stock solutions. Putrescine stock solution was prepared by dissolving 45 mg of putrescine in 20 ml of ethanol and adding 80 ml of distilled water. The final concentration of putrescine in the test medium was 0.45 mg/100 ml. Hypoxanthine solution consisted of 300 mg of hypoxanthine mixed with 50 ml of distilled water and dissolved by adding drops of 10 N sodium hydroxide while heating. The final concentration of hypoxanthine in the test medium was 6 mg/100 ml of broth. Vitamin solution was prepared by adding 2 mg of calcium pantothenate, 20 mg of pyridoxal HCl, 20 mg of thiamine HCl, 2 mg of niacin, 0.01 mg of *p*-aminobenzoic acid, and 0.1 mg of biotin to 10 ml of ethanol in 40 ml of distilled water acidified with 2 drops of concentrated hydrochloric acid; 0.01 ml of vitamin solution was added per ml of test broth. Original inoculum for the serial transfers was 0.03 ml from an overnight peptone-yeast extract (5) broth culture. Serial transfers were 0.03 ml of inoculum from an overnight culture with at least 2+ growth (moderate turbidity) or from a 36-h-old culture if growth was less than 2+ in 18 h.

Polyacrylamide gel electrophoresis of soluble proteins. Cultures grown for 18 h in 5 ml of supplemented brain heart infusion broth (5) with 0.1% calcium carbonate were sedimented, and 0.08 ml of pH 7.0 tris(hydroxymethyl)aminomethane-chloride buffer and 0.15 g of 74- to 110- μ m-diameter glass beads were added to the cell pellet. The cells were disrupted by shaking for two 2-min periods (16). Broken-cell preparations were heated at 55°C for 5 min. Cellular debris was sedimented by centrifugation, and one-third volume of powdered sucrose per volume of supernatant was added. Soluble proteins were separated by slab gel electrophoresis and stained as described previously (16).

DNA isolation. The test organisms were grown in a medium prepared as described previously (3); it contained mineral salts, 1% Trypticase, 0.5% yeast extract, 2% sodium lactate, 0.03% cysteine, 0.03% sodium formaldehyde sulfoxalate, and 0.05 M potassium phosphate buffer (pH 7.0). Flasks containing 1 liter of medium were inoculated with 20 ml of an overnight culture and incubated for 18 to 24 h at 37°C.

The harvested cells were suspended in 0.15 M NaCl and 0.01 M ethylenediaminetetraacetic acid salt solution (pH 8.0), incubated with 50 μ g of pronase per ml at 50°C for 30 min, and then lysed by adding 20%

sodium dodecyl sulfate to a final concentration of 1%. After a preliminary extraction with chromatography-grade liquid phenol, high-molecular-weight DNA (for in vitro labeling) was isolated by the method of Marmur (14). Other DNA preparations were isolated by a hydroxyapatite procedure (7). This procedure was slightly modified in that the lysate did not contain any sodium borate and contained 0.1 M phosphate buffer (pH 6.8).

Guanine-plus-cytosine (G+C) content of DNA. Thermal melting points (T_m) were used to determine the G+C contents of the DNA preparations, with DNA from *Escherichia coli* b used as the standard (9, 15).

Preparation of labeled DNA. The high-molecular-weight DNA (diluted to 200 μ g/ml) was nicked by incubating it for 1 min with 0.1 μ g of electrophoretically purified deoxyribonuclease per ml. The deoxyribonuclease was then inactivated by heating at 65°C for 10 min. The nicked DNA (10 μ g) was labeled with [³H]thymidine-triphosphate by means of a nick translation method (1). The specific activity of the labeled DNA preparations was about 100,000 cpm/ μ g.

DNA homology experiments. Homology experiments were done by a variation of the S1 nuclease procedure described by Crosa et al. (2, 10). The reassociation mixtures contained 10 μ l (ca. 0.025 μ g) of labeled DNA, 50 μ l (30 μ g) of unlabeled DNA, and 50 μ l of 0.88 M NaCl-10⁻³ M 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer (pH 7.0). Just prior to each experiment, the amount of labeled DNA needed for the experiment was denatured by heating in a boiling-water bath for 5 min. The reaction vials were incubated for 16 h at 60°C.

The unlabeled DNA used was either denatured and fragmented homologous or heterologous bacterial DNA or native fragmented salmon sperm DNA. Reaction vials containing native salmon sperm DNA were used to measure the amount of self-renaturation of labeled DNA during the incubation period.

RESULTS

The results of the G+C determinations and of the DNA experiments are listed in Table 2. The serological results in Table 2 were summarized in an earlier paper (19) and provided a major criterion used in the taxonomy of these organisms (20).

The moles percent G+C values obtained with the strains in this study are consistently 2 to 3% lower than those reported for the respective type strains in the 8th edition of *Bergey's Manual* (20). Those values were determined by CsCl buoyant density centrifugation. Small differences in G+C values obtained with buoyant density and T_m methods are not uncommon.

The DNA homology values reported here were determined by the S1 nuclease method. It is often difficult to obtain precisely reproducible results with this method. For example, when several preparations of DNA from a single strain were compared with labeled DNA from that strain, results varied by 10 to 15%. Probable reasons for this have been discussed previously (8).

TABLE 2. Homology values of *Veillonella* strains

Unlabeled DNA from strain	Sero- group	mol% G+C	Percent homology with								
			11221	11223	11220	12134	11222	11225	11226	3312A	12140
<i>V. parvula</i>											
11221	VI	38	(100)	35	20	14	21	4	5	2	9
0557				87	32			6		0	8
12093	II	39		81	24		14				
5452				79	36			5			
3822				78			7				
1724				78	41			9		5	10
5259				78			10				
12173				78	35	24	15		7	7	16
12169	IV	37		77	45	29	16		9	2	17
12109				77	40	25	21				
12161	II			76	41	24	20				
12107	II			76	37	21	21				
12119	II			76	41	23	15				
12092	IV			76	42	21	9				
9265				75	35	14	11		0	8	16
12126	II			75	44	23	21				
8463				75	39		6				
12156	II			74	39	23	23				
12125	II			74	39	25	13		8	9	20
12120	II			74	40		11				
11224	IV	37		74	34	12	6				
12103	II			73	40		12				
12076				72	36		9		3	7	15
8923				71	37		6				
3244A				71	43	27	6	5	7		
5788		37		70	40	12	5	3	5		
12154				70	37	26	24				
12168	II	37		69	31	21	9		3	6	13
12099	II			69	34	21	14				
12176	II	38		67	36	25	9				
12145	II			67	32	22	8				
12078	II			66	35	25	5				
12113	II			65	29	21	10				
12164	II	40		64	32	20	7				
10860A				64	31		9				
3420		37		63	40	12	7	2	5		
8524				63	42	8	8	1	6		
12123	II			62	43	26	18				
12197				60	33		6		3	8	13
12149	II	39		58	38	22	9				
12112	II	39		56	42	27	14		8	8	16
12091				55	38	19	3				
12111	II			55	42	25	6				
12117	II			53	29	24	10				
AVERAGE		38		70	38	22	16	3	5	7	15
<i>V. dispar</i>											
11223	VII	38		29	(100)	20	17	12	6	8	3
12114				29	89	28		5	3		13
6878		39		37	87	20		7			
10460				37	82	26		6			
8551		40		54	70	32		7	4	6	14
6950				36	68	27		7	9	11	18
5454		39		36	68	30		4			
11506				38	39	67	27	7			
AVERAGE		39		38	76	27	17	7	5	7	15
<i>V. atypica</i>											
11220	V	38		20	(100)	69	4		6	5	11
5877				22	26	92	73	1		8	8
12121	VI			20	22	92	79	5			17
12106	VI			18	22	90	64	4			
12170	VI	40		18	28	88	65	2	7	8	15
12148	V			25	23	88	75	4			

TABLE 2. *Continued*

Unlabeled DNA from strain	Sero- group	mol% G+C	Percent homology with								
			11221	11223	11220	12134	11222	11225	11226	3312A	12140
12118	VI		18	26	88	71	4				
12172	VI		23	23	87	71	5				
12157	VI		24	26	82	77	2				
12077			18	24	82	71	4				
12166	VI	39	23	24	79	78	9				
12132	VI		22	24	79	84	8				
12108	VI		7	28	78		3	4	5		
12162		39	19	24	78	71	6				
5456		39	21	25	78	79	10				
12917		40	15	17	78		6	2	1	9	14
12177	VI	39	30	24	77	66	3		8		
11560		39	17	22	77		5				
5418			21	27	77	57	9		5	9	17
12165	VI		22	25	77	79	9				
3821		37	20	35	76		8	3	6		
3885		36	17	23	75		8	3	6		
12105	VI		18	22	74	76	8		5	7	15
12916	VI	40	16	17	74		10	3	5	10	16
12138	VI		23	24	73	78	10				
12088	VI		21	24	72	75	7		9		
5451		37	41	37	72		15	4	7		
3240		36	18	23	72		8	3	2		
12159	VI	38	24	26	70	67	6	3	5	5	17
12102	VI		23	24	70	58	7				
12134		40	19	23	56	(100)	19		5	5	10
AVERAGE		39	20	24	78	72	7	5	6	8	15
<i>V. rodentium</i>											
11222	II	42	18	15	12	8	(100)	3	4		
12146	II		21	26	21		100	3	9		
12089			22	20	17		99	3	4	10	16
12155	II	43	20	24	17		97	6	10	10	19
AVERAGE		43	20	21	17	8	99	4	7	19	18
<i>V. ratii</i>											
11225	III	41	10	1	2	1	2	(100)	16	1	1
12147		43	5	5	2	11	1	88	14	1	
AVERAGE		42	8	3	2	6	2	88	15	1	1
<i>V. criceti</i>											
11226	I	38	3	1	4	4	9	12	(100)	3	3
12096	I	39	6	4			3	9	98	12	11
12175	I		6	1	4		3		91	9	8
12094	I		6	6	5		1	7	89	9	9
12141	I	40	5	5	8		2	12	88	10	10
12144	I		5	9	8		8	3	87	10	10
12158		39	6	3	4		2	11	85	11	13
12130	I		7	7	4		6	8	85	10	9
12127	I		5	4	7		2	7	84	11	7
12135	I		8	9	8			8	84	9	6
12101	I		5	6	5		3	8	80	9	8
12083	I		5	8	8		8	10	77	11	9
12085	I			3	10		1		77	9	6
12131	I	39	6	6	4	2	1	8	75	4	7
12174	I	39	5	4	3		2	10	72	8	6
AVERAGE		39	6	5	6	3	3	9	84	9	8
Group 3312A											
3312A		37	8	6	6	1	1	2	6	(100)	3
6788D		35	12	14	9	4	2	4	5	77	3
AVERAGE		36	10	10	8	3	2	3	6	77	3

TABLE 2. *Continued*

Unlabeled DNA from strain	Sero- group	mol% G+C	Percent homology with								
			11221	11223	11220	12134	11222	11225	11226	3312A	12140
<i>V. caviae</i> sp. nov.											
12140			17	16	21		21	2	9	11	(100)
12104		39	17	15	17		15	6	9	4	97
12086		38	13	16	19	12	6	4	2	10	96
12151		37	15	19	20	11	13	3	6	4	92
12136		39	15	14	19	11	6	7	6	3	91
12153		39	15	24	23	7	18	3	6	4	75
12100		39	16	18	22	11	18	2	10	5	71
AVERAGE		39	15	17	20	10	14	4	7	6	87
Other <i>Veillonella</i> strains											
8638			15	9	2	0	2	1	3	1	6
12095		40	7	5	6	1	6	9	31	4	3
7944		41	6	11	7	13	9	29	16	10	10
1148B		43	16	10	5	1	4	3	11	3	3

The inherent variation of results with the S1 nuclease method probably explains the lack of 100% homology among cultures reputedly derived from the same strain (e.g., Prévot Te3, *V. parvula* subsp. *parvula*). DNA reference strain 11221 was obtained from the American Type Culture Collection (ATCC) strain 10790, deposited by J. E. Faber, who in turn received it from Prévot (Prévot Te3). Our strain 0557 is strain Te3 obtained directly from Prévot. DNA from strain 11221 had 87% homology with DNA from strain 0557. Similar results were obtained with other reference strains received from both the ATCC and the Rogosa collection. The soluble-cell-protein electrophoretic patterns (16) of these pairs of strains were identical, indicating that the pairs of strains may belong to the same strain. Although there can be slight differences among soluble-cell-protein electrophoretic patterns for different strains within a homology group, different cultures of the same strain usually give identical patterns (16).

The average G+C values for the DNA preparations ranged from 36 to 43 mol%; the majority clustered around 39 mol% (Table 2). The organisms were separable into eight DNA homology groups. Each group is designated by the VPI number of the reference strain for the group. The groups contained from one (11225, *V. alcalescens* subsp. *ratti*) to 44 representatives (11221, *V. parvula* subsp. *parvula*). There was 20 to 40% intergroup homology among strains in homology groups 11221, 11223, and 11220 (*V. parvula* subsp. *parvula* and *atypica* and *V. alcalescens* subsp. *dispar*). Strains in the 11220 group also were tested with a second reference strain, 12134, to see if there were subgroups within the group. No subgroups were indicated. The guinea pig isolates, represented by DNA reference strain 12140, have 10 to 20% intergroup homology with the above-mentioned

groups. The heterogeneity within the groups appears to vary. For example, organisms within the 11221 (*V. parvula* subsp. *parvula*) DNA homology group have from 53 to 87% homology with the reference organism, whereas the organisms within the 11222 (*V. parvula* subsp. *rodentium*) DNA homology group have 97 to 100% homology with the reference strain. The four "other *Veillonella* strains" (8638, 12095, 7944, and 1148B) listed in Table 2 have only low levels of DNA homology with any of the other reference strains.

All strains tested reduced nitrate to nitrite, did not ferment glucose, and produced hydrogen. Nine of 10 strains tested in the 11226 (*V. alcalescens* subsp. *cricketi*) homology group lowered the pH of fructose medium by 0.4 to 1.0 (to pH 5.6 or lower) and produced increased amounts of acetic and propionic acids. The other *veillonellae* did not ferment carbohydrates.

Some cultures required additional putrescine to produce sufficient cell yield for harvesting DNA from 1-liter cultures in Trypticase-yeast extract-lactate medium. This was most noticeable in strain 11224 (type strain of *V. alcalescens* subsp. *alcalescens*) and was consistent with the reported putrescine requirement by this subspecies. The putrescine, hypoxanthine, and vitamin requirements of 100 strains were tested. There was no clear requirement for putrescine, hypoxanthine, or vitamins among any of the homology groups, although these additives stimulated growth of many strains. The type strain of *V. alcalescens* subsp. *alcalescens* and 38 other strains that had high DNA homology with *V. alcalescens* subsp. *parvula* grew well in CL medium supplemented with putrescine, hypoxanthine, and vitamins, and in CL medium supplemented with hypoxanthine and vitamins (without putrescine). Vitamins or hypoxanthine stimulated growth of about half of the strains.

There was no growth in CL without additives.

Polyacrylamide gel electrophoresis patterns for representatives of each of the homology groups are shown in Fig. 1.

DISCUSSION

Strains of *Veillonella* are exceedingly similar phenotypically. In general, they do not ferment the usual carbohydrates. Our preliminary studies of the fructose fermentation by some strains of *V. alcalescens* subsp. *criceti* suggest that this may be associated with the presence of a plasmid. In other rare instances (11, 12), ribose has been very weakly utilized for biosynthetic, non-fermentative purposes only. All strains in the genus similarly attack several metabolic intermediates (e.g., lactate and pyruvate). Cross-reactions among some of the serological groups, particularly serogroups II, IV, V, and VI, have been reported (19). However, these low-titer cross-reactions were reciprocally absorbed (19), and serological groups were obtained with the absorbed antisera at high titers (1:2560 to 1:40,960). Because serovars (serotypes) do not necessarily designate different species, the serovars were designated subspecies in two species. However, the separation of the species was difficult. The requirement for cadaverine or putrescine varied among strains in some of the

subspecies (20). The decomposition of hydrogen peroxide often varied among subcultures of the same strain.

The results of this study demonstrate that the genus *Veillonella* consists of at least eight genetically distinct groups. Of the 116 strains of *Veillonella* tested, 112 showed a high level of DNA homology with one or another of eight reference strains. There was negligible or low intergroup homology, a result that indicates species-level differences (6). In general, the homology groups are represented by the type strains of the subspecies given in the 8th edition of *Bergey's Manual* (20). Two of the DNA homology groups, 12140 and 3312A, have not been recognized previously as distinct entities. The 12140 homology group consists of seven strains, all of which were isolated from the mouths of guinea pigs. These organisms appear to represent a major group of veillonellae isolated from the guinea pig, and therefore we are recognizing this group as a new species (see below). The 3312A homology group consisted of two human clinical isolates, one from urine and one from blood. Because there are only two strains in this group and strains of other groups appear to be much more commonly isolated from humans, we are deferring proposing a name for this group until additional strains are available for study. DNA from three other human strains (8638, vagina; 7944, urine; and 1148B, feces) and strain 12095 (mouse) had no homology with any of the reference DNA preparations used. These obviously represent other *Veillonella* species, but their importance cannot be ascertained at this time.

We propose that the former subspecies of *V. parvula* and *V. alcalescens* be elevated to species rank as *V. parvula* (Veillon and Zuber, 1898) Prévot, *V. atypica* (Rogosa) comb. nov., *V. dispar* (Rogosa) comb. nov., *V. rodentium* (Rogosa) comb. nov., *V. ratti* (Rogosa) comb. nov., and *V. criceti* (Rogosa) comb. nov., and that the guinea pig isolates with significant homology with strain 12140 be named *V. caviae* sp. nov. with strain 12140 (= ATCC 33540) as the type strain. The type strain of *V. parvula* subsp. *parvula* had 74% homology with the type strain of *V. alcalescens* subsp. *alcalescens*; these are, therefore, the same species and are designated *V. parvula* by rule of priority. The relatedness of these two strains also has been indicated by Yoshimura et al. (26), who found that the acetate kinases from the two organisms have similar properties as measured by the effect of succinate on enzyme activity. Also, the purified enzymes gave serological cross-reactions and had similar electrophoretic migrations.

Sources of the strains of the species and the serogroups to which they belong, when known, are summarized in Table 3.

FIG. 1. Polyacrylamide gel electrophoretic patterns of soluble proteins from representative strains of *Veillonella* species. Lanes 1 and 2, *Streptococcus faecalis* standard reference strain U4-20. *V. parvula*: lane 3, ATCC 10790 (type); lane 4, VPI 12093. *V. atypica*: lane 5, ATCC 17744 (type); lane 6, VPI 12121. *V. dispar*: lane 7, ATCC 17748 (type); lane 8, VPI 6878. *V. rodentium*: lane 9, ATCC 17743 (type); lane 10, VPI 12146. *V. criceti*: lane 11, ATCC 17747 (type); lane 12, VPI 12096. *V. caviae*: lane 13, ATCC 33540 (type); lane 14, VPI 12104. *V. ratti*: lane 15, ATCC 17746 (type). Other veillonellae: lane 16, human isolate VPI 3312A.

TABLE 3. Type strains, sources, and serogroups of *Veillonella* species

Species	Type strain	Isolated from	Serogroup							Serogroup not tested
			I	II	III	IV	V	VI	VII	
<i>V. parvula</i>	ATCC 10790	Human	—	—	—	3 ^a	—	1*	—	13
		Rat	—	12	—	1	—	—	—	—
		Rabbit	—	10	—	—	—	—	—	4
		Unknown	—	—	—	1	—	—	—	1
<i>V. dispar</i>	ATCC 17748	Human	—	—	—	—	—	—	1*	6
<i>V. atypica</i>	ATCC 17744	Human	—	—	—	—	1*	15	—	7
		Guinea pig	—	—	—	—	—	—	—	1
		Unknown	—	—	—	—	—	2	—	4
<i>V. rodentium</i>	ATCC 17743	Hamster	—	3*	—	—	—	—	—	1
<i>V. rattii</i>	ATCC 17746	Rat	—	—	1*	—	—	—	—	1
<i>V. criceti</i>	ATCC 17747	Hamster	14*	—	—	—	—	—	—	1
<i>V. caviae</i>	ATCC 33540	Guinea pig	—	—	—	—	—	—	—	7

^aNumber of strains in serogroup; — = no strains; * = serogroup to which the type strain of the species belongs.

The description of the genus *Veillonella* presently states that carbohydrates and polyols are not fermented (20). Because fermentation of fructose occurs in some strains of *V. criceti*, the description of the genus is here emended.

Emendation of the description of the genus *Veillonella*. Carbohydrates and polyols are not fermented by most strains. Fructose fermentation has been observed with most strains of *V. criceti*.

Species and emendations of the descriptions of species in the genus. *Veillonella parvula* (Veillon and Zuber) Prévot 1933. Putrescine not required for growth in medium containing Casamino Acids, lactate, hypoxanthine, and vitamins. Strains are of human, rat, and rabbit origin. Most strains are from rats and rabbits and belong to serogroup II. Serogroups IV and VI are represented in the human strains. Type strain: ATCC 10790.

Veillonella dispar (Rogosa) comb. nov. (Basonym: *V. alcalescens* subsp. *dispar* Rogosa 1965, 708). Four of eight strains tested could be serially subcultured in a medium containing Casamino Acids, lactate, putrescine, hypoxanthine, and vitamins; four strains did not grow in the fourth serial transfer. Type strain: ATCC 17748 (24).

V. atypica (Rogosa) comb. nov. (*V. parvula* subsp. *atypica* Rogosa 1965, 707). Putrescine is not required for growth by the 29 strains tested in medium containing Casamino Acids, hypoxanthine, and vitamins. Isolated from the buccal cavity of man. Most strains belong to serogroup

VI; one strain belongs to serogroup V. Type strain: ATCC 17744 (24).

V. rodentium (Rogosa) comb. nov. (*V. parvula* subsp. *rodentium* Rogosa 1965, 707). Putrescine is not required for growth of the three strains tested in medium containing Casamino Acids, lactate, hypoxanthine, and vitamins. Vitamins were required by one of three strains tested. Isolated from the buccal cavity of the hamster. All strains tested belong to serogroup II. Type strain: ATCC 17743 (24).

V. rattii (Rogosa) comb. nov. (*V. alcalescens* subsp. *rattii* Rogosa 1965, 708). The type strain grew in a medium containing Casamino Acids and lactate supplemented with either hypoxanthine and vitamins or putrescine and vitamins. Type strain: ATCC 17746 (24).

V. criceti (Rogosa) comb. nov. (*V. alcalescens* subsp. *criceti* Rogosa 1965, 708). Only 3 of the 13 strains tested grew in a medium containing Casamino Acids, lactate, putrescine, hypoxanthine, and vitamins. Most strains fermented fructose. Type strain: ATCC 17747 (24).

V. caviae sp. nov. (M.L. noun *Cavia* genus of the guinea pig; M.L. gen. noun *caviae* of the guinea pig). Cells of the type strain and of six other strains tested are 0.2 to 0.3 µm in diameter and are arranged in masses and pairs. Cells are gram negative, nonmotile, and nonsporeforming. Surface colonies on blood agar plates (brain heart infusion agar supplemented with yeast extract and 5% defibrinated rabbit blood) are 1.0 to 2.0 mm in diameter, circular, and flat with an

entire edge after 48 h at 37°C in an anaerobic atmosphere. No surface growth is obtained on similar blood agar plates incubated aerobically or in an atmosphere enriched to 10% with carbon dioxide.

Broth growth in prereduced peptone-yeast extract medium (5) is lightly turbid, usually without sediment in 24 h. In peptone-yeast extract-lactate or peptone-yeast extract-pyruvate broths, growth is turbid, usually with a smooth sediment. Amygdalin, arabinose, cellobiose, erythritol, esculin, fructose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, ribose, sorbitol, starch, sucrose, trehalose, and xylose are not fermented. Neither esculin nor starch is hydrolyzed. Indole is not produced. Nitrate is reduced to nitrite. Small to moderate amounts of acetate and propionate are detected in peptone-yeast extract cultures. Lactate and pyruvate are metabolized to acetate, propionate, hydrogen, and carbon dioxide. The type strain and six other strains tested grew well in a medium containing Casamino Acids, lactate, putrescine, hypoxanthine, and vitamins. Growth was not regularly obtained if any of these constituents was deleted from this medium.

Isolated from the mouths of guinea pigs.

The G+C content of the DNA is 39 mol%.

Differentiated from other species in the genus by DNA/DNA homology studies and by electrophoretic mobilities of soluble cellular proteins.

Type strain: ATCC 33540 (= VPI 12140 from Rogosa PV1, guinea pig mouth).

Comments. According to our results, most of the human oral isolates belong to *V. atypica*, *V. parvula*, or *V. dispar*. Of the human strains examined serologically, most belonged to serogroup VI of *V. atypica*.

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REPRINT REQUESTS

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