Emended Descriptions of *Prevotella denticola*, *Prevotella loescheii*, *Prevotella veroralis*, and *Prevotella melaninogenica*

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During studies of human periodontal disease, a number of bacterial strains were encountered that, on the basis of results of standard biochemical tests, appeared to be *Prevotella buccalis*, *Prevotella denticola*, *Prevotella melaninogenica*, or *Prevotella loescheii*. However, use of the standard biochemical tests, cellular fatty acid analyses, and the polyacrylamide gel electrophoresis patterns of soluble proteins resulted in conflicting identifications of these strains. The results of tests for cellobiose fermentation, inulin fermentation, and pigment production were responsible for most of the discordant results. Cellular fatty acid analyses in which the Microbial Identification System was used did not differentiate these strains from validly described species, even though separate library entries were created for them. DNA reassociation determinations in which the S1 nuclease procedure was used showed that cellobiose fermentation and pigment production are variable among strains of *P. melaninogenica* and *P. denticola* and that fermentation of xylan is not a reliable characteristic for differentiating *P. buccalis* from *Prevotella veroralis*. In contrast to previous indications, most strains of *P. veroralis* do not ferment xylan. These species can be differentiated by DNA-DNA reassociation and by cellular fatty acid analysis, using the Microbial Identification System, but differentiation by currently described phenotypic characteristics is not reliable. Similarly, *P. loescheii* and the genetically distinct (but closely related) D1C-20 group cannot be distinguished reliably from each other or from *P. veroralis*, *P. denticola*, and *P. melaninogenica* on the basis of currently described phenotypic tests other than cellular fatty acid composition or, for some species, electrophoretic patterns of soluble whole-cell proteins.

Prevotella species are a major portion of the microflora of human gingival crevices in patients with periodontal disease (9). The similarity of the phenotypic characteristics of these species often makes their routine differentiation and identification difficult. A total of 19 strains of phenotypically similar *Prevotella* (*Bacteroides*) species were used in this study in an attempt to determine their DNA relatedness and to identify reliable phenotypic characteristics for their differentiation. As a result of these analyses, we propose emended descriptions of *Prevotella veroralis*, *Prevotella denticola*, *Prevotella melaninogenica* (2381 DNA-DNA similarity group that contains the type strain), *Prevotella loescheii*, and the genetically closely related D1C-20 group (3).

MATERIALS AND METHODS

The strains used in this study are shown in Table 1. Most of these strains were isolated from human gingival or subgingival plaque; the exceptions were *P. loescheii* VPI 9621, which came from a human cranial abscess, and *P. melaninogenica* VPI 15087, which came from sputum. Strain VPI D1C-20B was a recent single-colony isolate obtained from the original lyophilized culture of strain D1C-20, and strain VPI 15056 was strain D1C-20 accessioned under a different Virginia Polytechnic Institute and State University (VPI) number. Strains belonging to named species had characteristics that have been described previously (3, 4, 7, 16). Strains of phenospecies "Prevotella D70" were similar to *Prevotella buccalis* phenotypically but produced discordant cellular fatty acid and polyacrylamide gel electrophoresis profiles. Three nonpigmenting strains of phenospecies "Prevotella D71" were identified as *P. denticola* by cellular fatty

acid analysis but were unlike *P. denticola* in that cellobiose was fermented. Two pigmented strains that had characteristics of *P. loescheii* (acid production from cellobiose and pigmentation) also were included in phenospecies "Prevotella D71" because of their different cellular fatty acid profiles. Isolates VPI D136E-13a, VPI D136E-13b, and VPI D136E-13c were different single colonies that were picked from strain VPI D136E-13 in an effort to purify the culture, which was thought to be a mixture of *P. loescheii* and *P. melaninogenica*.

All of the strains listed in Table 1 were characterized phenotypically on at least two occasions, at the time of original isolation or receipt in our laboratory and from lyophilized cultures when this study was initiated, which was 5 to 13 years later.

The methods used for cultural tests and the conditions used were those described by Holdeman et al. (2); the tests included tube tests in prereduced media and analysis of short-chain fermentation acids by gas-liquid chromatography. The methods used for electrophoretic analysis of soluble cellular proteins were the methods described by Moore et al. (8), and the methods used for cellular fatty acid analysis were the methods described by Sasser (13). For some strains, 10% (vol/vol) sterile rabbit serum was added to differential media to enhance growth. Final pH values of cultures in fermentation broth media containing glucose, glycogen, maltose, starch, or trehalose also were determined without added serum to verify the fermentation of these substrates in the absence of serum amylase (3). Pigmentation of colonies was determined by examining growth on supplemented brain heart infusion agar (2) or Trypticase soy-yeast extract agar, both containing 5% (vol/vol) hemolyzed rabbit blood, following anaerobic incubation for at least 14 days. The susceptibilities of the strains to 12 µg of chloramphenicol per ml, 1.6 µg of clindamycin per ml, 3 µg of erythro-

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TABLE 1. Levels of DNA relatedness of *Prevotella* strains used in this study

Unlabeled DNA from:			% Relatedness to labeled DNA from:							
Species or genospecies	Strain (VPI no.)	<i>P. loeschii</i> VPI 9085 ^T	" <i>Prevotella</i> D1C-20" VPI 15056	<i>P. veroralis</i> VPI D22A-7 ^T	" <i>Prevotella</i> D70" VPI D150E-7	<i>P. denticola</i> VPI 14460 ^T	" <i>Prevotella</i> D71" VPI D96K-22A	<i>P. melaninogenica</i> VPI 15087 ^T	" <i>Prevotella</i> D71" VPI D136E-13A	
<i>P. loeschii</i>	9085 ^T	(100) ^a	45	15	13	16	15	8	9	
<i>P. loeschii</i>	9621	90	46	7	7	13	12	8	7	
" <i>Prevotella</i> D1C-20"	15056	49	(100)	7	6	10	8	8	7	
" <i>Prevotella</i> D1C-20"	D1C-20B	50	101	8	8	15	16	8	9	
<i>P. veroralis</i>	D22A-7 ^T	6	6	(100)	82	13	13	16	17	
" <i>Prevotella</i> D70"	D126E-4A	6	4	90	89	15	13	21	21	
" <i>Prevotella</i> D70"	D130H-30B	4	3	85	81	19	18	19	18	
" <i>Prevotella</i> D70"	D130H-13	13	9	89	89	16	15	22	21	
" <i>Prevotella</i> D70"	D150E-7	5	4	85	(100)	25	27	20	19	
<i>P. denticola</i>	14460 ^T	8	9	22	24	(100)	87	33	37	
<i>P. denticola</i>	12512	8	7	21	21	86	89	30	35	
" <i>Prevotella</i> D71"	D96K-22A	6	5	17	19	80	(100)	20	20	
" <i>Prevotella</i> D71"	D102A-17	10	8	32	30	84	89	38	44	
" <i>Prevotella</i> D71"	D145L-23	10	8	19	20	77	83	22	20	
" <i>Prevotella</i> D71"	D145M-4	7	6	24	25	77	82	21	20	
<i>P. melaninogenica</i>	15087 ^T	8	6	23	23	19	17	(100)	77	
" <i>Prevotella</i> D71"	D136E-13a	5	4	29	33	17	20	79	(100)	
" <i>Prevotella</i> D71"	D136E-13b	13	7	34	35	20	20	86	109	
" <i>Prevotella</i> D71"	D136E-13c	5	4	20	21	21	25	78	99	

^a Levels of relatedness with reference DNA were normalized to 100%.

mycin per ml, 2 U of penicillin G per ml, and 6 µg of tetracycline per ml were tested by using the broth disk method of Wilkins and Thiel (18).

For DNA preparations cultures were grown in the BHI-PYG medium of Holdeman and Johnson (3), which contained mineral salts (2 ppm of calcium chloride, 2 ppm of magnesium sulfate, 20 ppm of sodium chloride), 1% (wt/vol) pepticase, 0.5% (wt/vol) yeast extract, 1% (wt/vol) dehydrated brain heart infusion broth (Difco Laboratories, Detroit, Mich.), 0.05% (wt/vol) hemin, and 0.05 M potassium phosphate buffer. The medium was prepared in 300-ml quantities in 1-liter Erlenmeyer flasks as described by Cummins and Johnson (1). A 3-ml portion of 10% (wt/vol) sterile aqueous NaHCO₃ was added to each 300 ml of medium. The flasks were inoculated with 15-ml portions of 18-h cultures grown in chopped meat broth medium and were incubated at 37°C for 18 to 24 h.

DNA was isolated by using a variation of the procedure described by Saghai-Marooof et al. (12). Cells were harvested by centrifugation at 8,200 × g for 10 to 15 min and were resuspended in suspension buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8], 0.35 M sucrose). Lysozyme (2 mg/ml) was added, and the resulting suspension was incubated at 37°C until lysis was complete. The solution was mixed with an equal volume of 2× lysing solution (90 mM Tris-HCl [pH 8.0], 1.5 M NaCl, 20 mM EDTA, 2% CTAB, 2% [vol/vol] 2-mercaptoethanol, 200 µg of proteinase K per ml) and then incubated for about 4 h at 60°C. The lysate was extracted with chloroform-isopentanol until there was little or no protein at the interface. Then 0.6 volume of isopropanol was added to precipitate the DNA. The DNA was washed twice with a 76% aqueous ethanol-10 mM ammonium acetate mixture. The DNA clot was air dried in a 37°C incubator and was dissolved in 20 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). RNase A (1.0 ml of a 0.5-mg/ml solution) and phage RNase T₁ (5 µl of a 200-U/ml solution) were added, and the solution was incubated for 1 h and then extracted once with chloroform-isoamyl alcohol (25:1). A 0.1% volume of 3 M sodium acetate was added, and the preparation was overlaid with 2 volumes of 95% ethanol. The precipitated DNA was spooled onto a glass rod. The DNA was washed with 76% ethanol in water at 20°C and air dried. Finally, the DNA was dissolved in 3 to 5 ml of TE buffer and stored at -20°C.

The concentration of the DNA was determined by measuring A₂₆₀ (5, 10, 17). The purity of each preparation was determined by measuring the hyperchromic shift during thermal denaturation (5). Each DNA preparation was adjusted to a concentration of 0.4 mg/ml with TE buffer.

The DNA preparations were sheared by passing each of them three times through a French pressure cell at 16,000 lb/in², were denatured by heating them in a boiling water bath for 5 min, and then were cooled quickly by placing the tubes in ice water (6). The DNA preparations were then centrifuged at 12,000 × g for 15 min to remove particulate debris and were stored at -20°C. Small amounts (5 µg) from the fragmented and denatured DNA preparations were labeled with ¹²⁵I as described by Selin et al. (14).

The S1 nuclease procedure was used for the DNA similarity determinations (5, 6). Each of the reassociation vials contained 10 µl (10 to 20 ng; 30,000 cpm) of labeled DNA, 50 µl (20 µg) of unlabeled DNA, and 50 µl of 13.2× SSC (1× SSC is 0.15 M NaCl and 15 mM sodium citrate, pH 7.0) containing 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0). These vials were incubated at 65°C for 24 h. Labeled reference DNA prepa-

TABLE 2. Cellular fatty acid compositions of *P. denticola*, *P. loescheii*, genospecies "Prevotella D1C-20," *P. melaninogenica*, and *P. veroralis*

Compound ^a	Cellular fatty acid composition ^b				
	<i>P. loescheii</i>	"Prevotella D1C-20"	<i>P. denticola</i>	<i>P. melaninogenica</i>	<i>P. veroralis</i>
13:0 iso FAME			0.8 ± 0.2	0.8 ± 0.4	1.0 ± 0.3
13:0 anteiso FAME					0.4 ± 0.2
14:0 iso FAME	6.7 ± 2.4	7.1 ± 1.8	6.9 ± 3.4	5.4 ± 2.2	6.1 ± 3.8
14:0 FAME	9.1 ± 3.6	8.8 ± 3.7	1.1 ± 0.4	1.5 ± 1.0	0.9 ± 0.4
15:0 iso FAME	11.1 ± 2.5	10.1 ± 1.3	7.4 ± 1.5	8.2 ± 1.9	10.7 ± 2.7
15:0 anteiso FAME	22.3 ± 5.3	21.6 ± 3.5	42.3 ± 4.2	42.8 ± 6.3	37.4 ± 3.3
15:0 FAME	0.5 ± 0.4		0.3 ± 0.2	0.4 ± 0.2	
16:0 iso FAME	1.9 ± 0.9	2.2 ± 0.8	3.8 ± 0.9	3.4 ± 0.8	4.0 ± 0.9
16:1 <i>cis</i> -9 FAME	0.7 ± 0.8	1.1 ± 1.4			
16:0 FAME	24.0 ± 4.6	29.4 ± 3.3	9.9 ± 2.8	7.8 ± 3.0	5.9 ± 2.2
17:0 iso FAME			2.3 ± 0.7	1.8 ± 1.1	4.4 ± 1.6
17:0 anteiso FAME			3.7 ± 0.9	4.3 ± 2.0	4.6 ± 1.7
16:0 3OH FAME	11.8 ± 3.5	11.2 ± 2.3	6.5 ± 2.2	5.3 ± 1.6	3.5 ± 1.8
18:1 <i>cis</i> -9 FAME	1.3 ± 0.9	1.2 ± 0.6			
17:0 ante 3OH FAME			1.2 ± 0.6	1.8 ± 0.9	0.7 ± 0.6
13:1 <i>cis</i> -12 FAME, 14:0 alde, or 11:1 2OH FAME	2.2 ± 1.5	1.7 ± 1.4	1.4 ± 1.4	1.3 ± 0.8	0.9 ± 0.7
15:0 iso alde or un 13.570 ECL	1.2 ± 1.3		3.3 ± 4.1	5.4 ± 3.4	9.2 ± 5.1
16:0 iso 3OH FAME or un 17.157 DMA	0.8 ± 0.6	0.7 ± 0.6	1.1 ± 0.4	1.2 ± 0.5	0.6 ± 0.3
17:0 iso 3OH FAME or 18:2 DMA	4.7 ± 2.0	3.4 ± 1.4	6.7 ± 2.6	7.5 ± 2.7	8.8 ± 3.1

^a FAME, fatty acid methyl ester; 3OH, 3-hydroxy; 2OH, 2-hydroxy; alde, aldehyde; un, unknown; ECL, equivalent chain length; DMA, dimethylaldehyde. Compounds that occurred in less than one-half of the isolates of species are not shown and were not used in reference patterns for identification.

^b Mean percentage of the total sample area ± standard deviation.

rations reassociated in the presence of 20 µg of salmon sperm DNA resulted in background values which were 5 to 10% of the values obtained when the preparations were reassociated in the presence of unlabeled reference DNA.

RESULTS

The levels of DNA-DNA relatedness of the strains are shown in Table 1. Strains belonging to *P. loescheii* and the D1C-20 group were closely related, as reported previously (3), and were distinct from the other taxa tested. All four representative strains of "Prevotella D70" exhibited high levels of DNA relatedness to *P. veroralis*. Four of the five strains of "Prevotella D71" tested, which fermented cellobiose, exhibited high levels of relatedness to *P. denticola*, and one strain of "Prevotella D71," represented by three single colonies from the same preparation, was highly related to the type strain of *P. melaninogenica*.

The results of replicate phenotypic testing were similar to the original results. The strains of "Prevotella D71" all fermented cellobiose with different lots of cellobiose substrate, and the results of xylan fermentation were the same for replicate analyses of the same strains. Thus, cellobiose was fermented by some strains of *P. denticola*, as well as by some strains of *P. melaninogenica*, and xylan was or was not fermented by strains of *P. veroralis*.

When it became evident that cellobiose and xylan fermentations were not reliable characteristics for differentiating *P. veroralis*, *P. melaninogenica*, and *P. denticola*, we included the cellobiose-fermenting strains together with other strains in each DNA similarity group to restructure the Microbial Identification System cellular fatty acid reference patterns (Table 2) and used the new reference library to assign other "Prevotella D70" and "Prevotella D71" strains to the three previously described species (*P. melaninogenica*, *P. denticola*, and *P. loescheii*). All of the strains were then used to determine the phenotypic characteristics of these species, as

shown in Tables 3 and 4. In addition to the characteristics shown in Table 3, 70% of the 30 strains of *P. veroralis* tested did not ferment xylan, 20% fermented xylan only weakly, and 10% (the type strain and two other strains) produced strong acid (pH <5.5).

DISCUSSION

In previous work the phenotypic characteristics used to differentiate new isolates of *P. loescheii*, *P. denticola*, *P. melaninogenica*, *P. veroralis*, and the D1C-20 genospecies were based on reactions of strains that represented distinct DNA-DNA relatedness groups (3, 4, 7, 15, 16). However, only a limited number of such strains was available (9 strains of *P. loescheii* [3], 6 strains of the D1C-20 genospecies [3], 12 strains of *P. melaninogenica* [3], 1 strain of *P. veroralis* [16], and 20 strains of *P. denticola* [3, 15]). On the basis of the results obtained with these few strains, cellobiose fermentation and pigment production were thought to be key tests for differentiation as follows: acid production from cellobiose and pigment production, *P. loescheii* and the genospecies D1C-20; acid production from cellobiose and no pigment production, *P. veroralis*; no acid production from cellobiose and pigment production, *P. melaninogenica*; and no acid production from cellobiose and generally no pigment production, *P. denticola*. Although Holdeman and Johnson (3) reported cellobiose fermentation by 9% of 12 strains of *P. melaninogenica* that exhibited high levels of DNA relatedness, these authors did not detect cellobiose fermentation by any of 18 strains of *P. denticola* that exhibited high levels of DNA relatedness. Fermentation of xylan by *P. veroralis* was used to separate *P. veroralis* from *P. denticola*, *P. buccalis*, and *P. oralis* (16).

Phenospecies "Prevotella D70" was established in our laboratory to accommodate xylan-negative strains that corresponded to *Prevotella buccalis* on the basis of fermentation results but appeared to be *P. veroralis* on the basis of the

TABLE 3. Biochemical characteristics of *P. denticola*, *P. loescheii*, genospecies "*Prevotella* D1C-20," *P. melaninogenica*, and *P. veroralis*^a

Characteristic	% of isolates that are positive				
	<i>P. denticola</i> (95/60) ^b	<i>P. loescheii</i> (133/68)	" <i>Prevotella</i> D1C-20" (16/13)	<i>P. melaninogenica</i> (68/51)	<i>P. veroralis</i> (30/17)
Acid produced from:					
Amygdalin	8	14	0	2	1
Cellobiose	22	98	94	15	80
Esculin	57	40	50	15	23
D-Fructose	99	92	100	91	97
Gum arabic	9	6	0	0	0
Inulin	92	93	100	73	96
Larch arabinogalactan	0	5	6	11	17
D-(+)-Maltose	100	100	100	94	100
D-Mannitol	0	0	0	4	0
D-(+)-Mannose	100	99	100	99	100
Melezitose	2	0	0	1	0
Melibiose	14	71	56	3	7
Pectin	16	33	12	16	10
D-(+)-Raffinose	98	99	100	96	100
Rhamnose	1	16	6	1	0
D-Ribose	41	4	0	29	0
Salicin	16	37	31	3	7
Starch	99	95	100	96	100
Trehalose	2	16	25	0	0
Xylan	20	14	44	18	33
D-Xylose	0	4	3	4	6
Hydrolysis of:					
Esculin	96	98	100	84	100
Starch	89	89	88	96	83
Digestion of:					
Gelatin	98	98	100	93	73
Milk	5	12	19	6	7
Meat	15	6	0	6	0
Milk curd	100	100	100	97	100
Production of:					
β-Hemolysis	5	29	69	12	1
Lipase	0	62	67	0	0

^a All strains ferment glucose, glycogen, lactose, and sucrose; are inhibited by 20% bile; and produce little or no hydrogen or other gas. No strain ferments L-arabinose, DL-erythritol, inositol, or L-sorbose; produces indole, catalase, or lecithinase; reduces nitrate; or is motile.

^b Number of isolates studied/number of people from whom the isolates were obtained.

results of cellular fatty acid analyses. "*Prevotella* D71" resembled nonpigmenting or weakly pigmenting strains of *P. loescheii* or strains of *P. denticola* or *P. melaninogenica* that produced acid from cellobiose. On the basis of the properties of strains that exhibited high levels of DNA relatedness in this study, greater variation in cellobiose fermentation occurs among strains of *P. denticola* and *P. melaninogenica* than we thought previously and, unlike the type strain, most strains of *P. veroralis* do not ferment xylan. Thus, previous identification keys and charts of reactions are not adequate to differentiate these species because the key characteristics were selected on the basis of too few strains with known DNA relatedness.

Because *P. veroralis* descriptions were based on only one strain characterized by DNA sequence similarity, it was not surprising to find that there was more phenotypic variation than has been reported previously for the species and that other genetically similar strains did not ferment xylan, which unfortunately has been used as a key differential characteristic. Conversely, the description of *P. denticola* was based on 20 strains grouped by DNA similarity, and none of these strains was reported to ferment cellobiose (3, 15). Lack of cellobiose fermentation was considered to be a reliable characteristic for the species because so many strains had

been included in the study. This assumption, too, was erroneous.

Initially, we did not know whether to rely more upon differentiation by standard phenotypic tests or upon the cellular fatty acid identifications, which also had been developed by analyzing strains belonging to DNA similarity groups. Because the cellular fatty acid analytical procedure was new and the reliability of the identification library entries had not been proved, we followed the usual custom of considering the identifications based on cellular fatty acid analyses wrong and the identifications determined by our standard procedures correct when there was disagreement. However, the results of our DNA reassociation experiments confirmed that the Microbial Identification System identifications were correct, even though they did not agree with standard and accepted identification criteria. Thus, the following two principles were illustrated: (i) It is essential to have many strains of known DNA relatedness in order to select appropriate diagnostic and differential tests; and (ii) when strains that have not been analyzed by using DNA reassociation experiments are used to evaluate new differential tests, the identifications based on the new test(s) may very well be more nearly correct than identifications based on results obtained with the bench mark standard tests.

TABLE 4. Other characteristics of *P. denticola*, *P. loescheii*, genospecies "Prevotella D1C-20," *P. melaninogenica*, and *P. veroralis*

Characteristic	<i>P. loescheii</i> (133/68) ^a	"Prevotella D1C-20" (16/13)	<i>P. denticola</i> (95/60)	<i>P. melaninogenica</i> (68/51)	<i>P. veroralis</i> (30/17)
% of strains with colonies that are ^b :					
Black	11	0	4	29	0
Reddish brown	48	43	44	47	3
Tan	18	38	20	9	27
Colorless	23	19	30	15	70
Acid products (mean meq/100 ml ± SEM)					
Acetic acid	0.97 ± 0.04	0.95 ± 0.12	1.75 ± 0.07	1.41 ± 0.08	1.52 ± 0.11
Formic acid	Tr	Tr	Tr	Tr	Tr
Isovaleric acid	0	0	0.03 ± 0.005	0.04 ± 0.005	0.02 ± 0.003
Lactic acid	0.29 ± 0.04	0.22 ± 0.04	0.18 ± 0.02	0.08 ± 0.01	0.08 ± 0.02
Succinic acid	2.79 ± 0.14	2.56 ± 0.32	5.36 ± 0.30	4.52 ± 0.30	4.75 ± 0.31
% of strains resistant to:					
Chloramphenicol (12 µg/ml)	2	0	0	0	0
Clindamycin (1.6 µg/ml)	2	0	1	6	4
Erythromycin (3 µg/ml)	15	0	1	9	7
Penicillin G (2 U/ml)	21	12	39	41	7
Tetracycline (6 µg/ml)	28	6	8	9	4
Sources of strains used in this study					
Gingival crevices	+	+	+	+	+
Other oral or pleural sites			+	+	
Other body sites			+ ^c	+ ^d	

^a Number of isolates studied/number of people from whom the isolates were obtained.

^b Pigmentation generally increases as length of incubation increases, is detected more often and more rapidly on hemolyzed blood than on whole blood, and is produced more reliably on rabbit or human blood than on blood from other animal species, including sheep and horses. Nevertheless, some strains of *P. melaninogenica*, *P. denticola*, and *P. loescheii* produce black colonies in 48 h, whereas other strains do not produce pigmented colonies even after 14 to 21 days of incubation on agar containing hemolyzed rabbit blood. Pigmentation may be detected after initial isolation from mixed cultures but not subsequently in pure cultures.

^c Spinal fluid.

^d Abdominal abscess, Whipple's disease, and cystic acnes.

When cellobiose-fermenting strains are included in the phenotypic data base, *P. veroralis*, *P. denticola*, and *P. melaninogenica* cannot be differentiated with certainty by the phenotypic tests that were given in the initial descriptions of these species and that we have used previously (2). In the absence of cellular fatty acid information, only very tentative identifications can be made on the basis of a combination of colony pigmentation; cellobiose, esculin, inulin, and ribose fermentation; and meat digestion.

The cellular fatty acid patterns of *P. loescheii* and of the D1C-20 genospecies are quite distinct from those of the other three species, particularly in the smaller relative amounts of 15:0 anteiso fatty acid methyl ester and greater relative amounts of 16:0 fatty acid methyl ester (Table 2). The similarity between *P. loescheii* and the genospecies D1C-20 is not surprising since these species exhibit moderate levels of DNA relatedness (Table 1). The cellular fatty acid patterns of *P. denticola*, *P. melaninogenica*, and *P. veroralis* are similar to each other, and these three species also exhibit

measurable low levels of DNA relatedness to each other (Table 1). However, the Microbial Identification System can differentiate among these species, despite the similarity of the cellular fatty acid patterns.

Table 5 shows the incidence of the species discussed above among isolates taken from the gingival crevices of people with healthy or diseased gingiva. For these determinations 30 colonies were picked in a randomized manner to obtain a representative cross section of the composition of the flora in each sample taken from the depths of the gingival pockets. On the basis of the association of these species with the different disease states, it appears that, of the species considered in this paper, only *P. denticola* may have a significant relationship with periodontal health. The incidence of this species increased directly with the severity of disease. However, it is impossible to tell whether this increase represents a contributing cause of tissue destruction or whether it simply represents growth stimulation by increased serum and blood in affected sites.

TABLE 5. Incidence of *P. loescheii*, "Prevotella D1C-20," *P. denticola*, *P. melaninogenica*, and *P. veroralis* in the gingival crevices of subjects with different levels of periodontal health

Subjects with:	No. of people	No. of isolates	% of total isolates				
			<i>P. loescheii</i>	"Prevotella D1C-20"	<i>P. denticola</i>	<i>P. melaninogenica</i>	<i>P. veroralis</i>
Healthy gingiva	20	1,047	0.29	0	0.29	0.10	0
Adult gingivitis	11	680	0.59	0.44	0.74	0.29	0
Adult periodontitis	25	1,913	0.47	0.47	0.89	0.26	0.05
Severe periodontitis	21	1,315	0.30	0.38	1.98	0	0

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