Descriptions of *Prevotella tannerae* sp. nov. and *Prevotella enoeca* sp. nov. from the Human Gingival Crevice and Emendation of the Description of *Prevotella zoogleoformans*

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*Prevotella tannerae* sp. nov. and *Prevotella enoeca* sp. nov. from the human gingival crevice are described. These organisms are obligately anaerobic, non-spore-forming, nonmotile, gram-negative, rod-shaped bacteria that ferment carbohydrates and produce succinic and acetic acids. Bile inhibits growth. Some strains (38%) of *P. tannerae* produce colonies with a tan to black pigment when they are grown on rabbit blood agar. The type strains are *P. tannerae* ATCC 51259 and *P. enoeca* ATCC 51261. In addition, the description of *Prevotella zoogleoformans* is emended to exclude strains now recognized as members of *Prevotella heparinolytica*.

During quantitative and qualitative studies of the bacterial flora of samples obtained from the human gingival crevice, we isolated numerous strains of anaerobic, gram-negative, rod-shaped bacteria that represent species unlike any that have been described previously. Two of these organisms, designated "Bacteroides D28" and "Bacteroides D84" in our laboratory, were selected for further taxonomic study.

In addition, we propose that the description of *Prevotella zoogleoformans* should be emended to exclude strains now recognized as members of *Prevotella heparinolytica*. In 1938 Prévot described a new genus, *Capsularis*, with *Capsularis zoogleoformans* (Weinberg et al. 1937) as the type species (12). Because there was no strain to represent this species, it was not included on the 1980 Approved Lists of Bacterial Names (15). Following isolation from the gingival crevice of strains with the characteristics of "C. zoogleoformans," Cato et al. proposed the name *Bacteroides zoogleoformans* (Weinberg et al. 1937) (corrig.) comb. nov. (2). In 1988 Bailey et al. (1) showed that some strains originally thought to be *B. zoogleoformans* strains by Cato et al. and other strains whose characteristics corresponded to the characteristics of *Bacteroides zoogleoformans* (Weinberg et al. 1937) Cato et al. exhibited only 47 to 52% DNA relatedness with the type strain of *B. zoogleoformans* and concluded that these organisms should be members of a distinct species. Bailey et al. named the new species *Bacteroides heparinolyticus* and showed that strains of *B. heparinolyticus* produce indole and strains of *B. zoogleoformans* do not. Both species were transferred to the genus *Prevotella* (13), but an emended description of *P. zoogleoformans* that includes only the indole-positive strains has not been published previously. An emended description based on five indole-positive strains is given below.

**MATERIALS AND METHODS**

**Bacterial strains.** In this study we examined *Prevotella tannerae* ("Bacteroides D28") ATCC 51259T (American Type Culture Collection, Rockville, Md.) (T = type strain), which was isolated from the gingival crevice of an adult with gingivitis, and 47 other isolates obtained from 33 samples from the gingival crevices of 33 other people (10 juveniles or adults with healthy gingiva, 4 people with gingivitis, 16 people with adult periodontitis, 2 people with juvenile periodontitis, and 1 person with rapidly progressive periodontitis).

We also examined *Prevotella enoeca* ("Bacteroides D85") ATCC 51261T, which was isolated from the gingival crevice of a person with adult periodontitis, and seven other isolates obtained from six other people (four juveniles or adults with healthy gingiva, one person with adult periodontitis, and one person with juvenile periodontitis).

*P. zoogleoformans* ATCC 33285T and four other strains isolated from people with adult periodontitis were also included in this study.

Type strains of other species were also examined for comparison (Table 1).

**Characterization of strains.** Fermentation, enzymatic, and antimicrobial agent susceptibility tests in preduced media and polycrylamide gel electrophoresis of soluble proteins were performed as described previously (3, 9). The fatty acid methyl ester contents of whole-cell sediments obtained from preduced anaerobically sterilized peptone-yeast extract-glucose broth cultures were determined as described previously (8).

**DNA isolation and hybridization.** Strains were grown in

<table>
<thead>
<tr>
<th>TABLE 1. Levels of DNA relatedness among some <em>Prevotella</em> species</th>
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<tbody>
<tr>
<td><strong>Unlabeled DNA from:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>P. tannerae</em> strains</td>
</tr>
<tr>
<td>ATCC 51259T (= VPI N14B-15T)</td>
</tr>
<tr>
<td>VPI D212A-14</td>
</tr>
<tr>
<td><em>P. enoeca</em> strains</td>
</tr>
<tr>
<td>ATCC 51261T (= VPI D194A-25A)</td>
</tr>
<tr>
<td>VPI D42D-22</td>
</tr>
<tr>
<td><em>P. nigrescens</em> VPI 8944T</td>
</tr>
<tr>
<td><em>P. melaninogenicus</em> VPI 2381T</td>
</tr>
<tr>
<td><em>P. denticola</em> ATCC 35308T (= VPI 14460T)</td>
</tr>
<tr>
<td><em>P. oris</em> VPI D1A-1AT</td>
</tr>
<tr>
<td><em>P. oulora</em> ATCC 43324T</td>
</tr>
</tbody>
</table>

* Corresponding author. Phone: (703) 231-7110. Fax: (703) 231-7126.

* Value normalized to 100%.

* NT, not tested.
Acid produced from:

Dextrin
Fructose
Gum arabic
Glycogen
Inulin
Lactose
Larcharabino-galactan
Maltose
Mannose
Melibiose
Pectin
Raffinose
Salicin
Starch
Sucrose
Trehalose
Xylose

Hydrolysis of:

Esculin
Starch

Digestion of:

Gelatin
Milk
Meat
Milk curd
Nitrate reduction
H2S (Simmons indole motility medium)

Peptone-yeast extract-glucose agar

Gas
Reducing
Resistance to:

Chloramphenicol (12 μg/ml)
Clindamycin (1.6 μg/ml)
Erythromycin (3 μg/ml)
Penicillin G (2 U/ml)
Tetracycline (6 μg/ml)

RESULTS AND DISCUSSION

"Bacteroides D28" and "Bacteroides D84" are anaerobic, fermentative, gram-negative bacilli that do not grow well in the presence of 20% bile and have phenotypic characteristics similar to those of Prevotella species (Tables 2 and 3). The major cellular fatty acids of these organisms also are similar to those found in members of the genus Prevotella (Table 4), with anteiso-C15:0 fatty acid making up 30 to 36% of the total fatty acids detected. The G+C contents of the DNAs (45 and 47 mol%) are within the range found for other species in the genus Prevotella (5). These two closely related species exhibited negligible levels of DNA-DNA reassociation with Prevotella nigrescens, Prevotella melaninogenica, Prevotella denti-cola, and Prevotella oris (Table 1), species which are similar to them in cellular fatty acid content.

Because the characteristics of "Bacteroides D28" and "Bacteroides D84" are unlike the characteristics of previously described Prevotella species, we propose the following new species for these taxa: Prevotella tannerae and Prevotella enoeca, respectively.

**Description of Prevotella tannerae** sp. nov. Prevotella tannerae (tan’ne.rae N. L. gen. n. tannerae, of Tanner, in honor of Anne Moore)
TABLE 3. Differentiation of *P. tannerae*, *P. enoeca*, and *P. zoogloeformans* from indole-negative *Prevotella* speciesa

<table>
<thead>
<tr>
<th>Species</th>
<th>Cellulbiose acid</th>
<th>Esculin hydrolysis</th>
<th>Glycogen acid</th>
<th>Lactose acid</th>
<th>Sucrose acid</th>
<th>Meat digestion</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. disiens</em></td>
<td>−</td>
<td>−</td>
<td>A</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td><em>P. bivia</em></td>
<td>−</td>
<td>−</td>
<td>A</td>
<td>93</td>
<td>−</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td><em>P. enoeca</em></td>
<td>−</td>
<td>38</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td><em>P. tannerae</em></td>
<td>−</td>
<td>14</td>
<td>81</td>
<td>31</td>
<td>35</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td><em>P. oulora</em> b</td>
<td>+</td>
<td></td>
<td>A</td>
<td>+</td>
<td>A</td>
<td>−</td>
<td>16</td>
</tr>
<tr>
<td><em>P. zoogloeformans</em></td>
<td>83</td>
<td>+</td>
<td>−</td>
<td>17</td>
<td>67</td>
<td>−</td>
<td>16</td>
</tr>
<tr>
<td><em>P. veroralis</em></td>
<td>80</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>16, 17</td>
</tr>
<tr>
<td><em>P. oris</em></td>
<td>A</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>5</td>
</tr>
<tr>
<td><em>P. buccae</em></td>
<td>A</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>16</td>
</tr>
<tr>
<td><em>P. oralis</em></td>
<td>A</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>6</td>
</tr>
<tr>
<td><em>P. buccae</em></td>
<td>A</td>
<td>+</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>−</td>
<td>6</td>
</tr>
</tbody>
</table>

* a = 90 to 100% of the strains are negative; +, 90 to 100% of the strains are positive; A, 90 to 100% of the strains produce pH <5.7. The values are the percentages of strains that are positive.

b Contrary to the original description (14), we have not been able to demonstrate that the type strain of *Rikenella oulora* lowers the pH of media containing glycogen unless serum (which contains amylase) is added to the medium. We realize that there has been confusion concerning the type strain of this species (11), but the tests for fermentation of glycogen were performed with the correct type strain obtained from the American Type Culture Collection in 1993 rather than with the strain that has been shown to be *Rikenella microfusus* (11).

c This reaction may be acid when serum is added to the medium.

C. R. Tanner, a United States microbiologist. The description below is based on studies of the type strain and 47 other isolates obtained from 37 other people. Cells of the type strain from peptone-yeast extract-glucose broth cultures are 0.3 μm wide by 0.7 to 8.0 μm long; filaments up to 14 μm long are observed. Cells occur in short chains and are not motile. Surface colonies on blood agar plates incubated for 2 days are 1 mm in diameter, circular, low convex, and translucent to colorless after incubation for 5 days. Colonies in pure cultures of 2 of 41 strains are surrounded by small clear zones of hemolysis.

Broth cultures are cloudy with a smooth sediment. The terminal pH values of glucose broth cultures after incubation for 5 days are 4.8 to 5.3; 53% of the strains require the addition of 10% sterile serum to broth media for optimum growth and acid production.

The fermentation acids are succinic acid (2.6 ± 0.22 meq/100 ml of culture [mean ± standard error of the mean]), acetic acid (1.5 ± 0.11 meq/100 ml), formic acid (0.17 ± 0.03 meq/100 ml), isovaleric acid (0.03 ± 0.006 meq/100 ml). No hydrogen is produced.

Additional characteristics of the species are shown in Tables 2 and 4.

Isolated from the human gingival crevice (Table 5) (10). The type strain is ATCC 51259 (= VPI N14B-15).

The G+C content of the DNA of the type strain is 45 mol%.

Phenotypically, *P. tannerae* can be differentiated from many saccharolytic *Prevotella* species by the inability of most strains to hydrolyze esculin (86% of the strains are negative) and by the inability of most strains to ferment sucrose (69% of the strains are negative) (Table 3). It differs from *Prevotella bivia* by not digesting meat and from the new species *P. enoeca* by its cellular fatty acid profile (iso-C15:0 fatty acid content) (Table 4).

**Description of *Prevotella enoeca* sp. nov. *Prevotella enoeca* (e.no'eca. Gr. adj. enoikos, inhabiting; N.L. fem. adj. enoeca, inhabiting, because the organism is an inhabitant of the gingival crevice). The description below is based on studies of the type strain and seven other isolates obtained from six other people. Cells of the type strain are 0.5 μm wide by 2.2 to 4.6 μm long; filaments up to 7.7 μm long are observed. Cells are not motile and occur in pairs and short chains. Surface colonies on rabbit blood agar plates incubated for 2 days are 1 to 2 mm in diameter, circular, entire, convex, transparent to translucent, and not hemolytic. No pigment develops on either hemolyzed or whole rabbit blood agar plates or streak tubes incubated for 10 days, and there are no hemolytic zones around colonies on blood agar plates. Broth cultures are turbid with a smooth sediment. The terminal pH values of glucose broth cultures incubated for 5 days are 4.8 to 5.4.

The fermentation acids are succinic acid (3.2 ± 0.72 meq/100 ml of culture [mean ± standard error of the mean]) and acetic acid (1.4 ± 0.19 meq/100 ml); sometimes a trace of

<table>
<thead>
<tr>
<th>TOXON</th>
<th>Fatty acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>iso-C14:0</td>
</tr>
<tr>
<td><em>Prevotella spp.</em></td>
<td>5 (0-13)</td>
</tr>
<tr>
<td><em>P. tannerae</em></td>
<td>3</td>
</tr>
<tr>
<td><em>P. enoeca</em></td>
<td>3</td>
</tr>
<tr>
<td><em>P. zoogloeformans</em></td>
<td>0</td>
</tr>
</tbody>
</table>

a The values for *Prevotella* spp. are the means (and ranges) obtained for the following species and subspecies: *P. bivia*, *P. buccae*, *P. buccalis*, *P. corporis*, *P. denticola*, *P. disiens*, *P. heparinolytica*, *P. intermedia*, *P. loeschei*, *P. melaninogena*, *P. nigrescens*, *P. oralis*, *P. oulora*, *P. ruminicola* subsp. *ruminicola*, *P. veroralis*, and *P. zoogloeformans*. Data were obtained from reference 8.
formic acid is present. No hydrogen is detected in the head-space gas of glucose broth cultures.

Additional characteristics of the species are shown in Tables 2 and 4.

Isolated from the gingival crevices of humans with healthy gingiva and periodontitis (Table 5). The type strain is ATCC 51261 (= VPI D194A-25A).

The G+C content of the DNA of the type strain is 47 mol%.

The lack of sucrose fermentation and the inability of \( P. \) \( \text{tannerae} \) to digest gelatin differentiate this species from many \( \text{Prevotella} \) species (Table 3). It can be differentiated from \( P. \) \( \text{tannerae} \) by its cellular fatty acid profile (iso-C\(_{15:0}\) fatty acid content) (Table 4).

**Description of \( \text{Prevotella zoogleaformans} \) (Weinberg et al. 1937) Shah and Collins 1990.** The phenotypic characteristics of the type strain and four other strains of \( P. \) \( \text{zoogleaformans} \) are shown in Table 2, and the characteristic cellular fatty acids of the species are shown in Table 4. Characteristics that differentiate \( P. \) \( \text{zoogleaformans} \) from some other \( \text{Prevotella} \) species are shown in Table 3. Production of acid from cellobiose and usually from lactose and an inability to produce colonies with dark pigment on blood agar differentiate \( P. \) \( \text{zoogleaformans} \) from \( \text{Prevotella intermedia} \) and \( \text{P. nigrescens} \), the other indole-producing strains in the genus.

The G+C content of the DNA of the type strain, ATCC 33285, is 47 mol%.

The incidence of the species in periodontally healthy and diseased individuals is shown in Table 5.

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