Proposal for Reclassification of Bacteroides asaccharolyticus, Bacteroides gingivalis, and Bacteroides endodontalis in a New Genus, Porphyromonas

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The asaccharolytic, pigmented Bacteroides, Bacteroides asaccharolyticus, Bacteroides gingivalis, and Bacteroides endodontalis, form a group of relatively homogeneous species which differ markedly in biochemical and chemical properties from the type species of Bacteroides, Bacteroides fragilis (Castellani and Chalmers), such that they should not be retained within this genus. Therefore, we propose that Bacteroides asaccharolyticus (Holdeman and Moore) Finegold and Barnes, Bacteroides gingivalis Coykendall, Kaczmarek and Slots, and Bacteroides endodontalis van Steenbergen, van Winkelhoff, Mayrand, Gieren and de Graaff be reclassified in a new genus, Porphyromonas, as Porphyromonas asaccharolytica comb. nov., Porphyromonas gingivalis comb. nov., and Porphyromonas endodontalis comb. nov., respectively.

In Berger's Manual of Determinative Bacteriology, 8th ed. (12), the asaccharolytic, pigmented Bacteroides were regarded as a single homogeneous taxon, Bacteroides melaninogenicus subsp. asaccharolyticus. As the clinical significance of these microorganisms in oral cavities was recognized, extensive taxonomic studies were carried out. Heterogeneity was first demonstrated among these bacteria (29). These findings were substantiated by an analysis of their chemotaxonomic contents (52 to 54 mol%) and an electrophoretically fast-migrating malate dehydrogenase (MDH) was reclassified as a new species, Bacteroides asaccharolyticus (7, 29). Further differences between B. asaccharolyticus and the group of strains with low G+C contents (46 to 48 mol%) and a slow-migrating MDH (29) were revealed by an analysis of their lipids (26). Strains of B. asaccharolyticus possess very high levels of 13-methyl-tetradecanoic acid (iso-C_{15:0} acid) and menaquinones with 10 isoprene units (MK-10), whereas the group of strains with low G+C contents contains significantly lower levels of iso-C_{15:0} fatty acids and menaquinones with nine isoprene units (MK-9) (26). The latter group of strains was subsequently reclassified as Bacteroides gingivalis (4). It is generally believed that most oral, asaccharolytic, black-pigmented Bacteroides strains belong to B. gingivalis, whereas most nonoral or clinical isolates are B. asaccharolyticus strains. Recently, a third asaccharolytic species, Bacteroides endodontalis, which was isolated from infected dental root canals, has been proposed (37). Phenotypically, B. endodontalis appears to be more closely related to B. asaccharolyticus than to B. gingivalis and possesses a DNA base composition of ca. 50 to 51 mol% G+C (37). The MDH of B. endodontalis migrates faster than the MDHs of B. asaccharolyticus and B. gingivalis (Shah, unpublished data).

B. asaccharolyticus, B. gingivalis, and B. endodontalis are well-defined species and on the basis of biochemical and chemical properties form a relatively homogeneous group quite unrelated to the type species of the genus Bacteroides, Bacteroides fragilis. All of the asaccharolytic, pigmented bacteroides accumulate major levels of protophorophyrin rather than protoporphyrin when cells are cultured on blood agar (3, 25, 27). The three species are nonfermentative and utilize nitrogenous substrates such as Trypticase and Proteose Peptone as energy sources (28a, 28b). The metabolic end products from these substrates include significant levels of n-butyric acid in addition to other volatile fatty acids (11). Furthermore, particular amino acids, such as aspartate, are preferentially catabolized (28a, 28b), and strains generally possess proteolytic activities (16, 19). Unlike other members of the genus Bacteroides, which contain predominantly 12-methyl-tetradecanoic acid (anteiso-C_{15:0} acid) as their long-chain fatty acid, the asaccharolytic, pigmented species contain mainly 13-methyl-tetradecanoic acid (iso-C_{15:0} acid). The latter taxa also contain MDH and glutamate dehydrogenase but differ from the type species of the genus Bacteroides, B. fragilis, in lacking enzymes of the hexose monophosphate shunt-pentose phosphate pathway (viz., glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase) (27, 28). All of the asaccharolytic, pigmented species examined so far have DNA base compositions within the range from 46 to 54 mol% G+C. These taxa also differ from other Bacteroides species studied in lacking diaminopimelic acid in their cell walls (8, 29).

We have suggested previously that the genus Bacteroides should be restricted to the type species, B. fragilis, and related taxa (3, 27). These related taxa include Bacteroides distasonis, Bacteroides eggerthii, Bacteroides ovatus, Bacteroides thetaotaomicron, Bacteroides uniformis, and Bacteroides vulgatus and, more recently, Bacteroides caccae, Bacteroides merdae, and Bacteroides stercoris (14). The organisms in the "B. fragilis" group have relatively low G+C contents, ranging from 40 to 48 mol% (13), and the species are relatively homogeneous biochemically (27, 28). B. fragilis and related species are saccharolytic and generally produce acetic and succinic acids as the major end products of glucose metabolism (11). They also differ from the asaccharolytic, pigmented Bacteroides species in possessing enzymes of the hexose monophosphate shunt-pentose phosphate pathway (27, 28) and in their cellular fatty

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acids (23, 26). Furthermore, the dibasic amino acid of their peptidoglycan is meso-diaminopimelic acid (29).

Thus, there is now overwhelming biochemical and chemical evidence that the asaccharolytic, pigmented Bacteroides species differ so markedly from the type species of the genus Bacteroides, B. fragilis, as to warrant placement in a separate genus. Therefore, we formally propose that the species presently designated B. asaccharolyticus (7), B. gingivalis (4), and B. endodontalis (37) be reclassified in a new genus, Porphyromonas, as Porphyromonas asaccharolytica comb. nov., Porphyromonas gingivalis comb. nov., and Porphyromonas endodontalis comb. nov., respectively.

Description of Porphyromonas gen. nov. (Por. phy. ro.mo'nas. Gr. adj. porphyreos purple; Gr. n. monas unit; N. L. fem.n. Porphyromonas porphyrin cell). The description below is based on our own observations and previous descriptions of the asaccharolytic, pigmented Bacteroides species (2, 11, 16, 20, 22, 23, 26-33, 35-38, 42, 43, 45).

Gram-negative, obligately anaerobic, nonsporeforming, nonmotile rods or cocccobacilli. Most cells in broth are small (0.5 to 0.8 by 1.0 to 3.5 μm), but occasionally longer cells (4 to 6 μm) may be formed. Colonies on blood agar plates are smooth (rarely rough), shiny, convex, and 1 to 3 mm in diameter and darken progressively from the edge toward the center after 6 to 10 days. Eventually the whole colony becomes black due to protoheme production. Growth is not significantly affected by carbohydrates. Nitrogenous substrates such as Proteose Peptone, Trypticase, and yeast extract markedly enhance growth. The optimum temperature for growth is 37°C. Major fermentation products from BM or PYG (11) medium are n-butyric and acetic acids; lower levels of propionic, isobutyric, and isovaleric acids are also produced.

MDH and glutamate dehydrogenase are present; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are absent. Proteolytic activity is variable. Limited ability to ferment amino acids such as aspartate and arginine. Indole produced. Nitrate is not reduced to nitrite. Starch and esculin are not hydrolyzed.

Cell wall peptidoglycan contains lysine as the diamino acids; 2-keto-3-deoxyoctulosonic acid is absent. The principal respiratory quinones are unsaturated menaquinones with 9 or 10 isoprene units. Both nonhydroxylated and 3-hydroxylated long-chain fatty acids are present. The nonhydroxylated fatty acids are composed of predominantly iso-methyl branched types (iso-C₁₅₂₀ acid) and lower levels of straight-chain saturated acids. The 3-hydroxylated fatty acids are the straight-chain saturated types.

The DNA base compositions range between 46 to 54 mol% G+C. The type species is Porphyromonas asaccharolytica.

Description of Porphyromonas asaccharolytica (Holdeman and Moore) comb. nov. The description below is based on previous studies (3, 5–11, 15, 18, 23–29, 31, 35, 36, 44) and our own observations.

Gram-negative, obligately anaerobic, nonsporeforming, nonmotile rods or coccobacilli. Cells in broth are 0.8 to 1.0 by 1.5 to 3.5 μm, but occasionally longer cells are seen. Cells from solid media are often coccobacillar. Colonies on blood agar plates are smooth, shiny, convex, and 1 to 3 mm in diameter and darken progressively from the edge of the colony toward the center after 6 to 10 days. Eventually the whole colony becomes black due to protoheme production. Nitrogenous substrates such as Proteose Peptone, Trypticase, and yeast extract markedly enhance growth. Addition of 0.4 to 0.6% NaCl to media supplemented with the latter hydrolysates enhances growth.

Major fermentation products from BM or PYG medium are n-butyric and acetic acids; lower levels of propionic, isobutyric, and isovaleric acids are also produced. Phenylacetic acid is not produced. Proteolytic activity is low, but gelatin liquefaction is positive and fibrinolytic activity is present. Indole is produced. α-Fucosidase is produced. Nitrate is not reduced to nitrite. Starch and esculin are not hydrolyzed. Cells do not agglutinate sheep erythrocytes. MDH and glutamate dehydrogenase are present; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are absent.

The cell wall peptidoglycan contains lysine; 2-keto-3-deoxyoctulosonic acid is absent. The principal respiratory quinones are menaquinones with 10 isoprene units. Both nonhydroxylated and 3-hydroxylated fatty acids are present. The nonhydroxylated fatty acids are composed of predominantly iso-methyl branched types, with iso-C₁₅₂₀ acid predominating.

The DNA base compositions range between 52 and 54 mol% G+C.

The type strain is strain ATCC 25260.

Description of Porphyromonas gingivalis (Coykendall, Kaczmarek and Slots) comb. nov. The description below is based on previous studies (1, 3–5, 8–11, 15–19, 21, 23, 25–29, 31, 33, 35, 36, 38, 39; G. Sundqvist, Ph.D. thesis, Umea University of Odontology, Umea, Sweden, 1976) and our own observations.

Gram-negative, obligately anaerobic, nonsporeforming, nonmotile rods or cocccobacilli. Cells in broth are 0.5 by 1 to 2 μm. Cells from solid media are coccobacilli or very short rods. Colonies on blood agar plates are smooth (occasionally rough), shiny, convex, and 1 to 2 mm in diameter and darken from the edge of the colony toward the center between 4 and 8 days. Nonpigmented colonies rarely occur. Protoheme is the major porphyrin produced, but traces of protoporphyrin also occur. Growth is markedly affected by the presence of protein hydrolysates such as Trypticase, Proteose Peptone, and yeast extract. Several amino acids, such as aspartate, arginine, cystine, histidine, serine, tryptophan, leucine, methionine, phenylalanine, and isoleucine, are utilized. Growth is enhanced by 0.5 to 0.8% NaCl.

Major fermentation products from BM or PYG medium are n-butyric and acetic acids; lower levels of propionic, isobutyric, isovaleric, and phenylacetic acids are also produced. Proteases (for example, a trypsinlike enzyme and collagenase) are present. Indole is produced. α-Fucosidase is not produced. Nitrate is not reduced to nitrite. Starch and esculin are not hydrolyzed. Cells agglutinate sheep erythrocytes. MDH and glutamate dehydrogenase are present; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are absent.

Cell wall peptidoglycan contains lysine as the diamino acid; 2-keto-3-deoxyoctulosonic acid is absent. The principal respiratory quinones are menaquinones with nine isoprene units. Both nonhydroxylated and 3-hydroxylated fatty acids are present. The nonhydroxylated fatty acids are composed of predominantly iso-methyl branched types, with iso-C₁₅₂₀ acid predominating.

The DNA base compositions range between 46 to 48 mol% G+C.

The type strain is strain ATCC 33277.

Description of Porphyromonas endodontalis (van Steenhagen, van Winkelhoff, Mayrand, Grenier and de Graaff) comb. nov. The description below is based on previous studies (3, 37, 40–42; Sundqvist, Ph.D. thesis) and our own observations.
TABLE 1. Biochemical and chemical characteristics useful in distinguishing the genus Porphyromonas from the B. fragilis group and some other nonfermentive or weakly fermentative taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Metabolism*</th>
<th>Major end products from PYG*</th>
<th>Presence of:†</th>
<th>Major long-chain fatty acids</th>
<th>Menaquinones</th>
<th>G+C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyromonas</td>
<td>NF</td>
<td>A,B,iV,S,pA</td>
<td>MDH</td>
<td>iso-C15:0</td>
<td>+</td>
<td>46–54</td>
</tr>
<tr>
<td>Anaerorhabdus</td>
<td>NF</td>
<td>L,A</td>
<td>GDH</td>
<td>C10:0,C18:1</td>
<td>–</td>
<td>34</td>
</tr>
<tr>
<td>Bacteroides coagulans</td>
<td>NF</td>
<td>A</td>
<td>G6PDH</td>
<td>C10:0,C18:1</td>
<td>ND†</td>
<td>37</td>
</tr>
<tr>
<td>B. fragilis group</td>
<td>F</td>
<td>A</td>
<td>6PGDH</td>
<td>antiseiso-C15:0</td>
<td>+</td>
<td>40–48</td>
</tr>
<tr>
<td>Bacteroides putredinis</td>
<td>NF</td>
<td>S,iV,P</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Bacteroides ureolyticus</td>
<td>NF</td>
<td>S,A</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rikenella</td>
<td>NF</td>
<td>A,S,p</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Tissierella</td>
<td>NF</td>
<td>A,B,iV</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>28</td>
</tr>
</tbody>
</table>

* F, Fermentative; NF, nonfermentative.
† Abbreviations: A, acetic acid; B, butyric acid; L, lactic acid; P, propionic acid; iV, isovaleric acid; S, succinic acid; pA, phenylacetic acid. Data from reference 11.
‡ Production of acetic, phenylacetic, isovaleric, and succinic acids varies between species (see Table 2).
† ND, Not determined.

Gram-negative, obligately anaerobic, nonsporeforming, nonmotile rods or cocccobacilli. Most cells in broth are small, 0.4 to 0.6 by 1.0 to 2.0 μm. Colonies on blood agar plates are smooth, shiny, convex, entire, and circular and develop dark brown or black pigmentation between 7 and 14 days. Proto- heme is the major pigment, but protoporphyrin is also present. Most strains produce colonies which adhere strongly to blood agar plates, and growth in liquid media is slow. Proteose Peptone, Trypticase, and yeast extract are strongly to blood agar plates, and growth in liquid media is slow. Proteose Peptone, Trypticase, and yeast extract are good substrates for growth. Casitone and Casamino Acids are poor growth substrates. The optimum temperature for growth is 37°C. Major fermentation products are α-butyric and α-acetic acids; lower levels of propionic, isobutyric, and isovaleric acids are also produced. Phenylacetic acid is not produced. Except for gelatin hydrolysis, proteolytic activity is low. α-Fucosidase is not produced. Cells do not agglutinate sheep erythrocytes. Catalase is not produced. Indole and H2S are produced. Arginine is hydrolyzed; starch and esculin are not hydrolyzed. Nitrates are not reduced to nitrite. Three serotypes are present.

MDH and glutamate dehydrogenase are present; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are absent. Cell wall peptidoglycan is based on heme. The characterization of clinically important Gram-negative anaerobic bacilli by conventional bacteriological tests. J. Appl. Bacteriol. 40:165–188.

The type strain is strain ATCC 35406.

Biochemical and chemical characteristics useful in distinguishing the genus Porphyromonas from the B. fragilis group and some other nonfermentative or weakly fermentative taxa are shown in Table 1. The major distinguishing characteristics of the three species of the genus Porphyromonas are given in Table 2.

LITERATURE CITED


