Porphyromonas uenonis sp. nov., a Pathogen for Humans Distinct from *P. asaccharolytica* and *P. endodontalis*

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Three *Porphyromonas* species (*Porphyromonas asaccharolytica*, *P. endodontalis*, and the novel species that is the subject of the present report, *P. uenonis*) are very much alike in terms of biochemical characteristics, such as enzyme profiles and cellular fatty acid contents. *P. asaccharolytica* is distinguished from the other two species by virtue of production of α-fucosidase and glyoxylic acid positivity. The novel species is difficult to differentiate from *P. endodontalis* phenotypically and was designated a *P. endodontalis*-like organism for some time. However, *P. endodontalis* is recovered almost exclusively from oral sources and also grows poorly on Biolog Universal Agar, both characteristics that are in contrast to those of the other two organisms. Furthermore, *P. uenonis* is glycerol positive in the Biolog AN Microplate system. Both *P. asaccharolytica* and *P. uenonis* are positive by 13 other tests in the Biolog system, whereas *P. endodontalis* is negative by all of these tests. *P. asaccharolytica* grew well in both solid and liquid media without supplementation with 5% horse serum, whereas the other two species grew poorly without supplementation. Sequencing of 16S rRNA revealed about 10% divergence between the novel species and *P. endodontalis* but less than 2% sequence difference between the novel species and *P. asaccharolytica*. Subsequent DNA-DNA hybridization studies documented that the novel organism was indeed distinct from *P. asaccharolytica*. We propose the name *Porphyromonas uenonis* for the novel species. We have recovered *P. uenonis* from four clinical infections in adults, all likely of intestinal origin, and from the feces of six children.

*Porphyromonas endodontalis* has been recovered almost exclusively from oral sources (12), whereas a novel organism that we encountered and originally called the *P. endodontalis*-like organism appears to be found primarily in sources indicating an intestinal origin. The novel organism, *P. uenonis*, is phenotypically similar to *P. endodontalis* and *P. asaccharolytica*. The aim of this study was to describe the isolation and characterization of this novel organism, which is a species distinct from the other two species, and to describe tests useful in distinguishing between these three organisms. We also describe the types of infections in which we have found the novel species and its recovery from the feces of children.

**MATERIALS AND METHODS**

**Bacterial strains.** Six isolates of the novel species obtained from fecal specimens of young children in Helsinki, Finland, were included in the bacteriologic and genetic studies: four strains recovered from clinical infections (one each of appendicitis, peritonitis, pneumonial abscess, and an infected sacral decubitus ulcer; the four strains were from the Wadsworth Anaerobe Laboratory [WAL] collection), the ATCC 35406 strain of *P. endodontalis* (isolated from an infected root canal), and the ATCC 25260 strain of *P. asaccharolytica* (isolated from empyema fluid). Five additional *P. endodontalis* clinical isolates, all from oral sources, and five *P. asaccharolytica* clinical isolates, all from nonoral sources, were included in cellular fatty acid analyses.

The fecal strains were isolated during a microbiological study of antimicrobial agent-associated flora changes. The fecal samples were inoculated on various selective and nonselective media by quantitative culture techniques (6). For the isolation of the novel organism, brucella blood agar and kanamycin-sacomyycin laked blood agar (KVLB) and phenylethyl alcohol blood agar (PEA) plates were incubated anaerobically for up to 10 days and were then examined; all but one strain grew within 7 days. The clinical WAL strains were characterized as part of a comprehensive reevaluation of pigmented gram-negative rods; they generally grew within 3 days.

**Identification by conventional methods.** The strains were characterized by routine biochemical tests (5, 6) by using prereduced anaerobically sterilized biochemicals, gas-liquid chromatography for metabolic end products (6), API ZYM panels (BioMerieux, Marcy l’Etoile, France), the RapID ANA II system (Remel, Lenexa, Kans.), the AN Microplate system (Biolog, Hayward, Calif.), and Rosco (Taastrup, Denmark) diagnostic tablets. The production of β-lactamase was detected by the nitrocefin disk test (Biodisk). Antimicrobial susceptibili- ty studies were done with three strains and various antimicrobial agents by the NCCLS-approved Wadsworth agar dilution method (8).

**Cellular fatty acid analysis.** Cellular fatty acids were detected with a Hewlett-Packard 5890 series II gas chromatograph and Microbial Identification System software (MIDI, Newark, N.J.). The isolates were grown on supplemented brain heart infusion agar with blood, and the bacterial mass was harvested directly from the plates because of poor growth in liquid medium. The corresponding library (ANAEROBE, version Moore 5.0) was used in successive analyses.

**Genotypic characterization.** The mole percent G+C contents of the organism DNAs were determined by high-pressure liquid chromatography (7). The 16S rRNA genes were amplified by PCR, and the products were sequenced directly with a Biotech Diagnostic (Laguna Niguel, Calif.) Big Dye sequencing kit on an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.). The sequences obtained were compared with the sequences in the GenBank database by using BLAST software (1), and the percent similarity to other sequences was deter-
Colonies produced a black pigment (Fig. 1) after 3 days of incubation. One isolate that was the exception was sensitive to colistin and resistant to the kanamycin and colistin special-potency disks; all except one isolate were resistant to the kanamycin special-potency disk, and all except one isolate were N-glucosaminidase activity; Serum stim., growth stimulated by serum; BUA, growth on BUA; source, likely source as indigenous flora.

RESULTS

The novel species recovered from clinical infections were always isolated together with other anaerobes or aerobes. The mean number of accompanying anaerobes was 4.7, and the mean number of accompanying aerobes was 1.8.

The novel species was detected in fecal specimens of 6 of 30 children. It was isolated only from the area of heavy growth on brucella blood agar, KVLB, or PEA plates and not as single colonies. In the initial cultures, an incubation time of at least 7 days was required before the pigmentation of the novel species, which assisted in its recognition, was detected. On subculture, growth and colony pigmentation occurred in 3 days on laked rabbit blood agar. The counts of the novel species in the culture, growth and colony pigmentation occurred in 3 days on laked rabbit blood agar. The mean number of accompanying aerobes was 1.8. The novel species was tested negative by a number of other tests: P. endodontalis, P. asaccharolytica, and the type strains of all three species, also consistently showed a positive α-fucosidase test result only for P. asaccharolytica; but, in addition, it showed that P. asaccharolytica was the only one of the three species positive for glyoxylic acid, and among the three species, only P. uenonis was positive for glyceral. Both P. asaccharolytica and P. uenonis tested positive and P. endodontalis tested negative by a number of other tests: n-cellobiose, dextrin, n-galacturonic acid, gentibiose, α-n-glucosaminidase activity; Serum stim., growth stimulated by serum; BUA, growth on BUA; source, likely source as indigenous flora.

TABLE 1. Differential characteristics of selected Porphyromonas species

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<tr>
<th>Species</th>
<th>Reactiona</th>
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<tr>
<td></td>
<td>α-Fuc</td>
<td>β-NAG</td>
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<tr>
<td>P. asaccharolytica</td>
<td>+</td>
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<td>P. endodontalis</td>
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<tr>
<td>P. uenonis</td>
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a Data were compiled from references 3, 4, and 5 and the authors' own determinations.

b Symbols and abbreviations: +, positive reaction; -, negative reaction; ±, sometimes positive and sometimes negative reaction; α-Fuc, α-fucosidase activity; β-NAG, N-acetyl-β-glucosaminidase activity; Serum stim., growth stimulated by serum; BUA, growth on BUA; source, likely source as indigenous flora.

c Occasional exceptions were noted.
cose, glucose-6-phosphate, maltose, D-mannose, 3-methyl-D-glucose, /H9252-methyl-D-glucose, D-trehalose, turanose, and /H9251-ketoctyric acid (despite the designation “asaccharolytica” for P. asaccharolytica). Antimicrobial susceptibility tests, usually one strain per drug, revealed that the novel species was highly susceptible (MIC, /H11021g/ml) to amoxicillin-clavulanate, piperacillin-tazobactam, ticarcillin-clavulanate, imipenem, meropenem, ceftizoxime, clindamycin, trovafloxacin, gemifloxacin, and metronidazole. Two isolates produced /H9252-lactamases, and their susceptibilities to penicillin G and ampicillin were variable. The ciprofloxacin MIC for one strain was 2 μg/ml. One strain was highly resistant to trimethoprim-sulfamethoxazole. Testing with prereduced anaerobically sterilized biochemicals was not helpful in distinguishing the three species.

The main cellular fatty acid detected in the novel species was iso-C15:0 (50 to 60% of the total fatty acids); the four other cellular fatty acids were found only in amounts that ranged from 2.17 to 9.4% of the total acids. A cellular fatty acid found in cytophaga-flavobacter-bacteroides phylum. The tree was constructed by the maximum-parsimony method and is based on a comparison of approximately 1,400 nucleotides. Bootstrap values, expressed as a percentage of 1,000 replications, are given at the branching points. The scale bar indicates 1% sequence divergence. Superscript letters: a, “Porphyromonas canis” is not a valid species; Superscript letters: b, Porphyromonas salivosa is a junior synonym of Porphyromonas macacae (6a). Although P. crevioricanis and P. gingicanis are valid species (4), their 16S rRNA gene sequences are not available in public databases; therefore, they are not included in the phylogenetic tree.

FIG. 2. Unrooted tree showing the phylogenetic position of P. uenonis sp. nov. within the Bacteroides subgroup of the cytophaga-flavobacter-bacteroides phylum. The tree was constructed by the maximum-parsimony method and is based on a comparison of approximately 1,400 nucleotides. Bootstrap values, expressed as a percentage of 1,000 replications, are given at the branching points. The scale bar indicates 1% sequence divergence. Superscript letters: a, “Porphyromonas canis” is not a valid species; Superscript letters: b, Porphyromonas salivosa is a junior synonym of Porphyromonas macacae (6a). Although P. crevioricanis and P. gingicanis are valid species (4), their 16S rRNA gene sequences are not available in public databases; therefore, they are not included in the phylogenetic tree.
showed that they are quite closely related (98.2 to 98.9% sequence similarity). The latter sequence similarity would be consistent with the two species belonging to the same species; however, a DNA-DNA reassocation study between the proposed type strain (WAL 9902) of the novel species (P. uenonis) and the type strain of P. asaccharolytica (ATCC 25260) showed that the similarity was only 54.3% (59.9% on repeat analysis), documenting that they are distinct species. A phylogenetic tree is shown in Fig. 2.

DISCUSSION

The isolation and identification of P. endodontalis and the novel species, P. uenonis, are difficult. These organisms are highly sensitive to oxygen, grow slowly and poorly without supplementation (e.g., with horse serum), and produce pigment in the initial cultures sometimes only after 7 days or more of incubation. Furthermore, biochemically they are very inert, except in the Biolog AN Microplate system. The isolation of fecal strains of P. uenonis was difficult because of their presence in very small numbers; the colonies could not be detected before pigmentation became visible. P. endodontalis is recovered almost exclusively from oral sources. P. asaccharolytica, in contrast to the two previously mentioned organisms, grows well on both plated media and liquid media without horse serum supplementation. It is otherwise identical to the other two organisms by phenotypic tests, except that it is serum supplementation. It is otherwise identical to the other two species belonging to the same species; however, a DNA-DNA reassocation study between the proposed type strain (WAL 9902) of the novel species (P. uenonis) and the type strain of P. asaccharolytica (ATCC 25260) showed that the similarity was only 54.3% (59.9% on repeat analysis), documenting that they are distinct species. A phylogenetic tree is shown in Fig. 2.

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REFERENCES