***Porphyromonas endodontalis*–Like Organisms from Extraoral Sources.** M.-L. VÄISÄNEN, M. KIVIRANTA, P. SUMMANEN, S. M. FINEGOLD, AND H. R. JOUSIMIES-SOMER. *From the National Public Health Institute, Helsinki, Finland; and the Veterans Affairs Wadsworth Medical Center, Los Angeles, California, USA*

Porphyromonas endodontalis has been isolated almost exclusively from the oral cavity. *P. endodontalis* is a key pathogen in endodontic infections such as infected root canals and abscesses [1]. We report herein the isolation of *P. endodontalis*–like organisms (PELOs) from extraoral sources. These organisms have a close phenotypic resemblance to oral isolates of *P. endodontalis;* however, their taxonomic position warrants reassessment. The aim of this study was to describe the isolation and characterization of PELOs and the laboratory tests useful in their identification.

Six PELO isolates recovered in fecal specimens from young children, as well as six strains that originated from extraoral clinical specimens from adult patients (from the Wadsworth Anaerobic Bacteriology Laboratory [WAL; West Los Angeles, CA] collection) and *P. endodontalis* ATCC (American Type Culture Collection) 35406^T, were included in the bacteriologic study. Five other *P. endodontalis* strains isolated from oral sources, five *Porphyromonas asaccharolytica* strains, and *P. asaccharolytica* ATCC 25260^T were also included in the cellular-fatty-acid analyses (table 1). 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 with use of quantitative culture techniques [2]. To isolate PELOs, we used Brucella blood agar and kanamycin/vancomycin laked blood (KVLB) agar and phenylethyl alcohol (PEA) blood agar plates incubated anaerobically for 5–10 days. The clinical WAL strains were characterized during a comprehensive reevaluation of pigmented gram-negative rods.

We characterized the strains by using routine biochemical tests [2], special-potency antibiotic identification disks, prereduced anaerobically sterilized biochemicals, gas liquid chromatography, API ZYM (bioMérieux, Marcy l'Etoile, France) panels, and Rosco Diagnostic Tablets (Rosco, Taastrup, Denmark). The production of β-lactamase was detected with use of the nitrocefin disk test (Nitrocefin, BIODISK, Solna, Sweden). Cellular fatty acids were detected by a Hewlett Packard (Palo Alto, CA) 5890 series II gas chromatograph and the Microbial Identification System software (Microbial ID, Newark, DE). 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 media. The corresponding library (Microbial ID, ANAEROBE version 3.8) was used in successive analyses. The identity of fatty acids was confirmed by combined gas chromatography–mass spectrometry. A cluster analysis, expressed as a dendrogram, was run by the program included in the MIDI software.

The sources of PELOs in clinical specimens were appendiceal tissue or peritoneal fluid (three specimens), an infected sacral decubitus ulcer, an infected mastoid bone, and a pilonidal abscess (table 1). PELOs were always isolated together with other anaerobes or aerobes. The mean number of accompanying anaerobes per specimen was 4.7, and the mean number of accompanying aerobes was 1.8. The fecal specimens of four of 20 patients harbored PELOs. Fecal strains were isolated from Brucella blood agar, KVLB, or PEA in areas of heavy growth only, not as single colonies. An incubation time of at least 7 days was required before the pigmentation was visible in the primary cultures. In successive subcultures, colonies pigmented faster on laked rabbit blood agar. PELO counts were low ($<10^3$ cfu/g) in the fecal samples.

All PELOs were susceptible to the vancomycin special-potency disk and resistant to kanamycin and colistin (one isolate was susceptible to colistin). The colonies produced black pigment in 3 days on laked rabbit blood agar and had a red fluorescence when they were examined under long-wave ultraviolet light. The organisms were indole positive; lipase, catalase, and nitrate negative; bile sensitive; and asaccharolytic; and produced acetic acid, propi-

Table 1. Strains used in a study to identify *Porphyromonas endodontalis*–like organisms.

Organism	AHN no.	WAL no.	Source
PELO	11186	. . .	Feces
PELO	11239	. . .	Feces
PELO	11253	. . .	Feces
PELO	11260	. . .	Feces
PELO	11330	. . .	Feces
PELO	11972	. . .	Feces
PELO	10884	9902	Infected sacral decubitus ulcer
PELO	11017	10973	Mastoid bone
PELO	11109	11389	Pilonidal abscess
PELO	18382	11374	Peritoneal fluid (appendicitis)
PELO	18428	11296	Appendiceal tissue
PELO	18747	10677	Appendiceal tissue
Porphyromonas endodontalis	2987	. . .	Peritonsillar abscess
P. endodontalis	3027	. . .	Maxilla
P. endodontalis	9720	. . .	Peritonsillar abscess
P. endodontalis	10835	. . .	Gingival pocket
P. endodontalis	11385	. . .	Odontogenic abscess
P. endodontalis	ATCC 35406	. . .	Infected root canal
Porphyromonas asaccharolytica	10803	8203	Peritoneal fluid
P. asaccharolytica	10909	9119	Foot abscess
P. asaccharolytica	11006	11040	Osteomyelitis, bone, toe
P. asaccharolytica	11124	10894	Perirectal abscess
P. asaccharolytica	11258	. . .	Feces
P. asaccharolytica	ATCC 25260	. . .	Pleural empyema

NOTE. AHN = Anaerobe Reference Laboratory, National Public Health Institute (Helsinki, Finland); ATCC = American Type Culture Collection; PELO = *P. endodontalis*–like organism; WAL = Wadsworth Anaerobic Bacteriology Laboratory (Los Angeles, CA).

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Table 2. Major cellular fatty acids (>3%) among some *Porphyromonas* species.

Species	No. of strains	iso-$C_{13:0}$	$C_{14:0}$	iso-$C_{15:0}$	anteiso-$C_{15:0}$	3 OH iso-$C_{15:0}$	$C_{16:0}$	3 OH-$C_{16:0}$	iso-$C_{17:0}$	Sum 3*	Sum 11†
P. asaccharolytica	5 + 1‡	5.7§	3.3	57.7	. . .	4.5	5.3	. . .	. . .	. . .	11.0
P. canoris NCTC 12835	1	. . .	. . .	54.1	3.4	5.0	. . .	. . .	4.5	4.8	18.4
P. catoniae ATCC 51270	1	14.2	10.2	31.0	17.8	. . .	6.8	. . .	3.5	5.8	. . .
P. circumdentaria NCTC 12469	1	. . .	. . .	53.4	. . .	. . .	6.6	. . .	. . .	5.8	18.7
P. endodontalis	5 + 1	3.2	. . .	51.5	. . .	. . .	9.4	. . .	. . .	6.9	11.9
P. endodontalis–like	12	6.1	3.0	55.1	. . .	3.6	7.1	. . .	. . .	3.7	9.9
P. gingivalis ATCC 33277	1	3.2	3.1	27.2	12.1	. . .	9.2	5.0	. . .	8.0	19.7
P. levii ATCC 29147	1	3.7	3.3	24.7	21.7	4.6	4.8	. . .	5.7	. . .	13.8
P. levii–like	27	6.7	5.8	32.3	17.2	5.8	4.5	3.2	8.6	. . .	4.9
P. macacae ATCC 33141	1	7.2	. . .	42.7	13.6	. . .	4.1	3.9	. . .	9.1	10.2

NOTE. ATCC = American Type Culture Collection; NCTC = National Collection of Type Cultures.
* Summed feature 3 = unknown.
† Summed feature 11 = 3 OH iso-C_{17}.
‡ Number of type strain tested.
§ Fatty acid contents expressed as mean values.

onic acid, isobutyric acid, butyric acid, isovaleric acid, and succinic acid as metabolic end products from peptone yeast glucose extract. The API ZYM panel gave positive reactions for alkaline phosphatase, esterase, esterase lipase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase and variable reactions for leucine arylamidase. α-Fucosidase, the enzyme that distinguishes *P. asaccharolytica* from *P. endodontalis,* was found to be lacking with use of both the API ZYM panel and Rosco Diagnostic Tablets. Two isolates produced β-lactamase.

The main cellular fatty acid detected was iso-C_{15}, followed by 3 OH iso-C_{17}, C_{16}, iso-C_{13}, 3 OH iso-C_{15}, C_{14}, and minor amounts of anteiso-C_{15}; this profile was not distinctively different from those of *P. endodontalis* and *P. asaccharolytica* (table 2). Furthermore, the cluster analyses of the cellular fatty-acid profiles disclosed several subgroups of PELOs, which occurred either alone or among the *P. endodontalis* and the *P. asaccharolytica* isolates. No isolation site–specific associations among the subgroups were found. Table 3 lists the characteristics that are useful for identifying *P. endodontalis* and PELOs. The lack of glucose fermentation and susceptibility to the vancomycin special-potency disk differentiate PELOs from pigmented *Prevotella* species. As seen from table 3, PELOs cannot be distinguished from oral *P. endodontalis* by any phenotypic characteristics.

The isolation and identification of *P. endodontalis* and PELOs can pose problems, as these organisms are highly sensitive to oxygen, grow slowly and poorly without supplementation, and produce pigment in primary cultures only after ⩾7 days of incubation. Furthermore, they are biochemically very inert, asaccharo-

Table 3. Differential reactions among *Porphyromonas* species.

	Test												
Species	Pigmentation	Indole	Catalase	Lipase	PAA	Vancomycin*	Glucose	α-Fucosidase	β-NAG	Trypsin	Chymotrypsin	α-Galactosidase	β-Galactosidase
P. asaccharolytica	+	+	−	−	−	S	−	+	−	−	−	−	−
P. cangingivalis	+	+	+	−	−	S	−	−	−	−	+	−	−
P. cansulci	+	+	+w	−	+	S	−	−	−	−	−	−	−
P. canoris	+	+	+	−	−	S	−	−	+	−		−	+
P. catoniae	−	−	−	−	−	R	+	+	+	−+	+−	−+	+
P. circumdentaria	+	+	+	−	+	S	−	−	−	−	−	−	−
P. crevioricanis	+	+	−	NA	+	S	NA	−	−	−	NA	NA	NA
P. endodontalis	+	+	−	−	−	S	−	−	−	−	−	−	−
P. endodontalis–like	+	+	−	−	−	S	−	−	−	−	−	−	−
P. gingivalis	+	+	−	−	+	S	−	−	+	+	−	−	−
P. gingivicanis	+	+	+	NA	−	S	NA	−	−	−	NA	NA	NA
P. levii†	+	−	−	−	−	S	+w	−	+	−	+	−	+
P. macacae‡	+	+	+	+	+	S	+w	−	+	+	+	+	−

NOTE. Data are from [3–5] and authors' determinations. + = positive; − = negative; +w = weak reaction; NA = data not available; β-NAG = N-acetyl-β-glucosaminidase; PAA = phenylacetic acid production.
* Susceptibility to the vancomycin special-potency disk.
† *P. levii* American Type Culture Collection strain and *Porphyromonas levii*–like organisms display identical profiles.
‡ *P. macacae* presently includes former *P. salivosa.*

lytic, and positive for only a few enzymatic reactions. In this study, we found that the isolation of fecal PELO strains was problematic because they grew in very low numbers ($<10^3$ cfu/g) and appeared only in the heavy-growth area. Therefore, the colonies could not be detected before their pigmentation was visible. The cellular fatty-acid profile of *P. asaccharolytica* was found to be very similar to those of *P. endodontalis* and PELOs. It has been reported that a few strains of *P. asaccharolytica* may be α-fucosidase negative [3]. In the present study, all strains classified as *P. asaccharolytica* were α-fucosidase positive and grew well on plated media and in liquid media that was not supplemented with 5% horse serum. All strains designated as *P. endodontalis* or PELOs were α-fucosidase negative and grew very poorly without supplementation.

In conclusion, our findings suggest that *P. endodontalis* may be more common outside the oral cavity than has been previously appreciated. Biochemical characteristics, enzyme profiles, cellular fatty-acid content, and cluster analyses did not clearly separate our extraoral isolates from the type strain or from other clinical oral isolates. Therefore, further taxonomic analyses based on nucleic-acid relatedness are warranted to assess whether PELOs are genotypically similar to oral *P. endodontalis* strains.

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