Biochemical and Structural Characterization of an Unusual Group of Gram-negative, Anaerobic Rods from Human Periapical Osteitis

By Markus Haapasalo, Helena Ranta, Haroun Shah, Kari Ranta, Kari Lounatmaa and Reiner M. Kroppenstedt

Department of Cariology, Department of Bacteriology and Immunology, Department of General Microbiology and Electron Microscopy, University of Helsinki, Mannerheimintie 172, SF-00280, Helsinki 28, Finland,
National Public Health Institute, Mannerheimintie 166, SF-00280, Helsinki 28, Finland
Department of Oral Microbiology, London Hospital Medical College, London E1 2AD, UK
German Collection of Micro-organisms, 6100 Darmstadt, FRG

(Received 22 April 1985; revised 26 September 1985)

The biochemical and chemotaxonomic properties of three previously undescribed strains from human dental root canal infections are presented. The strains were obligately anaerobic Gram-negative rods with fimbriae and a thick capsule-like structure. Carbohydrates were not fermented and agglutination tests were negative. The presence of α-galactosidase, α- and β-glucosidase, β-N-acetylglucosaminidase and β-galactosidase was confirmed. The strains produced acetic and succinic acids as metabolic end products. They contained a peptidoglycan structure based upon meso-diaminopimelic acid (A1γ) and lacked respiratory quinones. The cellular fatty acids were mainly straight-chain saturated and methyl-branched molecules. High interstrain DNA homology was observed and the DNA base compositions were between 56 and 59 mol % G + C. These three strains appear to comprise the nucleus of a new genus of anaerobic, Gram-negative rods from odontogenic infections.

INTRODUCTION

Bacteriological studies on human root canal infections have revealed the presence of several anaerobic Gram-negative bacteria (Sundqvist, 1976). Of these, the genus Fusobacterium and bile sensitive pigmented and non-pigmented Bacteroides species are well characterized. However, isolates not belonging to any known species are frequently found. In the course of a clinical study on human periapical osteitis, three unusual Gram-negative anaerobes were isolated. Using conventional bacteriological tests such as physiological reactions, antibiotic sensitivity patterns and metabolic end product analysis as described by Holdeman et al. (1977) these strains appeared to belong to the genus Bacteroides.

One of the probable mechanisms of pathogenicity of some Bacteroides species is thought to be mediated by such structures as capsules and fimbriae (Sundqvist et al., 1982; Pruzzo et al., 1984). Ultrastructural studies were made on the three new isolates to see whether they shared common properties with one of the recognized periodontal pathogens such as Bacteroides gingivalis. Similar studies in this laboratory on other Bacteroides species from the oral cavity (B. capillus, B. buccae, B. pentosaceus, B. oris, B. oralis, and B. veroralis) revealed extracellular polysaccharides and, in some species, an external proteinaceous surface layer (S-layer) outside the outer membrane (Haapasalo et al., 1985). In this paper we report the ultrastructure and chemotaxonomic properties of the newly isolated strains and compare them to recognized taxa.
Patients and sampling. Three healthy adults with an acute dental root canal infection with bone destruction were referred to the Department of Endodontics, University of Helsinki. Routine endodontic therapy was performed. The treatment was successful, and the control radiographs taken one year later showed reappearance of normal bone structure. At the beginning of the treatment a bacteriological sample was taken as follows. The tooth was cleaned with pumice and isolated with a rubber dam. The tooth and the rubber dam were disinfected with 10% (v/v) H₂O₂ and 0.5% chlorhexidine gluconate in 70% (v/v) ethanol (Möller, 1966). Paper points for root canal sampling were washed with chloroform before sterilization to avoid the inhibitory effect of fatty acids in the paper point on bacterial growth. The paper point was inserted into the canal to the approximate apex region. The samples were inoculated onto the following media: kanamycin (75 μg ml⁻¹) and vancomycin (7.5 μg ml⁻¹) laked blood agar (KVLB agar); MCG agar, containing bacteriological agar no. 1 (Oxoid), 5% (v/v) horse blood, 0.5% (w/v) yeast extract, 0.5 mg menadione l⁻¹, 500 mg cysteine l⁻¹ and 0.2% (w/v) glucose; and chocolate agar. The plates were placed immediately in an anaerobic jar (BBL) and incubated in an anaerobic atmosphere (Gas Generating Kit, Oxoid) at 37 °C.

Identification of strains. Anaerobic jars were opened after 7 d and subcultures were made. Pure cultures were identified to the genus level when possible using routine tests to separate different genera of anaerobic Gram-negative bacteria (Holdeman et al., 1977). The strains were then kept at −70 °C for subsequent studies. Three strains, initially assigned to the genus Bacteroides, were studied in detail: ES2772, ES2645 and KR11. They were grown on MCG and HMCG agar (MCG agar with haemolysed blood) for biochemical tests and for ultrastructural studies. They were studied for growth in air and in 100% (v/v) CO₂ in air, for Gram-staining reaction, and for colony morphology on MCG and HMCG plates; hydrolysis of starch and aesculin and production of indole were also studied (Vera & Power, 1980). Carbohydrate fermentation and gelatin liquefaction were tested by the API 20A system in triplicate. Growth in API media was tested.

Sensitivities to antibiotics (rifampicin, penicillin G, colistin, vancomycin, kanamycin and erythromycin) were evaluated on blood agar plates (Difco) using M/D 8 Mastring discs (Mast Laboratories) as described previously (Knivet et al., 1983). Staphylococcus aureus NCTC 6571 was used as a reference strain.

Enzymes. The strains were studied for the presence of the following enzymes: α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, α-mannosidase, β-N-acetylglucosaminidase, and β-xidosidase. A heavy inoculum of cells grown on HMCG agar for 3 d was suspended in 0.25 ml physiological saline in a test tube. Diagnostic tablets (Rosco, Taastrup, Denmark) containing the chromogenic substrate for the appropriate enzyme were added. The tubes were vigorously shaken for a few seconds and incubated at 37 °C in air for 4 h. A colour change from light grey to bright yellow indicated a positive reaction. Duplicate tests were performed.

Malate and glutamate dehydrogenases were assayed in cell-free extracts according to Reeves et al. (1971). The assays were done at 25 °C in 3 ml volumes in 0-1 M-HEPES buffer (BDH) pH 7.5 containing 0-49 mm-NAD or NADP, with 5 mm-L-malate or 3-5 mm-glutamate as substrate. Enzyme activity was measured as the formation of NAD(P)H, assayed spectrophotometrically at 340 nm in a Gilford model 240 spectrophotometer. The protein concentration of the extracts was determined by the Lowry method. Electrophoretic methods were described by Shah & Williams (1982).

Analysis of acid end-products. Volatile and non-volatile fatty acid products of metabolism were analysed by GLC as described previously (Shah et al., 1976) with a Pye Unicam model 104 gas chromatograph, according to the method of Carlson (1973). Two volumes of culture supernatant of cells grown in either BM medium (Shah et al., 1976) or PYG medium (Holdeman et al., 1977) were shaken with one volume of Zerolit 225 (SRC16) in the acid form (BDH) and allowed to stand at 25 °C for at least 3 h.

Peptidoglycan analysis Cell walls prepared by the method of Schleifer & Kandler (1972) were hydrolysed with 4 M-HCl in sealed ampoules at 100 °C for 16 h. The constituent amino acids were separated by two-dimensional paper chromatography (Whatman no. 1) in ethanol/n-butanol/water/propanic acid (10:10:5:2, by vol.) and acetone/n-butanol/water/dicyclohexylamine (10:10:5:2, by vol.) according to Hardy et al. (1955). Diaminopimelic acid was identified after descending chromatography on Whatman no. 1 paper in a solvent system of methanol/pyridine/conc. HCl/water (32:8:1:7, by vol.) according to Rhuland et al. (1955).

Analysis of long-chain fatty acid methyl esters. Dry organisms (50 mg) were examined by the acid methanolysis and TLC procedure described by Minnikin et al. (1975). Fatty acid methyl esters were analysed on a Varian model 2700 gas chromatograph with a stream-splitter injection system fitted with polar (FFAP) (Macherey-Nagel) and non-polar (OV101) (Macherey-Nagel) wall-coated open tubular columns (50 m). Both columns were operated isothermally at 220 °C with helium as carrier gas. The identity of the hydroxy fatty acid esters was confirmed by conversion to their corresponding trimethylsilyl derivatives by adding dry fatty acid methyl esters to N-methyl-N-trimethylsilyl heptafluorobutyramide (Macherey-Nagel) under mild heating. The relative proportions of the fatty acid esters were determined with a Varian integrator (model CDS 101).

Extraction and analysis of isoprenoid quinones. Dry organisms (100 mg) were extracted and analysed as described by Collins et al. (1977).
Novel fimbriate anaerobic rods

Agglutination tests. Haemagglutination was performed with washed human A, B, and O, and sheep erythrocytes. A 2% (v/v) suspension of erythrocytes in PBS (25 μl) was pipetted onto glass slides (kept on ice) and bacteria collected with platinum loop from HMCG plates (3-day-old cultures, anaerobic incubation) were added. The slides were rotated at intervals and haemagglutination was registered after 30 min. Type 1 fimbriation was determined by using a 1% (w/v) suspension of *Saccharomyces cerevisiae* cells (OY ALKO AB, Helsinki, Finland) with and without 5% (w/v) methyl-α-D-mannoside (Sigma) in the medium (Korhonen, 1979). Agglutination tests were done in triplicate.

DNA base composition and DNA/DNA hybridization. Cells grown in BM medium for 7 d to the late exponential phase were harvested by centrifugation and washed twice in 0.05 M-Tris-buffer containing 0.01 M NaCl (pH 8.0) (TES). Cells were lysed in TES containing 50 μg proteinase K ml⁻¹ (Merck) and sodium dodecyl sulphate (final concentration, 1 mg ml⁻¹), incubated for 15 min at 45 °C. The lysate was deproteinized and treated with ribonuclease (Sigma) (Marmur, 1961). The DNA was then purified by column chromatography, using Fractogel 65 (BDH) as the column matrix. The DNA base composition was estimated with a Gilford model 240 spectrophotometer and a Gilford 2527 thermal programmer from its melting temperature in 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0. DNA from *Escherichia coli* strain B (Sigma) was used as a reference.

DNA/DNA hybridization between strains was estimated by the optical renaturation method of De Ley et al. (1970) and Huss et al. (1983) using the same equipment as for the DNA base composition. The renaturation temperature (T_{OR}) was calculated from the equation $T_{OR} = (0.51 \times \text{mol} \% G + C + 470)$ (Gillis et al., 1970). The degree of binding (%D) was calculated from the equation $%D = 100 \times (4V'_{a} - V'_{a} - V'_{b})/2V_{a} \times V_{b}$, where $V'$ is the renaturation rate of samples A and B or the mixture M (described in detail by De Ley et al., 1970). *Bacteroides capillosus* ATCC 29799, *Rikenella microfusus* ATCC 29728 and *Mitsuokella multiacidus* ATCC 27723 were used as reference strains for DNA base composition and homology studies.

Electron microscopy. The cells were collected with a platinum loop from MCG and HMCG plates (grown anaerobically for 3 d) into 0.1 M-sodium phosphate buffer (pH 7.2) and negatively stained with 2% (w/v) phosphotungstic acid (pH 6.5). In some preparations (not shown here) the cells were also washed with distilled water before staining, but the results were not as good as those with unwashed cells. The samples for thin sectioning were stained with tannic acid (Wagner, 1976) or ruthenium red (Luft, 1964) and prepared as previously described (Lounatmaa et al., 1976). The micrographs were taken with a JEM-100B electron microscope operating at 80 kV. As a control, *B. gingivalis* ATCC 35277 was used.

RESULTS

Strains ES2772, ES2645 and KR11 were obligately anaerobic, Gram-negative rods which were isolated from mixed anaerobic infections. Cells of all three strains were 1 to 2 μm long, had rounded ends and were usually oval-shaped. Their colonies were wet and translucent, irregular in shape and 1 to 2 mm in diameter. On MCG agar the strains showed weak α-haemolysis. Growth on HMCG agar was heavier than that on MCG agar. Strain ES2645 was always lost after repeated subcultures on MCG agar. None of the three strains grew on KVLB agar and their growth in API media was poor.

In common with most bacteroides-like organisms previously examined (Duerden et al., 1976; Knivett et al., 1983) the three strains were resistant to kanamycin, vancomycin and colistin but sensitive to erythromycin, penicillin and rifampicin. All the strains had moderate NAD-dependent malate dehydrogenase activity and much lower glutamate dehydrogenase activity (Table 1). Carbohydrates were not fermented and the agglutination and gelatin liquefaction tests were also negative. All three strains were negative for hydrolysis of starch and aesculin, and for production of indole, α-fucosidase, α-mannosidase and β-xyllosidase, and positive for α- and β-glucosidase, β-N-acetylglucosaminidase and β-galactosidase. Strains ES2645 and KR11 were also positive for β-galactosidase, but strain ES2772 gave only a weak reaction.

The major end products of metabolism from either PYG or BM media were acetic and succinic acids, although traces of lactic acid were detected in strain KR11. In general these metabolic end products were similar to those produced by most *Bacteroides* species (Shah & Collins, 1983).

Chromatographic analysis of cell wall hydrolysates revealed a peptidoglycan type based upon meso-diaminopimelic acid as the dibasic amino acid. Other amino acids detected were glycine, alanine, glutamic acid and aspartic acid, indicating an A1γ peptidoglycan chemotype (Schleifer & Kandler, 1972).
Table 1. Electrophoretic mobility of malate dehydrogenase (MDH), and MDH and glutamate dehydrogenase (GDH) activities of strains ES2772, ES2645 and KR11

Enzyme activities are given as nmol coenzyme reduced (mg protein)\(^{-1}\) min\(^{-1}\). tr, trace; ND, not done.

<table>
<thead>
<tr>
<th>Strain</th>
<th>MDH mobility (cm)</th>
<th>MDH: NAD</th>
<th>MDH: NADP</th>
<th>GDH: NAD</th>
<th>GDH: NADP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermedius T588*</td>
<td>4.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ES2772</td>
<td>5.1</td>
<td>137</td>
<td>tr</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>ES2645</td>
<td>5.2</td>
<td>146</td>
<td>–</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>KR11</td>
<td>5.1</td>
<td>128</td>
<td>–</td>
<td>8</td>
<td>tr</td>
</tr>
</tbody>
</table>

*Control strain for electrophoretic mobility.

Table 2. Percentage fatty acid composition of strain ES2772

<table>
<thead>
<tr>
<th>Fatty acid type*</th>
<th>%</th>
<th>Fatty acid type*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{12:0})</td>
<td>1.3</td>
<td>C(_{17:0})</td>
<td>1.8</td>
</tr>
<tr>
<td>iso-C(_{14:0})</td>
<td>15</td>
<td>iso-C(_{18:0})</td>
<td>1.0</td>
</tr>
<tr>
<td>C(_{14:0})</td>
<td>11</td>
<td>C(_{18:0})</td>
<td>7.0</td>
</tr>
<tr>
<td>anteiso-C(_{15:0})</td>
<td>6.0</td>
<td>C(_{18:1}) (\omega9)</td>
<td>9.4</td>
</tr>
<tr>
<td>C(_{15:0})</td>
<td>1.7</td>
<td>C(_{18:1}) (\omega7)</td>
<td>0.3</td>
</tr>
<tr>
<td>iso-C(_{16:0})</td>
<td>7.4</td>
<td>C(_{18:2})</td>
<td>1.1</td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>16</td>
<td>iso-3-OH-C(_{16:0})</td>
<td>1.6</td>
</tr>
<tr>
<td>C(_{16:1})</td>
<td>2.3</td>
<td>3-OH-C(_{16:0})</td>
<td>12.4</td>
</tr>
</tbody>
</table>

* Abbreviations for the fatty acids are illustrated by the following examples. C\(_{15:0}\), pentadecanoic acid; iso-C\(_{15:0}\), 13-methyltetradecanoic acid; anteiso-C\(_{15:0}\), 12-methyltetradecanoic acid; C\(_{18:1}\) \(\omega9\), cis-9,10-octadecenoic acid (oleic acid); C\(_{18:2}\), cyclopropanenonadecanoic acid; 3-OH-C\(_{16:0}\), 3-hydroxytetradecanoic acid (β-hydroxymyristic acid). Major components are shown in bold type.

Table 3. DNA base composition and homologies between strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mol % G + C</th>
<th>DNA homology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ATCC 29729* ATCC 29728† ATCC 27723‡</td>
</tr>
<tr>
<td>ES2772</td>
<td>59.7</td>
<td>29</td>
</tr>
<tr>
<td>KR11</td>
<td>56.4</td>
<td>100</td>
</tr>
<tr>
<td>ES2645</td>
<td>58.6</td>
<td>–</td>
</tr>
</tbody>
</table>

* Bacteroides capillosus. † Rikenella microfusus. ‡ Mitsuokella multiacidus.

TLC analysis of whole-organism methanolysates revealed the presence of both hydroxylated and non-hydroxylated long-chain fatty acid methyl esters. GLC analysis of one strain, ES2772, indicated that the 3-hydroxy acids were of the straight-chain saturated type and no 2-hydroxy acids were present. In contrast to results for other Gram-negative anaerobes (Shah & Collins, 1983), the major hydroxylated fatty acid was 3-hydroxyhexadecanoic acid (3-OH-C\(_{16:0}\)) (Table 2). The predominant fatty acids were hexadecanoic (C\(_{16:0}\)) and 12-methyltridecanoic (iso-C\(_{14:0}\)) acids (Table 2). Respiratory quinones were not detected in cell extracts analysed as described in Methods.

The DNA base compositions of the three test strains, and the results of DNA homology studies, are shown in Table 3. The DNA base compositions of the reference strains *Bacteroides capillosus*, *Rikenella microfusus* and *Mitsuokella multiacidus* were 60, 61 and 58 mol % G+C, respectively.

Electron microscopy

In negatively stained cells of strain ES2772 grown on MCG medium bundles of fimbriae could be seen (Fig. 1a). The fimbriae were about 1 μm long; they were peritrichous and most cells were...
Fig. 1 (a) Negatively stained cell of strain ES2772 (grown on MCG). Many fimbriae (F) are clearly seen. The bar in this and subsequent micrographs represents 0.2 μm. (b) Negatively stained cells of strain ES2772 (grown on HMCG). Numerous fimbriae and a thick capsule-like structure (C) can be seen.
Fig. 2(a) Thin-sectioned cell of strain ES2772 stained with ruthenium red. The electron-dense zone around the cell is very pronounced. (b) Thin-sectioned cell of strain ES2772 stained with tannic acid. The capsule-like structure is only partially stained. (c) Negatively stained cells of strain ES2645. A thick capsule-like structure (C) and numerous fimbriae (F) are visible. (d) Thin-sectioned cell of strain ES2645 stained with tannic acid. A thick capsule-like layer and bundles of fimbriae are seen.
Novel fimbriate anaerobic rods

Fig. 3(a) Negatively stained cells of strain KR11. Long capsular projections (CP) are visible. (b) Thin-sectioned cell of strain KR11 stained with tannic acid. A thick capsule-like layer is present. (c) Thin-sectioned cells of B. gingivalis ATCC 33277 stained with tannic acid.

covered by them. In cells grown on HMCG medium a thick capsule-like zone around the cell was seen (Fig. 1b). This zone, about 0.15 µm thick, was found in most cells of successive HMCG-grown preparations. The fimbriae ran without disruption from the OM through the capsule-like zone, thus excluding the possibility that this zone was an artefact caused by, for example, plasmolysis (Fig. 1b). In thin-sectioned cells of strain ES2772 stained with ruthenium
red the proximal part of the capsule-like zone was heavily but unevenly stained, whereas in the distal part the staining was much lighter (Fig. 2a). The opposite reaction was observed with tannic acid: an electron-translucent zone approximately 20 nm wide between the cell envelope and the thick capsule-like structure was always found (Fig. 2b). This translucent zone was present regardless of the thickness of the capsule-like structure and is therefore unlikely to be an artefact (Figs 2b, d; see also strain KR11 in Fig. 3b).

Negative staining of cells of strain ES2645 also revealed fimbriae (Fig. 2c). In thin-sectioned cells of this strain stained with tannic acid (a cross-linking agent for proteins), a very thick capsule-like zone was seen around the cell (Fig. 2d). Electron-dense lines within this zone were probably bundles of fimbriae. The fimbriae were apparently covered by the capsular material, even outside the main capsule-like zone (Fig. 2d).

Single, straight fimbriae as found in strains ES2772 and ES2645 could not be detected in negatively stained cells of strain KR11 (Fig. 3a). It is thus possible that the projections seen in this strain were composed of polymeric material from the capsular layer. The presence of projections was confirmed in thin-sectioned specimens stained with tannic acid (Fig. 3b).

Cells of *B. gingivalis* ATCC 33277 stained with tannic acid showed a thin layer of extracellular electron-dense material (Fig. 3c).

**DISCUSSION**

Anaerobic, Gram-negative rods comprise the major component of the bacterial flora associated with various forms of periodontal disease and dental root canal infections. However, many of the micro-organisms associated with these sites are still not well characterized. These organisms are often difficult to identify and can be misidentified using conventional tests for the routine identification of anaerobes from clinical specimens. This is particularly so for non-fermentative/non-pigmented strains, which are generally difficult to cultivate. These difficulties are highlighted in this study. Using characteristics given in *Berger's Manual of Systematic Bacteriology* (Krieg, 1984) such as morphology, growth requirements and metabolic end products, our three new isolates were initially assigned to the genus *Bacteroides*. They were non-fermentative and produced very low levels of acetic and succinic acids. However, they differed markedly from true members of the genus *Bacteroides* by other tests. *Bacteroides* spp. (e.g. *B. fragilis*, *B. melaninogenicus*, *B. oralis* groups) have high malate dehydrogenase and glutamate dehydrogenase activities (Shah & Collins, 1983) whereas our strains had only moderate malate dehydrogenase activity and a trace of glutamate dehydrogenase. Furthermore, the cellular fatty acids of most true *Bacteroides* spp. comprise primarily straight-chain saturated, iso- and anteiso-methyl branched-chain acids with 12-methyltetradecanoic acid (anteiso-C₁₅:₀) as the major acid (Shah & Collins, 1983). Weakly saccharolytic/non-fermentative species contain 13-methyltetradecanoic acid (iso-C₁₅:₀) as the major component. By contrast, one of the isolates we examined, ES2772, had only low levels of 12-methyltetradecanoic acid (6%) and lacked 13-methyltetradecanoic acid (Table 2).

The high mol % G + C of the DNA of the test strains (56.4–59.7) also indicates that they are not true members of the genus *Bacteroides*, which have a mol % G + C content of approximately 40–52 (Shah & Collins, 1983). The new isolates had a DNA base composition more compatible with *Bacteroides capillosus* (60 mol % G + C) and two other taxa (formerly *Bacteroides* spp.), *Mitsuokella multicidaus* (56–58%) and *Rikenella microfusus* (60–61%) (Shah & Collins, 1983; Collins et al., 1985). However, the three fimbriate strains formed a homogeneous group with high interstrain DNA homology and showed only low DNA/DNA relatedness with reference strains of the three taxa mentioned above.

The importance of a capsule to the virulence of bacteria in opportunistic infections has been shown in many studies (Peterson et al., 1978; Sundqvist et al., 1982). Sundqvist et al. (1982) studied the phagocytosis of black-pigmented *Bacteroides* in relation to the presence of a capsule, and found that species with a thicker capsule were more resistant. Although the capsule is reported to be very prominent in *B. gingivalis*, we were unable to demonstrate a distinct capsule in *B. gingivalis* ATCC 33277 (Fig. 3c). However, a thinner unorganized layer outside the
outer membrane was observed. We have studied five clinical isolates of \textit{B. gingivalis} and so far the presence of a well-defined capsule-like layer comparable to that of strains ES2772, ES2645 and KR11 has not been confirmed (unpublished observations).

The terminology of structures surrounding the outer membrane of \textit{Bacteroides} spp. is at present confusing. Methods such as immunoelectronmicroscopy are needed to clarify the precise nature and localization of the structural components. Nevertheless, comparison of our electron micrographs, obtained after different preparative techniques and showing similar structures, can offer valuable information when interpreted with caution.

Fimbriae were easily detected in negatively stained preparations of strains ES2772 and ES2645. They seemed to be thicker than those described in black-pigmented \textit{Bacteroides} and \textit{B. fragilis} (Okuda \textit{et al}., 1981; Pruzzo \textit{et al}., 1984), and they often seemed to coalesce and form bundles. In both negatively stained specimens and thin sections the capsule-like material appeared to cover the fimbriae. The negative agglutination tests further emphasize the possibility of receptor sites being hidden by the capsule-like material. On the other hand, not all fimbriae agglutinate erythrocytes (Pruzzo \textit{et al}., 1984). All three strains were isolated from necrotic infections of the dental root canal, where the defence mechanisms of the host are not effective. In this situation the fimbriae are probably not important in promoting the colonization of bacteria. However, in another environment, e.g. in the gingival crevice, they may play an important role.

The results reported demonstrate the biochemical and ultrastructural homogeneity of the new isolates from human periapical osteitis. They also indicate that these isolates comprise the nucleus of a new taxon distinct from other Gram-negative anaerobic rods such as \textit{B. capillosus}, \textit{Mitsuokella multiacidus} and \textit{Rikenella microfusus}, which have a similar DNA base composition. In addition their surface structure differs from other oral \textit{Bacteroides} species studied.

We thank Olli-Veikko Renkonen for helpful discussions and Tuire Koro for skilful technical assistance and Astra Pharmaceuticals for financial support.

\textbf{REFERENCES}


