**Eubacterium exiguum** sp. nov., Isolated from Human Oral Lesions

SERGIO E. POCO, JR., FUTOSHI NAKAZAWA, TETSURO IKEDA, MICHIKO SATO, TAKUCHI SATO, AND ETSURO HOSHINO*

Department of Oral Microbiology, Niigata University School of Dentistry, Gokkocho-dori 2, Niigata, 951, Japan

**Eubacterium exiguum** sp. nov. is the name proposed for organisms formerly described as *Eubacterium* group S strains and similar bacteria isolated from various types of oral lesions. This new species was established on the basis of the results of DNA-DNA hybridization experiments and DNA base composition determinations (G+C contents, 60 to 64 mol%). The results of an API ZYM analysis, Western blotting (immunoblotting) reactions, and phenotypic tests are also given. The type strain of *E. exiguum* is strain S-7.

*Eubacterium* spp. are nonsporing, nonmotile, gram-positive, strictly anaerobic bacilli which produce various combinations of butyric, lactic, acetic, and formic acids or no major acids (18, 24). *Eubacterium* spp., particularly asaccharolytic species, are frequently isolated from advanced periodontal pockets (5, 6, 15, 17, 28, 32), dental pulp (9, 22), carious dentin (1, 3, 8), acute dento-alveolar abscesses (31), and periapical infections (12). Several researchers have recently reported increased systemic antibiotic titers to these organisms compared with healthy controls (4, 23, 25–27), suggesting that they play potent pathogenic roles in oral infectious diseases.

There are six previously described oral asaccharolytic *Eubacterium* species, namely, *Eubacterium timidum*, *Eubacterium brachy*, *Eubacterium nodatum*, *Eubacterium lentum*, and the recently proposed species *Eubacterium saphenum* (29) and *Eubacterium minutum* (21). These species have proven to be difficult to culture and unreactive in conventional biochemical tests. However, by using better anaerobic techniques, we isolated several *Eubacterium* strains which could not be classified in any of the previously established species. These clinical isolates were found to be the predominant organisms among the asaccharolytic *Eubacterium* species in human necrotic pulp samples and were tentatively classified as members of *Eubacterium* group S (22). The phenotypic characteristics of *Eubacterium* group S resembled those of *E. lentum* when *Eubacterium* group S was compared with the previously established asaccharolytic *Eubacterium* species and unclassified *Eubacterium* group D-6 (17). They also resembled the characteristics of another unclassified group, *Eubacterium* cluster 2, which was described previously by Wade et al. (31, 33), although its characteristics were not given in detail.

In this paper we describe the characteristics of *Eubacterium* group S and other unclassified strains as determined by DNA-DNA hybridization, a DNA base composition analysis, a Western blotting (immunoblotting) analysis, a sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis, and an API ZYM analysis and propose a new taxonomic position for *Eubacterium* group S.

**MATERIALS AND METHODS**

**Bacterial strains.** The bacterial strains used in this study and their sources are listed in Table 1. The five *Eubacterium* group S strains (strains S-7**T** = type strain), S-4, S-6, S-8, and S-22) were isolated from human necrotic pulp samples (22). The type strains of previously established asaccharolytic *Eubacterium* species and other unclassified *Eubacterium* strains, such as *L. Acid Homo* (12), 108 and 142 (31, 33), and D136M-28 (17), were also included in this study.

**Culture conditions.** All of the strains were cultured on brain heart infusion (BHI)-blood agar plates under strictly anaerobic conditions for 7 to 10 days in an anaerobic glove box (model AZ-Hard; Hirasawa, Tokyo, Japan) containing 80% N₂, 10% H₂, and 10% CO₂. The bacterial cells were harvested by centrifugation, washed with 10 mM sodium phosphate-buffered saline (pH 7.2), and stored at −20°C until they were used. The *Eubacterium* strains were characterized by their ability to grow strictly anaerobic conditions were maintained in the glove box, the reduction of methyl viologen (at −466 mV) was carefully checked whenever experimental procedures were carried out.

**Morphological and biochemical characteristics.** Cellular morphology was determined by examining cells grown on BHI-blood agar plates. Biochemical reactions were determined by the methods described in the *Anaerobe Manual* (21). The end products produced in peptone-yeast extract-glucose broth were determined by gas chromatography, as described previously (10).

**API ZYM (Rapid ID 32A) analysis.** A Rapid ID 32A kit (API BioMerieux, Marcy l'Etoile, France) was used as recommended by the manufacturer, except that preparations were incubated under strictly anaerobic conditions.

**SDS-PAGE of cellular proteins.** Cellular proteins were extracted by using the SDS-PAGE procedure described by Laemmli (13). Approximately 50 mg (wet weight) of whole cells was suspended in 1 ml of distilled water. Samples were boiled in 150 μl of lysis buffer containing 4% (w/v) SDS, 40% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, and 0.25 M Tris-HCl (pH 6.8). The protein concentrations of the supernatants were determined with a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). About 7 to 10 μg of protein per lane was subjected to electrophoresis on a 12.5% polyacrylamide gel by using a model KS-8010 Micro slab gel electrophoresis system (Matsusato Co., Tokyo, Japan). The proteins were stained with Coomassie brilliant blue R-250 (Nakarai Chemicals, Ltd., Kyoto, Japan).

**Western immunoblotting analysis.** Proteins were transferred from SDS-PAGE gels to nitrocellulose membrane filters (pore size, 0.45 μm; Bio-Rad) by using the transfer buffer system described by Burnette (2) in conjunction with the Trans blot system (Matsusato) at a constant current of 350 mA for 4 h with cooling. The membrane filters were processed as described previously (21). Briefly, an immune rabbit antiserum (1:1,000) was used as the first antibody, and then the preparation was incubated with goat anti-rabbit immunoglobulin G conjugated with peroxidase (1:1,000) as the second antibody; finally, the color was developed.

**DNA isolation and purification.** DNA was isolated and purified by a modification of the procedure of Marmur (14), as described previously (21).

**DNA base composition.** The G+C content of DNA was determined by high-performance liquid chromatography (HPLC), as described previously (11).

**DNA-DNA hybridization.** DNA-DNA hybridization was performed by the membrane filter method as described previously (16). Briefly, reference DNA was labeled by using a multiprime DNA labeling kit (Amersham, Buckinghamshire, United Kingdom) and [α-32P]PCTP and then purified with a G-50 column (Pharmacia LKB Biotechnologie, Bromma, Sweden). About 40 μg of unlabeled single-stranded DNA immobilized on each nitrocellulose membrane filter and 0.015 μg of labeled reference DNA were reassociated in a solution containing 0.06% SDS, 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400, 0.02% bovine serum albumin (fraction V, Sigma), and 1 ml of 0.01 SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate). After incubation overnight at 60°C, the filters were washed and dried. The radioactivity was measured with a liquid scintillation counter. Triplicate tests were performed for each assay, and the results were normalized to 100% for the homologous DNA.

* Corresponding author. Phone: 81–25-223–6161, ext. 4165. Fax: 81–25-225–0513. Electronic mail address: hoshino@dent.niigata-u.ac.jp.
Eubacterium

Eubacterium

E. timidum

E. lenturn

E. nodatum

E. saphenurn

Unclassified

Eubacterium

unlike

unclassified organism

but the

dium was detected by gas chromatography. Thus, phenotypi-

prolonged incubation, the colonies were less than 1 mm in

metabolic end product from peptone-yeast extract-glucose me-

diameter.

used are shown in Table 2. The five

strains were nonreactive in most of the usual biochemical tests, and no

strains were nonfermentative, arginine hydrolysis positive, and

strains were gram negative. Growth in

moderately enhanced by the presence of 5% bovine serum,

0.2% lysine, or 0.2% arginine. Like other oral asaccharolytic

Eubacterium

Growing cells of these bacteria were gram positive, but some-

strains, strains these strains formed typical tiny colonies

on BHI-blood agar plates; the colonies were about 0.3 to 0.5

mm in diameter, circular, convex, and translucent. Even after

prolonged incubation, the colonies were less than 1 mm in diameter.

Phenotypic characteristics. The biochemical characteristics

of Eubacterium group S and the reference Eubacterium species

used are shown in Table 2. The five Eubacterium group S

strains were nonfermentative, arginine hydrolysis positive, and

nonreactive in most of the usual biochemical tests, and no

metabolic end product from peptone-yeast extract-glucose me-

dium was detected by gas chromatography. Thus, phenotypi-

cally, Eubacterium group S most closely resembled E. lenturn

among the previously established Eubacterium species and the

unclassified organism Eubacterium sp. strain D136M-28 (17),

but the Eubacterium group S strains did not reduce nitrate,

unlike E. lenturn and Eubacterium sp. strain D136M-28. The

Eubacterium group S strains also resembled the unclassified

organisms Eubacterium sp. strains 108 and 142, which produce

lactic acid and succinic acid as end products (33), but in this

study no lactic acid or succinic acid was detected as a metabolic

end product.

SDS-PAGE protein profile analysis. The protein profiles of

Eubacterium group S strains and asaccharolytic reference

strains are shown in Fig. 1. The Eubacterium group S strains

produced typical protein profiles, which had several major

bands at low molecular weights (20,000 to 45,000) and were

quite similar to the protein profiles of the unclassified organ-

isms Eubacterium sp. strains D136M-28, 108, and 142. The

profiles of the oral asaccharolytic species had no major bands in

common, which suggested that the protein components of

these organisms are very heterogeneous.

API ZYM (Rapid ID 32A) analysis. The enzymatic activities

of the Eubacterium group S strains and reference strains are

summarized in Table 3. The Eubacterium group S strains and

the unclassified organisms Eubacterium sp. strains 108 and 142

strains had an enzymatic profile that was quite distinct (API

code, 2000 0337 05). These organisms were positive for most

aminopeptidase activities, including arginine arylamidase, pro-

line arylamidase, leucyl glycine arylamidase, phenylalanine

arylamidase, tyrosine arylamidase, alanine arylamidase, glycine

arylamidase, histidine arylamidase, and serine arylamidase ac-

tivities.

Most of the previously described oral asaccharolytic Eubac-

terium species (namely, E. brachy, E. nodatum, E. lenturn, and

E. saphenurn) were not reactive in most of the aminopeptidase

tests. The recently proposed new species E. minuturn (API

code, 0000 0526 01) was positive in most of the aminopeptidase

strains and reference strains are

Table 2. Characteristics of Eubacterium group S, unclassified Eubacterium strains, and other oral asaccharolytic Eubacterium species

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Fermentation of glucose</th>
<th>End products of fermentation</th>
<th>Arginine hydrolysis</th>
<th>Nitrate reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eubacterium group S</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Unclassified Eubacterium strains</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>108</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>142</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D136M-28</td>
<td>–</td>
<td>B or b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. minuturn</td>
<td>–</td>
<td>ib, ic, iv, phe-p</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. brachy</td>
<td>–</td>
<td>a, b</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>E. lenturn</td>
<td>–</td>
<td>a, b</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>E. nodatum</td>
<td>–</td>
<td>a, b</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>E. saphenurn</td>
<td>–</td>
<td>a, b</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>E. timidurn</td>
<td>–</td>
<td>phe-a</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

| a | acetate; b and B, butyrate; ib, isobutyrate; ic, isocaproate; iv, isovalerate; phe-a, phenylacetate; phe-p, phenylpropionate. Uppercase letters indicate that the concentration is 10 mM or more, and lowercase letters indicate that the concentration is less than 10 mM.

| Eubacterium group S strains S-7T, S-4, S-6, S-8, and S-22 |
Our data clearly showed that oral asaccharolytic \textit{Eubacterium} species, including \textit{Eubacterium} group S, could be distinguished serologically, which indicates that there is immunological heterogeneity within the genus \textit{Eubacterium}. These data are consistent with the previously published data of Nakazawa and Hoshino (19).

**G+C contents.** The G+C contents of \textit{Eubacterium} group S strains and reference species, as determined by HPLC, are listed in Table 5. The \textit{Eubacterium} group S strains and \textit{Eubacterium} sp. strains L-24, 108, 142, and D136M-28 had high G+C contents, ranging from 60 to 64 mol\%. Most of the previously established \textit{Eubacterium} species had G+C contents of 38 to 50 mol\%, values which are significantly lower than the G+C contents of the \textit{Eubacterium} group S strains. Because of their high G+C contents, the \textit{Eubacterium} group S strains can be placed in the high G+C-content group described previously by Nakazawa and Hoshino (20). Of the previously established asaccharolytic \textit{Eubacterium} species, only \textit{E. lentum} had a high G+C content (62 mol\%) which was similar to the G+C contents of the \textit{Eubacterium} group S strains.

**DNA-DNA hybridization analysis.** The DNA-DNA hybridization experiments performed by the membrane filter method (Table 5) showed that the \textit{Eubacterium} group S strains were genetically related because of their very high levels of DNA reassociation. These experiments also revealed high levels of reassociation between the DNAs of the \textit{Eubacterium} group S strains and the DNAs of \textit{Eubacterium} sp. strains 108 (92\%), 142 (98\%), and D136M-28 (87\%). The hybridization analysis revealed that \textit{Eubacterium} group S strains and strains 108, 142, and D136M-28 exhibit very low levels of DNA homology with the previously established asaccharolytic \textit{Eubacterium} species (levels of homology, 1 to 20\%). These data clearly indicate that the \textit{Eubacterium} group S strains are completely distinct from the previously established \textit{Eubacterium} species. Consequently, we propose that \textit{Eubacterium} group S should be named \textit{Eubacterium exiguum} sp. nov. Three genetically related, previ-

---

**TABLE 3. Enzymatic profiles of \textit{Eubacterium} group S, unclassified \textit{Eubacterium} strains, and other oral asaccharolytic \textit{Eubacterium} strains**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Arginine dihydrolase</th>
<th>Nitrase</th>
<th>Arginine arylamidase</th>
<th>Proline arylamidase</th>
<th>Leucyl glycine arylamidase</th>
<th>Phenylalanine arylamidase</th>
<th>Leucine arylamidase</th>
<th>Pyroglutamic arylamidase</th>
<th>Tyrosine arylamidase</th>
<th>Alanine arylamidase</th>
<th>Glycine arylamidase</th>
<th>Histidine arylamidase</th>
<th>Serine arylamidase</th>
<th>Rapid ID 32A API ZYM code</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Eubacterium} group S'</td>
<td>w\textsuperscript{a}</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2000 0337 05</td>
</tr>
<tr>
<td>Unclassified \textit{Eubacterium} strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-24</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D136M-28</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2000 0337 05</td>
</tr>
<tr>
<td>108 and 142</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2000 0337 05</td>
</tr>
<tr>
<td>\textit{E. timidum} ATCC 33093\textsuperscript{T}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0000 0040 00</td>
</tr>
<tr>
<td>\textit{E. minutum} ATCC 70079\textsuperscript{T}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0000 0526 01</td>
</tr>
<tr>
<td>\textit{E. nodatum} ATCC 33099\textsuperscript{T}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0000 0100 00</td>
</tr>
<tr>
<td>\textit{E. saphenum} ATCC 49989\textsuperscript{T}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0000 0000 00</td>
</tr>
<tr>
<td>\textit{E. lentum} ATCC 25559\textsuperscript{T}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0000 1100 00</td>
</tr>
<tr>
<td>\textit{E. brachy} ATCC 33089\textsuperscript{T}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0000 4200 00</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Results obtained with the Rapid ID 32A (API BioMerieux) identification system for anaerobes.

\textsuperscript{b} Summary of test results.

\textsuperscript{c} \textit{Eubacterium} group S strains S-7T, S-4, S-6, S-8, and S-22.

\textsuperscript{d} w, weak; +, positive; –, negative.
nously unclassified strains, strains D136M-28, 108, and 142, should also be placed in this species.

**Description of Eubacterium exiguum sp. nov.** *Eubacterium exiguum* (ex. i.gu’um. L. adj. exiguum, scanty, small, referring to the scanty or poor growth of this organism). Cells are short gram-positive rods that are obligately anaerobic, nonmotile, and nonsporing. Individual cells are 0.5 by 1.0 μm, and the cells occur singly or in clumps. Sometimes cells from older cultures stain gram-negative. On BHI-blood agar plates, the cells form minute colonies that are less than 1 mm in diameter, circular, convex, and translucent even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor in the presence or in the absence of carbohydrates and is moderately enhanced in the presence of 5% bovine serum, 0.2% lysine, or 0.2% arginine. No hemolysis occurs around colonies on BHI-blood agar plates.

The cells are inert in most biochemical tests. Starch and esculin are not hydrolyzed, and nitrate is not reduced. No detectable metabolic end products are produced in peptone-yeast extract medium supplemented with glucose or peptone-yeast extract-glucose broth.

Isolated from human necrotic pulp samples, periapical infections, and acute dento-alveolar abscesses.

The G+C content is 60 to 64 mol%. The type strain is strain S-7.

**ACKNOWLEDGMENTS**

We are grateful to William G. Wade for strains 108 and 142, to L. V. H. Moore for strain D136M-28, to T. Kiryu for strain L-24, and to H. Uematsu for critical discussions.

This study was supported in part by grants-in-aid for scientific research 05807169, 06044082, 07457427, and 07807166 from the Ministry of Education, Science and Culture of Japan.

---

**TABLE 5.** DNA base compositions and levels of DNA relatedness among *Eubacterium* group S strains, unclassified *Eubacterium* strains, and other asaccharolytic *Eubacterium* strains

| Source of DNA | G+C content (mol%)* | % Homology with labeled DNA from:
|---------------|---------------------|--------------------------------------
|               |                     | S-7T | S-8 | S-4 | L-24 | 108 | 142 | D136M-28 | ATCC 33093T |
| *Eubacterium* group S strains |                     |      | 100 |     |     |     |     |     |     |
| S-7T          | 60                  | 100  |     | 100 |     |     |     |     |     |
| S-8           | 65                  | >100 | 100 |     | 100 |     |     |     |     |
| S-4           | 65                  | >100 | >100| >100| >100| 100 |     |     |     |
| Unclassified *Eubacterium* strains |                     |      |     |     |     |     |     |     |
| L-24          | 61                  | >100 | >100| >100| >100| >100| 100 |     |     |
| 108           | 62                  | >100 | 97  | 92  | >100| 100 |     |     |     |
| 142           | 61                  | >100 | 98  | 99  | >100| >100| >100| >100| >100 |
| D136M-28      | 64                  | 87   | 95  | 100 | >100| >100| >100| >100| >100 |
| *E. lentum* ATCC 25559T | 62                  | 10   | 13  | 10  | 4   | 20  | 13  | 10  | 4    |
| *E. timidum* ATCC 33093T | 50                  | 2    | 1   | 3   | 2   | 5   | 3   | 3   | 100  |
| *E. timidum* ATCC 33092 | 50                  | 5    | 1   | 6   | 6   | 5   | 12  | 3   | 96   |
| *E. minutum* ATCC 700079T | 38-40               | 6    | 5   | 6   | 4   | 13  | 12  | 6   | 2    |
| *E. saphenum* ATCC 49989T | 45                  | 3    | 7   | 6   | 5   | 3   | 11  | 2   | 4    |
| *E. nodatum* ATCC 33097T | 41                  | 1    | 1   | 2   | 2   | 2   | 2   | 2   | 8    |
| *E. brachy* ATCC 33089T | 39                  | 3    | 7   | 3   | 5   | 4   | 6   | 2   | 9    |
| *E. limosum* ATCC 8486T | 50                  | 2    | 3   | 4   | 3   | 6   | 6   | 2   | 5    |

* G+C contents were determined by HPLC.
* All values were normalized to 100% for the homologous reactions.
* Data from references 20, 21, and 29.
REFERENCES


