***Cryptobacterium curtum* gen. nov., sp. nov., a new genus of Gram-positive anaerobic rod isolated from human oral cavities**

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Novel *Eubacterium*-like isolates, strains 12-3^T and KV43-B, which were isolated from the periodontal pocket of an adult patient with periodontal disease and necrotic dental pulp, respectively, were studied taxonomically and phylogenetically. The morphological and differential biochemical characteristics of these organisms are also described in this paper. These organisms were Gram-positive, anaerobic, non-spore-forming, rod-shaped bacteria that were inert in most of the conventional biochemical tests and closely resembled members of asaccharolytic oral *Eubacterium* species. On the other hand, protein profiles of whole cells in SDS-PAGE and Western immunoblotting reaction analysis distinguished these isolates from strains of the previously described genus *Eubacterium*. The G+C content of the DNAs from the novel isolates was 50 and 51 mol%, respectively. The levels of DNA–DNA relatedness to other asaccharolytic oral *Eubacterium* species, including *Eubacterium brachy*, *Eubacterium lentum*, *Eubacterium nodatum*, *Eubacterium timidum*, *Eubacterium saphenum*, *Eubacterium minutum* and *Eubacterium exiguum*, was less than 11%. These organisms also exhibited a very low level of reassociation with the DNA of *Eubacterium limosum*, the type species of the genus *Eubacterium*. The results of 16S rDNA sequence comparisons revealed that these organisms represent a novel lineage distinct from all previously described genera of Gram-positive, rod-shaped bacteria. On the basis of our results, it is suggested that strains 12-3^T and KV43-B should be classified in a new genus and species, for which the name *Cryptobacterium curtum* gen. nov., sp. nov. is proposed. The type strain of *Cryptobacterium curtum* is 12-3^T (= ATCC 700683^T).

Keywords: *Cryptobacterium curtum* gen. nov., sp. nov., DNA–DNA relatedness, 16S rDNA sequence, phylogenetic analysis

INTRODUCTION

The genus *Eubacterium* comprises Gram-positive, anaerobic, non-spore-forming, rod-shaped bacteria (Moore & Moore, 1986). Several species of *Eubacterium* have been documented as the aetiological agents of chronic periodontitis (Hill *et al.*, 1987; Holdeman *et al.*, 1980; Martin *et al.*, 1986; Moore *et al.*, 1982, 1983, 1985; Slayne & Wade, 1994; Uematsu & Hoshino,

1992; Wade *et al.*, 1992) based on frequent isolation from diseased periodontal sites. The pathogenic role of these species was indicated by their incidence in cultures of subgingival periodontitis samples (as high as 54%; Tolo & Schenck, 1985) and in the cultivable flora in the subgingival sites (as high as 57%) and their infrequent isolation from adjacent supragingival areas (Hill *et al.*, 1987). In addition, antibody titres against *Eubacterium* species in the serum of patients with periodontitis are higher than those of healthy persons, suggesting that these bacteria cause immunological reactions in periodontal lesions (Gunsolley *et al.*, 1990; Sato *et al.*, 1998; Tew *et al.*, 1985; Tolo & Jorkjend,

The DDBJ/EMBL/GenBank accession number for the 16S rRNA gene sequence of strain 12-3^T is AB019260.

1990; Tolo & Schenck, 1985). Moreover, they have been recognized as pathogens in oral and dental infections, such as advanced caries (Ando & Hoshino, 1990; Edwardsson, 1974; Hoshino, 1985), pulpal infections (Hoshino *et al.*, 1992; Sato *et al.*, 1993) and dentoalveolar abscesses (Wade *et al.*, 1994) and also from non-oral, clinically significant infections, such as head and neck infections, abdominal infections (Brook & Frazier, 1993), bacteraemia associated with malignancy (Fanstein *et al.*, 1989) and genital tract infections associated with intrauterine devices (Brook, 1983).

Historically, the definition of genus *Eubacterium*, on the basis of negative fermentation characteristics, has provided a convenient classification for a collection of diverse organisms. From the nature of the definition, it was inevitable that there was considerable heterogeneity among the species which were placed in this genus and it now contains many species and groups which are phylogenetically unrelated (Cheeseman *et al.*, 1996; Moore & Moore, 1986; Nakazawa & Hoshino, 1994). In recent years, many oral asaccharolytic isolates have been described, some of which qualify as members of the genus *Eubacterium* but could not be assigned to any of the named species (Cheeseman *et al.*, 1996; Poco *et al.*, 1996a, b; Sato *et al.*, 1998).

A recent study in our laboratory has shown that reference strains, including strains 12-3^T and KV43-B, of oral asaccharolytic *Eubacterium* species are distinguished by RFLP analysis of 16S rDNA amplified by PCR, using endonucleases *Hpa*II and *Alu*I (Sato *et al.*, 1998). In this paper, a polyphasic approach was used to characterize these unnamed strains, 12-3^T and KV43-B, which could not be classified as any of the previously established species. We report here the results of conventional biochemical tests including whole-cell protein profile analysis, Western immunoblotting analysis, DNA base composition analysis, DNA-DNA hybridization studies and 16S rDNA sequence comparisons. On the basis of our results and the taxonomic properties of the organisms, we conclude that strains 12-3^T and KV43-B may represent members of a new genus and a new species, for which we propose the name *Cryptobacterium curtum*.

METHODS

Bacterial strains and culture conditions. The bacterial strain 12-3^T was isolated in our laboratory from a human periodontal pocket sample (Sato *et al.*, 1998). Strain KV43-B was isolated from necrotic dental pulp (Sundqvist, 1976). The type strains of previously established asaccharolytic *Eubacterium* species, together with the more recently proposed species *Eubacterium minutum* (Poco *et al.*, 1996a) and *Eubacterium exiguum* (Poco *et al.*, 1996b), were included in this study. *Eubacterium infirmum* and *Eubacterium tardum* (Cheeseman *et al.*, 1996) were not examined in this work but are discussed. All of the strains were cultured on brain-heart infusion (BHI)-blood agar plates for 7–10 d under strictly anaerobic conditions in an anaerobic glove box (model AZ-Hard; Hirasawa) containing 80% N₂, 10%

H₂ and 10% CO₂. The bacterial cells were harvested by centrifugation, washed with PBS (136 mM NaCl, 10 mM sodium phosphate, pH 7.2), and stored at –20 °C until use. To ensure strictly anaerobic conditions in the box, the reduction of methyl viologen (at –446 mV) was carefully checked when 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 tested by the methods described previously (Holdeman *et al.*, 1977). End products produced in peptone-yeast extract-glucose broth (PYG) were assayed by GC as described previously (Hoshino & Sato, 1986).

Electron microscopy. Transmission electron microscopy was used for cell wall characterization. Cultures (48 h) were fixed in a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 h at 4 °C. The cells were centrifuged and washed three times with the same buffer. They were post-fixed with 1% osmic acid for 18 h at 4 °C and dehydrated for 10 min each with 50, 75, 90, 95 and 100% ethanol. Preparations were washed with 33.3, 66.6 and 100% epoxy resin in propylene oxide for 60 min and then embedded in 100% epoxy resin. After hardening, ultrathin sections were cut with a diamond knife, and then stained with uranyl acetate and lead citrate. The thin sections were studied with a Hitachi H-600A electron microscope.

Whole-cell protein profiles. Cellular protein profiles were examined by SDS-PAGE analysis as described previously (Laemmli, 1970). Approximately 50 mg (wet wt) whole cells were washed three times in 1 ml distilled water and suspended in 150 µl 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 suspensions were boiled for 10 min and then centrifuged at 12000 g for 10 min. The protein concentrations of the supernatants were measured by a protein assay kit (Bio-Rad). The supernatants (10 µg of each) were subjected to electrophoresis on a 12.5% polyacrylamide gel by using a model KS-8010 Micro slab gel electrophoresis system (Mariesol). After electrophoresis, the gel was stained with Coomassie brilliant blue R-250.

Western immunoblotting analysis. Proteins were transferred from SDS-PAGE gels to a nitrocellulose membrane (pore size, 0.45 µm; Bio-Rad) by using the transfer buffer system described previously (Burnette, 1981), in conjunction with the transblot system (Mariesol) at a constant current of 350 mA with cooling for 4 h. Membranes were processed with slight modification as described previously (Nakazawa & Hoshino, 1993). Briefly, an immune rabbit antiserum (1:1000) was used as first antibody followed by incubation with goat anti-rabbit immunoglobulin G conjugated with peroxidase (1:1000) as the second antibody. The colour was then developed.

DNA isolation and base composition analysis. DNA was isolated and purified following a modification of the Marmur protocol (Marmur, 1961; Nakazawa & Hoshino, 1994). The G+C content of DNA was determined by HPLC, as described previously (Katayama-Fujimura *et al.*, 1984).

DNA-DNA hybridization analysis. DNA-DNA hybridization was performed by the membrane filter method as described previously (Meyer & Schleifer, 1978). Briefly, reference DNA was labelled by using a multiprimer DNA labelling kit (Amersham) and [α -³²P]dCTP, and then purified with a Nick column (Pharmacia LKB). About 40 µg unlabelled

single-stranded DNA immobilized on each nitrocellulose membrane filter and 0.015 µg labelled reference DNA were reassociated in a solution containing 0.08 % SDS, 0.02 % polyvinylpyrrolidone, 0.02 % Ficoll 400, BSA (Fraction V; Sigma) and 1 ml 6 × SSC (1 × 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.

Sequencing of the 16S rRNA gene and phylogenetic analysis.

The 16S rRNA gene was amplified by PCR with a nucleotide primer set [5'-AGA GTT TGA TCM TGG CTC AG-3', located at positions 8–27 (*Escherichia coli* numbering) and 3'-TTC AGC ATT GTT CCA TYG GCAT-5', located at positions 1492–1513 (*E. coli* numbering)]. The PCR reaction was performed with Premix Taq (Takara) according to the manufacturer's instructions (Fukunaga *et al.*, 1995) in a thermal controller (model PTC-100; MJ Research). The DNA fragment obtained by PCR amplification was sequenced by using a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham) and a series of custom-synthesized primers with ALFexpress DNA sequencer (Pharmacia LKB) according to the manufacturer's instructions. The sequences were aligned with each other and connected by using SEQMAN II of the LASERGENE program (DNASTAR). Programs MEGALIGN of LASERGENE, CLUSTAL W and NJPLOT were used to compare sequences and to construct an evolutionary tree by the neighbour-joining method. Also, confidence intervals were assessed by NJPLOT and CLUSTAL W with bootstrap analysis.

Nucleotide sequence accession numbers. The accession numbers for the reference sequences used in this study are shown in Fig. 3.

RESULTS AND DISCUSSION

Morphological and biochemical characteristics

Strains 12-3^T and KV43-B were non-spore-forming, non-motile, strictly anaerobic, very short rod-shaped (0.4 by 0.8–1.0 µm) organisms. Ultrathin sections showed a single-layered cell wall of about 10 nm in thickness (Fig. 1), which is typical of Gram-positive bacteria. The cell wall was tightly fitted to the cytoplasmic membrane. The cells divided after septum formation that was initiated peripherally. Pili- or flagella-like structures were not found. The growing cells were Gram-positive, but sometimes cells in older cultures were Gram-negative. Growth in broth media was very poor with or without carbohydrates. These organisms formed tiny colonies on BHI-blood agar plates of about 0.3–0.5 mm in diameter that were circular, convex and translucent. Even after prolonged incubation, the colonies were less than 1 mm in diameter, which is typical of oral asaccharolytic *Eubacterium* species. No haemolysis occurred around colonies on BHI-blood agar plates. Strains 12-3^T and KV43-B were asaccharolytic because they did not ferment glucose and other carbohydrates. They were positive for arginine hydrolysis and inert in most of the conventional tests (Table 1). Nitrate reduction was negative and they did not hydrolyse aesculin, starch or

Fig. 1. Transmission electron microphotograph of a thin section of cell of *Cryptobacterium curtum* 12-3^T (= ATCC 700863^T) showing the Gram-positive cell wall. Bar, 200 nm. Cell wall (CW), cytoplasmic membrane (CM) and invagination of cytoplasmic membrane before cell divisions (IVCM) are indicated.

gelatin. In addition, they did not produce indole, ammonia, urease or catalase. When assayed by GC, these strains did not produce any metabolic end product in PYG medium. Among the previously described oral asaccharolytic *Eubacterium* species, they phenotypically resembled *E. exiguum* and *Eubacterium lentum*, which also did not produce any end product (Poco *et al.*, 1996a, b). They are phenotypically distinct from the acetate- and butyrate-producing asaccharolytic *Eubacterium* species, such as *Eubacterium nodatum* (Moore & Moore, 1986), *Eubacterium saphenum* (Uematsu *et al.*, 1993), *E. minutum* (Poco *et al.*, 1996b), *E. tardum* and *E. infirmum* (Cheeseman *et al.*, 1996).

Whole-cell protein profiles and Western immunoblotting analysis

The protein profiles of the two strains, which were produced by SDS-PAGE analysis, had major bands in the region of 80, 50 and 45 kDa (Fig. 2). By visual analysis, the oral asaccharolytic *Eubacterium* species each showed typical profiles and no major bands were common indicating great heterogeneity in polypeptide components. Western blotting reactions (Table 2) with rabbit antisera revealed that antigens of strain 12-3^T did not react with the antisera raised against the reference oral asaccharolytic species, whereas antigens from the previously described species were recognized by their respective antisera.

Table 1. Comparison of biochemical characteristics and enzymic profiles

None of the strains fermented glucose.

Taxon	End product*	Arginine hydrolysis	Nitrate reduction
<i>Cryptobacterium curtum</i>	—	+	—
<i>Eubacterium lentum</i>	—	+	+
<i>Eubacterium timidum</i>	phe-a	—	—
<i>Eubacterium exiguum</i>	—	+	—
<i>Eubacterium minutum</i>	B or b	—	—
<i>Eubacterium nodatum</i>	a, B	+	—
<i>Eubacterium saphenum</i>	a, b	—	—
<i>Eubacterium brachy</i>	ib, ic, iv, phe-p	—	—

*End product when grown on PYG medium. a, Acetate; b, butyrate; ib, isobutyrate; ic, isocaproate; iv, isovalerate; phe-a, phenylacetate; phe-p, phenylpropionate. Upper-case letters represent an amount of product equal to or more than 10 mM and lower-case letters represent less than 10 mM.

Fig. 2. Protein profiles of *E. exiguum* ATCC 700122^T (lane 1), *E. lentum* ATCC 25559^T (lane 2), *E. brachy* ATCC 33089^T (lane 3), *E. nodatum* ATCC 33099^T (lane 4), *E. timidum* ATCC 33093^T (lane 5), *E. minutum* ATCC 700079^T (lane 6), *Cryptobacterium curtum* 12-3^T (= ATCC 700863^T, lane 7), *E. saphenum* ATCC 49989^T (lane 8) and molecular mass markers (lane 9; in kDa) on a 12.5% SDS-polyacrylamide gel.

The present study clearly showed that strains 12-3^T and KV43-B could be distinguished from the reference species by their protein components, including the structural proteins of the whole bacterial cells, and immunological reactions. These data are consistent with the previously published data of Nakazawa & Hoshino (1993).

DNA base composition

As previously reported (Nakazawa & Hoshino, 1993, 1994; Poco *et al.*, 1996a, b), the G + C contents of oral asaccharolytic *Eubacterium* species range from 38 to 62 mol%, as determined by HPLC (Table 3). The G + C content of strains 12-3^T and KV43-B were 50 and 51 mol%, respectively, which are at the same level as *Eubacterium timidum* or *Eubacterium limosum* (both 50 mol%), the latter of which is the type species of the genus *Eubacterium*.

DNA-DNA hybridization analysis

The DNA-DNA hybridization experiments as measured by the membrane filter method are listed in Table 3. The DNA of strain 12-3^T exhibited a high level of reassociation with the DNA of strain KV43-B (97%), suggesting that they are related genetically. Moreover, the DNAs of the other *Eubacterium* species, including the type species of the genus *Eubacterium*, *E. limosum*, showed very low levels of reassociation with the DNAs of strains 12-3^T and KV43-B (1–11%). Also, the DNAs of strains 12-3^T and KV43-B exhibited low levels (1–2%) of DNA relatedness to two previously unnamed asaccharolytic *Eubacterium*-like strains D2-18 and D5-2 (data not shown). These DNA-DNA hybridization data clearly showed that levels of homology among these strains were low indicating that all the species examined were not closely related, further confirming the data of Nakazawa & Hoshino (1994) and Sato *et al.* (1998).

16S rRNA gene sequence and phylogenetic analysis

The 16S rRNA gene sequence of strain 12-3^T (1429 bases in length) was determined in order to establish its precise phylogenetic affiliation. The 16S rRNA gene

Table 2. Western blotting reactions of *Cryptobacterium curtum* and other oral asaccharolytic *Eubacterium* taxa

Species: 1, *Eubacterium timidum*; 2, *Eubacterium minutum*; 3, *Eubacterium brachy*; 4, *Eubacterium lentum*; 5, *Eubacterium nodatum*; 6, *Eubacterium saphenum*. +, Positive; —, negative; w, weakly positive.

Antigen	Reaction with antisera against species:					
	1	2	3	4	5	6
<i>Cryptobacterium curtum</i> 12-3 ^T	—	—	—	—	—	—
<i>Eubacterium timidum</i> ATCC 33093 ^T	+	—	—	—	—	—
<i>Eubacterium minutum</i> ATCC 700079 ^T	—	+	—	—	—	—
<i>Eubacterium brachy</i> ATCC 33089 ^T	—	—	+	—	—	—
<i>Eubacterium lentum</i> ATCC 25559 ^T	—	—	w	+	—	—
<i>Eubacterium nodatum</i> ATCC 33099 ^T	—	—	—	—	+	—
<i>Eubacterium saphenum</i> ATCC 49989 ^T	—	—	—	—	—	+
<i>Eubacterium exiguum</i> ATCC 700122 ^T	—	—	—	w	w	—

Table 3. DNA base compositions and levels of DNA relatedness to oral asaccharolytic *Eubacterium* species

Source of DNA	G + C (mol%)*	Homology (%) with labelled DNA from:†	
		12-3 ^T	KV43-B
<i>Cryptobacterium curtum</i> 12-3 ^T	51	100	
<i>Cryptobacterium curtum</i> KV43-B	50	97	100
<i>Eubacterium exiguum</i> ATCC 700122 ^T	60	6	11
<i>Eubacterium minutum</i> ATCC 700079 ^T	38–40	2	4
<i>Eubacterium timidum</i> ATCC 33893 ^T	50	2	5
<i>Eubacterium nodatum</i> ATCC 33099 ^T	41	1	2
<i>Eubacterium lentum</i> ATCC 25559 ^T	62	5	9
<i>Eubacterium brachy</i> ATCC 33089 ^T	39	1	4
<i>Eubacterium saphenum</i> ATCC 49989 ^T	45	2	4
<i>Eubacterium limosum</i> ATCC 8486 ^T	50	2	4

* G + C contents were determined by HPLC.

† All values were normalized to 100 % for the homologous reactions.

sequence of *E. minutum* ATCC 700079^T (1456 bases in length) was also aligned for comparison together with other *Eubacterium* species. The sequences were compared with those of other asaccharolytic *Eubacterium* species, including *Eubacterium brachy*, *E. nodatum*, *E. timidum*, *E. saphenum*, *E. tardum* and *E. infirmum*, and other related Gram-positive bacteria selected from the GenBank database by the sequence similarity search using the BLAST and FASTA algorithms. A database search revealed that strain 12-3^T belongs to the actinomycetes of the Gram-positive bacteria and its 16S rRNA sequence may be most similar to that of members of the family *Actinomycetaceae* in the order *Actinomycetales*. On the basis of the sequence similarity matrix data, an evolutionary tree was constructed by using the MEGALIGN program (Fig. 3).

According to this tree, strains 12-3^T and KV43-B could be distinguished from these asaccharolytic *Eubacterium* species. These phylogenetic data completely agree with previous results which are obtained by RFLP analysis of 16S rDNA from asaccharolytic *Eubacterium* species (Sato *et al.*, 1998). Our analysis also indicates that the novel strain belongs to a large group of the Actinomycetes, although it does not belong to any taxa. Strain 12-3^T formed a cluster with *Coriobacterium glomerans* and *Denitrobacterium detoxificans*. However, it showed distinct lines exhibiting specific phylogenetic association with *C. glomerans* (86.6 % 16S rRNA sequence similarity; 14.8 % sequence divergence) or *D. detoxificans* (88.5 % 16S rRNA sequence similarity; 12.6 % sequence divergence). Bootstrap resampling values (100 and 92 %)

Fig. 3. Evolutionary tree based on 16S rRNA gene sequence comparison showing the phylogenetic position of *Cryptobacterium curtum* 12-3^T (= ATCC 700863^T). The dendrogram was created by using the neighbour-joining method. The numbers on the tree indicate bootstrap values (%) calculated from 1000 replications. The nucleotide sequence accession numbers are in parentheses.

demonstrated these associations to be statistically significant.

From the 16S rRNA gene sequence comparisons, it is evident that the bacterium has a close phylogenetic relationship with *D. detoxificans*. Although the association between these taxa is statistically significant, a sequence divergence of > 10% suggests two phylogenetically closely related but nevertheless different genera. Also, it was shown that strain 12-3^T had no close phylogenetic affinity with any of the other reference species examined (sequence similarities < 84%; sequence divergence values > 17%; individual data not shown). Our phylogenetic analysis based on comparisons of 16S rRNA gene sequences indicates that strains 12-3^T and KV43-B are clearly distinct not only from *E. limosum*, the type species of the genus

Eubacterium, but also from the other taxa established previously, which is consistent with the creation of a new genus.

It is noteworthy that all asaccharolytic *Eubacterium* species examined in this study, i.e. *E. timidum*, *E. infirmum*, *E. saphenum*, *E. nodatum*, *E. tardum*, *E. minutum* and *E. brachy*, showed no close phylogenetic affinity with *E. limosum* in the evolutionary tree. These results indicate that the genus *Eubacterium* may be incoherent and that these oral asaccharolytic *Eubacterium* species may need reassignment to different genera, as suggested by several authors (Cheeseman *et al.*, 1996; Nakazawa & Hoshino, 1993, 1994; Wade *et al.*, 1994). At present, we have no information on the distribution, incidence and virulence of the novel species.

On the basis of morphological and biochemical characteristics, SDS-PAGE and Western immunoblotting analysis, G+C content, DNA-DNA hybridization and 16S rRNA gene sequence data, we believe that this bacterium merits classification as a new genus, for which the name *Cryptobacterium curtum* gen. nov., sp. nov. is proposed. The taxonomic description below summarizes the properties of the species.

Description of *Cryptobacterium* gen. nov.

Cryptobacterium (Crypt.o.bac.te'ri.um. Gr. n. *kryptos* hidden; Gr. n. *bakterion* a small rod; M.L. neut. n. *Cryptobacterium* a hidden rod-shaped bacterium).

Cells are short Gram-positive rods. Occasionally Gram-variable in stationary phase. Obligately anaerobic, non-motile and non-sporing. Catalase-negative. Asaccharolytic and no volatile end products in PYG. The DNA G+C content is 50–51 mol%. According to the almost complete 16S rDNA sequence, the genus groups with the general cluster of actinomycetes of Gram-positive bacteria. Type species is *Cryptobacterium curtum*.

Description of *Cryptobacterium curtum* sp. nov.

Cryptobacterium curtum (cur'tum. L. neut. adj. *curtum* shortened, a shortened cell of this organism).

This description is based on the study of two strains isolated from oral cavities. Cells are very short, Gram-positive rods, obligately anaerobic, non-motile and non-sporing. Individual cells occur singly or in masses. Older cultures sometimes stain Gram-negative. On BHI-blood agar plates, minute colonies less than 1 mm in diameter are formed that are circular, convex and translucent even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor with or without carbohydrates. No haemolysis occurs on BHI-blood agar plates. The cells are inert in most biochemical tests. Starch and aesculin are not hydrolysed; nitrate is not reduced. No liquefaction of gelatin occurs. Indole, urease and catalase are also negative. Ammonia is produced from arginine. The strains are non-fermentative and do not utilize adonitol, amygdalin, arabinose, cellobiose, erythritol, aesculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose or xylose. No metabolic end product is detected in peptone-yeast extract medium supplemented with glucose or PYG broth. Isolated from the periodontal pocket of a adult human with periodontal disease. The DNA G+C content is 50–51 mol%. The type strain is 12-3^T (= ATCC 700863^T).

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