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Reclassification of *Bacteroides forsythus* (Tanner *et al.* 1986) as *Tannerella forsythensis* corrig., gen. nov., comb. nov.

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The characteristics of the fusiform species *Bacteroides forsythus*, isolated from human periodontal pockets, were examined. 16S rDNA sequence analysis confirmed that *B. forsythus* was not a species within the genus *Bacteroides sensu stricto.* Although *B. forsythus* was phylogenetically related to *Bacteroides distasonis* and *Bacteroides merdae* in the phylogenetic tree, the ratio of anteiso-15:0 to iso-15:0 in whole-cell methanolysates of *B. forsythus* was different from those of *B. distasonis*, *B. merdae* and other *Bacteroides* species. *B. forsythus* did not grow on medium containing 20% bile, but members of the *Bacteroides fragilis* group did. *B. forsythus* was the only species tested that was trypsin-positive in API ZYM tests. The dehydrogenase enzyme pattern was of no use for the differentiation of *B. forsythus* and the *B. fragilis* group. On the basis of these data, a new genus, *Tannerella*, is proposed for *Bacteroides forsythus*, with one species, *Tannerella forsythensis* corrig., gen. nov., comb. nov. The type strain of *Tannerella forsythensis* is JCM 10827^T (= ATCC 43037^T).

Keywords: *Tannerella forsythensis* gen. nov., comb. nov., *Bacteroides forsythus*, 16S rDNA sequence analysis, cellular fatty acid, bile

INTRODUCTION

Bacteroides forsythus, a fusiform, fastidious, anaerobic, Gram-negative organism, was isolated from deep periodontal pockets of humans (Tanner *et al.*, 1979) and was proposed by Tanner *et al.* (1986) as a novel species in the genus *Bacteroides*. In the past, because of poor definition of the genus, more than 50 species of *Bacteroides* have been included in *Bergey's Manual of Systematic Bacteriology* (Holdeman *et al.*, 1984) and the Approved Lists of Bacterial Names (Moore *et al.*, 1985). Later, Shah & Collins (1989) formally proposed that the genus *Bacteroides* be restricted to *Bacteroides fragilis* and related taxa, and the description of the genus *Bacteroides* was emended accordingly. Consequently, *B. forsythus* is regarded as an outmember of the genus *Bacteroides* and the generic position of this organism is uncertain. The 16S rRNA sequence analysis by Paster *et al.* (1994) led to the suggestion that *B. forsythus* was not a species within the genus *Bacteroides*. *B. forsythus* was related to members of the first subcluster of the *Porphyromonas* cluster, with a mean sequence similarity of about 86%. However, because of this phylogenetic depth, it is uncertain whether *B. forsythus* should be considered a species of *Porphyromonas* (Paster *et al.*, 1994; Tanner *et al.*, 1994). Paster *et al.* (1994) have already suggested that other criteria will have to be used to determine the taxonomic status of this species.

In this study, we attempted to determine the taxonomic status of *B. forsythus*. Based on the results presented, we propose that this organism should be classified as *Tannerella forsythensis* corrig., gen. nov., comb. nov.

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Abbreviations: G6PDH, glucose-6-phosphate dehydrogenase; GDH, glutamate dehydrogenase; MDH, malate dehydrogenase; NAM, *N*-acetylmuramic acid; 6PGDH, 6-phosphogluconate dehydrogenase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of the following *Tannerella forsythensis* strains are AB053938 (strain FJ1), AB053939 (G9), AB053940 (HA3), AB053941 (HG3), AB053942 (KM3), AB053943 (Ko3), AB053944 (KS16), AB053945 (L7), AB053946 (Sai5) and AB053947 (TR6).

METHODS

Bacterial strains and growth conditions. The strains used in this study are listed in Table 1. These organisms were maintained on Trypticase soy agar plates supplemented with 5% (v/v) sheep blood (TSBA; Becton Dickinson) at 37 °C in an atmosphere containing 10% (v/v) H_2, 10% (v/v) CO_2 and 80% (v/v) N_2. *B. forsythus* strains were also isolated from subgingival plaque samples from patients with various forms of periodontal disease. These strains were maintained on TSBA plates on which a piece of filter disk (Braham & Moncla, 1992) containing *N*-acetylmuramic acid (NAM) was placed. *B. forsythus* requires exogenous NAM as a growth factor (Wyss, 1989). Bacteroides bile aesculin agar (Shah, 1992) was used to check whether *B. forsythus* is a member of the '*B. fragilis* group'.

Cellular fatty acids. Fatty acid methyl esters (FAMEs) were obtained from about 40 mg wet cells by saponification, methylation and extraction using minor modifications (Kuykendall *et al.*, 1988) of the method of Miller (1982). Cellular fatty acid profiles were determined by the MIDI microbial identification system (Microbial ID). Peaks were automatically integrated, fatty acids were identified by equivalent chain-length (ECL) and percentages of the total peak area were calculated. External calibration was done by using MIDI calibration mixture 1 (FAMEs of straight-chain saturated fatty acids from 9 to 20 carbons in length and five hydroxy acids).

Isoprenoid quinones. Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and were analysed by HPLC with a Cosmosil $5C_{18}$ column (4·6 × 150 mm; Nacalai Tesque). The elution solvent was a mixture of methanol and isopropyl ether (4:1, v/v).

Enzyme assays. Cells were suspended in 1 ml Tris/HCl (pH 7·8), lysed by sonication at 4 °C and centrifuged at 17000 ***g*** for 20 min at 4 °C to remove cellular debris. Protein was measured by the dye-binding method (Bradford, 1976) using BSA as a standard. Glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) activities were determined spectrophotometrically as described previously (Gharbia & Shah, 1991; Bailey & Love, 1995).

API enzyme substrate tests. API ZYM and API An-Ident enzymic substrate tests were performed in duplicate as recommended by the manufacturer (bioMérieux). Cells from agar plates were suspended in sterile water, inoculated onto the test strips and incubated in air for 4 h at 37 °C.

16S rDNA sequence analysis. The 16S rDNA genes of *B. forsythus* strains were amplified by PCR with universal primers 27F and 1492R (Lane, 1991). Amplification was carried out with a DNA thermal cycler (TaKaRa PCR Thermal Cycler MP; Takara Shuzo) according to the following program: 95 °C for 3 min, followed by 30 cycles consisting of 95 °C for 0·5 min, 60 °C for 0·5 min and 72 °C for 1·5 min, with a final extension period at 72 °C for 10 min. The amplified 16S rDNA genes were purified by using an UltraClean PCR Clean-up DNA purification kit (MO BIO Laboratories). Cycle-sequencing reactions were performed using an ABI PRISM BigDye Terminator Cycle Sequencing ready reaction kit (Applied Biosystems). An ABI PRISM 310 Genetic Analyzer (Applied Biosystems) was used for sequence determination. Previously determined 16S rRNA/rDNA sequences used for comparisons in this study were retrieved from the DDBJ, EMBL and GenBank nucleotide sequence databases. Sequence data were aligned with CLUSTAL W (Thompson *et al.*, 1994) and corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980) after gaps and unknown bases were eliminated. The phylogenetic tree was constructed by

Table 1. Strains used in this study

Strain	16S rDNA accession no.	Source
Tannerella forsythensis JCM 10827^T	L16495	Periodontal pocket
Tannerella forsythensis FJ1	AB053938	Periodontal pocket
Tannerella forsythensis G9	AB053939	Periodontal pocket
Tannerella forsythensis HA3	AB053940	Periodontal pocket
Tannerella forsythensis HG3	AB053941	Periodontal pocket
Tannerella forsythensis KM3	AB053942	Periodontal pocket
Tannerella forsythensis Ko3	AB053943	Periodontal pocket
Tannerella forsythensis KS16	AB053944	Periodontal pocket
Tannerella forsythensis L7	AB053945	Periodontal pocket
Tannerella forsythensis Sai5	AB053946	Periodontal pocket
Tannerella forsythensis TR6	AB053947	Periodontal pocket
Bacteroides distasonis JCM 5825^T	M86695	Human faeces
Bacteroides merdae JCM 9497^T	X83954	Human faeces
Bacteroides acidifaciens JCM 10556^T	AB021164	Mouse caecum
Bacteroides caccae JCM 9498^T	X83951	Human faeces
Bacteroides ovatus JCM 5824^T	L16484	Human faeces
Bacteroides stercoris JCM 9496^T	X83953	Human faeces
Bacteroides thetaiotaomicron JCM 5827^T	L16489	Human faeces
Bacteroides uniformis JCM 5828^T	L16486	Human faeces
Bacteroides vulgatus JCM 5826^T	M58762	Human faeces

the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of tree topologies.

RESULTS AND DISCUSSION

Morphological and physiological characteristics

All strains of *B. forsythus* were Gram-negative fusiforms. Colonies were pale speckled-pink, circular, entire and slightly convex on TSBA plates with an NAM disk. *B. forsythus* strains and the other *Bacteroides* species tested hydrolysed aesculin. The growth of *B. forsythus* strains was inhibited on Bacteroides bile aesculin agar. On the other hand, growth of the other *Bacteroides* species tested was not inhibited on medium containing 20% bile. In addition, *B. forsythus* strains required NAM for growth, whereas other strains tested did not. Wyss (1989) has reported that *B. forsythus* requires exogenous NAM as a growth factor. These characteristics are very important features that differentiate *B. forsythus* from the *B. fragilis* group. Braham & Moncla (1992) reported that *B. forsythus* strains isolated from subgingival plaque samples from monkeys (*Macaca fascicularis*) also required NAM for growth. However, *B. forsythus* strains recovered from cat and dog bite-wound infections in humans did not require NAM for growth (Hudspeth *et al.*, 1999). These results suggest that there are host-specific biotypes within the species *B. forsythus*.

Cellular fatty acid compositions

The cellular fatty acid composition of *Bacteroides* species has been determined (Mayberry *et al.*, 1982; Miyagawa *et al.*, 1979; Shah & Collins, 1980) and reviewed for the classification of the genus *Bacteroides* (Shah & Collins, 1983). In this study, the cellular fatty acid compositions of *B. forsythus* strains were similar to those of other *Bacteroides* species except for the ratio of anteiso-15:0 to iso-15:0 (Table 2). *B. forsythus* strains contain predominantly anteiso-15:0 and 3-OH-16:0 as their long-chain fatty acids, while other *Bacteroides* species possess significant levels of anteiso-15:0 and iso-3-OH-17:0. The ratio of anteiso-15:0 to

Table 2. Cellular fatty acid compositions of *Tannerella forsythensis* and related species

Values are percentages of total fatty acids. Strains are identified as: 1–11, *Tannerella forsythensis* strains JCM 10827^T (1), FJ1 (2), G9 (3), HA3 (4), HG3 (5), KM3 (6), Ko3 (7), KS16 (8), L7 (9), Sai5 (10) and TR6 (11); 12, *Bacteroides distasonis* JCM 5825^T; 13, *Bacteroides merdae* JCM 9497^T; 14, *Bacteroides acidifaciens* JCM 10556^T; 15, *Bacteroides caccae* JCM 9498^T; 16, *Bacteroides ovatus* JCM 5824^T; 17, *Bacteroides stercoris* JCM 9496^T; 18, *Bacteroides thetaiotaomicron* JCM 5827^T; 19, *Bacteroides uniformis* JCM 5828^T; 20, *Bacteroides vulgatus* JCM 5826^T; 21, *Bacteroides fragilis* NCTC 9343^T (data from Miyagawa *et al.*, 1979). tr, Trace amount (< 0·5%).

Fatty acid by chemical class	1	2	3	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Saturated straight-chain																			
14:0	1·5	0·6	1·0	0·7	1·2	1·6	1·3	1·0	1·2	3·7	0·8	2·5	1·3	6·4		2·6	4·6	9·8	
15:0			tr			tr			0·7	1·7	0·6		tr	1·4		0·8		2·4	2·0
16:0	9·1	4·6	5·4	5·7	9·1	4·6	5·9	5·8	9·4	10·4	3·9	10·4	8·5	14·0	5·7	8·5	12·8	10·8	14·6
17:0			tr	tr															
18:0	3·3	1·2	1·2	2·0	1·2	1·4	1·8	1·8	3·3		1·1	2·0	1·1	0·9	1·4	1·0		1·6	
Unsaturated straight-chain																			
16:1ω9*c*											0·5								
18:2ω9,12*c*		0·7	0·7	1·2	1·0	0·9	0·7	1·1	1·2	2·9	1·8	2·4	1·0	1·9	1·2	1·4	2·9	2·7	
18:1ω9*c*	2·7	2·2	1·6	2·2	1·9	1·4	1·3	1·9	2·1	8·1	6·5	5·8	2·2	7·0	3·3	5·9	7·4	10·6	
18:1ω11*t*		tr	tr	tr															
Hydroxy acids																			
3-OH-14:0		tr	tr	tr		tr	tr	tr	0·7					0·7					
iso-3-OH-15:0	1·1	tr	0·6	tr	0·5	0·5	0·7	tr	1·0		0·8		tr		0·7	0·6			
2-OH-15:0			tr						tr										
iso-3-OH-16:0			0·7	0·8					0·9		tr		0·7			1·2		1·1	
3-OH-16:0	15·8	17·5	14·4	15·0	15·5	19·0	15·3	20·6	18·4	7·5	5·6	6·1	7·0	10·4	4·0	7·9	12·5	15·9	8·0
iso-3-OH-17:0	7·7	6·4	7·4	17·7	12·8	7·0	9·2	9·7	7·7	19·2	23·5	14·5	18·5	10·1	27·3	16·5	18·6	13·1	18·1
anteiso-3-OH-17:0	4·2	7·8	3·7	5·7	3·1	4·2	5·2	5·1	3·1	2·1	5·6	3·0	3·4	2·4	2·8	4·2	1·7	2·5	
3-OH-17:0			tr	tr							tr								
Saturated branched-chain																			
iso-13:0			tr	tr		tr					tr		tr	0·5	0·6	tr			
anteiso-13:0													tr	0·7					
iso-14:0			tr	tr	tr						tr		1·0	0·9		1·1		1·4	
iso-15:0	1·7	0·6	1·4	1·7	1·8	1·5	1·7	1·5	2·1	8·8	6·4	13·5	10·6	7·1	12·7	9·7	12·8	4·4	10·5
anteiso-15:0	53·0	57·1	57·6	43·5	50·1	56·5	56·4	49·5	47·8	35·8	40·1	39·9	38·9	35·1	30·9	37·0	26·8	23·8	41·5
iso-16:0			tr	tr	tr								1·2	0·5	1·0	0·7			0·7
iso-17:0													0·7		3·8				
anteiso-17:0		tr	tr	tr	tr			tr			0·7		1·8		4·7	0·5			3·5
Unsaturated branched chains																			
anteiso-13:1			tr					tr											
anteiso-17:1			1·0	tr	tr	tr	tr	tr											
Ratio of anteiso-15:0/iso-15:0	31·2	95·2	41·1	25·6	27·8	37·7	33·2	33·0	22·8	4·1	6·3	3·0	3·7	4·9	2·4	3·8	2·1	5·4	4·0

Table 3. Menaquinone compositions of *Tannerella forsythensis* and related species

Values are percentages of total menaquinones.

Strain	MK-9	MK-10	MK-11	MK-12
Tannerella forsythensis JCM 10827^T	13	48	33	6
Bacteroides distasonis JCM 5825^T	10	75	9	
Bacteroides merdae JCM 9497^T	45	45		
Bacteroides acidifaciens JCM 10556^T		47	50	
Bacteroides caccae JCM 9498^T		48	51	
Bacteroides ovatus JCM 5824^T		49	43	
Bacteroides stercoris JCM 9496^T	2	66	29	
Bacteroides thetaiotaomicron JCM 5827^T		49	48	
Bacteroides uniformis JCM 5828^T		48	49	
Bacteroides vulgatus JCM 5826^T	6	80	7	

iso-15:0 in whole-cell methanolysates of *B. forsythus* was very much higher than that of other *Bacteroides* species. The ratios of anteiso-15:0 to iso-15:0 ranged from 22·8 to 95·2 % in *B. forsythus* strains and from 2·1 to 6·3 % in the other *Bacteroides* species. This finding is in accord with the report of Brondz & Carlsson (1990). In addition, although *B. forsythus* strains contain predominantly anteiso-15:0 as their long-chain fatty acid, *Porphyromonas* species have mainly iso-15:0 (Brondz *et al.*, 1989; Shah & Collins, 1980, 1983). These data support the conclusion that *B. forsythus* should not be a member of either of the genera *Bacteroides* or *Porphyromonas*.

Menaquinone analysis

The menaquinone compositions of the strains studied are shown in Table 3. The major menaquinones of *B. forsythus* JCM 10827^T and the other *Bacteroides* species tested were MK-10 and MK-11 (except for *B. merdae* JCM 9497^T, which lacked MK-11 and had elevated MK-9). This result supports results reported in previous papers (Shah & Collins, 1980, 1983). In this study, only *B. forsythus* JCM 10827^T possessed a menaquinone with MK-12 (6 %); the *B. fragilis* group did not. The reference strains of *B. fragilis* and *Bacteroides thetaiotaomicron*, however, contained smaller amounts of MK-12 (Shah & Collins, 1980).

Enzymes

The dehydrogenase enzyme pattern is an important criterion that differentiates the genus *Bacteroides sensu stricto* from other Gram-negative, anaerobic, non-spore-forming rods (Shah & Collins, 1989; Shah, 1992). *B. fragilis* and related species possess enzymes of the hexose monophosphate shunt/pentose phosphate pathway such as G6PDH, 6PGDH, MDH and GDH. Shah & Collins (1988, 1990) have proposed new genera, *Porphyromonas* and *Prevotella*, on the basis of the absence of enzymes G6PDH and 6PGDH. In this study, the type strain of *B. forsythus* (JCM 10827^T) and three other strains of this organisms (FJ1, G9 and KM3) had all of the above enzymes. In addition, all enzymes were detected in the other *Bacteroides* species tested. Consequently, the dehydrogenase enzyme pattern is of no use for differentiation of *B. forsythus* and the *B. fragilis* group. Bailey & Love (1995) have dealt with the usefulness of G6PDH and 6PGDH activities as key determinants of species identity in the genera *Bacteroides*, *Prevotella* and *Porphyromonas* as currently defined.

API ZYM and API An-Ident reactions

The API ZYM and API An-Ident systems have been reported to be useful in the identification of oral and non-oral Gram-negative bacteria (Laughon *et al.*, 1982; Slots, 1981; Tanner *et al.*, 1985). The biochemical characteristics of *B. forsythus* strains and the other *Bacteroides* species tested are shown in Table 4. Eleven strains of *B. forsythus* (including the type strain) were tested with API ZYM and API An-Ident. *B. forsythus* was the only species that gave a positive result for trypsin. Tanner *et al.* (1985) reported that *B. forsythus* was the only species tested that was trypsin-positive in API ZYM tests. Trypsin activity has been detected in *Porphyromonas gingivalis* (formerly *Bacteroides gingivalis*) and *Treponema denticola* (Laughon *et al.*, 1982; Slots, 1981). The presence of trypsin activity is regarded as an important factor for tissue destruction in periodontal disease. In this study, *B. forsythus* strains (human oral isolates) were catalase-negative, whereas *B. forsythus* strains recovered from cat and dog bite wounds in humans were catalase-positive (Hudspeth *et al.*, 1999). In addition, human oral isolates of *B. forsythus* were negative for indole production, whereas the monkey oral isolates and bite-wound isolates were positive for indole production (Braham & Moncla, 1992; Hudspeth *et al.*, 1999). 16S rDNA sequence analysis indicated that the three bite-wound isolates had 99·3 % similarity to each other and 98·9 and 99·22 % similarity, respectively, to the human strain *B. forsythus* ATCC 43037^T and monkey oral

Table 4. API ZYM and API An-Ident test results

Reactions are scored as: +, positive reaction (colour range 3–5 on API colour charts); −, negative reaction; w, weak reaction (colour range 1–2 on API colour charts). Strains are identified as: 1–11, *Tannerella forsythensis* strains; 12, *Bacteroides distasonis* JCM 5825^T; 13, *Bacteroides merdae* JCM 9497^T; 14, *Bacteroides acidifaciens* JCM 10556^T; 15, *Bacteroides caccae* JCM 9498^T; 16, *Bacteroides ovatus* JCM 5824^T; 17, *Bacteroides stercoris* JCM 9496^T; 18, *Bacteroides thetaiotaomicron* JCM 5827^T; 19, *Bacteroides uniformis* JCM 5828^T; 20, *Bacteroides vulgatus* JCM 5826^T. All strains were positive in API ZYM tests for alkaline phosphatase and *N*-acetyl-*β*-glucosaminidase activities and in API An-Ident tests for *N*-acetyl-*β*-D-glucosaminidase, alkaline phosphatase, *β*-galactosidase, indoxyl-acetate and alanine aminopeptidase activities. All strains were negative in API ZYM tests for lipase (C4), valine and cystine arylamidase and α-mannosidase activities and in API An-Ident tests for arginine and tyrosine aminopeptidase activities.

Test	1–11	12	13	14	15	16	17	18	19	20
API ZYM										
Esterase (C4)	w	w	w	−	−	w	w	−	w	−
Esterase lipase (C8)	w	w	w	−	w	w	w	w	w	−
Leucine arylamidase	w	+	+	w	w	−	−	+	−	−
Trypsin	+	−	−	−	−	−	−	−	−	−
Chymotrypsin	−	−	+	−	−	−	−	−	w	−
Acid phosphatase	+	+	+	+	w	+	+	+	w	+
Naphthol-AS-BI-phosphohydrolase	+	w	w	w	w	+	+	w	−	−
α-Galactosidase	−	+	+	+	+	+	−	+	+	w
β-Galactosidase	−	+	+	+	+	+	+	+	+	+
β-Glucuronidase	+	−	−	−	−	+	−	+	−	+
α-Glucosidase	+	+	w	+	−	+	w	+	w	w
β-Glucosidase	−	+	−	−	−	−	−	−	+	−
α-Fucosidase	+	−	−	+	w	w	w	+	w	w
API An-Ident										
Indole	−	−	−	−	−	+	+	+	+	−
α-Glucosidase	+	+	−	+	+	+	+	+	+	+
α-Arabinofuranosidase	−	w	w	+	+	+	−	+	+	+
β-Glucosidase	+	+	−	−	+	+	+	+	+	−
α-L-Fucosidase	+	−	−	+	+	+	+	+	+	+
α-Galactosidase	−	+	+	+	+	+	−	+	+	+
Leucine aminopeptidase	−	+	+	−	+	−	−	+	−	−
Proline aminopeptidase	−	−	w	−	−	−	−	−	−	−
Pyroglutamic acid arylamidase	−	w	w	−	−	−	−	−	−	−
Arginine aminopeptidase	+	+	+	−	+	−	−	+	−	−
Histidine aminopeptidase	+	+	+	−	+	−	−	+	−	−
Phenylalanine aminopeptidase	−	w	w	−	−	−	−	−	−	−
Glycine aminopeptidase	−	+	+	−	−	−	−	−	−	+
Catalase	−	+	−	−	−	+	−	+	−	−

strains (Hudspeth *et al*., 1999). The high similarity (⩾ 98·9 %) of 16S rDNA sequences from all the strains tested suggests that they belong to a single species; however, DNA–DNA homology studies will be necessary for this to be confirmed. Recently, a novel species, *Porphyromonas gulae* sp. nov. (formerly *Porphyromonas gingivalis* animal biotype), was proposed to include strains isolated from the gingival sulcus of various animal hosts that are distinct from related strains of *Porphyromonas gingivalis* strains of human origin (Fournier *et al*., 2001). While this novel species is catalase-positive, *Porphyromonas gingivalis* is catalase-negative. In addition, although *Porphyromonas gulae* shared 98·1 % sequence similarity with *Porphyromonas gingivalis* of human origin, *Porphyromonas gulae* strains shared a DNA homology of only 53–65 % with the type strain of *Porphyromonas gingivalis*. *B. forsythus* strains do not react in the API 20A or API 20E test series. There is no detectable pH decrease in media supplemented with carbohydrates (Tanner *et al*., 1985, 1986). However *B. forsythus* strains gave positive results for α-glucosidase, *β*-glucosidase, α-fucosidase and *β*-glucuronidase. These results suggest that *B. forsythus* may have trouble transporting the sugars across their membranes or metabolizing them in the glycolytic pathway.

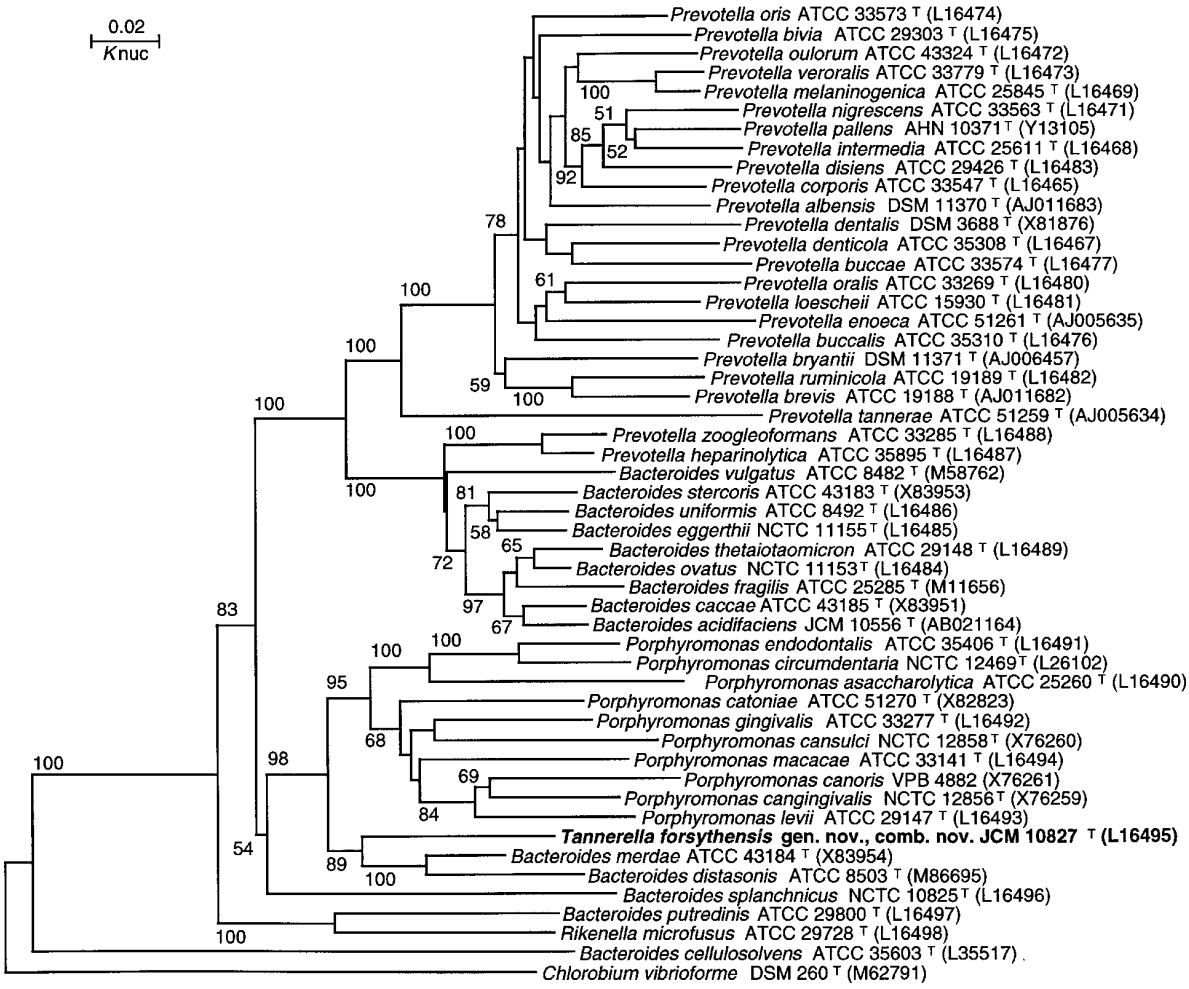

Fig. 1. Phylogenetic tree showing the position of *Tannerella forsythensis* within the *Bacteroides* subgroup of the phylum *Cytophaga–Flavobacterium–Bacteroides* (CFB). The tree was constructed by the neighbour-joining method based on 16S rRNA/rDNA sequences. The scale bar represents 0·02 substitutions per nucleotide position. The numbers at the nodes of the tree indicate bootstrap values for each node out of 100 bootstrap resamplings (values below 50 are not shown).

Phylogenetic analysis

In the phylogenetic tree (Fig. 1), two species of *Prevotella*, *Prevotella heparinolytica* and *Prevotella zoogleoformans* (Shah & Collins, 1990) [formerly *Bacteroides heparinolyticus* (Okuda *et al.*, 1985) and *Bacteroides zoogleoformans* (Cato *et al.*, 1982)], fell outside the generic grouping and clustered with *Bacteroides sensu stricto*. This has been pointed out previously (Paster *et al.*, 1994; Tanner *et al.*, 1994). Paster *et al.* (1994) have already suggested that it will be necessary to modify the current descriptions of the genera *Bacteroides* and *Prevotella* with respect to certain phenotypic criteria in order to accommodate *Prevotella heparinolytica* and *Prevotella zoogleoformans* within the genus *Bacteroides*. For example, although *Bacteroides* species are presently described as growing in the presence of 20 % bile (Shah & Collins, 1989), the growth of *Prevotella heparinolytica* and *Prevotella zoogleoformans* is inhibited by 20 % bile and these species exhibit G6PDH and 6PGDH activities (Bailey & Love, 1995; Cato *et al.*, 1982; Okuda *et al.*, 1985).

Approximately 1500 bases of the 16S rDNA sequence were determined for each of the 10 strains of *B. forsythus* isolated from periodontal pockets. The 16S rRNA sequence for the type strain of *B. forsythus* has been determined previously (Paster *et al.*, 1994). For the phylogenetic analysis, 1370 bp (positions 34–1375; *Escherichia coli* numbering system) sequences of each species were used. 16S rDNA sequence analysis showed that *B. forsythus* was not a species of *Bacteroides sensu stricto* affiliated with the type species (Fig. 1). In addition, 11 isolates and the type strain of *B. forsythus* formed a single cluster at a high bootstrap level. The levels of sequence similarity among the 11 strains of *B. forsythus* were more than 99·1 % (99·1–100 %). These results are in good agreement with the report of Paster *et al.* (1994). *B. forsythus* was related to *Bacteroides distasonis* and *Bacteroides merdae* at about 88 and 91 % similarity, respectively. These data

Table 5. Differential characteristics of *Tannerella* gen. nov. and some related taxa

Data were taken from Braham & Moncla (1992), Collins *et al.* (1994), Fournier *et al.* (2001), Hirasawa & Takada (1994), Hudspeth *et al.* (1999), Johnson (1978), Johnson *et al.* (1986), Love (1995), Love *et al.* (1992, 1994), Moore *et al.* (1994), Shah & Collins (1988, 1989, 1990), Shah *et al.* (1995), Tanner *et al.* (1986), Willems & Collins (1995a, b) and this study. Abbreviations: NF, non-fermentative; F, fermentative; MF, moderately fermentative; A, acetic acid; B, butyric acid; IV, isovaleric acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid; V, variable reaction.

Characteristic	*Tannerella*	[*Bacteroides*] *distasonis*	[*Bacteroides*] *merdae*	*Porphyromonas*	*Bacteroides sensu stricto*	*Prevotella*
Growth in bile	−	+	+	−	+	−
NAM required	+*	−	−	−	−	−
Indole produced	V	−	−	V	V	V
Catalase produced	V	+	−	V	V	V
Aesculin hydrolysed	+	+	+	−	+	V
Pigment produced	−	−	−	+†	−	V
Metabolism	NF	F	F	NF‡	F	MF
Major end-products	A, B, IV, P, PA	A, S	A, S	A, B, IV, P, PA, S	A, S	A, S
Presence of:						
G6PDH	+	+	+	V	+	−
6PGDH	+	+	+	V	+	−
Proteolytic activity	+	−	−	V	−	V
Major cellular fatty acids	anteiso-$C_{15:0}$	anteiso-$C_{15:0}$	anteiso-$C_{15:0}$	iso-$C_{15:0}$§	anteiso-$C_{15:0}$	anteiso-$C_{15:0}$
Ratio of anteiso-$C_{15:0}$ to iso-$C_{15:0}$	22·8–95·2	4·1	6·3	< 1	2·1–5·4	1·2–11·3
Predominant menaquinones	MK-10, MK-11	MK-10	MK-9, MK-10	MK-9, MK-10	MK-10, MK-11	MK-10, MK-11, MK-12, MK-13‖
G+C content (mol%)	44–48	43–45	43–46	40–55	40–48	40–60
Principal habitat	Periodontal pockets	Faeces	Faeces	Oral cavities	Faeces	Oral cavities

* Bite-wound isolates do not require NAM for growth.

† *Porphyromonas catoniae* does not produce a black pigment on blood agar.

‡ Some species are weakly saccharolytic.

§ *Porphyromonas catoniae* contains approximately equal amounts of iso- and anteiso-$C_{15:0}$ as the predominant fatty acids.

‖ *Prevotella dentalis* lacks menaquinones.

also agree with previous findings from 16S rRNA sequence analysis (Paster *et al.*, 1994; Tanner *et al.*, 1994). Although *B. forsythus* was related to *B. distasonis* and *B. merdae*, the ratio of anteiso-15:0 to iso-15:0 in whole-cell methanolysates of *B. forsythus* was different from those of *B. distasonis* and *B. merdae*. While the ratios of anteiso-15:0 to iso-15:0 ranged from 22·8 to 95·2 in *B. forsythus* strains, those of *B. distasonis* JCM 5825^T and *B. merdae* JCM 9497^T were respectively 4·1 and 6·3. These results suggest that a novel genus should be established to accommodate *B. forsythus* and foreshadow the need for nomenclatural change for the latter two species.

Based on the above-mentioned findings and the 16S rDNA sequence analysis, we propose that *Bacteroides forsythus* be reclassified as the first species of a new genus, *Tannerella*, as *Tannerella forsythensis* corrig., gen. nov., comb. nov. Differential characteristics of *Tannerella* gen. nov. and some related taxa are shown in Table 5.

Description of *Tannerella* gen. nov.

Tannerella (Tan.ne.rel′la. L. dim. suffix *-ella*; N.L. fem. n. *Tannerella* of Tanner, after the American microbiologist Anne C. R. Tanner, for her contributions to research on periodontal disease).

Cells are Gram-negative, obligately anaerobic, non-motile fusiforms (0·3–0·5 × 1–30 μm). The major end-products are acetic acid, butyric acid, isovaleric acid, propionic acid and phenylacetic acid; smaller amounts of isobutyric acid and succinic acid may be produced (Braham & Moncla, 1992; Tanner *et al.*, 1986). While most strains require NAM for growth, some strains do not. Growth is inhibited in the presence of 20% bile. Aesculin is hydrolysed. Indole variable. Trypsin activity is positive. G6PDH, 6PGDH, MDH and GDH are present. The principal respiratory quinones are menaquinones MK-10 and MK-11. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The non-hydroxylated acids are predominantly of the saturated straight-chain and anteiso-methyl branched-chain types. The ratio of anteiso-15:0 to iso-15:0 is very much higher ($\geqslant 20$) than that for members of *Bacteroides sensu stricto*. The G+C content is 44–48 mol% (Tanner *et al.*, 1986). The genus *Tannerella* is a member of the *Bacteroides* subgroup of the phylum *Cytophaga–Flavobacterium–Bacteroides* (CFB) and exhibits a close phylogenetic association with *B. distasonis* and *B. merdae*. The type species is *Tannerella forsythensis*.

Description of *Tannerella forsythensis* (Tanner *et al.*, 1986) corrig., comb. nov.

Tannerella forsythensis (for.sy.then′sis. N.L. adj. *forsythensis* pertaining to the Forsyth Dental Center, where the species was first isolated).

Basonym: *Bacterioides forsythus* Tanner *et al.*, 1986.

Cells are Gram-negative, obligately anaerobic, non-motile fusiforms (0·3–0·5 × 1–30 μm). Colonies are pale speckled-pink, circular, entire and slightly convex on TSBA plates with an NAM disk. Aesculin is hydrolysed. Indole is not produced. Catalase is not produced. α-Glucosidase, β-glucosidase, α-fucosidase, β-glucuronidase and trypsin activities are positive. The predominant respiratory quinones are menaquinones MK-10 and MK-11. Minor menaquinones are MK-9 and MK-12. The predominant cellular fatty acid is anteiso-15:0. The ratio of anteiso-15:0 to iso-15:0 of the type strain is 31.2. The G+C content of the type strain is 46 mol% (Tanner *et al.*, 1986). The type strain is JCM 10827^T (= ATCC 43037^T = FDC 338^T), isolated from human periodontal pockets.

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