Barnesiella viscericola gen. nov., sp. nov., a novel member of the family *Porphyromonadaceae* isolated from chicken caecum

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Two bacterial strains isolated from chicken caecum, C46^T and C47, were characterized using a polyphasic taxonomic approach that included analysis of the phenotypic and biochemical features, cellular fatty acid profiles, menaquinone profiles and phylogenetic position (using 16S rRNA gene sequence analysis). The 16S rRNA gene sequence analysis showed that these strains belonged to the family *Porphyromonadaceae*. These strains shared 100 % 16S rRNA gene sequence similarity with each other and were related to *Parabacteroides distasonis* (showing 86 % sequence similarity). The strains were found to be obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative rods. Growth of the strains was inhibited on medium containing 20 % bile. The major menaquinones of the isolates were MK-11 and MK-12. This menaquinone composition was different from those of other genera of the family *Porphyromonadaceae*, such as *Parabacteroides* (in which the predominant menaquinones are MK-9 and MK-10), *Porphyromonas* (MK-9 and MK-10) and *Tannerella* (MK-10 and MK-11). This is an important chemotaxonomic characteristic of these micro-organisms. The DNA G + C content of strain C46^T is 52.0 mol%. On the basis of these data, strains C46^T and C47 represent a novel genus and species, for which the name *Barnesiella viscericola* gen. nov., sp. nov. is proposed. The type strain of *Barnesiella viscericola* is C46^T (=JCM 13660^T=DSM 18177^T).

Previous phylogenetic analyses of chicken caecal microbiota, based on 16S rRNA gene clone library analyses, have revealed a large number of novel phylotypes (Lan *et al.*, 2002; Zhu *et al.*, 2002). Using a special anaerobic culture technique, referred to as the 'plate-in-bottle' method (Mitsuoka *et al.*, 1969), attempts were made to cultivate bacteria from chicken caecum. We found that many of the isolates obtained were members of the order 'Bacteroidales'. From these isolates, we recently proposed three novel *Bacteroides* species: *Bacteroides barnesiae*, *Bacteroides gallinarum* and *Bacteroides salanitronis* (Lan *et al.*, 2006). Analyses of the 16S rRNA gene sequences of two other isolates, which could not grow on medium containing 20 % bile, showed that these strains represented a new subline within the family *Porphyromonadaceae*. These strains shared 100 % 16S rRNA gene sequence similarity with each other and were related to *Parabacteroides distasonis* (86 % sequence similarity). In the present study, we have attempted to determine the taxonomic status of these strains.

Strains C46^T and C47 used in the present study were isolated from chicken caecum (Lan *et al.*, 2002) and were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 2 days at 37 °C in an atmosphere comprising 100 % CO₂. The ability to tolerate bile was tested using *Bacteroides* bile aesculin agar (Shah, 1992). Physiological reactions were determined with an API 20A anaerobe test kit, in duplicate, as recommended by the manufacturer (bioMérieux). The metabolic end products were prepared as described previously (Holdeman *et al.*, 1977) and were analysed as described by Sakamoto *et al.* (2005). Fatty acid methyl esters were obtained from about 40 mg wet cells by saponification, methylation and extraction using the method of Miller (1982) but with minor modifications as described by Kuykendall *et al.* (1988). Cellular fatty acid profiles were determined using the MIDI microbial identification system (Microbial ID). Isoprenoid quinones were extracted (Komagata & Suzuki, 1987) and analysed (Sakamoto *et al.*, 2002) as described previously. Biochemical reactions were determined with the Rapid ID 32A anaerobe identification kit, in duplicate, as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated by means of previously described methods (Marmur, 1961; Saito & Miura, 1963), with

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain C46^T is AB267809.

some modifications. The DNA G+C content was determined by using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M $\text{NH}_4\text{H}_2\text{PO}_4$ and acetonitrile (20:1, v/v). The 16S rRNA gene sequence was analysed as described previously (Sakamoto *et al.*, 2002). Related sequences were aligned with the CLUSTAL W program (Thompson *et al.*, 1994) and corrected by manual inspection. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980) after gaps and unknown bases had been eliminated. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap resampling analysis (Felsenstein, 1985) was performed to estimate the confidence of the tree topologies.

Strains C46^T and C47 were obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. The growth of these isolates was inhibited on a medium containing 20 % bile. Cells on EG agar were 0.8–1.6 × 1.7–11 µm in size and occurred singly. Colonies on EG agar plates were 1–2 mm in diameter, grey to off-white–grey, circular, entire, slightly convex and smooth. The physiological and biochemical characteristics are given in the species description.

The major cellular fatty acids of the two novel strains were anteiso-C_{15:0} and iso-C_{15:0} (41–42 % and 16–18 %, respectively; Table 1). The amount of anteiso-C_{15:0} was relatively high in comparison with amounts reported for members of the genera *Bacteroides* (27–42 %), *Parabacteroides*

(25–32 %) and *Prevotella* (7–37 %) (Sakamoto & Benno, 2006; Sakamoto *et al.*, 2002, 2005). Although *Proteiniphilum acetatigenes* also possessed a large proportion of anteiso-C_{15:0} (46.21 %), the proportion of iso-C_{15:0} was low (3.77 %) (Chen & Dong, 2005).

The major menaquinones of strains C46^T and C47 were MK-11 (65–66 %) and MK-12 (21–24 %). MK-10 was also present (10–11 %). This menaquinone composition differed from those of members of the genera *Parabacteroides* (in which the predominant menaquinones are MK-9 and MK-10), *Porphyromonas* (MK-9 and MK-10) and *Tannerella* (MK-10 and MK-11) (Sakamoto & Benno, 2006; Sakamoto *et al.*, 2002). Among the strains isolated from chicken caecum, *B. salanitronis* BL78^T also exhibited MK-11 (43 %) and MK-12 (43 %) as the main components (Lan *et al.*, 2006).

The DNA G+C contents of strains C46^T and C47 were in the range 52.0–52.2 mol%. These values are somewhat higher than those for the members of other genera in the family *Porphyromonadaceae* (Table 2).

Approximately 1500 bases of the 16S rRNA gene sequence were determined for the novel isolates. Strains C46^T and C47 shared 100 % sequence similarity with each other and were most closely related (96 % sequence similarity) to uncultured bacterial clones isolated in the course of a human caecum mucosal biopsy (Eckburg *et al.*, 2005). In addition, the novel strains were related to *Parabacteroides distasonis* (86 % sequence similarity). For the phylogenetic analysis, 1340 bp (positions 61–1375; *Escherichia coli* numbering system) sequences of each species were used. 16S rRNA gene sequence analyses of the two novel strains showed that the strains represented a novel subline within the family *Porphyromonadaceae* (Fig. 1). Distantly related taxa included members of the genera *Dysgonomonas* (84.0–85.9 % sequence similarity), *Paludibacter* (84.4 % sequence similarity), *Parabacteroides* (84.4–86.0 % sequence similarity), *Porphyromonas* (81.4–84.2 % sequence similarity), *Proteiniphilum* (83.8 % sequence similarity) and *Tannerella* (84.1–85.1 % sequence similarity).

Although strains C46^T and C47 were included in the family *Porphyromonadaceae*, their menaquinone composition (MK-11 and MK-12) differed from those of members of other genera of the family *Porphyromonadaceae*, i.e. *Paludibacter* (MK-8), *Parabacteroides* (MK-9 and MK-10), *Porphyromonas* (MK-9 and MK-10) and *Tannerella* (MK-10 and MK-11). The menaquinone composition, therefore, is an important chemotaxonomic characteristic of the isolates. In addition, the high G+C content of the DNA of the two strains is also useful for differentiating them from members of known genera. These results suggest that a novel genus should be established to accommodate strains C46^T and C47.

On the basis of the above-mentioned findings and the results of the 16S rRNA gene sequence analysis, strains C46^T and

Table 1. Cellular fatty acid compositions of strains C46^T and C47

Summed feature 3 contained an unknown fatty acid (equivalent chain length 13.570) and/or iso-C_{15:0} ALDE. Values shown are percentages of total fatty acids.

Fatty acid	C46 ^T	C47
Saturated straight-chain		
C _{14:0}	1.3	1.0
C _{16:0}	4.8	4.6
C _{18:0}	1.0	1.0
Unsaturated straight-chain		
C _{18:1ω9c}	1.4	1.3
C _{18:2ω6,9c}	1.5	1.4
Hydroxy		
iso-C _{15:0} 3-OH	4.4	4.7
C _{16:0} 3-OH	2.4	2.3
iso-C _{17:0} 3-OH	6.1	6.0
anteiso-C _{17:0} 3-OH	1.9	1.8
Saturated branched-chain		
iso-C _{15:0}	16.4	17.7
anteiso-C _{15:0}	41.6	41.3
iso-C _{17:0}	3.9	4.0
anteiso-C _{17:0}	4.8	4.9
Summed feature 3	2.8	3.1

Table 2. Differential characteristics of strains C46^T and C47 and some related taxa

Taxa: 1, strains C46^T and C47 (*Barnesiella* gen. nov.); 2, *Dysgonomonas*; 3, *Paludibacter*; 4, *Parabacteroides*; 5, *Porphyromonas*; 6, *Proteiniphilum*; 7, *Tannerella*. Data were taken from Chen & Dong (2005), Hofstad *et al.* (2000), Lawson *et al.* (2002), Sakamoto & Benno (2006), Sakamoto *et al.* (2002), Ueki *et al.* (2006) and this study. Abbreviations: NF, non-fermentative; F, fermentative; A, acetic acid; B, butyric acid; IV, isovaleric acid; L, lactic acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid; NT, not tested; v, variable; UASB, upflow anaerobic sludge blanket.

Characteristic	1	2	3	4	5	6	7
Growth with bile	—	+	—	+	—	—	—
Aerobic growth	—	+	—	—	—	—	—
N-Acetylmuramic acid requirement	—	—	—	—	—	—	+*
Pigment production	—	—	—	—	+†	—	—
Metabolism	F	F	F	F	NF‡	NF	NF
Major end products	A, S	P, L, S	A, P	A, S	A, B, IV, P, PA, S	A, P	A, B, IV, P, PA
Major cellular fatty acids	anteiso-C _{15:0}	iso-C _{14:0} , anteiso-C _{15:0} , iso-C _{16:0} 3-OH	anteiso-C _{15:0} , C _{15:0} , anteiso-C _{17:0} 3-OH	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}	2.3–2.5	6.0–8.8	28	3.1–10.3	<1	12.3	22.8–95.2
Predominant menaquinone(s)	MK-11, MK-12	NT	MK-8	MK-9, MK-10	MK-9, MK-10	NT	MK-10, MK-11
Growth at 37 °C	+	+	—	+	+	+	+
DNA G + C content (mol%)	52	38–38.5	39.3	43–46	40–55	46.6	44–48
Principal habitat	Faeces	Human clinical specimen	Irrigated rice-field soil	Faeces	Oral cavities	Sludge from UASB reactor	Periodontal pockets

*Bite-wound isolates do not require N-acetylmuramic acid 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.

C47 represent a novel genus and species, for which the name *Barnesiella viscericola* gen. nov., sp. nov. is proposed. Differential characteristics of *Barnesiella* gen. nov. and some related taxa are shown in Table 2.

Description of *Barnesiella* gen. nov.

Barnesiella (Bar.ne.si.el'la. N.L. dim. fem. n. *Barnesiella* named after the British microbiologist Ella M. Barnes, who

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain C46^T and some related taxa. Accession numbers for the 16S rRNA gene sequences are given for each strain. Numbers at nodes indicate bootstrap percentages from 1000 replicates. Bar, 0.02 substitutions per nucleotide position.

has contributed much to our knowledge of intestinal bacteriology and anaerobic bacteriology in general).

Cells are Gram-negative, obligately anaerobic, non-spore-forming, non-motile, rod-shaped and $0.8\text{--}1.6 \times 1.7\text{--}11\text{ }\mu\text{m}$ in size. On EG agar plates, colonies are 1–2 mm in diameter, grey to off-white–grey, circular, entire, slightly convex and smooth. Saccharolytic. Growth is inhibited on a medium containing 20 % bile. Aesculin is hydrolysed. Indole is not produced. The DNA G + C content is 52 mol%. The genus *Barnesiella* is a member of the family *Porphyromonadaceae*. The type species is *Barnesiella viscericola*.

Description of *Barnesiella viscericola* sp. nov.

Barnesiella viscericola [vis.ce.ri'co.la. L. neut. n. *viscus*, *visceris* intestine; L. suff. n. *-cola* (from L. n. *incola*) inhabitant; N.L. fem. n. *viscericola* inhabitant of the intestine].

Exhibits the following characteristics in addition to those given in the description of the genus. Urease is not produced. Catalase is not produced. Gelatin is digested. Acid is produced from D-cellobiose, glucose, maltose, D-mannose and sucrose, but not from L-arabinose, glycerol, lactose, D-mannitol, D-melezitose, D-raffinose, L-rhamnose, salicin, D-sorbitol, D-trehalose or D-xylose. Positive reactions are obtained using the Rapid ID 32A tests for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, glutamic acid decarboxylase, α -fucosidase, alkaline phosphatase, leucyl glycine arylamidase and alanine arylamidase. Raffinose is fermented. All of the other tests give negative results. The major end products are acetic acid and succinic acid; lower levels of other acids may be produced. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0}. The predominant respiratory quinones are MK-11 (65–66 %) and MK-12 (21–24 %). MK-10 is present as a minor menaquinone (10–11 %). The DNA G + C content of the type strain is 52 mol%.

The type strain, C46^T (=JCM 13660^T = DSM 18177^T), was isolated from chicken caecum. One additional strain, C47 (=JCM 13661), is included in this species.

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