Butyricimonas synergistica gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov., butyric acid-producing bacteria in the family 'Porphyromonadaceae' isolated from rat faeces

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Two bacterial strains, designated MT01^T and MT12^T, isolated from rat faeces were characterized by using a polyphasic taxonomic approach that included analysis of their phenotypic and biochemical features, cellular fatty acid profiles, menaquinone profiles and phylogeny based on 16S rRNA gene sequences. The 16S rRNA gene sequence analysis showed that these strains were members of the family 'Porphyromonadaceae'. The strains shared 94 % 16S rRNA gene sequence similarity with each other and were related to *Odoribacter splanchnicus* NCTC 10825^T (86–87 % sequence similarity). The strains consisted of obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative rods. Growth of the strains was inhibited on medium containing 20 % bile. The two strains produced significant levels of butyric and isobutyric acids as end products from glucose. Although the major cellular fatty acid of these two strains and *O. splanchnicus* JCM 15291^T was iso-C_{15:0}, strains MT01^T and MT12^T showed a higher level of iso-C_{15:0} (66 and 74 %, respectively) than did *O. splanchnicus* JCM 15291^T (48 %). In addition, the ratios of iso-C_{15:0} to anteiso-C_{15:0} in whole-cell methanolsates of the two isolates were very much higher than that of *O. splanchnicus* JCM 15291^T. The major menaquinone of the isolates was MK-10. This menaquinone composition was different from those of other genera of the family 'Porphyromonadaceae', such as *Barnesiella* (predominant menaquinones: MK-11 and MK-12), *Odoribacter* (MK-9), *Paludibacter* (MK-8), *Parabacteroides* (MK-9 and MK-10), *Porphyromonas* (MK-9 and MK-10) and *Tannerella* (MK-10 and MK-11). Menaquinone composition is therefore an important chemotaxonomic characteristic of these micro-organisms. Strains MT01^T and MT12^T have DNA G + C contents of 46 mol%. On the basis of these data, strains MT01^T and MT12^T represent two novel species of a novel genus, for which the names *Butyricimonas synergistica* gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov., respectively, are proposed. The type strains of *B. synergistica* and *B. virosa* are MT01^T (=JCM 15148^T =CCUG 56610^T) and MT12^T (=JCM 15149^T =CCUG 56611^T), respectively.

Tea catechins belong to the flavan-3-ols, a class of flavonoids, and are the major components in tea (*Camellia*

Abbreviation: EGC, (–)-epigallocatechin.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain MT01^T and strain MT12^T are AB443948 and AB443949, respectively.

Supplementary tables showing the results of physiological and biochemical analyses are available with the online version of this paper.

sinensis). It is well recognized that tea catechins have many physiological functions, including antioxidant, blood cholesterol-lowering, blood sugar-lowering and anticancer properties. Studies on the biotransformation and metabolism of tea catechins are considered to be important to our understanding of the potential health benefits of this tea.

We have studied the metabolism of tea catechins by rat intestinal microbiota and have proposed a metabolic

pathway for (–)-epigallocatechin gallate in the intestinal tract (Takagaki *et al.*, 2007). Some types of enteric bacterium, i.e. *Enterobacter aerogenes*, *Klebsiella planticola*, *Klebsiella pneumoniae* subsp. *pneumoniae* and *Bifidobacterium infantis*, were found to be capable of hydrolysing (–)-epigallocatechin gallate to yield (–)-epigallocatechin (EGC) and gallic acid. Furthermore, we isolated *Adlercreutzia equolifaciens* (Maruo *et al.*, 2008) strain MT4s-5, which is capable of catabolizing EGC to 1-(3',4',5'-trihydroxyphenyl)-3-(2',4',6'-trihydroxyphenyl) propan-2-ol (A. Takagaki, D. Arai, S. Ishikawa & F. Nanjo, unpublished data). Subsequent research revealed that *A. equolifaciens* strain MT4s-5 could convert 1-(3',4',5'-trihydroxyphenyl)-3-(2',4',6'-trihydroxyphenyl) propan-2-ol to 1-(3',5'-dihydroxyphenyl)-3-(2',4',6'-trihydroxyphenyl) propan-2-ol in the presence of three types of symbiotic bacterium, namely *Escherichia coli* and two isolates designated MT01^T and MT12^T (unpublished data). 16S rRNA gene sequence analyses of strains MT01^T and MT12^T, which could not grow on medium containing 20 % bile, showed that these strains represented a novel subline within the family 'Porphyromonadaceae'. They shared 94 % 16S rRNA gene sequence similarity with each other and were related to *Odoribacter splanchnicus* NCTC 10825^T (86–87 % sequence similarity). In this study, we attempted to determine the taxonomic status of these strains.

The strains used in the present study were maintained on Eggerth–Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 2–4 days at 37 °C in an atmosphere containing 100 % CO₂. As strains MT01^T and MT12^T were isolated from rat faeces, the strains' ability to tolerate bile was tested on 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). 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 approximately 40 mg wet cells by using the saponification, methylation and extraction method of Miller (1982), but with the minor modifications described by Kuykendall *et al.* (1988). Cellular fatty acid profiles were determined by using the MIDI microbial identification system (Microbial ID). Isoprenoid quinones were extracted as described by Komagata & Suzuki (1987) and analysed as described by Sakamoto *et al.* (2002). Biochemical reactions were determined with the Rapid ID 32A anaerobe identification kit, in duplicate, as recommended by the manufacturer (bioMérieux). Chromosomal DNA was isolated according to previously described methods (Marmur, 1961; Saito & Miura, 1963), with some modifications. The DNA G+C contents were determined by using the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M NH₄H₂PO₄ and acetonitrile (20:1, v/v). The 16S rRNA gene sequences were analysed as described previously (Sakamoto *et al.*, 2002). Related sequences were aligned

with the CLUSTAL_X 2.0 program (Larkin *et al.*, 2007) 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 topology.

Approximately 1500 bases of the 16S rRNA gene sequence were determined for the isolates. Strains MT01^T and MT12^T were related most closely (98–100 % 16S rRNA gene sequence similarity) to uncultured bacterial clones isolated from human faeces (Li *et al.*, 2008). In addition, strains MT01^T and MT12^T were related to *O. splanchnicus* NCTC 10825^T (Hardham *et al.*, 2008), with 87.0 and 85.6 % 16S rRNA gene sequence similarity, respectively. The next most closely related species was *Odoribacter denticanis* B106^T (83.3 % 16S rRNA gene sequence similarity) (Hardham *et al.*, 2008). The level of sequence similarity between strains MT01^T and MT12^T was 94.0 %. For the phylogenetic analysis, 1337 bp sequences (positions 61–1375; *Escherichia coli* numbering system) were used for each taxon. 16S rRNA gene sequence analyses of strains MT01^T and MT12^T showed that these strains represented a novel subline within the family 'Porphyromonadaceae' (Fig. 1). The 16S rRNA gene sequence for the type strain of *O. denticanis* (ATCC PTA-3625^T) was shown to be similar to that of *Porphyromonas macacae* ATCC 33141^T. Consequently, we could not use *O. denticanis* ATCC PTA-3625^T as a reference strain.

Strains MT01^T and MT12^T comprised obligately anaerobic, non-spore-forming, non-motile, Gram-negative rods. Cells on EG agar were 1.0 × 1.5 µm in size and occurred singly. Colonies on EG agar plates after 72 h incubation at 37 °C under anaerobic conditions were 0.5–1.0 mm in diameter, opalescent to yellowish, circular, entire, slightly convex and smooth. On Bacteroides bile aesculin agar, growth of the isolates was inhibited and aesculin hydrolysis was negative. Results from physiological and biochemical analyses are given in the species description and in Supplementary Tables S1 and S2 (available in IJSEM Online). In API 20A tests, strain MT01^T could be differentiated from *O. splanchnicus* JCM 15291^T on the basis of the former's inability to ferment lactose, maltose, L-arabinose, glycerol and raffinose and inability to hydrolyse aesculin. Strain MT12^T could be differentiated from *O. splanchnicus* JCM 15291^T on the basis of the former's inability to ferment lactose, maltose, L-arabinose, D-mannose and raffinose, inability to hydrolyse aesculin, inability to liquefy gelatin and the presence of catalase activity. In API Rapid ID 32A tests, strain MT01^T differed from *O. splanchnicus* JCM 15291^T: the former lacked β-galactosidase, α-glucosidase and arginine, phenylalanine, leucine, tyrosine and histidine arylamidase activities and was negative for raffinose fermentation. The enzymic pattern of strain MT12^T differed from that of *O. splanchnicus* JCM 15291^T: the former strain was negative for α-galactosidase, α-glucosidase, α-fucosidase

and arginine, phenylalanine, leucine, pyroglutamic acid, tyrosine and histidine arylamidase activities and was negative for mannose and raffinose fermentation.

The major cellular fatty acid of *O. splanchnicus* JCM 15291^T was iso-C_{15:0} (Table 1). The cellular fatty acid composition of *O. splanchnicus* JCM 15291^T corresponded with that reported previously for *Bacteroides* (*O.*) *splanchnicus* 1651/6^T (Miyagawa & Suto, 1980). The major cellular fatty acid of strains MT01^T and MT12^T was also iso-C_{15:0}. However, strains MT01^T and MT12^T showed higher levels of iso-C_{15:0} (66 and 74 %, respectively) than did *O. splanchnicus* JCM 15291^T (48 %). In addition, the ratios of iso-C_{15:0} to anteiso-C_{15:0} in whole-cell methanolysates of MT01^T and MT12^T were very much higher (30.0 and 35.3, respectively) than that in *O. splanchnicus* JCM 15291^T (2.9). The ratio of fatty acids has previously been used as a criterion to establish a novel genus, *Tannerella* (Sakamoto *et al.*, 2002). Consequently, these results suggest that a novel genus should be established to accommodate strains MT01^T and MT12^T.

Table 1. Cellular fatty acid compositions of strain MT01^T, strain MT12^T and *O. splanchnicus* JCM 15291^T

Fatty acid	1	2	3
Saturated straight-chain			
C _{12:0}	—	—	tr
C _{14:0}	—	1.8	6.5
C _{16:0}	1.3	1.9	3.0
Unsaturated straight-chain			
C _{14:1} <i>ω</i> 5 <i>c</i>	—	—	tr
C _{16:1} <i>ω</i> 7 <i>c</i>	—	—	1.0
C _{18:1} <i>ω</i> 9 <i>c</i>	14.1	4.4	5.3
Hydroxy acids			
iso-C _{15:0} 3-OH	—	1.5	—
C _{16:0} 3-OH	2.4	4.0	6.8
iso-C _{17:0} 3-OH	10.1	6.8	6.1
Saturated branched-chain			
iso-C _{13:0}	—	—	tr
iso-C _{15:0}	66.1	74.1	48.4
anteiso-C _{15:0}	2.2	2.1	16.5
iso-C _{16:0}	—	—	tr
Summed features*			
1	—	1.1	1.8
3	3.7	2.2	1.7

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previously (Collins & Jones, 1981; Shah & Collins, 1983) (Table 2). The menaquinone compositions of these two isolates were different from those of members of the genera *Barnesiella* (predominant menaquinones: MK-11 and MK-12), *Paludibacter* (MK-8), *Parabacteroides* (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, 2007; Ueki *et al.*, 2006). This feature is therefore an important chemotaxonomic characteristic of these micro-organisms.

The DNA G+C contents of strains MT01^T and MT12^T were 46.4 and 46.5 mol%, respectively, whereas that of *O. splanchnicus* JCM 15291^T was 45.9 mol%.

On the basis of the above-mentioned findings and the results of the 16S rRNA gene sequence analysis, strains MT01^T and MT12^T represent two novel species of a novel genus, for which the names *Butyricimonas synergistica* gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov. are proposed, respectively. Differential characteristics of *Butyricimonas* gen. nov. and some related taxa are shown in Table 3.

Description of *Butyricimonas* gen. nov.

Butyricimonas (Bu.ty.ri.ci.mo'nas. N.L. n. *acidum butyricum* butyric acid; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Butyricimonas* a butyric acid-producing monad).

Cells are Gram-negative, obligately anaerobic, non-spore-forming, non-motile, rod-shaped and $1.0 \times 1.5 \mu\text{m}$. Colonies on EG agar plates after 72 h incubation at 37 °C under anaerobic conditions are 0.5–1.0 mm in diameter, opalescent to yellowish, circular, entire, slightly convex and smooth. Saccharolytic. The major end products are butyric and isobutyric acids; smaller amounts of acetic, propionic and succinic acids are produced. Growth is inhibited on medium containing 20 % bile. Aesculin is not hydrolysed. Indole and hydrogen are produced. The principal respiratory quinone is MK-10. Both non-hydroxylated and 3-hydroxylated long-chain fatty acids are present. The non-hydroxylated acids are predominantly of the isomethyl branched type (iso-C_{15:0}), with smaller amounts of straight-chain saturated acids. The genus is a

member of the family 'Porphyromonadaceae'. The type species is *Butyricimonas synergistica*.

Description of *Butyricimonas synergistica* sp. nov.

Butyricimonas synergistica [sy.ner.gis'ti.ca. N.L. fem. adj. *synergistica* (from Gr. *sunergēs* working with, co-operating) synergistic].

Exhibits the following characteristics in addition to those given in the description of the genus. Urease and catalase are not produced. Gelatin is digested. Acid is produced from glucose and D-mannose, but not from L-arabinose, cellobiose, glycerol, lactose, maltose, D-mannitol, melezitose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose. Positive reactions are obtained using Rapid ID 32A for α -galactosidase, *N*-acetyl- β -glucosaminidase, glutamic acid decarboxylase, α -fucosidase, indole, alkaline phosphatase and leucyl glycine, pyroglutamic acid, alanine and glutamyl glutamic acid arylamidases. Mannose is fermented. All other tests in Rapid ID 32A are negative. The major end product of the type strain is butyric acid; smaller amounts of acetic, propionic, succinic and isobutyric acids are produced. The major cellular fatty acid of the type strain is iso-C_{15:0} (66 %). The predominant respiratory quinone of the type strain is MK-10 (42 %); minor amounts of MK-8 (22 %), MK-9 (9 %) and MK-11 (27 %) are present. The DNA G+C content of the type strain is 46.4 mol%.

The type strain, MT01^T (=JCM 15148^T=CCUG 56610^T), was isolated from rat faeces.

Description of *Butyricimonas virosa* sp. nov.

Butyricimonas virosa (vi.ro'sa. L. fem. adj. *virosa* fetid, stinking).

Exhibits the following characteristics in addition to those given in the description of the genus. Urease is not produced. Catalase is produced. Gelatin is not digested. Acid is produced from glucose and glycerol, but not from L-arabinose, cellobiose, lactose, maltose, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose. Positive reactions are obtained using Rapid ID 32A for β -galactosidase, *N*-acetyl- β -glucosaminidase, glutamic acid decarboxylase, indole, alkaline phosphatase and leucyl glycine, alanine and glutamyl glutamic acid arylamidases. All the other tests in Rapid ID 32A are negative. The major end products of the type strain are butyric and isobutyric acids; smaller amounts of acetic, propionic and succinic acids are produced. The major cellular fatty acid of the type strain is iso-C_{15:0} (74 %). The predominant respiratory quinone of the type strain is MK-10 (50 %); minor amounts of MK-8 (28 %), MK-9 (4 %) and MK-11 (18 %) are present. The DNA G+C content of the type strain is 46.5 mol%.

Table 2. Menaquinone compositions of strain MT01^T, strain MT12^T and *O. splanchnicus* JCM 15291^T

Taxa: 1, strain MT01^T; 2, strain MT12^T; 3, *O. splanchnicus* JCM 15291^T. Values shown are percentages of total menaquinones.

Menaquinone	1	2	3
MK-8	22	28	35
MK-9	9	4	40
MK-10	42	50	13
Mk-11	27	18	12

Table 3. Differential characteristics of strains MT01^T and MT12^T and some related taxa

Data were taken from Brondz & Olsen (1991), Chen & Dong (2005), Collins *et al.* (1994), Hofstad *et al.* (2000), Lawson *et al.* (2002), Love *et al.* (1992, 1994), Sakamoto & Benno (2006), Sakamoto *et al.* (2002, 2007), Summanen *et al.* (2005), Ueki *et al.* (2006) and this study. NT, Not tested; +, positive; −, negative; UASB, upflow anaerobic sludge blanket.

Characteristic	<i>Butyricimonas</i>	<i>Barnesiella</i>	<i>Dysgonomonas</i>	<i>Odoribacter</i>	<i>Paludibacter</i>	<i>Parabacteroides</i>	<i>Porphyrimonas</i>	<i>Proteiniphilum</i>	<i>Tannerella</i>
Growth in bile	−	−	+	+	−	+	−	−	−
Aerobic growth	−	−	+	−	−	−	−	−	−
<i>N</i> -Acetylmuramic acid required	−	−	−	−	−	−	−	−	+*
Pigment produced	−	−	−	−	−	−	+†	−	−
Metabolism‡	F	F	F	F	F	F	NF§	NF	NF
Major end products	B, IB	A, S	P, L, S	A, S	A, P	A, S	A, B, IV, P, PA, S	A, P	A, B, IV, P, PA
Major cellular fatty acids	iso-C _{15:0}	anteiso-C _{15:0}	iso-C _{14:0} , anteiso-C _{15:0} and iso-C _{16:0} 3-OH	iso-C _{15:0}	anteiso-C _{15:0} , C _{15:0} and ante-iso-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}	<1	2.3–2.5	6.0–8.8	<1	28	3.1–10.3	<1	12.3	22.8–95.2
Ratio of iso-C _{15:0} to anteiso-C _{15:0}	30.0–35.3	<1	<1	2.9	<1	<1	1.1–40.5	<1	<1
Predominant menaquinone(s)	MK-10	MK-11, MK-12	NT	MK-9	MK-8	MK-9, MK-10	MK-9, MK-10	NT	MK-10, MK-11
Growth at 37 °C	+	+	+	+	−	+	+	+	+
DNA G+C content (mol%)	46	52	38.0–38.5	46	39.3	43–46	40–55	46.6	44–48
Isolation source	Faeces	Faeces	Human clinical specimen	Faeces	Irrigated rice-field soil	Faeces	Oral cavities	Sludge from UASB reactor	Periodontal pockets

*The bite-wound isolates do not require *N*-acetylmuramic acid for growth.

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

‡Abbreviations: NF, non-fermentative; F, fermentative.

§Some species are weakly saccharolytic.

||A, Acetic acid; B, butyric acid; IB, isobutyric acid; IV, isovaleric acid; L, lactic acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid.

¶*P. catoniae* contains approximately equal amounts of iso- and anteiso-C_{15:0} as it does of the predominant fatty acids.

The type strain, MT12^T (=JCM 15149^T=CCUG 56611^T), was isolated from rat faeces.

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