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Butyricimonas faecalis sp. nov., isolated from human faeces and emended description of the genus *Butyricimonas*

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Abstract

A Gram-negative, strictly anaerobic, non-spore forming, non-motile, non-pigmented bacterial strain, designated H184^T, was isolated from human faeces. 16S rRNA gene sequence analysis showed that strain H184^T represents a member of the genus *Butyricimonas*. Strain H184^T is related to but distinct from *Butyricimonas virosa* JCM 15149^T and *Butyricimonas paravirosa* JCM 18677^T, with 16S rRNA gene sequence similarities of 96.32 and 96.24 %, respectively. Strain H184^T shared 90.50 % *hsp60* gene sequence similarity to B. *virosa* JCM 15149^T and *B. paravirosa* JCM 18677^T. Growth occurs between 25 and 42 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–3.75 % and 0–1.8 %, respectively. pH range for growth is 5.5–8. The strain produced propionate as the major end product from glucose. The major cellular fatty acids of strain H184^T were iso-$C_{15:0}$ (63.5 %) and iso-$C_{17:0}$ 3-OH (12.8%). The major menaquinone of the strain was MK-10 (86 %). DNA G+C content of the isolate H184^T was 44.2 mol%. The genome-based comparison between strain H184^T and *B. virosa* JCM 15149^T by pairwise average nucleotide identity indicated a clear distinction with a score of 87.22. On the basis of these data, strain H184^T represents a novel species of the genus *Butyricimonas*, for which the name *Butyricimonas faecalis* sp. nov. is proposed. The type strain of *B. faecalis* is H184^T (DSM 106867^T, LMG 30602^T).

The ability to sequence 16S rRNA gene and metagenome transformed our perception of the diversity of the human gut microbiota more than a decade ago [1, 2]. However and despite the significant progress recently made in the cultivation of human gut bacteria [3], a large proportion of species still escape taxonomic description. Sequencing-based studies repeatedly identify bacterial taxa associated with either health or disease, but the absence of cultivable strains representative of those taxa prevents the demonstration of the causal impact of those bacteria on human health.

In this study, strain H184^T was isolated from a faecal sample of a healthy 31 year old female, as part of the Walloon Excellence in Life Sciences and Biotechnology project (WELBIO; http://welbio.org/cms/c_5039/en/about-welbio). One of the goals of the project is to identify potential new beneficial microbes isolated from the human gut. The 16S rRNA gene sequence of this particular isolate indicate that it belongs to the genus *Butyricimonas*. Considering the relatively low 16S rRNA gene sequence similarity of strain H184^T to its closest cultured organism *Butyricimonas virosa* JCM 15149^T [4] (96.32 %) we proceeded to a more detailed study of the isolate. Based on the results presented here, we propose that this strain should be classified as representing novel species of the genus *Butyricimonas*.

The faecal sample was kept in a sealed container with an O_2-absorbing and CO_2-generating agent (Genbox Anaer, Biomérieux) and isolation was performed within 2 h after collection. The samples were transferred into an anaerobic chamber (Coy) containing 100 % N_2 as gas atmosphere and immediately diluted 1/10 in modified YCFA (yeast extract–casein hydrolysate–fatty acids) [5] enriched in antioxidants [6] (Table S1). Faecal suspension was then transferred in tubes hermetically closed with butyl rubber under an atmosphere of 20 % CO_2 – 80 % N_2. Then, single-cell cultivation

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Keywords: *Butyricimonas*; human intestinal microbiota; human faeces; porphyromonadaceae.
Abbreviations: ANI, average nucleotide identity; YCFA, yeast extract–casein hydrolysate–fatty acid.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and *hsp60* gene sequence of *B. faecalis* strain H184^T are MG963286 and MH255795. The whole genome sequence of *B. faecalis* strain H184^T has been deposited into GenBank/EMBL/DDBJ under accession number CP032819-CP032820.
One supplementary figure and one supplementary table are available with the online version of this article.

Table 1. Strains : 1, *B. faecalis* H184^{T}sp. nov.; 2, *B. synergistica* JCM 15148^T; 3, *B. virosa* JCM 15149^T; 4, *B. faecihominis* JCM 18676^T; 5, *B. paravirosa* JCM 18677^T

TR, Trace amount (<1 %) ; −, not detected.

Characteristic	1	2	3	4	5
Source of isolation	Human gut	Rat gut	Rat gut	Human gut	Human gut
G+C content (mol%)	44.2	44.8	42.3	45.2	44.9
Catalase	+	−	+	+	+
Growth bile concentration range	0–3.75 %	0–3.5	0–3.5	0–4.5	0–4.5
Growth NaCl concentration range	0–1.8 %	0–1.8 %	0–2.2 %	0–2 %	0–2 %
Growth pH range	5.5–8	6–7.5	6–7.5	5.5–8	5.5–8
Growth temperature range (optimum)	25–42 °C (37)	25–38 °C (37)	25–38 °C (37)	35–42 °C (37)	35–38 °C (37)
Production of acid from:					
Glycerol	−	−	+	+	+
Enzymatic activity:					
α-galactosidase	−	+	−	−	−
Pyroglutamic acid arylamidase	+	+	−	+	+
Gelatinase	−	+	−	−	−
Arginine dihydrolase	+	−	−	−	−
Menaquinone composition (%):					
MK-8	−	22	28	26	18
MK-9	3	4	4	6	12
MK-10	86	42	50	66	67
MK-11	8	27	18	4	3

was performed using extinction dilution technique, i.e. we diluted and aliquoted the faecal suspension in 300 vials such that a single vial received on average one cell [7]. Positive cultures after 48 h to 7 days were spread onto solid modified YCFA and incubated 72 h to 7 days in anaerobic jars (Merck) with an O_2-absorbing and CO_2-generating agent (Genbox Anaer, Biomérieux). Single colonies were picked and transferred to fresh medium and the process was repeated until the cultures were deemed pure. We obtained more than 200 cultures, of which one, designated H184^T, was selected for further study.

Type strains *B. synergistica* JCM 15148^T, *B. virosa* JCM 15149^T, *B. paravirosa* JCM 18677^T and *B. faecihominis* JCM 18676^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Forty-eight-hour-old cultures in modified YCFA medium were used for routine incubation, growth tests and biochemical analyses. The strain was stored at −80 °C in 20 % glycerol.

An almost-complete (1399 bp) 16S rRNA sequence of strain H184^T was obtained using the universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [NCBI accession number MG963286] [8]. We also analysed the partial *hsp60* gene sequence as previously described (NCBI accession number MH255795) [9, 10]. 16S rRNA sequences of the closest previously identified relatives of strain H184^T were determined and retrieved using EzBioCloud's Identify service (database updated 2017.10.23)[11] and the GenBank database.

Multiple alignment of the sequences was performed using MUSCLE [12]. Distances were computed using the maximum-composite-likelihood method [13] and the phylogenetic tree based on 16S rRNA gene sequence was reconstructed by the neighbour-joining method [14] in MEGA 7.0 [15] after gaps and unknown bases were eliminated (Fig. 1a). Strain H184^T was found to be related to *B. virosa* JCM 15149^T (96.32 % sequence similarity), *B. paravirosa* JCM 18677^T (96.24 %) and *B. faecihominis* JCM 18676^T (96.22 %).

Likewise, *hsp60* gene sequence analysis showed that strain H184^T is related but different from *B. virosa* JCM 15149^T, *B. paravirosa* JCM 18677^T and *B. faecihominis* JCM 18676^T, with gene sequence similarities of 90.50 and 89.05 %, respectively (Fig. 1b). Strain H184^T is also related but more distantly to *B. synergistica* JCM 15148^T with 84.23 % sequence similarity.

For whole genome sequence analysis, high molecular weight DNA was extracted using Qiagen DNeasy UltraClean Microbial kit. Long reads were obtained using PacBio technology at Eurofins GATC company. Assembly was performed using the hierarchical genome-assembly process as previously described [16] and produced a 4 976 430 base pair complete genome divided into two contigs of 4 925 408 and 51 022 bp (NCBI accession number CP032819-CP032820), whose depth of coverage were 172.8 and 191.7, respectively. Genome analysis using the ContEst16S algorithm indicated that the genome of strain H184^T was not

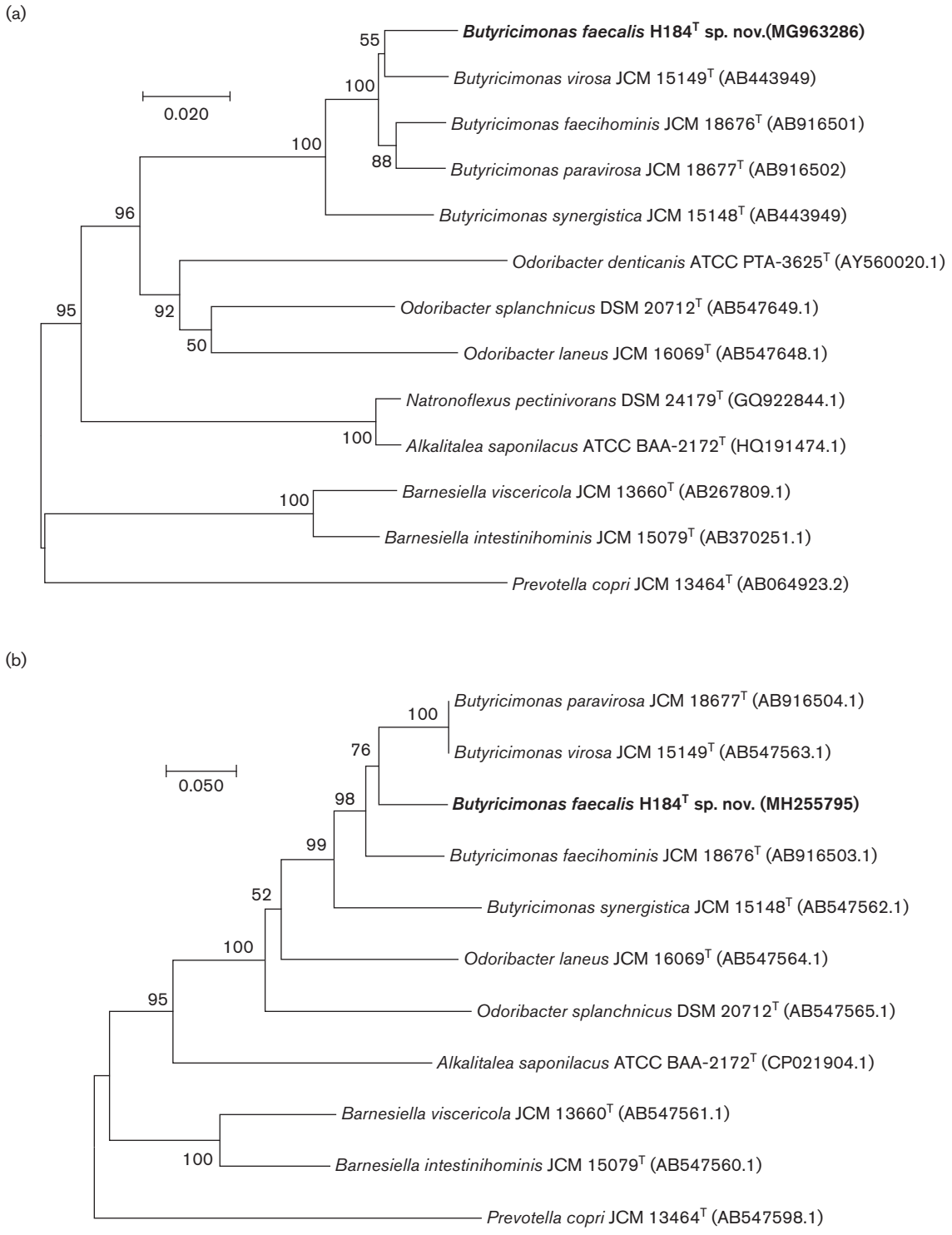

Fig. 1. Phylogenetic trees reconstructed by the neighbour-joining method based on 16S rRNA (Bar indicates 0.02 substitutions per nucleotide position) (a) and *hsp60* (bar, 0.05 substitutions per nucleotide position) (b) gene sequences, showing the connections between strain H184^T, the four *Butyricimonas* species and some related taxa. GenBank accession numbers are shown in parentheses.

contaminated [17]. The sequences of five 16S rRNA gene copies were retrieved and compared to the 16S rRNA gene sequence obtained by PCR and Sanger sequencing. The five copies were not all strictly identical, but had a similarity to

Table 2. Strains : 1, *B. faecalis* H184^{T}sp. nov. ; 2, *B. synergistica* JCM 15148^T; 3, *B. virosa* JCM 15149^T; 4, *B. faecihominis* JCM 18676^T; 5, *B. paravirosa* JCM 18677^T

TR, Trace amount (<1 %) ; –, not detected.

Fatty acid	1	2	3	4	5
Saturated straight-chain:					
$C_{12:0}$	–	–	TR	–	–
$C_{14:0}$	1.7	TR	1.3	1.0	TR
$C_{15:0}$	3.3	–	–	–	–
$C_{16:0}$	1.8	1.0	2.6	2.3	1.0
$C_{18:0}$	–	TR	TR	–	–
Unsaturated straight-chain:					
$C_{18:1}\omega 9c$	–	TR	TR	–	–
Hydroxy:					
iso-$C_{15:0}$ 3-OH	1.0	1.0	1.0	TR	1.0
$C_{16:0}$ 3-OH	6.4	3.2	6.5	5.3	4.6
iso-$C_{17:0}$ 3-OH	12.8	14.2	9.9	11.5	12.2
Saturated branched-chain:					
iso-$C_{11:0}$	–	–	–	–	TR
iso-$C_{13:0}$	TR	TR	TR	TR	TR
iso-$C_{15:0}$	63.5	70.2	69.4	70.0	71.8
anteiso-$C_{15:0}$	2.8	3.0	3.0	3.9	2.8
iso-$C_{17:0}$	1.2	1.2	1.0	1.7	1.1
Summed features:*					
1	TR	–	–	–	–
3	1.3	TR	TR	TR	TR
9	Tr	2	1.3	1.7	1.2

*Summed features represent groups of two or three fatty acids that could not be separated using the MIDI Sherlock system. Summed feature 1 contains $C_{13:1}$cis12 and/or $C_{14:0}$ ALDE. Summed feature 3 contains one or more of an unknown fatty acid of ECL 13.570 and/or iso-$C_{15:0}$ ALDE. Summed feature 9 contains iso-$C_{16:0}$ 3-OH and/or an unknown fatty acid 17.157 DMA. Summed feature 10 contains one or more of an unknown fatty acid 17.834 and/or $C_{18:0}\omega 11c/9$ t/6 t fatty acid methyl ester.

the sequence obtained by PCR ranging between 99.68 and 100 %. The genomes of reference strains *B. synergistica* JCM 15148^T and *B. virosa* JCM 15149^T were obtained from GenB ank (accession numbers GCA_000379665.1 and GCA _000519105.1). At the time of writing, the genomes of reference strains *B. paravirosa* JCM 18677^T and *B. faecihominis* JCM 18676^T are not available in public databases. Average nucleotide identity (ANI) scores between strain H184^T and available reference strains genomes were calculated using OAT standalone 0.93.1 software [18]. The ANIs of strain H 184^T to *B. synergistica* JCM 15148^T and *B. virosa* JCM 15149^T were 76.31 and 87.22, respectively, confirming that strain H184^T is a new species.

Scanning electron microscopy (Philips Electron Microscope CM12/STEM) of cultures showed that cells were straight rods, approximately 0.4–0.5×1.4–2.0 µm in size and occurred singly (Fig. S1a, b, available in the online version of this article). H184^T colonies on solid modified YCFA after 72 h of incubation at 37 °C were 1–2 mm in diameter, opalescent, beige and slightly translucent, circular, entire, slightly convex and smooth. The strain was negative for motility when stab inoculated into semi-solid modified YCFA (0.5 % agar) and anaerobically incubated at 37 °C for 72 h. The ability to tolerate bile and NaCl was tested in liquid modified YCFA containing increasing concentration of bovine bile (Sigma; 1 % w/v of dehydrated bile corresponding to 10 % w/v fresh bile) or NaCl (VWR). Growth of the strain was inhibited on medium whose concentration of bile and NaCl was 4 % or above and 2 % or above, respectively. Growth was tested at 20, 25, 30, 35, 38, 40, 42 and 44 °C and occurred at each temperature except 20 and 44 °C. pH range for growth was tested between 4 and 8.5 with 0.5 increments and was observed at pH between 5.5 and 8.

Gram staining and KOH tests (3 %, w/v) were negative. No spore formation was observed in transmission electron microscopy (Philips Electron Microscope CM12/STEM), no spores were observed after malachite green staining and no growth occurred after a 30 min treatment with 70% ethanol. Catalase test using 3 % w/v H_2O_2 is positive for strain H184^T. A comparison of the morphological, biochemical and physiological properties can be found in Table 1.

We used the rapid ID 32A anaerobe identification kit (bioMérieux) according to the manufacturer's instruction and the API 20A anaerobe test kit and the API 50CH carbohydrates kit (bioMérieux) with modified YCFA without glucose. Tests were performed three times on three separate cultures. Strain H184^T could be differentiated from *B. virosa* JCM 15149^T by its activity for arginine dihydrolase and pyroglutamic acid arylamidase (Table 1) in addition to its inability to ferment glycerol (Table 1). Strain H184^T could also be differentiated from *B. paravirosa* JCM 18676^T by its activity for arginine dihydrolase and its inability to ferment glycerol. All strains produced acid from D-glucose, D-mannose, D-galactose, lactose and N-acetylglucosamine. None of the strains produced acid from erythrol, D or L-arabinose, D-ribose, D or L-xylose, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, myo-inositol, methyl α-D-mannopyranoside, amygdalin, arbutin, melibiose, inulin, starch, glycogen, xylitol, gentibiose, turanose, D or L-lyxose, D-tagatose, D or L-fucose, D or L-arabitol, potassium gluconate and potassium 5-cetogluconate.

Respiratory lipoquinones were analysed by the Identification Service of the DSMZ, Braunschweig, Germany. Briefly, quinones were extracted from 100 mg freeze-dried cells using methanol:hexane, followed by phase separation into hexane according to Tindall's method [19, 20]. Quinones were then separated by thin layer chromatography on silica gel, removed from the plate and analysed by HPLC (LDC Analytical; Thermo Separation Products). The major menaquinone of strain H184^T was MK-10 with 86 % (Table 1), which is notably higher than the four other species of the genus (42 to 67 %). Lower amounts of MK-11 and MK-9, respectively 3 and 8 %, were also measured. Although the menaquinone of this strain was overall similar to those of

the four previously described *Butyricimonas* type strains, H184^T menaquinone composition differed from its closest relatives *B. virosa* JCM 15149^T and *B. paravirosa* JCM 18677^T by the absence of MK-8.

Cellular fatty acids were analysed by the Identification Service of the DSMZ, Braunschweig, Germany from 30 mg freeze-dried cells by saponification, methylation and extraction using minor modifications of the method of Miller [21] and Kuykendall *et al.* [22]. The major cellular fatty acid of strain H184^T is iso-$C_{15:0}$ (63.5 %) (Table 2). These results are in agreement with the four described species belonging to the genus *Butyricimonas* [4, 23] (57.6–68.6 %) although the ratio of iso-$C_{15:0}$ to anteiso-$C_{15:0}$ is distinctly lower being respectively 22.5 while the four other *Butyricimonas* species fall within a 34–46 range. Like the four other species of the genus, the second major cellular fatty acid for strain H184^T is iso-$C_{17:0}$ 3-OH (12.8 %).

The major end products produced by the previously described *Butyricimonas* species from glucose are butyrate, isobutyrate and isovalate; smaller amounts of acetate, propionate and succinate are also produced. On the other hand, strain H184^T produced large amounts of propionate and moderate amounts of acetate, four carbons acids (butyrate and isobutyrate) and five carbons acids (valerate and isovalerate).

Analysis of DNA base composition was carried out by the Identification Service, DSMZ, Braunschweig, Germany. The DNA G+C content of strain H184^T was 44.2 mol%. Those of *B. virosa* JCM 15149^T, *B. paravirosa* JCM 18677^T and *B. faecihominis* JCM 18676^T were similar, with 42.3, 44.9 and 45.2 mol%, respectively [23, 24].

Based on the above-mentioned findings, we propose that strain H184^T represents a novel species of the genus *Butyricimonas*, for which the name *Butyricimonas faecalis* sp. nov. is proposed.

EMENDED DESCRIPTION OF THE GENUS *BUTYRICIMONAS* SAKAMOTO *ET AL.* 2014

The description is as given by Sakamoto *et al.* with the following modification. Acid is not produced from erythrol, D/L-arabinose, D-ribose, D/L-xylose, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, myo-inositol, methyl α-D-mannopyranoside, amygdalin, arbutin, melibiose, inulin, starch, glycogen, xylitol, gentibiose, turanose, D/L-lyxose, D-tagatose, D/L-fucose, D/L-arabitol, potassium gluconate and potassium 5-cetogluconate.

EMENDED DESCRIPTION OF *BUTYRICIMONAS SYNERGISTICA* SAKAMOTO *ET AL.* 2009

The description is as given by Sakamoto *et al.* with the following modifications. Growth occurs between 25 and 38 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–3.5 % and 0–1.8 %, respectively. pH range for growth is pH 6–7.5. Positive reaction is obtained for β-galactosidase. Acid is produced from lactose.

EMENDED DESCRIPTION OF *BUTYRICIMONAS VIROSA* SAKAMOTO *ET AL.* 2009

The description is as given by Sakamoto *et al.* with the following modifications. Growth occurs between 25 and 38 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–3.5 % and 0–2.2 %, respectively. pH range for growth is pH 5.5–7.5. Positive reaction is obtained for α-fucosidase. Acid is produced from mannose.

EMENDED DESCRIPTION OF *BUTYRICIMONAS FAECIHOMINIS* SAKAMOTO *ET AL.* 2014

The description is as given by Sakamoto *et al.* with the following modifications. Growth occurs between 35 and 42 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–4.5 % and 0–2 %, respectively. pH range for growth is pH 5.5–8.

EMENDED DESCRIPTION OF *BUTYRICIMONAS PARAVIROSA* SAKAMOTO *ET AL.* 2014

The description is as given by Sakamoto *et al.* with the following modifications. Growth occurs between 35 and 38 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–4.5 % and 0–2 %, respectively. pH range for growth is pH 5.5–8. Acid is produced from mannose.

DESCRIPTION OF *BUTYRICIMONAS FAECALIS* SP. NOV.

Butyricimonas faecalis [fae.ca'lis. N.L. fem. adj. faecalis faecal].

Cells are obligatory anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-stain-negative rods (0.4–0.5×1.4–2.0 µm). Colonies on solid modified YCFA after 72 h of incubation at 37 °C under anaerobic conditions were 1–2 mm in diameter, opalescent, beige and slightly translucent, circular, entire, slightly convex and smooth. Growth occurs between 25 and 42 °C with an optimum at 37 °C. Bile and NaCl concentration range allowing growth are 0–3.75 % and 0–1.8 %, respectively. pH range for growth is pH 5.5–8.

Aesculin is not hydrolysed. Indole is produced. Nitrate is not reduced. Gelatin is not digested. Urease is not produced. Catalase is produced. Acid is produced from D-glucose, D-galactose, lactose, D-mannose and *N*-acetylglucosamine, but not from erythrol, D/L-arabinose, D-ribose, D/L-xylose, D-adonytol, D-fructose, L-sorbose, L-rhamnose, D-mannitol, D-sorbitol, inositol, cellobiose, maltose, cellobiose, melibiose, sucrose, trehalose or D-melezitol. Positive reactions are obtained for arginine dihydrolase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, indole, glutamic acid decarboxylase, leucyl glycine arylamidase, pyroglutamic

acid arylamidase, alanine arylamidase and glutamyl glutamic acid arylamidase. All the other tests are negative. The major fermentation end-product is propionate. The major cellular fatty acid is iso-$C_{15:0}$. The predominant respiratory quinone is MK-10. Minor respiratory quinones are MK-9 and MK-11. The DNA G+C content of the type strain is 44.2 mol%.

Type strain, H184^T (=DSM 106867^T=LMG 30602^T), was isolated from human faeces. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and *hsp60* gene sequence of *B. faecalis* strain H184^T are MG963286 and MH255795. The whole genome sequence of strain H184^T has been deposited into GenBank/EMBL/DDBJ under accession number CP032819-CP032820.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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