International Journal of Systematic and Evolutionary Microbiology (2014), **64**, 000–000 **DOI** 10.1099/ijs.0.061739-0

Dysgonomonas macrotermitis sp. nov., isolated from the hindgut of a fungus-growing termite

Ying-jie Yang,[1] Ning Zhang,[1] Shi-qi Ji,[2] Xin Lan,[3] Kun-di Zhang,[2] Yu-long Shen,[1] Fu-li Li[2] and Jin-feng Ni[1]

Correspondence
Jin-feng Ni
jinfgni@sdu.edu.cn
Fu-li Li
lifl@qibebt.ac.cn

[1]State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

[2]Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, PR China

[3]Department of Biochemistry, Qingdao Vocational and Technology College, Qingdao 266555, PR China

A Gram-stain-negative, facultatively anaerobic, non-motile and coccoid- to short-rod-shaped bacterium, designated strain Dys-CH1^T, was isolated from the hindgut of a fungus-growing termite *Macrotermes barneyi*. The optimal pH and cultivation temperature of strain Dys-CH1^T were pH 7.2–7.6 and 35–37 °C, respectively. Sequence analysis of 16S rRNA gene showed that Dys-CH1^T shared 94.6 % and 90.9 % similarity with *Dysgonomonas capnocytophagoides* JCM 16697^T and *Dysgonomonas gadei* CCUG 42882^T, respectively. Strain Dys-CH1^T was found to be different from other species of the genus *Dysgonomonas* with validly published names with respect to taxonomically important traits, including habitat, biochemical tests, DNA G+C content, bile resistance, fatty-acid composition and susceptibility to antimicrobial agents. On the basis of these characteristics, strain Dys-CH1^T represents a novel species of the genus *Dysgonomonas* for which the name *Dysgonomonas macrotermitis* sp. nov. is proposed. The type strain is Dys-CH1^T (=JCM 19375^T=DSM 27370^T).

The genus *Dysgonomonas*, a member of the family *Porphyromonadaceae* in the phylum *Bacteroidetes*, was established by Hofstad *et al.*, (2000). The type species of the genus is *Dysgonomonas gadei* (Hofstad *et al.*, 2000). At the time of writing, the genus *Dysgonomonas* includes five species with validly published names: *D. gadei*, *D. capnocytophagoides*, *D. mossii*, *D. hofstadii* and *D. oryzarvi*. The first four species were isolated from human clinical specimens (Hofstad *et al.*, 2000; Lawson *et al.*, 2002, 2010) and last one, *D. oryzarvi*, was from a microbial fuel cell (Kodama *et al.*, 2012). To investigate the symbiotic roles of the gut microbiota in fungus-growing termites, we isolated bacterial strains from the hindgut of the fungus-growing termite *Macrotermes barneyi*. One strain, Dys-CH1^T, (closely related to *D. capnocytophagoides* JCM 16697^T, 94.6 % 16S rRNA gene sequence similarity), whose 16S rRNA gene sequence was nearly identical to uncultured bacterial clone BOf7-08 from another fungus-growing termite *Odontotermes formosanus*, was characterized.

Strain Dys-CH1^T was isolated from the hindgut homogenate samples under strictly anaerobic conditions. The sample was serially diluted with pre-reduced 0.1 mol PBS l^{-1} (0.145 mol NaCl l^{-1}, 0.15 mol sodium phosphate l^{-1}; pH 7) and spread onto modified Gifu Anaerobic Medium (GAM) agar supplemented with antibiotics (norfloxacin 40 μg ml^{-1}, Sigma) (Nagai *et al.*, 2010). The plates were incubated for three days at 37 °C in anaerobic jars sealed in an anaerobic glove box (Coy Laboratory Products) which contained 88 % nitrogen, 7 % hydrogen and 5 % CO_2. Resulting colonies were picked and further purified by restreaking on new plates, then identified preliminarily by 16S rRNA gene sequencing. Among ten investigated colonies, seven colonies were closely related to species of the genus *Dysgonomonas*. One colony (later named as strain Dys-CH1^T, closely related to *D. capnocytophagoides* JCM 16697^T; 94.6 % 16S rRNA gene sequence similarity), had a nearly identical 16S rRNA gene sequence to that of the uncultured bacterial clone BOf7-08 from another fungus-growing termite *Odontotermes formosanus*. Routine cultivation of the isolate was performed at 37 °C in a modified medium 14 (MM14; Japan Collection of Microorganisms), 50 ml l^{-1} horse blood was replaced by 5 g l^{-1} digested serum powder and 5 mg l^{-1} haem. Bacterial cells were stored at −80 °C in MM14 supplemented with 15 % (v/v) glycerol.

Phenotypic features of the isolate Dys-CH1^T were determined by cultivating the isolate on MM14 at 35 °C. Cell

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Dys-CH1^T is HG315620.

Three supplementary tables are available with the online version of this paper.

morphology was determined by using an optical microscope (BX51; Olympus). Gram staining and motility tests were conducted according to standard procedures (Smibert & Krieg, 1994). Oxidase activity was determined from oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined from bubble production in 3 % (v/v) H_2O_2 solution. Growth requirements for X and V factors were examined using discs impregnated with X factor (haem), V factor (NAD), or both and trypticase soy agar as basal medium. Bile sensitivity was determined by adding bile salts (Oxoid) (0.5 %, w/v) to liquid medium MM14. The effects of different temperatures on growth were assessed on liquid medium MM14 incubated at 15, 20, 25, 28, 30, 35, 37, 40 and 42 °C. The pH range for growth was determined for the culture in MM14 at various pH values, adjusted with HCl or NaOH (1 mol l^{-1}). *D. capnocytophagoides* JCM 16697^T was used as a reference strain.

For physiological and biochemical characterizations, API ZYM, API 20E and API 20NE systems (bioMérieux) were used according to the manufacturer's instructions. Reference strain *D. capnocytophagoides* JCM 16697^T was also characterized using the API systems in triplicate. To investigate the assimilation of single carbon sources, fermentative growth was examined in the basal peptone (BP) medium that contained the inorganic ingredients of the modified 1/10 PYGV medium, 0.5 g trypticase peptone l^{-1} and 10 mmol HEPES l^{-1} (pH 7.3) (Kodama *et al.*, 2012). The concentration of each added carbon source was 0.5 % (w/v). The medium without inoculation and without additional carbon source was used as a negative control and the medium with inoculation and with glucose as a positive control. Growth was monitored by measuring OD_{600}. Utilization of different electron acceptors was tested in the BP medium supplemented with 1.8 g glucose l^{-1} as the sole carbon source and sodium sulfite, sodium sulfate, sodium thiosulfate, sodium nitrate, or O_2 (the head-space N_2 gas was replaced with air) as the sole electron acceptor according to the protocol of Kodama *et al.* (2012). Organic acids were analysed by HPLC. Antibiotic sensitivity was assessed as follows: 0.1 ml cell suspension (OD_{600} ~0.1) was inoculated into 5 ml MM14 liquid medium supplemented with antibiotic. The inoculated media were incubated overnight at 35 °C. *D. capnocytophagoides* JCM 16697^T was used as a reference under the same culture conditions. All tests were performed in triplicate.

DNA G+C content was determined by reversed-phase HPLC as described by (Mesbah *et al.*, 1989). The 16S rRNA gene was amplified by PCR using the universal primers, 27f (5′-AGAGTTTGATCCTGGCT-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The 16S rRNA gene sequence of strain Dys-CH1^T was submitted to GenBank and similar sequences were searched for in public databases using the BLAST algorithm and sequence similarity was analysed with the EzTaxon-e server (Kim *et al.*, 2012). Multiple alignments of the sequences and reconstruction of the phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) were performed using MEGA5.1 software (Saitou & Nei, 1987; Tamura *et al.*, 2011).

Long-chain cellular fatty acid analysis was performed using a chloroform/methanol system. Cells were anaerobically cultured on MM14 agar base containing 0.5 % serum powder and 5 mg haem l^{-1} for 48 h at 37 °C and centrifuged. Saponification, methanolysis, extraction and identification of the fatty acid methyl esters were performed using the Microbial Identification System (Microbial ID) as described previously (Debelian *et al.*, 1997; Moore *et al.*, 1994).

Strain Dys-CH1^T was able to grow in modified GAM, MM14, modified 1/10 PYGV medium and BP medium supplemented with glucose. Colonies of strain Dys-CH1^T on modified GAM agar were circular, more translucent and larger than on MM14. Cells were Gram-stain-negative, non-motile, coccobacillus-shaped (approximately 1 μm × 2 μm). Growth was observed on nutrient agar around X discs but not around V discs, indicating a requirement for haem for its growth. Strain Dys-CH1^T could not grow in the presence of 0.5 % (w/v) bile salts, indicating that it was sensitive to bile. The complete morphological and biochemical data for strain Dys-CH1^T are given in the species description.

Results of physiological and biochemical properties by using the API 20E, API 20NE and API ZYM systems are included in the species description. The activity of *N*-acetyl-*β*-glucosaminidase was detected in strain Dys-CH1^T, but not detected in *D. capnocytophagoides* JCM 16697^T by the API ZYM and API 20NE systems. Sucrose was not fermented to produce acids by Dys-CH1^T but was fermented to produce acids by *D. capnocytophagoides* JCM 16697^T, according to API 20E tests. The activities of *β*-galactosidase and *α*-glucosidase from *D. capnocytophagoides* JCM 16697^T were stronger than those of strain Dys-CH1^T according to API ZYM tests. However, *β*-galactosidase was not detected from *D. capnocytophagoides* JCM 16697^T by the API 20E system. Data on assimilation of single carbon sources including monosaccharides, disaccharides, polysaccharides, amino acids, organic acids and organic alcohols are given in the species description. The carbohydrates described above were also used to perform fermentation tests with the reference strain. *D. capnocytophagoides* JCM 16697^T was able to ferment sucrose and raffinose, but strain Dys-CH1^T was not. L-Cysteine was utilized by strain Dys-CH1^T, but not by *D. capnocytophagoides* JCM 16697^T. Interestingly, birchwood xylan, beechwood xylan and oatmeal xylan could be fermented by *D. capnocytophagoides* JCM 16697^T, while only birchwood xylan and beechwood xylan could be used by strain Dys-CH1^T.

The major fermentation products of Dys-CH1^T from glucose (10 mmol l^{-1}) in the BP medium were acetate (14.8 mmol l^{-1}), lactate (11.3 mmol l^{-1}), propionate (2.4 mmol l^{-1}), formate (2.4 mmol l^{-1}) and succinate (2.0 mmol l^{-1}) under anaerobic conditions. Under the same conditions, major products of *D. capnocytophagoides*

JCM 16697^T were acetate (20.8 mmol l^{-1}), lactate (3.9 mmol l^{-1}), propionate (2.2 mmol l^{-1}), formate (2.0 mmol l^{-1}) and succinate (2.0 mmol l^{-1}). Thus, the production order of major acids obtained by us from *D. capnocytophagoides* JCM 16697^T was identical to that for Dys-CH1^T. Acetate and lactate are also the main products for other species of the genus *Dysgonomonas*. For both Dys-CH1^T and *D. capnocytophagoides* JCM 16697^T, oxygen served as an electron acceptor in the presence of glucose, while sulfite, sulfate, thiosulfate and nitrate did not. The major product of strain Dys-CH1^T were lactate (5.6 mmol l^{-1}), acetate (5.0 mmol l^{-1}), propionate (0.9 mmol l^{-1}), formate (0.7 mmol l^{-1}) and succinate (0.6 mmol l^{-1}) when oxygen served as the electric acceptor. As for *D. capnocytophagoides* 16697^T, the major products were lactate (10.6 mmol l^{-1}), acetate (24.2 mmol l^{-1}), propionate (2.0 mmol l^{-1}), formate (1.4 mmol l^{-1}) and succinate (1.2 mmol l^{-1}).

The fatty acids of strain Dys-CH1^T were anteiso-$C_{15:0}$ (31.8 %), iso-$C_{17:0}$ 3-OH (12.4 %), $C_{16:0}$ (7.8 %), $C_{17:0}$ 3-OH (7.3 %), iso-$C_{16:0}$ 3-OH (7.0 %), iso-$C_{14:0}$ (6.1 %), $C_{18:0}$ (4.8 %), $C_{16:0}$ 3-OH (4.5 %), iso-$C_{15:0}$ (3.8 %), $C_{17:0}$ 2-OH (3.5 %), iso-$C_{16:1}$ /$C_{14:0}$ 3-OH (3.4 %), $C_{15:0}$ 3-OH (4.0 %), anteiso-$C_{13:0}$ (1.9 %) and $C_{17:0}$ (1.7 %). This fatty acid profile was similar to those of species of the genus *Dysgonomonas*, although fatty acids were present in different proportions. Detailed fatty acid compositions of the novel isolate and related strains are given in Table S1 (available in the online Supplementary Material). The genomic DNA G+C content of strain Dys-CH1^T was 40.0 mol%.

The susceptibility of strain Dys-CH1^T to antimicrobial agents was investigated and compared with data for the reference strain *D. capnocytophagoides* JCM 16697^T by the same method and data for *D. gadei* CCUG 42882^T from Hofstad *et al.* (2000; Table S2). Dys-CH1^T was resistant to clindamycin phosphate (MIC, 100 μg ml^{-1}). In contrast, *D. capnocytophagoides* JCM 16697^T was sensitive to clindamycin phosphate (MIC 20 μg ml^{-1}). The MICs of antibiotics for strain Dys-CH1^T are given in the species description and Table S2.

Phylogenetic comparison of the 16S rRNA gene sequence from strain Dys-CH1^T revealed that it was most closely related to *D. capnocytophagoides* JCM 16697^T (94.6 % 16S rRNA gene sequence similarity) (Fig. 1). Compared with *D. mossii* CCUG 43457^T, *D. oryzarvi* JCM 16859^T, *D. gadei* CCUG 42882^T and *D. hofstadii* CCUG 54731^T, 16S rRNA gene sequence similarities of Dys-CH1^T were 92.3 %, 92.2 %, 90.9 % and 90.3 %, respectively. In addition to these type strains, strain Dys-CH1^T showed 16S rRNA gene sequence similarity (>97 %) to some uncultured bacterial clones that were retrieved from the guts of some fungus-growing termites which included BOf7-08 (GenBank accession number AB288912, 99.6 %) from *Odontotermes formosanus* (Shinzato *et al.*, 2007). Comparison of 16S rRNA gene sequences with those of other uncultured clones are given in Table S3. These data indicate that relatives of strain Dys-CH1^T are widely distributed in the guts of invertebrate animals, especially fungus-growing termites, cockroaches and beetles.

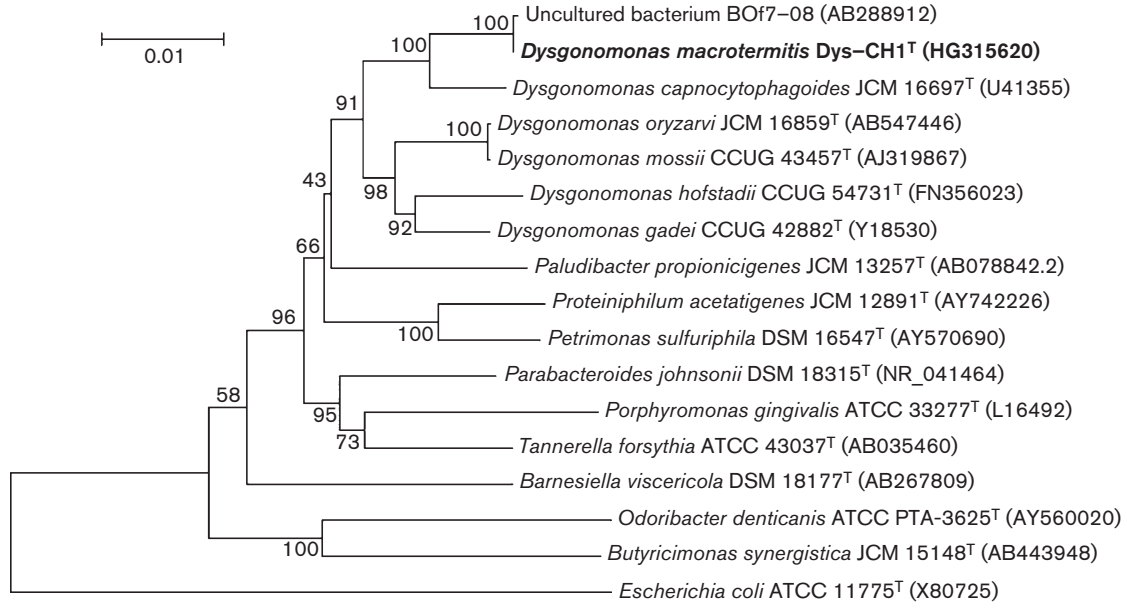

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain Dys-CH1^T in the family *Porphyromonadaceae*. *Escherichia coli* ATCC 11775^T was used as the outgroup. GenBank accession numbers of the sequences are given in parentheses. Numbers at branch nodes are bootstrap values (per 100 trials). Bar, 0.01 substitutions per site.

Table 1. Differential characteristics of strain Dys-CH1^T and members of the genus *Dysgonomonas*

Strains: 1, Dys-CH1^T; 2, *D. capnocytophagoides* JCM 16697^T (unless indicated otherwise, data were taken from Hofstad *et al.*, 2000); 3, *D. mossii* CCUG 43457^T (Lawson *et al.*, 2002); 4, *D. oryzarvi* JCM 16859^T (Kodama *et al.*, 2012); 5, *D. gadei* CCUG 42882^T (Hofstad *et al.*, 2000); 6, *D. hofstadii* CCUG 54731^T (Lawson *et al.*, 2010). +, Positive; −, negative; ND, no data available; L, lactate; A, acetate; P, propionate; S, succinate.

Characteristic	1	2	3	4	5	6
Isolation source	Termite hindgut	Human clinical sample	Human clinical sample	Microbial fuel cell	Human clinical sample	Human clinical sample
16S rRNA gene sequence similarity with strain Dys-CH1^T (%)	100	94.6	92.3	92.2	90.9	90.3
DNA G+C content (mol%)	40.0	39.5	38.5	37.5	39.9	ND
Resistance to bile	−	+	+	−	+	+
Aerobic growth	+	+	+	+	+ (microaerobic)	+
Production of:						
Indole	−	−	+	+/−	+	+
α-Arabinosidase	+	+	+/−	+	+	+
N-acetyl-β-glucosaminidase	+	−	+	+	+	+
β-Glucuronidase	−	−	+	−	ND	−
β-Galactosidase	+	+	+	+	−	+
α-Fucosidase	−	−	+	+/−	+	+
Fermentative growth on:						
Sucrose	−	+	+	+	+	+
Trehalose	−	−*	−	−	+	+
L-Arabinose	+	+*	−	+	+	−
L-Rhamnose	+	+*	−	+	+	−
Raffinose	−	+*	−	+	+	ND
Major fermentation products from glucose†	L, A	L, A* (P, L, S)	L, A, P	L, A	ND	ND
Major fatty acids	anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:0}$,	iso-$C_{14:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ 3-OH	anteiso-$C_{15:0}$, $C_{15:0}$, iso-$C_{14:0}$	anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:0}$ 3-OH	anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{14:0}$	anteiso-$C_{15:0}$, iso-$C_{14:0}$, iso-$C_{16:0}$ 3-OH

*Determined during this study.

†Major fermentation products when grown on glucose (10 mmol l^{-1}) in the modified 1/10 PYGV medium.

Features of strain Dys-CH1^T in comparison with those of species of the genus *Dysgonomonas* with validly published names are summarized in Table 1. Strains of species of the genus *Dysgonomonas* were mostly isolated from human clinical specimens except a bioreactor of microbial fuel cell inoculated with paddy field soil (Hofstad *et al.*, 2000; Kodama *et al.*, 2012; Lawson *et al.*, 2002, 2010). In addition to 16S rRNA gene sequence and habitats, other distinguishing features include bile resistance, substrate utilization patterns, fatty acid composition and susceptibility to antimicrobial agents. Based on the physiological and biochemical properties, phenotypic, chemotaxonomic and genotypic characteristics described above, strain Dys-CH1^T represents as a novel species of the genus *Dysgonomonas* for which the name *Dysgonomonas macrotermitis* sp. nov. is proposed.

Description of *Dysgonomonas macrotermitis* sp. nov.

Dysgonomonas macrotermitis (ma.cro.ter'mi.tis. N.L. gen. n. *macrotermitis* of the termite *Macrothermes*, where the organism was first isolated).

Cells are Gram-stain-negative, non-motile, coccoid- to short-rod-shaped. Colonies on modified GAM agar are 1–2 mm in diameter, circular, milky white after incubation for 48 h at 37 °C. Catalase-positive and oxidase-negative. Facultatively anaerobic. The pH and temperature ranges for growth are pH 5.0–9.0 and 20–40 °C (optimum at pH 7.2 and 35–37 °C). No growth is detected below 15 °C or above 42 °C. Growth is observed on nutrient agar around X but not V discs, indicating a requirement for haem. Does not grow in the presence of bile salts, indicating sensitivity to bile. Glucose is fermented with production of acid but no gas. ONPG positive. Negative for citrate utilization, H_2S production, arginine dihydrolase and indole production. Aesculin is hydrolysed, but gelatin and urea are not. Nitrate is not reduced to nitrite. Does not catalyse reaction of acetonate to produce 3-hydroxyethyl ketone. Decarboxylation reactions of arginine, lysine and ornithine are all negative. Activity is detected for alkaline phosphatase, butyrate esterase, acid phosphatase, phosphoamidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Caprylate esterase lipase, myristate lipase, leucine arylamidase, valine arylamidase, cystine arylamidases, trypsin, chymotrypsin, β-glucuronidase, α-mannosidase, α-fucosidase and tryptophan deaminase are not produced. The following substrates are utilized as sole carbon sources: glucose, mannitose, *N*-acetyl-β-glucosaminidase, D-galactose, L-arabinose, D-xylose, cellobiose, maltose, lactose, D-mannose, D-ribose, L-rhamnose, fructose, melibiose, saligenin, methionine, L-cysteine, yeast extract, birchwood xylan, beechwood xylan and starch. The following substrates are not utilized as sole carbon sources: trehalose, sucrose, sorbose, raffinose, D-sorbitol, D-mannitol, inositol, glycine, arginine, asparagine, leucine, lysine, histidine, threonine, tryptophan, tyrosine, methanol, ethanol, 2-propanol, formate, acetate, citrate, gluconate, decanoic acid, hexanedioic acid, malic acid, phenylacetic acid, peptone (Sango Biotech), trypticase peptone, oatmeal xylan, CM-cellulose and crystalline cellulose. Acids are produced from glucose, rhamnose, melibiose, amygdalin and arabinose. Major products of glucose fermentation are acetate and lactic acid. Resistant to the following antibiotics: levofloxacin hydrochloride, azithromycin, erythromycin, chloramphenicol, thiamphenicol, penicillin G, ampicillin, norfloxacin, clindamycin phosphate, spectinomycin and gentamicin sulfate. The major whole-cell fatty acids are anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:0}$, $C_{17:0}$ 3-OH and iso-$C_{16:0}$ 3-OH.

Strain Dys-CH1^T (=JCM 19375^T=DSM 27370^T) was isolated from the hindgut of a fungus-growing termite. The genomic DNA G+C content of the type strain is 40.0 mol%.

Acknowledgements

This work was supported by grants from the National Basic Research Program of China (973 program: 2011CB707402), the National Natural Science Foundation of China (31272370, 30870085) and Key Laboratory of Biofuel, Chinese Academy of Sciences (CASKLB201305).

References

Debelian, G. J., Olsen, I. & Tronstad, L. (1997). Distinction of *Prevotella intermedia* and *Prevotella nigrescens* from endodontic bacteremia through their fatty acid contents. *Anaerobe* **3**, 61–68.

Hofstad, T., Olsen, I., Eribe, E. R., Falsen, E., Collins, M. D. & Lawson, P. A. (2000). *Dysgonomonas* gen. nov. to accommodate *Dysgonomonas gadei* sp. nov., an organism isolated from a human gall bladder, and *Dysgonomonas capnocytophagoides* (formerly CDC group DF-3). *Int J Syst Evol Microbiol* **50**, 2189–2195.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kodama, Y., Shimoyama, T. & Watanabe, K. (2012). *Dysgonomonas oryzarvi* sp. nov., isolated from a microbial fuel cell. *Int J Syst Evol Microbiol* **62**, 3055–3059.

Lawson, P. A., Falsen, E., Inganäs, E., Weyant, R. S. & Collins, M. D. (2002). *Dysgonomonas mossii* sp. nov., from human sources. *Syst Appl Microbiol* **25**, 194–197.

Lawson, P. A., Carlson, P., Wernersson, S., Moore, E. R. & Falsen, E. (2010). *Dysgonomonas hofstadii* sp. nov., isolated from a human clinical source. *Anaerobe* **16**, 161–164.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic by high performance liquid chromatography. *Int J Syst Evol Microbiol* **39**, 159–167.

Moore, L. V., Bourne, D. M. & Moore, W. E. (1994). Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic Gram-negative bacilli. *Int J Syst Bacteriol* **44**, 338–347.

Nagai, F., Morotomi, M., Watanabe, Y., Sakon, H. & Tanaka, R. (2010). *Alistipes indistinctus* sp. nov. and *Odoribacter laneus* sp. nov.,

common members of the human intestinal microbiota isolated from faeces. *Int J Syst Evol Microbiol* **60**, 1296–1302.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Shinzato, N., Muramatsu, M., Matsui, T. & Watanabe, Y. (2007). Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Odontotermes formosanus*. *Biosci Biotechnol Biochem* **71**, 906–915.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–655. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Dear Authors,

Please find enclosed a proof of your article for checking.

When reading through your proof, please check carefully authors' names, scientific data, data in tables, any mathematics and the accuracy of references. Please do not make any unnecessary changes at this stage. All necessary corrections should be marked on the proof at the place where the correction is to be made; please mark up the correction in the PDF and return it to us (see instructions on marking proofs in Adobe Reader).

Any queries that have arisen during preparation of your paper for publication are listed below and indicated on the proof.

Please provide your answers when returning your proof.

Please return your proof by email (sgmprod@charlesworth-group.com) within 2 days of receipt of this message.

Query no.	Query
1	Please check and ensure the copyright for this paper (bottom right of first page) is updated as per the editorial office's request.
2	The sentence describing MM14 has been edited for clarity. Please check and confirm edits are ok and that the intended meaning has been retained.
3	Please give the conditions for the PCR or a reference from which they can be obtained.
4	In Table 1, the row header '16S rRNA gene sequence similarity' has been expanded to include the strain details. Please check and confirm ok.

Ordering reprints for SGM journals

As a result of declining reprint orders and feedback from many authors who tell us they have no use for reprints, **SGM no longer provides free reprints to corresponding authors**; instead, corresponding authors will receive two emails:

i) An email including a link to download the published PDF of their paper. You can forward this link to co-authors or others, and they can also use it to download the published PDF. The link can be used up to 25 times. This email will be sent out at around the time your article is published online.

ii) An email including a link to the SGM Reprint Service. You can forward this email to your co-authors if you wish, so that they can order their own reprints directly, or to your finance or purchasing department, if orders are placed centrally. This email will be sent out at around the time that your article is finalized for printing.

When you click on the link in this second email, you will be taken to an order page to place your reprint order. Like most online ordering sites, it is necessary to set up an account and provide a delivery address while placing your order, if you do not already have an account. Once an account and delivery address have been set up, these details will be stored by the system for use with future orders. Payments can be made by credit card, PayPal or purchase order.

As reprint orders are despatched by courier, there is a charge for postage and packing.

SUMMARY

- You can create or update your reprint account at any time at http://sgm-reprints.charlesworth.com/
- You will be sent an email when the reprints of this paper are ready for ordering
- **You cannot order reprints of this paper before this email has been sent**, as your paper will not be in the system
- Reprints can be ordered at any time after publication
- You will also receive an email with a link to download the PDF of your published paper

The reprint ordering details will be emailed to the author listed as the corresponding author on the journal's manuscript submission system. If your paper has been published (the final version, not the publish-ahead-of-print version) but you have not received this notification, email reprints@sgm.ac.uk quoting the journal, paper number and publication details.

If you have any questions or comments about the reprint-ordering system or about the link to your published paper, email reprints@sgm.ac.uk