INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Byun *et al.*, *Int. J. Syst. Evol. Microbiol.* 2023;73:006187
DOI 10.1099/ijsem.0.006187

MICROBIOLOGY SOCIETY

OPEN ACCESS

Parabacteroides leei sp. nov., isolated from human blood

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Abstract

An obligate anaerobic, Gram-negative, rod-shaped and non-spore-forming bacterium, designated as strain GYB001^T, was isolated from the blood of a patient with a sigmoid colon perforation. Taxonomic characterization of the novel isolate was performed using a polyphasic approach. A phylogenetic analysis based on 16S rRNA gene and whole genome sequences revealed that GYB001^T represented a member of the genus *Parabacteroides*, in the family *Tannerellaceae*. The closest species, based on 16S rRNA sequence, was *Parabacteroides gordonii* DSM 23371^T with 97.4% similarity. Average nucleotide identity and digital DNA–DNA hybridization values between strain GYB001^T and *P. gordonii* DSM 23371^T were 86.7 and 28.7% and between GYB001^T and *Parabacteroides faecis* JCM 18682^T were 86.6 and 27.7%, respectively. The genome was 6.57 Mbp long with 43.3 mol% G+C content. Colonies on Brucella blood agar (BBA) were circular, convex, smooth, grey and small in size. Growth was observed on trypticase soy agar (TSA), TSA +5% sheep blood and *Euglena gracilis* agar. Growth occurred at 18–42 °C on BBA in the presence of 0–3% NaCl (w/v) and at pH 6.0–8.5. The major polar lipids were phosphatidylethanolamine and phospholipids. The major fatty acids in strain GYB001^T were anteiso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH, and the predominant respiratory quinones were menaquinone-10 (MK-10) and MK-9. The cell wall contained *meso*-diaminopimelic acid. Considering these phenotypic features and comparative genome analyses, we propose strain GYB001^T as the type strain of *Parabacteroides leei* sp. nov. (=KCTC 25738^T=KBN12P06525^T=LMG 32797^T).

INTRODUCTION

The genus *Parabacteroides* was proposed with the reclassification of three *Bacteroides* species [1]. At the time of writing this paper, 11 species have been designated with validly published names in *Parabacteroides*, including *Parabacteroides distasonis* [1, 2], *Parabacteroides goldsteinii* [3], *Parabacteroides merdae* [1], *Parabacteroides johnsonii* [4], *Parabacteroides gordonii* [5], *Parabacteroides chartae* [6], *Parabacteroides chinchilla* [7], *Parabacteroides faecis* [8], *Parabacteroides chongii* [9], *Parabacteroides acidifaciens* [10] and the most recently reported *Parabacteroides hominis* [11]. The members of *Parabacteroides* are isolated from human clinical specimen including stool, and considered as members of the gut microbiota.

Members of the genus *Parabacteroides* are obligate anaerobic, non-motile, non-sporulating and Gram-negative rods. Members of this genus are mainly isolated from human faeces and other clinical specimens, such as peritoneal fluids and intra-abdominal abscess fluid [3, 11]. Chemotaxonomically, the predominant respiratory quinones of *Parabacteroides* include menaquinone-9 (MK-9) and MK-10, and the major fatty acids are iso-$C_{17:0}$ 3-OH and iso-$C_{15:0}$ [1, 3, 4, 6–10]. The G+C content ranges from 37.2 to 47.6 mol%. Here, we report the isolation of a novel bacterial strain GYB001^T of *Parabacteroides* from the blood specimen of a patient with a sigmoid colon perforation. The chemotaxonomic, phenotypic and genomic traits were investigated. The outcomes of these analyses indicate that the strain GYB001^T should be considered as the type strain of a new *Parabacteroides* species.

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Keywords: 16S rRNA; blood; genomics; *Parabacteroides*; phylogeny.
Abbreviations: ANI, average nucleotide identity; AP, alignment percentage; BBA, Brucella blood agar; dDDH, digital DNA–DNA hybridization; EG, *Euglena gracilis*; ML, maximum-likelihood; NJ, neighbour-joining; UPGMA, unweighted pair group method with arithmetic mean.
The 16S rRNA gene sequence of strain GYB001^T is available at GenBank under ON362233. This Whole Genome Shotgun project has been deposited under the accession JAMBQG000000000. The version described in this paper is version JAMBQG000000000.1.
Three supplementary figures and three supplementary tables are available with the online version of this article.

STRAIN ISOLATION

A novel species belonging to the genus *Parabacteroides* was isolated from the blood of an 83-year-old Korean female patient with a sigmoid colon perforation. Strain GYB001^T was detected in an anaerobic blood culture bottle, FN, using a BacT/Alert Virtuo instrument (bioMérieux) and subcultured on Brucella blood agar (BBA). The plates were incubated in an anaerobic chamber at 35°C. After 24 h of incubation, the colonies were found to be non-haemolytic, circular, convex, small in size (diameter, 1 mm) and grey in colour. For further study, the isolate was purified via repeated subculture on BBA in an anaerobic chamber at 35°C and stored in 20% skim milk at −70°C.

PHENOTYPIC AND BIOCHEMICAL CHARACTERISTICS

Gram staining was performed as described previously [12]. Catalase activity was determined based on the production of effervescence after addition of 3% (v/v) hydrogen peroxide. Cell size, shape and flagella were determined using a Libra 120(120 kV) transmission electron microscope (Carl Zeiss) and a Nikon light microscope (×1000 magnification) after culturing cells for 2 days at 35°C on BBA. Cell motility was assessed using Brucella broth supplemented with 0.2% agar [13].

The growth of strain GYB001^T was evaluated at different temperatures, pH, sodium chloride (NaCl) concentration and media, including Reasoner's 2A agar, nutrient agar, trypticase soy agar (TSA), TSA +5% sheep blood, Luria–Bertani agar, de Man–Rogosa–Sharpe agar and *Euglena gracilis* (EG) agar (all from BD Difco). Growth was assessed using measurements or colony-forming units after 7 days of incubation at 4C, 10, 18, 25, 30, 37, 42, 45 and 50°C. Growth at various pH values (pH 4–10 at intervals of 0.5 pH units) was monitored after 7 days of incubation at 37°C. The following three buffers at 20 mM final concentration were used to adjust the pH of the Brucella broth: acetate buffer for pH 4.0–5.5, phosphate buffer for pH 6.0–8.0 and Tris buffer for pH 8.5–10.0. Salt tolerance was tested in Brucella blood broth, which was made with casein peptone, meat peptone, yeast extract (BD Difco), dextrose (Samchun Chemicals) and sodium bisulfite (Sigma-Aldrich), where the concentration of NaCl was adjusted from 1–10.0% (w/v at intervals of 1% unit). The bile tolerance test was performed using BBA containing 20% bile.

Biochemical reactions were tested using the API 20A kit (bioMérieux) and Rapid ID 32A kit (bioMérieux) according to the manufacturer's instructions. The differences in phenotypic and biochemical characteristics between strain GYB001^T and related species in the genus *Parabacteroides* are listed in Table 1.

CHEMOTAXONOMIC ANALYSIS

Cellular fatty acid profiles were determined for strains cultured on BBA for 2 days at 35°C. Cellular fatty acids were saponified, methylated and extracted according to the Sherlock Microbial Identification System protocol. The fatty acid methyl esters were identified according to the standard protocol of the Sherlock Microbial Identification System version 6.01 (MIS, database TSBA6, MIDI Inc.) [14]. Isoprenoid quinones were extracted from cells grown on BBA for 2 days using chloroform–methanol (2:1, v/v), evaporated under vacuum conditions, and extracted again in n-hexane–water (1:1, v/v). The crude n-hexane–quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and then analysed via high-performance liquid chromatography, as previously described [15]. Polar lipids were extracted from freeze-dried cells, examined using two-dimensional thin-layer chromatography (TLC) and identified as previously described [16]. Diaminopimelic acid content was analysed using two-dimensional TLC [17]. The fatty acid profiles of strain GYB001^T and the reference strains are presented in Table S1 (available in the online version of this article). The major cellular fatty acids were anteiso-$C_{15:0}$ (46.7%), iso-$C_{17:0}$ 3-OH (10.7%), $C_{18:1}\omega9c$ (8.4%) and $C_{16:0}$ 3-OH (8.4%). Also, $C_{17:0}$ 2-OH (6.1%), iso-$C_{15:0}$ (5.1%) $C_{17:0}$ 3-OH (1.4%) and summed feature 5 (2.4%) were identified. Comparative fatty acid profiles of the strain and other species of the genus *Parabacteroides* indicated that strain GYB001^T could be differentiated from closely related species based on the relatively higher amounts of anteiso-$C_{15:0}$ and $C_{18:1}\omega9c$. Strain GYB001^T had a lower amount of iso-$C_{17:0}$ 3-OH than that in other *Parabacteroides* species. The predominant respiratory quinones were identified as menaquinones MK-10 and MK-9. The polar lipids detected were phosphatidylethanolamine, unknown phospholipids, unknown aminophospholipids, unknown glycolipids (glycolipid) and unknown lipids (Fig. S1, available in the online version of this article). The cell wall contained *meso*-diaminopimelic acid.

PHYLOGENETIC ANALYSIS

Genomic DNA was extracted using the commercial FastDNA Soil Kit (MP Biomedicals). The 16S rRNA gene was amplified with two universal primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGGTTACCTTGTTACGACTT-3′). Then, purified PCR products were sequenced using universal primers 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTCCTTTRAGTTT-3′). The sequence of the 16S rRNA gene (1454 bp, GenBank accession no. ON362233) was determined using the ABI PRISM 3730XL Analyzer (Macrogen, Seoul, Republic of Korea). The sequence was compared with the 16S rRNA gene sequences of related taxa obtained from EzBioCloud (www.ezbiocloud.net/identify) database and showed highest sequence similarity to *P. gordonii* strain DSM 23371^T with 97.4% similarity and *P. chongii* strain KACC 19034^T with 97.2% similarity.

Table 1. Characteristics of strain GYB001^T and other *Parabacteroides* type strains

Strains: 1, GYB001^T; 2, *Parabacteroides gordonii* JCM 15724^T; 3, *Parabacteroides goldsteinii* JCM 13446^T; 4, *Parabacteroides chongii* B3181^T; 5, *Parabacteroides faecis* JCM 18682^T; 6, *Parabacteroides merdae* NCTC 13052^T; 7, *Parabacteroides johnsonii* DSM 18315^T; 8, *Parabacteroides acidifaciens* NBRC 113433^T; 9, *Parabacteroides chinchillae* DSM 29073^T; 10, *Parabacteroides distasonis* ATCC 8503^T; 11, *Parabacteroides chartae* DSM 24967^T. All the strains were positive for aesculin hydrolysis, *N*-acetyl-*β*-glucosaminidase, leucine arylamidase, glutamyl glutamic acid arylamidase, and acid production from lactose, sucrose, mannose and raffinose. All strains were negative for indole production, urease and gelatin hydrolysis. +, Positive; –, negative; ND, no data. The results for strains 1, 2, 3, 4, and 5 were from this study while other data were taken from previous studies [1, 4, 6, 7, 10].

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Temperature for growth:											
Range	18–42	20–40	18–40	20–40	25–40	25–45	ND	20–45	ND	20–40	10–40
Optimum	37	37	37	37	37	30–37	37	37–40	37	37	35–37
pH for growth:											
Range	6.0–8.5	4.5–8.5	5.5–9.0	5.5–8.5	4.5–9.0	5.5–8.0	ND	6–10	ND	5.5–8.0	5.5–8.5
Optimum	7.0	7.0–7.5	7.0–7.5	7.0	7.0–7.5	ND	ND	6.0–8.0	ND	ND	7.0–7.5
NaCl for growth (%):											
Range	0–3	0–3	0–5	0–2	0–3	0–4	ND	0–2.5	ND	0–2	0–2
Optimum	0–1	0	0–1	0–1	1	0.5–1.0	ND	ND	ND	0.5	0
Catalase	+	+	+	+	+	–	+	–	–	+	–
Acid production from:											
D-Glucose	–	+	–	+	+	+	+	+	+	+	+
D-Mannitol	+	–	–	+	+	–	–	+	–	–	+
Maltose	+	+	+	+	+	+	+	+	+	ND	+
Salicin	–	–	–	–	–	–	–	–	–	+	–
D-Xylose	+	+	+	+	+	+	+	+	–	+	+
L-Arabinose	–	+	–	+	–	–	+	+	–	–	+
Glycerol	–	–	–	–	–	–	–	+	–	–	–
Cellobiose	+	–	–	–	+	–	–	+	–	+	+
Melezitose	–	–	–	–	+	–	–	–	–	+	+
D-Sorbitol	–	–	–	–	–	–	–	–	–	–	+
L-Rhamnose	–	–	–	–	+	–	+	+	–	+	–
Trehalose	+	–	+	–	+	+	+	+	–	+	+
Gelatin hydrolysis	–	–	–	–	–	–	–	–	–	–	–
Arginine dihydolase	–	–	–	–	–	ND	–	–	–	ND	ND
α-Galactosidase	+	+	+	+	+	+	+	+	–	+	+
β-Galactosidase	+	+	+	+	+	+	+	+	–	+	+
β-Galactosidase-6-phosphate	+	–	+	+	+	+	–	–	–	+	–
α-Glucosidase	+	–	+	+	+	+	+	+	+	+	+
β-Glucosidase	+	–	+	+	+	+	–	–	+	+	+
α-Arabinosidase	+	+	–	–	+	+	+	+	–	+	–
β-Glucuronidase	–	–	+	+	–	+	+	+	–	–	–
Glutamic acid decarboxylase	–	–	–	+	+	+	+	+	+	+	–
α-Fucosidase	–	–	–	+	–	–	–	+	+	–	+

Continued

Table 1. Continued

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Alkaline phosphatase	+	−	−	+	+	+	+	+	+	+	+
Arginine arylamidase	+	+	+	+	+	+	+	+	+	+	ND
Proline arylamidase	+	+	+	+	−	ND	−	−	−	−	ND
Leucyl glycine arylamidase	+	+	+	+	+	+	+	+	+	+	ND
Phenylalanine arylamidase	+	+	+	+	+	+	+	+	+	+	−
Pyroglutamic acid arylamidase	+	+	+	+	−	+	−	−	−	−	−
Tyrosine arylamidase	+	+	+	+	+	+	+	+	+	+	−
Alanine arylamidase	+	+	+	+	+	+	+	+	+	+	ND
Glycine arylamidase	+	+	+	+	+	+	+	+	−	+	−
Histidine arylamidase	+	+	+	+	+	+	+	+	+	+	−
Serine arylamidase	+	+	+	+	+	+	+	+	+	+	−
DNA G+C content (mol%)	43.3	44.6	43	42.3	41.8	44	47.6	45.9	42.8	44	37.2

Phylogenetic analysis based on 16S rRNA gene sequences was performed using MEGA version 7.0 [18, 19] via the neighbour-joining (NJ) [20], maximum-likelihood (ML) [21] and maximum-parsimony (MP) [22] methods. An NJ tree was generated using Kimura's two-parameter method [23]. Branching patterns of the trees were evaluated by bootstrapping with 1000 replicates [21]. The bootstrap consensus trees resulting from the ML and MP methods supported the NJ tree (Fig. S2). Based on phylogenetic analysis, strain GYB001^T was closely related to and was considered a member of the genus *Parabacteroides*.

Genome-based identification was performed. Whole-genome sequencing was performed at ChunLab Inc. (Seoul, Republic of Korea) using an Illumina MiSeq system and a 300 bp paired-end reads sequencing kit (MiSeq Reagent Kit version 3; Illumina). Obtained raw data (GenBank accession no. NZ_JAMBQG000000000.1) were uploaded to the TrueBac ID cloud system (www.truebacid.com) and analysed using the TrueBac ID-Genome system (version 1.93). The N50 value of the genome was 270349 bp. The G+C content of strain GYB001^T was 43.3 mol%, which is in the range reported for members of the genus *Parabacteroides*. Average nucleotide identity (ANI) values were 86.7 and 86.6% between the strains GYB001^T and *P. gordonii* DSM 23371^T and between the strains GYB001^T and *P. faecis* JCM 18682^T, respectively. The genome sequences of the strain GYB001^T and *Parabacteroides* species were obtained using the TrueBac ID genome system and are listed in Table S2. DNA–DNA hybridization (DDH) was calculated using GGDC 3.0 [24]. DDH values of the genome were 28.7 and 27.7% between strains GYB001^T and *P. gordonii* DSM 23371^T and between GYB001^T and *P. faecis* JCM 18682^T, respectively. The ANI and digital DDH (dDDH) values between GYB001^T and 11 *Parabacteroides* species were 83.61–86.77% and 18.2–28.7%, respectively (Table S2). These values are significantly lower than the accepted threshold values for delineating prokaryotic species using ANI (94–96%) and dDDH (70%) [25, 26]. The ANI comparison was used to generate a set of whole genome phylogenetic trees from both alignment percentage (AP) and ANI calculations using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and (NJ using CLC Genomics Workbench version 22 (Qiagen). The phylogenetic tree based on whole-genome sequences showed that GYB001^T clustered with *P. gordonii* DSM 23371^T, *P. goldsteinii* DSM 19448^T, *P. hominis* KCTC 25129^T, *P. chongii* KACC 19034^T and *P. faecis* JCM 18682^T (Fig. 1). Only the tree generated using ANI and the UPGMA method is shown here as the result of the genome-based phylogenetic tree reconstruction is the same as that using either AP or ANI.

Genome annotations of the strain based on Rapid Annotation using Subsystem Technology are presented in Table S3 [27].

DESCRIPTION OF *PARABACTEROIDES LEEI* SP. NOV.

Parabacteroides leei (lee'i. N.L. gen. n. *leei*, named in honour of the Korean clinical microbiologist Kyungwon Lee).

Cells are Gram-negative, obligate anaerobic, non-motile and non-spore forming rods (2.1–4.7 µm long and 0.4–1.5 µm in diameter) (Fig. S3). Colonies on BBA plates after 24 h of incubation at 35°C under anaerobic conditions are 1 mm in diameter, grey, circular and convex. Catalase test is positive. Cells grow at 18–42°C (optimum, 37°C), at pH 6.0–8.5 (optimum, pH 7.0) and with 0–3.0% (w/v) NaCl (optimum, 0–1.0%) (Table 1). Growth occurs on TSA, TSA+5% sheep blood, EG agar and medium containing 20% (w/v) bile. Aesculin is hydrolysed and gelatin is not digested based on the API 20A system. Acid is produced from D-mannitol, lactose, sucrose, maltose, D-xylose, cellobiose, D-mannose, raffinose and trehalose, but not from D-glucose, salicin, L-arabinose, glycerol, melezitose, D-sorbitol or L-rhamnose based on the API 20A kit. Positive reactions are obtained using the

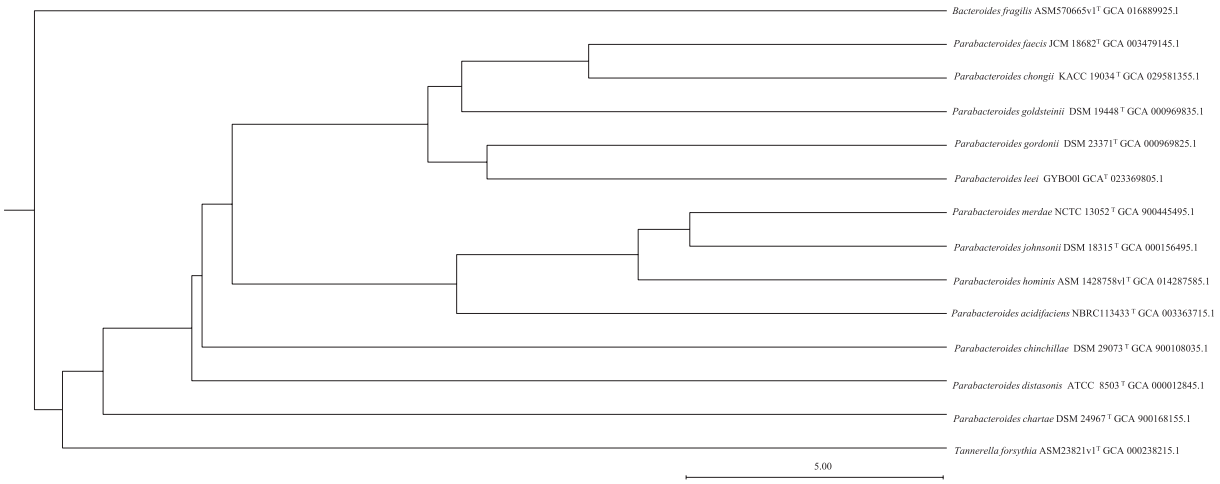

Fig. 1. Genome-based phylogenetic tree reconstruction (ANI UPGMA) showing the phylogenetic relationships between strain GYB001^T and related species. The scale bar indicates the difference (%) of ANI values among strains. The accession numbers are shown.

Rapid ID system 32A for α- and *β*-galactosidase, *β*-galactosidase-6-phosphate, α- and *β*-glucosidases, α-arabinosidase, *N*-acetyl-*β*-glucosaminidase, alkaline phosphatase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase, and serine arylamidase. Negative reactions are obtained using the Rapid ID 32A system for urease, arginine dihydolase, *β*-glucuronidase, glutamic acid decarboxylase, α-fucosidase, nitrate reduction and indole production. The major cellular fatty acids are anteiso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH. The polar lipids detected include phosphatidylethanolamine, unknown phospholipids, unknown aminophospholipids, unknown glycolipids and unknown lipids. The predominant respiratory quinones are menaquinones MK-10 and MK-9, consistent with the description of the quinone composition of the genus *Parabacteroides* [1]. Meso-diaminopimelic acid is detected in the cell wall. The genomic DNA G+C content of the type strain is 43.3 mol%.

The type strain, GYB001^T (=KCTC 25738^T=KBN12P06525 ^{T}=LMG 32797^T), was isolated from the blood sample of a patient with a colon perforation. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and whole-genome sequences of strain GYB001^T are ON362233 and JAMBQG000000000, respectively.

Funding information
This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea Government (Ministry of Science and ICT) (NRF-2020R1F1A1066895).

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

Ethical statement
According to the Gyeongsang National University Hospital Institutional Review Board (IRB) policy, IRB review of the study and the need for obtaining informed consent from the patient for the publication were waived (IRB No. 2022-10-005).

References

1. Sakamoto M, Benno Y. Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *nov Int J Syst Evol Microbiol* 2006;56:1599–1605.
2. Eggerth AH, Gagnon BH. The *Bacteroides* of human feces. *J Bacteriol* 1933;25:389–413.
3. Song Y, Liu C, Lee J, Bolanos M, Vaisanen M-L, *et al*. "*Bacteroides goldsteinii* sp. nov." isolated from clinical specimens of human intestinal origin. *J Clin Microbiol* 2005;43:4522–4527.
4. Sakamoto M, Kitahara M, Benno Y. *Parabacteroides johnsonii* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2007;57:293–296.
5. Sakamoto M, Suzuki N, Matsunaga N, Koshihara K, Seki M, *et al*. *Parabacteroides gordonii* sp. nov., isolated from human blood cultures. *Int J Syst Evol Microbiol* 2009;59:2843–2847.
6. Tan H-Q, Li T-T, Zhu C, Zhang X-Q, Wu M, *et al*. *Parabacteroides chartae* sp. nov., an obligately anaerobic species from wastewater of a paper mill. *Int J Syst Evol Microbiol* 2012;62:2613–2617.
7. Kitahara M, Sakamoto M, Tsuchida S, Kawasumi K, Amao H, *et al*. *Parabacteroides chinchillae* sp. nov., isolated from chinchilla (*Chincilla lanigera*) faeces. *Int J Syst Evol Microbiol* 2013;63:3470–3474.
8. Sakamoto M, Tanaka Y, Benno Y, Ohkuma M. *Parabacteroides faecis* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2015;65:1342–1346.

9. Kim H, Im W-T, Kim M, Kim D, Seo YH, *et al*. *Parabacteroides chongii* sp. nov., isolated from blood of a patient with peritonitis. *J Microbiol* 2018;56:722–726.
10. Wang Y-J, Xu X-J, Zhou N, Sun Y, Liu C, *et al*. *Parabacteroides acidifaciens* sp. nov., isolated from human faeces. *Int J Syst Evol Microbiol* 2019;69:761–766.
11. Liu C, Du M-X, Abuduaini R, Yu H-Y, Li D-H, *et al*. Enlightening the taxonomy darkness of human gut microbiomes with a cultured biobank. *Microbiome* 2021;9:119.
12. Buck JD. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* 1982;44:992–993.
13. Weon H-Y, Kim B-Y, Joa J-H, Son J-A, Song M-H, *et al*. *Methylobacterium iners* sp. nov. and *Methylobacterium aerolatum* sp. nov., isolated from air samples in Korea. *Int J Syst Evol Microbiol* 2008;58:93–96.
14. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. In: *MIDI Technical Note*, vol. 101. Newark, DE: MIDI Inc, 1990
15. Hiraishi A, Ueda Y, Ishihara J, Mori T. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* 1996;42:457–469.
16. Minnikin DE, Patel PV, Alshamaony L, Goodfellow M. Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Evol Microbiol* 1977;27:104–117.
17. Suzuki K-I, Komagata K. *Pimelobacter* gen. nov., a new genus of coryneform bacteria with LL-diaminopimelic acid in the cell wall. *J Gen Appl Microbiol* 1983;29:59–71.
18. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
19. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, *et al*. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007;23:2947–2948.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
21. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
22. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Biol* 1971;20:406–416.
23. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
24. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2021;50:D801–D807.
25. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:1–14.
26. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
27. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, *et al*. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.