ORIGINAL ARTICLE

Parabacteroides pekinense sp. nov.: a new bacterium isolated from the stool of a healthy man living in China

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Abstract

Strain Quantibio-BCGUT is a new species from the genus Parabacteroides that was isolated from a stool sample of a 49-year-old healthy Chinese male adult. Cells are Gram-negative and obligate anerobic bacilli. Strain Quantibio-BCGUT exhibits 95.86% 16S rRNA gene sequence similarity to Parabacteroides merdae strain JCM 9497 (NR_041343.1), the phylogenetically closely related species with standing in nomenclature. Major fatty acids are C16:0, C18:0 and C19:0-IS. Quantibio-BCGUT exhibits a high level of resistance to aztreonam. Growth occurred at pH 5.5–9.0. Optimal growth was observed at 35 °C in YCFA medium in anerobic condition, no growth occurs at 25 °C or 50 °C. Strain grows in YCFA medium in the presence of 0.1%–2.0% (w/v) NaCl (optimum 1.0%). Based on the phenotypic and phylogenetic evidence, OrthoANI values and results of the biochemical tests, the new species is named Parabacteroides pekinense sp. nov., for which strain Quantibio-BCGU T (= CGMCC = QHBCGU) is proposed as the type strain.

Keywords: Culturomics, gut, human microbiota, Parabacteroides pekinense sp. nov, taxonogenomics
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Introduction

The human gut microbiome has high impact on human health and disease [1,2]. Although it is important in maintain health, there is a big gap between the knowledge of microbial diversity and the understanding on the role of individual microbiome species, their interactions and functions [3]. With the development of culturomics, our knowledge of the human microbiota has been greatly enlarged through the discovery of previously uncultured bacteria [4,5]. Besides, multiple methods are now available to identify every isolated bacterium, which include matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), genome sequencing, phylogenetic analysis, and main phenotypic description [6,7].

Parabacteroides genus, a kind of gram-reaction-negative obligate anaerobes with no spore forming, can colonize the intestine, oral cavity, upper respiratory tract and the genital tract, mainly in the gut with a straight rods, arcs, spirals, or polymorphic shape. The genus *Parabacteroides*, proposed by Sakamoto & Benno [8], contains strain *Parabacteroides goldsteinii*, *Parabacteroides distasonis*, *Parabacteroides johnsonii*, *Parabacteroides merdae*, *Parabacteroides gordonii*, *Parabacteroides chartae* and so on [9]. The major products of *Parabacteroides* genus are beneficial acetic acid and succinic acid, which participate in ameliorating obesity, metabolic dysfunctions, cancer, and neuropathy diseases [10,11]. For this reason, *Parabacteroides* genus is accepted as beneficial bacterium.

In this work, we isolated a strain Quantibio-BCGU ^{T} from the stool of a healthy male Chinese adult as part of an exploration of the gut microbial diversity in Chinese adults. Combination of genotypic and phenotypic characteristics, the strain

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Quantibio-BCGU^T appeared to be closely related to species of the genus Parabacteroides. We then determined the taxonomic status of this novel strain. Based on the results presented here, we proposed that this strain should be classified as a novel species of the genus Parabacteroides, and nominated it as Parabacteroides pekinense sp. nov.

Isolation and growth conditions

In 2020, as part of an exploration of the gut microbial diversity in Chinese adults, a strain was isolated from the stool of a healthy male Chinese adult of 49 years of age living in Hainan province of China. The stool sample was initially enriched for 35 days in an anaerobic blood bottle (Autobio, Zhengzhou, China) with 5 mL of sheep's blood and 10 mL of 0.22 μm filtered rumen fluid (ELITE Biotech, Shanghai, China) in anaerobic conditions at 37 °C. Then the isolated strain Quantibio-BCGU^T was observed after a 48-h incubation at 37 °C on BD Columbia agar with 5% sheep blood medium (Heidelberg, Germany) under anerobic conditions. Screening with MALDI-TOF MS was performed on an Autof ms1000 (Autobio, Zhengzhou, China). The obtained spectra (Fig.1) were automatically imported into Autof ms1000 database 1.10.

Phenotypic characteristics of strain

Colonies were white and circular with a mean diameter of 1 mm. Bacterial cells were Gram-negative and rod-shaped, ranging in length from 1.75 to 2.88 μm and in width from 0.25 to 0.48 μm as revealed by scanning electron microscope (SEM) examination (TM4000 instrument, Hitachi Group, Krefeld, Germany) (Fig.2). The Parabacteroides pekinense sp. nov. strain Quantibio-BCGU^T shows catalase-negative activity. API 20A was performed at 37°C under anaerobic conditions. Positive reactions were observed for D-glucose, D-lactose, D-xylose, L-arabinose, esculin, D-mannose, D-raffinose, and L-rhamnose, whereas negative reactions were obtained for L-tryptophnen, urea, D-mannitol, D-maltose, salicin, gelatin, glycerol, D-cellobiose, D-melezitose, D-sorbitol, D-trehalse (Table 1).

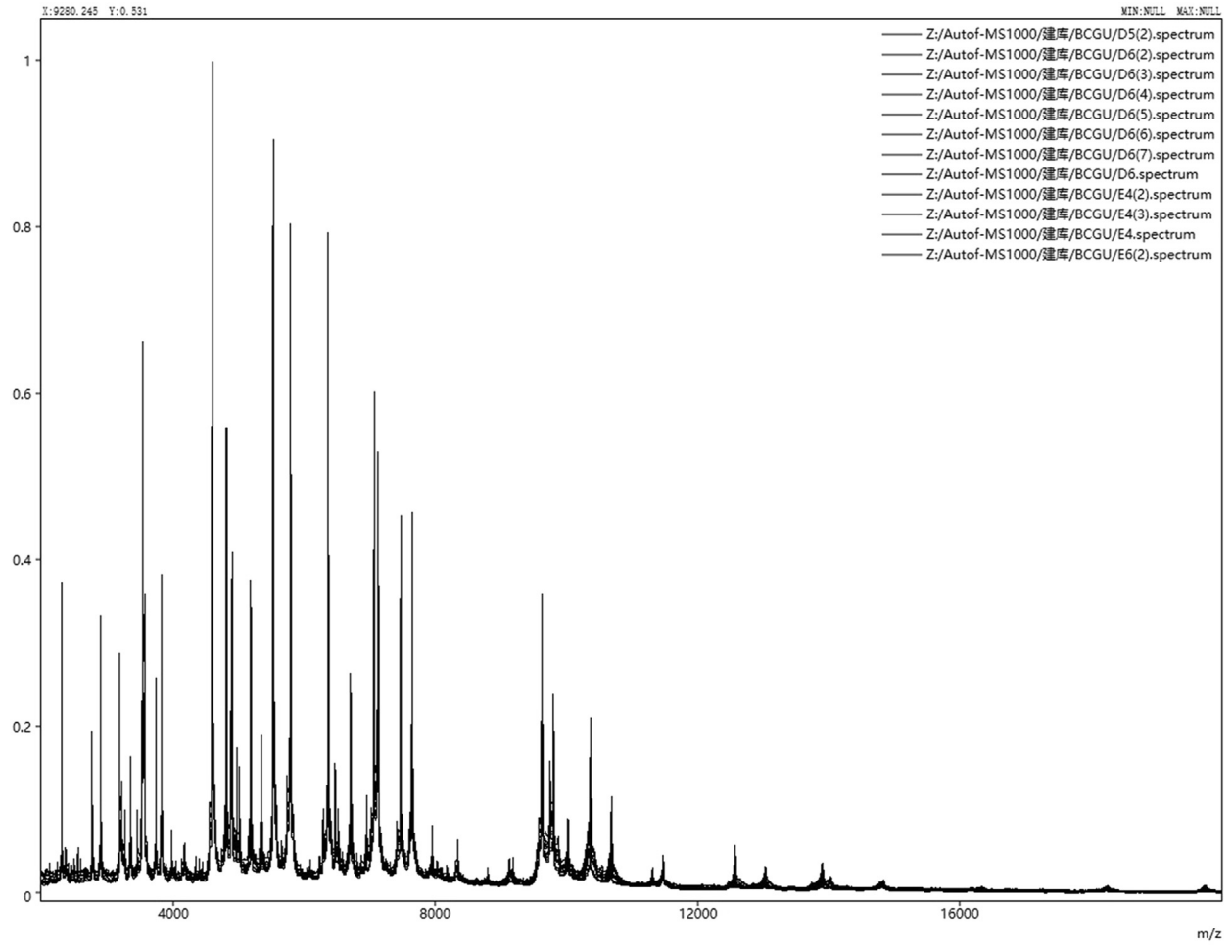

FIG. 1. Reference mass spectrum generated by MALDI-TOF MS. The reference spectrum was based on spectra from 12 individual colonies.

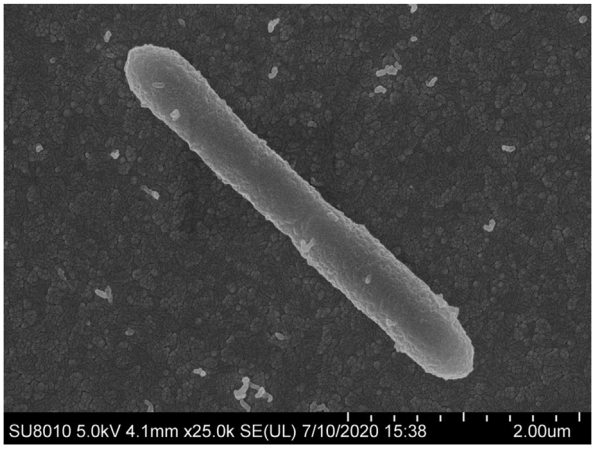

FIG. 2. Scanning electron micrograph image of Parabacteroides pekinense strain Quantibio-BCGU^T collected by Hitachi SU8010 microscope. Scale bar and acquisition settings are shown on original micrograph.

TABLE 1. **Phenotypic characterization of *Parabacteroides* pekinense based on analytical profile index (API 20A)**

Characteristic	Result
L-tryptophen	—
Urea	—
D-glucose	+
D-mannitol	—
D-lactose	+
D-saccharose	+
D-maltose	—
Salicin	—
D-xylose	+
L-arabinose	+
Gelatin	—
Esculin	+
Glycerol	—
D-cellobiose	—
D-mannose	+
D-melezitose	—
D-raffinose	+
D-sorbitol	—
L-rhamnose	+
D-trehalose	—
CAT	—
GRAM	—
COCC	—

Physiological and biochemical characteristics of strain

Growth of strain Quantibio-BCGU^T was assessed at various pH values, temperatures and salt concentrations in tubes with YCFA growth medium (1 L medium contains 10 g tryptone, 2.5 g yeast extract, 4 g $NaHCO_3$, 1 g cysteine, 1 g inulin, 0.45 g K_2HPO_4, 0.9 g NaCl, 0.09 g $MgSO_4 \bullet 7H_2O$, 0.09 g $CaCl_2$, 1 mg resazurin, 10 mg haemin, 10 µg biotin, 10 µg cobalamin, 30 µg p-aminobenzoic acid, 50 µg folic acid, 150 µg pyridoxamine, 50 µg thiamine, 50 µg vibioflain, 25 mM glucose.) with H_2/CO_2 in the gas phase. All of the analytical assays were performed in triplet. Growth was measured by INFINITE 200 PRO (Tecan) and evaluating OD at 600nm. The pH value was adjusted to the desired value by the addition of anaerobic, sterile solutions containing 10% (w/v) $NaHCO_3$ or 0.1 M HCl. Growth occurred at pH 5.5–9.0. Subsequently, growth of strain Quantibio-BCGU^T was tested at temperatures between 30 and 50 °C in YCFA medium at pH 7.5, optimal growth was observed at 35 °C; no growth occurred at 25 °C or 50 °C. To investigate salt tolerance, NaCl was directly weighed into YCFA tubes to give 0.1%–15% (w/v). Strain Quantibio-BCGU^T grew in the YCFA medium in the presence of 0.1%–2.0% (w/v) NaCl (optimum 1.0%).

We tested the susceptibility of isolate Quantibio-BCGU^T to a range of antibiotics using the disc diffusion assay. To this end, the strain was grown for 2 days on YCFA agar medium, discs containing defined amounts of antibiotics (Oxoid) were put on top and inhibition zones around discs were measured after 2 days of growth. The inhibition zones were determined for antibiotics to which strain Quantibio-BCGU^T had shown resistance in the disc diffusion assay. Strain Quantibio-BCGU^T was found to be resistant to aztreonam (30 µg/disc), but no resistant to avermectin (50 µg/disc), cefepime (30 µg/disc), ceftriaxone (30 µg/disc), meropenem (10µg/disc) and levofloxacin (5 µg/disc). These results, including the size of clearance zones and the defined amounts of antibiotics are summarized in Table 2.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done by using the primer pair 27F and 1492R and sequencing on 3730xl DNA Analyzer (Sangon, Shanghai, China). The 16S rRNA nucleotide sequences were assembled and corrected using Seqman software (DNASTAR,

TABLE 2. **The susceptibility of *Parabacteroides* pekinense to a range of antibiotics using the disc diffusion assay**

Antibiotics	Concentration (µg/L)	Clone diameter (mm)
Aztreonam	30	0
Clindamycin	2	25
Amikacin	30	25
Gentamicin	10	25
Sulfamethoxazole	25	28
Chloramphenicol	30	31
Tetracycline	30	31
Ampicillin	10	34
Ceftazidime	30	35
Ciprofloxacin	5	35
Linezolid	30	35
Piperacillin	110	35
Erythromycin	15	35
Cefuroxime	30	36
Amoxicillin	30	36
Penicillin	10	36
Avermectin	50	40
Cefepime	30	40
Ceftriaxone	30	40
Cefoxitin	30	40
Meropenem	10	40
Levofloxacin	5	40

Inc., Madison, USA). Strain BGCU exhibited a 95.86% sequence identity with Parabacteroides merdae strain JCM 9497 (Genbank accession number NR041343.1), the phylogenetically closest species with standing in nomenclature (Fig.3). We consequently classified this strain as a member of a new species within the genus *Parabacteroides*, family Porphyromonadaceae, phylum Bacteroidetes as the type strain of the new species *Parabacteroides* pekinense.

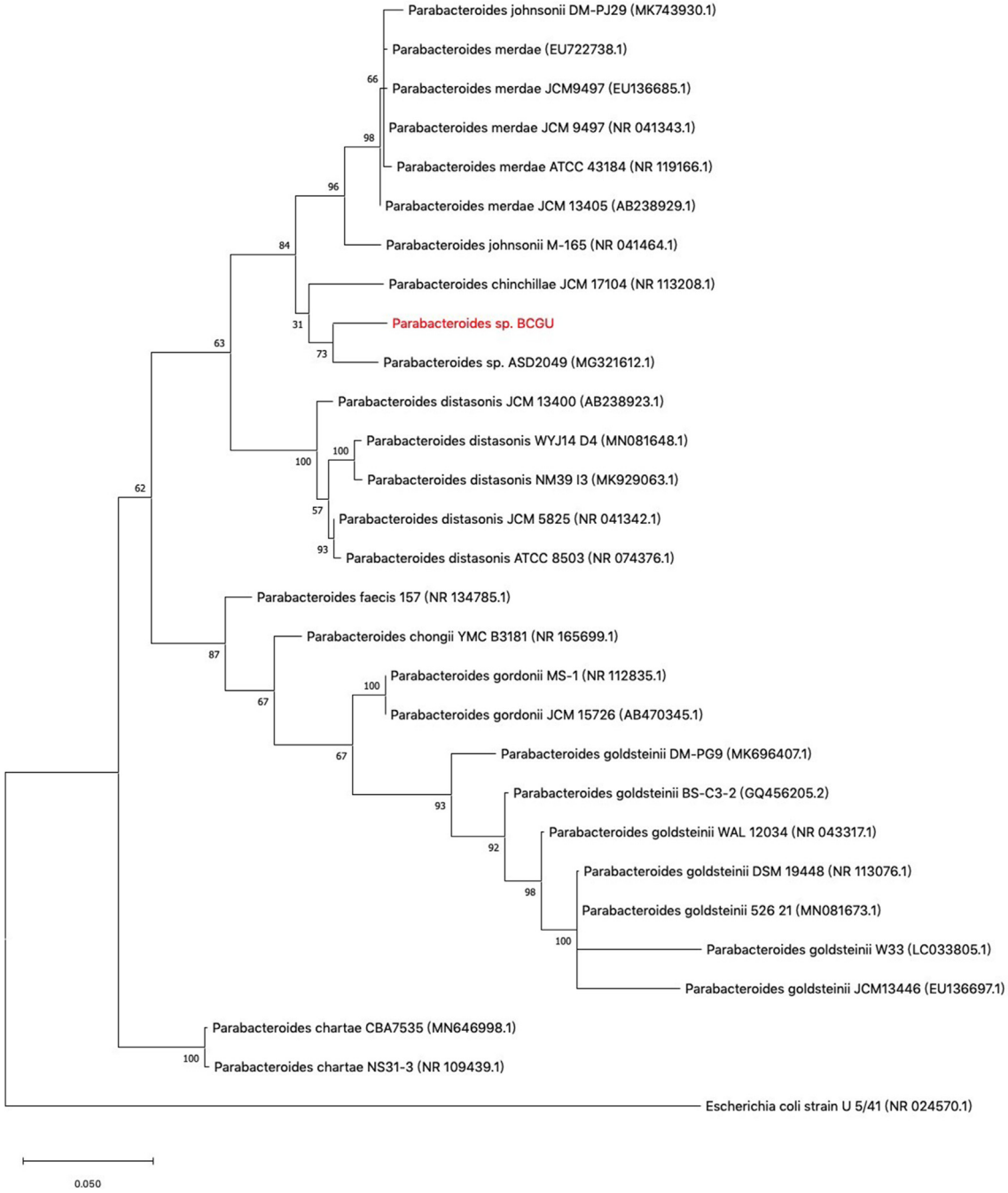

FIG. 3. Phylogenetic tree showing the position of Parabacteroides pekinense strain Quantibio-BCGU^T relative to other phylogenetically close neighbors. Respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences alignment and phylogenetic inferences were obtained using the maximum likelihood method within MEGA X software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree.

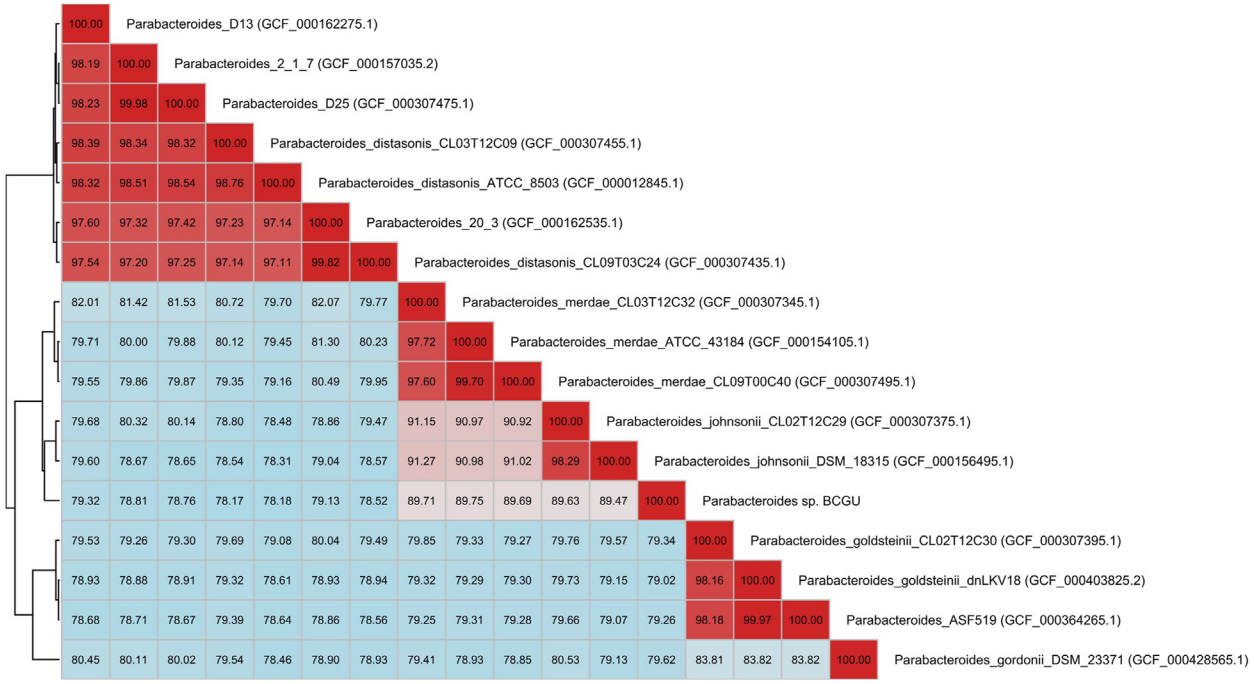

FIG. 4. Heat map generated with FastANI values. Whole-genome Average Nucleotide Identity (ANI) values were calculated using FastANI software between *Parabacteroides* pekinense and other closely related species with standing in nomenclature.

TABLE 3. Details about the fatty acid composition of *Parabacteroides* pekinense based on HPLC-MS

Fatty acids	Peak area	Concentration (nmol/L)
FA16:0	345677.688	11.10932443
FA18:0	182285.063	5.858243022
FA19:0_IS	137260.625	4.411256114
FA15:0	58142.578	1.868575221
FA18:1	46047.141	1.479854345
FA14:0	37025.973	1.189933747
FA17:0	23241.268	0.746923494
FA16:1	16753.104	0.538408101
FA12:0	14830.655	0.476624797
FA18:2	14046.508	0.451424029
FA 20:5	9311.931	0.299265085
FA11:0	4717.601	0.151613372
FA17:1	4432.369	0.14244664
FA14:1	4236.576	0.136154282
FA18:3	2838.193	0.091213312
FA 20:0	2221.003	0.071378176
FA 20:4	2203.481	0.070815057
FA 22:5	1839.17	0.059106899
FA 20:3	1773.702	0.057002901
FA 20:1	1180.279	0.037931584
FA 22:0	1158.413	0.037228859
FA 24:0	946.24	0.030410083
FA 21:0	900.368	0.028935857
FA 20:2	753.569	0.024218059
FA 26:0	665.665	0.021393016
FA 22:4	629.902	0.020243672
FA 22:1	592.402	0.019038504
FA 23:0	498.316	0.016014786
FA 25:0	342.98	0.011022627
FA 24:1	255.92	0.008224709
FA 22:2	NA	NA
FA 22:3	NA	NA
FA 22:6	NA	NA
FA 25:1	NA	NA
FA 26:1	NA	NA
FA18:4	NA	NA

Genome sequencing and comparison

Genomic DNA was extracted using the DNeasy PowerSoil Pro Kti (Qiagen, Germany) and then sequenced on the NovaSeq 6000 (Illumina, USA). The assembly was performed with a pipeline incorporating software Metawrap. Scaffolds <800 base pairs (bp) and scaffolds with a depth value lower than 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of strain Quantibio-BCGUT is 4 862 217 bp long with a 46.55 mol% G + C content. The closely related species of assemblies was estimated using GTDB-TK pipeline, where ANI was calculated by fastANI tool [12–14] (Fig.4).

Chemotaxonomic characterization of strain

For fatty acid analysis, 0.1–0.2 g healthy growing cells were harvested by centrifugation (13000 rpm for 15 min at 4 °C). Cellular fatty acids were separated and identified by Waters ACQUITY UPLC I-CLASS and Waters XEVO TQ-S Micro. Mass spectrometry data acquisition software was TargetLynx (Waters). Quinones were extracted with a chloroform/methanol (2:1, v/v) mixture and analysed by HPLC. Major fatty acids of Quantibio-BCGU^T were $C_{16:0}$, $C_{18:0}$ and $C_{19:0\text{-IS}}$. Details about the fatty acid composition are shown in Table 3. Methylnaphthoquinone present in the strain, and the concentration was 70.7731 μg/mL. Based on the phylogenetic inference

supported in genomic, biochemical and chemotaxonomy, we consequently describe a new species within the genus *Parabacteroides*, family *Porphyromonadaceae,* phylum *Bacteroidetes* as the type strain of the new species *Parabacteroides pekinense*.

Conclusion

Strain Quantibio-BCGUT, exhibiting a 16S rRNA sequence divergence < 98.65% with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species Parabacteroides pekinense sp. nov.

Description of Parabacteroides pekinense sp. nov

Parabacteroides pekinense sp. nov., is a Gram-negative and motile bacterium. Bacterial cells were rod-shaped with a length ranging from 1.75 to 2.88 μm and a width ranging from 0.25 to 0.48 μm. Cells exhibit catalase-positive and oxidase-negative activities. Colonies of strain Quantibio-BCGU^T have a mean diameter of 1 mm with regular edges and a white aspect on BD Columbia agar with 5% sheep blood medium (Heidelberg, Germany). The strain grows under anerobic conditions at 30–45 °C. Major fatty acids are $C_{16:0}$, $C_{18:0}$ and $C_{19:0\text{-}IS}$. Quantibio-BCGU^T exhibited a high level of resistance to aztreonam. Growth occurred at pH 5.5–9.0. Strain grew in YCFA medium in the presence of 0.1%–2.0% (w/v) NaCl (optimum 1.0%). The potential pathogenicity of the type strain Quantibio-BCGU^T (= CGMCC = QHBCGU) is unknown. This strain has a genome size of 4 862 217 bp long with a 46.55 mol % G + C content. The 16S rRNA gene sequence of Quantibio-BCGU^T was deposited in Genbank under accession number MT756977. Quantibio-BCGU^T is the type strain of Parabacteroides pekinense sp. nov. isolated from stool of a healthy Chinese adult living in Hainan province of China.

Credit author statement

Zhuanyu Li: Methodology, Investigation, Resources, Writing – Original Draft. Xingfan Zhou: Investigation, Resources, Funding acquisition. Wenyi Xu: Investigation, Validation, Writing – Original Draft. Rui Chen: Investigation, Resources. Bowen Zhao: Conceptualization, Investigation, Supervision, Writing – Review & Editing. Chongming Wu: Conceptualization, Investigation, Supervision, Formal analysis, Writing – Original Draft, Writing – Review & Editing.

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in Genbank under accession number MT756977 (https://www.ncbi.nlm.nih.gov/search/all/?term=MT756977).

Deposit in culture collections

Strain Quantibio-BCGU^T was deposited in two different strain collections under number (= CGMCC = QHBCGU).

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Ethics and consent

This study was approved by the ethics committee of the Third Affiliated Hospital of Qiqihar Medical University under the reference 2020LL-3. The volunteers gave a written consent.

Conflict of interest

ZYL,WYX, YHZ, and BWZ are employees of Beijing Quanti-Health Technology Co., Ltd. The other authors declare they have no competing interests.

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None

References

[1] Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun 2015;6:6528.

[2] Zhang Q, Yang Y, Ren R, Chen Q, Wu J, Zheng Y, et al. Self-assembled aggregations in Coptis chinensis decoction dynamically regulate intestinal tissue permeability through Peyer's patch-associated immunity. Chin Herbal Medicines 2021;13(3):370–80.
[3] Thomas AM, Segata N. Multiple levels of the unknown in microbiome research. BMC Biol 2019;17(1):48.
[4] Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28(1):237–64.
[5] Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.
[6] Baxter NT, Zackular JP, Chen GY, Schloss PD. Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. Microbiome 2014;2:20.
[7] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.
[8] 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. Int J Syst Evol Microbiol 2006;56(Pt 7):1599–605.
[9] Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science 2016;352(6285):560–4.
[10] Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, et al. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. Cell Rep 2019;26(1):222–235 e5.
[11] Wu TR, Lin CS, Chang CJ, Lin TL, Martel J, Ko YF, et al. Gut commensal Parabacteroides goldsteinii plays a predominant role in the anti-obesity effects of polysaccharides isolated from Hirsutella sinensis. Gut 2019;68(2):248–62.
[12] Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 2018;36(10):996–1004.
[13] Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 2019;36(6):1925–7.
[14] Parks DH, Chuvochina M, Chaumeil PA, Rinke C, Mussig AJ, Hugenholtz P. A complete domain-to-species taxonomy for Bacteria and Archaea. Nat Biotechnol 2020;38(9):1079–86.