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ORIGINAL PAPER

Bacteroides rhinocerotis sp. nov., isolated from the fresh feces of rhinoceros in Beijing Zoo

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Abstract

A Gram-negative strain, anaerobic, non-motile, non-spore-forming, rod-shaped bacterial strain named as NGMCC 1.200684 ^{T} was isolated from the fresh feces of rhinoceros in Beijing Zoo. Based on 16S rRNA gene sequences, phylogenetic analysis indicated that strain NGMCC 1.200684 ^{T} belonged to the genus *Bacteroides* and was most strongly related to the type strain of *Bacteroides uniformis* ATCC 8492 ^{T} (96.88%). The G + C content of the genomic DNA was determined to be 46.62%. Between strains NGMCC 1.200684 ^{T} and *B. uniformis* ATCC 8492 ^{T}, the average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) were 93.89 and 67.60%, respectively. Strain NGMCC 1.200684 ^{T} can produce acid from fermentation of several substrates, including glucose, mannitol, lactose, saccharose, maltose, salicin, xylose, cellobiose, mannose, raffinose, sorbitol, trehalose, D-galactose, and maltotriose. The major cellular fatty acids (> 10%) were identified as anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{14:0}$, and iso-$C_{17:0}$ 3-OH. The polar lipid profiles of strain NGMCC 1.200684 ^{T} were determined to contain diphosphatidyl glycerol, phosphatidylglycerol, phosphatidylethanolamine, three unknown phospholipids, and two unknown amino-phospholipids. Based on phenotypic, phylogenetic, and chemotaxonomic characteristics, a novel species of the genus *Bacteroides*, *Bacteroides rhinocerotis* sp. nov. is proposed. The type strain is NGMCC 1.200684 ^{T} (= CGMCC 1.18013 ^{T} = JCM 35702 ^{T}).

Keywords Rhinoceros feces · 16S rRNA phylogeny · Polyphasic taxonomy · *Bacteroides rhinocerotis* sp. nov

Introduction

Bacteroides are anaerobic and mostly found in the gastrointestinal tract of animals and humans, besides Firmicutes, as well as control the gut microflora of mammals (Smith et al. 2006; Ley et al. 2008; Thomas et al. 2011). In addition to human fecal samples (Kim et al. 2022; Sun et al. 2022), a large number of novel *Bacteroides* species isolated from animals have been described recently. These include those found in the cecum of wild-derived house mice (Fokt et al. 2022; Clavel et al. 2010), the gut of a subterranean termite (*Reticulitermes speratus*) (Sakamoto and Ohkuma 2013), caecum of chicken (Irisawa et al. 2016; Saputra et al. 2015), a methanogenic reactor treating waste from cattle farms (Nishiyama et al. 2009; Ueki et al. 2008, 2011), and chinchilla feces (Kitahara et al. 2011). *Bacteroides* spp. play diverse functions role as gut commensals, inducing both health-promoting and disease-promoting effects (e.g., *Bacteroides fragilis*) (Wexler 2007; Wang et al. 2021; Tan et al. 2019). In addition, *Bacteroides* species have an excellent ability to utilize the nutrients at hand. *Bacteroid* fermentation of carbohydrates produces a pool of volatile fatty acids, which are then reabsorbed through the large intestine and used as an energy source by the host, meeting a substantial amount of the host's daily energy needs (Hooper et al. 2002). As well, *Bacteroides* species have a tremendous capacity to use a wide range of dietary polysaccharides. Many dietary

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plant polysaccharides that are normally indigestible can be broken down by *Bacteroides* (e.g., amylose, amylopectin, and pullulan). Other organisms in the intestine do not have a series of sugar-utilizing enzymes owned by *Bacteroides*. But they can benefit from the presence of *Bacteroides* using sugars (generated by the glycosylhydrolases) (Sonnenburg et al. 2004). The wild animal intestinal microbiome, in particular, was recognized as an undisclosed environment with great bacterial diversity, and each animal can develop its own microbiota signature (Endo et al. 2010; Tsuchida and Ushida 2015). Nevertheless, there hasn't been much research done on rhinoceros' fresh feces. Only three new species have been isolated from rhinoceros' feces in the last 20 years that do not belong to the genus *Bacteroides* (Chen et al. 2017; Li et al. 2015, 2016). At the time of writing on 10th, December 2022, the genus *Bacteroides* comprised 106 species with validly published names (https://lpsn.dsmz.de/genus/bacteroides). We obtained three representative strains in this study investigating the microbial composition of rhinoceros' feces, NGMCC 1.200682, NGMCC 1.200684 ^{T}, and NGMCC 1.200685. Strains NGMCC 1.200682 and NGMCC 1.200685 separately showed 99.71% and 99.71% 16S rRNA gene similarity to *Bacteroides stercoris* ATCC 43183 ^{T} and *Bacteroides fragilis* NCTC 9343 ^{T}, thus were considered new isolates of them. Strain NGMCC 1.200684 ^{T} was considered to belong to a potential novel species within the genus *Bacteriodes*. In this paper, we describe its taxonomic position from a polyphasic perspective.

Materials and methods

Isolation and growth conditions

The strain NGMCC 1.200684 ^{T} was isolated from rhinoceros' feces. The feces samples were collected, immediately placed in anaerobic PBS solution containing 1% cysteine, and transferred into an anaerobic glove box (Shanghai Longyue Co., Ltd) that was 90% N_2, 5% H_2, and 5% CO_2. Pipetting was used to disperse the suspended feces, which were then filtered through 70 µm and 40 µm cell sieves. Following that, the filtrate was serially diluted up to 10^{-7}, and 100 µl of each of the last four dilutions was respectively plated on modified Gifu anaerobic broth (mGAM; HB8518, Hopebio) agar plates and YCFA agar plates. As described above, plates were incubated for 3 days at 37 ℃ in an anaerobic glove box. Strain NGMCC 1.200684 ^{T} was isolated from an mGAM agar plate of 10^{-4} series diluted fecal samples, which were heat-treated. Single colonies were picked and grown on modified GAM agar plates. This procedure was repeated until pure cultures were obtained and stored at − 80 °C in mGAM broth supplemented with 20% glycerol (w/v). Reference strains *Bacteroides fluxus* DSM 22534 ^{T}, *Bacteroides rodentium* DSM 26882 ^{T}, and *B. uniformis* DSM 6597 ^{T} were obtained from DSMZ, and maintained under the same conditions.

Morphological, physiological, and biochemical characterization

For the purposes of phenotypic, chemotaxonomic, and phylogenetic characterization, the strain NGMCC 1.200684 ^{T} was grown on mGAM agar or in liquid medium at 37 °C and anaerobic cultivation 3 days, unless otherwise stated. Gram staining was carried out using a Gram staining kit (G1060, Solarbio), and optical microscopy (CX-31, Olympus) was used to evaluate the results. Cellular morphology and the presence of spores were examined by scanning electron microscopy (Merlin compact, ZEISS). Growth was examined in environments that were aerobic, anaerobic, and microaerophilic, which were produced using a bio-incubator, AnaeroPackTM-Anaero, and MicroAeroTM-MicroAero (Mitsubishi Gas Chemical Co, Inc.). Cell motility was performed depending on the development of turbidity in an anaerobic tube containing mGAM semisolid medium (Tittsler and Sandholzer 1936). The activities of catalase and oxidase were investigated with 3% (v/v) hydrogen peroxide solution and oxidase test strips (M153, LAND BRIDGE), respectively. According to the manufacturer's instructions, physiological and biochemical tests were conducted using the VITEK 2 ANC card of anaerobic bacteria identification test (bioMérieux), API ZYM Kit (bioMérieux), and API 20A systems (bioMérieux). Other phenotypic traits, including temperature, pH for growth, and salt tolerance, were evaluated using the methods previously described (Sun et al. 2022; Yu et al. 2019). Cellular polar lipids were extracted with chloroform–methanol filtration and identified by two-dimensional TLC (Minnikin and Abdolrahimzadeh 1974). The processes of saponification, methylation, extraction, and measurement of cellular fatty acids followed those previously reported (Sakamoto et al. 2002). Using the Microbial Identification System (MIDI) (Sasser 1990), the fatty acid composition of strain NGMCC 1.200684 ^{T} was examined as per Sasser's (1990) instructions.

Phylogenetic and genome sequencing analyses

The 16S rRNA gene of strain NGMCC 1.200684 ^{T} was amplified using universal primers: 27F (5′-AGAGTTTGATCCTGGCTCA-3′), 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR products were sequenced using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM 3730 XL Genetic Analyzer (Applied Biosystems). The closest recognized relatives of the novel isolates were identified and downloaded by comparing the 16S rRNA gene sequence (1412 bp) of strain

NGMCC 1.200684 [T] to those available in the EzBioCloud database (www.ezbiocloud.net). The isolate sequences were aligned with 16S rRNA gene sequences obtained from EzBioCloud using the multiple sequence alignment program Clustal_X software (version 2.0) (Thompson et al. 1997). The trimmed alignment was converted to mega format for phylogenetic analyses. Phylogenetic consensus trees were constructed using the neighbor-joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) methods with MEGA_X (Kumar et al. 2018; Felsenstein 1981) and evaluated using 1000 bootstrap replicates (Saitou and Nei 1987; Kluge and Farris 1969). Evolutionary distance was obtained by the two-parameter method of Kimura (Kimura 1980). The genomic DNA from pure cultures of strain NGMCC 1.200684 [T] was extracted using the TIANamp Bacteria DNA Kit (DP302, Tiangen) following the manufacturer's instructions. The Illumina PE150 platform was used to sequence the genome. Using the algorithm outlined by Yoon et al. (Yoon et al. 2017), the OrthoANI determined the average nucleotide identity (ANI) values using EzBioCloud (www.ezbiocloud.net/tools/ani). Version 3.0 of the Genome-to-Genome Distance Calculator (GGDC) (http://ggdc.dsmz.de/ggdc.php) was used to determine the digital DNA-DNA hybridization (dDDH) values (Auch et al. 2010; Meier-Kolthoff et al. 2013). The phylo-genomic tree is constructed using a concatenated alignment of 120 conserved bacterial single-copy genes with GTDB-Tk v. 1.5.1(Parks et al. 2018; Chaumeil et al. 2020).

Results and discussion

Morphological, physiological, and biochemical characteristics

Strain NGMCC 1.200684 [T] were anaerobic, Gram-stain-negative, non-spore-forming, non-motile, and rod-shaped (0.5–1 μm width and vary in length, mostly 1.5–7.5 μm) (Supplementary Fig. S1). The novel strain was consistent with the estimated 0.5–1.5 μm wide and 1.5–11 μm long cell sizes of members of the genus *Bacteroides* (Shah and Collins 1989). Colonies on mGAM agar plates were 1–3 mm in diameter, translucent, whitish, circular, convex, and neat edges after 3 days of cultivation. Growth occurred at temperatures ranging from 25 to 45 °C, with 37 °C being the optimum. The pH range for growth was from pH 5.0 to 7.0 (optimum, pH 7.0). Isolate grew at 0–2.0 NaCl% (w/v), with the optimum at 0.5–1 NaCl% (w/v). Isolate was anaerobic according to an oxygen tolerance test. Anaerobic and microaerophilic growth was seen; however, after two days of exposure to air at 37 °C, no colonies developed on the plates. Table 1 compares the strain NGMCC 1.200684 [T] physiological and biochemical characteristics to those of the type strains of strongly related *Bacteroides* species. Supplementary Table S1 lists the results from the three API systems. As determined by the API 20A test, strain NGMCC 1.200684 [T] can ferment a variety of substrates to produce acid, but not arabinose, glycerol, melezitose, and L-rhamnose. These substrates include glucose, mannitol, lactose, saccharose, maltose, salicin, xylose, cellobiose, mannose, raffinose, sorbitol, and trehalose. Gelatin is not hydrolyzed, whereas esculin is. Indole is generated rather than urease. The incapacity of strain NGMCC 1.200684 [T] to ferment L-arabinose allows it to be distinguished from the other three type strains. Following the API ZYM test, the results were positive for α- and β-galactosidase, alkaline phosphatase, esterase (C4), chymotrypsin, acid phosphatases, Naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-Acety-β-glucosaminidase, α-fucosidase, but negative for lipoidase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, β-glucuronidase, α-glucosidase, and α-mannosidase. The result for lipid esterase (C8) is weakly. Strain NGMCC 1.200684 [T] was different from other type strains in the chymotrypsin, α-glucosidase. According to the API VITEK 2 ANC card test, strain NGMCC 1.200684 [T] was positive for alanine–phenylalanine–proline arylamidase, 5-Bromo-4-Chloro-3-indole-β-D-glucoside, β-D-Fucosidase, 5-Bromo-4-Chloro-3-hydroxyindole-b-N-acetylglucosamine, 5-Bromo-4-Chloro-3-Indole-β-D-glucuronide, α-L-arabinoside, β-Galactopyranosyl glucosidase indole phenol, α-Arabinosidase, 5-Bromo-4-chloro-3-indole-α-D-galacto pyranoside, α-L-fucosidase, Phosphatase, the fermentative production of acids from D-galactose, maltotriose, and negative for ELLMAN, Phenylalanine arylaminase, L-Proline arylaminase, L-Pyrrolidone arylaminase, Tyrosine arylamidase, Arbutin, N-Acetyl-D-glucosamine, β-mannosidase, Arginine, Pyruvate, 5-Bromo-4-Chloro-3-Indolyl-α-D-Mannopyranoside, phenylphosphonate, D-Ribose2. Strain NGMCC 1.200684 [T] differed from the three others in acid production from N-Acetyl-D-glucosamine and arbutin. To sum up, the physiological characteristics of strain NGMCC 1.200684 [T] enabled it to distinguish it from recognized *Bacteroides* species.

Phylogenetic and genomic analyses

The 16S rRNA gene sequences of strain NGMCC 1.200684 [T] (1412 bp) were determined. The 16S rRNA gene sequences of strain NGMCC 1.200684 [T] and related type species of the genus *Bacteroides* were aligned, and a phylogenetic tree was constructed using *Parabacteroides distasonis* ATCC 8503 [T] as an outgroup (Fig. 1). According to the findings of phylogenetic analyses based on 16S rRNA gene sequences using the NJ, ML, and MP techniques, strain NGMCC 1.200684 [T] and the closely related species formed a separate branch within the genus *Bacteroides*. The phylogenetic analysis and

Table 1 Different characteristics of strain NGMCC 1.200684 [T] and related type strains of species of the genus *Bacteroides*

Characteristics	1	2	3	4
API ZYM results				
Chymotrypsin	+	−	−	ND
Acid phosphatases	+	+	+	ND
β-glucuronidase	−	+	−	−
α-glucosidase	−	+	+	+
α-fucosidase	+	+	−	+
API 20A results				
Indole production	+	+	−	+
D-mannitol	+	+	−	−
Glycerol	−	+	−	−
D-sorbitol	+	+	−	−
L-rhamnose	−	+	−	−
D-trehalose	+	+	−	−
VITEK2 ANC card				
D-galactose	+	+	+	ND
Leucine arylamidase	−	+	−	−
ELLMAN	−	−	+	ND
L-Pyrrolidone arylaminase	−	−	−	ND
Tyrosine arylamidase	−	w	−	−
Alanine–phenylalanine–proline arylamidase	+	+	+	ND
Arbutin	−	+	+	ND
N-Acetyl-D-glucosamine	−	+	+	+
5-Bromo-4-chloro-3-indole-β-D-glucoside	+	−	+	ND
5-Bromo-4-chloro-3-indole-β-D-glucuronide	+	+	−	ND
α-Arabinosidase	+	−	+	+
5-Bromo-4-chloro-3-indole-α-D-galactopyranoside	+	−	+	ND
β-mannosidase	−	w	−	ND
β-D-Fucosidase	+	w	+	ND
5-Bromo-4-chloro-3-hydroxyindole-b-N-acetylglucosamine	+	−	+	ND
L-arabinose	−	v	+	+
D-ribose2	−	+	−	ND
Phenylphosphonate	−	−	+	ND
α-L-arabinoside	+	−	+	ND

Strains: 1, NGMCC 1.200684 [T]; 2, *B. fluxus* DSM 22534 [T]; 3, *B. rodentium* DSM 26882 [T]; 4, *B. uniformis* DSM 6597 [T] (data from Kitahara et al. 2011). Data were obtained in this study unless indicated

+Positive, - negative, *w* weakly, *v* variable, *ND* no data available

EzBioCloud database searches indicated that the type strains of *B. uniformis* ATCC 8492 [T], *B. rodentium* JCM 16496 [T], and *B. fluxus* YIT 12057 [T] had similar sequences to NGMCC 1.200684 [T], with approximate similarity values of 96.88%, 95.56%, and 93.45%, respectively. A phylo-genomic tree based on whole genomes was reconstructed (Fig. 2). The result showed that NGMCC 1.200684 [T] was clustered with the type strains of *B. uniformis* ATCC 8492 [T] in the same clade, and they have a high bootstrap value (98%). The average nucleotide identity (ANI) and the digital DNA–DNA hybridization (dDDH) results showed that *B. uniformis* ATCC 8492 [T] was the closest strain, with values of 93.89% and 67.60%, which were lower than the classification limits of 95% and 70% of international standards (Wayne 1988) (Table 2). We concluded that strain NGMCC 1.200684 [T] represented a novel species within the genus *Bacteroides*.

The TIANamp Bacteria DNA Kit was used to extract genomic DNA from cells cultured in the mGAM broth (DP302, Tiangen). The genome was sequenced by the Illumina PE150 platform. The size of the strain NGMCC 1.200684 [T] genome was 4.88 Mb. 76 high-quality scaffolds were produced from 1,126 Mb of clean readings after de novo assembly. The isolate's DNA G + C content was 46.62%, which was within the range (40–48%) previously described for the genus *Bacteroides* (Shah 1992). In the draft genome, the genome carried 62 ncRNA genes, including

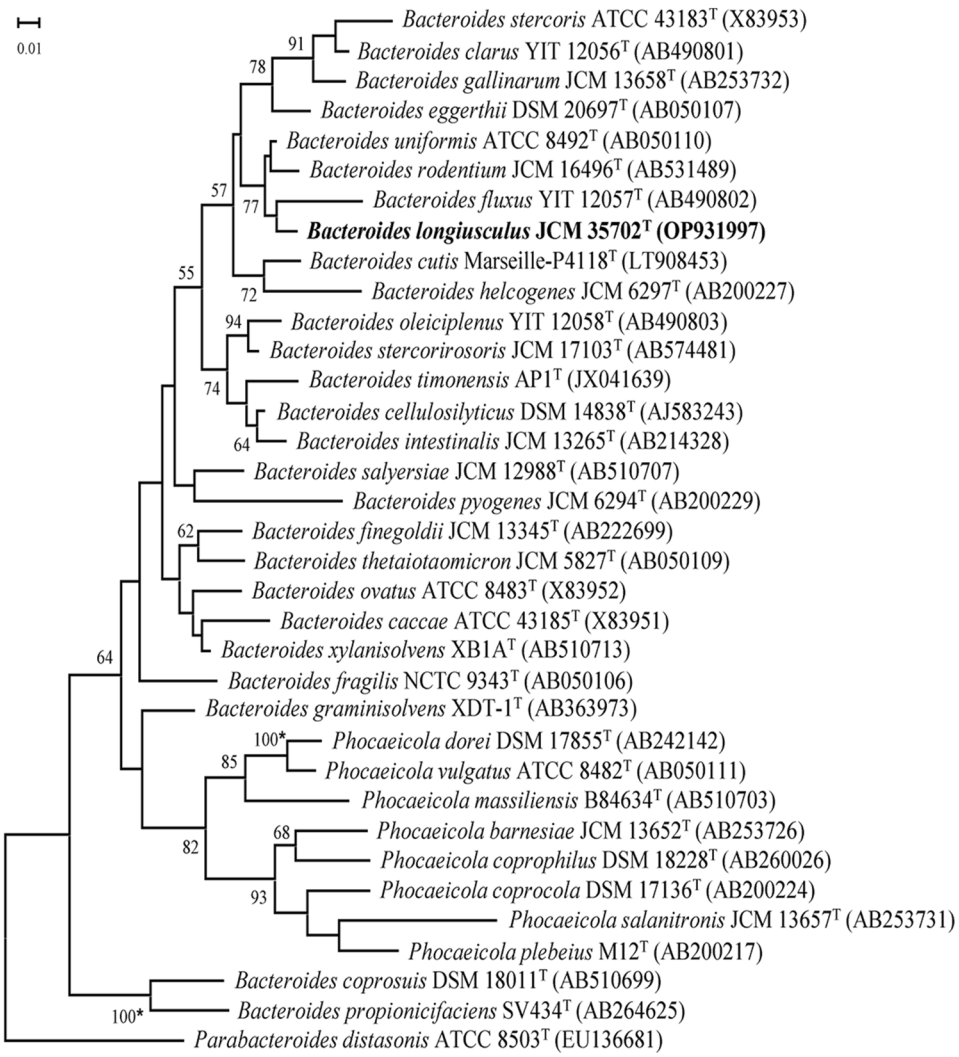

Fig. 1 Phylogenetic and phylo-genomic trees of strain NGMCC 1.200684 T and closely related type strains within the genus *Bacteroides*. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strains NGMCC 1.200684 T. Bootstrap values (> 50%) based on 1000 replicates for the maximum-likelihood method are shown at branch nodes. Bar, 0.01 substitutions per site. GenBank accession numbers are given in parentheses. Asterisks denote nodes that were also recovered using the maximum-parsimony and neighbor-joining methods

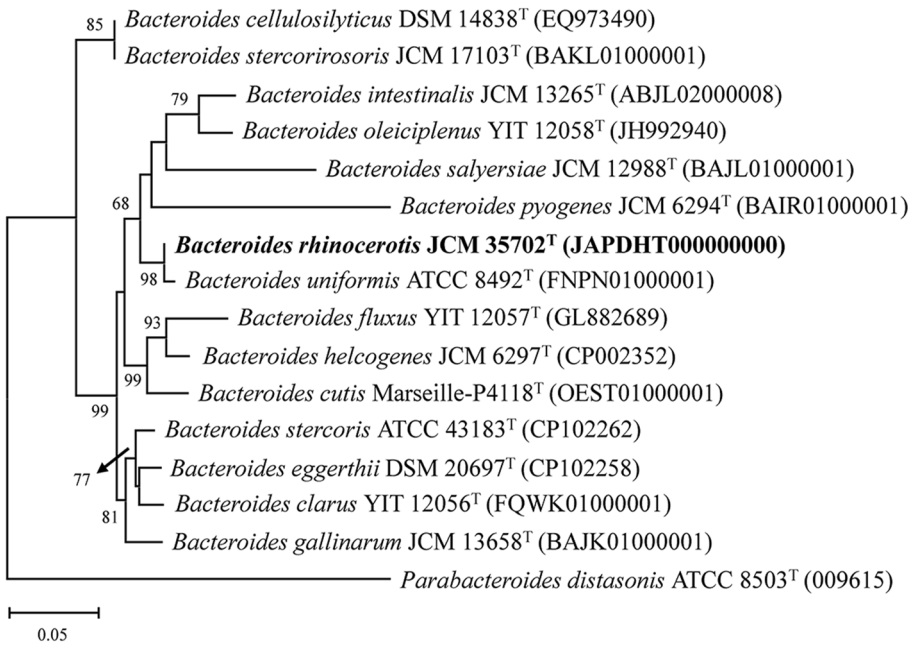

Fig. 2 Genome phylo-genomic tree showing the position of strain NGMCC 1.200684 T. Bootstrap values are indicated at branch points based on 1000 iterations. *Parabacteroides distasonis* ATCC 8503 T (EU136681) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position

Table 2 Average nucleotide identity and levels of DNA–DNA hybridization among the strain NGMCC 1.200684 [T] and related strains

Query genome	Reference genome	ANI (%)	dDDH (%)
NGMCC 1.200684	*B. uniformis* ATCC 8492 [T] (AAYH02000049)	93.89	67.60
NGMCC 1.200684	*B. fluxus* YIT 12057 [T] (AB490802)	79.84	22.90
NGMCC 1.200684	*B. cuits* Marseille-P4118[T] (OEST01000016)	77.86	22.80

3 rRNA genes, 59 tRNA genes and 0 sRNA genes. Strain NGMCC 1.200684 [T] sequenced genomes were subjected to coding gene prediction using GeneMarkS (Version 4.17) software, and 4,212 coding sequences (CDS) were predicted. *Bacteroides* were the top two most closely related species in the top 20 of the predicted strain NGMCC 1.200684 [T] genome based on species annotated with genes in the Non-Redundant Protein Database (NR) database. For assessment of the function of predicted coding genes, the NCBI-NR, Swiss-Prot, KEGG, COG, GO, Pfam, PHI, VFDB, CARD, and CAZy databases were used. Analysis of gene functions with KEGG resulted in an allocation of the majority of the genes to carbohydrate metabolism (205 genes), amino acid metabolism (132 genes), metabolism of cofactors and vitamins (111 genes), energy metabolism (91 genes), and nucleotide metabolism (67 genes) (Supplementary Fig. S2). Further genome mining revealed strain NGMCC 1.200684 [T] genome sequence encodes the starch utilization system (Sus), which is made up of susABCDEFG genes and can degrade various oligosaccharides into monosaccharides or disaccharides by periplasmic glycan-degrading enzymes like susA and susB. The sus system was also found in Kim et al.'s study (Kim et al. 2022) (Supplementary Table S2).

Chemotaxonomic characteristics

In 1980, Shah and Collins evaluated the cellular fatty acid profiles of *Bacteroides* species and reassessed their genus taxonomy in 1983, and revealed the majority of cellular fatty acids were straight-chain, anteiso- and iso-methyl branched-chain fatty acids (Shah and Collins 1980, 1983). Table 3 lists detailed results of the cellular fatty acid study of strain NGMCC 1.200684 [T] and its phylogenetically adjacent neighbors. In this work, the isolate's major cellular fatty acids were anteiso-$C_{15:0}$ (29.87%), iso-$C_{15:0}$ (17.86%), iso-$C_{14:0}$ (15.41%), and iso-$C_{17:0}$ 3-OH (10.43%) (Table 3). All strains, including the reference species, contain the primary components iso-$C_{15:0}$ and anteiso-$C_{15:0}$. While, $C_{18:1}$ ω9c was not observed in strain NGMCC 1.200684 [T], but present in the reference species (Table 3). It was proven that these contents differed somewhat between each other, yet followed a pattern that was comparable to those of other *Bacteroides* species. The polar lipids' profile of strain NGMCC 1.200684 [T] was determined to contain diphosphatidyl glycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), three unidentified phospholipids (PL1–3), and two unidentified amino-phospholipids (APL1–2) (Fig. 3).

Table 3 Cellular fatty acid compositions of strains NGMCC 1.200684 [T] and three related *Bacteroides* species

Fatty acid	1	2	3	4
Saturated straight chain				
$C_{14:0}$	1.42	–	–	–
$C_{15:0}$	–	–	–	2.4
$C_{16:0}$	5.25	–	12.9	7.5
$C_{18:0}$	1.41	–	–	–
Unsaturated straight chain				
$C_{18:1}$ ω9c		14.0	13.5	10.3
$C_{18:2}$ ω6,9c			1.7	1.4
Hydroxy				
$C_{15:0}$ 3-OH	–	–	–	–
$C_{16:0}$ 3-OH	3.51	–	12.7	7.0
$C_{17:0}$ 3-OH	–	–	–	1.3
iso-$C_{17:0}$ 3-OH	10.43	–	19.4	20.0
anteiso-$C_{17:0}$ 3-OH	–	–	2.7	3.0
Saturated branched chain				
iso-$C_{13:0}$	5.46	–	–	–
iso-$C_{14:0}$	15.41	–	–	–
iso-$C_{15:0}$	17.86	9.3	9.9	10.9
anteiso-$C_{13:0}$	2.35	–	–	–
anteiso-$C_{15:0}$	29.87	28.8	27.3	35.8
anteiso-$C_{17:0}$	–	–	–	1.0
Summed features*				
3	–	–	–	–
9	–	–	–	–
10	–	–	–	–
11	–	27.8	–	–

Strains: 1, NGMCC 1.200684 [T]; 2, *Bacteroides fluxus* DSM 22534 [T]; 3, *Bacteroides rodentium* DSM 26882 [T] (data from Kitahara et al. 2011); 4, *Bacteroides uniformis* DSM 6597 [T] (data from Kitahara et al. 2011). Values ≥ 1% are shown

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of iso-$C_{15:0}$ ALDE and/or an unknown fatty acid ECL 13.570. Summed feature 9 consisted of iso-$C_{16:0}$ 3-OH and/or an unknown fatty acid ECL 17.157. Summed feature 10 consisted of one or more of iso-$C_{18:1}$ω11c/9t/6t and an unknown fatty acid ECL 17.834

Taxonomic conclusion

On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic studies, we propose that strain NGMCC 1.200684 [T] be classified as representing a novel species of the genus *Bacteroides*, for which the name *Bacteroides rhinocerotis* sp. nov. is proposed.

Description of Bacteroides rhinocerotis sp. nov.

Bacteroides rhinocerotis ('*rhinocerotis*' is. L. gen. n. *rhinocerotis* of rhinoceros, referring to the isolation source of type strain NGMCC 1.200684 [T]).

Anaerobic, Gram-negative, non-spore-forming, non-motile, and rod-shaped, 0.5–1 μm width and variable in length, mostly 1.5–7.5 μm. After cultivation on mGAM medium at 37 °C for 3 days, colonies are translucent, whitish, circular, convex, and neat edges and 1–3 mm in diameter, grow at 20–45 °C (optimum 37 °C), at pH 5.0–7.0 (optimum, 7.0) and tolerate up to 2% (w/v) NaCl (optimum, 0.5–1%), oxidase-, catalase-, and urease-negative and production of indole positive, hydrolyzes aesculin, but not gelatin, produces acid from glucose, mannitol, lactose, saccharose, maltose, salicin, xylose, cellobiose, mannose, raffinose, sorbitol, trehalose, D-galactose, and maltotriose, but not from arabinose, glycerol, melezitose, and L-rhamnose.

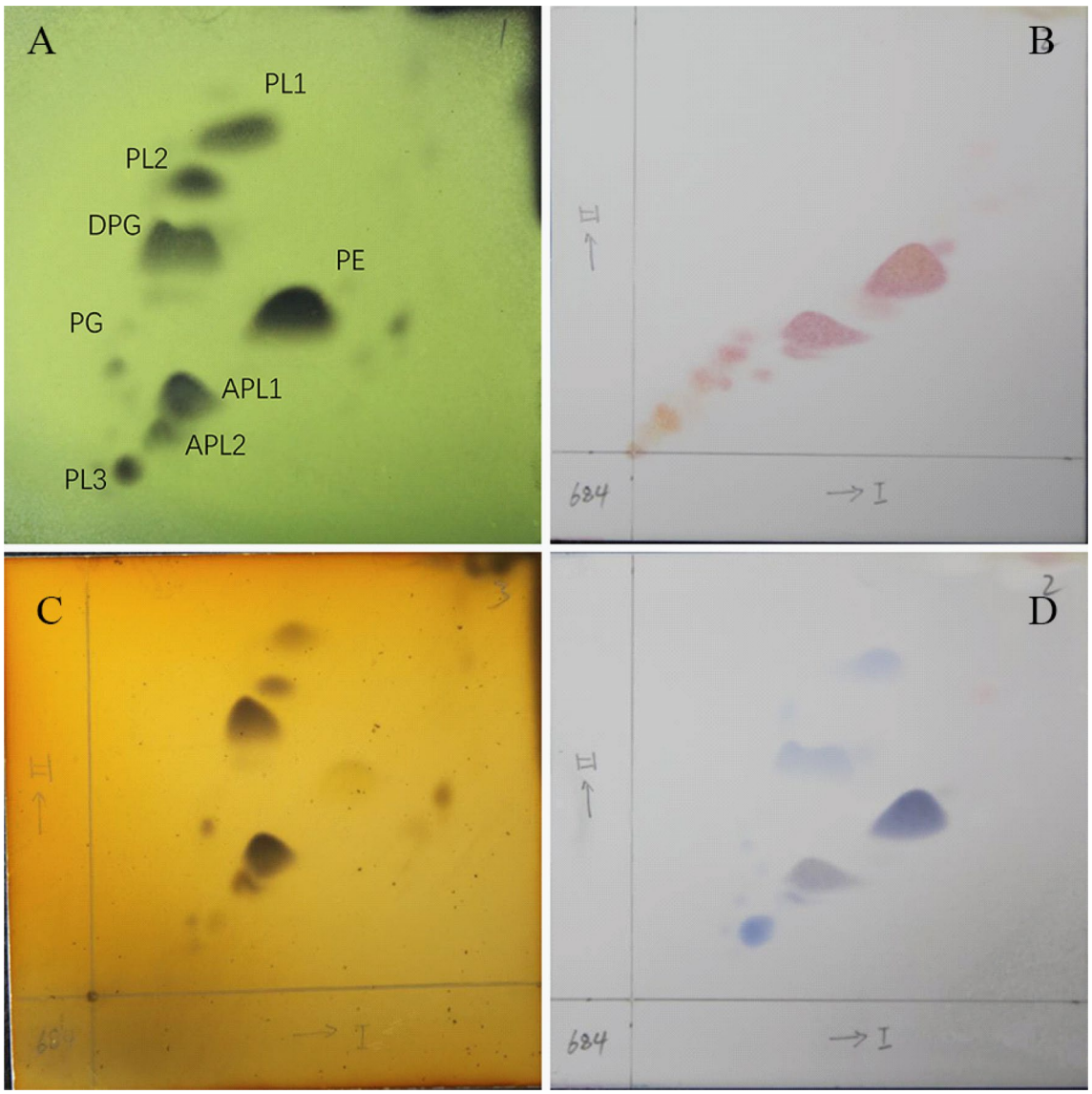

Fig. 3 Polar lipids' profile of strain NGMCC 1.200684 [T]. The different pictures of staining agent to show all polar lipids as **A**, molybdophosphoric acid, **B**, ninhydrin, **C**, α-naphthol and **D**, molybdenum blue, respectively. *DPG* diphosphatidyl glycerol, *PG* phosphatidylglycerol; PE, phosphatidylethanolamine, *PL1-3* unidentified phospholipids, *APL1-2* unidentified amino-phospholipids

The major fatty acids are anteiso-$C_{15:0}$ (29.87%), iso-$C_{15:0}$ (17.86%), iso-$C_{14:0}$ (15.41%), and iso-$C_{17:0}$ 3-OH (10.43%). The polar lipids' profile was determined to contain diphosphatidyl glycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), three unidentified phospholipids (PL1–3), and two unidentified amino-phospholipids (APL1–2). The DNA G + C content of the type strain is 46.62%.

The type strain, NGMCC 1.200684 ^{T} (= CGMCC 1.18013 ^{T} = JCM 35702 ^{T}), was isolated from rhinoceros' feces. The DDBJ/ENA/GenBank accession numbers for the 16S rRNA gene and genome sequences of the type strain are OP931997 and JAPDHT000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-023-03513-z.

Author contributions XL and LS carried out the data analysis, wrote, and revised the manuscript. XL, PLS, LG, and WXS performed the experiments. ZGX and ML participated in the data analysis. LS and CQ supervised the project. All authors reviewed and approved the final manuscript.

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Data availability The GenBank accession number for 16S rRNA gene sequences of strains NGMCC 1.200684 ^{T} is OP931997. The draft genome sequences of strains NGMCC 1.200684 ^{T} have been deposited at NCBI under the accession no. JAPDHT000000000.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethics approval and consent to participate In this study, the collection and the analysis of animal feces did not involve animal ethics.

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