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ORIGINAL PAPER

Description of *Anaerostipes faecalis* sp. nov., a new segmented filamentous bacterium isolated from swine faeces

Seung-Hyeon Choi · Ji Young Choi · Jam-Eon Park · Ji-Sun Kim · Se Won Kang · Jiyoung Lee · Mi-Kyung Lee · Jung-Sook Lee · Ju Huck Lee · Hyunjung Jung · Tai-Young Hur · Hyeun Bum Kim · Ju-Hoon Lee · Jae-Kyung Kim · Yeongjin Hong · Seung-Hwan Park

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Abstract A novel, strictly anaerobic, gram-negative, segmented filamentous bacterium strain AGMB03513^{T}, was isolated from the faeces of a 5-month-old pig. Phylogenetic analysis based on the 16S rRNA gene indicated that the isolate was a member of the family *Lachnospiraceae*, and the closest strain was *Anaerostipes butyraticus*. Strain AGMB03513^{T} formed a lineage within the genus *Anaerostipes* and was closely related to *A. butyraticus* DSM 22094 ^{T} (= KCTC 15125 ^{T}, 95.8%), *Anaerostipes hadrus* DSM 3319 ^{T} (= KCTC 15606 ^{T}, 95.5%), *Anaerostipes caccae* DSM 14662 ^{T} (= KCTC 15019 ^{T}, 94.0%), and *Anaerostipes rhamnosivorans* DSM 26241 ^{T} (= KCTC 15316 ^{T}, 93.4%). Strain AGMB03513^{T} grew at temperatures between 30 and 45 °C, within a pH range of 7.0–9.0, and in medium containing up to 1.5% NaCl. Cells were found to utilise D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, D-xylose, L-arabinose, D-mannose, and D-sorbitol, and acetate was identified as the major end product of metabolism. The major components of the cellular fatty acids were $C_{12:0}$, $C_{16:0}$, and $C_{18:0}$. In addition, the bacterium contained *meso*-diaminopimelic acid in the cell wall. According to the comparative analysis of the whole genome sequence, the DNA G + C content of strain AGMB03513 was 37.0 mol%. In addition, Average nucleotide identity

Seung-Hyeon Choi and Ji Young Choi have contributed equally to this study.

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S.-H. Choi · J. Y. Choi · J.-E. Park · J.-S. Kim · S. W. Kang · J. Lee · M.-K. Lee · J.-S. Lee · J. H. Lee · S.-H. Park (✉)
Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 181 Ipsin-gil, Jeongeup-si, Jeollabuk-do 56212, South Korea
e-mail: biopark@kribb.re.kr

H. Jung · T.-Y. Hur
Swine Science Division, National Institute of Animal Science, # 114, Shinbang 1 gil, Seonghwan-eup, Seobuk-gu, Cheonan-si, Chungcheongnam-do 31000, South Korea

H. B. Kim
Department of Animal Resources Science, Dankook University, Cheonan 31116, South Korea

J.-H. Lee
Department of Food Science and Animal Biotechnology, Seoul National University, Seoul, South Korea

J.-H. Lee
Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea

J.-H. Lee
Center for Food and Bioconvergence, Seoul National University, Seoul, South Korea

J.-K. Kim
Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup-Si 56212, South Korea

(ANI), average amino acid identity (AAI), and digital DNA-DNA hybridisation (dDDH) values were obtained in comparisons of strain AGMB03513^T with reference strains of species in the genus *Anaerostipes*. ANI values were found to be between 71.0 and 75.7%, AAI values between 66.6 and 73.2%, and dDDH values between 19.5 and 21.4%. All the data were below the threshold range for species determination. Based on phenotypic, phylogenetic, biochemical, chemotaxonomic, and genomic characteristics, we considered it reasonable to assign a novel species status to strain AGMB03513^T, for which we propose the name *Anaerostipes faecalis* sp. nov. The type strain is AGMB03513^T (= KCTC 25020 ^{T} = NBRC 114896 ^{T}).

Keywords *Anaerostipes faecalis* · Faeces · Segmented filamentous bacterium · Swine

Abbreviations

AGMB	Animal gut microbiome bank
KCTC	Korean collection for type cultures

Introduction

The swine gut microbiota plays an important role in regulating health and performance through its effects on the physiological, nutritional, and immunological systems (Richards et al. 2005). From the perspective of livestock rearing, these gut microbiota are important, given their close association with growth rate, weight gain, mortality, and production of pigs (Ahasan et al. 2015; Dowarah et al. 2017; Nowland et al. 2019). As a good example, analysis of the intestinal contents of piglets has revealed that the dominant genus, *Lactobacillus*, plays an important role in disease prevention by reducing gut populations of pathogenic bacteria (Beasley 2004). After transplanting faeces containing healthy microbiota from growing pigs into weaning pigs, the growth performance of weaning pigs was remarkably improved (Wang et al. 2019). Although previous studies have emphasized the importance of swine gut microbiota, we still lack an understanding of the gut microbiota. Therefore, further studies that can be developed and applied to pig farming are needed based on the analysis and understanding of the swine gut microbiome (Nowland et al. 2019).Using metagenomic analysis, we analysed more than 120 samples of faeces from swine raised at the National Institute of Animal Science in Korea. In this study, we discovered a novel bacterium, AGMB03513^T.

The genus *Anaerostipes*, within the phylum Firmicutes, was initially proposed by Schwiertz et al. (2002), who classified *Anaerostipes caccae* within this new genus and assigned it to the family *Lachnospiraceae*. At the time of writing, the genus *Anaerostipes* comprises four species with validly published names (www.bacterio.net/anaerostipes.html) (Euzeby 1997). Members of *Anaerostipes* are non-motile, rod-shaped, gram-variable obligate anaerobes (Schwiertz et al. 2002). In addition, all strains produce butyrate (Schwiertz et al. 2002, Eeckhaut et al. 2010, Allen-Vercoe et al. 2012, Bui et al. 2014). The range of genomic DNA G + C contents in this genus is 37.0–46.0 mol% (Bui et al. 2014). In this study, we isolated a novel strain, AGMB03513^T, from swine faeces, which was characterised through phenotypic, biochemical, phylogenetic, and chemotaxonomic analyses; based on our findings, we propose that this strain represents a novel species within the genus *Anaerostipes*.

Materials and methods

Isolation of the bacterial strain and culture conditions

Strain AGMB03513^T was isolated from swine faeces raised at the National Institute of Animal Science (Wanju, South Korea). The collected samples were immediately maintained using BD GasPak EZ anaerobic pouch system (BD Biosciences, New Jersey, USA) and subsequently transported to the laboratory. Isolation was performed using an anaerobic chamber (Coy Laboratory Products, Michigan, USA) containing an atmosphere of 86% nitrogen, 7% hydrogen, and 7% carbon dioxide. The sample was serially diluted in a saline solution (0.85% [w/v] NaCl) and spread on tryptic soy broth (BD Bioscience; 17.0 g tryptone, 3.0 g soytone, 2.5 g glucose, 5.0 g sodium chloride, 2.5 g dipotassium phosphate per litre) containing 15.0 g agar (MB cell, Seoul, South Korea) and 5%

Y. Hong (✉)
Department of Microbiology, Chonnam National University Medical School, Gwangju, South Korea
e-mail: yjhong@chonnam.ac.kr

(v/v) sheep blood (Kormed, Seongnam, South Korea) (TSAB). After incubation for 72 h at 37 °C, single colonies were isolated. Identification was performed based on 16S rRNA gene sequencing, and taxonomic analysis was performed based on phylogenetic, phenotypic, biochemical, chemotaxonomic, and genomic analyses. The isolate was suspended in 10% (w/v) skim milk (BD Bioscience) and stored in a deep freezer (− 80 °C) under aerobic conditions. For comparative studies, we used strains of the four existing members of the genus *Anaerostipes butyraticus* KCTC 15125 ^{T}, *Anaerostipes hadrus* KCTC 15606 ^{T}, *Anaerostipes caccae* KCTC 15019 ^{T}, and *Anaerostipes rhamnosivorans* KCTC15316^{T} as reference species.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA was extracted from strain AGMB03513^{T} grown on Reinforced clostridial medium (RCM) (MB Cell; 10.0 g peptone, 5.0 g sodium chloride, 10.0 g beef extract, 3.0 g yeast extract, 5.0 g dextrose, 1.0 g starch, 0.5 g L-cysteine hydrochloride monohydrate, and 3.0 g sodium acetate per litre) supplemented with agar (15.0 g per litre) using the phenol:chloroform:isoamyl alcohol method (Wilson et al. 1990). The extracted genomic DNA was used to amplify the near complete sequence of the 16S rRNA gene using the universal primer pair 785F (5-GGATTAGATACCCTGGTA-3) and 907R (5-CCGTCAATTCMTTTRAGTTT-3). The amplified 16S rRNA gene was sequenced commercially by Macrogen Inc. (South Korea). The complete 16S rRNA sequence was assembled by comparison with the whole-genome sequence of AGMB03513^{T} and identified using the EZBioCloud (Yoon et al. 2017) and GenBank/EMBL/DDBJ (http://www.ncbi.nlm.nih.gov/blast) databases. Respective sequences of the 16 s rRNA of the isolate and related strains were aligned using CLUSTAL W (Thompson et al. 1997), and phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) 7.0.26 software (Kumar et al. 2016). Evolutionary distances were calculated using Kimura's (1980) two-parameter model. Phylogenetic trees based on sequences of the 16S rRNA gene were reconstructed according to the neighbour-joining (NJ) (Saitou and Nei 1987)), maximum-likelihood (ML) (Fitch 1971), and maximum-parsimony (MP) (Felsenstein 1981) algorithms with bootstrap analysis (1000 replications).

Phenotypic and biochemical analyses

For phenotypic and biochemical analyses, the AGMB03513^{T} isolate was grown on RCM agar for 24–48 h at 37 °C. Cell morphology was observed using an Eclipse 80i phase-contrast microscope (Nikon), a SUPRA 55VP scanning electron microscope (Carl Zeiss, Germany), and a Tecnai 10 transmission electron microscope (FEI, USA). Gram staining was performed using a Gram stain kit (Difco) according to the manufacturer's instructions. KOH tests were based on determination of the formation of viscous and mucoid strings within 15 s (Suslow TV, 1982). To determine the optimal growth conditions, cells were incubated at 37 °C, over a temperature range of 10–50 °C (at 5 °C intervals), and at eight different pH values (4, 5, 6, 7, 8, 9, 10, and 11). Salt tolerance was determined by growing cells in media with NaCl concentrations ranging from 0.5 to 4.0% (at 0.5% intervals). Growth was measured at 600 nm using a DU 700 UV–visible spectrophotometer (Beckman Coulter, CA, USA). Catalase test was confirmed based on bubble formation using a catalase reagent (bioMérieux, # 55,561), and oxidase test was verified based on the production of a purple colouration by using an oxidase reagent (bioMérieux, # 55,635). Spore formation was assessed using the Schaeffer–Fulton method with malachite green (Schaeffer and Fulton 1933), whereas for characterisation of biochemical properties, cells were analysed using API 20A, Rapid ID 32A, and ZYM strips (bioMérieux, Marcy-l'Étoile, France).

Chemotaxonomic and genomic characteristics

Fermentation end products were characterised in cell-free supernatants derived from cells cultured for 2 days at 37 °C (Stationary phase) in RCM broth by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) equipped with Aminex^{TM} Organic Acid Columns (Bio-Rad, CA, USA). The diamino acid content in the cell walls was determined using a previously described method (Komagata and Suzuki 1988). Fatty acid profiles were determined in cells grown on RCM agar at 37 °C for 28 h (Log phase). The cellular fatty acids were saponified, methylated, and

extracted according to the instructions of the Chemical Analysis System (MIDI, DE, USA), and subsequently identified using gas chromatography (GC-2010; Shimadzu) and Sherlock™ Chromatographic Analysis System software package (Anaerobe Database version 6.4). Diamino acids in cell wall peptidoglycans were analysed as described previously (Schleifer and Kandler 1972). Polar lipids were extracted using a chloroform/methanol method and analysed by two-dimensional thin-layer chromatography following a previously described method (Kates 1986). Briefly, after extracting polar lipids from 100 mg freeze-dried cells, silica gel 60 F_{254} aluminium-backed thin layer plates (Merck) were dotted with samples and subjected to two-dimensional development, with the first mobile phase solvent of chloroform/methanol/distilled water (65:25:4, v/v) followed by a second mobile phase solvent of chloroform/methanol/acetic acid/distilled water (40:7.5:6:2, v/v). Whole-genome sequencing using the NovaSeq 6000 system (Illumina, San Diego, CA, USA) was performed at Macrogen Inc. to determine the G + C content of genomic DNA and for genomic analysis. The average nucleotide identity (ANI) and the average amino acid identity (AAI) were calculated using ChunLab's online ANI calculator with the OrthoANI algorithm (Lee et al. 2016) and AAI calculator tools (http://enve-omics.ce.gatech.edu/aai/) (Rodriguez-R and Konstantinidis 2016). Digital DNA–DNA hybridisation (dDDH) was calculated using the Genome-to-Genome Distance Calculator (GGDC) version 2.1 (Meier-Kolthoff et al. 2013). Whole-genome analysis was performed using CLgenomics™ software (ChunLab, South Korea) and the UniProt database (https://www.uniprot.org).

Results and discussion

Phylogenetic analysis

Approximately 1459 bases of the 16S rRNA gene were sequenced, and comparative analysis of the sequence indicated that strain AGMB03513^T is closely related to species in the genus *Anaerostipes*. AGMB03513^T showed sequence similarities between 93.3 and 95.8% with the reference bacteria, with the highest similarity to *A. butyraticus* 35-7 ^{T} (KCTC 15,125; 95.8%). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain AGMB03513^T is a species within the family *Lachnospiraceae* (Fig. 1).

Phenotypic and biochemical characteristics

Cells of strain AGMB03513^T were found to be strictly anaerobic, gram-negative, non-motile, and did not form spores. The strain failed to grow on RCM agar incubated in air or in an atmosphere containing 5% CO_2, whereas under anaerobic conditions, cells grew in several long chains of connected rods, referred to as segmented filamentous bacteria (SFB) (Figs. S1 and S2). Colonies grown on RCM agar were circular, convex, white, opaque, and shiny, and grew at temperatures between 35 and 45 °C (optimum at 37 °C). In RCM broth, cells were found to grow at pH values ranging from 7 to 9 (optimum pH 7) and NaCl concentrations up to 1.5%. The isolate was observed to utilise carbon sources such as D-glucose, D-mannitol, D-lactose, D-saccharose, D-mannose, D-sorbitol, and D-raffinose, and to a limited extent, L-leucine. As the final product of fermentation, strain AGMB03513^T produces acetate and small amounts of propionate and butyrate, the latter of which is the final fermentation product of the reference strain of *A. caccae* used in the present study (Table 1). However, none of the four reference strains produced acetate as the final fermentation product. Furthermore, strain AGMB03513^T showed no evidence of catalase or oxidase activity.

Chemotaxonomic and genomic characteristics

The major cellular fatty acids (> 10%) of strain AGMB03513^T were $C_{12:0}$ (20.8%), $C_{16:0}$ (16.8%), and $C_{18:0}$ (11.9%). The major fatty acids of the four reference strains in this study were as follows: *A. caccae* DSM 14662 ^{T}: $C_{12:0}$ (29.7%), $C_{18:0}$ DMA (12.5%), and $C_{18:0}$ ALDE (19.5%); *A. butyraticus* DSM 22094 ^{T}: $C_{12:0}$ (32.0%), $C_{18:0}$ (12.1%), and $C_{18:0}$ ALDE (12.2%); *A. rhamnosivorans* DSM 26241 ^{T}: $C_{12:0}$ (32.1%), $C_{16:0}$ (9.4%), $C_{18:0}$ ALDE (12.6%), and *A. hadrus* DSM 3319 ^{T}: $C_{11:0}$ DMA (13.2%), $C_{12:0}$ (24.5%), $C_{18:0}$ DMA (15.4%), and $C_{18:0}$ ALDE (22.7%). The details of the cellular fatty acid profiles of strain AGMB03513^T and the reference strains are shown in Table 2. Strain AGMB03513^T contained the following polar lipids: three glycophosphoaminolipids, four glycolipids, four unidentified lipids, one

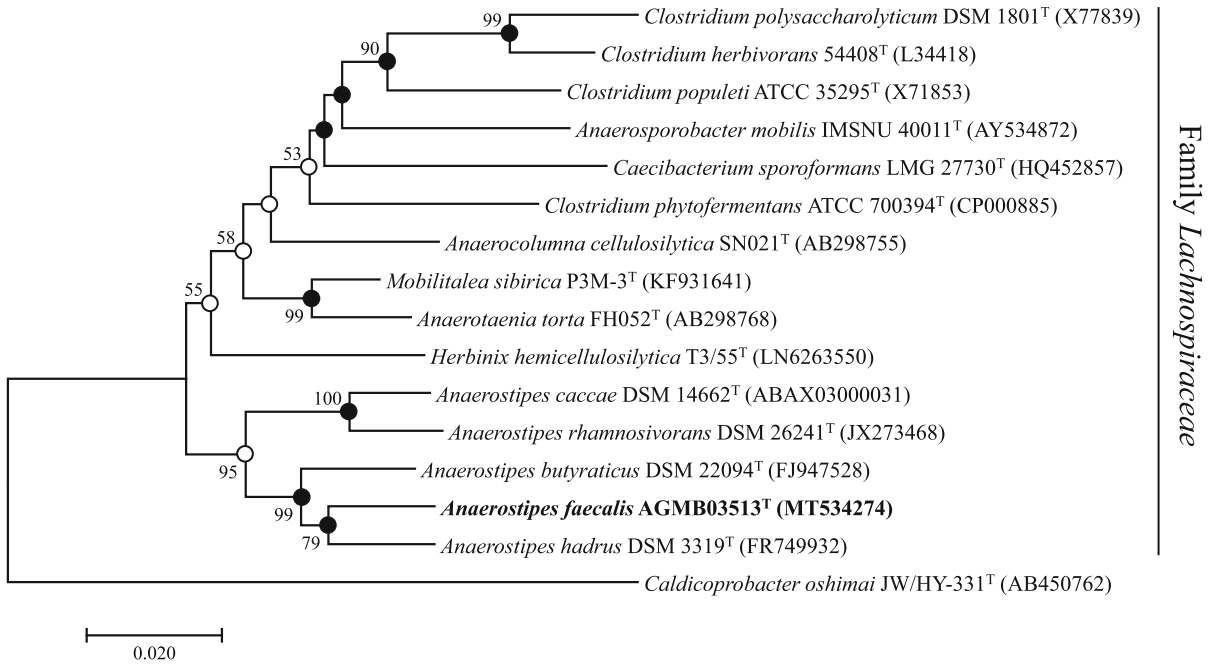

Fig. 1 Phylogenetic tree based on the near full-length 16S rRNA gene sequence, showing the placement of strain AGMB03513^T and relationships between strain AGMB03513^T and related taxa among species in the family *Lachnospiraceae*. Phylogenetic trees were constructed using neighbour-joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) methods, with bootstrap values obtained from 1000 replicates. Bootstrap values > 50% are shown at the nodes. Filled circles indicate that the corresponding nodes (groupings) were recovered in trees generated using the NJ, ML, and MP methods. The open circles indicate that the corresponding nodes are also depicted by NJ and ML. Bars: 0.02 substitutions per nucleotide position

glycophosphoaminolipid, one glycoaminolipid, one phospholipid, and an aminolipid (Fig. S4).

The genome of strain AGMB03513^T is 2,544,126 bp in length and contains 2,492 coding sequences and genes encoding 10 rRNA and 59 tRNAs. The ANI and AAI values obtained based on comparisons between strain AGMB03513^T (JABRXE000000000) and strains of the four congeneric species *A. butyraticus* JCM 17,466 (BLYI00000000), *A. caccae* NCIMB 13811 ^{T} (CP036345), *A. hadrus* ATCC 29173 ^{T} (AMEY00000000), and *A. rhamnosivorans* 1y-2 ^{T} (CP040058) were 75.3%, 71.0%, 75.5%, and 71.2% and 73.2%, 66.6%, 72.5%, and 66.7%, respectively, and the respective dDDH values were 19.5%, 20.2%, 20.1%, and 21.4%. These values were notably lower than the threshold values of ANI and AAI (95%–96%) and dDDH (70%) for differentiating bacterial species.

Strain AGMB03513^T also contained *meso*-diaminopimelic acid (DAP) in the cell wall (Fig. S3), which is synthesised from L-aspartate and L-aspartate via tetrahydrodipicolinate (THDPA) as an intermediate product (Rodionov et al. 2003; Xu et al. 2019). First, we confirmed that strain AGMB03513^T has the *lysC*, *asd*, *dapA*, and *dapB* genes required for conversion from L-aspartate to THDPA. Next, there are several pathways that convert THDPA to *meso*-DAP (Xu et al. 2019), among which the isolate has *the dapDH* gene that is involved in the succinylase pathway. In addition, the cells have the *dapL* and *dapF* genes that play roles in another synthetic pathway from THDPA to *meso*-DAP, called the *meso*-DAP/L-lysine biosynthetic pathway. Strain AGMB03513^T contains all the key genes required for the pathway mentioned above.

DNA G + C content

The G + C content of strain AGMB03513^T genomic DNA was found to be 37.0 mol%, which compared with the values of 45.5–46.0 mol%, 44.0 mol%, 44.5 mol%, and 37.0 mol% obtained for the reference

Table 1 Differential characteristics of strain AGMB03513^T and the phylogenetically related type species in the genus *Anaerostipes*

Characteristic	1	2	3	4	5
Source	Swine faeces	Human faeces[a]	Chicken caecal[b]	Human intestinal[c]	Human faeces[d]
Cell morphology	Segmented filaments	Rods[a]	Large rods[b]	Curved rods[c]	Rods[d]
Size (μm)	NA	0.5–0.6 × 2.0 − 4.0[a]	5.0–15.0[b]	0.7–0.8 × 3.0–6.0[c]	4.8 × 0.8[d]
Temperature range for growth (°C)	30–45	30–45	35–45	30–45	30–45
pH range for growth	7–9	6–10	6–8	6–9	6–9
NaCl concentration for growth (%)	0.5–1.5	0.5–3.5	0.5–3.0	0.5–3.0	0.5–4.0
Gram reaction	−	v[a]	+[b]	v[c]	+[d]
Spore formation	−	−[a]	+[b]	+[C]	−[d]
Enzyme activity	−				
Arginine arylamidase	−	W	−	W	−
Leucine arylamidase	W	+	−	+	−
Acid production					
D-saccarose	−	+	W	+	−
Esculin	−	+	+	−	−
D-cellobiose	−	−	+	+	W
D-rhamnose	−	−	−	+	W
D-trehalose	−	+	+	+	−
End products of fermentation	A, p, b	A, B[a]	B[b]	A, B[c]	B[d]
DNA G + C content (mol%)	37.0	45.5–46.0[a]	44.0[b]	44.5[c]	37.0[d]

Strains: 1, *Anaerostipes faecalis* AGMB03513^T; 2, *Anaerostipes caccae* KCTC 15019 ^{T}; 3, *Anaerostipes butyraticus* KCTC 15125 ^{T}; 4, *Anaerostipes rhamnosivorans* KCTC 15316 ^{T}; and 5, *Anaerostipes hadrus* KCTC 15606 ^{T}

Unless otherwise stated, all presented data were obtained in the present study

+ , Positive; −, negative; W, weakly positive. NA, not available; v, gram variable; A, acetate; P, propionate; B, butyrate. Upper- and lower-case letters indicate the major and minor end products of fermentation, respectively

[a]Data from Schwiertz et al. (2002), [b]Eeckhaut et al. (2010), [c]Bui et al. (2014), and [d]Allen-Vercoe et al. (2012)

strains of *A. caccae*, *A. butyraticus*, *A. rhamnosivorans*, and *A. hadrus*, respectively (Allen-Vercoe et al. 2012) (Table 1).

Taxonomic conclusions

Phylogenetic tree analysis based on 16S rRNA gene sequences revealed that strain AGMB03513^T belongs in the family *Lachnospiraceae* and is closely related to species in the genus *Anaerostipes*. The strain AGMB03513^T showed 93.3–95.5% identity to the four reference strains with respect to the 16S rRNA gene sequence and showed clear similarities as well as differences with respect to phenotypic, biochemical, chemotaxonomic, and genomic characteristics. Based on this evidence, we consider it reasonable to designate strain AGMB03513^T as a novel species in the genus *Anaerostipes*, for which the name *Anaerostipes faecalis* sp. nov. is proposed.

Description of *Anaerostipes faecalis* sp. nov.

Anaerostipes faecalis sp. nov. (fae.ca'lis. L. fem. adj. *faecalis* derived from faeces).

Cells are long, rod-shaped, gram-negative, non-motile, and non-spore-forming obligate anaerobes. SEM images revealed segmented filamentous bacterial morphology. Colonies cultured for 24–48 h on RCM agar were circular, convex, white, opaque, and shiny. Growth occurred at temperatures between 30

Table 2 Cellular fatty acid profiles (% of total) of strain AGMB03513^T and the type strains of closely related species in the genus *Anaerostipes*

Fatty acid	1	2	3	4	5
$C_{10:0}$	TR	1.6	–	1.7	1.1
$C_{11:0}$ DMA	1.8	TR	5.2	1.1	**13.3**
$C_{12:0}$	**20.8**	**29.7**	**32.0**	**32.12**	**24.5**
$C_{14:0}$	8.0	6.5	5.6	4.2	2.7
$C_{14:0}$ DMA	1.6	TR	1.4	TR	TR
anteiso-$C_{15:0}$	–	TR	2.5	TR	–
iso-$C_{15:0}$	–	TR	1.6	–	–
$C_{16:0}$	**16.4**	5.2	7.2	9.4	3.5
$C_{16:0}$ DMA	5.8	1.8	2.8	5.4	1.8
$C_{16:0}$ ALDE	5.6	2.1	2	5.6	2.0
$C_{17:0}$ *cyc*	1.5	3.0	1.9	2.2	3.8
$C_{18:0}$	**12.0**	2.3	**12.1**	2.5	4.8
$C_{18:0}$ DMA	4.7	**12.5**	7.2	5.9	**15.4**
$C_{18:0}$ ALDE	7.1	**19.5**	**12.7**	**12.6**	**22.7**
$C_{18:1}$ DMA	1.1	2.2	1.5	1.7	2.5
$C_{18:1}$ *cis* 9	1.8	TR	–	TR	–
$C_{18:1}$ *cis* 9 DMA	3.2	4.0	–	4.3	–
Summed features*					
1	TR	TR	1.1	–	TR
7	3.7	6.4	–	6.0	–

Strains: 1, *Anaerostipes faecalis* AGMB03513^T; 2, *Anaerostipes caccae* KCTC 15019 ^{T}; 3, *Anaerostipes butyraticus* KCTC 15125 ^{T}; 4, *Anaerostipes rhamnosivorans* KCTC 15316 ^{T}; 5, *Anaerostipes hadrus* KCTC 15606 ^{T}

All presented data were obtained in the present study. Values are percentages of total cellular fatty acids

TR, trace amount (< 1%); –, not detected. Only fatty acids accounting for more than 1% in at least one of the strains are shown

Major components (> 10%) are highlighted in bold

*Summed features: 1, $C_{13:1}$ *cis* 12 and/or $C_{14:0}$ ALDE; 7, $C_{17:2}$ and/or $C_{17:1}$ *cis* 8

and 45 °C (optimum 37 °C) within a pH range of 7–9 (optimum pH 7). API 20A strip analysis indicated that cells produce acid from D-glucose, D-mannitol, D-lactose, D-saccharose, D-mannose, and D-sorbitol, whereas acid production from D-maltose, D-xylose, and L-arabinose was weakly positive, and no acid was produced from salicin, glycerol, D-cellobiose, D-melezitose, D-raffinose, D-rhamnose, and D-trehalose. Neither indole nor urease were detected. Additionally, esculin and gelatin hydrolysis was not observed. API Rapid ID 32A strip analysis revealed positive reactions for alkaline phosphatase and the fermentation of D-mannose and D-raffinose, whereas negative reactions were detected for urease, arginine dihydrolase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, nitrate reduction, indole production, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, leucine arylamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase, and serine arylamidase. However, leucine arylamidase activity was weakly positive. API ZYM strip analysis indicated positive reactions for alkaline phosphatase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, whereas esterase (C4) and leucine arylamidase showed weakly positive activity. In contrast, negative reactions were observed for esterase lipase (C8), lipase (C14), valine arylamidase, crystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. As end products of fermentation, cells produce acetate, propionate, and butyrate. *meso*-DAP was identified as the diagnostic cell-wall diamino acid. The cell polar lipid profile comprised three glycophosphoaminolipids, four glycolipids, four unidentified lipids, one glycophosphoaminolipid, one glycoaminolipid, one phospholipid and an aminolipid; the major cellular fatty acids (> 10%) were $C_{12:0}$, $C_{16:0}$, and $C_{18:0}$. The G + C content of genomic DNA was 37.0 mol%.

The type strain AGMB03513^T (= KCTC 25020 ^{T} = NBRC 114896 ^{T}) was isolated from swine faeces. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AGMB03513^T is MT534274, and the GenBank/EMBL/DDBJ accession number for the whole genome sequence of strain AGMB03513^T is JABRXE000000000.

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Authors' contribution SHP, YH, SHC, JYC designed and coordinated this study; SHC and JYC performed the experiments and drafted the manuscript; JEP and JSK helped with the experiments and interpreted the results; SWK, JL,

MKL, JSL, and JHL collected the samples and helped in the experiments; SHP, HJ, TH, HBK, JHL, and JKK revised the manuscript.

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Declarations

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

Ethical approval The experimental protocols for this study were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (NIAS-2019-1731).

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