Anaerostipes Faecalis Sp. Nov. Isolated from Swine Faeces

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Research Article
Abstract

A novel, strictly anaerobic, gram-negative, segmented filamentous bacterium (SFB), strain AGMB03513\textsuperscript{T}, was isolated from the faeces of a 5-month-old pig. Comparative analysis of 16S rRNA gene sequences indicated that strain AGMB03513\textsuperscript{T} forms a lineage within the genus \textit{Anaerostipes} and is most closely related to \textit{Anaerostipes butyraticus} DSM 22094\textsuperscript{T} (= KCTC 15125\textsuperscript{T}, 95.8\%), \textit{Anaerostipes hadrus} DSM 3319\textsuperscript{T} (= KCTC 15606\textsuperscript{T}, 95.5\%), \textit{Anaerostipes caccae} DSM 14662\textsuperscript{T} (= KCTC 15019\textsuperscript{T}, 94.0\%), and \textit{Anaerostipes rhamnosivorans} DSM 26241\textsuperscript{T} (= KCTC 15316\textsuperscript{T}, 93.4\%). Phylogenetic analysis based on the 16S rRNA gene and whole genome sequencing analysis revealed that its closest relatives are members of the family \textit{Lachnospiraceae} and that the closest related is \textit{Anaerostipes butyraticus}. Strain AGMB03513\textsuperscript{T} grows at temperatures of between 30 and 45°C within a pH range of 7.0 to 9.0, and in medium containing up to 1.5% NaCl. Cells were found to utilize 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 DNA G+C content of the strain is 37.0 mol\%. Average nucleotide identity (ANI) values obtained in comparisons of strain AGMB03513\textsuperscript{T} with reference strains of species in the genus \textit{Anaerostipes} were between 71.0\% and 75.7\%, which are below the ANI criterion for interspecies identity. The major components of cellular fatty acids were C\textsubscript{12:0}, C\textsubscript{16:0}, and C\textsubscript{18:0}. On the basis of phenotypic, phylogenetic, biochemical, chemotaxonomic, and genomic characteristics, we consider it reasonable to assign novel species status to strain AGMB03513\textsuperscript{T}, for which we propose the name \textit{Anaerostipes faecalis} sp. nov. The type strain AGMB03513\textsuperscript{T} (=KCTC 25020\textsuperscript{T}=NBRC 114896\textsuperscript{T}).

Introduction

In the past few decades, antibiotics have been used indiscriminately to prevent diseases and promote livestock growth. However, this indiscriminate usage has given rise to the increasing emergence of antibiotic-resistant bacteria, which affects not only livestock but also humans (Gong et al., 2014). In recent years, antibiotic alternatives such as prebiotics and probiotics have been used with increasing frequency (Mingmongkolchai and Panbangred, 2018). Identifying viable alternatives to antibiotics is of particular importance in the pork industry, in which premature mortality accounts for approximately 15\% of the stock. In this regard, the use of probiotics and prebiotics as antibiotic alternatives can improve health and limit disease by nurturing healthy intestinal microbiota through the promotion of optimal initial microbial colonization (Nowland et al., 2019). In suckling piglets, the bacterial genera \textit{Bacteroides}, \textit{Oscillibacter}, \textit{Escherichia}, \textit{Shigella}, \textit{Lactobacillus}, and unclassified \textit{Ruminococcaceae} are the primary components of the intestinal microbiota. In contrast, after weaning, species in the genera \textit{Acetivibrio}, \textit{Dialister}, \textit{Oribacterium}, \textit{Succinivibrio}, and \textit{Prevotella} become increasingly prominent (Mach et al., 2015). Analysis of the intestinal contents of piglets, has revealed that the dominant genus \textit{Lactobacillus} plays an important role in disease prevention by reducing gut populations of pathogenic bacteria (Beasley, 2004). Furthermore, a higher abundance of \textit{Prevotella} has been found to be associated with increased body weight, which is correlated with the production of luminal secretory IgA by \textit{Prevotella} within the gut (Mach et al., 2015). However, our current understanding of the role of the gut microbiota in promoting
livestock health is notably limited, and therefore, there is a pressing need for further studies on the characterization of intestinal microorganisms, the effects of these microorganisms on host health, and microbial community manipulation (Nowland et al., 2019).

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*. The members of *Anaerostipes* are non-motile, rod-shaped, gram-variable obligate anaerobes (Schwiertz et al., 2002). At the time of writing, the genus *Anaerostipes* comprised four species with validly published names (www.bacterio.net/anaerostipes.html) (Euzéby, 1997). In this study, we isolated a novel strain, AGMB03513\(^T\), from swine faeces, which was characterized through phenotypic, biochemical, phylogenetic, and chemotaxonomic analyses, and on the basis of 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 faeces of swine raised in the National Institute of Animal Science (Wanju, Republic of Korea). The collected samples were immediately maintained under anaerobic conditions 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 agar containing 5% (v/v) sheep blood (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 stored at -80°C in 10% (w/v) skim milk. For the purpose of comparative studies, we used strains of the four existing members of the genus, namely, *Anaerostipes butyricus* 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, Republic of Korea; 10.0 g peptone, 5.0 g NaCl, 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 by 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. (Republic of Korea). The complete 16S rRNA sequence was assembled by comparison with the whole-genome sequence 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 16s 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 two-parameter model (Kimura, 1980). 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 the purposes of phenotypic and biochemical analyses, the AGMB03513\(^T\) isolate was grown on RCM agar for 24–48 h. 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 microscopy (FEI, USA). Gram staining was performed using a Gram stain kit (Difco) according to the manufacturer's instructions. KOH tests were based on determinations of the formation of viscous and mucoid strings within 15 s (Suslow, 1982). To determine the optimal growth conditions, cells were incubated at a temperature of 37°C, over a temperature range of 10 to 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 using a DU 700 UV-visible spectrophotometer (Beckman Coulter, CA, USA). Catalase activity was confirmed based on bubble formation using a catalase reagent (bioMérieux, #55561), and oxidase activity was verified based on the production of a purple colouration by using an oxidase reagent (bioMérieux, #55635). Spore formation was assessed using the Schaeffer–Fulton method with malachite green (Schaeffer and Fulton, 1933), whereas for characterization 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 characterized in cell-free supernatants derived from cells cultured for 2 days in RCM broth by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) equipped with Aminex™ Organic Acid Columns (Bio-Rad, CA, USA). The diamino acid in 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 24 h. The cellular fatty acids were saponified, methylated, and extracted according to 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 a 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 and Konstantinidis, 2016). Digital DNA–DNA hybridization (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, Republic of 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 AGMB03513T is closely related to species in the genus *Anaerostipes*. AGMB03513T showed sequence similarities of between 93.3% and 95.8% with the reference bacteria, with highest similarity to *A. butyraticus* 35-7T (KCTC 15125; 95.8%). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain AGMB03513T is a species within the family *Lachnospiraceae* (Fig. 1).

**Phenotypic and biochemical characteristics**

Cells of strain AGMB03513T were found to be strictly anaerobic, gram-negative, non-motile, and formed spores. The strain failed to grow on RCM agar incubated in air or in an atmosphere containing 5% CO₂, 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 of 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 utilize 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 final products of fermentation, strain AGMB03513T 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 were found to produce acetate as the final fermentation product. Furthermore, strain AGMB03513T showed no evidence of either catalase or oxidase activity.

**Chemotaxonomic and genomic characteristics**
The major cellular fatty acids (>10%) of strain AGMB03513<sup>T</sup> were C<sub>12:0</sub> (20.8%), C<sub>16:0</sub> (16.8%), and C<sub>18:0</sub> (11.9%). Comparatively, the major fatty acids of the four reference strains in this study are as follows: A. caccae DSM 14662<sup>T</sup>: C<sub>12:0</sub> (29.7%), C<sub>18:0</sub> DMA (12.5%), and C<sub>18:0</sub> ALDE (19.5%); A. butyraticus DSM 22094<sup>T</sup>: C<sub>12:0</sub> (32.0%), C<sub>18:0</sub> (12.1%), and C<sub>18:0</sub> ALDE (12.2%); A. rhamnosivorans DSM 26241<sup>T</sup>: C<sub>12:0</sub> (32.1%), C<sub>16:0</sub> (9.4%), and C<sub>18:0</sub> ALDE (12.6%); and A. hadrus DSM 3319<sup>T</sup>: C<sub>11:0</sub> DMA (13.2%), C<sub>12:0</sub> (24.5%), C<sub>18:0</sub> DMA (15.4%), and C<sub>18:0</sub> ALDE (22.7%). Details of the cellular fatty acid profiles of strain AGMB03513<sup>T</sup> and the reference strains are shown in Table 2. Strain AGMB03513<sup>T</sup> was found to contain the following polar lipids: three glycpophosphoaminolipids, four glycolipids, four unidentified lipids, one glycpophosphoaminolipid, one glycoaminolipid, one phospholipid, and an aminolipid (Fig. S4).

The genome of strain AGMB03513<sup>T</sup> 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<sup>T</sup> (JABRXE000000000) and strains of the four congeneric species A. butyraticus JCM 17466 (BLYI000000000), A. caccae NCIMB 13811<sup>T</sup> (CP036345), A. hadrus ATCC 29173<sup>T</sup> (AMEY000000000), and A. rhamnosivorans 1y-2<sup>T</sup> (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 found to be notably lower than the threshold values of ANI and AAI (95%–96 %) and dDDH (70 %) for differentiating bacterial species.

Strain AGMB03513<sup>T</sup> was also found to contain meso-diaminopimelic acid (DAP) in the cell wall (Fig. S3), which is synthesized from l-aspartate, and l-aspartate via tetrahydrodipicolinate (THDPA) as an intermediate product (Rodionov et al., 2003, Xu et al., 2019). Within cells, l-aspartate is converted to THDPA via activity of the lysC, asd, dapA, and dapB gene products (Rodionov et al., 2003), and there are several pathways whereby THDPA is converted to DAP (Xu et al., 2019), among which is the succinylase pathway containing the enzyme encoded by dapDH. In addition, the new isolate expresses the dapL and dapF genes that play roles in the meso-DAP/L-lysine biosynthetic pathway.

**DNA G+C content**

The G + C content of strain AGMB03513<sup>T</sup> 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 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<sup>T</sup> is grouped in the family Lachnospiraceae and closely related to species in the genus Anaerostipes. The strain AGMB03513<sup>T</sup> 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. On the basis of this evidence, we consider it reasonable to designate strain AGMB03513ᵀ 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 were long rod-shaped, gram-negative, non-motile, and non-spore forming obligate anaerobes. SEM images revealed a 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 and 45°C (optimum 37°C) within a pH range from 7 to 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-xylene, and l-arabinose was weakly positive, and no acid was produced from salicin, glycerol, d-cellobiose, d-melezitose, d-raffinose, d-rhamnose, or d-trehalose. Neither indole nor urease was detected. Additionally, esculin and gelatin hydrolysis were absent. 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, phenylalanine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase, and serine arylamidase. Leucine arylamidase activity was, however, found to be 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, cystine 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 glycoprophosphoaminolipids, four glycolipids, four unidentified lipids, one glycoprophosphoaminolipid, one glycoaminolipid, one phospholipid, and an aminolipid, and the major cellular fatty acids (>10%) were C₁₂:₀, C₁₆:₀, and C₁₈:₀. The G + C content of genomic DNA was 37.0 mol%.

The type strain AGMB03513ᵀ (=KCTC 25020ᵀ = NBRC 114896ᵀ) was isolated from swine faeces. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AGMB03513ᵀ is MT534274, and the GenBank/EMBL/DDBJ accession number for the whole-genome sequence of strain AGMB03513ᵀ is JABRXE0000000000.

**Abbreviations**
SFB  segmented filamentous bacterium  
ANI  average nucleotide identity  
AGMB  animal gut microbiome bank  
KCTC  Korean Collection for Type Cultures  
TSAB  Tryptic soy agar containing 5% (v/v) sheep blood  
RCM  reinforced clostridial medium  
DAP  diaminopimelic acid

Declarations

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Author contributions

Seung-Hwan Park, Seung-Hyeon Choi, Ji Young Choi designed and coordinated this study; Seung-Hyeon Choi, Ji Young Choi performed experiments and draft manuscript; Jameon Park, Ji-Sun Kim helped in experiments and interpreting the results; Se Won Kang, Jiyoung Lee, Mi-Kyung Lee, Jung-Sook Lee, Ju Huck Lee collected the samples and helped in experiments; Seung-Hwan Park, Hyunjung Jung, Tai-Young Hur, Hyeun Bum Kim, Ju-Hoon Lee, Jae-Kyung Kim revised the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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**Tables**
Table 1. Differential characteristics of strain AGMB03513\textsuperscript{T} and the phylogenetically related type species in the genus *Anaerostipes*

| Characteristic                          | 1                          | 2                          | 3                          | 4                          | 5                          |
|----------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Source                                 | Swine faeces                | Human faeces\textsuperscript{a} | Chicken caecal\textsuperscript{b} | Human intestinal\textsuperscript{c} | Human faeces\textsuperscript{d} |
| Cell morphology                        | Segmented filaments         | Rods\textsuperscript{a}     | Large rods\textsuperscript{b} | Curved rods\textsuperscript{c} | Rods\textsuperscript{d}     |
| Size (\(\mu\))                        | NA                          | 0.5−0.6 × 2.0−4.0\textsuperscript{a} | 5.0−15.0\textsuperscript{b} | 0.7−0.8 × 3.0−6.0\textsuperscript{c} | 4.8 × 0.8\textsuperscript{d} |
| Temperature range for growth (ºC)      | 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                          | -                           | +\textsuperscript{a}        | +\textsuperscript{b}        | +\textsuperscript{c}        | +\textsuperscript{d}        |
| Spore formation                        | -                           | -\textsuperscript{a}        | +\textsuperscript{b}        | +\textsuperscript{c}        | -\textsuperscript{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                     | b                           | NA                          | NA                          | NA                          |
| DNA G + C content (mol\%)              | 37.0                        | 45.5−46.0\textsuperscript{a} | 44.0\textsuperscript{b}     | 44.5\textsuperscript{c}     | 37.0\textsuperscript{d}     |

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

\textsuperscript{a}Data from Schwirtz et al. (2000); \textsuperscript{b}Eeckhaut et al. (2010); \textsuperscript{c}Bui et al. (2014); and \textsuperscript{d}Allen-Vercoe et al. (2012).

**Table 2.** Cellular fatty acid profiles (% of total) of strain AGMB03513\textsuperscript{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\textsuperscript{T}; 2, *Anaerostipes caccae* KCTC 15019\textsuperscript{T}; 3, *Anaerostipes butyraticus* KCTC 15125\textsuperscript{T}; 4, *Anaerostipes rhamnosivorans* KCTC 15316\textsuperscript{T}; 5, *Anaerostipes hadrus* KCTC 15606\textsuperscript{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.
Figures

Figure 1

Phylogenetic tree based on the near full-length 16S rRNA gene sequence, showing the placement of strain AGMB03513T and relationships between strain AGMB03513T 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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryfiguresAnaerostipesChoietal.pdf