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Anaerococcus faecalis sp. nov., Isolated from Swine Faeces

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Abstract

An obligate anaerobic, Gram-stain-positive, non-spore forming, non-motile, catalase and oxidase-negative, coccoid-shaped bacterium designated AGMB00486^T was isolated from swine faeces. The optimal growth of the isolate occurred at pH 8.0 and 37 ℃. Furthermore, the growth was observed in the presence of up to 4% (w/v) NaCl but not at salinity levels higher than 5%. The phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain AGMB00486^T was a member of the genus *Anaerococcus* and that the isolate was most closely related to *Anaerococcus vaginalis* KCTC 15028^T (96.7% 16S rRNA gene sequence similarity) followed by *Anaerococcus hydrogenalis* KCTC 15014^T (96.7%) and *Anaerococcus senegalensis* KCTC 15435^T (96.3%). Whole-genome sequence analysis determined that the DNA G+C content of strain AGMB00486^T was 30.1 mol%, and the genome size, numbers of tRNA and rRNA genes were 2,268,866 bp, 47 and 8, respectively. The average nucleotide identity values between strain AGMB00486^T and the three related type strains were 77.0, 77.4 and 77.2%, respectively. The major cellular fatty acids (> 10%) of strain AGMB00486^T were $C_{14:0}$, $C_{16:0}$ and $C_{16:0}$ DMA. Accordingly, these distinct phenotypic and phylogenetic properties revealed that strain AGMB00486^T represents a novel species, for which the name *Anaerococcus faecalis* sp. nov. is proposed. The type strain is AGMB00486^T (= KCTC 15945^T = CCTCC AB 202009^T).

Introduction

The gut microbiota refers to a population of microbes that inhabit the gastrointestinal tract, and they are symbiotic through interaction with the host [1]. It has been known that the gut microbiota are essential to promote host health, controlled and maintained by various factors such as eating habits, environment and heredity; the miscontrolled gut microbiota called gut dysbiosis has been, however, linked to a variety of diseases in the host [2]. Therefore, the understanding of the intestinal microbiota can help to improve the health of host. Recently, with the improvement of 16S rRNA sequencing technology, the metagenomic analysis of human gut microbiota has been actively progressing, but compared to this, swine gut microbiota are relatively less analysed although it was also suggested that gut flora were likely to play a role in pig physiology [3]. To understand the gut microbiota of swine in Korea, more than 100 faeces samples from healthy pigs raised in the National Institute of Animal Science in Korea were employed for metagenomic analysis. At the same time, the gut microbes of pigs were

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isolated, leading to the discovery of novel strains including strain AGMB00486^T.

The genus *Anaerococcus* was reclassified from the genus *Peptostreptococcus* by Ezaki, in which six species were included in the genus *Anaerococcus* based on phylogenetic and metabolic features [4]. At the time of writing, the genus *Anaerococcus* includes 13 validly named species (https://lpsn.dsmz.de/genus/peptoniphilus). All of them were isolated from human, and mostly from infectious site, such as peritoneal, vaginoses, ovarian, digital and cervical abscesses, bacteremias, sacral, foot ulcers, arthritic knee and sternal wound [4–10]. This study describes a new member of the genus *Anaerococcus*, which is first animal origin, isolated from faecal samples of industrial swine faeces.

Materials and Methods

Isolation of Bacterial Strain and Culture Conditions

Strain AGMB00486^T was isolated from swine faeces, which was collected from swine farm located in the National Institute of Animal Science in Cheonan, Republic of Korea. The geographic coordinates are 36.93 N, 127.11 E. The isolation and culture of bacteria from faecal sample were performed in an anaerobic chamber (Coy Laboratory Products) filled with 86% N_2, 7% CO_2 and 7% H_2. For isolation, the faecal sample was suspended and serially diluted in a sterilized saline solution (0.85% NaCl), followed by cultivation onto reinforced clostridial agar (MB cell) supplemented with 5% horse blood (RCAB). After 3 days of incubation at 37 ℃, single colonies were transferred on new RCAB plates under the anaerobic conditions. A flat, circular and slimy texture colony of strain AGMB00486^T was obtained and subjected to taxonomic analysis based on phenotypic, physiological and phylogenetic studies. The isolate was routinely cultured on RCAB plates at 37 ℃ in an anaerobic chamber for 48 h and preserved at − 80 ℃ in 10% (w/v) skim milk. Strain AGMB00486^T was deposited in KCTC and CCTCC culture collections.

Phenotypic and Biochemical Analyses

The cell morphology of strain AGMB00486^T was observed by a phase-contrast microscope (Eclipse 80i, Nikon), a scanning electron microscopy (SEM; CX-200TA, Coxem) and a transmission electron microscope (TEM; CM120, Philips) using the cells cultivated on RCAB for 3 days at 37 ℃ in anaerobic chamber. For SEM analysis, the cells were fixed and dehydrated with gradient ethanol solutions, followed by isoamyl acetate and hexamethyldisilazane. Then, the sample was dried and coated with gold in a sputter coater (SPT-20, Coxem) and observed using the SEM. For TEM analysis, the fixed cells were applied to carbon-coated grids that had been glow-discharged for 3 min in air. The grids were then negatively stained using 1% uranyl acetate and observed using the TEM (Supplementary data Fig. S1). Gram-staining was performed as described by Buck [11]. Bacterial growth was tested in aerobic, microaerophilic (CO_2 incubator with 5% O_2) and anaerobic condition (anaerobic chamber) for 3 days at 37 ℃. The optimal temperature for growth was determined at various temperature ranges (10–50 ℃ using increments of 5 ℃ units and 37 ℃) and NaCl concentration (0.5, 1, 2, 3, 4 and 5%, w/v) was also determined on RCAB. The bacterial growth at various pH range (4–10 using an increment of 1 pH unit) was determined by inoculating strain AGMB00486^T in pH-adjusted GAM (MB cell) broth. The pH range was adjusted using appropriate biological buffers [12] as follows: 100 mM acetate buffer (for pH 4.0–5.0), 100 mM phosphate buffer (pH 6.0–8.0) or 100 mM $NaHCO_3$/Na_2CO_3 buffer (pH 9.0–10.0). The pH values were measured after autoclaving and only minor change was confirmed. The growth was monitored by measuring OD_{600} using a DU 700 UV–visible spectrophotometer (Beckman Coulter). Catalase and oxidase activity tests were performed using a commercial dropper reagent (bioMérieux). In addition, the spore production ability was evaluated with 70% (v/v) ethanol test [13]. The biochemical properties of strain AGMB00486^T were determined using the API 20A and rapid ID 32A (bioMérieux). For profiling of the fermentation end products, the cultured medium was analysed using a high-performance liquid chromatography (HPLC) system (Shimadzu) with Aminex™ Organic Acid Columns (Bio-Rad).

16S rRNA Gene Sequencing and Phylogenetic Analysis

For phylogenetic analysis, the gDNA of AGMB00486^T was used as a template for the amplification of 16S rRNA gene using the universal primers as follows [14]: 518F (5′- CCA GCAGCCGCGGTAATACG -3′), 800R (5′- TACCAGGGT ATCTAATCC -3′), 785F (5′- GGATTAGATACCCTGGTA -3′) and 907R (5′- CCGTCAATTCMTTTRAGTTT -3′) (Macrogen Inc.). The complete 16S rRNA gene sequence was confirmed in the result of whole-genome sequence. The 16S rRNA gene sequence of strain AGMB00486^T was compared with the sequences of the related strains obtained from EzBioCloud database (http://www.ezbiocloud.net) and GenBank/EMBL/DDBJ database (http://www.ncbi.nlm.nih.gov/blast) to find the taxonomic position of the isolate [15]. To construct a phylogenetic tree based on the 16S rRNA gene sequences, the sequences of all *Anaerococcus* species were edited and aligned manually using the BioEdit program using CLUSTAL W [16]. The phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis 7 (MEGA 7.0) software [17]. Phylogenetic trees based on 16S rRNA gene sequences were

reconstructed using the neighbour-joining (NJ; Saitou and Nei, 1987) and maximum-likelihood (ML; Fitch, 1971) methods, with the bootstrap analysis performed based on 1000 replications. Evolutionary distances were calculated using Kimura 2-parameter method [18].

Chemotaxonomic and Genomic Analysis

The cellular fatty acid profiles were determined with strain AGMB00486^T and reference strains, *A. vaginalis* KCTC 15028^T, *A. hydrogenalis* KCTC 15014^T and *A. senegalensis* KCTC 15435^T, grown on RCAB for 48 h. The reference strains used in this study were obtained from KCTC. The fatty acids were saponified, methylated and extracted according to the manufacturer's instructions of MIDI/Hewlett Packard Microbial Identification System [19]. The fatty acids were analysed by gas chromatography (model 6890 N; Agilent) and identified using the Microbial Identification Sherlock software package with the Anaerobe database version 6.1.

Genomic DNA (gDNA) was extracted from the cells cultured on RCAB plates using a phenol:chloroform:isoamyl alcohol method described by Wilson et al. [20]. To determine the DNA G+C content and genomic similarity between strain AGMB00486^T and closely related species, the whole-genome sequencing of the extracted gDNA was performed using Illumina NovaSeq technology (Illumina) at Macrogen, Inc. De novo assembly for AGMB00486^T genome was performed using SPAdes (version 3.13.0) after quality trimming. Completeness and contamination of the assembled genome were examined using ContEst16S and CheckM tools. Prodigal and tRNAscan-SE were used to search the coding DNA sequences (CDSs) and tRNA, respectively. The rRNAs were found by covariance model search with inference of Rfam 12.0. Each of the CDSs was annotated by homology search against EggNOG 4.5, SEED and KEGG databases. Average nucleotide identity (ANI) values were calculated using ChunLab's online ANI calculator [21].

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AGMB00486^T is MT568623.

The GenBank/EMBL/DDBJ accession number for the whole-genome sequence of strain AGMB00486^T is NZ_JABVBA000000000.

Results and Discussion

Phylogenetic Analysis

The comparative analysis of 16S rRNA gene sequence (1463 bp) of strain AGMB00486^T indicated that the isolate was closely related to *Anaerococcus vaginalis* KCTC 15028^T (96.7%), *Anaerococcus hydrogenalis* KCTC 15014^T (96.7%) and *Anaerococcus senegalensis* KCTC 15435^T (96.3%). The phylogenetic analysis based on 16S rRNA gene sequences

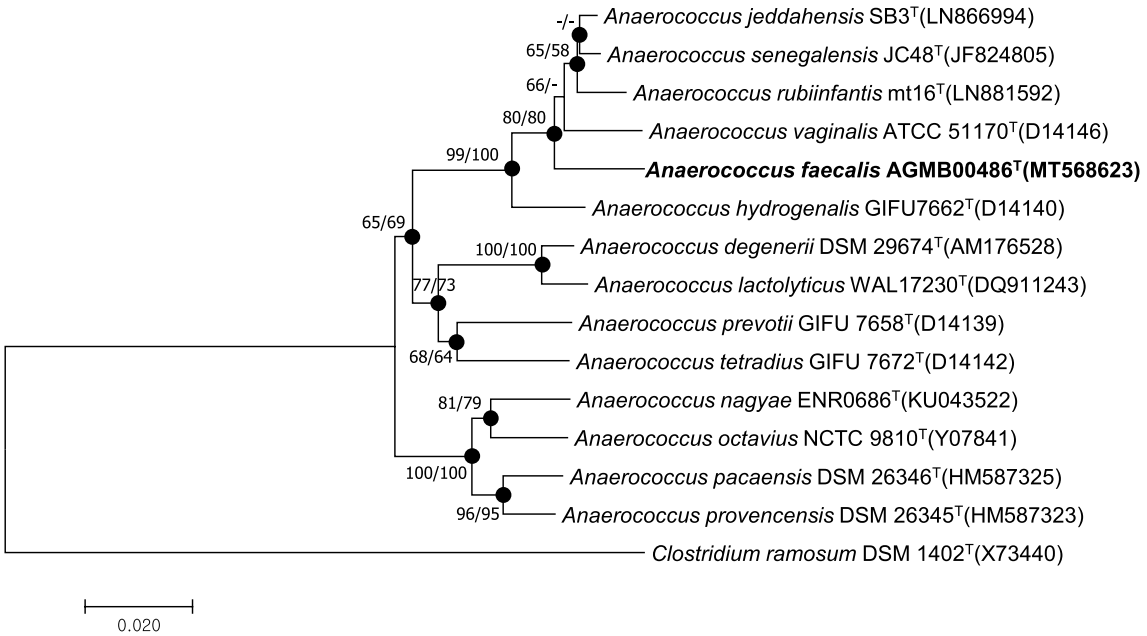

Fig. 1 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain AGMB00486^T and closely related taxa. Numbers at nodes refer to bootstrap values (based on 1000 replicates, only values > 50% are shown at branch points and values ≦50% are displayed as –). Filled circles indicate that the corresponding nodes (groupings) were recovered by the maximum-likelihood and neighbour-joining. Bar, 2% sequence divergence

Table 1 Differential characteristics of strain AGMB00486^T and closely related type species

Characteristics	1	2	3	4
Isolation source	Swine faeces	Vaginal discharges†	Vaginal discharges†	Human faeces‡
Growth temperature range °C	20–45	20–40	25–40	30–37‡
Optimum temperature °C	37	37	37	37‡
Growth pH range	7.0–9.0	NA	NA	NA
Salinity (%)	4	NA	NA	5‡
Cell shape	Coccoid	Coccoid	Coccoid	Coccoid
DNA G+C content (mol%)	30.1	28.9	29.6	28.6‡
Motility	Nonmotile	Nonmotile†	Nonmotile†	Nonmotile‡
Acid production (API 20A) from:				
D-mannitol	+	−	w	−
D-maltose	+	w	w	−
D-xylose	w	−	−	−
Esculin	+	+	−	−
D-trehalose	w	−	w	−
Enzymatic activity (rapid ID 32A)				
Arginine dihydrolase	−	+	−	+
α-glucosidase	w	−	−	−
β-glucuronidase	+	−	−	+
Nitrate reduction	−	w	−	−
Indole production	−	−	−	+
Alkaline phosphatase	w	+	w	−
Arginine arylamidase	w	+	−	+
Leucine arylamidase	−	+	−	+
Alanine arylamidase	w	w	−	−
Glycine arylamidase	−	w	−	−
Histidine arylamidase	−	+	−	+

Strains: 1, AGMB00486^T; 2, *A. vaginalis* KCTC 15028^T; 3, *A. hydrogenalis* KCTC 15014^T; 4, *A. senegalensis* KCTC 15435^T. All these organisms are saccharolytic

+positive, − negative, *w* weakly positive, *NA* no data available

†Data from[4]

‡Data from[22]

showed that strain AGMB00486^T belonged to the genus *Anaerococcus* within family *Peptoniphilaceae*, and that strain AGMB00486^T and the closely related species formed a separate branch of the *Anaerococcus* (Fig. 1). Furthermore, the ANI values between strain AGMB00486^T and the related species, *Anaerococcus vaginalis* KCTC 15028^T (GenBank assembly Accession No. ACXU00000000), *Anaerococcus hydrogenalis* KCTC 15014^T (ABXA00000000) and *Anaerococcus senegalensis* KCTC 15435^T (CAEK00000000) were 77.0, 77.4 and 77.2%, respectively. These ANI values were significantly lower than the proposed cut-off values for bacterial species delineation (95–96%) [21]. Based on the phylogenetic analysis, these three strains were used as reference species for further comparative test.

Phenotypic and Biochemical Characteristics

Strain AGMB00486^T was an obligate anaerobic, Gram-stain-positive, non-spore-forming, non-motile and coccoid-shaped (1.2×1.4 μm). The colonies were flat, circular and slimy texture. The temperature range for growth was 20–45 ℃ (optimum, 37 ℃) and pH range for growth was 7.0–9.0 (optimum, pH 8). Strain AGMB00486^T was negative for oxidase and catalase. The end product of glucose fermentation of strain AGMB00486^T was propionate. The biochemical and phenotypic characteristics based on the API 20A and rapid ID 32A results were summarized in Table 1 (differences) and Supplementary Table S3 (all). Because *A. vaginalis* KCTC 15028^T is the closest species based on 16S similarity, it shows physiological characteristics very similar

Table 2 Cellular fatty acid compositions of strain AGMB00486^T and closely related type species grown on RCAB

Fatty acids	1	2	3	4
Saturated				
$C_{10:0}$	–	1.4	–	–
$C_{12:0}$	2.6	–	–	–
$C_{14:0}$	**22.5**	3.0	3.8	–
$C_{16:0}$	**17.8**	**23.0**	**29.8**	**27.7**
$C_{18:0}$	**10.5**	7.2	9.7	3.2
Unsaturated				
$C_{16:1}$ *ω9c*	7.0	–	–	–
$C_{18:1}$ *ω9c*	4.7	**29.2**	**22.1**	**13.3**
$C_{18:2}$ *ω9,12c*	–	7.8	5.4	**12.2**
Dimethyl acetal (DMA)				
$C_{16:0}$	**21.8**	4.3	2.4	6.5
$C_{16:1}$ *ω9c*	1.8	–	–	–
$C_{18:0}$	3.6	5.1	2.3	6.8
$C_{18:1}$ *ω9c*	2.6	**11.7**	5.7	**11.6**
$C_{18:1}$ *ω11c*	4.3	–	–	–
Aldehyde				
$C_{16:0}$ ALDE	5.1	–	–	–
Summed features*				
10	2.4	–	7.0	–
11	–	3.8	1.9	**12.3**

All data were obtained from this study. Cells were grown on RCMB for 3 days at 37 ℃ in anaerobic condition. Data are reported as the percentage of total fatty acid. Fatty acids that represented < 1.0% are not shown in this table

Major components (> 10%) are highlighted in bold

Strains: 1, AGMB00486^T; 2, *A. vaginalis* KCTC 15028^T; 3, *A. hydrogenalis* KCTC 15014^T; 4, *A. senegalensis* KCTC 15435^T

– not detected

*Summed feature composition is as follows: 10, $C_{18:1}$*ω7c* and/or $C_{18:1}$*ω9t* and/or $C_{18:1}$*ω12t*; 11, iso-$C_{17:0}$ 3–OH and/or $C_{18:2}$ DMA. ALDE, aldehyde; DMA, dimethyl acetal

to those of strain AGMB00486^T, except for α-glucosidase, β-glucuronidase, D-mannitol, D-xylose and D-trehalose, which were positive in strain AGMB00486^T but negative in *Anaerococcus vaginalis* KCTC 15028^T.

Chemotaxonomic Characteristics

The cellular fatty acids of strain AGMB00486^T and closely related type species are listed in Table 2. The major cellular fatty acids (> 10%) of strain AGMB00486^T were $C_{14:0}$ (22.5%), $C_{16:0}$ DMA (21.8%), and $C_{16:0}$ (17.8%). The fatty acid profile of strain AGMB00486^T was distinguishable from the profiles of the reference strains. $C_{16:0}$ DMA was present as a major fatty acid (21.8%) in strain AGMB00486^T, but as a minor fatty acid in reference species; the $C_{16:0}$ DMA of *A. vaginalis* KCTC15028^T, *A. hydrogenalis* KCTC 15014^T and *A. senegalensis* KCTC 15435^T were 4.3, 2.4 and 6.5%, respectively.

Genomic Analysis

The draft genome of strain AGMB00486^T showed that the whole-genome size was 2,268,866 bp chromosome, which contained 2,228 open reading frames, 8 rRNA and 47 tRNA genes (Supplementary Table S1). In addition, 1995 coding sequences (CDS) assigned by clusters of orthologous group of proteins (COG) discovered that the majority of the genes are functional unknown and followed by carbohydrate transport and metabolism (177 genes, 8.9%), replication, recombination and repair (170 genes, 8.5%) and amino acid transport and metabolism (168 genes, 8.4%) (Supplementary Table S2). The DNA G+C content of strain AGMB00486^T based on whole-genome sequence analysis was 30.1 mol%, which is similar to those of reference species (Table 1).

Taxonomic Conclusion

The phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain AGMB00486^T is a member of genus *Anaerococcus*. However, the phenotypic characteristics and genomic similarity showed that strain AGMB00486^T is distinguishable from the closely related species as shown in Fig. 1 and Table 1. On the basis of the phylogenetic, physiological and chemotaxonomic analyses, we suggest that strain AGMB00486^T represents a novel species of genus *Anaerococcus* for which the name *Anaerococcus faecalis* sp. nov. is proposed.

Description of *Anaerococcus faecalis* sp. nov.

Anaerococcus faecalis sp. nov. (fae.ca'lis. N.L. masc. adj. faecalis, derived from faeces).

Cells are coccoid-shaped (1.2×1.4 μm), obligate anaerobic, Gram-positive, non-motile, non-spore-forming, catalase and oxidase-negative. Colonies grown on RCAB plate under anaerobic conditions are flat, circular and slimy texture. Growth occurs at 20–45 ℃ (optimum, 37 ℃) and at pH 7.0–9.0 (optimum, pH 8). The type strain was saccharolytic in API test systems. Propionate is the glucose fermentation end product. The major fatty acids (> 10%) are $C_{14:0,}$ $C_{16:0}$ and $C_{16:0}$ DMA. The DNA G+C content is 30.1 mol%.

The type strain, AGMB00486^T (= KCTC 15945^T = CCTCC AB 202009^T), was isolated from swine faeces.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-021-02497-7.

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Declarations

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

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