

Peptoniphilus faecalis sp. nov., isolated from swine faeces

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Abstract

An obligately anaerobic, Gram-positive, non-motile, coccus-shaped bacterial strain designated AGMB00490^T was isolated from swine faeces. 16S rRNA gene sequence-based phylogenetic analysis indicated that the isolate belongs to the genus *Peptoniphilus* and that the most closely related species is *Peptoniphilus gorbachii* WAL 10418^T (=KCTC 5947^T, 97.22% 16S rRNA gene sequence similarity). Whole genome sequence analysis determined that the DNA G+C content of strain AGMB00490^T was 31.2 mol% and moreover that the genome size and numbers of tRNA and rRNA genes were 2129517 bp, 34 and 10, respectively. Strain AGMB00490^T was negative for oxidase and urease; positive for catalase, indole production, arginine arylamidase, leucine arylamidase, tyrosine arylamidase and histidine arylamidase; and weakly positive for phenylalanine arylamidase and glycine arylamidase. The major cellular fatty acids (>10%) of the isolate were determined to be C_{16:0} and C_{18:1} ω9c. Strain AGMB00490^T produced acetic acid as a major end product of metabolism. Accordingly, phylogenetic, physiologic and chemotaxonomic analyses revealed that strain AGMB00490^T represents a novel species for which the name *Peptoniphilus faecalis* sp. nov. is proposed. The type strain is AGMB00490^T (=KCTC 15944^T=NBRC 114159^T).

The swine is the one of the major livestock used for food. It is also used as model species for analysis of human physiological functions and diseases [1] because swine species are closely comparable with humans in physiology, organ development and disease progression [2–5]. Furthermore, 96% of the functional pathways in the human catalogue are present in the pig catalogue [6]. The gut microbiota have been considered an important environmental factor that influences human metabolism and correlated pathological conditions [7]. Because of the functional similarities of gut between swine and humans [4, 5], swine gut microbiota have also been shown to be important factors in swine health, affecting nutritional, physiological and immunological processes [8, 9]. It has been reported that probiotics treatment confers a protective effect against the opportunistic pathogen *Treponema* and

ameliorated swine growth and feed intake [10, 11]. Moreover, the antibiotic tylosin received swine faecal samples produced compositional shifts of microbiota, resulting in beneficial growth-promoting effects [12].

The genus *Peptoniphilus* belongs to the Gram-positive anaerobic cocci (GPAC). GPAC are the most prominent (approximately 25–30%) Gram-positive anaerobic bacteria associated with clinical infections [13]; however, they are also considered commensal bacteria, including most studied species of the genus *Peptostreptococcus* and rarely studied groups in the genera such as *Coprococcus* and *Sarcina* [14]. Recently, metagenomic data indicated that GPAC, especially members in the genus *Peptoniphilus*, increased in an impaired healing group of diabetic foot ulcers [15]. The genus *Peptoniphilus*, which belongs to the family *Peptoniphilaceae* of the

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Abbreviations: ANI, average nucleotide identity; CDS, coding DNA sequence; GPAC, Gram-positive anaerobic cocci; meso-DAP, meso-diaminopimelic acid; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PY, peptone, yeast extract; PYG, peptone, yeast extract and glucose; RCM, reinforced clostridial medium; TSAB, tryptic soy agar with 5% sheep blood.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the whole genome sequences of strain AGMB00490^T are MT396160 and NZ_JABDSR000000000, respectively. The strain is available from the Korean Collection for Type Culture (KCTC 15944^T) and the NITE Biological Resource Center (NBRC 114159^T).

Two supplementary tables and three supplementary figures are available with the online version of this article.

order *Eubacteriales*, was originally classified as *Peptococcus* and then reclassified as the genus *Peptoniphilus* in 2001 [16]. At the time of writing, the genus *Peptoniphilus* includes 17 validly published species (<https://lpsn.dsmz.de/genus/peptoniphilus>). Most *Peptoniphilus* species were isolated from human clinical samples, with the exception of *Peptoniphilus indolicus*, *Peptoniphilus methioninivorax* and *Peptoniphilus stercorisuis*, which were isolated from cattle, retail ground beef and a swine manure storage tank, respectively. To investigate the relationship between swine health and gut microbiota and obtain swine gut microbiota resources for research and development, we have isolated and characterized microbiota from swine faecal samples. In this study, we characterized strain AGMB00490^T by using a polyphasic taxonomic approach and proposed that strain AGMB00490^T represents a novel species of the genus *Peptoniphilus*.

ISOLATION AND ECOLOGY

Strain AGMB00490^T was isolated from the faeces of a 2-week-old piglet, which did not receive any antibiotics and was raised in the farm located in the National Institute of Animal Science in Cheonan, Republic of Korea. The geographic coordinates are 36.93° N, 127.11° E, approximately. The experimental protocols for this research were reviewed and approved by the Institutional Animal Care and Use Committee at the National Institute of Animal Science (NIAS-2019-1731). All procedures, including bacterial isolation, were performed in an anaerobic chamber (Coy Laboratory Products) filled with 86% N₂, 7% CO₂ and 7% H₂. To isolate gut microbiota, the faecal sample was suspended and diluted serially up to 10⁻⁶ in a phosphate buffered saline (PBS) solution, followed by spreading it onto tryptic soy agar containing 5% sheep blood (TSAB). After 2–3 days of incubation at 37 °C, single colonies were picked and streaked to new TSAB plates under the anaerobic condition. A circular, entire, ivory and convex colony of strain AGMB00490^T was obtained and subjected to taxonomic study by employing a polyphasic taxonomic approach. The isolate was routinely cultivated on TSAB plates at 37 °C in an anaerobic chamber for 48 h and preserved at –80 °C in 10% (w/v) skimmed milk. Strain AGMB00490^T was deposited in the KCTC and NBRC culture collections.

16S rRNA GENE PHYLOGENY

For 16S rRNA sequencing, genomic DNA (gDNA) was extracted using a Wizard Genomic DNA Purification Kit (Promega) and following the manufacturer's instructions, and the 16S rRNA gene of strain AGMB00490^T was amplified and sequenced using two universal primers, 785F (5'-GGATTA-GATACCCTGGTA-3') and 907R (5'-CCGTCAATTC-MTTTTRAGTTT-3') at Macrogen (Seoul, Republic of Korea). The nearly complete 16S rRNA gene sequence was assembled using BioEdit software [17]. To find the taxonomic position of strain AGMB00490^T, the assembled sequence was compared with the 16S rRNA gene sequences of other related strains

acquired from the EzBioCloud database (www.ezbiocloud.net) [18] and the GenBank/EMBL/DDBJ databases (www.ncbi.nlm.nih.gov/blast) using ClustalW [19]. The compared sequences were used to draw phylogenetic trees created using Molecular Evolutionary Genetics Analysis 7 (MEGA7.0) software [20]. The phylogenetic trees based on 16S rRNA gene were reconstructed using the neighbour-joining (NJ) [21], maximum-likelihood (ML) [22] and maximum-parsimony (MP) [23] methods, with bootstrap analysis performed based on 1000 replications. Evolutionary distances were calculated using Kimura's two-parameter model [24].

Comparative analysis using the 16S rRNA gene sequence (1456 bp) of strain AGMB00490^T revealed that the isolate was closely related to the species in the genus *Peptoniphilus* of the family *Peptoniphilaceae* within the phylum Firmicutes. The novel strain had 97.2% sequence similarity to *Peptoniphilus gorbachii* WAL 10418^T (accession No. DQ911241). The 16S rRNA gene similarities of the other reference strains were under 97%. The phylogenetic trees drawn by using the NJ, ML and MP methods determined that strain AGMB00490^T clustered with *P. gorbachii* WAL 10418^T, *Peptoniphilus harei* DSM 10020^T and *Peptoniphilus timonensis* JC401^T (Figs 1 and S1). Based on the results of phylogenetic analysis, *P. gorbachii* WAL 10418^T [25], *P. harei* DSM 10020^T [26] and *P. timonensis* JC401^T [27] were selected as reference species for further comparative tests. For physiological and biochemical analysis, *P. gorbachii* KCTC 5947^T (=WAL 10418^T), *P. harei* KCTC 5952^T (=DSM 10020^T) and *P. timonensis* KCTC 15412^T (=JC401^T) were obtained from the Korean Collection for Type Cultures (KCTC).

GENOME FEATURES

Whole genome sequencing to determine the G+C content and genomic similarity was performed using Illumina NovaSeq technology (Illumina) at Macrogen (Seoul, Republic of Korea). Obtained paired-end reads were assembled with SPAdes (version 3.13.0) after quality trimming. Completeness and contamination of the assembled genome were examined by ContEst16S and CheckM tools. The coding DNA sequences (CDSs) and tRNA were predicted using prodigal and tRNAscan-SE, respectively. The rRNA genes were searched by covariance model search with inference of Rfam 12.0. The annotation of each CDS was performed by homology search against the Swiss-prot, EggNOG 4.5, SEED and KEGG databases. For *in silico* prediction of the average nucleotide identity (ANI), values were calculated using Chun-Lab's online ANI calculator [28].

The draft genome of strain AGMB00490^T was determined; sequencing depth of coverage was 1449.3× and 38 contigs were obtained. Based on the genome sequence analysis of strain AGMB00490^T, the length of the genome is 2129517 bp, the DNA G+C content is 31.2 mol% and the genome contains 2075 open reading frames, 10 rRNA genes and 34 tRNA genes (Table S1, available in the online version of this article). Moreover, the genome has 18 clusters of orthologous groups of

Table 1. Differential characteristics of strain AGMB00490^T and closely related species

Strains: 1, AGMB00490^T; 2, *Peptoniphilus gorbachii* KCTC 5947^T; 3, *Peptoniphilus harei* KCTC 5952^T; 4, *Peptoniphilus timonensis* KCTC 15412^T. Rapid ID 32A of reference strains are taken from this study. +, Positive; -, negative; w, weakly positive; NA, no data available.

Characteristics	1	2	3	4
Isolation source	Swine faeces	Human clinical specimens ^a	Human clinical infections ^b	Healthy human faeces ^c
Cell size (diameter, µm)	0.6	0.7 ^a	0.5–1.5 ^b	NA
DNA G+C content (mol%)	31.2	NA	25 ^b	30.7 ^c
Enzymatic activities (Rapid ID 32A):				
Indole production	+	+	-	+
Phenylalanine arylamidase	w	+	-	w
Leucine arylamidase	+	+	+	w
Glycine arylamidase	w	+	-	-
Glutamyl glutamic acid arylamidase	-	+	-	-
Serine arylamidase	-	+	-	w
End products (PY and PYG broth)*	A, b, ib	A, b, ib	A, b, ib	A, ib

a, Data from Song et al. [25]; b, data from Murdoch et al. [26]; c, data from and Mishra et al. [27].

*A, acetic acid; B, butyric acid; ib, iso-butyric acid; Upper case letters represent major products, lowercase letters represent minor products.

end products were examined using strain AGMB00490^T and reference strains grown in RCM, peptone and yeast extract (PY) and PYG (PY with glucose) broth under anaerobic conditions. The cultured medium was analysed using a high-performance liquid chromatography system (Shimadzu) with Aminex Organic Acid Columns (Bio-Rad).

Strain AGMB00490^T was an obligately anaerobic, non-motile, Gram-positive, coccus-shaped bacteria (Fig. S2). The cell diameter was approximately 0.6 µm. Colonies were circular, entire, ivory and convex. The growth ranges of the strain were 20–45 °C (optimum, 37 °C) and pH 6.0–9.0 (optimum, pH 7.0). The isolate was found to be resistant to erythromycin, lincomycin and tetracycline, but susceptible to amoxicillin, metronidazole and ampicillin. Strain AGMB00490^T was catalase-positive and oxidase- and urease-negative. Based on the Rapid ID 32A results, strain AGMB00490^T and the reference strains were positive for arginine arylamidase, leucine arylamidase, tyrosine arylamidase and histidine arylamidase, whereas all species were negative for arginine dihydrolase, α-galactosidase, β-galactosidase, β-galactosidase 6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, N-acetyl-β-glucoaminidase, mannose fermentation, raffinose fermentation, glutamic acid decarboxylase, α-fucosidase, reduction of nitrates, alkaline phosphatase, proline arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase and alanine arylamidase. While the major metabolic end product in RCM broth was iso-butyric acid (strain AGMB00490^T 2.26 mM, *P. gorbachii* KCTC 5947^T 2.67 mM, *P. harei* KCTC 5952^T 1.98 mM and *P. timonensis* KCTC 15412^T 2.02 mM), all strains including the isolate produced acetic acid as a major end product even

though the amount was less than in the rich medium RCM broth (strain AGMB00490^T 1.43 µM, *P. gorbachii* KCTC 5947^T 1.82 µM, *P. harei* KCTC 5952^T 0.75 µM and *P. timonensis* KCTC 15412^T 0.11 µM) in PY and PYG broth, confirming that strain AGMB00490^T has a saccharolytic property like the genus *Peptoniphilus*. Moreover, the isolate produced butyric acid and iso-butyric acid as minor end products in PY and PYG broth. The phenotypic and biochemical differences between strain AGMB00490^T and its phylogenetic neighbours, *P. gorbachii* KCTC 5947^T, *P. harei* KCTC 5952^T and *P. timonensis* KCTC 15412^T, are summarized in Table 1.

For the analysis of chemotaxonomic characteristics, the cellular fatty acid profiles were analysed using strain AGMB00490^T and reference strains grown on TSAB plates for 48 h. After harvesting the cells, fatty acids were saponified, methylated and extracted according to the protocol of the MIDI/Hewlett Packard Microbial Identification System [30]. The fatty acid compositions were analysed by gas chromatography (model 6890 N, Agilent) and identified using Microbial Identification Sherlock software with the Anaerobe database version 6.1. The diamino acid in the cell-wall peptidoglycan was determined using previously described methods [31].

The cellular fatty acid compositions of strain AGMB00490^T and the related reference strains are shown in Table 2. The major cellular fatty acids (>10%) in strain AGMB00490^T were C_{18:1} ω9c (25.7%) and C_{16:0} (22.9%), and the fatty acid profile of strain AGMB00490^T was different from those of the reference strains. C_{16:0} DMA was present as a major or nearly major fatty acid in the reference strains, but it was a minor fatty acid in strain AGMB00490^T. Furthermore, C_{15:0}, C_{16:1} ω7c and

Table 2. Cellular fatty acid profiles of strain AGMB00490^T and related type strains

Strains: 1, AGMB00490^T; 2, *Peptoniphilus gorbachii* KCTC 5947^T; 3, *Peptoniphilus harei* KCTC 5952^T; 4, *Peptoniphilus timonensis* KCTC 15412^T. All data were obtained from the present study. Cells were grown on TSAB plates for 2 days at 37 °C in anaerobic conditions. Data are reported as the percentage of total fatty acid. Fatty acids that represented <1.0% are not shown. –, Not detected. Major components (>10%) are highlighted in bold.

Fatty acids	1	2	3	4
Saturated:				
C _{10:0}	2.7	6.0	2.6	6.2
C _{12:0}	4.1	4.2	4.5	4.9
C _{14:0}	8.1	5.5	4.8	4.8
C _{15:0}	1.2	–	–	–
C _{16:0}	23.0	24.0	25.6	16.6
<i>anteiso</i> -C _{17:0}	–	1.3	–	2.4
C _{18:0}	6.7	6.3	6.7	2.7
Unsaturated:				
C _{16:1} ω7c	1.9	–	–	–
C _{16:1} ω9c	6.1	1.8	1.5	1.6
C _{18:2} ω9,12c	1.9	6.7	7.1	4.2
C _{18:1} ω9c	25.7	14.8	15.0	6.9
Hydroxy:				
C _{13:0} iso 3-OH	–	1.4	–	1.1
Dimethyl acetal (DMA):				
C _{16:0}	3.4	9.1	11.3	15.2
C _{18:1} ω9c	9.9	9.6	10.9	17.0
C _{18:0}	1.9	1.4	2.3	2.7
Aldehyde:				
C _{16:0}	–	1.7	1.7	3.0
C _{18:0}	–	–	–	1.2
Summed features:*				
7	–	1.8	2.3	3.3
10	1.2	–	–	–
11	1.6	3.6	3.6	5.5

*Summed feature composition is as follows: 7, C_{17:2} and/or C_{17:1} ω8c; 10, C_{18:1} ω6t/9t/11c and/or unknown 17.834; 11, iso C_{17:0} 3-OH and/or C_{18:2} DMA.

summed feature 10 (unidentified constituent with equivalent chain length of 17.834 and/or C_{18:1} ω6t/9t/11c) were present in strain AGMB00490^T, but these were absent in all reference strains. The cell-wall peptidoglycan of strain AGMB00490^T

and the reference strains contained *meso*-diaminopimelic acid (*meso*-DAP) as the diagnostic diamino acid (Fig. S3).

In summary, the phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain AGMB00490^T is a member of the genus *Peptoniphilus*. However, the phenotypic characteristics and genomic similarity showed that strain AGMB00490^T is distinguishable from the closely related species, *P. gorbachii* KCTC 5947^T, *P. harei* KCTC 5952^T and *P. timonensis* KCTC 15412^T. However, because *P. gorbachii* KCTC 5947^T is the closest species based on 16S similarity, it shows physiological characteristics very similar to those of strain AGMB00490^T, except for glutamyl glutamic acid arylamidase and serine arylamidase, which were both negative in strain AGMB00490^T but positive in *P. gorbachii* KCTC 5947^T. Other physiological characteristics of strain AGMB00490^T and the reference strains are listed in Table 1. On the basis of the results of phylogenetic, physiological and chemotaxonomic analyses, we suggest that strain AGMB00490^T represents a novel species of the genus *Peptoniphilus*, for which the name *Peptoniphilus faecalis* sp. nov. is proposed.

DESCRIPTION OF *PEPTONIPHILUS FAECALIS* SP. NOV.

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

Cells are Gram-stain-positive, obligately anaerobic, coccus-shaped and non-motile. They are catalase-positive and oxidase- and urease-negative. Colonies grown on TSAB under anaerobic conditions are circular, entire, ivory and convex. Growth occurs within 20–45 °C and at pH 6.0–9.0, with an optimum temperature and pH of 37 °C and pH 7.0, respectively. Resistant to erythromycin, lincomycin and tetracycline, and susceptible to amoxicillin, metronidazole and ampicillin. Acetic acid is the major metabolic end product and butyric acid and *iso*-butyric acid are minor end products in PY media. In Rapid ID 32A strips, cells are positive for indole production, arginine arylamidase, leucine arylamidase, tyrosine arylamidase and histidine arylamidase; weakly positive for phenylalanine arylamidase and glycine arylamidase; and negative for arginine dihydrolase, α-galactosidase, β-galactosidase, β-galactosidase 6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucoaminidase, mannose fermentation, raffinose fermentation, glutamic acid decarboxylase, α-fucosidase, reduction of nitrates, alkaline phosphatase, proline arylamidase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, alanine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase. The major fatty acids are C_{16:0} and C_{18:1} ω9c. The diamino acid in the cell-wall peptidoglycan is *meso*-DAP.

The type strain, AGMB00490^T (=KCTC 15944^T=NBRC 114159^T), was isolated from swine faeces. The DNA G+C content of strain AGMB00490^T is 31.2 mol%.

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Author contributions

Conceptualization: S. W. R. and J. H. L. Data curation: S. W. R. Funding acquisition: J. H. L. Methodology: J. S. K., B. S. O., S. Y. Y. and J. S. L. Software: S. H. P. and S. W. K. Investigation: S. W. R., J. L., M. K. L., M. S. R., H. B. K., J. K. K. and J. H. L. Resources: H. J. and T. Y. H. Writing – original draft: S. W. R. Writing – review and editing: J. H. L.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

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

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