INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Jiang *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004721

DOI 10.1099/ijsem.0.004721

MICROBIOLOGY SOCIETY

Peribacillus faecalis sp. nov., a moderately halophilic bacterium isolated from the faeces of a cow

Lingmin Jiang[1], Won Yong Jung[2], Zhun Li[1], Mi-Kyung Lee[1], Seung-Hwan Park[1], Se Won Kang[1], Jung-Sook Lee[1], Hyunjung Jung[3], Tai-Young Hur[4], Hyeun Bum Kim[5], Jae-Kyung Kim[6], Ju-Hoon Lee[7], Ju Huck Lee[1,*] and Jiyoung Lee[1,*]

Abstract

A Gram-stain-positive, facultatively anaerobic, endospore-forming, rod-shaped strain, AGMB 02131^T, which grew at 20–40 °C (optimum 30 °C), pH 3.0–11.0 (optimum pH 4.0) and in the presence of 0–18% (w/v) NaCl (optimum 10%), was isolated from a cow faecal sample and identified as a novel strain using a polyphasic taxonomic approach. The phylogenetic analysis based on 16S rRNA gene sequences along with the whole genome (92 core gene sets) revealed that AGMB 02131^T formed a group within the genus *Peribacillus*, and showed the highest sequence similarity with *Peribacillus endoradicis* DSM 28131^T (96.9%), following by *Peribacillus butanolivorans* DSM 18926^T (96.6%). The genome of AGMB 02131^T comprised 70 contigs, the chromosome length was 4038965 bp and it had a 38.5% DNA G+C content. Digital DNA–DNA hybridization revealed that AGMB 02131^T displayed 21.4% genomic DNA relatedness with the most closely related strain, *P. butanolivorans* DSM 18926^T. AGMB 02131^T contains all of the conserved signature indels that are specific for members of the genus *Peribacillus*. The major cellular fatty acids (>10%) of AGMB 02131^T were $C_{18:1}\omega 9c$, $C_{18:0}$ and $C_{16:0}$. The major polar lipids present were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. On the basis of the phenotypic, phylogenetic, genomic and chemotaxonomic features, AGMB 02131^T represents a novel species of the genus *Peribacillus*, for which the name *Peribacillus faecalis* sp. nov. is proposed. The type strain is AGMB 02131^T (=KCTC 43221^T=CCTCC AB 2020077^T).

Recently, the genus *Bacillus*, of 293 species and subspecies, was reclassified on the basis of phylogenomic and comparative genomic frameworks, six genera, *Peribacillus*, *Cytobacillus*, *Mesobacillus*, *Neobacillus*, *Metabacillus* and *Alkalihalobacillus,* were separated from the genus *Bacillus* [1]. The genus *Bacillus* has been limited to only the members of the Subtilis and Cereus clade of species [2]. There are 16 species in the genus *Peribacillus* with validly published names (https://lpsn.dsmz.de/genus/peribacillus), most species of this genus have been isolated from soil [3–10] and plant tissues [11, 12]. Members of this genus are generally Gram-positive or Gram-variable, facultatively anaerobic or aerobic, cells are motile, the temperature range at which growth can occur is 3–45 °C. Of the species with validly published names, the whole-genome sequences are available for 10 of them. The whole genomes of members of this genus feature genome sizes of 4.1–5.7 Mbp, and genomic DNA G+C contents of 37.5–43.0%. The members of this genus can be distinguished from all other species of the family *Bacillaceae* by three conserved signature indels (CSIs) that are exclusively shared by either all or most members of this genus, namely HAMP domain-containing protein, phospho-*N*-acetylmuramoylpentapeptide-transferase, and stage II sporulation protein E [1]. The major respiratory quinone is menaquinone (MK-7); the major polar lipids are

Author affiliations: [1]Korean Collection for Type Cultures (KCTC), Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup 56212, Republic of Korea; [2]Korean Bioinformation Center (KOBIC), Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea; [3]Swine Science Division, National Institute of Animal Science, Cheonan 31000, Republic of Korea; [4]Division of Animal Disease and Health, National Institute of Animal Science, Wanju-Gun 55365, Republic of Korea; [5]Department of Animal Resources Science, Dankook University, Cheonan 3116, Republic of Korea; [6]Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 56212, Republic of Korea; [7]Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea.

***Correspondence:** Jiyoung Lee, jiyoung1@kribb.re.kr; Ju Huck Lee, juhuck@kribb.re.kr

Keywords: polyphasic taxonomy; novel species; phylogeny; *Peribacillus faecalis* sp. nov.

Abbreviations: ANI, average nucleotide identity; CSI, conserved signature indel; dDDH, digital DNA–DNA hybridization; GGDC, genome-to-genome distance calculation; UBCG, up-to-date bacterial core gene.

The GenBank accession numbers of the 16S rRNA gene and the whole-genome sequences of AGMB 02131^T are MW009695 and JACXSI000000000, respectively. The strain is available from the Korea Collection for Type Cultures (KCTC 43221^T) and the China Center for Type Culture Collection (CCTCC AB 2020077^T).

One supplementary table and five supplementary figures are available with the online version of this article.

phospholipid, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. In the present study, the strain AGMB 02131^T was isolated from the faeces of a Korean cow during a cattle gut microbiome study. Using polyphasic taxonomic analyses, AGMB 02131^T was characterized by its taxonomic position as a representative of a novel species of the genus *Peribacillus*.

ISOLATION AND ECOLOGY

A 1 g sample of faeces of a Korean cow (6 months old and 158 kg) was mixed with 10 ml phosphate-buffered saline (PBS; 100 mM Tris-HCl pH 8.0, 150 mM NaCl), and then serially diluted (10^{-1}–10^{-4}) with PBS. A total of 100 µl of the diluted solution was spread on tryptic soy agar (Difco) with 5% sheep blood (Synergy Innovation)(TSAB). The plates were cultured at 25 °C for 7 days in an anaerobic chamber (Coy Scientific) filled with 86% N_2, 7% CO_2, and 7% H_2 atmosphere. A single circular and white pigmented colony, designated as strain AGMB 02131^T, was preserved at −80 °C in a suspension containing 10% (w/v) skim milk, was selected for polyphasic taxonomy and routinely cultured on TSAB at 28 °C. *Peribacillus endoradicis* HAMBI 3097^T (=DSM 28131^T; Basonym: *Bacillus endoradicis*) and *Peribacillus butanolivorans* K9^T (=DSM 18926^T; Basonym: *Bacillus butanolivorans*) were obtained from the corresponding culture collections as closely related strains to compare the taxonomic characteristics under similar culture conditions.

16S rRNA GENE PHYLOGENY

The 16S rRNA gene of AGMB 02131^T was amplified by polymerase chain reaction from bacterial DNA using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [13]. The 16S rRNA fragments were then sequenced using 27F, 1492R, 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′) (Macrogen). The almost full length of 16S rRNA gene sequence (1485 bp) of AGMB 02131^T was obtained after assembly using the Vector NTI software (1.6.1). The sequence was then compared with the 16S rRNA gene sequences which can be obtained from the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzBioCloud server (http://www.ezbiocloud.net) [14]. Multiple sequences were aligned using BioEdit 7.0.5 software [15]. On the basis of the aligned sequence of AGMB 02131^T and closely related type strains, the phylogenetic trees were reconstructed using the neighbor-joining (NJ), minimum evolution (ME), and maximum likelihood (ML) algorithms with 1000 bootstrap iterations using the Molecular Evolutionary Genetics Analysis programme (MEGA, v.7.0) [16]. NJ analysis was calculated using Kimura's two-parameter model. ML was calculated using the general time-reversible model with gamma-distribution with invariant sites (G+I).

Comparative analysis based on 16S rRNA (1485 bp) gene sequences by the Sanger sequencing method revealed that AGMB 02131^T showed the highest similarity to *P. endoradicis* DSM 28131^T (96.9%) and *P. butanolivorans* DSM 18926^T (96.6%). Besides, the similarity of AGMB 02131^T was less than 96.5% when compared with the remaining species of the genus *Peribacillus* with validly published names. Thus, AGMB 02131^T was regarded as representing a novel species of the genus *Peribacillus* as the proposal novel species recognition threshold is 98.6% [17]. The phylogenetic tree based on 16S rRNA gene sequences indicated that AGMB 02131^T formed a distinct phylogenetic lineage with *P. endoradicis* DSM 28131^T. Similar results were obtained from the trees reconstructed using the ML, ME and NJ algorithms (Fig. 1). Thus, *P. endoradicis* DSM 28131^T and *P. butanolivorans* DSM 18926^T were used as reference strains for the comparisons of phenotypic properties and chemotaxonomic analyses.

GENOMIC FEATURES

The genomic DNA was extracted by using phenol: chloroform: isoamyl alcohol method described by Wilson *et al.* [18], and then PicoGreen and Nanodrop were used to measure the quantity and quality of the extracted genomic DNA. A DNA library was prepared using a TruSeq Nano DNA kit (Illumina) and validated with an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies). Using the NovaSeq™ 6000 sequencing system (Illumina), the whole genome of AGMB 02131^T was sequenced. *De novo* assembly was performed using the St. Petersburg genome assembler (SPAdes) Version 3.12.0. Genome annotation was then performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [19]. Digital DDH values and average nucleotide identity (ANI) values were calculated using the Genome-to-Genome Distance Calculation web server (http://ggdc.dsmz.de/distcalc2.php) [20] and ANI calculator (https://www.ezbiocloud.net/tools/ani) [21] respectively. Whole-genome information for all closely related strains which were publicly available in NCBI, was collected and reconstructed using an up-to-date bacterial core gene set (UBCG) consisting of 92 core genes, as described by Na *et al.* [22]. Briefly, Prodigal (v2.6.3) was used to predict CDSs, then 92 core genes were identified using hmmsearch (v3.1b2) from CDSs. Each core gene was multiply aligned by MAFFT (v7.310), positions filtered and gene trees inferred using FastTree (v2.1.10), gene support index (GSI) was calculated, and finally a UBCG tree (FastTree; v2.1.10) was inferred. The CSIs as one of the primary criteria for describing taxa, indicate genes and/or proteins that are important category markers, are well suited for classification. As described by Patel *et al.* [1], members of the genus *Peribacillus* have three specific CSIs, which can be used to distinguish them from members of all other genera. The three specific CSIs were extracted from the whole-genome to better elucidate the taxonomic position of AGMB 02131^T, and then compared with CSIs of species of the genus *Peribacillus* and other genera in the family *Bacillaceae* with validly published names which were well identified by Patel *et al.* [1].

The draft genome of AGMB 02131^T consisted of 70 contigs, with a total length of 4038965 bp (the N_{50} value was 90803 bp). The DNA G+C-content, calculated from the whole-genome,

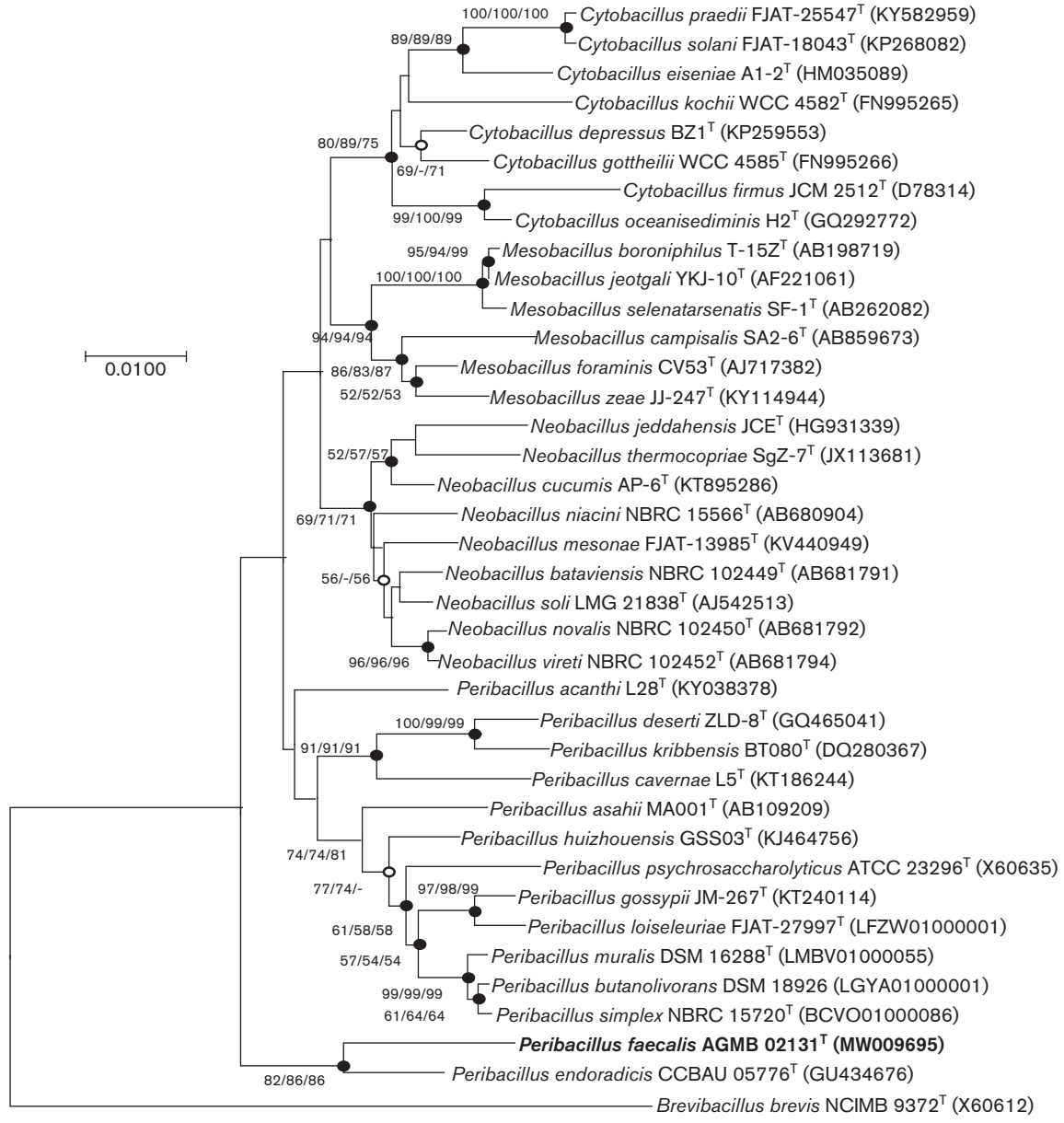

Fig. 1. Phylogenetic tree reconstructed using the neighbor-joining (NJ) algorithm based on the 16S rRNA gene of AGMB 02131^T and closely related strains. Bootstrap values (>50%) calculated using the NJ, maximum likelihood (ML) and minimum evolution (ME) algorithms are indicated at the nodes. Filled circles on the nodes indicated that the relationships were also recovered by ML and ME algorithms, whereas open circles indicate nodes recovered by either the ML or ME algorithm. Bar, 0.01000 changes per nucleotide position.

was 38.5%. After annotation with PGAP (GenBank accession number: JACXSI010000000), there were 3806 protein-coding genes, 90 transfer RNA genes, 17 ribosomal RNA genes including 7 5S rRNA genes, 7 16S rRNA genes, 3 23S rRNA genes, 5 ncRNA genes, and 51 pseudogenes. Of theses, 3203 genes were functionally assigned to categories based on clusters of orthologous groups (COG) assignments, 26.7% of the total assigned COGs were classified as unknown and the next largest categories were amino acid transport and metabolism (8.3%), transcription (7.4%) and inorganic ion transport and metabolism (6.7%). The bacteria possess the ability to utilize amino acids, the building blocks of proteins, as nitrogen and/or carbon sources. Genome analysis indicated that AGMB 02131^T might play an important role in nitrogen and carbon cycles. The digital DDH values and average nucleotide identity (ANI) values revealed that AGMB 02131^T showed 23.6, 21.4, 22.1 and 70.4, 70.6, 69.2% to the closely related strains *Peribacillus acanthi* L28^T and *P. butanolivorans* DSM 18926^T as well as type species *Peribacillus simplex* NBRC 15720^T, respectively. Considering that the values obtained were significantly lower than the proposed ANI cutoff values (95–96%) and dDDH values (<70%) for bacterial species delineation [23],

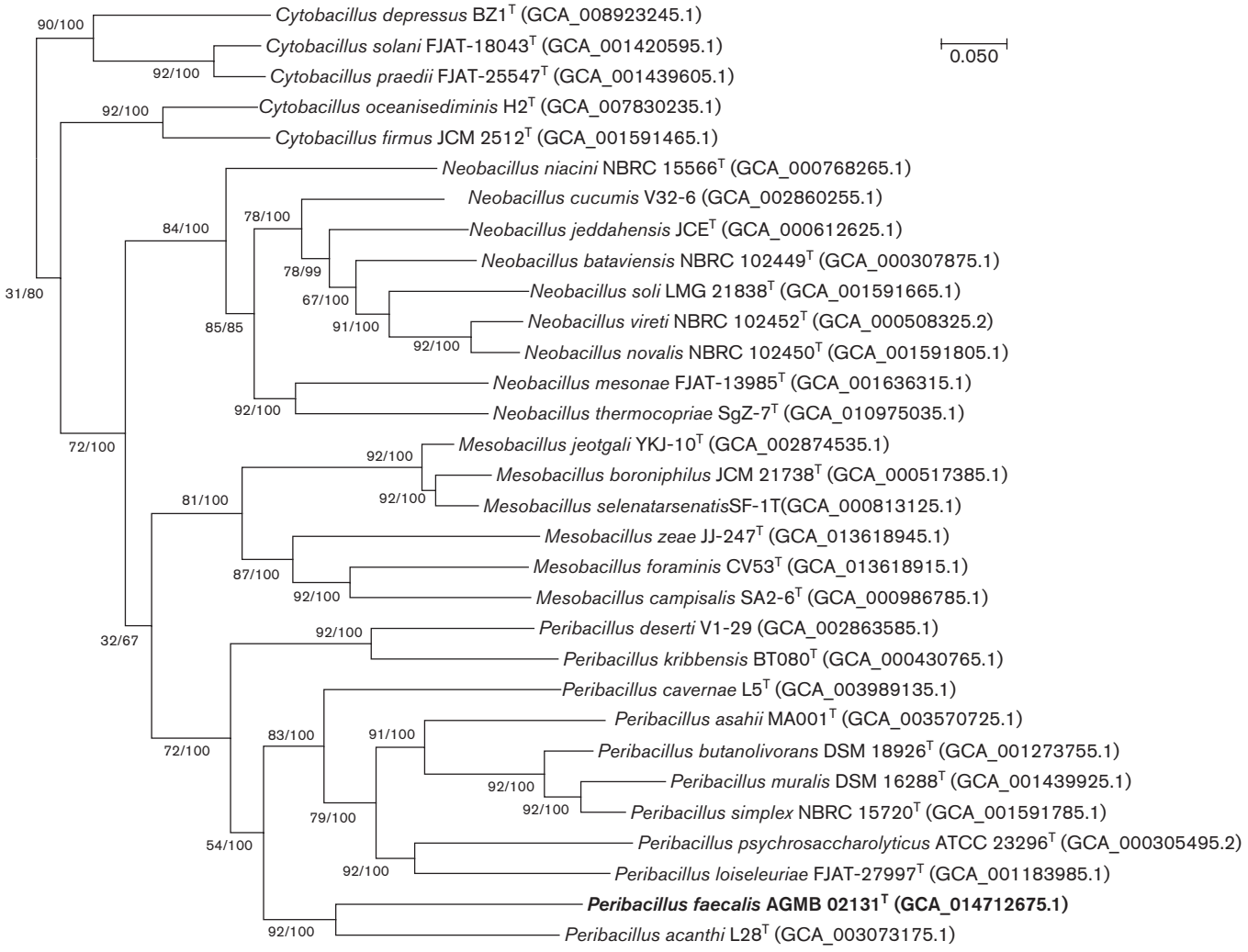

Fig. 2. Phylogenomic tree reconstructed using the ML algorithm based on UBCGs (concatenated alignment of 92 core genes) of AGMB 02131^T and strains of related species. Gene support index (GSI, left) and bootstrap values (right) are indicated at the nodes. Bar, 0.050 substitutions per position. The 92 bacterial core genes were *alaS*, *argS*, *aspS*, *cgtA*, *coaE*, *cysS*, *dnaA*, *dnaG*, *dnaX*, *engA*, *ffh*, *fmt*, *frr*, *ftsY*, *gmk*, *hisS*, *ileS*, *infB*, *infC*, *ksgA*, *lepA*, *leuS*, *ligA*, *nusA*, *nusG*, *pgk*, *pheS*, *pheT*, *prfA*, *pyrG*, *recA*, *rbfA*, *rnc*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplI*, *rplJ*, *rplK*, *rplL*, *rplM*, *rplN*, *rplO*, *rplP*, *rplQ*, *rplR*, *rplS*, *rplT*, *rplU*, *rplV*, *rplW*, *rplX*, *rpmA*, *rpmC*, *rpmI*, *rpoA*, *rpoB*, *rpoC*, *rpsB*, *rpsC*, *rpsD*, *rpsE*, *rpsF*, *rpsG*, *rpsH*, *rpsI*, *rpsJ*, *rpsK*, *rpsL*, *rpsM*, *rpsO*, *rpsP*, *rpsQ*, *rpsR*, *rpsS*, *rpsT*, *secA*, *secG*, *secY*, *serS*, *smpB*, *tig*, *tilS*, *truB*, *tsaD*, *tsf*, *uvrB*, *ybeY* and *ychF*.

AGMB 02131^T represents a distinct species of the genus *Peribacillus*. Three exclusive CSIs were extracted from the genome of AGMB 02131^T, and compared with those of the species of the genus *Peribacillus* with validly published names, which have been well identified by Patel *et al.* [1]. The results indicated that AGMB 02131^T shared the three exclusive CSIs of this genus. Namely: HAMP domain-containing protein, phospho-*N*-acetylmuramoylpentapeptide-transferase, and stage II sporulation protein E (Table S1, Figs S1–S3, available in the online version of this article). The whole genome-based phylogenetic tree based on 92 core gene sets also supported that AGMB 02131^T formed a phylogenetic lineage within the genus *Peribacillus* (Fig. 2), consistent with the 16S rRNA-based phylogenetic tree.

PHYSIOLOGY AND CHEMOTAXONOMY

Morphological features of cells were observed by field-emission scanning electron microscopy (JEOL). Various media, namely Luria-Bertani agar (LB, Difco), Reasoner's 2A agar (R2A, MB cell), marine agar 2216 (MA, Difco), tryptic soy agar (TSA, Difco), TSAB, potato dextrose agar (PDA, Difco), and malt extract agar (MEA, Difco) were used for identifying the optimal medium; the strain was cultured at different temperatures (4, 10, 15, 20, 25, 30, 37, 40, 45, 50, and 60 °C) on TSAB medium for 2 days. A pH range of 3.0–12.0 (1.0 pH unit intervals, adjusted with 1 M HCl and 1 M NaOH) was tested in TSAB medium, which was prepared using 10 mM acetate or 10 mM Tris-HCl buffer

instead of distilled water [24]. Salt concentrations of 0–20% (w/v, 1% concentration increments) in tryptic soy broth (TSB, Difco) with 5% sheep blood were used to determine the salt tolerance. The pH and NaCl tolerance were monitored by measuring the OD_{600} using a DU 700 UV–visible spectrophotometer (Beckman Coulter). Gram staining was tested using a Gram Staining Kit (Difco), while endospore formation was examined by staining with a Schaeffer and Fulton spore stain kit (Sigma). A 0.4% (w/v) soft agar tube with the stabbing technique was used to estimate motility. Bubble production after adding a drop of 3% (v/v) hydrogen peroxide solution was used to determine catalase activity, while oxidase activity was determined by analysing the change in the violet colour of colonies after using oxidase reagent (bioMérieux). Antibiotic resistance was determined with discs (BD BBL™) impregnated with the following antibiotics (μg/disc unless indicated): chloramphenicol (30), kanamycin (30), nalidixic acid (30), nitrofurantoin (300), tetracycline (30), and penicillin (10 U). Additional biochemical features were assayed using API 20NE (bioMérieux), API 50CH (bioMérieux), and API ZYM with NaCl 0.85% medium (bioMérieux) according to the manufacturer's instructions.

AGMB 02131^T only grew on TSAB medium. Colonies grown on TSAB medium were circular, white, smooth and 1–3 mm in diameter after three days of culture. AGMB 02131^T was found to grow at a temperature range of 20–40 °C (optimum 30 °C), NaCl of 0–18% (optimum 10%) and a pH of 3–11 (optimum 4) and was facultatively anaerobic, Gram-stain-positive, motile, catalase-positive and oxidase-negative. Endospores were produced at the termini in non-swollen sporangia. The strain was found to be sensitive to tetracycline, penicillin, chloramphenicol, kanamycin and nalidixic acid and resistant to nitrofurantoin. While the closely related strains *P. endoradicis* DSM 28131^T and *P. butanolivorans* DSM 18926^T showed resistance to nalidixic acid, and sensitivity to nitrofurantoin. Cells were rod-shaped, 0.7–0.9 μm in width, and 1.9–10.3 μm in length (Fig. S4). AGMB 02131^{T}could be differentiated physiologically and phenotypically from the other closely related strains. For example, only AGMB 02131^T was negative for the activity of aesculine hydrolysis, glucose assimilation, N-acetylglucosamine, and malate glusomine which is obviously different from the other strains. Other characteristics that differentiate AGMB 02131^T from closely related strains are listed in Tables 1 and 2.

For the analysis of chemotaxonomic characteristics including polar lipids [25] and quinones [26], AGMB 02131^T was cultured in liquid TSB medium with 5% sheep blood and closely related strains were cultured in a TSB medium, respectively, and then harvested after 5 days. 100 mg freeze-dried cells were used for identifying the phospholipids and quinones. Polar lipid spots were subsequently separated using two-dimensional thin-layer chromatography, and then identified by spraying with 0.2% ninhydrin (Sigma-Aldrich), α-naphthol, molybdenum blue (Sigma-Aldrich), 4% phosphomolybdic acid and Dragendorff's solution, which were used to identified amino group-containing lipids, sugar-containing lipids, phosphorus-containing lipids, total lipids and quaternary nitrogen-containing lipids,respectively. Isoprenoid quinones were extracted from 20 ml chloroform/methanol (2:1, v/v), subsequently analysed by reverse-phase high-performance liquid chromatography under UV absorbance detection at 270 nm. According to the instructions of the MIDI/Hewlett Packard Microbial Identification System, the cellular fatty acid profiles were analysed. The profile of fatty

Table 1. Differential physiological and biochemical comparison of AGMB 02131^T and other closely related strains of species of the genus *Peribacillus*

1, *Peribacillus faecalis* AGMB 02131^T; 2, *P. endoradicis* DSM 28131^T; 3, *P. butanolicorans* DSM 18926^T; 4, *P. acanthi* L28^T; ND, not determined.

Characteristics	1	2*	3†	4‡
Isolation source	Cow faeces	Soil	Soybean root	Soil
Metabolism	Facultatively anaerobic	Strictly aerobic	Aerobic	Aerobic
Colony morphology	Circular, white, smooth	Round, tawny, slightly raised and opaque	Transparent, white with a slightly irregular edge	Circular, smooth, convex, regular, white pigment
Growth temperature range (optimum, °C)	20–40 (30)	5–45 (25)	15–45 (28–37)	20–45 (37)
Motile	Motile	ND	ND	Non-motile
Cell size (μm)	(0.7–0.9)×(1.9–10.3)	(0.8–1.3)×(2.5–5.1)	(0.7–0.9)×(1.9–2.7)	(0.5–1)×(1.5–5)
pH range (optimum)	3.0–11.0 (4.0)	6–8.8 (7.0)	6–10 (7–8)	6.5–9 (7.5)
NaCl concentration range (%)	0–18 (10)	0.5–5 (1)	0–7 (0–0.5)	0–5 (0.5)
Nalidixic acid resistance	Negative	Positive	Positive	ND
Nitrofurantoin resistance	Positive	Negative	Negative	ND

*Data from Zhang *et al.* [11]
†Data from Kuisiene *et al.* [7]
‡Data from Ma *et al.* [10].

Table 2. Differential biochemical comparison of AGMB 02131^T and other closely related strains of species of the genus *Peribacillus*

1, *Peribacillus faecalis* AGMB 02131^T; 2, *P. butanolicorans* DSM 18926^T; 3, *P. endoradicis* DSM 28131^T; All data are from the present study obtained in parallel experiments under certain specified conditions. +, Positive; –, negative; w, weakly positive.

Characteristics	1	2	3
API 20NE:			
Nitrate reduction	–	+	–
Arginine hydrolysis	–	–	+
Urease hydrolysis	–	+	–
Aesculin hydrolysis	–	+	+
Glucose assimilation	–	+	+
Mannose assimilation	–	–	+
Mannitol assimilation	–	+	–
N-acetylglucosamine	–	+	+
Gluconate glusomine	–	+	–
Malate glusomine	–	+	+
Citrate glusomine	–	+	–
Phenylacetic glusomine	–	+	+
Acid production (API 50CH)			
D-ribose	–	–	+
D-galactose	–	–	+
D-glucose	+	–	+
D-fructose	+	–	+
L-sorbose	+	–	+
N-acetylglucosamine	+	–	+
Arbutin	–	–	+
Aesculin ferric citrate	+	–	–
Salicin	–	–	+
Cellobiose	+	–	–
Maltose	+	–	w
Starch	–	–	+
API ZYM			
Esterase lipase (C8)	–	+	+
Lipase (C14)	–	–	w
Leucine arylamidase	w	w	+
Valine arylamidase	w	w	+
Cystine arylamidase	–	–	+
Trypsin	–	–	w
α-chymotrypsin	w	w	+
α-galactosidase	–	–	w
β-galactosidase	–	–	w
β-glucuronidase	–	–	+
α-glucosidase	–	–	+
β-glucosidase	–	–	+

acids was identified by comparison with the software package (database TSAB 6.0) [27] using gas chromatography (model 6890 N; Agilent).

The polar lipids profile of AGMB 02131^T consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and two phospholipids (PL1 and PL2). The polar lipid profiles were similar to those of other closely related type strains (Fig. S5). The predominant respiratory quinone of AGMB 02131^T was menaquinone 7 (MK-7), which was the same as for other members of the genus *Peribacillus*. These results indicate that AGMB 02131^T was affiliated with the genus *Peribacillus*. AGMB 02131^T contained $C_{18:1}\omega 9c$ (29.7%), $C_{18:0}$ (15.3%), and $C_{16:0}$ (14.8%) as the major fatty acids (>10%) (Table 3), which were the most obvious difference from other closely related type strains under similar culture conditions, and can be used to distinguish AGMB 02131^T from the closely related strains.

In summary, the phylogenetic and chemotaxonomic analysis indicate that strain AGMB 02131^T represents a member of the genus *Peribacillus*. However, 16S rRNA similarity (below 96.9%), ANI, dDDH values and fatty acid composition, as well as some physiological characteristics (such as API 20NE, API 50CH and API ZYM test results), indicate that AGMB 02131^T is different from the closely related strains. Thus, we suggest that strain AGMB 02131^T represents a novel species of the genus *Peribacillus*, and the name of *Peribacillus faecalis* sp. nov. is proposed.

DESCRIPTION OF *PERIBACILLUS FAECALIS* SP. NOV.

Peribacillus faecalis (fae.ca′lis. N.L. masc. adj. *faecalis*, faecal, referring to faeces as the source of isolation).

Colonies are circular, white, smooth, and 1–3 mm in diameter when cultured on TSAB medium at 25 °C for 3 days. TSAB was the sole medium in which growth occurred, the temperature ranged from at 20 to 40 °C (optimum 30 °C), at pH 3.0–11.0 (optimum pH 4.0), and in the presence of 0–18% (optimum 10%). Cells are facultatively anaerobic, Gram-stain-positive, rod-shaped (0.7–0.9 µm×1.9–10.3 µm), motile, endospore-forming, catalase-positive and oxidase-negative. Sensitive to tetracycline, penicillin, chloramphenicol, kanamycin and nalidixic acid and resistant to nitrofurantoin. API 20NE tests indicated all negative reactions for nitrate reduction,

Table 3. Cellular fatty acid contents (>1%) of the AGMB 02131^T and closely related species of the genus *Peribacillus*

1, *Peribacillus faecalis* AGMB 02131^T; 2, *P. endoradicis* DSM 28131^T; 3, *P. butanolicorans* DSM 18926^T. All data are from the present study. All closely related strains were cultured on tryptic soy agar (TSA) at 25 °C for 2 days. Values are presented as the percentages of total fatty acids. ND, not detected. Major components (>10%) are shown in bold type.

Fatty acids	1	2	3
Saturated			
$C_{12:0}$	3.7	0.3	ND
$C_{14:0}$	6.2	2.0	4.1
$C_{16:0}$	**14.8**	8.7	4.4
$C_{17:0}$	1.2	ND	4.2
$C_{18:0}$	**15.3**	3.8	ND
Branched			
iso-$C_{13:0}$	ND	5.3	ND
iso-$C_{14:0}$	2.45	2.4	ND
anteiso-$C_{13:0}$	0.7	4.99	ND
iso-$C_{15:0}$	2.0	**30.6**	0.6
iso-$C_{16:0}$	1.3	2.0	3.9
anteiso-$C_{15:0}$	1.1	**12.7**	**16.2**
iso-$C_{17:0}$	0.7	3.1	2.0
anteiso-$C_{17:0}$	1.1	4.3	1.7
Unsaturated			
$C_{16:1}\omega7c$ alcohol	0.3	3.6	**53.1**
$C_{16:1}\omega11c$	3.9	6.1	2.5
iso-$C_{17:0}\omega10c$	ND	5.2	2.8
$C_{18:1}\omega9c$	**29.7**	0.4	ND
Summed feature*			
3	1.1	0.4	0.6
4	ND	2.6	ND
5	6.5	0.5	3.6
8	4.6	0.5	ND

*Summed features indicate groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 3 contains $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; Summed feature 4 contains $C_{17:1}$ iso/anteiso B; Summed feature 5 contains $C_{18:2}\omega6c$ and/or $C_{18:2}\omega9c$, $C_{18:0}$ ante; Summed feature 8 contains $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

indoleproduction, glucose fermentation, arginine dihydrolase, urease hydrolysis, aesculinhydrolysis, gelatine hydrolysis, β-galactosidase, assimilationactivity for glucose, arabinose, mannose, mannitol, N-acetylglucosamine, glusomineassimilation for maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. API 50CH tests showed the production of acid from D-glucose, D-fructose, L-sorbose, *N*-acetylglucosamine, aesculin ferric citrate, cellobiose and maltose. In the API ZYM kit, alkaline phosphate, esterase (C4), acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are positive, and esterase lipase (C8), lipase(C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase are negative. The polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and two phospholipids (PL1 and PL2). Menaquinone 7 (MK-7) is the predominant respiratory quinone. The principal fatty acids are $C_{18:1}\omega9c$, $C_{18:0}$ and $C_{16:0}$.

The type strain is *Peribacillus faecalis* sp. nov AGMB 02131^T (=KCTC 43221^T=CCTCC AB 2020077^T), which was isolated from the faeces of a Korean cow. The GenBank accession numbers for the 16S rRNA gene and the whole-genome sequence of strain AGMB02131^T are MW009695 and JACXSI000000000, respectively.

Funding information
This research was supported by the Bio & Medical Technology Development Programme of the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT) of the Republic of Korea (Project No. NRF-2019M3A9F3065226) and a grant from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) research initiative programme (KGM5282021).

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

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

References

1. Patel S, Gupta RS. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *Int J Syst Evol Microbiol* 2020;70:406–438.
2. Gupta RS, Patel S, Saini N, Chen S. Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the Subtilis and Cereus clades of species. *Int J Syst Evol Microbiol* 2020;70:5753–5798.
3. Feng L, Liu D, Sun X, Wang G, Li M. *Bacillus cavernae* sp. nov. isolated from cave soil. *Int J Syst Evol Microbiol* 2016;66:801–806.
4. Zhang L, Wu G-L, Wang Y, Dai J, Fang C-X. *Bacillus deserti* sp. nov., a novel bacterium isolated from the desert of Xinjiang, China. *Antonie Van Leeuwenhoek* 2011;99:221–229.
5. Yumoto I, Hirota K, Yamaga S, Nodasaka Y, Kawasaki T *et al*. *Bacillus asahii* sp. nov., a novel bacterium isolated from soil with the ability to deodorize the bad smell generated from short-chain fatty acids. *Int J Syst Evol Microbiol* 2004;54:1997–2001.
6. Lim J-M, Jeon CO, Lee JR, Park D-J, Kim C-J. *Bacillus kribbensis* sp. nov., isolated from a soil sample in Jeju, Korea. *Int J Syst Evol Microbiol* 2007;57:2912–2916.

7. Kuisiene N, Raugalas J, Spröer C, Kroppenstedt RM, Chitavichius D. *Bacillus butanolivorans* sp. nov., a species with industrial application for the remediation of n-butanol. *Int J Syst Evol Microbiol* 2008;58:505–509.
8. Li J, Yang G, Wu M, Zhao Y, Zhou S. *Bacillus huizhouensis* sp. nov., isolated from a paddy field soil. *Antonie van Leeuwenhoek* 2014;106:357–363.
9. Liu B, Liu G-H, Zhu Y-J, Wang J-P, Che J-M *et al*. *Bacillus loiseleuriae* sp. nov., isolated from rhizosphere soil from a loiseleuria plant. *Int J Syst Evol Microbiol* 2016;66:2678–2683.
10. Ma K, Yin Q, Chen L, Lai Q, Xu Y. *Bacillus acanthi* sp. nov., isolated from the rhizosphere soil of a mangrove plant *Acanthus ilicifolius*. *Int J Syst Evol Microbiol* 2018;68:3047–3051.
11. Zhang YZ, Chen WF, Li M, Sui XH, Liu H-C *et al*. *Bacillus endoradicis* sp. nov., an endophytic bacterium isolated from soybean root. *Int J Syst Evol Microbiol* 2012;62:359–363.
12. Kämpfer P, Busse H-J, McInroy JA, Glaeser SP. *Bacillus gossypii* sp. nov., isolated from the stem of *Gossypium hirsutum*. *Int J Syst Evol Microbiol* 2015;65:4163–4168.
13. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (editors). *Nucleic acid techniques in bacterial systematics*. New York: John Wiley and Sons; 1991.4.19. pp. 115–175.
14. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
15. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for window 95/98/NT. In nucleic acids symposium series 1999;41:95–98.
16. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
17. Kim M, Oh H-S, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.
18. Wilson KH, Blitchington RB, Greene RC. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. *J Clin Microbiol* 1990;28:1942–1946.
19. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP *et al*. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.
20. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
21. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
22. Na S-I, Kim YO, Yoon S-H, Ha S-M, Baek I, SI N, *et al*. UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* 2018;56:280–285.
23. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
24. Hwang SH, Hwang WM, Kang K, Ahn T-Y. *Gramella fulva* sp. nov., isolated from a dry surface of tidal flat. *J Microbiol* 2019;57:23–29.
25. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
26. Collins MD, Shah HN, Minnikin DE. A note on the separation of natural mixtures of bacterial menaquinones using reverse phase thin-layer chromatography. *J Appl Bacteriol* 1980;48:277–282.
27. Sasser M. *Bacterial Identification by Gas Chromatographic Analysis of Fatty Acids Methyl Esters (GC-FAME*, 19713, MIDI Technical Note #101. MIDI Inc: 125 Sandy Drive Newark, DE; 2006.