# *Chungangia koreensis* gen. nov., sp. nov., isolated from marine sediment

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A Gram-staining-positive, strictly aerobic, non-spore-forming, rod-shaped bacterial strain, CAU 9163$^T$, was isolated from marine sediment collected in the Republic of Korea and its taxonomic position was investigated using a polyphasic approach. The novel strain grew optimally at 30 °C and pH 8.0. In phylogenetic analysis based on 16S rRNA gene sequences, strain CAU 9163$^T$ formed a hitherto unknown lineage within the order *Bacillales*, which contains the genera *Planomicrobium*, *Planococcus*, *Sporosarcina*, *Rummeliibacillus, Viridibacillus, Lysinibacillus* and *Bacillus*. The levels of 16S rRNA gene sequence similarity between the novel strain and any established bacterial species were all <95.7 %. The major isoprenoid quinines of strain CAU 9163$^T$ were MK-8 (65.2 %) and MK-7 (22.8 %) and the predominant fatty acid was anteiso-$C_{15:0}$. The peptidoglycan was of the A4$\alpha$ type and based on L-Lys-D-Asp. The major whole-cell sugars were ribose and glucose. The polar lipid profile mainly consisted of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid and an unidentified polar lipid. The genomic DNA G+C content of the novel strain was 44.3 mol%. These data were sufficient to differentiate the novel strain from established genera in the phylum *Firmicutes*. Based on the phenotypic, chemotaxonomic and genotypic evidence, strain CAU 9163$^T$ represents a novel species in a new genus for which the name *Chungangia koreensis* gen. nov., sp. nov. is proposed. The type strain of *Chungangia koreensis* is 9163$^T$ (=KCTC 13729$^T$ =CCUG 59778$^T$).

Recent studies on marine samples from the Yellow Sea have revealed a considerable diversity of micro-organisms (Kim *et al.*, 2010; Jung *et al.*, 2011; Park *et al.*, 2011; Traiwan *et al.*, 2011). In the course of screening such samples for micro-organisms with biotechnological potential, a strain of rod-shaped bacteria, designated CAU 9163$^T$, was isolated from sediment collected at a shrimp aquafarm on Sukmo Island (37° 40′ 54.84″ N 126° 22′ 15.14″ E) in the Republic of Korea. Comparative 16S rRNA gene sequence analysis indicated that strain CAU 9163$^T$ formed a distinct phylogenetic lineage within the order *Bacillales*. The purpose of the present study was to establish the taxonomic position of this bacterial strain by following a polyphasic approach that included the determination of phenotypic and chemotaxonomic properties, a detailed phylogenetic investigation based on 16S rRNA gene sequences and a genetic analysis.

The novel strain was isolated by using the method of Gordon & Mihm (1962) using glucose–yeast extract agar [GYEA; containing ($1^{-1}$) 10 g yeast extract, 10 g glucose and 15 g agar] supplemented with cycloheximide (50 mg $l^{-1}$) and nalidixic acid (20 mg $l^{-1}$). The sediment sample was diluted with sterilized distilled water so that appropriate dilutions could be spread on plates of GYEA and incubated aerobically at 30 °C for 3 days. Pure cultures of the novel strain were preserved at −70 °C in GYE broth supplemented with 25 % (v/v) glycerol. The type species of six closely related genera that were used as reference strains in the phenotypic and chemotaxonomic analysis (*Sporosarcina ureae* KCTC 3856$^T$, *Falsibacillus pallidus* KCTC 13200$^T$, *Planomicrobium koreense* KCTC 3684$^T$, *Lysinibacillus boronitolerans* KACC 15323$^T$, *Viridibacillus arvi* KCTC

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain CAU 9163$^T$ is GU937385.

Four supplementary figures are available with the online version of this paper.

13115$^T$ and *Rummeliibacillus stabekisii* KCTC 13805$^T$) were obtained from the Korean Collection for Type Cultures (KCTC; Taejon, Republic of Korea) or the Korean Agricultural Culture Collection (KACC; Suwon, Republic of Korea).

For the investigation of most of its morphological, physiological and biochemical characteristics, strain CAU 9163$^T$ was cultivated on tryptic soy agar (TSA; Difco) at 30 °C. Cell morphology was investigated under a light microscope (DM 1000; Leica) and in a scanning electron microscope (JSM-5410LV; JEOL). Gram staining was carried out using the bioMérieux Gram staining kit according to the manufacturer's instructions. The novel strain was incubated on a nutrient sporulation medium (NSM) for 5 days, to induce the production of spores (Schaeffer *et al.*, 1965; Nicholson & Setlow, 1990), before any spores were stained with malachite green as described by Conn *et al.* (1957). Flagellum type was examined by transmission electron microscopy (JEM 1010; JEOL) using cells from an exponentially growing culture. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Growth of turbidity at various temperatures was determined on TSA at temperatures between 5 and 55 °C in both an aerobic incubator (MIR-253; Sanyo) and an anaerobic chamber (Bactron; Sheldon). The pH range for growth was investigated in nutrient broth (NB; Difco) that had been adjusted to pH 4.5–10.0 (at intervals of 0.5 pH unit) by using sodium acetate/acetic acid and $Na_2CO_3$ buffers. Growth in the absence of NaCl and in the presence of 0–15.0 % (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded and 0.45 % (w/v) $MgCl_2 . 6H_2O$ or 0.06 % (w/v) KCl was added.

Catalase activity was determined by bubble production in 3 % (v/v) $H_2O_2$ solution. Oxidase activity was evaluated from the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Hydrolysis of casein, starch and urea were determined, on brain heart infusion agar, by using the methods of Lányí (1987) and Smibert & Krieg (1994). Acid production from carbohydrates and other enzyme activities were tested as described by Leifson (1963), using the API 50CHB, API 20E and API ZYM strips (bioMérieux) according to the manufacturer's instructions (with incubation times of up to 3 days at 30 °C).

For the determination of fatty acid composition, cell mass was harvested from TSA after cultivation for 3 days at 30 °C. Cellular fatty acid methyl esters were obtained by using the method of Minnikin *et al.* (1980) and separated in a 6890N gas chromatograph (Agilent) fitted with a 7683 autosampler (Agilent). Peaks were identified by following the standard protocol of the Sherlock Microbial Identification System (MIDI) and using the Moore library. Menaquinones were extracted by reverse-phase HPLC, as described by Komagata & Suzuki (1987). Polar lipids were extracted and analysed by 2D TLC (Minnikin *et al.*, 1984). Whole-cell sugars were also analysed by TLC, using the method described by Komagata & Suzuki (1987). Peptidoglycan analysis was performed by the identification service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), as described by Schleifer (1985), with the modification that TLC was substituted for paper chromatography. The molar amino acid ratio was determined by GC and GC-MS, according to the method of MacKenzie (1987).

Genomic DNA was extracted according to the method of Marmur & Doty (1961). PCR amplification and sequencing of the 16S rRNA gene of strain CAU 9163$^T$ was carried out following established procedures (Cho *et al.*, 2008). The amplified 16S rRNA gene was sequenced directly by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic 3730 DNA sequencer (Applied Biosystems). Multiple alignments of the 16S rRNA gene sequence of strain CAU 9163$^T$ with the corresponding sequences from a broad selection of closely related strains, and calculations of the levels of sequence similarity, were made using the EzTaxon server (Chun *et al.*, 2007) and CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distance matrices were generated by the neighbour-joining method described by Jukes and Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms and the PHYLIP suite of programs (Felsenstein, 1989). Branch support in the neighbour-joining tree was evaluated by the bootstrap resampling method, with 1000 replicates (Felsenstein, 1985). Genomic DNA G+C content was determined following a modification of the method of Tamaoka & Komagata (1984); specifically, the DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC.

The morphological, cultural, physiological and biochemical characteristics of strain CAU 9163$^T$ are shown in Table 1 or given in the genus and species descriptions. Colonies of the strain were creamy in colour, smooth and circular, with diameters of 0.1–0.2 mm after 3 days of cultivation on TSA at 30 °C. No colonies formed under anaerobic growth conditions. The strain grew as Gram-staining-positive rods that each measured approximately 1.1–1.5 × 0.3–0.4 μm (see Fig. S1, available in IJSEM Online). Endospores were not detected but the bacterium was motile by means of a peritrichous flagellum (Fig. S2). Strain CAU 9163$^T$ grew at 30–45 °C (optimum 30 °C), at pH 5.5–9.5 (optimum pH 8.0) and with 0–9.0 % (w/v) NaCl (optimum 0 %).

Cells of the novel strain were positive for catalase, oxidase and hydrolysis of casein and weakly positive for acid production from ribose, aesculin, D-fucose and 5-ketogluconate. Positive for alkaline phosphatase and naphthol-AS-BI-phosphohydrolase activities but negative for nitrate reduction, hydrolysis of gelatin and starch, production of acids from glycerol, glucose and turanose, and esterase (C4),

**Table 1.** Differential phenotypic properties of strain CAU 9163$^T$ and the type species of closely related genera

Strains: 1, CAU 9163$^T$; 2, *Sporosarcina ureae* KCTC 3856$^T$; 3, *Falsibacillus pallidus* KCTC 13200$^T$; 4, *Planomicrobium koreense* KCTC 3684$^T$; 5, *Lysinibacillus boronitolerans* KACC 15323$^T$; 6, *Viridibacillus arvi* KCTC 13115$^T$; 7, *Rummeliibacillus stabekisii* KCTC 13805$^T$. All data are from this study. +, Positive; −, negative; w, weakly positive.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Cell morphology | Rods | Short rods | Rods | Cocci, short rods | Rods | Rods | Rods |
| Colony colour | Cream | White | Light pink | Orange | Opaque | Opaque | Opaque |
| Motility | + | + | + | + | + | − | + |
| Growth at: | | | | | | | |
| 4 °C | − | − | + | + | − | + | − |
| 45 °C | + | − | − | − | − | + | + |
| Optimum growth temperature (°C) | 30 | 25 | 30 | 30 | 30 | 30 | 30 |
| Catalase | + | + | w | + | + | + | + |
| Oxidase | + | + | − | − | − | + | − |
| Nitrate reduction | − | + | − | − | − | + | − |
| Hydrolysis of: | | | | | | | |
| Casein | + | − | + | + | w | + | − |
| Gelatin | − | − | + | + | + | + | + |
| Starch | − | − | − | − | − | − | + |
| Acid production from: | | | | | | | |
| Glycerol | − | − | − | − | − | + | − |
| Ribose | w | − | + | − | − | − | + |
| Glucose | − | − | + | w | − | − | + |
| Aesculin | w | − | + | + | − | w | − |
| Turanose | − | − | + | − | − | − | − |
| D-Fucose | w | − | + | − | − | − | − |
| 5-Ketogluconate | w | − | − | + | − | − | − |
| Enzyme activity: | | | | | | | |
| Alkaline phosphatase | + | − | − | − | − | − | − |
| Esterase (C4) | − | − | + | − | − | + | − |
| Esterase lipase (C8) | − | − | + | − | − | − | − |
| Leucine arylamidase | − | − | − | − | − | + | − |
| α-Chymotrypsin | − | − | − | − | − | w | − |
| Naphthol-AS-BI-phosphohydrolase | w | − | w | − | − | + | − |
| DNA G+C content (mol%) | 44.3 | 40–42 | 42.3 | 47 | 36.5 | 49.6 | 34.3 |

esterase lipase (C8), lipase (C14), leucine arylamidase, trypsin, α-chymotrypsin and acid phosphatase activities.

The nearly complete 16S rRNA gene sequence of strain CAU 9163$^T$ (1440 bp) was determined and compared with the corresponding sequences of other bacterial strains in the GenBank database. In the phylogenetic analysis based on the 16S rRNA gene sequences and the neighbour-joining algorithm (Fig. 1), the novel strain appeared distinct from a clade represented by members of the genera *Planomicrobium*, *Planococcus*, *Sporosarcina*, *Rummeliibacillus*, *Viridibacillus*, *Lysinibacillus*, *Bacillus* and *Geobacillus*. In the pairwise analyses, *Bacillus ginsengi* ge14$^T$ (95.7 % 16S rRNA gene sequence similarity), *Bhargavaea cecembensis* DSE10$^T$ (95.7 %), *Bacillus beijingensis* ge10$^T$ (95.6 %), *Sporosarcina koreensis* F73$^T$ (95.3 %), *Sporosarcina saromensis* HG645$^T$ (95.2 %), *Planomicrobium flavidum* ISL-4$^T$ (95.2 %), *Planococcus maitriensis* S1$^T$ (95.2 %), *Falsibacillus pallidus* CW 7$^T$ (95.0 %) and *Bacillus cohnii* DSM 6307$^T$ (94.1 % similarity) were the established species that appeared most closely related to strain CAU 9163$^T$. The trees generated using the maximum-likelihood and least-squares algorithms showed similar topologies to the neighbour-joining tree (Figs 1 and S3). The genomic DNA G+C content of the novel strain, 44.3 mol%, was higher than those recorded for the type strains of the type species of the genera *Sporosarcina*, *Falsibacillus*, *Lysinibacillus* and *Viridibacillus* (Table 1).

The peptidoglycan of strain CAU 9163$^T$ contained alanine, glycine, lysine and glutamic acid in approximate molar ratios of 2.7 : 0.3 : 1.0 : 3.0. This observation indicated that strain CAU 9163$^T$ had peptidoglycan that was of the A4α type and based on L-Lys-D-Asp (Schleifer & Kandler, 1972). The predominant menaquinone of the novel strain was MK-8 (65.2 %) but a major amount of MK-7 (22.8 %) was also detected. The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid and an unidentified polar lipid. Minor amounts of two unidentified aminophospholipids and an unidentified phosphoglycolipid were also

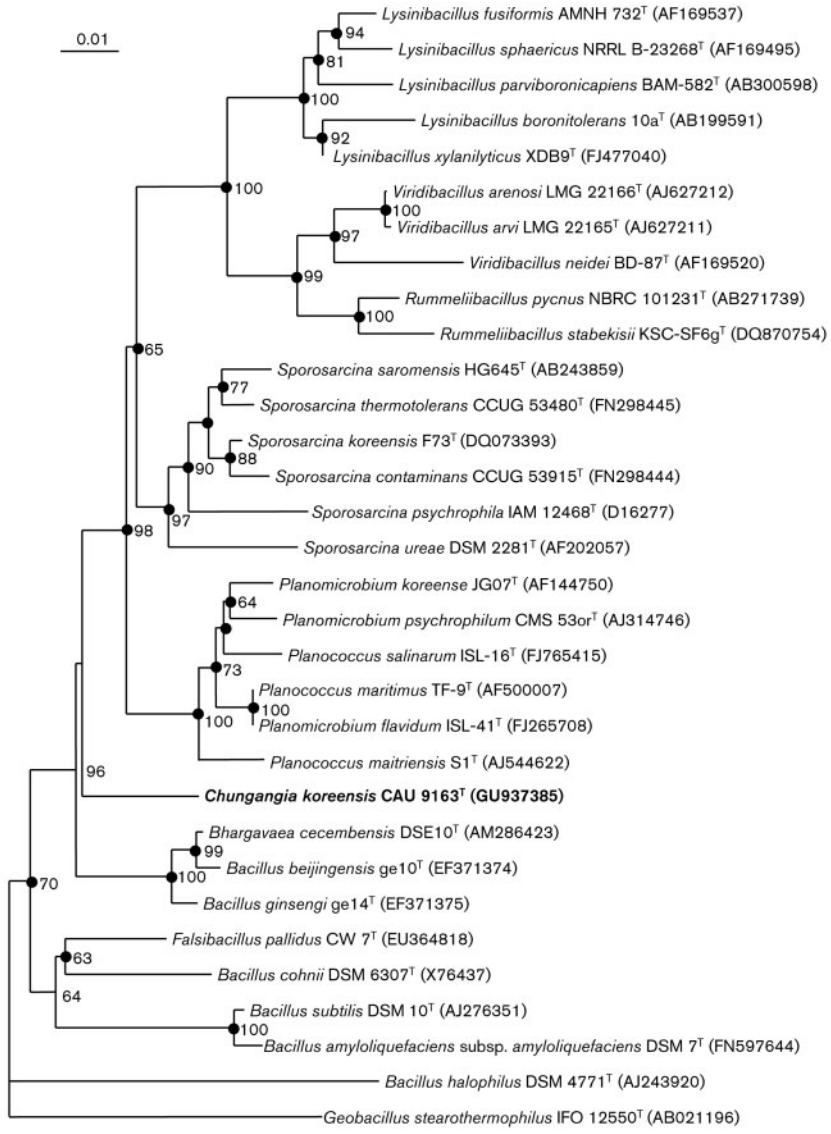


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain CAU 9163$^T$ and some closely related species. Bootstrap values >50 % (based on 1000 replications) are shown at branch points. Filled circles indicate nodes that were also recovered in the maximum-likelihood and least-squares trees. *Geobacillus stearothermophilus* IFO 12550$^T$ was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

detected (Fig. S4). TLC analysis of whole-cell hydrolysates of the novel strain revealed the presence of ribose and glucose. The cellular fatty acid profile contained branched-chain, saturated and unsaturated fatty acids, with anteiso-$C_{15:0}$ and iso-$C_{14:0}$ predominant (Table 2).

Strain CAU 9163$^T$ could be clearly distinguished from established members of the *Bacillaceae* and *Planococcaceae* by some of its chemotaxonomic properties. The novel strain differed from most species in the genera *Planomicrobium*, *Planococcus*, *Sporosarcina*, *Rummeliibacillus*, *Viridibacillus*, *Lysinibacillus*, *Bacillus* and *Geobacillus* (i.e. the established species that appeared to be the strain's closest phylogenetic neighbours) in the type of its predominant menaquinone (MK-8) and/or the type of its cell-wall peptidoglycan (L-Lys-D-Asp) (Table 3). For example, species in the genera *Sporosarcina* and *Planomicrobium* have either MK-7 as their predominant menaquinone (*Sporosarcina*) or similar, major amounts of both MK-7 and MK-8 (*Planomicrobium*), as well as peptidoglycans based on L-Lys-D-Glu or L-Lys-D-Asp (An *et al.*, 2007; Jung *et al.*, 2009; Kwon *et al.*, 2007; Tominaga *et al.*, 2009). Species in the genus *Falsibacillus* also have MK-7 as their predominant menaquinone but have *meso*-diaminopimelic acid in their peptidoglycan (Zhou *et al.*, 2009). Cells of the species in the genera *Lysinibacillus*, *Viridibacillus* and *Rummeliibacillus* either contain major amounts of both MK-7 and MK-8 or have MK-7 as their predominant menaquinone, as well as peptidoglycans based on L-Lys-D-Glu or L-Lys-D-Asp (Ahmed *et al.*, 2007; Albert *et al.*, 2007; Vaishampayan *et al.*, 2009). Strain CAU 9163$^T$ could also be distinguished from closely related species by its major fatty acids (Table 2).

On the basis of the genotypic, phenotypic, biochemical and chemotaxonomic evidence presented above, strain CAU 9163$^T$ represents a novel species in a new genus for which the name *Chungangia koreensis* gen. nov., sp. nov. is proposed.

**Table 2.** Cellular fatty acid contents (%) of strain CAU 9163$^T$ and the type species of closely related genera

Strains: 1, CAU 9163$^T$; 2, *Sporosarcina ureae* KCTC 3856$^T$; 3, *Falsibacillus pallidus* KCTC 13200$^T$; 4, *Planomicrobium koreense* KCTC 3684$^T$; 5, *Lysinibacillus boronitolerans* KACC 15323$^T$; 6, *Viridibacillus arvi* KCTC 13115$^T$; 7, *Rummeliibacillus stabekisii* KCTC 13805$^T$. All data are from this study. Fatty acids that represented <1 % of the total in all seven strains are not shown.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Saturated | | | | | | | |
| $C_{14:0}$ | | | | | | 1.6 | 3.2 |
| $C_{16:0}$ | | 1.1 | | 5.3 | | 1.2 | 2.1 |
| Unsaturated | | | | | | | |
| $C_{16:1}\omega7c$ alcohol | 1.7 | 2.0 | 6.3 | 9.0 | 9.4 | 2.3 | |
| $C_{16:1}\omega11c$ | | 1.5 | 1.4 | 5.2 | 1.4 | 3.7 | |
| Branched-chain | | | | | | | |
| anteiso-$C_{13:0}$ | 1.4 | | | | | | |
| anteiso-$C_{15:0}$ | 60.3 | 69.3 | 22.5 | 40.8 | 13.8 | 19.8 | 34.6 |
| anteiso-$C_{17:0}$ | 1.4 | 8.8 | 3.1 | 5.5 | 2.4 | 5.7 | 4.4 |
| iso-$C_{14:0}$ | 18.6 | 2.8 | 9.3 | 6.5 | 1.9 | 1.7 | 2.6 |
| iso-$C_{15:0}$ | 9.6 | 7.8 | 46.3 | 7.7 | 56.6 | 52.7 | 47.2 |
| iso-$C_{16:0}$ | 4.1 | 1.4 | 2.8 | 8.7 | 7.1 | 1.8 | 2.2 |
| iso-$C_{17:0}$ | | | 1.8 | 4.3 | 3.2 | 4.1 | |
| iso-$C_{17:1}\omega10c$ | | | 2.3 | 2.4 | 1.7 | 2.6 | |
| Summed feature 4* | | 2.7 | 2.8 | 2.6 | 1.3 | 2.6 | |

*Summed features consist of two or more fatty acids that could not be separated by GLC using the MIDI system. Summed feature 4 comprised iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

## Description of *Chungangia* gen. nov.

*Chungangia* (Chung.ang′i.a. N.L. fem. n. *Chungangia* after Chung-Ang University, Seoul, Republic of Korea, where the initial taxonomic studies on this genus were performed).

Cells are Gram-staining-positive, rod-shaped, obligately aerobic, non-spore-forming and catalase- and oxidase-positive. They have cell-wall peptidoglycan of the A4α type, based on L-Lys-D-Asp. The predominant menaquinone is MK-8 but MK-7 is also present. The whole-cell sugars are ribose and glucose. The polar lipid profile consists mainly of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, an unidentified glycolipid and an unidentified polar lipid. The predominant fatty acids are anteiso-$C_{15:0}$ and iso-$C_{14:0.}$ The type species is *Chungangia koreensis.*

## Description of *Chungangia koreensis* sp. nov.

*Chungangia koreensis* (ko.re.en′sis. N.L. fem. adj. *koreensis* pertaining to Korea, where the type strain was isolated).

Displays the following properties in addition to those given for the genus. Cells are strictly aerobic, Gram-staining-positive,

**Table 3.** Characteristics that can be used to differentiate strain CAU 9163$^T$ from species in closely related genera

Taxa: 1, strain CAU 9163$^T$; 2, genus *Sporosarcina*; 3, genus *Falsibacillus*; 4, genus *Planomicrobium*; 5, genus *Lysinibacillus*; 6, genus *Viridibacillus*; 7, genus *Rummeliibacillus*. AL, Aminolipid; APL, aminophospholipid; BPG, bisphosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid. *meso*-DAP, *meso*-diaminopimelic acid.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Major quinine(s) | MK-7, MK-8 | MK-7, MK-8 | MK-7 | MK-7, MK-8 | MK-7 | MK-7, MK-8 | MK-7 |
| Polar lipids | DPG, PG, PE, PGL, APL, L and GL | DPG, PG and PL or PG, DPG, PE and PL | DPG, PG and PE | PE, PG and BPG or PG, DPG and PE | DPG, PG and NPG or DPG, PG and PE | DPG, PE, PG, APL and two PL | PG, PE, PG, APL, two PL and AL |
| Peptidoglycan type | L-Lys-D-Asp | L-Lys-D-Glu | *meso*-DAP | L-Lys-D-Glu or L-Lys-D-Asp | L-Lys-D-Asp | L-Lys-D-Glu or L-Lys-D-Asp | L-Lys-D-Glu or L-Lys-D-Asp |

motile, short rods (1.1–1.5 × 0.3–0.4 μm). Endospores are not observed. Colonies that develop after incubation on TSA for 3 days at 30 °C are cream-coloured, smooth and circular. Growth occurs optimally at 30 °C and pH 8.0. Cells are catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Positive for the hydrolysis of casein. Weakly positive for acid production from ribose, aesculin, D-fucose and 5-ketogluconate. Positive for alkaline phosphatase activity and (weakly) for naphthol-AS-BI-phosphohydrolase activities.

The type strain, CAU 9163$^T$ (=KCTC 13729$^T$ =CCUG 59778$^T$), was isolated from the marine sediment of a shrimp farm at Sukmo Island in the Republic of Korea. The genomic DNA G+C content of the type strain is 44.3 mol%.

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