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NOTE

Cohnella soli sp. nov. and *Cohnella suwonensis* sp. nov. Isolated from Soil Samples in Korea

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Two bacterial isolates from soil samples taken in Korea, strains YM2-7^T and WD2-19^T, were characterized using a polyphasic approach. The cells were strictly aerobic, Gram-positive, motile with peritrichous flagella, and rod-shaped. Both strains formed ellipsoidal bulging positioned subterminal spores. Phylogenetic analysis of their 16S rRNA gene sequences revealed a clear affiliation with the *Firmicutes*. The 16S rRNA gene sequence similarity between YM2-7^T and WD2-19^T was 96.5%. Strains YM2-7^T and WD2-19^T showed 16S rRNA gene sequence similarities of 93.0-96.5% to type strains of recognized *Cohnella* species. The G+C contents of the DNA of strains YM2-7^T and WD2-19^T were 52.2 and 55.6 mol%, respectively. The major fatty acids of strains YM2-7^T and WD2-19^T were anteiso-C15:0 (44.4%), C16:0 (19.2%), and iso-C16:0 (16.8%) and anteiso-C15:0 (46.5%), iso-C16:0 (21.8%), and C16:0 (11.2%), respectively. Both strains contained menaquinone with seven isoprene units (MK-7) as the predominant quinone. Both strains had diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysophosphatidylglycerol as the major polar lipids. Comparative analysis of phenotypic and phylogenetic traits indicated that strains YM2-7^T and WD2-19^T represented two novel species of the genus *Cohnella*. The names *Cohnella soli* sp. nov. (type strain YM2-7^T =KACC 13346^T =NBRC 106486^T), and *Cohnella suwonensis* sp. nov. (type strain WD2-19^T =KACC 13347^T =NBRC 106485^T) are proposed for these organisms.

Keywords: *Cohnella soli*, *Cohnella suwonensis*, 16S rRNA gene sequence, taxonomy

The genus *Cohnella* was first proposed for two strains, one isolated from hygiene control checks at a starch-producing company and the other '*Paenibacillus hongkongensis*' strain (Kämpfer *et al*., 2006). This genus was one member of the family *Paenibacillaceae*, and its description was amended by García-Fraile *et al*. (2008). It was characterized by Gram-positive, spore-forming, aerobic, non-motile or motile, and rod-shaped cells, which had menaquinone-7 as the predominant quinone. The predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. At present, the genus comprises 12 species with the validly published names including *Cohnella thermotolerans* (Kämpfer *et al*., 2006), *Cohnella hongkongensis* (Teng *et al*., 2003), *Cohnella laeviribosi* (Cho *et al*., 2007), *Cohnella phaseoli* (García-Fraile *et al*., 2008), *Cohnella fontinalis* (Shiratori *et al*., 2010), *Cohnella luojiensis* (Cai *et al*., 2010), *Cohnella yongneupensis* (Kim *et al*., 2010), *Cohnella ginsengisoli* (Kim *et al*., 2010), *Cohnella thailandensis* (Khianngam *et al*., 2009), *Cohnella xylantilytica* (Khianngam *et al*., 2010), *Cohnella terrae* (Khianngam *et al*., 2010), and *Cohnella damuensis* (Luo *et al.*, 2010). These species have been isolated from a wide variety of ecological niches including clinical samples, volcanic areas, root nodules, water, and soil.

During the course of a study on bacterial diversity in Korea, several novel bacterial strains were isolated from a soil sample taken on Yeogi Mountain (37°16′46″N 126°59′5″E) and a field soil sample (37°16′29″N 126°59′32″E) from Suwon, Republic of Korea. The soil samples were serially diluted with 0.85% (w/v) NaCl and suitable tenfold dilutions were plated onto R2A agar (Difco, USA). The plates were incubated at 28°C for 5 days. The YM2-7^T and WD2-19^T colonies were distinguishable on R2A medium because of their white color and subjected to taxonomic investigation. Routine cultivation was conducted at 28°C on R2A agar. Maintenance in glycerol served as a medium-term preservation method. Cultures were preserved in liquid N_2 and freeze-dried. Phenotypic, chemotaxonomic, and phylogenetic analyses revealed that strains YM2-7^T and WD2-19^T were members of the genus *Cohnella*. On the basis of the substantial evidence from polyphasic taxonomic studies, we propose that YM2-7^T and WD2-19^T are the type strains of novel species *Cohnella soli* and *Cohnella terrae* belonging to the genus *Cohnella*.

After cultivation of strains YM2-7^T and WD2-19^T on R2A at 28°C for 3 days, cell morphology and motility were observed using phase-contrast (AXIO; Zeiss, USA) and transmission electron (912AB; LEO) microscopes. Gram staining, presence of catalase and oxidase activities, and hydrolysis of casein, xanthine, hypoxanthine, Tween 80, and starch were determined as described previously (Smibert and Krieg,

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Table 1. Phenotypic comparison of YM2-7^T and WD2-19^T and six *Cohnella* species
Strains: 1, YM2-7^T; 2, WD2-19^T; 3, *Cohnella yongneupensis* KACC 11768^T; 4, *Cohnella ginsengisoli* KACC 11771^T; 5, *Cohnella thermotolerans* KACC 11643^T; 6, *Cohnella hongkongensis* KACC 11644^T; 7, *Cohnella laeviribosi* KACC 13447^T; 8, *Cohnella phaseoli* KACC 13436^T. All strains were positive for aesculin hydrolysis, β-galactosidase, esterase (C4), and naphthol-AS-BI-phosphohydrolase. All strains were negative for glucose fermentation, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. None assimilated adipic acid, malic acid, trisodium citrate, phenylacetic acid, itaconic acid, sodium malonate, sodium acetate, lactic acid, potassium 5-ketogluconate, propionic acid, l-histidine, 3-hydroxybutyric acid, or l-proline. +, positive; (+), weakly positive; -, negative.

Characteristics	1	2	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]
Catalase/oxidase	-/+	-/+	(+)/+	+/+	+/+	(+)/+	+/-	(+)/+
Nitrate reduction	-	-	-	+	-	+	-	-
Indole production	-	+	-	-	-	-	-	-
Urease	-	-	-	-	-	-	+	-
Gelatin hydrolysis	-	-	-	-	-	-	(+)	-
Assimilation:								
D-Glucose	+	+	-	+	+	+	+	+
L-Arabinose	+	+	-	+	+	+	+	+
D-Mannose	-	+	-	+	+	+	+	+
D-Mannitol	-	-	-	+	+	+	+	-
N-Acetylglucosamine	+	-	-	-	-	+	-	+
D-Maltose	+	+	-	+	+	+	+	+
Potassium gluconate	-	-	-	-	+	-	-	-
L-Rhamnose	+	+	-	-	+	+	+	-
D-Ribose	+	-	-	-	+	+	+	+
Inositol	-	-	-	-	+	+	-	-
D-Saccharose	+	+	-	-	+	+	+	+
Glycogen	+	+	-	-	+	-	+	+
Salicin	+	+	-	+	+	+	-	+
D-Melibiose	+	+	-	+	+	+	+	+
L-Fucose	-	+	-	-	+	+	+	+
D-Sorbitol	-	-	-	-	+	+	+	-
Potassium 2-ketogluconate	-	-	-	-	+	-	-	+
Enzymatic activities: (API ZYM):								
Alkaline phosphatase	+	+	+	-	+	+	-	-
Esterase (C4)	+	-	+	+	+	+	+	+
Esterase lipase (C8)	+	+	+	+	+	+	(+)	-
Leucine arylamidase	-	-	-	-	+	+	(+)	(+)
Acid phosphatase	+	+	+	-	-	-	-	-
α-Galactosidase	+	-	+	-	+	-	+	-
α-Glucosidase	+	-	+	-	+	-	+	+
β-Glucosidase	+	+	+	-	+	+	+	+
α-Fucosidase	-	-	+	-	-	+	-	-
DNA G+C content (mol%)	52.2	55.6	58.8	61.3	59	60.9	51	60.3

[a] Data were obtained from Kim *et al*. (2010).

1994). Hydrolysis of arboxymethylcellulose (0.1%, w/v), chitin from crab shells (1%, w/v), pectin (0.5%, w/v), and tyrosine (0.5%, w/v) were also examined. Temperature tolerance was tested by growing cells at 10, 15, 20, 25, 30, 35, 37, and 40°C. The pH range (pH 4-10, using increments of 1 pH unit) for growth was determined after 9 days of incubation in R2A broth buffered with citrate/phosphate or Tris-HCl buffers (Breznak and Costilow, 1994). Salt tolerance was tested on liquid R2A medium supplemented with 0-5% NaCl (0, 0.5, 1, 1.5, 2, 3, 4, and 5%, w/v) after incubation for 7 days at 30°C. Additional enzyme activities and biochemical features were tested using API kits (API 20NE, API ID 32GN, and API ZYM; bioMérieux, France) according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 30°C, and the others after 21 days at 30°C. Cells of strains YM2-7^T and WD2-19^T were aerobic, Gram-positive, motile with peritrichous flagella, and rod-shaped. Both strains formed ellipsoidal bulging positioned subterminal spores. Strains YM2-7^T and WD2-19^T were able to grow on nutrient agar (NA; Difco), but not on Luria-Bertani (LB; Difco), trypticase soy (TSA; Difco), or MacConkey (Difco) agars. The phenotypic features and description of the new species are presented in Table 1.

Genomic DNA was isolated by the method of Ausubel *et al*. (1987), except that lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA genes

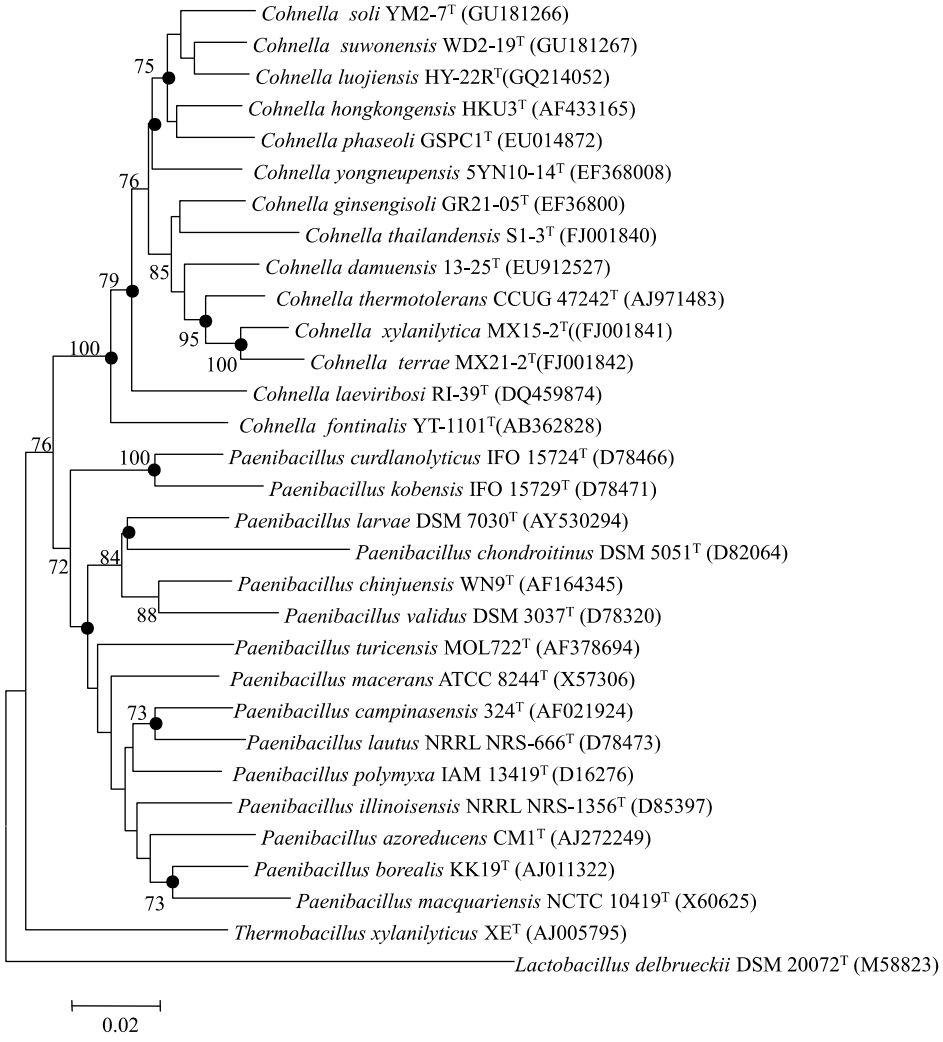

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among strains YM2-7^T, WD2-19^T, and closely related species. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap values (expressed as percentages of 1,000 replications) greater than 70% are indicated at the nodes. Bar=0.02 changes per nucleotide position.

were amplified using the universal primers fD1 and rP2 (Weisburg *et al*., 1991) and sequenced as described by Kwon *et al*. (2003). Phylogenetic neighbors were identified, and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (http://www.eztaxon.org/; Chun *et al*., 2007). After multiple alignments of data using CLUSTAL W (Thompson *et al.*, 1994), the MEGA software package (ver. 3.1; Kumar *et al.*, 2004) was used for all analyses. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values based on 1,000 replications (Felsenstein, 1985). Strains YM2-7^T and WD2-19^T were affiliated with the genus *Cohnella* and were closely related to *C. luojiensis* HY-22R^T with 96.5% sequence similarity. The 16S rRNA gene sequence similarity between YM2-7^T and WD2-19^T was 96.5%. The neighbor-joining tree showed that strains YM2-7^T and WD2-19^T formed a compact cluster with members of the genus *Cohnella*, with 100% bootstrap support (Fig. 1). The tree obtained using the maximum parsimony method also showed that strains YM2-7^T and WD2-19^T were members of the genus *Cohnella*.

After the growth of strains YM2-7^T and WD2-19^T on R2A for 3 days at 30°C, fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones were analyzed by HPLC as described by Groth *et al*. (1996). Extraction and analysis of polar lipids by two-dimensional TLC was performed according to Minnikin *et al.* (1984). Polar lipids were characterized with spray reagents: acid alcohol (for total lipids), ninhydrin (for aminolipids), molybdenum blue (for phospholipids), and dragendorff (quaternary nitrogen compounds). The G+C contents (mol%) of strains YM2-7^T and WD2-19^T were determined by HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al*. (1989), using a reverse-phased column (Supelcosil LC-18-S; Supelco). The predominant fatty acids of strain YM2-7^T were anteiso-$C_{15:0}$

Table 2. Cellular fatty acid compositions (%) of YM2-7^T and WD2-19^T and *Cohnella* species
Strains: 1, YM2-7^T; 2, WD2-19^T; 3, *Cohnella yongneupensis* KACC 11768^T; 4, *Cohnella ginsengisoli* KACC 11771^T; 5, *Cohnella thermotolerans* KACC 11643^T; 6, *Cohnella hongkongensis* KACC 11644^T; 7, *Cohnella laeviribosi* KACC 13447^T; 8, *Cohnella phaseoli* KACC 13436^T. All strains were cultivated on R2A medium at 30°C for 3 days prior to harvesting of the cell mass. -, not detected or <1%.

Fatty acid	1	2	3	4	5	6	7	8
iso-$C_{12:0}$	-	-	-	-	-	1.0	-	-
$C_{13:0}$	-	-	-	-	-	1.5	-	-
anteiso-$C_{13:0}$	1.7	1.2	2.2	-	-	3.3	-	2.2
$C_{14:0}$	4.4	2.0	3.2	3.1	2.0	6.0	1.7	1.7
iso-$C_{14:0}$	4.6	4.1	2.4	8.2	4.1	9.1	7.7	5.1
anteiso-$C_{15:0}$	44.4	46.5	51.1	48.9	35.2	35.6	28.6	46.0
iso-$C_{15:0}$	3.5	5.5	-	12.2	2.7	1.4	7.2	3.4
$C_{16:0}$	19.2	11.2	13.2	6.7	5.6	12.6	6.2	8.9
iso-$C_{16:0}$	16.8	21.8	18.5	15.0	30.6	17.6	40.9	17.0
C $_{16:1}$ ω11*c*	-	-	-	-	1.2	1.9	-	2.3
$C_{16:1}$ *ω7c* alcohol	-	-	-	-	1.6	3.3	-	4.0
iso-$C_{17:0}$	-	1.9	-	-	-	-	-	2.1
anteiso-$C_{17:0}$	2.9	4.7	4.2	1.8	5.7	1.7	3.7	3.4
$C_{17:1}$ *ω6c*	-	-	-	-	2.7	-	-	-
$C_{18:1}$ *ω7c*	-	-	-	-	5.9	-	-	-
Summed feature 3[a]	-	-	1.0	-	-	1.3	-	-
Summed feature 4[a]	-	-	-	1.7	-	-	-	-
Summed feature 5[a]	-	-	-	-	1.3	-	-	-

[a] Summed features represent groups of two fatty acids that could not be separated by gas-liquid chromatography with the MIDI system. Summed features: 3, $C_{16:1}$ *ω7c*/iso-$C_{15:0}$ 2-OH; 4, iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B; 5, ante-$C_{18:0}$/$C_{18:2}$ *ω6,9c*.

(44.4%), $C_{16:0}$ (19.2%), and iso-$C_{16:0}$ (16.8%), and those of strain WD2-19^T were anteiso-$C_{15:0}$ (46.5%), iso-$C_{16:0}$ (21.8%), and $C_{16:0}$ (11.2%) (Table 2). MK-7 was the predominant isoprenoid menaquinine of both strains YM2-7^T and WD2-19^T. Both strains had diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and lysophosphatidylglycerol as the major polar lipids (Fig. 2). The DNA G+C contents of strains YM2-7^T and WD2-19^T were 52.2 and 55.6 mol%, respectively.

Strains YM2-7^T and WD2-19^T can be differentiated from related *Cohnella* species based on the absence of traits such as catalase production. Strain WD2-19^T can be differentiated from strain YM2-7^T and related *Cohnella* species on the basis of indole production and the absence of esterase (C4). On the basis of the polyphasic study data, including analyses of physiological traits, fatty acid compositions, polar lipid profiles, and 16S rRNA gene sequences, we suggest that strains YM2-7^T and WD2-19^T represent two novel species within the genus *Cohnella*, for which the names *Cohnella soli* sp. nov. and *Cohnella suwonensis* sp. nov. are proposed.

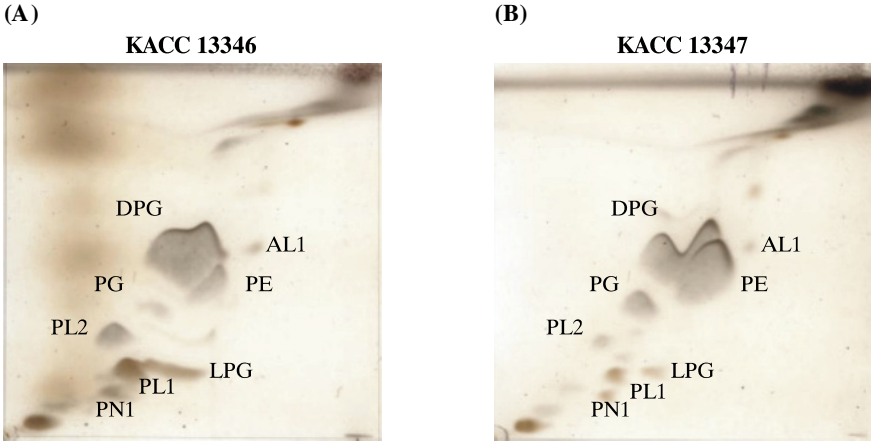

Fig. 2. Two-dimensional thin-layer chromatograms of polar lipids of strains YM2-7^T (A) and WD2-19^T (B) after separation by two-dimensional thin-layer chromatography (TLC). Chloroform/methanol/water (65:25:4) was used in the first dimension, and chloroform/methanol/acetic acid/water (80:12:15:4) in the second dimension. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; LPG, lysophosphatidylglycerol; PL, unknown phospholipids; PN, unknown aminophospholipid.

Description of *Cohnella soli* sp. nov.

Cohnella soli (so'li. L. gen. n. *soli* of the soil)

Cells are strictly aerobic, Gram-positive, motile with peritrichous flagella, and rod-shaped (0.6-0.7 μm in width and 1.8-3.5 μm in length). This strain forms ellipsoidal bulging positioned subterminal spores. Catalase is negative and oxidase is positive. The strain grows on R2A and NA, but not TSA, LB, or MacConkey. Colonies are white-colored and circular. Growth occurs at temperatures in the range of 15-37°C (optimum 30°C) and pH 5.0-7.0 (optimum pH 7.0). It does not tolerate salt concentrations above 1.5%. The strain is capable of hydrolyzing starch, but not casein, chitin, CM-cellulose, hypoxanthine, pectin, tyrosine, xanthine, or Tween 80. It is positive (by API ZYM strips) for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and β-glucosidase but negative for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The major fatty acids are anteiso-$C_{15:0}$ (44.4%), $C_{16:0}$ (19.2%), and iso-$C_{16:0}$ (16.8%) (>10% of the total fatty acids). Menaquinone-7 is the predominant quinone. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and lysophosphatidylglycerol. The DNA G+C content of the type strain is 52.2 mol%.

The type strain YM2-7^T (=KACC 13346^T =NBRC 106486^T) was isolated from soil on Yeogi Mountain, Republic of Korea.

Description of *Cohnella suwonensis* sp. nov.

Cohnella suwonensis (su.won.en'sis. N.L. masc. adj. *suwonensis* referring to Suwon region, Republic of Korea, where the type strain was first identified).

Cells are strictly aerobic, Gram-positive, motile with peritrichous flagella, and rod-shaped (0.6-0.7 μm in width and 2.0-4.9 μm in length). This strain forms ellipsoidal bulging positioned subterminal spore. Catalase is negative and oxidase is positive. Growth occurs on R2A and NA, but not TSA, LB, or MacConkey. Colonies are white-colored and circular. The strain grows at temperatures in the range of 10-35°C (optimum 30°C) and pH 5.0-8.0 (optimum pH 7.0). It does not tolerate salt concentrations above 1%. The strain is capable of hydrolyzing starch and CM-cellulose, but not casein, chitin, hypoxanthine, pectin, tyrosine, xanthine, or Tween 80. It is positive (by API ZYM strips) for alkaline phosphatase, esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α-galactosidase,* β-galactosidase, and β-glucosidase, but negative for esterase (C4), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The major fatty acids are anteiso-$C_{15:0}$ (46.5%), iso-$C_{16:0}$ (21.8%), and $C_{16:0}$ (11.2%) (>10% of the total fatty acids). Menaquinone-7 is the predominant quinone. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and lysophosphatidylglycerol. The DNA G+C content of the type strain is 55.6 mol%.

The type strain, WD2-19^T (=KACC 13347^T =NBRC 106485^T), was isolated from field soil in the Republic of Korea.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YM2-7^T and WD2-19^T are GU181266 and GU181267, respectively.

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