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Spirosoma daeguensis sp. nov., isolated from beach soil[§]

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A Gram-stain-negative, non-motile, non-spore-forming, rod-shaped, aerobic bacterium, designated 15J9-6^T, was isolated from beach soil on Jeju Island, South Korea. Strain 15J9-6^T, grew at 10–30°C (optimum growth at 25°C) and pH 7–8 (optimum growth at pH 7) on R2A, NA, and TSA agar. Phylogenetically, the strain was closely related to members of the genus *Spirosoma* (92.3–90.1% 16S rRNA gene sequence similarities) and showed highest sequence similarity to *Spirosoma panaciterrae* DSM 21099^T (92.3%). The G+C content of the genomic DNA of strain 15J9-6^T was 45.7 mol%. The strain contained phosphatidylethanolamine, two unidentified aminophospholipids, an unidentified phospholipid, and an unidentified lipid as the major polar lipids; menaquinone MK-7 as the predominant respiratory quinone and summed feature 3 ($C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c*; 30.1%), $C_{16:1}$ *ω5c* (23.1%), iso $C_{15:0}$ (13.3%), and $C_{16:0}$ (8.4%) as the major fatty acids which supported the affiliation of strain 15J9-6^T to the genus *Spirosoma*. The results of physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 15J9-6^T from recognized *Spirosoma* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain 15J9-6^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma daeguensis* sp. nov. is proposed. The type strain is 15J9-6^T (=KCTC 52036^T =JCM 31995^T)

Keywords: *Spirosoma*, *Bacteroidetes*, polyphasic taxonomy

Introduction

The genus *Spirosoma* was originally proposed with *Spirosoma linguale* as the type species (Larkin and Borrall, 1984), and the genus description was later emended by Finster *et al.* (2009) and Ahn *et al.* (2014). At the time of writing, the genus *Spirosoma* comprised fifteen species with validly published names, thirteen of which were directly listed in LPSN (http://www.bacterio.net/spirosoma.html), with the remaining two are awaiting notification (Joo *et al.*, 2017; Lee *et al.*, 2017a). Moreover, several other strains, such as '*Spirosoma fluminis*' 15J17T (Lee *et al.*, 2016), '*Spirosoma lacussanchae*' CPCC 100624 (Li *et al.*, 2017), and '*Spirosoma luteolum*' 16F6E (Lee *et al.*, 2017b), have also recently been proposed as members of novel *Spirosoma* species and are awaiting validation. The type strains of *Spirosoma* species have been recovered from air, dust, soil, fresh water, plant xylem sap, and extreme environments such as high Arctic glacial till, or Arctic permafrost soil (Baik *et al.*, 2007; Finster *et al.*, 2009; Fries *et al.*, 2013; Ahn *et al.*, 2014; Chang *et al.*, 2014; Joo *et al.*, 2015, 2017; Kim *et al.*, 2016; Yang *et al.*, 2016). Members of the genus *Spirosoma* are Gram-negative, yellow- or orange-pigmented, strictly aerobic or facultatively anaerobic, ring- or rod-shaped bacteria that are characterized chemotaxonomically as having menaquinone MK-7 as the predominant quinone, and summed feature 3 ($C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c*), $C_{16:1}$ *ω5c*, iso-$C_{15:0}$, and $C_{16:0}$ as the major fatty acids. The primary characteristics that can be used to differentiate the genus *Spirosoma* from other closely related genera were listed by the Editorial Board in Bergey's Manual of Systematic Bacteriology (2010) and McBride *et al.* (2014).

In the course of screening for novel bacteria, strain 15J9-6^T was isolated from a beach soil sample collected on Jeju Island, Korea. On the basis of a 16S rRNA gene sequence analysis, this isolate was considered to be a *Spirosoma*-like strain. The aim of the present work was to determine the exact taxonomic position of strain 15J9-6^T using a polyphasic taxonomic approach that included genotypic, chemotaxonomic, and phenotypic analyses.

Materials and Methods

Isolation of the bacterial strain and culture conditions

Strain 15J9-6^T was isolated from a beach soil sample collected in Jeju Island (30°57′19″ N, 126°30′44″ E), South Korea. The sample was serially diluted in distilled water. One hundred microliters of each dilution was spread onto R2A agar plates (Difco) and incubated at 25°C for 1 week (Srinivasan *et al.*, 2015). On the 10^7-diluted plate, 30–40 colonies appeared, of which one yellow colony, designated 15J9-6^T, was purified by transferring it onto fresh plate and incubating again under

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the same conditions. Strain 15J9-6^T was routinely cultured on R2A agar at 25°C and was maintained as a glycerol suspension (20%, w/v) at -70°C. The isolate was deposited in the Korean Collection for Type Cultures (KCTC) and the Japan Collection of Microorganisms (JCM). The reference type species *Spirosoma linguale* KACC 12156^T and *Spirosoma panaciterrae* KCTC 22263^T were obtained from the Korean Agricultural Culture Collection and the Korean Collection for Type Cultures, respectively.

Phenotypic and biochemical characteristics

Gram reaction of strain 15J9-6^T was examined using a Gram-staining standard method (Smibert and Krieg, 1994). Cell morphology was observed under an Olympus light microscope (×1,000 magnification) and a Hitachi HT7700 transmission electron microscope using cells grown on R2A agar for 3 days at 25°C. Cell motility was investigated on 0.5% (w/v) semi-solid R2A agar (Tittsler and Sandholzer, 1936) and gliding motility was assessed by the microscopic hanging drop technique (Agarwal *et al*., 1997). Catalase activity was determined by assessing the production of bubbles in 3% (v/v) H_2O_2, and oxidase activity was tested using 1% (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino and Sherman, 2010). Growth was assessed on R2A agar (Difco), Luria-Bertani agar (LB; Difco), nutrient agar (NA; Difco), and trypticase soy agar (TSA; Difco). The effect of pH on growth was evaluated in R2A broth using three different buffers (final concentration, 100 mM): sodium acetate buffer (for pH 4.0–6.0), potassium phosphate buffer (for pH 7.0–8.0), and Tris buffer (for pH 9.0–10.0). Growth at 4, 10, 15, 20, 25, 30, 37, and 42°C was assessed on R2A agar after 7 days of incubation. Salt tolerance was tested in R2A broth supplemented with 0.5, 1, 2, 3, 4, 5, and 10% (w/v) NaCl after 7 days of incubation. Enzyme activities, assimilation of carbon sources, acid production from substrates, and other physiological characteristics were determined by inoculating API ZYM, API 20 NE, API ID 32 GN, and API 50 CH strips according to the manufacturer's instructions (bioMérieux).

Sequencing of the 16S rRNA gene and phylogenetic analysis

Genomic DNA was extracted from strain 15J9-6^T using a commercial genomic DNA extraction kit (Kwak *et al*., 2016). The 16S rRNA gene was amplified using the universal bacterial primers 27F and 1492R as described previously (Weisburg *et al*., 1991), and the purified PCR products were sequenced by Genotech. Phylogenetic neighbors were identified, and pairwise 16S rRNA gene sequence similarities were calculated using both the EzBioCloud server (Yoon *et al*., 2016) and BLAST search program at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequences of related taxa were obtained from GenBank and aligned with that of strain 15J9-6^T using the Clustal X program (Thompson *et al*., 1997). Gaps and the 5′ and 3′ ends of the alignment were edited manually in BioEdit (Hall, 1999). Tree topologies were inferred by neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-likelihood (ML) (Felsenstein, 1981), and maximum-parsimony (MP) (Fitch, 1971) methods in the program MEGA7 (Kumar *et al*., 2016). The NJ tree was constructed using Kimura's two-parameter model with pairwise deletion (Kimura, 1980). The ML tree was inferred using the nearest neighbour interchange as the maximum-likelihood heuristic search method. The MP tree was inferred using subtree-pruning and regrafting. The option of complete deletion of gaps was applied for ML and MP tree construction. A bootstrap analysis with 1,000 replicate data sets was performed to assess support for clusters (Felsenstein, 1985).

Chemotaxonomic analyses

The fatty acids of strain 15J9-6^T, *S. linguale* KACC 12156^T and *S. panaciterrae* KCTC 22263^T were analyzed using cells grown on R2A agar for 3 days at 25°C. The cellular fatty acids were saponified, methylated, and extracted according to a Sherlock Microbial Identification System (MIDI) protocol. Fatty acid methyl esters were then analyzed by gas chromatography and Microbial Identification Software (Sherlock TSBA, version 6.0) (Sasser, 1990). Polar lipids were extracted using the procedure described by Minnikin *et al*. (1984) and identified by two-dimensional thin layer chromatography (TLC), followed by spraying with the appropriate detection reagents (Komagata and Suzuki, 1987). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum, and re-extracted in n-hexane/water (1:1, v/v). The extract was purified using Sep-Pak Silica Vac cartridges (Waters) and then analyzed by high performance liquid chromatography (HPLC) as described previously (Hiraishi *et al*., 1996).

DNA G+C content

Genomic DNA of strain 15J9-6^T was extracted according to the standard CTAB/NaCl protocol (Wilson, 1997). The genomic DNA G+C content of strain 15J9-6^T was determined by reverse-phase HPLC analysis of individual nucleosides resulting from DNA hydrolysis and dephosphorylation using nuclease P1 and alkaline phosphatase (Mesbah *et al*., 1989). Single-stranded DNA from salmon testes (D7656; Sigma; DNA G+C content, 41.2 mol%) was used as a standard.

Nucleotide sequence accession numbers

The 16S rRNA gene sequence of strain 15J9-6^T obtained in this study has been deposited in NCBI GenBank/EMBL/DDBJ under the accession number LC221819. The accession numbers of the reference strains closely related to strain 15J9-6^T are indicated in Fig. 1.

Results and Discussion

Phylogenetic analysis

The 16S rRNA gene sequence of strain 15J9-6^T was a continuous stretch of 1,424 bp. The sequence similarity search in the EzBioCloud database revealed that this strain had highest similarity with *Spirosoma panaciterrae* Gsoil 1519^T (92.29%) (Ten *et al*., 2009), followed by *S. pulveris* JSH 5-14^T (92.13%) (Joo *et al*., 2015), *S. aerolatum* PR1012K^T (91.99%) (Kim *et al*., 2015), and *S. linguale* DSM 74^T (91.93%) (Larkin and Borrall, 1984). Levels of sequence similarity to other genera (*Fibrisoma*, *Huanghella*, and *Rudanella*) were less than 88.4%.

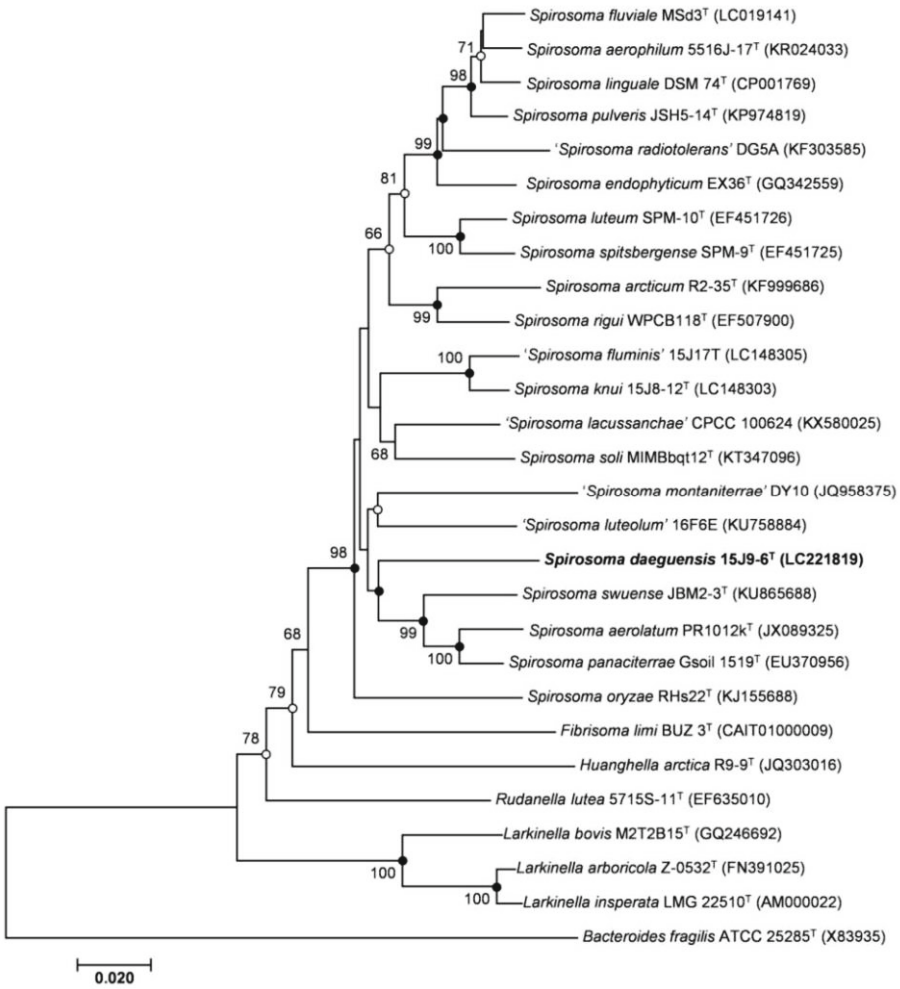

Fig. 1. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of *Spirosoma daeguensis* 15J9-6^T among related strains in the genus *Spirosoma* and representatives of other members of the phylum *Bacteroidetes*. Bootstrap values (based on 1,000 replications) greater than 60% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. The tree was rooted using *Bacteroides fragilis* ATCC 25285^T (X83935) as an outgroup. Bar, 0.02 substitutions per nucleotide position

The phylogenetic position of the new isolate, determined by various tree-making algorithms (neighbor joining, maximum likelihood, and maximum parsimony), revealed that strain 15J9-6^T clustered with members of the genus *Spirosoma*, forming a subgroup with *S. panaciterrae* Gsoil 1519^T, *S. aerolatum* PR1012K^T, and *S. swuense* JBM2-3^T (Joo *et al.*, 2017) (Fig. 1). The generally accepted criteria for delineating bacterial species state that strains showing 16S rRNA gene sequence dissimilarity above 3% are considered to belong to separate species (Stackebrandt and Goebel, 1994). Based on this definition, the above data indicate that strain 15J9-6^T could not be assigned to any recognized species within the genus *Spirosoma* and should be considered to represent a novel species of the genus *Spirosoma*.

Morphological and phenotypic characteristics

Cells of strain 15J9-6^T were Gram-stain-negative, aerobic, non-motile, non-spore-forming, and yellow pigment-producing rods (0.8–1.1 × 3.9–6.1 mm) without flagella (Supplementary data Fig. S1). Cells were mesophilic, growing at 10–30°C, but not at 4 or 37°C, with an optimum temperature of around 25°C. The isolate did not require NaCl for growth, but tolerated it up to 1%. Growth occurred on NA, R2A, and TSA agar, but not on LB agar. Other physiological and biochemical properties of strain 15J9-6^T are given in the species description. Phenotypic and chemotaxonomic characteristics that differentiated strain 15J9-6^T from its relatives in the genus *Spirosoma* are listed in Table 1. In particular, strain 15J9-6^T could be differentiated from *S. panaciterrae* KCTC 22263^T and *S. linguale* KACC 12156^T by its ability to grow at 10°C, to produce β-glucuronidase and lipase (C14), to utilize gluconate, D-mannitol, L-proline, and D-ribose, to produce acid from glucose, and to hydrolyze weakly carboxymethylcellulose, and by its inability to grow at 37°C.

Chemotaxonomic characteristics

The major fatty acids of strain 15J9-6^T were summed feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$; 30.1%), $C_{16:1}$ $\omega5c$ (23.1%), iso $C_{15:0}$ (13.3%), and $C_{16:0}$ (8.4%), representing 74.9% of the total fatty acids (Table 2). This cellular fatty acid profile is characteristic of members of the genus *Spirosoma* (Finster *et al.*, 2009; Joo *et al.*, 2015; Kim *et al.*, 2015), supporting an affiliation of strain 15J9-6^T with the genus *Spirosoma*. Under the same growth conditions, strain 15J9-6^T differed from *S. panaciterrae* KCTC 22263^T and *S. linguale* KACC 12156^T in terms of the proportions of major and some minor fatty acids. The major phospholipid identified in strain 15J9-6^T was phosphatidylethanolamine (PE), similar to that in other *Spiro-*

Table 1. Differential phenotypic characteristics of strain 15J9-6^T and phylogenetically related species of the genus *Spirosoma*
Strains: 1, 15J9-6^T; 2, *S. panaciterrae* KCTC 22263^T; 3, *S. linguale* KACC 12156^T.
All data were obtained in this study, unless otherwise noted. All strains grew on NA, R2A, and TSA agar, but not on LB agar. All strains were positive for *N*–acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, catalase, esterase (C4), esterase lipase (C8), *α*-galactosidase, *α*-glucosidase, *β*-glucosidase, leucine arylamidase, naphtol-AS-BI-phosphohydrolase, oxidase, and hydrolysis of Tween 80. All species were found to be negative for nitrite reduction, gelatin and chitin hydrolysis, arginine dihydrolase, *α*-fucosidase, and urease activities. All strains produced acid from *N*-acetyl-glucosamine, aesculin, D-cellobiose, D-galactose, D-maltose, D-mannose, salicin, and D-trehalose, but not from D-adonitol, dulcitol, erythritol, glycerol, inositol, D-mannitol, or L-sorbose. All strains utilized D-glucose, D-maltose, D-mannose, D-melibiose, and salicin, but not acetate, adipate, L-alanine, caprate, citrate, L-fucose, glycogen, L-histidine, 4-hydroxybenzoate, DL-3-hydroxybutyrate, *m*-inositol, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, malonate, phenyl acetate, propionate, L-serine, D-sorbitol, suberate, or *n*-valerate.
+, Positive reaction; –, negative reaction; w, weak positive reaction

Characteristic	1	2	3
Cell shape	Rods	Rods[a]	Rings, coils[b]
Growth at 10°C	+	–	–
Growth at 37°C	–	+	+
Indole production	–	–	+
Hydrolysis of:			
Starch	+	w[a]	+[c]
Tween 20	–	–	+[c]
Casein	–	–[a]	w
CM-cellulose	w	–[a]	–
Enzyme activity:			
α-Chymotrypsin, cystine arylamidase, *β*-galactosidase	+	–	w
β-Glucuronidase, lipase (C14)	w	–	–
α-Mannosidase	+	–	–
Trypsin, valine arylamidase	+	–	+
Assimilation of:			
N-Acetyl-glucosamine	+	+	–
L-Arabinose	w	+	–
D-Mannitol, L-proline, D-ribose	w	–	–
Gluconate	+	–	–
3-Hydroxybenzoate, L-rhamnose	+	–	w
Itaconate	–	–	+
D-Sucrose	+	+	–
Acid production from:			
Amygdalin, 5-ketogluconate, D-tagatose	w	–	+
D-Arabinose, L-arabinose, arbutin, D-fructose, D-fucose, gentiobiose, glycogen, D-lactose, D-melibiose, D-melezitose, methyl-*α*-D-glucopyranoside, methyl-*α*-D-mannopyranoside, D-raffinose, starch, D-sucrose, D-xylose	+	–	+
D-Arabitol, L-arabitol, xylitol	–	–	+
D-Glucose	+	–	–
Inulin, 2-ketogluconate, gluconate	–	–	w
D-Lyxose, methyl-*β*-D-xylopyranoside, L-rhamnose, L-xylose, D-turanose	+	–	w
L-Fucose, D-ribose	w	–	w
D-Sorbitol	–	+	–
DNA G+C content (mol%)	45.7	50.1[a]	l50.2[b]

[a] Data from Ten *et al.* (2009).
[b] Data from Lail *et al.* (2010).
[c] Data from Vancanneyt *et al.* (2006).

soma species (Baik *et al.*, 2007; Chang *et al.*, 2014; Kim *et al.*, 2016). In addition, the polar lipid profile of the isolate included major amounts of unidentified aminophospholipids (APL_1 and APL_2), an unidentified lipid (L_1), an unidentified phospholipid (PL) and minor amounts of three unidentified lipids (L_2–L_4) (Supplementary data Fig. S2). The predominant isoprenoid quinone in strain 15J9-6^T was menaquinone MK-7, which is also the major respiratory quinone found in other members of the genus *Spirosoma* (Larkin and Borrall, 1984; Baik *et al.*, 2007; Ten *et al.*, 2009; Ahn *et al.*, 2014; Joo *et al.*, 2017).

DNA G+C content

The DNA G+C content of strain 15J9-6^T was 45.7 mol%, which is lower than values reported for *Spirosoma* species (47.2–57.0 mol%) (Fries *et al.*, 2013; Ahn *et al.*, 2014). However, the value still lies within the range expected for members of the same genus and the G+C content range of the genus *Spirosoma* should be extended to take into account this result.

Taxonomic conclusion

On the basis of phylogenetic analysis and phenotypic data,

Table 2. Cellular fatty acid profiles of strain 15J9-6^T and phylogenetically related members of the genus *Spirosoma*

Strains: 1, 15J9-6^T; 2, *S. panaciterrae* KCTC 22263^T; 3, *S. linguale* KACC 12156^T.

All data are from the present study. All strains were grown on R2A agar at 25°C for 3 days. Values are percentages of total fatty acids, and only fatty acids accounting for more than 1% in at least one of the strains are indicated.

nd, not detected; tr, trace (<1.0%)

*Summed features contained two or three fatty acids that could not be separated by gas-liquid chromatography (GLC) with the Sherlock Microbial Identification (MIDI) System. Summed feature 3 comprised $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*. Summed feature 4 comprised iso $C_{17:1}$ I and/or anteiso $C_{17:1}$ B. Summed feature 9 comprised iso $C_{17:1}$ *ω9c* and/or $C_{16:0}$ 10-methyl.

Fatty acids	1	2	3
Saturated			
Iso $C_{13:0}$	nd	nd	2.6
$C_{14:0}$	1.6	2.0	tr
Iso $C_{15:0}$	13.3	5.9	12.1
Anteiso $C_{15:0}$	2.9	tr	3.9
$C_{16:0}$	8.4	14.3	5.7
Iso $C_{17:0}$	tr	nd	1.3
$C_{18:0}$	4.8	9.2	1.2
Hydroxy			
Iso $C_{15:0}$ 3-OH	1.8	2.7	3.7
$C_{16:0}$ 3-OH	1.5	3.7	1.1
Iso $C_{17:0}$ 3-OH	6.1	3.1	6.2
Unsaturated			
$C_{16:1}$ *ω5c*	23.1	24.6	23.2
Summed Feature 3*	30.1	30.6	32.1
Summed Feature 4*	tr	nd	1.1
Summed Feature 9*	tr	nd	1.7

strain 15J9-6^T is a member of the genus *Spirosoma*. However, the strain is distinguishable from recognized *Spirosoma* species by a combination of physiological and biochemical features. Therefore, on the basis of the data presented, strain 15J9-6^T should be classified as a representiative of a novel species of the genus *Spirosoma*, for which the name *Spirosoma daeguensis* sp. nov. is proposed.

Description of *Spirosoma daeguensis* sp. nov.

Spirosoma daeguensis (dae.gu.en'sis. N.L. fem. adj. *daeguensis* of Daegu, a city in South Korea, where the taxonomic study was performed).

Cells are Gram-stain-negative, non-motile, aerobic rods that are 0.8–1.1 mm wide and 3.9–6.1 mm long. After 3 days of incubation at 25°C on R2A agar, colonies are convex, smooth, circular, yellow, and approximately 2 mm in diameter. Grows at 10 and 30°C but not at 4 and 37°C, optimum growth occurs at 25°C. The pH range for growth is pH 7–8. Tolerate 1% NaCl but not 2%. Cells are positive for catalase and oxidase activities. Able to hydrolyze starch, Tween 80, and weakly carboxymethylcellulose, but not casein, chitin, Tween 20. In API 20 NE tests, positive for aesculin hydrolysis, acid production from glucose, and *β*-galactosidase, but negative for gelatin hydrolysis, nitrate reduction, indole production, urea hydrolysis, and arginine dihydrolase activity. Utilizes for growth *N*-acetyl-D-glucosamine, L-arabinose (weakly, w), D-glucose, gluconate, 3-hydroxybenzoate, D-maltose, D-mannitol (w), D-mannose, D-melibiose, L-proline (w), L-rhamnose, D-ribose (w), salicin, and D-sucrose but other substrates in API 32 GN and API 20 NE systems are not utilized. In the API ZYM kit, positive for N-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, cystine arylamidase, esterase (C4) (w), esterase lipase (C8) (w), *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, *β*-glucuronidase (w), leucine arylamidase, lipase (C14) (w), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase, but negative for *α*-fucosidase. Acid is produced from *N*-acetyl-glucosamine (weakly, w), aesculin, amygdalin (w), L-arabinose, D-arabinose, arbutin, D-cellobiose, D-fructose, D-fucose, L-fucose (w), D-galactose, gentiobiose, D-glucose, glycogen, 5-ketogluconate (w), D-lactose, D-lyxose, D-maltose, D-mannose, D-melibiose, D-melezitose, methyl-*α*-D-glucopyranoside, methyl-*α*-D-mannopyranoside, methyl-*β*-D-xylopyranoside, D-raffinose, L-rhamnose, D-ribose (w), salicin, starch, D-sucrose, D-tagatose (w), D-trehalose, D-turanose, D-xylose, and L-xylose, but not from other substrates tested in the API 50CH system. The major cellular fatty acids are summed feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*), $C_{16:1}$ *ω5c*, iso $C_{15:0}$, and $C_{16:0}$. The predominant isoprenoid quinone is MK-7. The major polar lipids are phosphatidylethanolamine, two unidentified aminophospholipids, an unidentified phospholipid and an unidentified polar lipid. The DNA G+C content of the type strain is 45.7 mol%. The type strain 15J9-6^T (=KCTC 52036^T =JCM 31995^T) was isolated from a beach soil sample collected on Jeju Island (29°51′61″ N, 126°27′08" E), South Korea.

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Conflict of Interest

The authors declare that there are no conflict of interest.

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