Current Microbiology (2018) 75:694–700
https://doi.org/10.1007/s00284-018-1434-z

Spirosoma agri sp. nov., Isolated from Apple Orchard Soil

Weilan Li[1] · Seung-Yeol Lee[1] · In-Kyu Kang[2] · Leonid N. Ten[1] · Hee-Young Jung[1,3]

Received: 3 October 2017 / Accepted: 5 January 2018 / Published online: 23 January 2018

Abstract
A Gram-negative, non-motile, rod-shaped, aerobic bacterial strain, designated S7-3-3^T, was isolated from apple orchard soil in Gyeongsangnam-do province, South Korea, and was characterized taxonomically using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain S7-3-3^T belonged to the family C*ytophagaceae* in the phylum *Bacteroidetes* was most closely related to *Spirosoma rigui* WPCB118^T (94.3%), *Spirosoma pulveris* JSH5-14^T (93.9%), and *Spirosoma linguale* DSM 74^T (93.7%). The strain showed typical chemotaxonomic characteristics of the genus *Spirosoma* with a predominant respiratory quinone of menaquinone MK-7 and the major fatty acids of summed feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*; 43.9%) and $C_{16:1}$ *ω5c* (25.6%). The G+C content of genomic DNA was 49.6 mol%. The polar lipid profile contained major amounts of phosphatidylethanolamine, an unidentified aminophospholipid, and an unidentified polar lipid. Phenotypic and chemotaxonomic data supported the affiliation of strain S7-3-3^T with the genus *Spirosoma*. The results of physiological and biochemical tests showed the genotypic and phenotypic differentiation of the isolate from recognized *Spirosoma* species. On the basis of its phenotypic properties, genotypic distinctiveness, and chemotaxonomic features, strain S7-3-3^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma agri* sp. nov. is proposed. The type strain is S7-3-3^T (= KCTC 52727^T = JCM 32199^T).

Introduction

The genus *Spirosoma* in the family *Cytophagaceae* of phylum *Bacteroidetes* was first proposed by Larkin and Borrall [22]. At the time of writing, the genus *Spirosoma* comprised seventeen species with validly published names, including four recently described species, *Spirosoma knui* [23], *Spirosoma lacussanchae* [25], *Spirosoma luteolum* [24, 28], and *Spirosoma swuense* [12]. The type strains of *Spirosoma* species have been isolated from various natural sources including air [14, 16], fresh water [2, 23], soil [6, 12, 33], glacier till [4], dust [13], and Zn/Cd-accumulating *Salix caprea* [8]. Members of the genus *Spirosoma* were Gram-stain-negative, yellow or orange in colony color, catalase-positive, non-motile or motile, morphologies of rods, filaments and coils, strictly aerobic or facultatively anaerobic bacteria [1] that characterized chemotaxonomically by MK-7 as the predominant respiratory quinone [10], and phosphatidylethanolamine as the major polar lipid. The major fatty acids were summed feature 3 (composed of $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c*) and $C_{16:1}$ *ω5c*, [2, 33], and DNA G+C content ranged from 47.2 to 57.0 mol% [14]. To determine its exact taxonomic position, strain S7-3-3^T was investigated in detail following 16S rRNA gene sequence analysis and polyphasic taxonomic approach that including phenotypic, chemotaxonomic, and genotypic analyses. All these data suggested that strain S7-3-3^T represents a novel species of *Spirosoma* species.

Materials and Methods

Isolation of Bacterial Strain and Culture Condition

Strain S7-3-3^T was isolated from the apple orchard soil in Gyeongsangnam-do province, South Korea (35°35′20″N, 127°51′29″E). One gram of soil was suspended in 10 ml

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-018-1434-z) contains supplementary material, which is available to authorized users.

✉ Hee-Young Jung
heeyoung@knu.ac.kr

[1] School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea

[2] Department of Horticultural Science, Kyungpook National University, Daegu 41566, Republic of Korea

[3] Institute of Plant Medicine, Kyungpook National University, Daegu 41566, Republic of Korea

saline [0.85% (w/v) NaCl] and serially diluted. One hundred microliters of each dilution was spread onto R2A agar plates (Difco, USA) and incubated at 25 °C for 1 week. On the 10^7-diluted plate, 30–40 colonies appeared, of which one yellow colony, designated S7-3-3^T, was purified by transferring it onto fresh plate and incubating again under the same conditions. Strain S7-3-3^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). *Spirosoma pulveris* KCTC 42550^T, *Spirosoma rigui* KACC 13387^T, and *Spirosoma linguale* KACC 121565^T were obtained from KCTC and the Korean Agricultural Culture Collection (KACC), respectively, and used as reference strains.

Phenotypic and Biochemical Characteristics

The cell morphology and motility of strain S7-3-3^T were observed under a light microscope (BX50, Olympus, Japan; 1000X) and a transmission electron microscope (HT7700, Hitachi, Japan), with cells grown for 3 days at 25 °C on R2A agar. Gram test was operated using Gram staining [31]. Catalase and oxidase tests were performed according to the procedures outlined by Cappuccino and Sherman [3]. Growth was assessed on R2A agar (Difco), Luria–Bertani agar (LB; Difco), nutrient agar (NA; Difco), and trypticase soy agar (TSA; Difco). The pH range for growth (pH 4.0–10.0) was assessed in R2A broth (MB Cell, Seoul, Korea) medium using three different buffers: 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 tested on R2A agar after 7 days of incubation. Salt tolerance was determined by amending R2A broth with NaCl to final concentrations of 0.5, 1, 2, 3, 4, 5, and 10% (w/v) NaCl, and the growth was checked after 7 days of incubation. Enzyme activities, assimilation of carbon sources, acid production from substrates, and other physiological characteristics were determined by using API ZYM, API 20 NE, API ID 32 GN, and API 50CH strips according to the manufacturer's instructions (bioMérieux).

16S rRNA Gene Sequencing and Phylogenetic Analysis

For the phylogenetic analysis, genomic DNA was extracted and purified using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) and a PowerClean DNA Clean-Up Kit (MO BIO Laboratories, Carlsbad, CA, USA) as described previously [15, 20]. The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primers 27F and 1492R [36], and the purified PCR products were sequenced by Genotech (Daejeon, South Korea). The obtained partial 16S rRNA sequence was assembled using SeMan software (DNASTAR, Madison, WI, USA). Phylogenetic neighbors were identified, and pairwise 16S rRNA gene sequence similarities were calculated using EzBioCloud server [38] and NCBI BLAST search program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequences of 30 related taxa were obtained from the GenBank. The recovered sequences were aligned with the sequence of strain S7-3-3^T using the program Clustal X [34]. Gaps and 5′ and 3′ ends of the alignment were edited manually using the BioEdit program [9]. Evolutionary distance matrices for the neighbor-joining algorithm were calculated using Kimura's two-parameter model [17]. Tree topologies were inferred by the neighbor-joining (NJ) [29], maximum-likelihood (ML) [5], and maximum-parsimony (MP) [7] methods using the program MEGA7 [19]. A bootstrap analysis with 1000 replicate datasets was performed to assess the support of clusters.

Chemotaxonomic Analyses

The fatty acid profiles of strain S7-3-3^T and three reference strains see above were analyzed using cells grown on R2A agar for 3 days at 25 °C. Two or three loops of fresh cells were harvested, and then the fatty acid were saponified, extracted, and methylated according to a Sherlock Microbial Identification System (MIDI) protocol [30]. Fatty acid methyl esters were analyzed by gas chromatography using the Microbial Identification software package (TSBA, version 6.0) [30]. Polar lipids were extracted using the procedure described by Minnikin et al. [27] and examined by two-dimensional thin layer chromatography (TLC), followed by spraying with appropriate detection reagents [18]. 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 [11].

DNA G+C Content

To determine the DNA G+C content, the genomic DNA of strain S7-3-3^T was extracted according to the standard cetyltrimethylammonium bromide/NaCl protocol [37]. Individual nucleosides were obtained by digesting the genomic DNA using nuclease P1 and alkaline phosphatase. Single-stranded DNA from salmon testes (D7656; DNA G+C content, 41.2 mol%, Sigma-Aldrich, St. Louis, MO, USA) was used as a standard. The nucleosides were analyzed by reverse-phase high-performance liquid chromatography as described previously [26].

DPD TaxonNumber and Nucleotide Sequence Accession Numbers

The Digital Protologue database TaxonNumber for strain S7-3-3^T is TA00286. The 16S rRNA gene sequence of strain S7-3-3^T in this study was deposited in NCBI GenBank/EMBL/DDBJ under the accession number LC269320. The accession numbers of the reference strains that closely related to strain S7-3-3^T are indicated in Fig. 1.

Results and Discussion

Phylogenetic Analysis

A nearly complete 16S rRNA gene sequence of strain S7-3-3^T (1420 bps) was obtained. The sequence similarity search in the EzBioCloud database revealed that the isolate had the highest similarity with *Spirosoma rigui* WPCB118^T (94.3%), followed by *Spirosoma pulveris* JSH5-14^T (93.9%) and *Spirosoma linguale* DSM 74^T (93.7%). Sequence similarities to other genera, including *Fibrisoma, Huanghella, Nibrella, Larkinella, Rudanella*, and *Fibrella*, were less than 90.0%. The phylogenetic position of the new isolate, determined using various tree-making algorithms (ML, MP, and NJ), revealed that strain S7-3-3^T appeared within the genus *Spirosoma* (Fig. 1). As mentioned above, the level of 16S rRNA gene sequence similarity between strain S7-3-3^T and three closest *Spirosoma* species is lower than the threshold generally employed for the delineation of novel species (i.e., 97% similarity or below) [32, 35]. Thus, strain S7-3-3^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*.

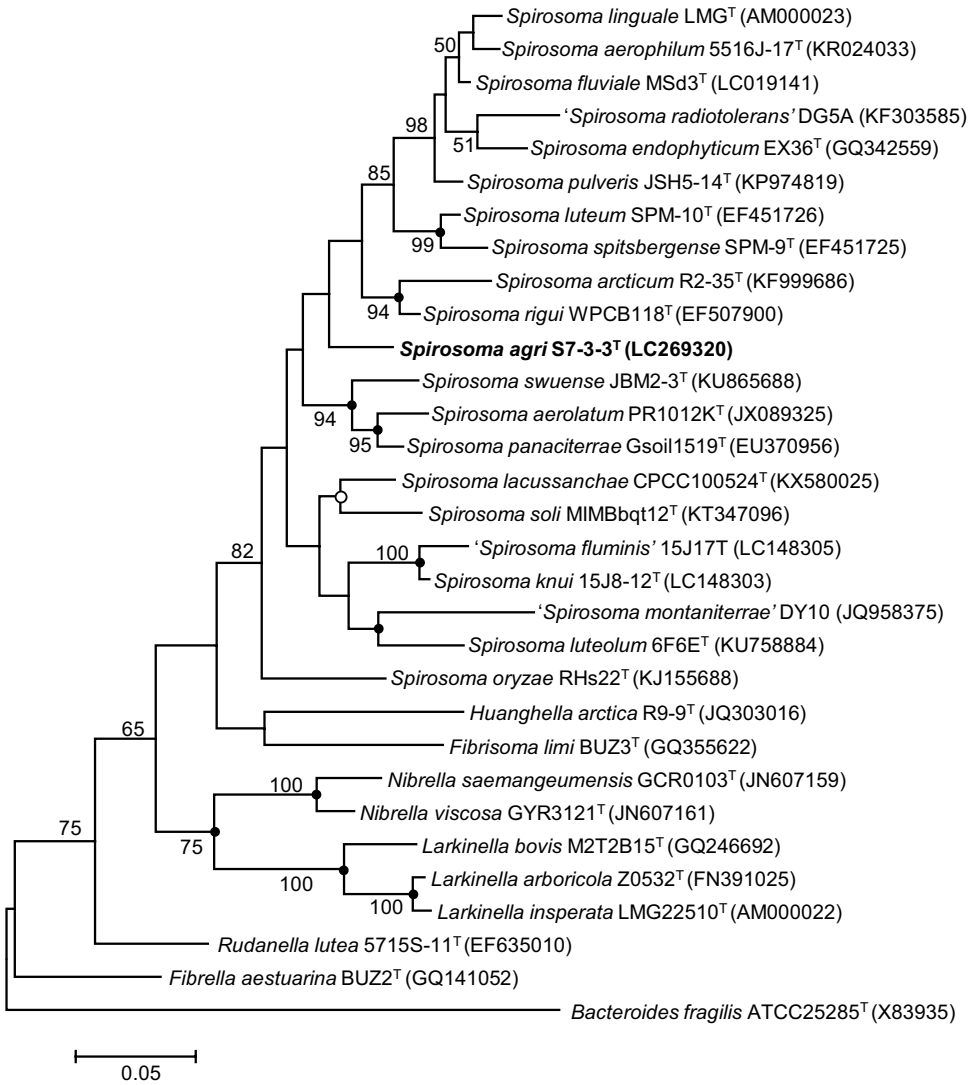

Fig. 1 Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain S7-3-3^T among related strains in the genus *Spirosoma* and representatives of other members of the family *Cytophagaceae*. Bootstrap values (based on 1000 replications) greater than 50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the neighbor-joining and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the neighbor-joining algorithm. The tree was rooted using *Bacteroides fragilis* ATCC 25285^T (X83935) as an outgroup. Bar, 0.05 substitutions per nucleotide position

Morphological and Phenotypic Characteristics

The morphological, physiological, and biochemical properties of strain S7-3-3^T are given in the species description and its negative characteristics in API 20 NE, API 32 GN, API ZYM, and API 50CH tests are listed in Supplementary Table S1. Comparison of differential characteristics with closely related *Spirosoma* species is shown in Table 1. In particular, strain S7-3-3^T could be differentiated from three reference strains based on its abilities hydrolyze gelatin, to produce *β*-glucuronidase, and to utilize gluconate, D-mannitol, L-proline, L-serine, and D-ribose, and by its inability to produce acid production from amygdalin and starch. There are several other phenotypic features, which could be used to distinguish the novel isolate from its closest phylogenetic neighbor *Spirosoma rigui* and the type strains of other closely related members of the genus *Spirosoma*.

Chemotaxonomic Characteristics

The cellular fatty acid profile of strain S7-3-3^T is characteristic of members of the genus *Spirosoma* [2, 13], supporting an affiliation of the isolate with the genus *Spirosoma* (Table 2). However, some qualitative and quantitative differences in fatty acid content could be observed between strain S7-3-3^T and its closest neighbors. In particular, strain S7-3-3^T could be differentiated from its above-mentioned phylogenetically closest relatives by the absence of summed feature 9 ($C_{17:1}$ iso ω9c/$C_{16:0}$ 10-methyl) and by the presence of $C_{17:1}$ iso ω5c. The major polar lipids of strain S7-3-3^T were phosphatidylethanolamine (PE), which was detected in other *Spirosoma* species as the main component [4], an unidentified aminophospholipid (APL_1), and an unidentified polar lipid (L_2). In addition, the polar lipid profile of the isolate included moderate amount of an unidentified polar lipid (L_3) and minor amounts of an unidentified aminophospholipid (APL_2), an unidentified aminolipid (AL), an unidentified phospholipid (PL), and an unidentified polar lipid (L_1) (Supplementary Fig. S1). The predominant isoprenoid quinone of stain S7-3-3^T was MK-7, which is the major respiratory quinone found in other members of the genus *Spirosoma* [13, 23].

DNA G+C Content

The genomic DNA G+C content of strain S7-3-3^T was 49.6 mol%, which lies within the range of those reported for other *Spirosoma* species (47.2–57.0 mol%) [1, 8].

Taxonomic Conclusion

All of the characteristics determined for strain S7-3-3^T are in accordance with those of the genus *Spirosoma*. However, there are several phenotypic differences between strain S7-3-3^T and its phylogenetically closest relatives (Table 1). The phylogenetic distinctiveness of strain S7-3-3^T confirmed that this isolate is distinct from recognized *Spirosoma* species. Therefore, on the basis of the data presented, strain S7-3-3^T should be classified as a novel species of the genus *Spirosoma*, for which the name *Spirosoma agri* sp. nov. is proposed.

Description of *Spirosoma agri* sp. nov.

Spirosoma agri (a'gri. L. gen. n. *agri* of a field). Cells are Gram-negative, non-motile, rod-shaped, aerobic, 0.8–1.2 μm wide, and 1.2–5.3 μm long. After 2 days of incubation at 25 °C on R2A agar, colonies are convex, smooth, circular, yellow, and slimy. Cells grow on R2A, NA, and TSA (weak growth) agar, but not on LB agar. Growth occurs at 10–30 °C and pH 6–8, with an optimal temperature of 25 °C and pH 7. Cells tolerate NaCl at a concentration of 1% but not 2%. Catalase and oxidase activities are positive. In API 20 NE tests, positive for *β*-glucosidase, *β*-galactosidase, gelatin hydrolysis, but negative for arginine dihydrolase, glucose fermentation, indole production, nitrate reduction, or urease activity. Utilizes for growth *N*-acetyl-D-glucosamine, L-arabinose (weakly, w), D-glucose, gluconate, D-maltose, D-mannose, D-melibiose, L-proline, D-ribose, salicin, L-serine (w), and D-sucrose, but other substrates in API 32 GN and API 20 NE systems are not utilized. In the API ZYM tests, positive for *N*-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, cystine arylamidase, esterase (C4), esterase (C8), *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase (w), *α*-glucosidase, *β*-glucosidase, leucine arylamidase, *α*-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. Acid is produced from *N*-acetyl-glucosamine (w), D-arabinose (w), L-arabinose (w), arbutin, D-cellobiose, esculin, D-fructose, L-fucose (w), D-galactose, gentiobiose, D-glucose, inulin (w), 5-ketogluconate, D-lactose, D-lyxose, D-maltose, D-mannose, D-melezitose, D-melibiose, methyl-*α*-D-glucopyranoside (w), methyl-*α*-D-mannopyranoside (w), methyl-*β*-D-xylopyranoside (w), D-raffinose, D-ribose (w), D-sucrose, salicin, D-tagatose (w), D-trehalose, D-turanose (w), D-xylose, and L-xylose (w), but not from other substrates tested in the API 50CH system. The major fatty acids are summed feature 3 ($C_{16:1}$ ω7*c*/$C_{16:1}$ ω6*c*) and $C_{16:1}$ ω5*c*. The predominant menaquinone is MK-7. Phosphatidylethanolamine, an unidentified aminophospholipid, and an unidentified lipid are the major polar lipids. The DNA G+C content is 49.6 mol%. The type strain S7-3-3^T (= KCTC 52727^T = JCM 32199^T) was isolated from apple orchard soil in Gyeongsangnam-do province (35°35′20″N, 127°51′29″E), South Korea.

Table 1 Differential phenotypic characteristics between strain S7-3-3^T and its phylogenetically closest relatives in the genus *Spirosoma*

Characteristic	1	2	3	4
Cell shape	Rods	Rods[a] Filaments[a]	Rods[b]	Rings[c] Coils[c]
Growth on/at/				
LB agar	−	+	w	−
10 °C	w	−	+	−
37 °C	−	−	+	w
Gelatin hydrolysis	+	−	−	−
Oxidase	+	−	−	+
Indole production	−	−	−	+
Enzyme activity				
N-Acetyl-*β*-glucosaminidase, acid phosphatase, *β*-galactosidase	+	+	+	w
Alkaline phosphatase	+	−	+	+
α-Chymotrypsin, cystine arylamidase, esterase (C4), *β*-glucosidase, naphthol-AS-BI-phosphohydrolase	+	−	w	w
Esterase (C8), *α*-galactosidase, trypsin	+	−	w	+
β-Glucuronidase	w	−	−	−
α-Mannosidase	+	−	w	−
Assimilation of				
N-Acetyl-D-glucosamine	+	+	w	−
L-Arabinose	w	w	w	−
Gluconate, D-ribose	+	−	−	−
3-Hydroxybenzoate	−	−	−	w
Itaconate	−	−	−	+
D-Mannitol, L-proline, L-serine	w	−	−	−
L-Rhamnose	−	w	−	w
Salicin	+	+	w	w
D-Sucrose	+	+	+	−
Acid production from				
N-Acetylglucosamine, turanose	w	+	w	w
Amygdalin	−	w	w	+
D-Arabinose, methyl-*α*-D-glucopyranoside, methyl-*α*-D-mannopyranoside	w	+	+	+
L-Arabinose	w	+	w	+
D-Arabitol, D-fucose, glycogen	−	w	−	+
L-Arabitol, xylitol	−	−	−	+
Arbutin, D-fructose, D-galactose, gentiobiose, D-mannose, melezitose, D-melibiose, D-raffinose, D-trehalose	+	+	w	+
Erythritol, L-sorbose	−	w	−	−
L-Fucose	w	w	−	w
Gluconate, 2-ketogluconate	−	−	−	w
Inulin, methyl-*β*-D-xylopyranoside, D-ribose, L-xylose	w	+	−	w
5-Ketogluconate	+	+	−	+
D-Lyxose	+	+	w	w
L-Rhamnose	−	w	−	w
Starch	−	+	w	+

Table 1 (continued)

Characteristic	1	2	3	4
D-Tagatose	w	+	−	+
DNA G+C content (mol%)	49.6	53.3[a]	49.2[b]	50.2[c]

Strains 1, S7-3-3^T, 2, *Spirosoma rigui* KACC13387^T, 3, *Spirosoma pulveris* KCTC 42550^T, 4, *Spirosoma linguale* KACC 121565^T

All data were obtained in this study, unless otherwise noted

+ positive, − negative, *w* weakly positive reaction

All the strains were Gram-negative and grew on NA, TSA, and R2A agar. All strains were positive for catalase, *α*-glucosidase, *β*-glucosidase, leucine arylamidase, and valine arylamidase, but negative for arginine dihydrolase, *α*-fucosidase, lipase (C14), or urease. All strains were positive for acid production from D-cellobiose, esculin, D-lactose, D-maltose, salicin, and D-sucrose, but not from D-adonitol, dulcitol, glycerol, inositol, D-mannitol, or sorbitol. All strains utilized D-glucose, D-maltose, D-mannose, and D-melibiose, but not acetate, adipate, L-alanine, caprate, citrate, L-fucose, glycogen, L-histidine, 4-hydroxybenzoate, DL-3-hydroxybutyrate, 2-ketogluconate, 5-ketogluconate, DL-lactate, L-malate, malonate, *myo*-inositol, phenyl acetate, propionate, D-sorbitol, suberate, or *n*-valerate

[a]Data from Ref. [2]

[b]Data from Ref. [13]

[c]Data from Ref. [21]

Table 2 Cellular fatty acid profiles of strain S7-3-3^T and phylogenetically related members of the genus *Spirosoma*

Fatty acids	1	2	3	4
Saturated				
$C_{13:0}$ iso	nd	nd	2.3	2.6
$C_{15:0}$ iso	4.7	3.4	9.5	12.1
$C_{15:0}$ anteiso	5.0	3.9	3.6	3.9
$C_{15:0}$ iso 3-OH	2.7	nd	3.8	3.7
$C_{16:0}$	4.9	5.8	4.0	5.7
$C_{16:0}$ 3-OH	2.6	nd	2.0	1.1
$C_{17:0}$ iso	nd	nd	tr	1.3
$C_{17:0}$ iso 3-OH	3.7	nd	6.1	6.2
$C_{18:0}$	nd	1.9	nd	1.2
Unsaturated				
$C_{13:1}$ at 12–13	nd	1.7	tr	nd
$C_{15:1}\omega6c$	1.2	tr	nd	tr
$C_{16:1}\omega5c$	25.6	31.8	26.5	23.2
$C_{17:1}$ iso $\omega5c$	2.3	nd	nd	nd
Summed feature 3[a] ($C_{16:1}\omega7c/C_{16:1}\omega6c$)	43.9	48.5	35.4	32.1
Summed Feature 4[a] ($C_{17:1}$ iso I/$C_{17:1}$ anteiso B)	nd	nd	1.1	1.1
Summed feature 9[a] ($C_{17:1}$ iso $\omega9c/C_{16:0}$ 10-methyl)	nd	tr	1.0	1.7

Strains 1, S7-3-3^T, 2, *Spirosoma rigui* KACC 13387^T, 3, *Spirosoma pulveris* KCTC 42550^T, 4, *Spirosoma linguale* KACC 121565^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%)

[a]Summed feature contained two or three fatty acids that could not be separated by gas–liquid chromatography (GLC) with the Sherlock Microbial Identification (MIDI) System

Acknowledgements This work was supported by the Brain Pool Program of 2016 (Grant 162S-4-3-1727) through the Korean Federation of Science and Technology Societies (KOFST) funded by the Ministry of Science, ICT and Future Planning, Republic of Korea.

Compliance with Ethical Standards

Conflict of interest No conflict of interest is declared.

References

1. Ahn JH, Weon HY, Kim SJ, Hong SB, Seok SJ, Kwon SW (2014) *Spirosoma oryzae* sp. nov., isolated from rice soil and emended description of the genus *Spirosoma*. Int J Syst Evol Microbiol 64:3230–3234
2. Baik KS, Kim MS, Park SC, Lee DW, Lee SD, Ka JO, Choi SK, Seong CN (2007) *Spirosoma rigui* sp. nov., isolated from fresh water. Int J Syst Evol Microbiol 57:2870–2873
3. Cappuccino JG, Sherman N (2010) Microbiology: a Laboratory Manual, 9th edn. Benjamin Cummings, San Francisco
4. Chang X, Jiang F, Wang T, Kan W, Qu Z, Ren L, Fang C, Peng F (2014) *Spirosoma arcticum* sp. nov., isolated from high arctic glacial till. Int J Syst Evol Microbiol 64:3230–3234
5. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
6. Finster KW, Herbert RA, Lomstein BA (2009) *Spirosoma spitsbergense* sp. nov. and *Spirosoma luteum* sp. nov., isolated from a high Arctic permafrost soil, and emended description of the genus *Spirosoma*. Int J Syst Evol Microbiol 59:839–844
7. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416
8. Fries J, Pfeiffer S, Kuffner M, Sessitsch A (2013) *Spirosoma endophyticum* sp. nov., isolated from Zn- and Cd-accumulating *Salix caprea*. Int J Syst Evol Microbiol 63:4586–4590
9. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
10. Hatayama K, Kuno T (2015) *Spirosoma fluviale* sp. nov., isolated from river water. Int J Syst Evol Microbiol 65:3447–3450
11. Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high performance liquid chromatography and photodiode array detection. J Gen Appl Microbiol 42:457–469
12. Joo ES, Kim EB, Jeon SH, Srinivasan S, Kim MK (2017) *Spirosoma swuense* sp. nov., a bacterium isolated from wet soil. Int J Syst Evol Microbiol 67:532–536
13. Joo ES, Lee JJ, Cha S, Jheong W, Seo T, Lim S, Jeong SW, Srinivasan S (2015) *Spirosoma pulveris* sp.nov., a bacterium isolated from a dust sample collected at Chungnam province, South Korea. J Microbiol 53:750–755
14. Kim DU, Lee H, Kim SG, Ahn JH, Park SY, Ka JO (2015) *Spirosoma aerolatum* sp. nov., isolated from a motor car air conditioning system. Int J Syst Evol Microbiol 65:4003–4007
15. Kim M, Srinivasan S, Back CG, Joo E, Lee SY, Jung HY (2015) Complete genome sequence of *Deinococcus swuensis*, a bacterium resistant to radiation toxicity. Mol Cell Toxicol 11:315–321
16. Kim SJ, Ahn JH, Weon HY, Hong SB, Seok SJ, Kim JS, Kwon SW (2016) *Spirosoma aerophilum* sp. nov., isolated from an air sample. Int J Syst Evol Microbiol 66:2342–2346
17. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
18. Komagata K, Suzuki KI (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–205
19. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
20. Kwak Y, Park GS, Shin JH (2016) High quality draft genome sequence of the type strain *Pseudomonas lutea* OK2^T, a phosphate-solubilizing rhizospheric bacterium. Stand Genom Sci 11:51
21. Lail K, Sikorski J, Saunders E, Lapidus A, Glavina del Rio T (2010) Complete genome sequence of *Spirosoma linguale* type strain (1^T). Stand Genom Sci 2:176–185
22. Larkin JM, Borrall R (1984) Family 1. *Spirosomaceae* Larkin and Borrall 1978 595AL. Bergey's Man Syst Bacteriol 1:125–132
23. Lee JJ, Lee YH, Park SJ, Lee SY, Kim BO, Ten LN, Kim MK, Jung HY (2017) *Spirosoma knui* sp. nov., a radiation-resistant bacterium isolated from the Han River. Int J Syst Evol Microbiol 67:1359–1365
24. Lee JJ, Park SJ, Lee YH, Lee SY, Park S, Cho YJ, Kim MK, Ten LN, Jung HY (2017) *Spirosoma luteolum* sp. nov. isolated from water. J Microbiol 55:247–252
25. Li Y, Ai MJ, Sun Y, Zhang YQ, Zhang JQ (2017) *Spirosoma lacussanchae* sp. nov., a phosphate-solubilizing bacterium isolated from a fresh water reservoir. Int J Syst Evol Microbiol 67:3144–3149
26. Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167
27. Minnikin DE, O'Donnella AG, Goodfellowb M, Aldersonb G, Athalyeb M, Schaala A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
28. Oren A, Garrity GM (2017) List of novel names and novel combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 67:2075–2078
29. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
30. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note. MIDI Inc, Newark, p 101
31. Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RG E, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654
32. Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
33. Ten LN, Xu JL, Jin FX, Im WT, Oh HM, Lee ST (2009) *Spirosoma panaciterrae* sp. nov., isolated from soil. Int J Syst Evol Microbiol 59:331–335
34. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
35. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464
36. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703
37. Wilson K (1997) Preparation of genomic DNA from bacteria. In: Ausubel FM et al (eds) Current protocols in molecular biology, no. Supplement 27. Wiley, New York, pp 2.4.1–2.4.5
38. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617