

A report of 7 unrecorded bacterial species isolated from several Jeju soil samples in 2016

Ju-Young Kim¹, Jun Hwee Jang¹, Soohyun Maeng², Myung-Suk Kang³ and Myung Kyum Kim^{1,*}

¹Department of Bio & Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea

²Department of Public Health Sciences, Graduate School, Korea University, Seoul 02841, Republic of Korea

³Biological Resources Utilization Department, National Institute of Biological Resources, Incheon 22689, Republic of Korea

*Correspondent: biotech@swu.ac.kr

Seven bacterial strains, 15J4M-1, 15J13-8, 16MFM10, 15J1-8, SR1-5-4, 15J13-6, and 15J8-11 assigned to the phylum *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* were isolated from soil samples collected from Jeju, Korea. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strains 15J4M-1, 15J13-8, 16MFM10, 15J1-8, SR1-5-4, 15J13-6, and 15J8-11 were most closely related to *Bacillus selenatarsenatis* SF-1^T (with 99.4% similarity), *Brevibacterium luteolum* CF87^T (99.5%), *Carnobacterium iners* CCUG 62000^T (99.6%), *Exiguobacterium profundum* 10C^T (99.3%), *Larkinella insperata* LMG 22510^T (99.3%), *Pseudokineococcus lusitanus* CECT 7306^T (99.4%), and *Spirosoma endophyticum* EX36^T (99.3%), respectively. This is the first report of these seven species in Korea.

Keywords: 16S rRNA, *Actinobacteria*, bacterial diversity, *Bacteroidetes*, *Firmicutes*, unreported species

© 2018 National Institute of Biological Resources
DOI:10.12651/JSR.2018.7.2.151

INTRODUCTION

In 2016, we collected diverse soil samples and isolated unrecorded bacterial species in Jeju, Korea. The present report focuses on the isolation and description of unrecorded radiation-resistant species belonging to the phylum *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*.

Actinobacteria represents one of the largest phyla among bacteria (Stackebrandt, 2000) and also exhibits a diverse physiological and metabolic properties, such as the production of extracellular enzymes and secondary metabolites (Schrempf, 2001). *Actinobacteria* characterized as a gram-positive bacterial group include microorganisms exhibiting a wide spectrum of morphologies (Ventura, 2007). *Actinobacteria* are widely isolated in both terrestrial and aquatic ecosystems. The phylum *Actinobacteria* mainly employ varied metabolic mechanisms. In addition to utilizing some various biochemical pathways, they also synthesize many macromolecules, such as unique cell wall peptidoglycans (Gokhale *et al.*, 2007).

Bacteroidetes are known to be distributed from many distinct habitats, including temperate, tropical, and polar ecosystems (Krieg *et al.*, 2010; Thomas *et al.*, 2011).

Bacteroides are Gram-negative, anaerobic, non-sporing and rod-shaped bacteria. They were mostly found in the gastrointestinal tract of animals and humans, and even dominated the gut microflora of mammals (Smith *et al.*, 2006; Turnbaugh *et al.*, 2009; Thomas *et al.*, 2011).

Firmicutes are a phenotypically diverse prokaryotic taxon. The division *Firmicutes* encompassed Gram-positive bacteria, when the phylum was first described by Gibbons and Murray (Gibbons and Murray, 1978). But only Gram-positive bacteria with a low DNA mol% G+C is included in this phylum in the current classification system (Roller *et al.*, 1992).

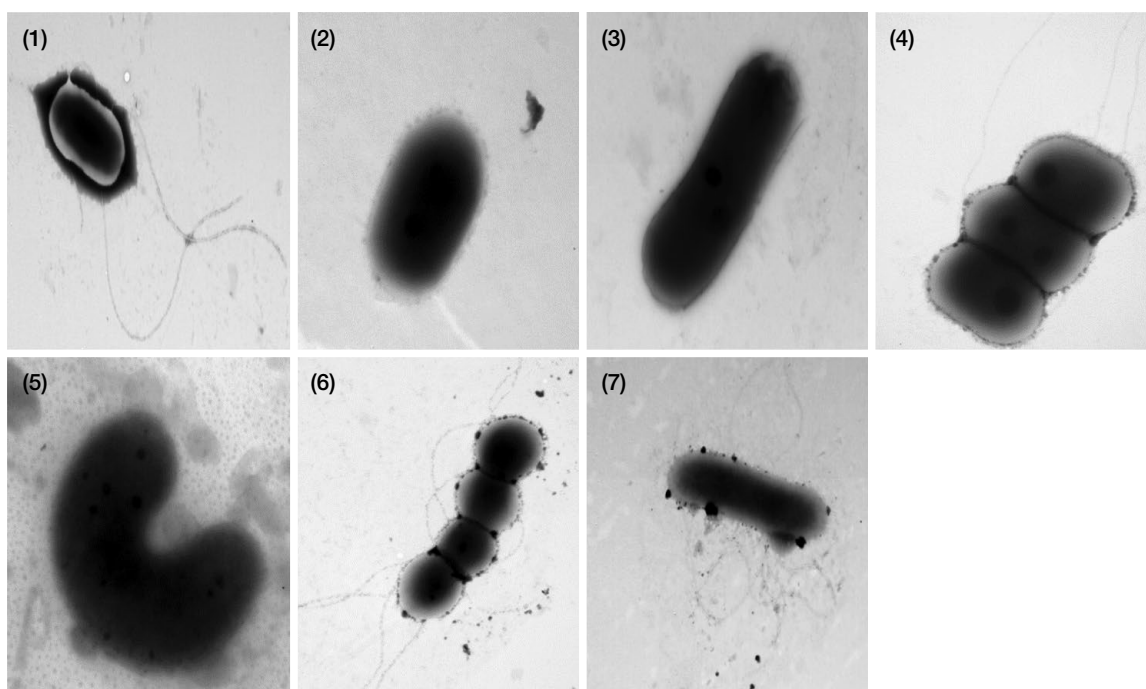
In this study, the present report focuses on the description of bacterial species belonging to two of *Actinobacteria*, two of *Bacteroidetes*, and three of *Firmicutes* that have not officially reported in Korea. Here we report 7 unrecorded species.

MATERIALS AND METHODS

Various soil samples were suspended in distilled water and serially diluted. The aliquot was inoculated onto R2A agar and incubated at 25°C for 3 days (Table

Table 1. List of 16S rRNA gene sequence similarity, isolation source, medium, and incubation conditions of unrecorded strains.

Strain ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
15J4M-1	<i>Bacillus selenatarsenatis</i>	99.4	Soil of Jeju	R2A	25°C, 3d
15J1-8	<i>Exiguobacterium profundum</i>	99.3	Soil of Jeju	R2A	25°C, 3d
15J13-8	<i>Brevibacterium luteolum</i>	99.5	Soil of Jeju	R2A	25°C, 3d
16MFM10	<i>Carnobacterium iners</i>	99.6	Soil of Jeju	R2A	25°C, 3d
15J13-6	<i>Pseudokineococcus lusitanus</i>	99.4	Soil of Jeju	R2A	25°C, 3d
SR1-5-4	<i>Larkinella insperata</i>	99.3	Soil of Jeju	R2A	25°C, 3d
15J8-11	<i>Spirosoma endophyticum</i>	99.3	Soil of Jeju	R2A	25°C, 3d

**Fig. 1.** Transmission electron micrographs of the strains isolated in this study. Strains: 1, 15J4M-1; 2, 15J13-8; 3, 16MFM10; 4, 15J1-8; 5, SR1-5-4; 6, 15J13-6; 7, 15J8-11.

1). The designated strain IDs, sources, culture media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and stored as 20% glycerol suspension at -80°C as well as lyophilized ampoules.

Colony morphology and cell size of the strains were observed on R2A agar after cells were grown for 3 days at 25°C by using transmission electron microscopy (LIBRA 120, Carl Zeiss). Transmission electron micrograph of the strains are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochemical characteristics were tested by using Biolog Microstation with GEN III microplate system. A single colony was selected and emulsified into 'inoculating fluid A' (Biolog) for subsequent inoculation on to the MicroPlate test plate (Biolog). More fastidious organisms, including capnophilic

strains, were cultured in alternative media, according to the manufacturer's instructions, and the inocula prepared to a specified transmittance using a turbidimeter, as specified in the user guide. For each isolate, $100\ \mu\text{L}$ of the cell suspension was inoculated into each well of the MicroPlate, using a multichannel pipette and incubated at 37°C for 24 h, according to growth characteristics. MicroPlates were read in the MicroStation semi-automated reader after 24 h and the results interpreted by the identification system's software (GEN III database, version 5.2.1). The system indicated the isolates which could not be identified after 20 h and required further incubation. Such isolates were re-incubated and re-read between 3 and 6 h later. (Wragg *et al.*, 2014) Genomic DNA was extracted and 16S rRNA gene was amplified by PCR with 9F and 1492R universal bacterial primers (Weisburg *et al.*, 1991). The 16S rRNA gene sequenc-

es of the related taxa were obtained from EzTaxon-e (<http://eztaxon-e.ezbiocloud.net>) (Kim *et al.*, 2012) and edited using the BioEdit program (Hall, 1999). Multiple alignments were performed with the MUSCLE program (Edgar, 2004). Using the two-parameter model (Kimura, 1983) calculated the evolutionary distances. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) in MEGA5 program (Tamura, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

Based on the comparative 16S rRNA gene sequence analyses and phylogeny, 7 strains, designated 15J4M-1, 15J13-8, 16MFM10, 15J1-8, SR1-5-4, 15J13-6, and 15J8-11 were assigned to species level. Morphology and physiological characteristics are shown in the species description section.

Strains 15J4M-1, 15J13-8, 16MFM10, 15J1-8, SR1-5-4, 15J13-6, and 15J8-11 were most closely related to *Bacillus selenatarsenatis* SF-1^T (AB262082; 99.4% 16S rRNA gene sequence similarity), *Brevibacterium luteolum* CF87^T (AJ488509; 99.5% 16S rRNA gene sequence similarity), *Carnobacterium iners* CCUG 62000^T (S86170; 99.6% 16S rRNA gene sequence similarity), *Exiguobacterium profundum* 10C^T (AY818050; 99.3% 16S rRNA gene sequence similarity), *Larkinella insperata* LMG 22510^T (AM000022; 99.3% 16S rRNA gene sequence similarity), *Pseudokineococcus lusitanus* CECT 7306^T (FN824365; 99.4% 16S rRNA gene sequence similarity) and *Spirosoma endophyticum* EX36^T (GQ342559; 99.3% 16S rRNA gene sequence similarity), respectively (Table 1).

As expected from high 16S rRNA gene sequence similarities of the 7 strains with their closest relatives, each strain formed a robust phylogenetic clade with the most closely related species (Figs. 2-4). From the high 16S rRNA gene sequence similarity and robust formation of phylogenetic clade, it is concluded that strains 15J4M-1 is a member of the species *Bacillus selenatarsenatis* (Yamamura *et al.*, 2007) and 15J13-8 is a member of the species *Brevibacterium luteolum* (Wauters *et al.*, 2003), respectively (Fig. 2). Strain 16MFM10 is a member of the species *Carnobacterium iners* (Snauwaert *et al.*, 2013), 15J1-8 is a member of the species *Exiguobacterium profundum* (Crapart *et al.*, 2007), and SR1-5-4 is a member of the species *Larkinella insperata* (Vancanneyt *et al.*, 2006), respectively (Fig. 3). Strain 15J13-6 is a member of the species *Pseudokineococcus lusitanus* (Jurado *et al.*, 2011) and 15J8-11 is a member of the species *Spirosoma endophyticum* (Fries *et al.*, 2013), respectively (Fig. 4).

There is no report on these 7 species in the phylum *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* have been isolated in Korea. The strains 15J4M-1, 15J13-8, 16MFM10, 15J1-8, SR1-5-4, 15J13-6, and 15J8-11 are unreported strains of *Bacillus selenatarsenatis*, *Brevibacterium luteolum*, *Carnobacterium iners*, *Exiguobacterium profundum*, *Larkinella insperata*, *Pseudokineococcus lusitanus*, and *Spirosoma endophyticum*.

Description of *Bacillus selenatarsenatis* 15J4M-1

Cells are Gram-stain-positive, flagellated, and rod-shaped. Colonies are orange-colored after 3 days of incubation on R2A agar at 25°C. In the GN3 microplates, acetic acid, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, D-arabitol, L-arginine, D-aspartic acid, L-aspartic acid, D-cellobiose, D-fructose, D-fructose 6-PO₄, D-fucose, L-fucose, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gelatin, gentiobiose, D-gluconic acid, α -D-glucose, D-glucose-6-PO₄, glucuronamide, D-glucuronic acid, L-glutamic acid, α -keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, α -D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, 3-methyl glucose, myo-inositol, pectin, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, L-rhamnose, D-salicin, D-serine, L-serine, D-sorbitol, stachyose, sucrose, D-trehalose, and D-turanose were utilized as sole carbon source. But *N*-acetyl-neuraminic acid, *N*-acetyl-D-glucosamine, L-alanine, γ -amino-butyric acid, bromo-succinic acid, citric acid, dextrin, formic acid, glycerol, L-histidine, α -hydroxybutyric acid, β -hydroxy-D, L-butyric acid, *p*-hydroxy-phenylacetic acid, inosine, α -keto-butyric acid, D-malic acid, L-malic acid, methyl pyruvate, mucic acid, D-saccharic acid, and tween 40 were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, 4% NaCl, 8% NaCl, lithium chloride, potassium tellurite, rifamycin SV, and sodium butyrate. But not 1% sodium lactate, aztreonam, fusidic acid, guanidine HCl, lincomycin, minocycline, nalidixic acid, niaproof, pH 5, pH 6, D-serine, sodium bromate, tetrazolium blue, tetrazolium violet, troleandomycin, and vancomycin. Strain 15J4M-1 (=NIBRBAC 000499684) was isolated from a soil sample, Jeju, Korea.

Description of *Brevibacterium luteolum* 15J13-8

Cells are Gram-stain-positive, flagellated, and rod-shaped. Colonies are yellow-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, acetic acid, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, *N*-acetyl-neuraminic acid, *N*-acetyl-D-glucosamine, L-alanine, γ -amino-butyric acid,

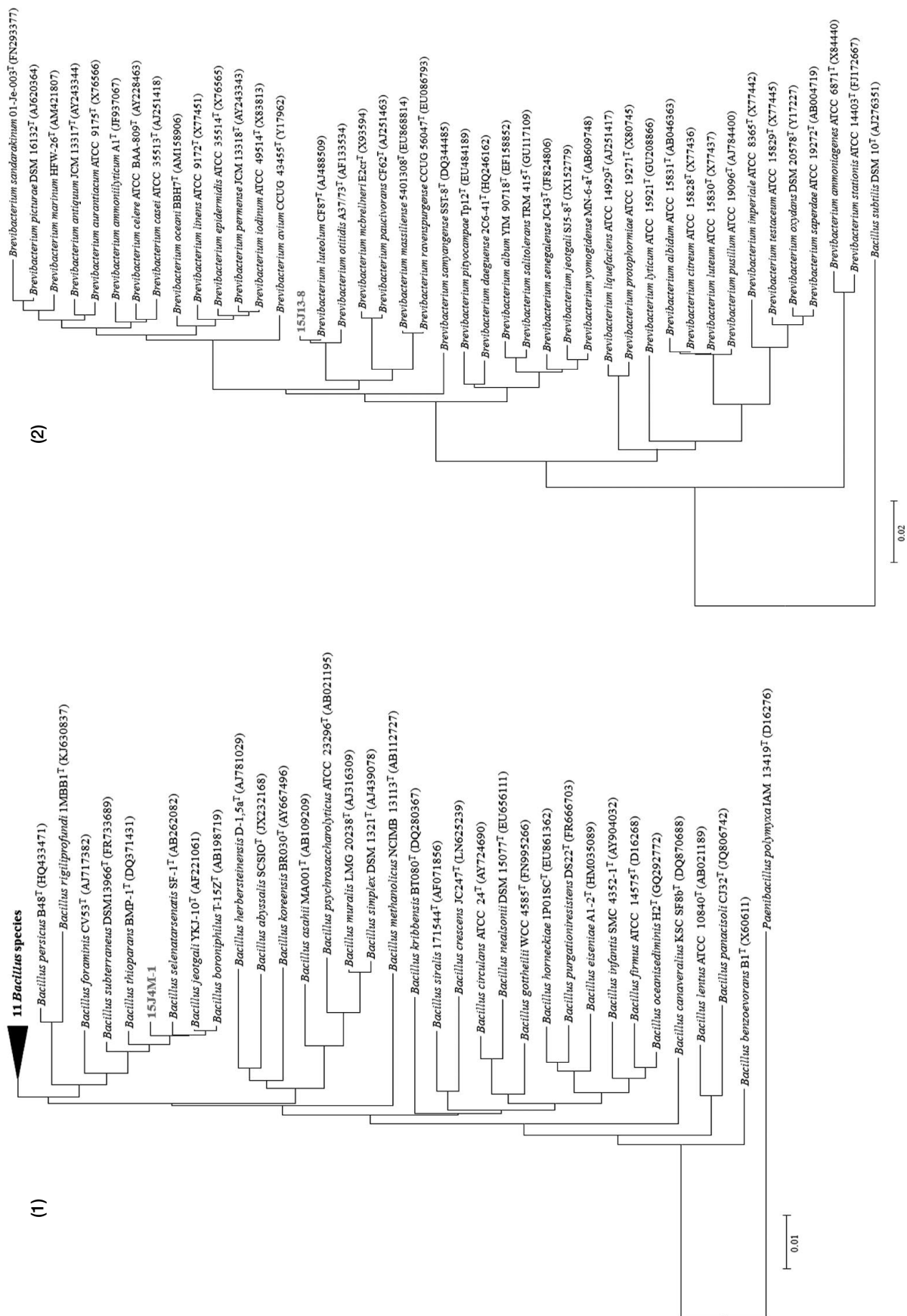


Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Bacillus* (1) and *Brevibacterium* (2). Bootstrap values (> 70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

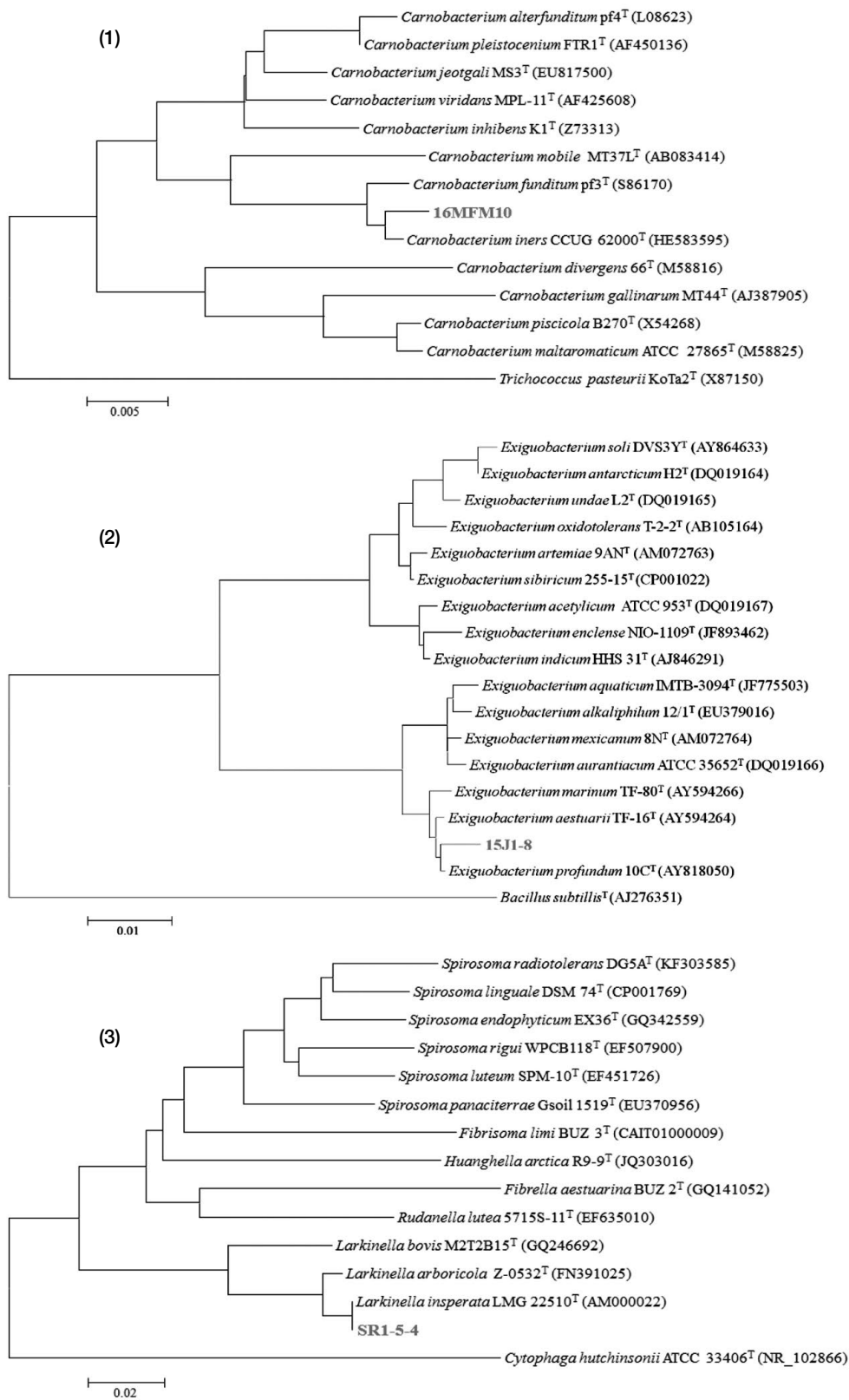


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Carnobacterium* (1), *Exiguobacterium* (2) and *Larkinella* (3). Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.005, 0.01 and 0.02 substitutions per nucleotide position, respectively.

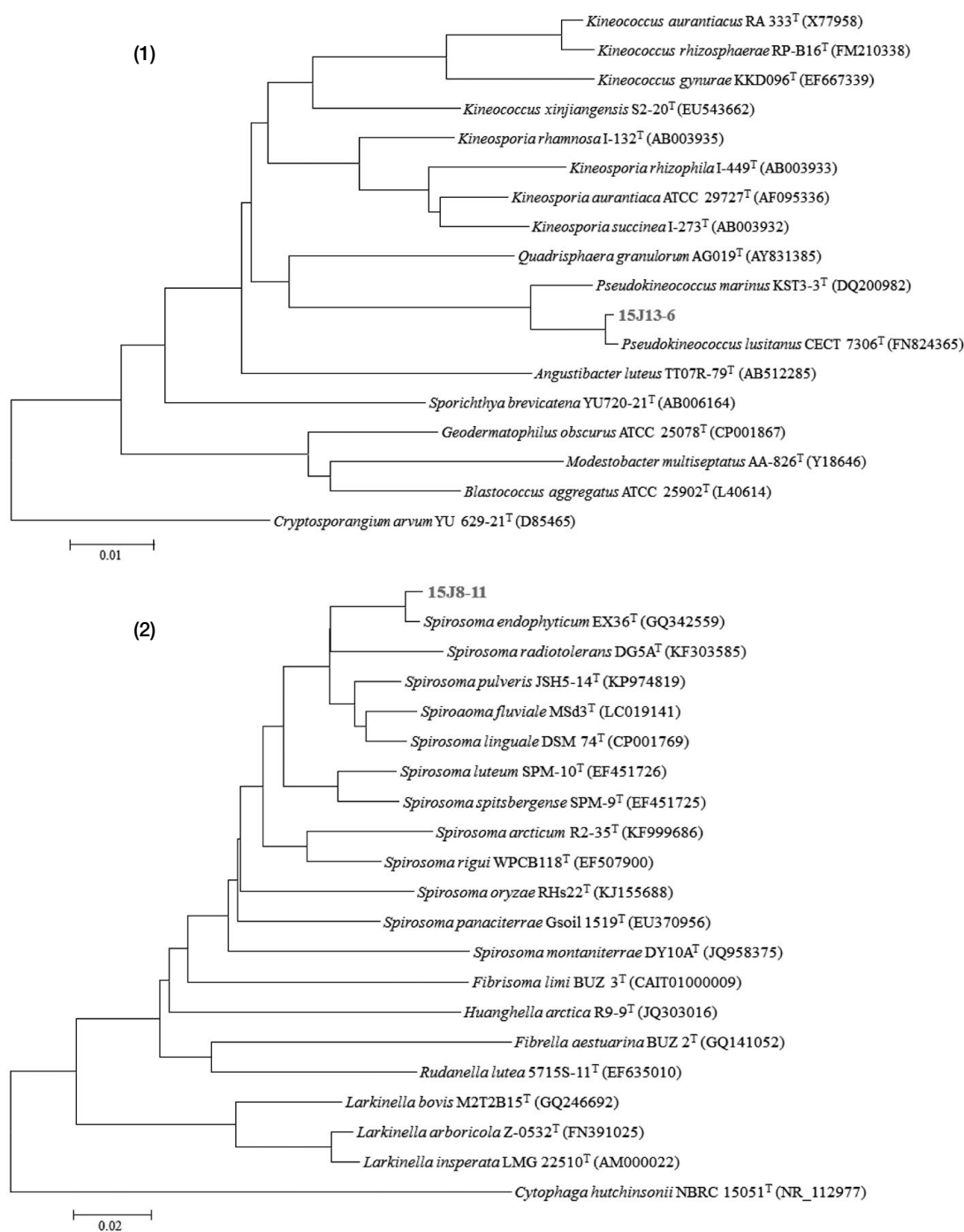


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Pseudokineococcus* (1) and *Spirosoma* (2). Bootstrap values (> 70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

D-arabitol, L-arginine, L-aspartic acid, D-cellobiose, citric acid, dextrin, formic acid, D-fructose, D-fructose 6-PO₄, D-fucose, L-fucose, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gelatin, gentiobiose, D-gluconic acid, α -D-glucose, D-glucose-6-PO₄, gluco-

ronamide, D-glucuronic acid, L-glutamic acid, glycerol, L-histidine, α -hydroxybutyric acid, *p*-hydroxy-phenyl-acetic acid, α -keto-butyric acid, α -keto-glutaric acid, D-lactic acid methyl ester, α -D-lactose, D-malic acid, L-malic acid, D-mannitol, D-mannose, D-melibiose, β -

methyl-D-glucoside, 3-methyl glucose, methyl pyruvate, mucic acid, myo-inositol, pectin, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, L-rhamnose, D-saccharic acid, D-salicin, L-serine, D-sorbitol, stachyose, sucrose, D-trehalose, D-turanose, and tween 40 were utilized as sole carbon source. But D-aspartic acid, bromo-succinic acid, β -hydroxy-D,L-butyric acid, inosine, L-lactic acid, D-maltose, and D-serine were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, 1% sodium lactate, 4% NaCl, 8% NaCl, aztreonam, lithium chloride, nalidixic acid, niaproof, pH 6, potassium tellurite, D-serine, and sodium butyrate. But not fusidic acid, guanidine HCl, lincomycin, minocycline, pH 5, rifamycin SV, sodium bromate, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin. Strain 15J13-8 (=NIBRBAC 000499671) was isolated from a soil sample, Jeju, Korea.

Description of *Carnobacterium iners* 16MFM10

Cells are Gram-stain-negative and rod-shaped. Colonies are pale yellow-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, *N*-acetyl-neuraminic acid, *N*-acetyl-D-glucosamine, L-arginine, dextrin, D-fructose, D-fructose 6-PO₄, D-fucose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, α -D-glucose, glucuronamide, D-glucuronic acid, glycerol, α -keto-butyric acid, α -D-lactose, D-maltose, D-mannose, 3-methyl glucose, pectin, L-rhamnose, D-saccharic acid, stachyose, sucrose, and D-trehalose were utilized as sole carbon source. But acetic acid, L-alanine, γ -amino-butyric acid, D-arabitol, D-aspartic acid, L-aspartic acid, bromo-succinic acid, D-cellobiose, citric acid, formic acid, L-galactonic acid lactone, gelatin, D-gluconic acid, D-glucose-6-PO₄, L-glutamic acid, L-histidine, α -hydroxybutyric acid, β -hydroxy-D,L-butyric acid, *p*-hydroxy-phenylacetic acid, inosine, α -keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, D-mannitol, D-melibiose, β -methyl-D-glucoside, methyl pyruvate, mucic acid, myo-inositol, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, D-salicin, D-serine, L-serine, D-sorbitol, D-turanose, and tween 40 were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, 1% sodium lactate, 4% NaCl, 8% NaCl, aztreonam, fusidic acid, lincomycin, lithium chloride, minocycline, nalidixic acid, pH 6, sodium butyrate, tetrazolium violet and troleandomycin. But not guanidine HCl, niaproof, pH 5, potassium tellurite, rifamycin SV, D-serine, sodium bromate, tetrazolium

blue and vancomycin. Strain 16MFM10 (=NIBRBAC 000499687) was isolated from a soil sample, Jeju, Korea.

Description of *Exiguobacterium profundum* 15J1-8

Cells are Gram-stain-positive, flagellated, and rod-shaped. Colonies are pale lemon-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, acetoacetic acid, *N*-acetyl-D-mannosamine, L-alanine, L-arginine, L-aspartic acid, D-cellobiose, dextrin, D-fructose, D-fructose 6-PO₄, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gelatin, gentiobiose, D-gluconic acid, α -D-glucose, glucuronamide, D-glucuronic acid, L-glutamic acid, inosine, α -keto-glutaric acid, L-malic acid, D-maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, 3-methyl glucose, myo-inositol, pectin, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, L-rhamnose, L-serine, stachyose, sucrose, D-trehalose, and D-turanose were utilized as sole carbon source. But acetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, *N*-acetyl-D-glucosamine, γ -amino-butyric acid, D-arabitol, D-aspartic acid, bromo-succinic acid, citric acid, formic acid, D-fucose, L-fucose, D-glucose-6-PO₄, glycerol, L-histidine, α -hydroxybutyric acid, β -hydroxy-D, L-butyric acid, *p*-hydroxy-phenylacetic acid, α -keto-butyric acid, L-lactic acid, D-lactic acid methyl ester, α -D-lactose, D-malic acid, methyl pyruvate, mucic acid, D-saccharic acid, D-salicin, D-serine, D-sorbitol, and tween 40 were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, 1% sodium lactate, 4% NaCl, 8% NaCl, guanidine HCl, lithium chloride, pH 6, potassium tellurite, D-serine, sodium butyrate, and tetrazolium violet. But not aztreonam, fusidic acid, lincomycin, minocycline, nalidixic acid, niaproof, pH 5, rifamycin SV, sodium bromate, tetrazolium blue, troleandomycin, and vancomycin. Strain 15J1-8 (=NIBRBAC 000499686) was isolated from a soil sample, Jeju, Korea.

Description of *Larkinella insperata* SR1-5-4

Cells are Gram-stain-positive and coil-shaped. Colonies are pale pink-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, *N*-acetyl-D-glucosamine, D-cellobiose, dextrin, D-fructose, D-fructose 6-PO₄, D-fucose, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α -D-glucose, D-glucose-6-PO₄, glucuronamide, D-glucuronic acid, α -D-lactose, D-maltose, D-mannose, D-melibiose, β -methyl-D-glucoside, pectin, D-raffinose, L-rhamnose, D-salicin, stachyose, sucrose, D-trehalose, and D-tura-

nose were utilized as sole carbon source. But acetic acid, acetoacetic acid, *N*-acetyl-neuraminic acid, L-alanine, γ -amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, L-aspartic acid, bromo-succinic acid, citric acid, formic acid, L-fucose, gelatin, L-glutamic acid, glycerol, L-histidine, α -hydroxybutyric acid, β -hydroxy-D,L-butyrac acid, *p*-hydroxy-phenylacetic acid, inosine, α -keto-butyric acid, α -keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, D-mannitol, 3-methyl glucose, methyl pyruvate, mucic acid, myo-inositol, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-saccharic acid, D-serine, L-serine, D-sorbitol, and tween 40 were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, aztreonam, nalidixic acid, pH 6, tetrazolium blue, tetrazolium violet, and vancomycin. But not 1% sodium lactate, 4% NaCl, 8% NaCl, fusidic acid, guanidine HCl, lincomycin, lithium chloride, minocycline, niaproof, pH 5, potassium tellurite, rifamycin SV, D-serine, sodium bromate, sodium butyrate and troleandomycin. Strain SR1-5-4 (= NIBRBAC 000499673) was isolated from a soil sample, Jeju, Korea.

Description of *Pseudokineococcus lusitanus* 15J13-6

Cells are Gram-stain-positive and coccus-shaped. Colonies are orange-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, acetic acid, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-alanine, D-arabitol, L-arginine, D-aspartic acid, D-cellobiose, dextrin, formic acid, D-fructose, D-fructose 6-PO₄, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, α -D-glucose, D-glucose-6-PO₄, glucuronamide, D-glucuronic acid, L-glutamic acid, glycerol, inosine, α -keto-butyric acid, α -keto-glutaric acid, α -D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, 3-methyl glucose, pectin, propionic acid, L-rhamnose, D-saccharic acid, D-salicin, L-serine, D-sorbitol, stachyose, sucrose, D-trehalose, D-turanose, and tween 40 were utilized as sole carbon source. But *N*-acetyl-D-mannosamine, *N*-acetyl-neuraminic acid, γ -amino-butyric acid, L-aspartic acid, bromo-succinic acid, citric acid, D-fucose, L-fucose, gelatin, L-histidine, α -hydroxybutyric acid, β -hydroxy-D,L-butyrac acid, *p*-hydroxy-phenylacetic acid, L-lactic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, methyl pyruvate, mucic acid, myo-inositol, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, and D-serine were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced at 1% NaCl, 1% sodium lactate, 4% NaCl, aztreo-

nam, lithium chloride, nalidixic acid, pH 5, pH 6, potassium tellurite, sodium bromate, sodium butyrate, tetrazolium blue, tetrazolium violet, and vancomycin. But not in the presence of 8% NaCl, fusidic acid, guanidine HCl, lincomycin, minocycline, niaproof, rifamycin SV, D-serine, and troleandomycin. Strain 15J13-6 (= NIBRBAC 000499670) was isolated from a soil sample, Jeju, Korea.

Description of *Spirosoma endophyticum* 15J8-11

Cells are Gram-stain-positive, flagellated and rod-shaped. Colonies are pale lemon-colored after 3 days of incubation on R2A at 25°C. In the GN3 microplates, *N*-acetyl-D-glucosamine, D-fructose 6-PO₄, D-fucose, L-fucose, L-galactonic acid lactone, D-galacturonic acid, gentiobiose, glucuronamide, D-glucuronic acid, α -keto-butyric acid, methyl pyruvate, L-rhamnose, and D-salicin were utilized as sole carbon source. But acetic acid, acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-mannosamine, *N*-acetyl-neuraminic acid, L-alanine, γ -amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, L-aspartic acid, bromo-succinic acid, D-cellobiose, citric acid, dextrin, formic acid, D-fructose, D-galactose, gelatin, D-gluconic acid, α -D-glucose, D-glucose-6-PO₄, L-glutamic acid, glycerol, L-histidine, α -hydroxybutyric acid, β -hydroxy-D, L-butyrac acid, *p*-hydroxy-phenylacetic acid, inosine, α -keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, α -D-lactose, D-malic acid, L-malic acid, D-maltose, D-mannitol, D-mannose, D-melibiose, β -methyl-D-glucoside, 3-methyl glucose, mucic acid, myo-inositol, pectin, propionic acid, glycyl-L-proline, L-pyroglutamic acid, quinic acid, D-raffinose, D-saccharic acid, D-serine, L-serine, D-sorbitol, stachyose, sucrose, D-trehalose, D-turanose, and tween 40 were not utilized.

In sensitivity tests, the tetrazolium redox dye was reduced in the presence of 1% NaCl, 1% sodium lactate, 4% NaCl, 8% NaCl, pH 6, potassium tellurite, tetrazolium blue, and tetrazolium violet. But not aztreonam, fusidic acid, guanidine HCl, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof, pH 5, rifamycin SV, D-serine, sodium bromate, sodium butyrate, troleandomycin, and vancomycin. Strain 15J8-11 (= NIBRBAC 000499674) was isolated from a soil sample, Jeju, Korea.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Seoul Women's University (2018) and by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the

Republic of Korea (NIBR201601113).

REFERENCES

- Crapart, S., M.-L. Fardeau, J.-L. Cayo, P. Thomas, C. Sery, B. Ollivier and Y. Combet-Blanc. 2007. *Exiguobacterium profundum* sp. nov., a moderately thermophilic, lactic acid-producing bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* 57:287-292.
- Doetsch, R.N. 1981. Determinative methods of light microscopy. *Manual of Methods for General Bacteriology*, pp. 21-33. In: P. Gerhardt, R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood, N.R. Krieg and G.H. Phillips (eds.), American Society for Microbiology. Washington, DC, USA. Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792-1797.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evol* 39:783-791.
- Fries, J., S. Pfeiffer, M. Kuffner and A. Sessitsch. 2013. *Spirosoma endophyticum* sp. nov., isolated from Zn- and Cd-accumulating *Salix caprea*. *Int J Syst Evol Microbiol* 63:4586-4590.
- Gibbons, N.E. and R.G.E. Murray. 1978. Proposals concerning the higher taxa of bacteria. *Int J Syst Bacteriol* 28:1-6.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95-98.
- Jurado, V., L. Laiz, A. Ortiz-Martinez, I. Groth and C. Saiz-Jimenez. 2011. *Pseudokineococcus lusitanus* gen. nov., sp. nov., and reclassification of *Kineococcus marinus* Lee 2006 as *Pseudokineococcus marinus* comb. nov. *Int J Syst Evol Microbiol* 61:2515-2519.
- Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won and J. Chun. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716-721.
- Kimura, M. 1983. *The neutral theory of molecular evolution*. Cambridge University Press, New York.
- Krieg, A.J., E.B. Rankin, D. Chan, O. Razorenova, S. Fernandez and A.J. Giaccia. 2010. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. *Mol Cell Biol* 30:344-353.
- Roller, C., W. Ludwig and K.H. Schleifer. 1992. Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes. *J Genet Microbiol* 138:1167-1175.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406-425.
- Schrempf, H. 2001. Recognition and degradation of chitin by streptomycetes. *Antonie Van Leeuwenhoek*. 79:285-289.
- Smith, M.A., N.E. Woodley, D.H. Janzen, W. Hallwachs and P.D.N. Hebert. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies. *Nat Acad Sci, USA* 103:3657-3662.
- Snauwaert, I., B. Hoste, K. De Bruyne, K. Peeters, L. De Vuyst, A. Willems and P. Vandamme. 2013. *Carnobacterium iners* sp. nov., a psychrophilic, lactic acid-producing bacterium from the littoral zone of an Antarctic pond. *Int J Syst Evol Microbiol* 63:1370-1375.
- Stackebrandt, S.P. 2000. *The prokaryotes: an evolving electronic resource for the microbiological community*. Springer-Verlag, New York, NY.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Ne and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731-2739.
- Thomas, F., J.-H. Hehemann, E. Rebuffet, M. Czjzek and G. Michel. 2011. Environmental and gut *Bacteroidetes*: the food connection. *Front Microbiol* 2:93.
- Turnbaugh, P.J., M. Hamady, T. Yatsunenkov, B.L. Cantarel, A. Duncan, R.E. Ley, M.L. Sogin, W.J. Jones, B.A. Roe, J.P. Affourtit, M. Egholm, B. Henrissat, A.C. Heath, R. Knight and J.I. Gordon. 2009. A core gut microbiome in obese and lean twins. *Nat* 457:480-484.
- Vancanneyt, M., O. Nedashkovskaya, C. Snauwaert, S. Mortier, K. Vandemeulebroecke, B. Hoste, P. Dawyndt, G.M. Frolova, D. Janssens and J. Swings. 2006. *Larkinella insperata* gen. nov., sp. nov., a bacterium of the phylum 'Bacteroidetes' isolated from water of a steam generator. *Int J Syst Evol Microbiol* 56:237-241.
- Ventura, M., C. Anchaya, A. Tauch, G. Chandra, G.F. Fitzgerald, K.F. Chater and D. van Sinderen. 2007. Genomics of Actinobacteria: Tracing the Evolutionary History of an Ancient Phylum. *Microbiol Mol Biol* 71:495:548.
- Wauters, G., V. Avesani, K. Laffineur, J. Charlier, M. Janssens, B. Van Bosterhaut and M. Delme e. 2003. *Brevibacterium lutescens* sp. nov., from human and environmental samples. *Int J Syst Evol Microbiol* 53:1321-1325.
- Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697-703.
- Wragg, P., L. Randall and A. Whatmor. 2014. Comparison of Biolog GEN III MicroStation semi-automated bacterial identification system with matrix-assisted laser desorption ionization-time of flight mass spectrometry and 16S ribosomal RNA gene sequencing for the identification of bacteria of veterinary interest. *J Microbiol Methods* 105: 16-21.
- Yamamura, S., M. Yamashita, N. Fujimoto, M. Kuroda, M. Kashiwa, K. Sei, M. Fujita and M. Lke. 2007. *Bacillus*

selenatarsenatis sp. nov., a selenate- and arsenate-reducing bacterium isolated from the effluent drain of a glass-manufacturing plant. Int J Syst Evol Microbiol 57:1060-1064.

Submitted: March 14, 2018

Revised: April 9, 2018

Accepted: May 16, 2018