Journal of Species Research 7(3):210-221, 2018

A report of eight unrecorded radiation resistant bacterial species in Korea isolated in 2018

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Eight bacterial strains assigned to the phylum *Firmicutes* were isolated from the soil samples in Korea. Phylogenetic analysis based on 16S rRNA gene sequences showed that strains 18JY14-16, 18JY14-35, 18JY42-5, 18JY12-20, 18JY35-8, 18JY76-9, 18JY39-1 and 18JY54-12 were most closely related to *Paenibacillus lupini* (MH497638; 99.4%), *Paenibacillus illinoisensis* (MH497643; 99.8%), *Paenibacillus tundrae* (MH497658; 99.7%), *Paenibacillus selenitireducens* (MH497639; 99.4%), *Paenibacillus eucommiae* (MH 497640; 99.9%), *Paenibacillus vini* (MH497654; 99.4%), *Paenibacillus gorillae* (MH497647; 99.5%), and *Paenibacillus macquariensis* (MH497649; 99.9%) respectively. These *Paenibacillus* species were Gram-stain-positive, rod-shaped and radiation resistant bacteria. This is the first report of these nine bacterial species in Korea.

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

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DOI:10.12651/JSR.2018.7.3.210

Introduction

The Bergey's Manual of Systematic Bacteriology divides the phylum *Firmicutes* into three classes, *Clostridia*, *Bacilli*, and *Erysipelotrichia*, based on 16S rRNA gene sequence comparison (Ludwig *et al*., 2009). Currently, the *Firmicutes* includes four additional classes, *Limnochordia*, *Negativicutes*, *Thermolithobacteria*, and *Tissierellia*.

The genus *Paenibacillus* in the phylum *Firmicutes*, class *Bacilli*, was first proposed by Ash *et al*. (1994) and amended by Shida *et al*. (1997). Currently, this genus comprises 238 recognized species and four subspecies (www.bacterio.net/paenibacillus.html). Cells are Gram-stain-positive or Gram-stain-negative, facultatively anaerobic or strictly aerobic, non-pigmented, rod-shaped, endospore-forming and chemoheterotrophic (Chen *et al*., 2017). The *Paenibacillus* are widespread, and have been isolated from a variety of sources, including rhizospheres of trees (Jin *et al*., 2011; Jiang *et al*., 2015), flowers (Siddiqi *et al*., 2015), gamma-irradiated Antarctic soil (Dsouza *et al*., 2014), duckweed (Kittiwongwattana and Thawai, 2015), nodules of *Lupinus albus* (Carro *et al*., 2014), tidal flats (Wang *et al*., 2012), water springs (Tang *et al*., 2011), rice seed (Liu *et al*., 2010), iron mineral soil (Cao *et al*., 2015) and necrotic wounds (Glaeser *et al*., 2013). Among the endospore-forming bacilli, members of the genus *Paenibacillus* are also of interest because of their ability to promote plant growth (Grady *et al*., 2016).

In this study, we described eight unreported bacterial strains 18JY14-16, 18JY14-35, 18JY42-5, 18JY12-20, 18JY35-8, 18JY76-9, 18JY39-1, and 18JY54-12 belonging to the genus *Paenibacillus* (family *Paenibacillaceae*, order *Bacillales* in the phylum *Firmicutes*) isolated from soil in Korea.

Materials and Methods

The soil samples were collected on Jeju island (33.

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

Strain ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
18JY14-16	*Paenibacillus lupini*	99.4	Soil	R2A	25°C, 3d
18JY14-35	*Paenibacillus illinoisensis*	99.8	Soil	1/10LB	25°C, 3d
18JY42-5	*Paenibacillus tundrae*	99.7	Soil	R2A	25°C, 3d
18JY12-20	*Paenibacillus selenitireducens*	99.4	Soil	1/10LB	25°C, 3d
18JY35-8	*Paenibacillus eucommiae*	99.9	Soil	R2A	25°C, 3d
18JY76-9	*Paenibacillus vini*	99.4	Soil	1/10LB	25°C, 3d
18JY39-1	*Paenibacillus* gorilla	99.5	Soil	R2A	25°C, 3d
18JY54-12	*Paenibacillus macquariensis*	99.9	Soil	R2A	25°C, 3d

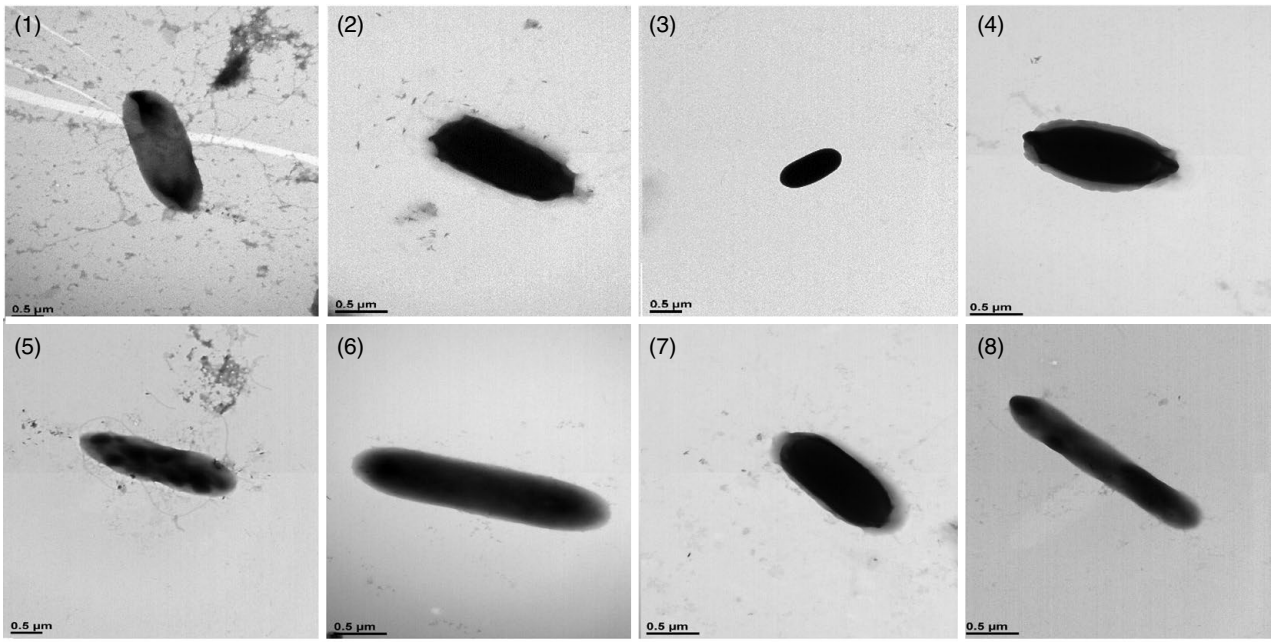

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: 1, 18JY14-16; 2, 18JY14-35; 3, 18JY42-5; 4, 18JY12-20; 5, 18JY35-8; 6, 18JY76-9; 7, 18JY39-1; 8, 18JY54-12.

4890° N, 126.4983° E), Republic of Korea. Samples were suspended in distilled water and serially diluted. 100 μL aliquots from each sample were inoculated onto 1/10 LB or R2A agar and incubated at 25°C for three days. The designated strain IDs, isolation sources, growth 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 freeze-dried ampoules.

The colony morphology and cell size of the strains were observed by transmission electron microscopy (LIBRA 120, Carl Zeiss) using cells grown for 3 days at 25°C on 1/10LB or R2Aagar. A transmission electron micrograph of the strains is shown in Fig. 1. Gram reaction was performed following the standard 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 on alternative media according to the manufacturer's instructions, and inocula were prepared to a specified transmittance using a turbidimeter, as specified in the user guide. For each isolate, 100 μL 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 were interpreted by the system's identification software (GEN III database, version 5.2.1). When the system which indicated that the isolates could not be identified after 20 h and required further incubation, isolates were re-incubated

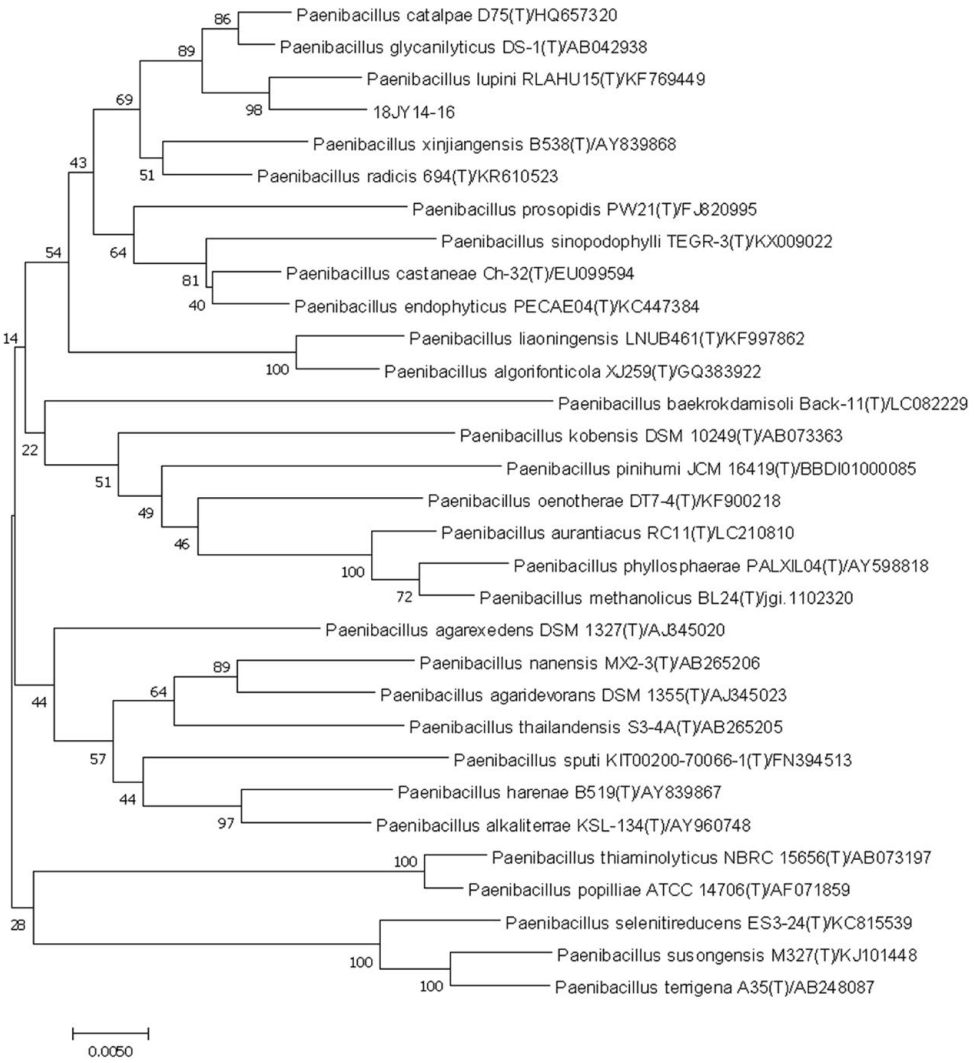

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY14-16 and its close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

and re-read between three and six h later (Wragg *et al*., 2014). Genomic DNA was extractedand16S rRNA gene was amplified by PCR with 9F and 1492R universal bacterial primers (Weisburg *et al*., 1991). The 16SrRNA gene sequences of the closely related strains 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 using the MUSCL program (Edgar, 2004) and the evolutionary distances were calculated using the two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei, 1987) using MEGA v. 5 program with bootstrap values based on 1,000 replications (Felsenstein, 1985).

The survival rate after exposure to UV radiation was measured using cells at the early stationary phase (~10^9 c.f.u. mL^{-1}) grown in tryptone glucose yeast broth (Difco). Cells were irradiated with a UVC UV cross-linker (UVP, CX-2000) at 254 nm and used with different dose adjustments (Im *et al*., 2013; Selvam *et al*., 2013). After irradiation, the cell suspensions were diluted and plated on tryptone glucose yeast agar plates in triplicate. Controls, *Deinococcus radiodurans* R1T (=DSM 20539T) and *Escherichia coli* K-12 (=KCTC 1116) were used for comparison as a positive and a negative control, respectively. The number of colony-forming units of the strains were counted, and the survival rate was calculated.

Results and Discussion

Based on 16S rRNA gene sequence similarity, eight

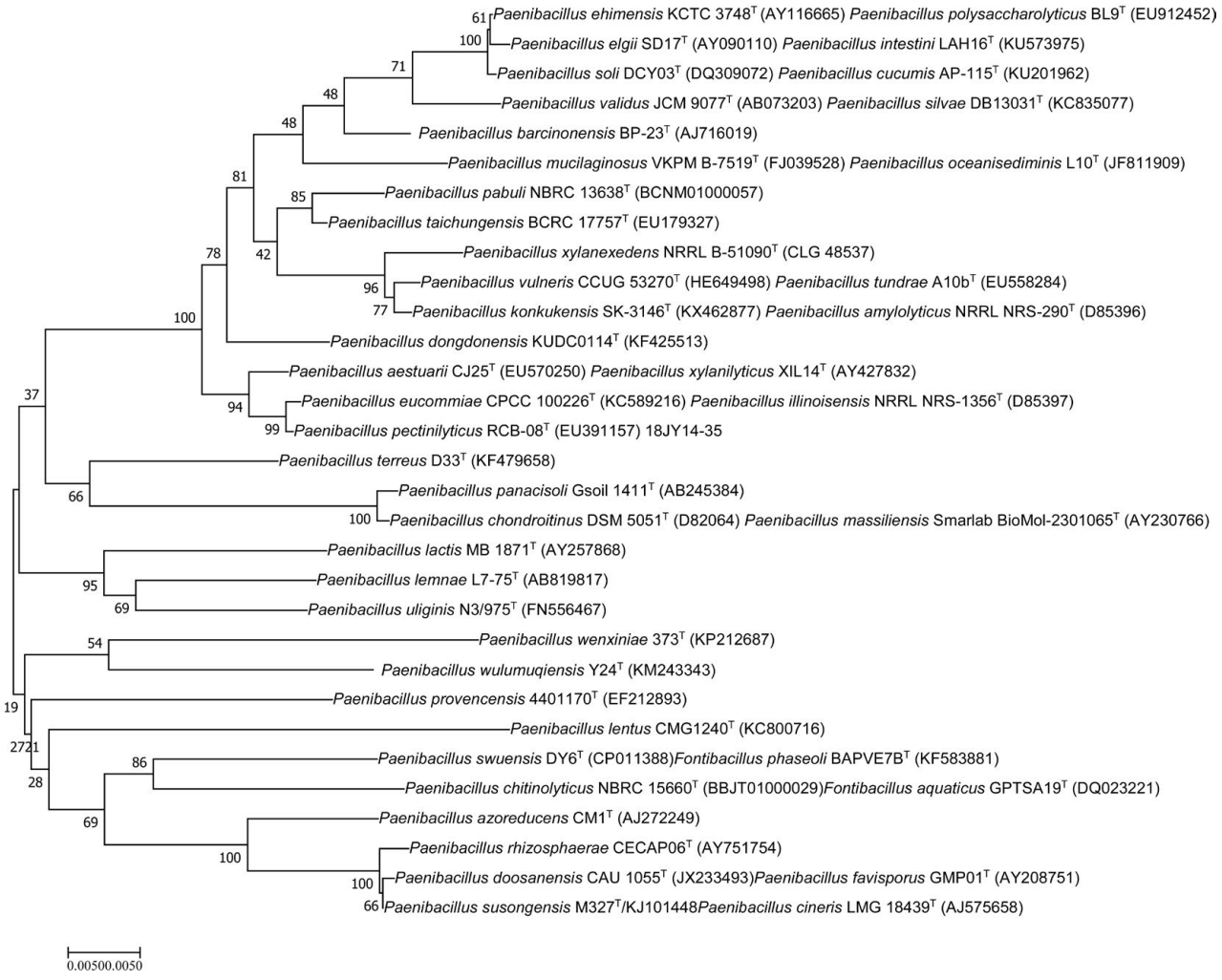

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY14-35 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

previously unreported bacterial species were identified from soil. The taxonomic identification results are summarized in Table 1. Nine strains 18JY14-16, 18JY14-35, 18JY42-5, 18JY12-20, 18JY35-8, 18JY76-9, 18JY39-1 and 18JY54-12 were assigned to the family *Paenibacillaceae* of the phylum *Firmicutes*. Phylogenetic analysis based on 16S rRNA gene sequence showed that strains were most closely related to *Paenibacillus lupini* (99.4%), *Paenibacillus illinoisensis* (99.8%), *Paenibacillus tundrae* (99.7%), *Paenibacillus selenitireducens* (99.4%), *Paenibacillus eucommiae* (99.9%), *Paenibacillus vini* (99.4%), *Paenibacillus gorillae* (99.5%), and *Paenibacillus macquariensis* (99.9%) respectively. The neighbor-joining trees showed a close relationship of the isolates and type strains of published species (Figs. 2-9). The detailed morphological and physiological characteristics are given in the strain description section. All nine isolates were shown to be radiation-resistant (Fig. 10). These species have not been previously reported in Korea.

Description of *Paenibacillus lupini* 18JY14-16

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped. Colonies are white-colored after 3 days of incubation on R2A at 25°C. In API 20NE system, positive for arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Weak positive for D-glucose, D-mannose, and adipic acid. Negative for nitrate reduction, glucose fermentation, indole production, gelatin hydrolysis, capric acid and phenylacetic acid as carbon sources.

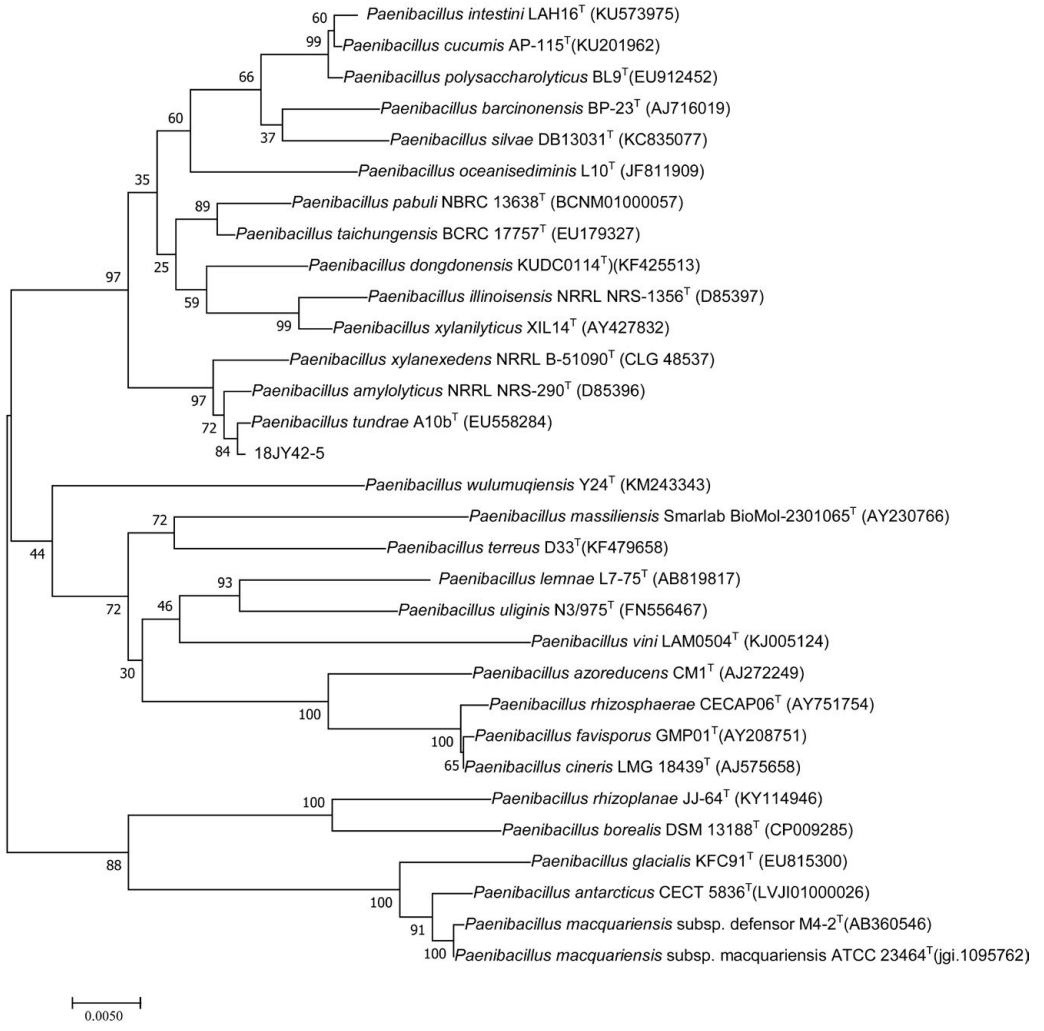

Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY42-5 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

In API 32GN system, positive for L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), D-maltose, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, trisodium citrate, L-histidine, potassium 2-ketogluconate and 3-hydroxybutyric acid. Negative for suberic acid, itaconic acid, sodium malonate, L-serine, propionic acid, capric acid, 4-hydroxybenzoic acid, and L-proline. 3-Hydroxybenzoic acid, valeric acid. Cells show resistance to UV radiation. Strain 18JY14-16 (=NIBRBAC0000116016) was isolated from a soil sample in Korea.

Description of *Paenibacillus illinoisensis* 18JY14-35

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped. Colonies are yellow-colored after 3 days of incubation on 1/10LB at 25°C. In API 20NE system, positive for arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, D-glucose, β-galactosidase, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid. Weak positive for L-arabinose, trisodium citrate, D-maltose, D-mannitol and D-mannose. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, capric acid, adipic acid and phenylacetic acid.

In API 32GN system, positive for *N*-acetyl-glucosamine, D-ribose, D-saccharose (sucrose), D-maltose, sodium acetate, lactic acid, L-alanine, glycogen, D-mannitol, D-glucose, salicin, D-melibiose, L-arabinose, trisodium citrate and L-histidine. Negative for itaconic acid, suberic acid, propionic acid, 3-hydroxybutyric acid,

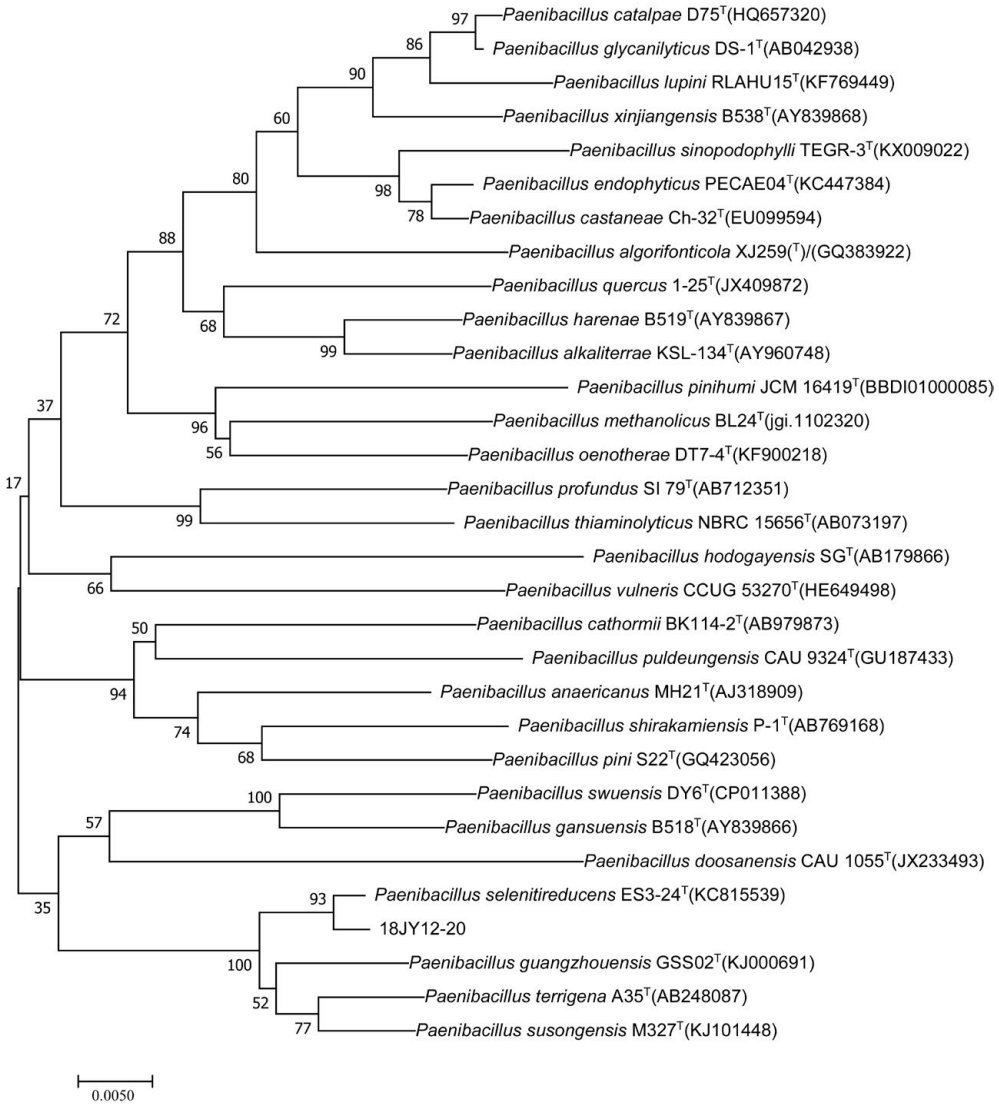

Fig. 5. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY12-20 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

L-rhamnose, *N*-acetyl-glucosamine, inositol, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, D-fucose, D-sorbitol, capric acid, valeric acid, potassium 2-ketogluconate, 4-hydroxybenzoic acid, and L-proline. Cells showed resistance to UV radiation. Strain 18JY14-35 (=NIBRBAC0000116019) was isolated from a soil sample in Korea.

Description of *Paenibacillus tundrae* 18JY42-5

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped. Colonies are yellow-colored after 3 days of incubation on R2A at 25°C. In API 20NE system, positive for the esculin hydrolysis, β-galactosidase, D-glucose, L-arabinose, D-mannose, D-maltose, potassium gluconate and malic acid. Weak positive for D-mannitol. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), nitrates (NO_3) to nitrogen (N_2), arginine dihydrolase, urease, gelatin hydrolysis, *N*-acetyl-D-glucosamine, adipic acid, trisodium citrate, phenylacetic acid, indole production on tryptophan, glucose fermentation, and capric acid.

In API 32GN system, positive for D-mannitol, D-glucose, D-sorbitol, valeric acid, L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), D-maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine,

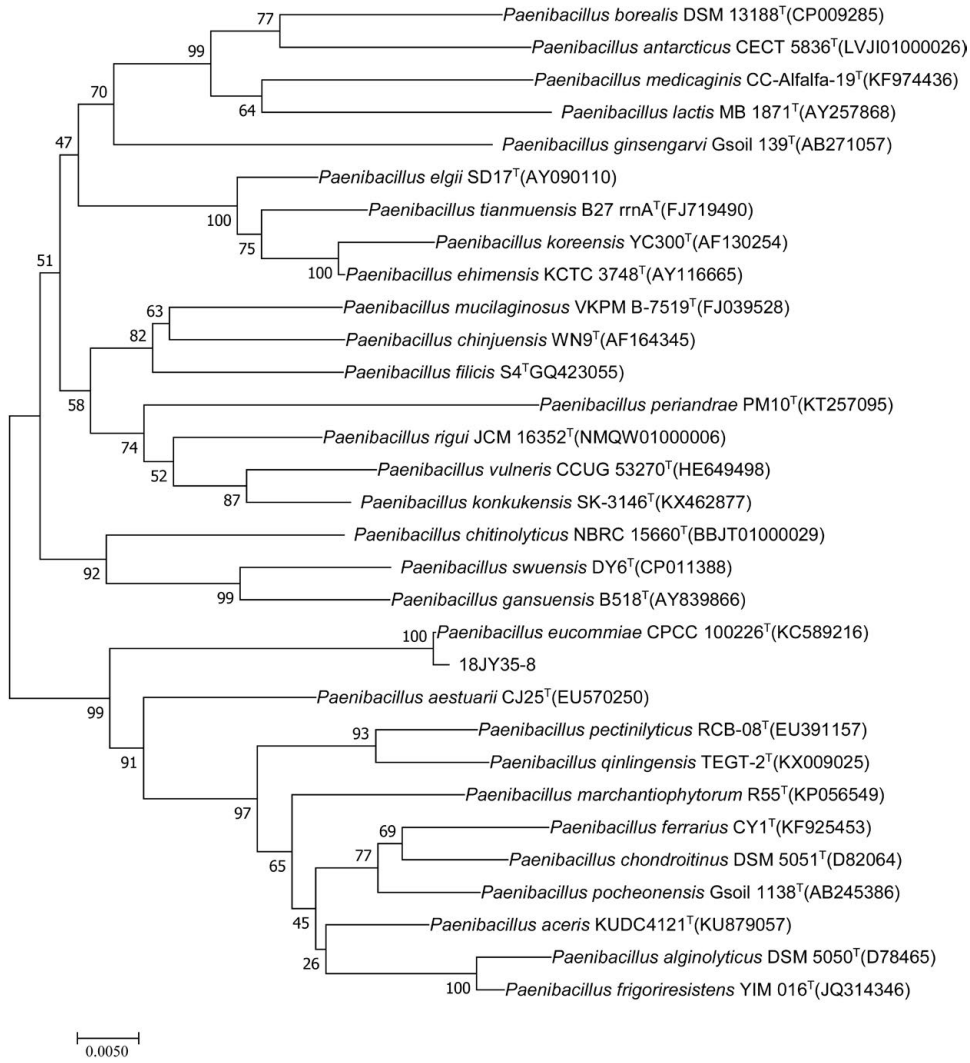

Fig. 6. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY35-8 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

salicin, D-melibiose, D-fucose, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, L-serine, 4-hydroxybenzoic acid, L-proline and 3-hydroxybutyric acid. Negative for capric acid. Cells showed resistance to UV radiation. Strain 18JY42-5 (=NIBRBAC0000116024) was isolated from a soil sample in Korea.

Description of *Paenibacillus selenitireducens* 18JY12-20

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped. Colonies are yellow-colored after 3 days of incubation on 1/10LB at 25°C. In API 20NE system, positive for the reduction of nitrates (NO_3) to nitrite (NO_2^-), esculin hydrolysis, β-galactosidase, D-glucose, potassium gluconate and malic acid. Weak positive for L-arabinose, *N*-acetyl-D-glucosamine, and D-maltose. Negative for the reduction of nitrates (NO_3) to nitrogen (N_2), urease, D-mannose, adipic acid, phenylacetic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid, indole production on tryptophan, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, D-mannitol, capric acid and trisodium citrate.

In API 32GN system, positive for D-saccharose (sucrose), itaconic acid, sodium malonate, lactic acid, D-fucose, L-arabinose, L-rhamnose, suberic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, sodium acetate, D-melibiose, trisodium ci-

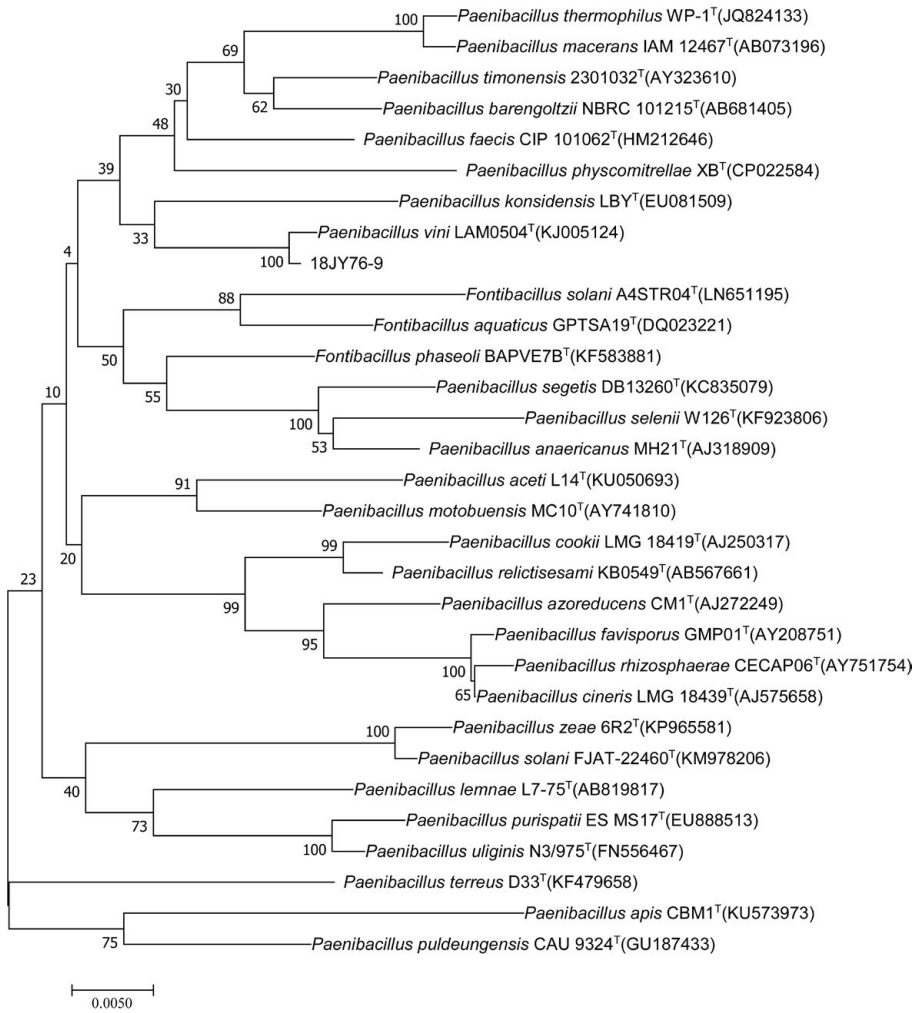

Fig. 7. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY76-9 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

trate, L-histidine, 4-hydroxybenzoic acid, and L-proline. Negative for *N*-acetyl-glucosamine, D-ribose, inositol, D-maltose, L-serine, D-mannitol, D-glucose, salicin, D-sorbitol, propionic acid, capric acid and valeric acid. Cells showed resistance to UV radiation. Strain 18JY12-20 (=NIBRBAC0000116025) was isolated from a soil sample in Korea.

Description of *Paenibacillus eucommiae* 18 JY35-8

Cells are Gram-stain-negative, flagellated, and rod-shaped. Colonies are yellow-colored after 3 days of incubation on R2A at 25°C. In API 20NE system, positive for arginine dihydrolase, urease, β-galactosidase, D-maltose, and potassium gluconate. Weak positive for trisodium citrate. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), nitrates (NO_3) to nitrogen (N_2), D-glucose, L-arabinose, D-mannose, D-mannitol, adipic acid, malic acid, indole production on tryptophan, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, *N*-acetyl-D-glucosamine, capric acid, and phenylacetic acid.

In API 32GN system, positive for D-ribose, itaconic acid, L-alanine, L-rhamnose, inositol, *N*-acetyl-glucosamine, D-saccharose (sucrose), D-maltose, sodium acetate, lactic acid, L-serine, D-melibiose, D-sorbitol, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, L-serine, 4-hydroxybenzoic acid, and L-proline. Negative for suberic acid, sodium malonate, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, D-mannitol, D-glucose, salicin, D-fucose, L-arabinose, propionic acid, capric acid and valeric acid. Cells showed resistance to UV radiation. Strain 18JY35-8 (=NIBRBAC0000116026) was isolat-

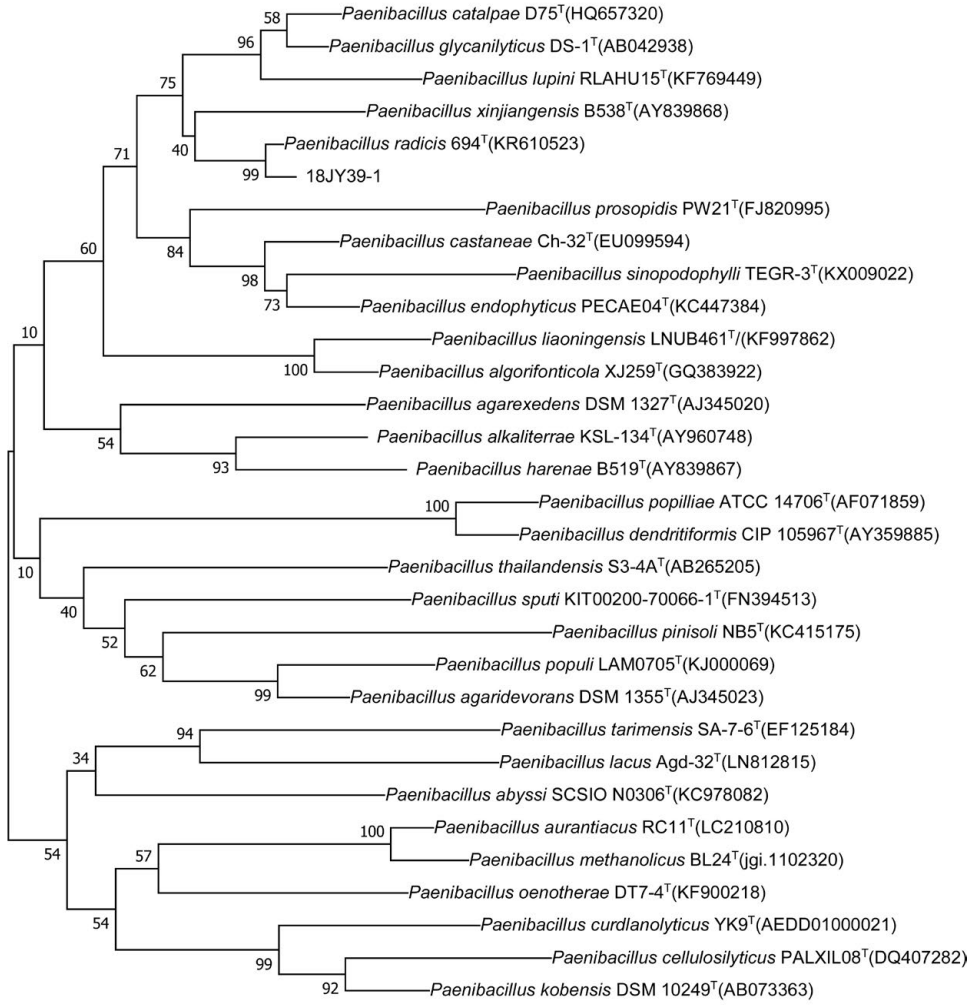

Fig. 8. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY39-1 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

ed from a soil sample in Korea.

Description of *Paenibacillus vini* 18JY76-9

Cells are Gram-stain-negative, non-flagellated, and rod-shaped. Colonies are white-colored after 3 days of incubation on 1/10LB at 25°C. In API 20NE system, positive for the reaction of nitrates (NO_3) to nitrite (NO_2^-), urease, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid. Weak for β-galactosidase, D-glucose, L-arabinose, gelatin hydrolysis and trisodium citrate. Negative for the reduction of reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, esculin hydrolysis, capric acid, adipic acid, arginine dihydrolase, D-mannitol and phenylacetic acid. In API 32GN system, positive for *N*-acetyl-glucosamine, inositol, D-glucose, salicin, D-sorbitol, L-arabinose, potassium 2-ketogluconate, L-proline, L-rhamnose, D-ribose, D-saccharose (sucrose), D-maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, potassium 5-ketogluconate, glycogen, D-melibiose, D-fucose, trisodium citrate, L-histidine, and 3-hydroxybutyric acid. Negative for L-alanine, 3-hydroxybenzoic acid, L-serine, D-mannitol, propionic acid, capric acid, valeric acid and 4-hydroxybenzoic acid. Cells showed resistance to UV radiation. Strain 18JY76-9 (=NIBRBAC0000116027) was isolated from a soil sample in Korea.

Description of *Paenibacillus gorilla* 18JY39-1

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped. Colonies are white-colored after 3 days of incubation on R2A at 25°C. In API 20NE system, positive for reduction of nitrates (NO_3) to nitrogen (N_2), arginine dihydrolase, urease, esculin hydrolysis,

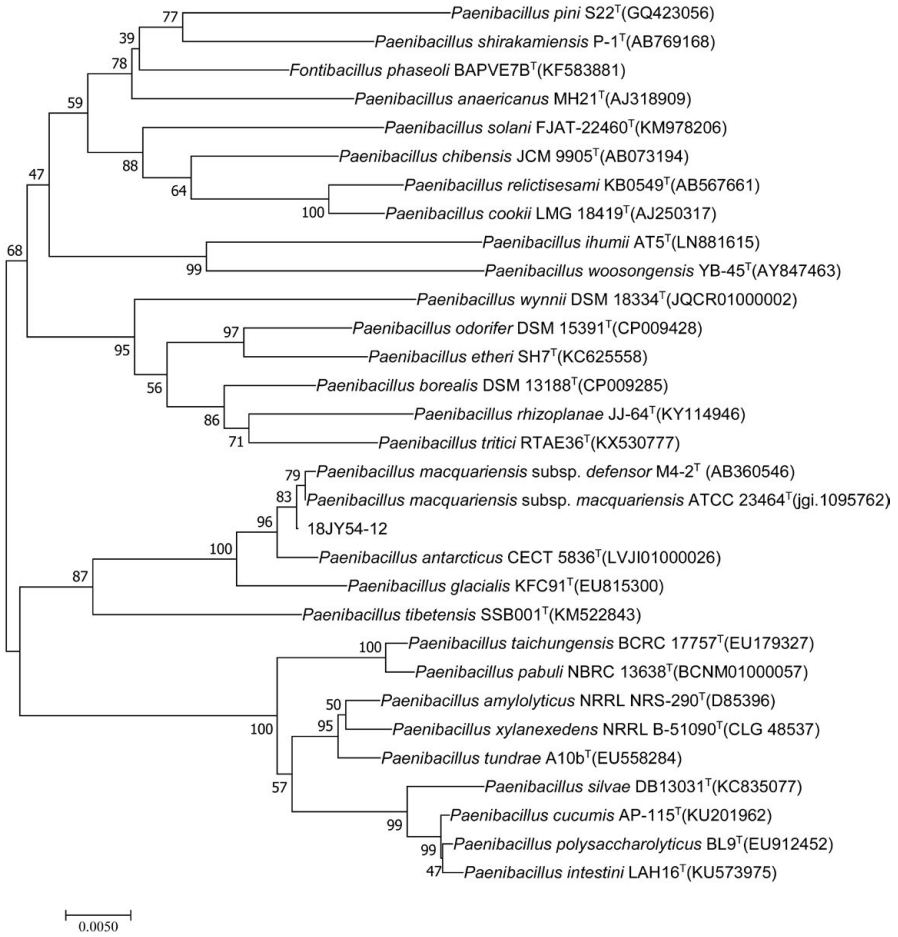

Fig. 9. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strain 18JY54-12 and their close relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively.

D-glucose, D-mannose, *N*-acetyl-D-glucosamine, β-galactosidase, D-maltose, malic acid and potassium gluconate. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production on tryptophan, glucose fermentation, gelatin hydrolysis, L-arabinose, D-mannitol, capric acid, adipic acid, trisodium citrate and phenylacetic acid.

In API 32GN system, positive for L-rhamnose, *N*-acetyl-glucosamine, D-ribose, D-saccharose (sucrose), D-maltose, glycogen, D-mannitol, D-glucose, salicin, D-melibiose, D-sorbitol, L-arabinose, propionic acid and L-histidine. Negative for suberic acid, lactic acid, 4-hydroxybenzoic acid, inositol, itaconic acid, sodium malonate, sodium acetate, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, D-fucose, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate, 3-hydroxybutyric acid, and L-proline. Cells showed resistance to UV radiation. Strain 18JY39-1 (=NIBRBAC0000116029) was isolated from a soil sample in Korea.

Description of *Paenibacillus macquariensis* 18JY54-12

Cells are Gram-stain-negative, flagellated and rod-shaped. Colonies are white-colored after 3 days of incubation on R2A at 25°C. In API 20NE system, positive for the reduction of nitrates (NO_3) to nitrogen (N_2), arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, D-glucose, *N*-acetyl-D-glucosamine, D-mannitol, potassium gluconate and malic acid. Weak positive for D-mannose, D-maltose, and trisodium citrate. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production on tryptophan, glucose fermentation, gelatin hydrolysis, L-arabinose, adipic acid, capric acid, and phenylacetic acid.

In API 32GN system, positive for *N*-acetyl-glucosamine, D-saccharose (sucrose), D-maltose, sodium acetate, lactic acid, glycogen, D-glucose, salicin, D-melibiose, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxy-

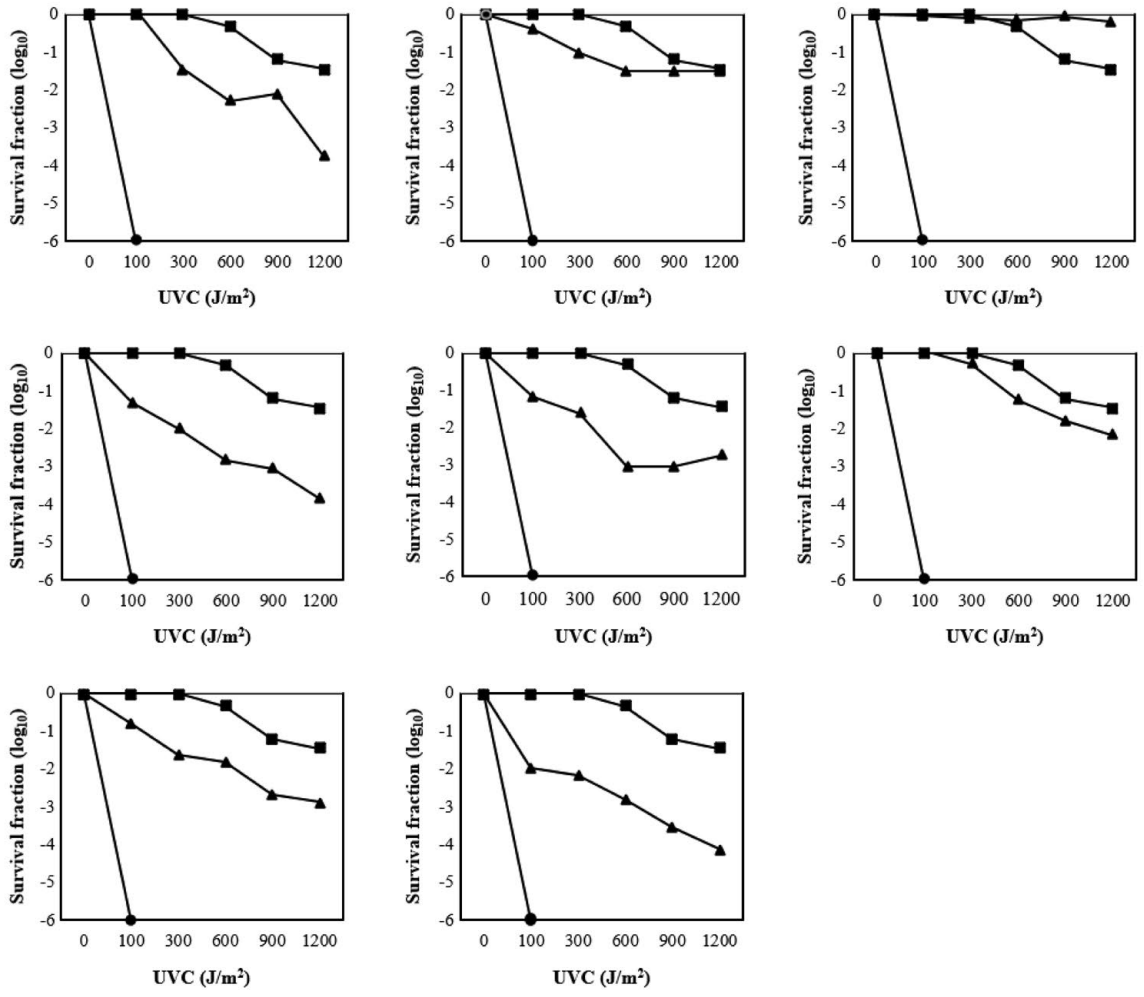

Fig. 10. Survival rate of nine radiation-resistant strains after exposure to UV radiation (▲)18JY14-16, 18JY14-35, 18JY42-5, 18JY12-20, 18JY35-8, 18JY76-9, 18JY39-1 and 18JY54-12 to UV radiation. Survival rates of *D. radiodurans* R1 (■) and *E. coli* (●) are also shown. The survival rate after UV radiation was measured by a number of cells in R2A (18JY14-16, 18JY42-5, 18JY35-8, 18JY39-1, and 18JY54-12) or 1/10LB (18JY14-35, 18JY12-20 and 18JY76-9) agar.

benzoic acid, L-rhamnose, D-ribose, D-mannitol, and L-proline. Negative for inositol, itaconic acid, suberic acid, sodium malonate, L-alanine, potassium 5-keto-gluconate, 3-hydroxybenzoic acid, L-serine, D-fucose, D-sorbitol, L-arabinose, propionic acid, and capric acid. Cells showed resistance to UV radiation. Strain 18JY54-12 (=NIBRBAC0000116033) was isolated from a soil sample in Korea.

Acknowledgments

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 (NIBR201801106).

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Submitted: June 29, 2018
Revised: August 2, 2018
Accepted: August 7, 2018