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A report of six unrecorded radiation-resistant bacterial species isolated from soil in Korea in 2018

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Six bacterial strains 18JY42-3, 18SH, 18JY76-11, 17J11-11, 18JY14-14, and 18JY15-11 assigned to the phylum *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were isolated from soil samples in Korea. The *Cohnella* species, strain 18JY42-3 was Gram-stain-positive, short rod-shaped and beige-colored. The *Methylobacterium* species, strains 18SH and 18JY76-11 were Gram-stain-negative, short rod-shaped and pink-colored. The *Microterricola* species, strain 17J11-11 was Gram-stain-positive, short rod-shaped and yellow-colored. The *Paenarthrobacter* species, strains 18JY14-14 and 18JY15-11 were Gram-stain-positive, short rod-shaped and white-colored. Phylogenetic analysis based on 16S rRNA gene sequence showed that strains 18JY42-3, 18SH, 18JY76-11, 17J11-11, 18JY14-14, and 18JY15-11 were most closely related *Cohnella rhizosphaerae* (MH497628; 98.8%), *Methylobacterium goesingense* (MH497632; 99.1%), *Methylobacterium populi* (MH497635; 99.9%), *Microterricolagilva* (MH504108; 98.4%), *Paenarthrobacter nicotinovorans* (MH497641; 100%), and *Paenarthrobacter nitroguajacolicus* (MH497646; 99.2%), respectively. All the six unrecorded strains showed resistance to UV radiation. This is the first report of these six species in Korea.

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

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Introduction

In 2018, six unreported bacterial species were isolated from diverse soil samples collected in Korea. The current report focuses on the description of six unreported bacterial species belonging to phylum *Proteobacteria*, *Firmicutes*, and *Actinobacteria*, that have not been previously reported in Korea.

The two unreported bacterial strains, 18SH and 18JY76-11 belong to the family *Methylobacteriaceae* in the phylum *Proteobacteria*, which currently contains five genera-*Methylobacterium*, *Microvirga*, *Protomonas*, *Enterovirga* and *Meganema*. The genus *Methylobacterium* is one of the largest genera containing 53 validated species (http://www.bacterio.net/). Initially, the phylum *Proteobacteria* was separated into four bacterial groups (alpha, beta, gamma, and delta) based on 16S rRNA gene sequence structures (Woese, 1987). The phylum was further established, using phylogenetic analysis of 16S rRNA gene sequences, by Garrity *et al*. (2005a), into five constituent classes containing all known Gram-negative bacteria, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria*. Recent molecular analyses using complete multigenome/multiprotein alignment studies, a sixth class, the *Zetaproteobacteria* and a seventh class, the *Oligoflexis* were established (Williams *et al*., 2007; Yutin *et al*., 2012). Currently, the *Proteobacteria* comprise seven classes, 50 orders and 116 validated families (http://www.bacterio.net/) and is highly abundant in various ecological niches such as soil, plants, the atmosphere, seawater and freshwater (Shin *et al*., 2015).

The unreported bacterial strains, 18JY14-14, 18JY15-11, and 17J11-11 belong to the phylum *Actinobacteria*. The strains 18JY14-14 and 18JY15-11 related to *Micrococcaceae*, which is one of the major families of *Actino-*

bacteria, comprise of at least 21 genera. Within the family, the genus *Paenarthrobacter* contain six validated species. The strain 17J11-11 is related to the *Microbacteriaceae* which are in the order *Burkholderiales*, which contains 49 genera (http://www.bacterio.net/). In terms of number and variety of identified species, the phylum *Actinobacteria* represents one of the largest taxonomic units among the 18 major lineages recognized within the domain *Bacteria* (Stackebrandt *et al*., 1997). At present, *Actinobacteria* includes six classes, 12 orders, 14 suborders and 61 families (http://www.bacterio.net). *Actinobacteria* display a wide variety of morphologies, from coccoid (*Micrococcus*) or rod-coccoid (e.g., *Arthrobacter*) to fragmenting hyphal forms (e.g., *Nocardia* sp.) or highly differentiated branched mycelium (e.g., *Streptomyces* sp.) (Atlas, 1997). They also exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of a wide variety of secondary metabolites (Schrempf, 2001).

The strain, 18JY42-3 belongs to the family *Paenibacillaceae* in the order *Bacillales*, phylum *Firmicutes*. The phylum *Firmicutes* consisted of seven classes, 13 orders and 45 families (http://www.bacterio.net/). These genera form a phylogenetic cluster based on 16S rRNA gene sequence similarity of 93-97% yet, have diverse phenotypic characteristics that includes aerobic organotrophs, anaerobic denitrifies, Fe^{3+}-reducing bacteria, hydrogen oxidizers, photoautotrophic and photoheterotrophic bacteria, and fermentative bacteria (Willems *et al*., 1991).

Therefore, the isolation of unreported strains belonging to the phyla *Proteobacteria*, *Actinobacterium*, and *Firmicutes* has the potential to advance human application of bioresources, as they can be exploited by the clinical and pharmaceutical industries.

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

Strain No.	Most closely related species	Accession number	Similarity (%)	Isolation source	Medium	Incubation conditions
18JY42-3	*Cohnella rhizosphaerae*	MH497628	98.8	Soil	R2A	25°C, 3d
18SH	*Methylobacterium goesingense*	MH497632	99.1	Soil	R2A	25°C, 3d
18JY76-11	*Methylobacterium populi*	MH497635	99.9	Soil	R2A	25°C, 3d
17J11-11	*Microterricola gilva*	MH504108	98.9	Soil	R2A	25°C, 3d
18JY14-14	*Paenarthrobacter nicotinovorans*	MH497641	100	Soil	R2A	25°C, 3d
18JY15-11	*Paenarthrobacter nitroguajacolicus*	MH497646	99.2	Soil	R2A	25°C, 3d

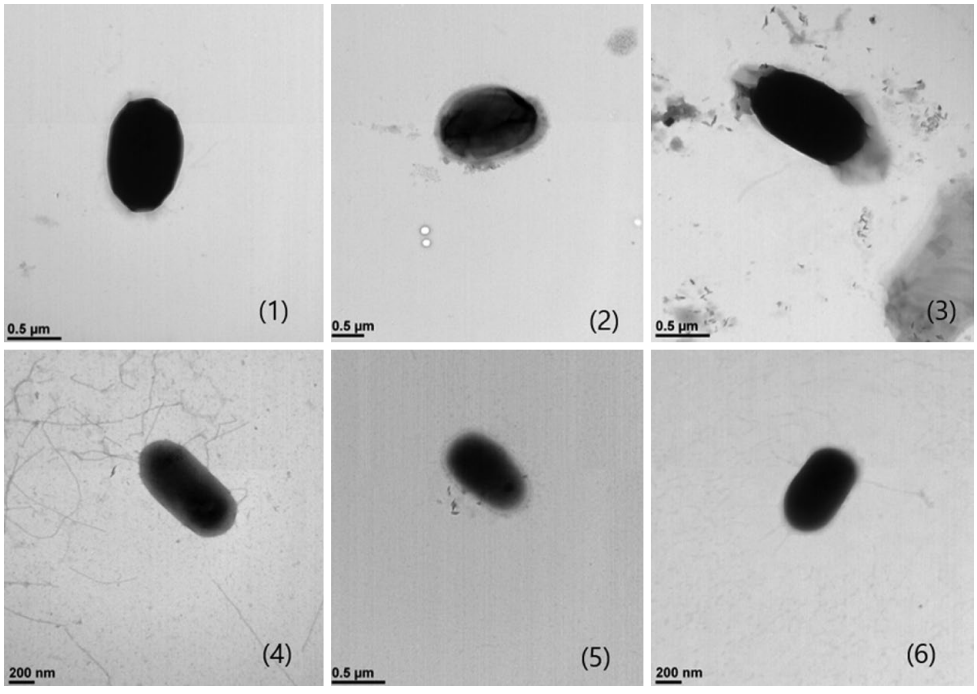

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: 1, 18JY42-3; 2, 18SH; 3, 18JY76-11; 4, 17J11-11; 5, 18JY14-14; 6, 18JY15-11.

Materials and Methods

Different soil samples were collected, serially diluted in distilled water and spread on to R2A agar (Difco, Beckton, Dickinson and Co., Spake, MD, U.S.A.) and incubated at 25°C for 3 days. The designated strain IDs, isolation sources, growth media, and incubation conditions are summarized in Table 1. All strains were purified as a single strain and stored in 20% glycerol suspension at −80°C as well as freeze-dried ampoules.

The cell size and morphology of the strains were observed by transmission electron microscopy (LIBRA 120, Carl Zeiss using cells grown for 3 days at 25°C on R2A agar. TEM images of the strains are shown in Fig. 1. Gram reaction was performed using a Gram staining kit following the manufacturer's instructions (BioMérieux). 16S rRNA gene was extracted and amplified by PCR with 518F, 785F, 800R and 907R universal primers (Weisburg *et al*., 1991). The 16S rRNA gene sequences were aligned using SeqMan software (DNASTAR Inc., USA) and 16S rRNA gene sequences of related taxa were obtained using Ezbioclud and analyzed by EZeditor2 program. Multiple alignments were performed with the Clustal_W program. The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) in the MEGA7 program (Tamura, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

The survival rate after exposure to UV radiation was measured in the early stationary phase of the cells (≈10^9 c.f.u. mL^{-1}) in tryptone glucose yeast broth (Difco). Cells

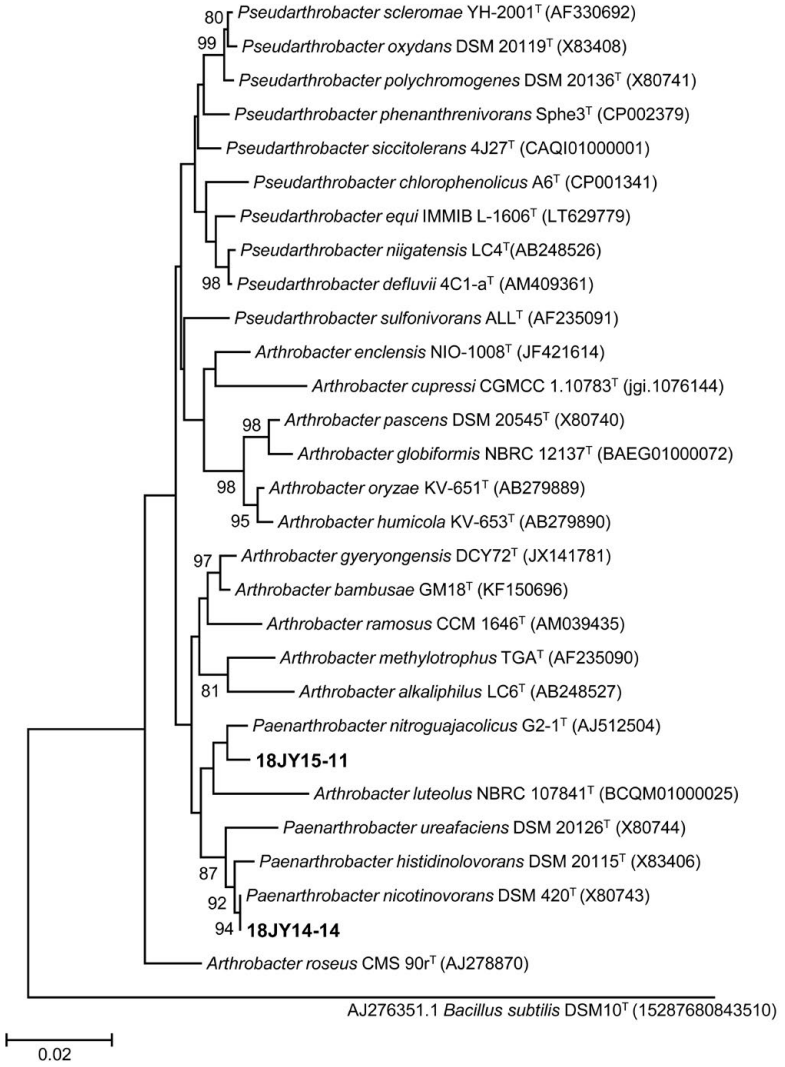

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Paenarthrobacter*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively. *Bacillus subtilis* DSM10T is used as an outgroup.

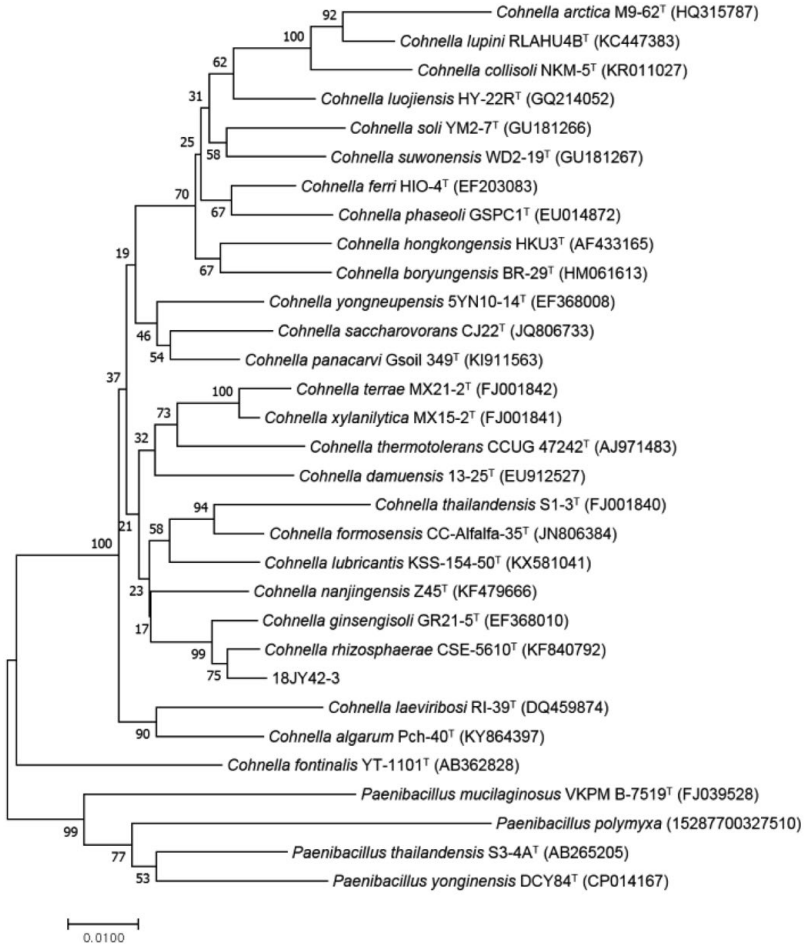

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Cohnella*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively. *Paenibacillus polymyxa* IAM 13419^T is used as an outgroup.

were irradiated with a UVC UV cross-linker (UVP, CX-2000) at 254 nm was 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. A positive control, *Deinococcus radiodurans* R1^T (DSM 20539^T), and a negative control, *Escherichia coli* K-12 (=KCTC 1116), were used for comparison (Kämpfer *et al*., 2008). The numbers 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, six previously unreported bacterial species were identified. The taxonomic composition and identification results are summarized in Table 1. The six strains were assigned to the family *Paenibacillaceae* (18JY42-3), *Methylobacteriaceae* (18SH and 18JY76-11), *Microbacteriaceae* (17J11-11), and *Micrococcaceae* (18JY14-14 and 18JY15-11). At the generic level, the strains 18JY42-3, 18SH, 18JY76-11, 17J11-11, 18JY14-14, and 18JY15-11 were closely related to seven different genera, *Cohnella rhizosphaerae* (MH497628; 98.8%), *Methylobacterium goesingense* (MH497632; 99.1%), *Methylobacterium populi* (MH497635; 99.9%), *Microterricola gilva* (MH504108; 98.4%), *Paenarthrobacter nicotinovorans* (MH497641; 100%), and *Paenarthrobacter nitroguajacolicus* (MH497646; 99.2%), respectively. The identification of the isolates based on 16S rRNA sequence similarity was supported by the phylogenetic trees. The neighbor-joining trees with the closely related type strains of validly published species are given in the Fig. 1. The cells of the strains 18JY42-3, 18SH, 18JY76-11, 17J11-11, 18JY14-14, and 18JY15-11 showed resistance to UV radiation (Fig. 6). The detailed morphological and physiological characteristics are specified in the strain

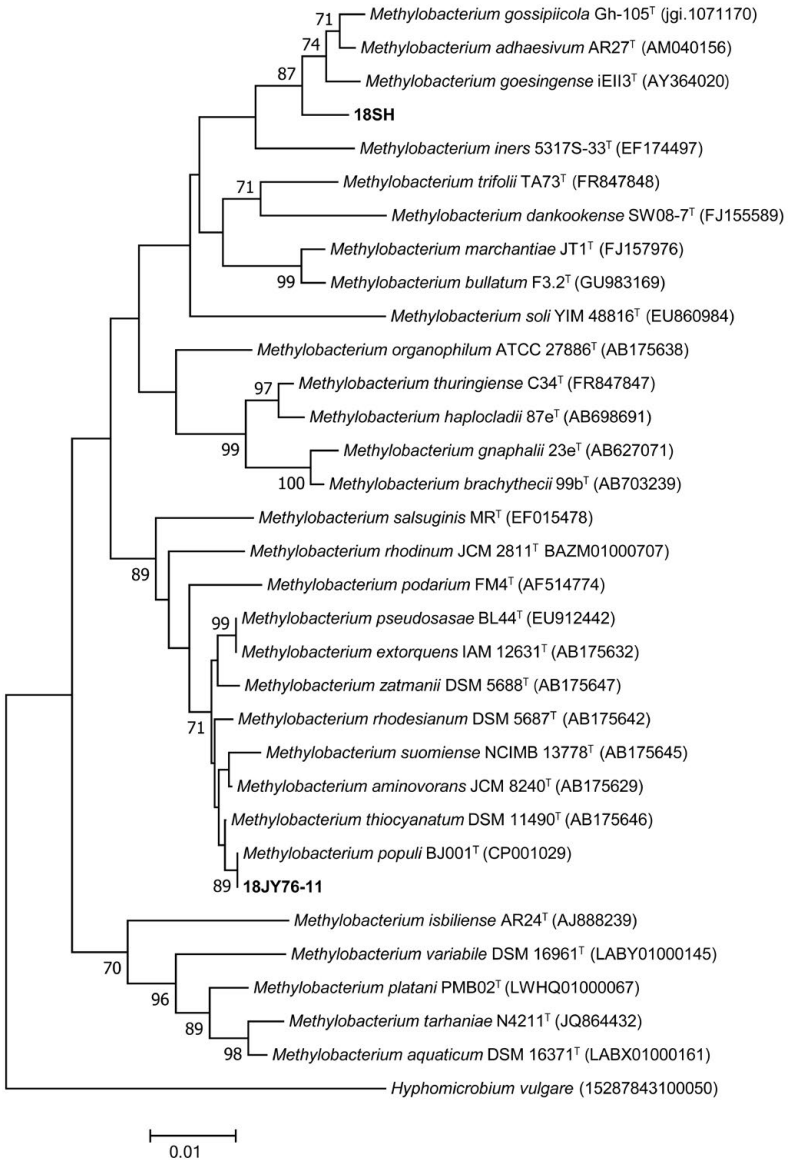

Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Methylobacterium*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions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

description section.

Description of *Cohnella rhizosphaerae* 18JY42-3

Cells are Gram-stain-positive, aerobic and short rod-shaped. Colonies grown on R2A agar are circular, convex and beige after 3 days of incubation on R2A at 25°C.

In API 20NE system, positive for the reduction of urease, esculin hydrolysis, β-galactosidase, D-glucose, D-mannose, and D-mannitol. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), Indole production on tryptophan, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, L-arabinose, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Weak positive for D-maltose.

In API 32GN system positive for L-rhamnose, D-maltose, suberic acid, sodium malonate, L-alanine, glycogen, 3-hydroxybenzoic acid, D-mannitol, D-glucose, salicin, D-melibiose, L-arabinose, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid, and L-proline.

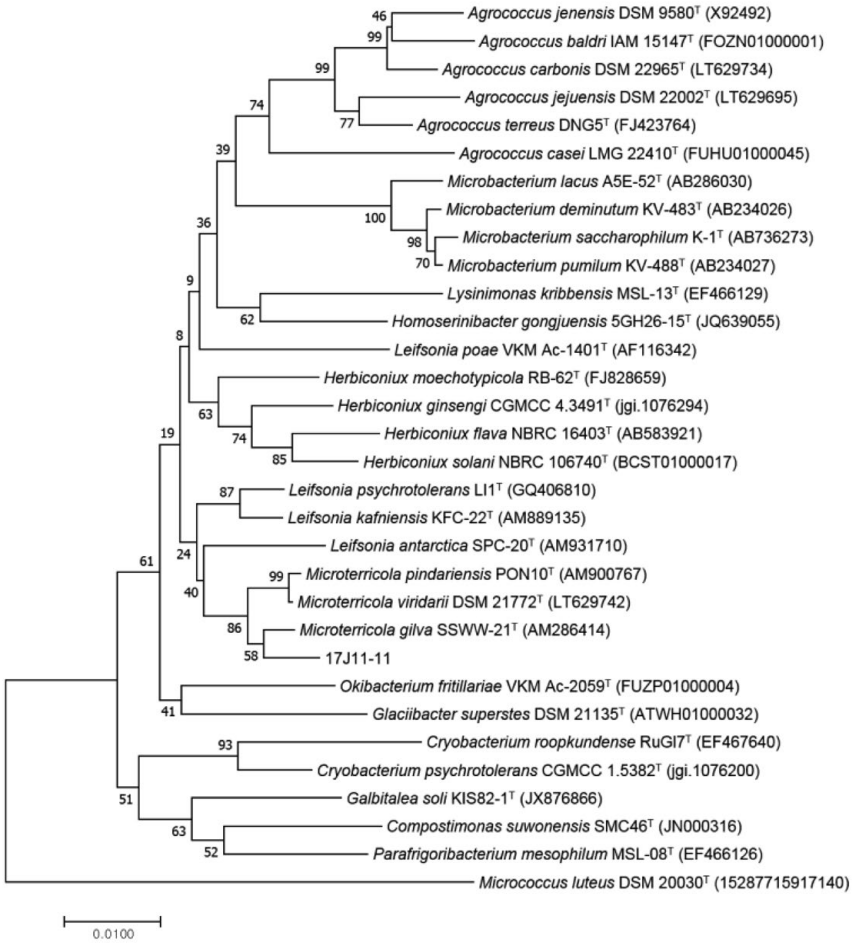

Fig. 5. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Microterricola*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 and 0.02 substitutions per nucleotide position, respectively. *Micrococcus luteus* DSM 20030^T is used as an outgroup.

Negative for *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), itaconic acid, sodium acetate, lactic acid, potassium 5-ketogluconate, L-serine, D-fucose, D-sorbitol, and 3-hydroxybutyric acid. G+C mol for strain 18JY42-3 is 59.8%. Cells showed resistance to UV radiation. Strain 18JY42-3 (=NIBRBA0000116021) was isolated from a soil sample in Korea.

Description of *Methylobacterium goesingense* 18SH

Cells are Gram-stain-negative, aerobic and short rod-shaped. Colonies are pink-colored and circular after 3 days of incubation on R2A at 25°C.

In API 20NE system, positive for the reduction of nitrates (NO_3) to nitrite (NO_2^-), arginine dihydrolase, urease, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, and trisodium citrate. Negative for reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, gelatin hydrolysis, β-galactosidase and capric acid. Weak positive for esculin hydrolysis and phenylacetic acid.

In API 32GN system, positive for 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, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, and 3-hydroxybutyric acid. Negative for 3-hydroxybenzoic acid, capric acid, 4-hydroxybenzoic acid, and L-proline. G+C mol for strain 18SH is 66.7%. Cells showed resistance to UV radiation. Strain 18SH (=NIBRBA0000116023) was isolated from a soil sample in Korea.

Description of *Methylobacterium populi* 18JY76-11

Cells are Gram-stain-negative, aerobic, and short rod-shaped. Colonies are pink-colored and circular after 3

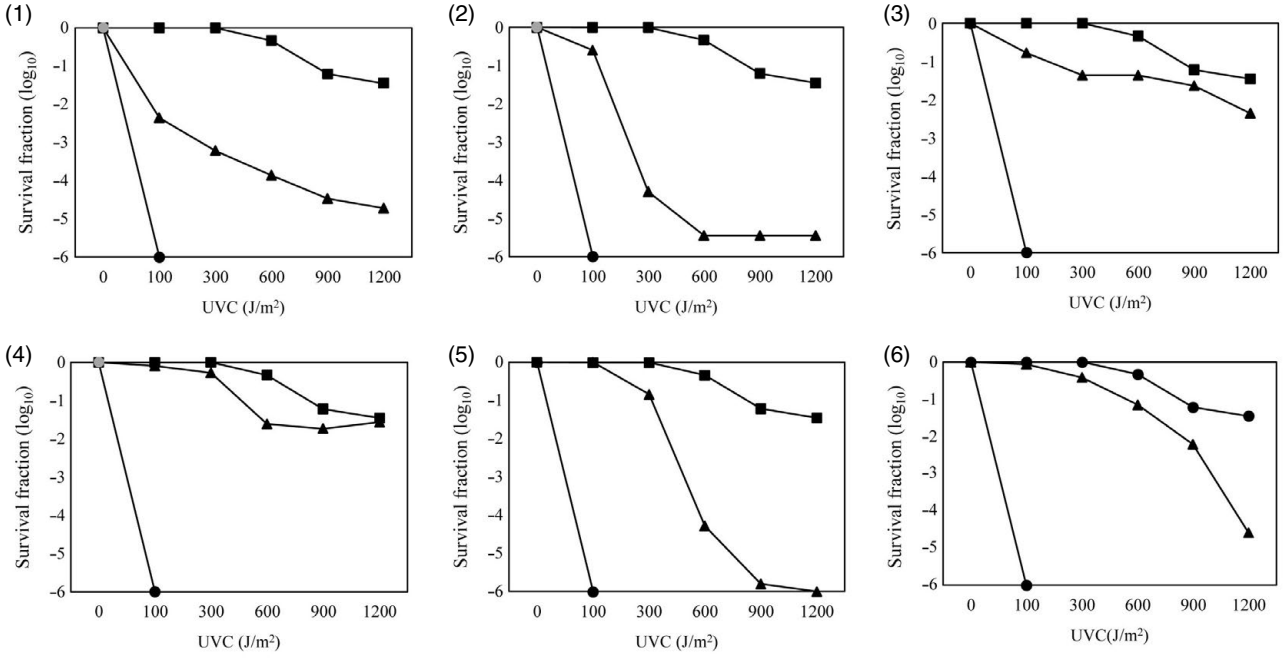

Fig. 6. Representative survival curve of strains (▲) following exposure to UV radiation, with a positive control, *D. radiodurans* R1 (●) and a negative control, *Escherichia coli* (■). Each increment on the y-axis represents a tenfold reduction in viability. (1) 17JY11-11; (2) 18JY14-1; (3) 18JY42-3; (4) 18JY76-11; (5) 18JY15-11 and (6) 18SH.

days of incubation on R2A at 25°C.

In API 20NE system, positive for the reduction of nitrates (NO_3) to nitrite (NO_2^-), urease, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, and trisodium citrate. Negative for the reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and capric acid. Weak positive for assimilation of phenylacetic acid.

In API 32GN system, posiive for L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), D-maltose, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline. Negative for capric acid and 4-hydroxybenzoic acid. G+C mol for strain 18JY76-11 is 70.4%. Cells showed resistance to UV radiation. Strain 18JY76-11 (=NIBRBA0000116030) was isolated from a soil sample in Korea.

Description of *Microterricola gilva* 17J11-11

Cells are Gram-stain-positive, aerobic, 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 reduction of nitrates (NO_3) to nitrogen (N_2), arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, D-glucose, L-arabinose, *N*-acetyl-D-glucosamine, D-maltose, and potassium gluconate. Negative for the reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production on tryptophan, glucose fermentation, gelatin hydrolysis, D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Weakly positive for assimilation of D-mannose.

In API 32GN system, positive for 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, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid and trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. Negative for L-proline. G+C mol for strain 17J11-11 is 69.2%. Cells showed resistance to UV radiation. Strain 17J11-11 (=NIBRBA0000116032) was isolated from a soil sample in Korea.

Description of *Paenarthrobacter nicotinovorans* 18JY14-14

Cells are Gram-stain-positive, aerobic, and short rod-shaped. Colonies are white-colored after 3 days of incu-

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

In API 32GN system, positive for L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), D-maltose, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline. Negative for itaconic acid and capric acid. G+C mol for strain 18JY14-14 is 59.8%. Cells showed resistance to UV radiation. Strain 18JY14-14 (=NIBRBA0000116008) was isolated from a soil sample in Korea.

Description of *Paenarthrobacter nitroguajacolicus* 18JY15-11

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

In API 32GN system, positive for L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose (sucrose), D-maltose, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, and L-proline. Negative for itaconic acid and capric acid. G+C mol for strain 18JY15-11 is 62.6%. Cells showed resistance to UV radiation. Strain 18JY15-11 (=NIBRBA0000116011) was isolated from a soil sample in Korea.

Acknowledgements

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