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

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Eight bacterial strains, 18JY8-13, 18JY13-16, 18JY43-7, 18JY12-7, 18JY1-1, 18JY1-7, 18JY15-3, and 18JY7-2 assigned to the phylum *Firmicutes* were isolated from a variety of soil samples collected in the Jeju Island, Korea. Cells of the eight strains were Gram-positive, aerobic and showed resistant to UV-radiation. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strains 18JY8-13, 18JY13-16, 18JY43-7, 18JY12-7, 18JY1-1, 18JY1-7, 18JY15-3, and 18JY7-2 were most closely related to *Bacillus paranthracis* (99.9%), *Bacillus paramycoides* (99.6%), *Bacillus australimaris* (99.9%), *Bacillus wiedmannii* (100%), *Bacillus halosaccharovorans* (99.6%), *Bacillus deserti* (98.7%), *Bacillus cereus* (99.8%), and *Bacillus albus* (100%), respectively. This is the first report of these eight species in Korea.

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

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Introduction

The eight unrecorded bacterial strains, 18JY8-13, 18JY 13-16, 18JY43-7, 18JY12-7, 18JY1-1, 18JY1-7, 18JY15-3, and 18JY7-2 were isolated from soil on Jeju Island, Korea. These eight strains were assigned to the genus *Bacillus* in the family *Bacillaceae*, phylum *Firmicutes*. The *Firmicutes* are a phenotypically diverse prokaryotic taxon. The *Firmicutes* are Gram-positive, endospore-forming bacteria with genomes characterized by low DNA G+C content (Ludwig *et al*., 2009). Until now (June 2018), the phylum *Firmicutes* consisted of seven classes (*Bacilli, Clostridia, Erysipelotrichia, Limnochordia, Negativicutes, Thermolithobacteria*, and *Tissierellia*), 13 orders, 45 families, and 421 genera (http://www.bacterio.net/-classifphyla.html#bacillus). In Korea, *Firmicutes* species isolated from various environments in Korea have been reported from 2000 and 187 species are validated until 2017 (Seong *et al*., 2018). All reported *Firmicutes* species in Korea, were affiliated with three classes (*Bacilli, Clostridia*, and *Erysipelotrichia*), four orders (*Bacillales, Lactobacillales, Clostridiales*, and *Erysipelotrichales*), 17 families, and 54 genera (Seong *et al*., 2018).

The *Bacillus* species are Gram-positive, rod-shaped and strictly aerobic or facultatively anaerobic bacteria that form heat-resisting endospores. Currently, there are 377 described species and eight subspecies belonging to the phylum *Firmicutes* (http://www.bacteria.net/bacillus.html). Most of the *Bacillus* species are ubiquitous and have been isolated from a wide variety of aquatic and terrestrial environments, ranging from sewage sludge (Demharter and Hensel, 1989), ocean sediments (Ru?ger *et al*., 2000) and saline water (Smibert and Krieg, 1994) to desert soils (Roberts *et al*., 1994). Among the *Firmicutes*, the family *Bacillaceae* was the most abundant family with 57 genera (http://www.bacteria.net/). Here we briefly described eight unreported bacterial strains belong to the family *Bacillaceae*, isolated from the soil on Jeju Island, Republic of Korea.

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

Strain ID	Most closely related species	Accession number	Similarity (%)	Isolation source	Medium	Incubation conditions
18JY8-13	*Bacillus paranthracis*	(KJ812420)	99.9	Soil	R2A	25°C, 3 d
18JY13-16	*Bacillus paramycoides*	(KJ812444)	99.6	Soil	R2A	25°C, 3 d
18JY43-7	*Bacillus australimaris*	(NZ_LGYN00000000)	99.9	Soil	R2A	25°C, 3 d
18JY12-7	*Bacillus wiedmannii*	(KU198626)	100	Soil	R2A	25°C, 3 d
18JY1-1	*Bacillus halosaccharovorans*	(HQ433447)	99.6	Soil	R2A	25°C, 3 d
18JY1-7	*Bacillus deserti*	(GQ465041)	98.7	Soil	R2A	25°C, 3 d
18JY15-3	*Bacillus cereus*	(AE016877)	99.8	Soil	R2A	25°C, 3 d
18JY7-2	*Bacillus albus*	(KJ812440)	100	Soil	R2A	25°C, 3 d

Materials and Methods

The bacteria isolated from the soil samples collected on Jeju Island (GPS: 33°30′35.0″N 126°31′19.0″E), Korea. The 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. The designated strain name, isolation sources, growth media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and stored in 20% glycerol suspension at −80°C as well in freeze-dried ampoules. 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 R2A agar. Transmission electron micrograph of the strains are shown in Fig. 1. Gram reaction was tested following the classic Gram procedure described by Doetsch (1981).

Biochemical characteristics were performed using Biolog Microstation with the 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 the inoculate was 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 the growth characteristics. MicroPlates were read in the MicroStation semi-automated reader after 24 h and the results were interpreted using identification system's software (GEN III database, version 5.2.1). The system indicates the isolates which could not be identified after 20 h, are subjected to further incubation. Consequently, 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 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 with the MUSCL program (Edgar, 2004). Using the two-parameter model (Kimura, 1983), the evolutionary distances were calculated. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) in the MEGA5 program (Tamura, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

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

The eight unreported bacterial species were identified from the soil on Jeju Island, Korea. The taxonomic composition and identification results are summarized in Table 1. Based on 16S rRNA gene sequence similarity, the nine strains were belonging to the genus *Bacillus*, to the family *Bacillaceae* in the phylum *Firmicutes*. The neighbor-joining trees showed the close relationship between the new strains and the type strains of validly published species in Fig. 2.

The 16S rRNA similarity of the strains, 18JY8-13, 18JY 13-16, 18JY43-7, 18JY12-7, 18JY1-1, 18JY1-7, 18JY15-3, and 18JY7-2 were closely related to be *Bacillus paranthracis* (KJ812418; 99.9%), *Bacillus paramycoides* (KJ 812444; 99.6%) *Bacillus australimaris* (NR148787; 99.9%) *Bacillus wiedmannii* (KU198626; 100%), *Bacil-*

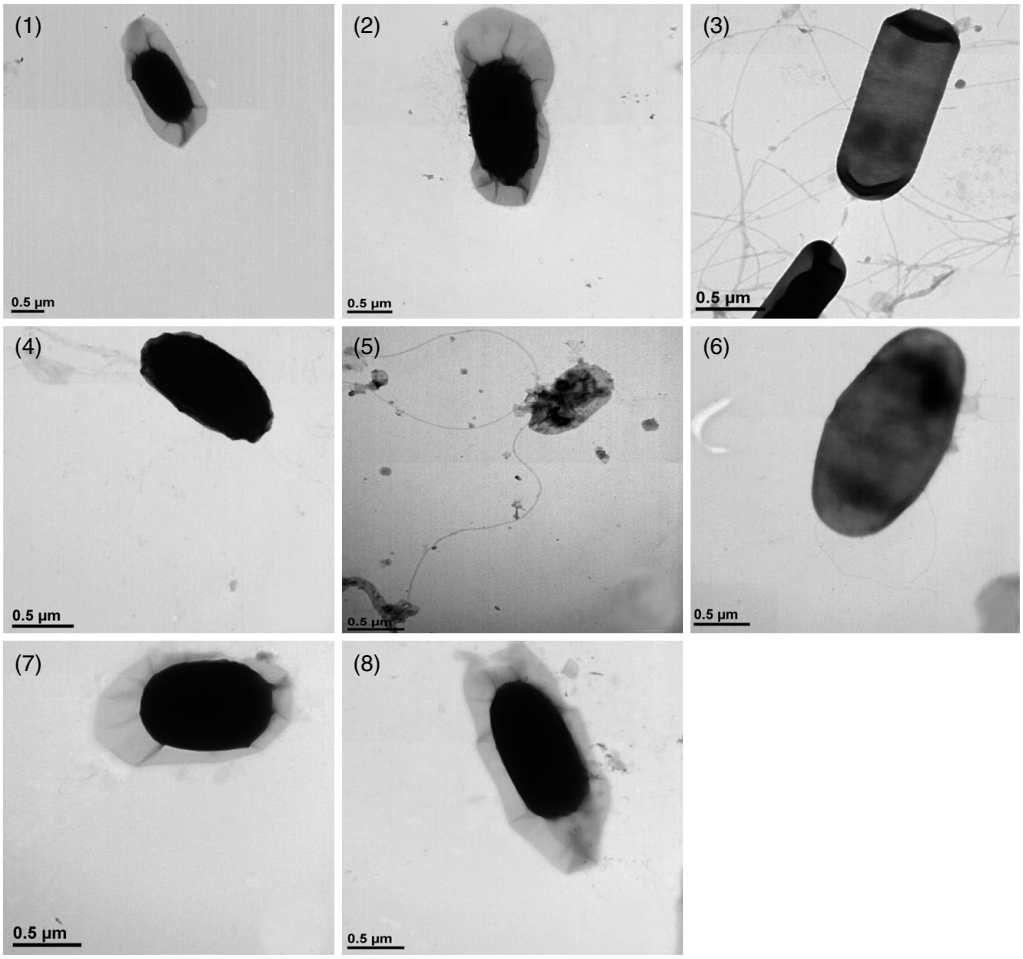

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: 1, 18JY8-13; 2, 18JY13-16; 3, 18JY43-7; 4, 18JY12-7; 5, 18JY1-1; 6, 18JY1-7; 7, 18JY15-3; 8, 18JY7-2.

lus halosaccharovorans (HQ433447; 99.6%) *Bacillus deserti* (GQ465041; 98.7%), *Bacillus cereus* (AE016877; 99.8%) and *Bacillus albus* (KJ812440; 100%), respectively. The strains 18JY8-13, 18JY13-16, 18JY43-7, 18JY12-7, 18JY1-1, 18JY1-7, 18JY15-3, and 18JY7-2, showed resistance to UV radiation (Fig. 3). The detailed morphological and physiological characteristics of all the isolates are given in the strain description section. There are no previous official reports of these nine bacterial strains having been isolated in Korea.

Description of *Bacillus paranthracis* 18JY8-13

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, utilization of *N*-acetyl-D-glucosamine, arginine dihydrolase, D-glucose, esculin hydrolysis, gelatin hydrolysis, malic acid, D-maltose, potassium gluconate, trisodium citrate and urease are positive. L-arabinose, capric acid, β-galactosidase, glucose fermentation, indole production on tryptophan, D-mannitol, D-mannose, reduction of nitrates (NO_3) to nitrite (NO_2^-) and reduction of nitrates (NO_3) to nitrogen (N_2) are negative. In the API 32GN systems, utilization of L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, 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, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline are positive. capric acid was not utilized. G+C content for strain 18JY8-13 is 42.7%. Cells showed resistance to UV radiation.

Strain 18JY8-13 (=NIBRBA0000116012) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus paramycoides* 18JY13-16

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of

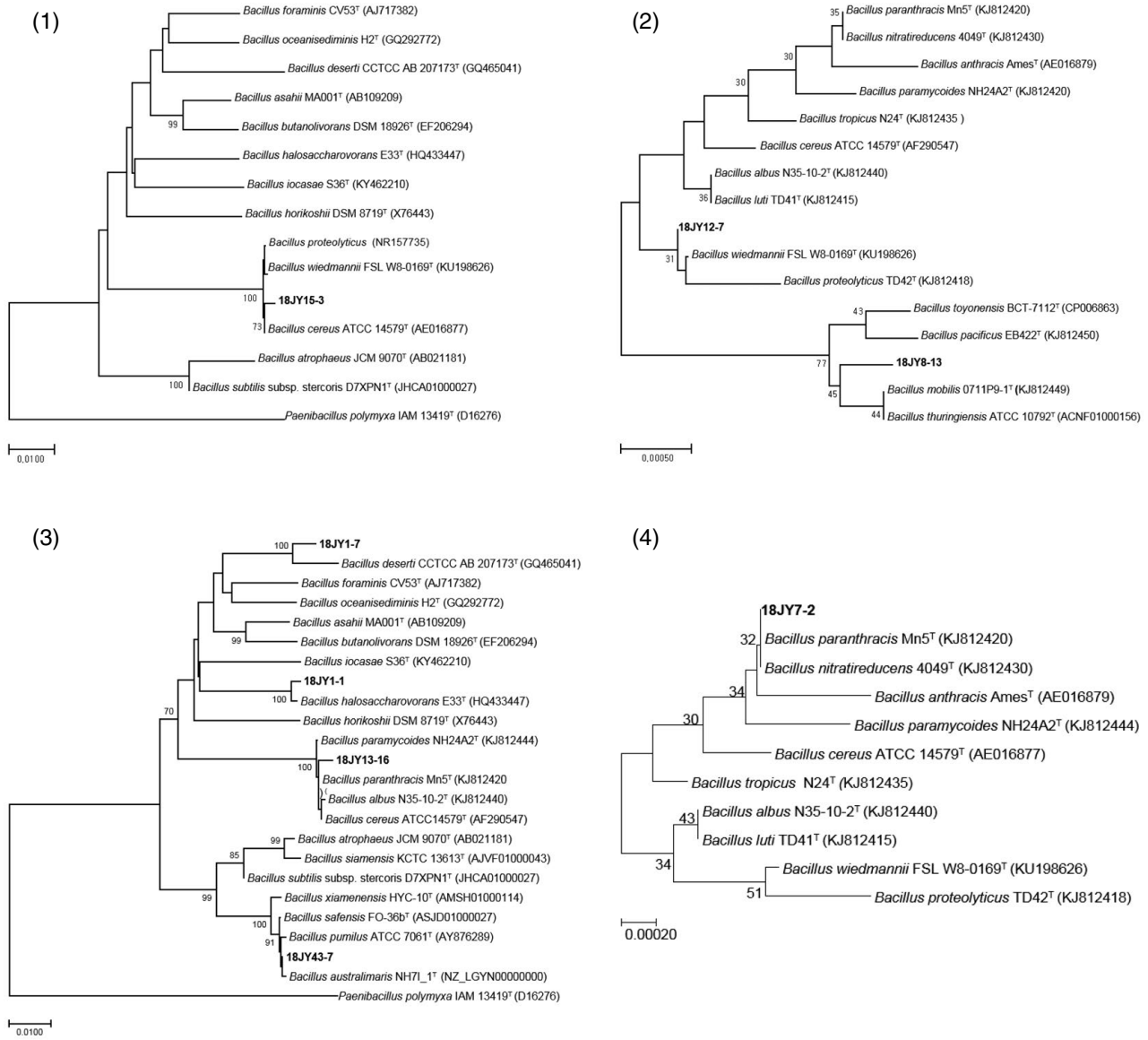

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Bacillus* in the phylum *Firmicutes*. Bootstrap values are shown above nodes for the neighbor-joining methods. Bar: 0.005 substitutions per nucleotide position, respectively. (1) 18JY15-3; (2) 18JY12-7, 18JY8-13; (3) 18JY1-1, 18JY13-16, 18JY43-7, 18JY1-7; (4) 18JY7-2.

incubation on R2A at 25°C. In the API 20NEsystems, utilization of arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, D-glucose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are positive. Reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, β-galactosidase, L-arabinose, D-mannose, D-mannitol, capric acid, adipic acid and phenylacetic acid are negative. In the API 32GN systems, utilization of capric acid, itaconic acid, D-mannitol, capric acid, potassium 2-ketogluconate and 4-hydroxybenzoic acid are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, D-saccharose, D-maltose, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, valeric acid, trisodium citrate, L-histidine, 3-hydroxybutyric acid and L-proline are utilized. G+C content for strain 18JY13-16 is 41.6%. Cells showed resistance to UV radiation.

Strain 18JY13-16 (=NIBRBA0000116013) was isolated from a soil sample, Jeju, Korea.

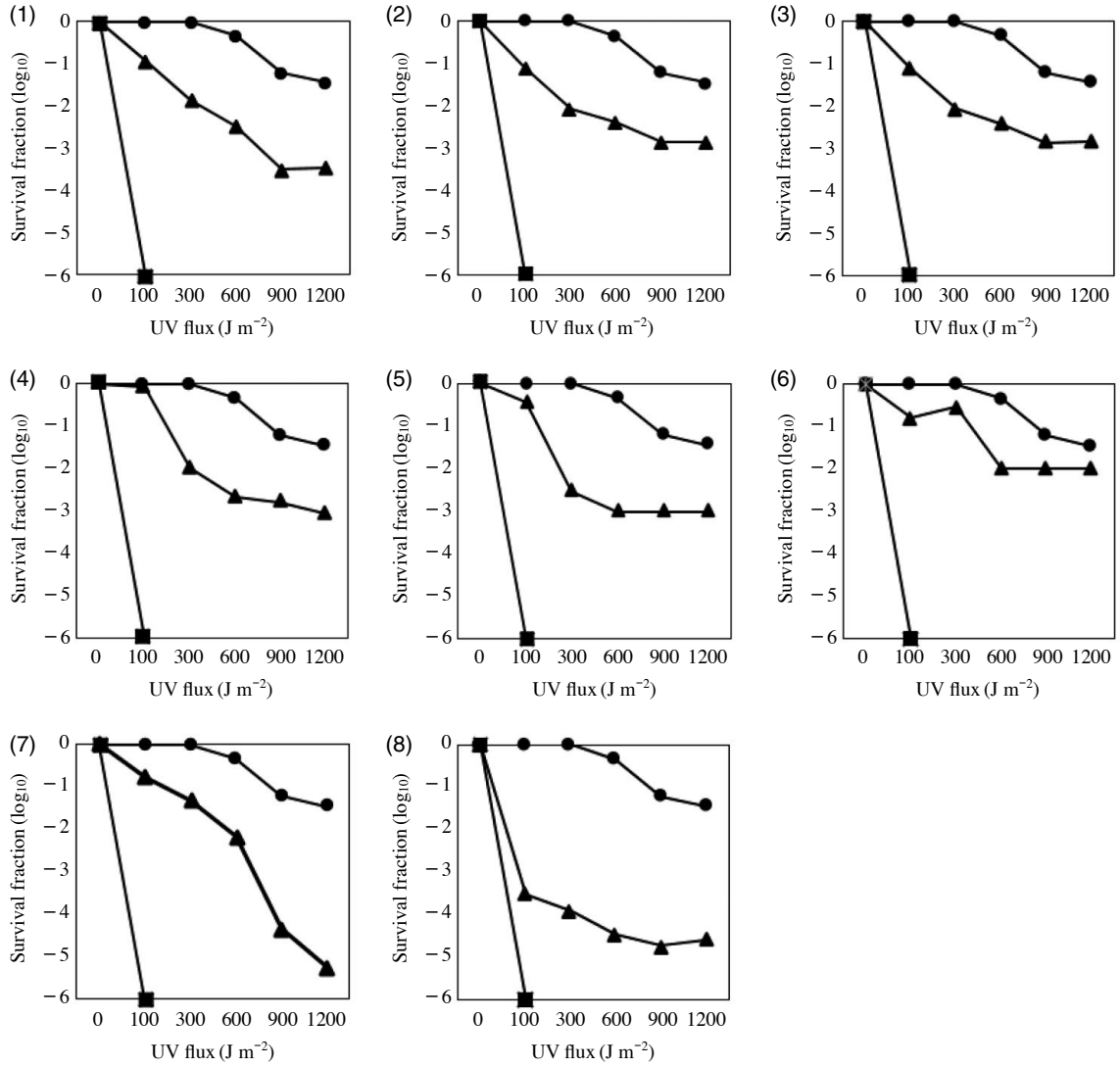

Fig. 3. Representative survival curve of strains (▲) following exposure to UV radiation (0-1,200 J m^{-2}), with a positive control, *D. radiodurans* R1 (●) and a negative control, *Escherichia coli* (■). Each increment on the y-axis represents a tenfold reduction in viability. (1) 18JY8-13; (2) 18JY13-16; (3) 18JY43-7; (4) 18JY12-7; (5) 18JY1-1; (6) 18JY1-7; (7) 18JY15-3; (8) 18JY7-2.

Description of *Bacillus australimaris* 18JY43-7

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine and potassium gluconate are positive. Reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, D-maltose, capric acid, adipic acid and phenylacetic acid are negative. In the API 32GN systems, utilization of itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, D-fucose, D-sorbitol, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, D-maltose, L-serine, D-mannitol, D-glucose, salicin, D-melibiose, L-arabinose, propionic acid, capric acid and valeric acid are utilized. G+C content for strain 18JY43-7 is 40%. Cells showed resistance to UV radiation.

Strain 18JY43-7 (=NIBRBA0000116014) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus wiedmannii* 18JY12-7

Cells are Gram-stain-positive and rod-shaped. Colonies

creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, reduction of nitrates (NO_3) to nitrogen (N_2) and utilization of esculin hydrolysis, gelatin hydrolysis, D-glucose, *N*-acetyl-D-glucosamine, D-maltose, malic acid and trisodium citrate are positive. Reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production on tryptophan, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, L-arabinose, D-mannitol, capric acid and adipic acid are not utilized. In the API 32GN systems, utilization of 3-hydroxybenzoic acid, capric acid, 4-hydroxybenzoic acid are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, 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, 3-hydroxybutyric acid and L-proline are utilized. G + C content for strain 18JY12-7 is 42.4%. Cells showed resistance to UV radiation.

Strain 18JY12-7 (= NIBRBA0000116017) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus halosaccharovorans* 18JY1-1

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, reduction of nitrates (NO_3) to nitrite (NO_2^-), and utilization of esculin hydrolysis, β-galactosidase, L-arabinose, D-mannose, D-mannitol and potassium gluconate are positive. Reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, D-glucose, *N*-acetyl-D-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. In the API 32GN systems, utilization of L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine and L-proline are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, D-maltose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, glycogen, D-mannitol, D-glucose, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid are utilized. G + C content for strain 18JY1-1 is 46.3%. Cells showed resistance to UV radiation.

Strain 18JY1-1 (= NIBRBA0000116018) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus deserti* 18JY1-7

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, utilization of arginine dihydrolase, urease, esculin hydrolysis, D-glucose, D-mannose, D-mannitol, D-maltose are positive. Reduction of nitrates (NO_3) to nitrite (NO_2^-), Reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, gelatin hydrolysis, β-galactosidase, L-arabinose, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid are not utilized. In the API 32GN systems, utilization of itaconic acid, suberic acid, sodium malonate, lactic acid, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, D-fucose, D-sorbitol, L-arabinose, propionic acid, capric acid, valeric acid, 4-hydroxybenzoic acid and L-proline are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, D-maltose, sodium acetate, L-alanine, glycogen, D-mannitol, D-glucose, trisodium citrate, L-histidine, potassium 2-ketogluconate and 3-hydroxybutyric acid are utilized. G + C content for strain 18JY1-7 is 43.0%. Cells showed resistance to UV radiation.

Strain 18JY1-7 (= NIBRBA0000116022) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus cereus* 18JY15-3

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of incubation on R2A at 25°C. In the API 20NE systems, utilization of arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, D-glucose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid andtrisodium citrate are positive. Reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, β-galactosidase, L-arabinose, D-mannose, D-mannitol, capric acid, adipic acid, phenylacetic acid are negative. In the API 32GN systems, D-melibiose, D-fucose, L-arabinose, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, L-proline are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, 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-sorbitol and propionic acid are utilized. G + C content for strain 18JY15-3 is 35.2%. Cells showed resistance to UV radiation.

Strain 18JY15-3 (= NIBRBA0000116028) was isolated from a soil sample, Jeju, Korea.

Description of *Bacillus albus* 18JY7-2

Cells are Gram-stain-positive and rod-shaped. Colonies creamy white, mucoid, and translucent after 3 days of

incubation on R2A at 25°C. In the API 20NE, reduction of nitrates (NO_3) to nitrite (NO_2^-) and utilization of esculin hydrolysis, gelatin hydrolysis, β-galactosidase, D-glucose, L-arabinose, D-mannitol, D-maltose, potassium gluconate, malic acid andtrisodium citrate are positive. Reduction of nitrates (NO_3) to nitrogen (N_2), indole production on tryptophan, glucose fermentation, arginine dihydrolase, capric acid, adipic acid and phenylacetic acid are not utilized. In the API 32GN systems, utilization of 3-hydroxybenzoic acid, capric acid and 4-hydroxybenzoic acid are negative. L-rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-saccharose, 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, 3-hydroxybutyric acid and L-proline are utilized. G+C content for strain 18JY7-2 is 43.2%. Cells showed resistance to UV radiation.

Strain 18JY7-2 (=NIBRBA0000116031) was isolated from a soil sample, Jeju, Korea.

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