A report of 38 unrecorded bacterial species in Korea, belonging to the phylum Actinobacteria

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As a subset work for the collection of indigenous prokaryotic species in Korea, 38 actinobacterial strains were isolated from various environmental samples obtained from plant root, ginseng cultivating soil, mud flat, freshwater and seawater. Each strain showed higher 16S rRNA gene sequence similarity (>99.1%) and formed a robust phylogenetic clade with closest actinobacterial species which were defined and validated with nomenclature, already. There is no official description on these 38 actinobacterial species in Korea. Consequently, unrecorded 37 species of 24 genera in the 12 families belonging to the order Actinomycetales of the phylum Actinobacteria were found in Korea. Morphological properties, basic biochemical characteristics, isolation source and strain IDs are described in the species descriptions.

Keywords: 16S rRNA sequence, Actinobacteria, Actinomycetales, unrecorded species

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INTRODUCTION

In 2013, the authors isolated a great number of novel and unrecorded prokaryotic species from diverse environmental samples in Korea. The identified species belonged to the phyla Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Deinococci and Verrucomicrobia. As a subset of this study, the present report focuses on the description of unrecorded species belonging to the phylum Actinobacteria.

Actinobacteria is a phylum of Gram-stain-positive bacteria with high G+C content in their DNA (Ventura et al., 2007). Members of Actinobacteria are distributed in terrestrial or aquatic environments (Servin et al., 2008). Actinobacteria is one of the dominant phyla and contains one of the largest genera, Streptomyces (LPSN; http://www.bacterio.net/). Most Actinobacteria of medical or economic significance belong to the order Actinomycetales (Miao and Davies, 2010). While many of these cause disease in humans, Streptomyces is notable as a source of antibiotics (Gupte et al., 2002; Mahajan, 2012). Actinobacteria, especially Streptomyces spp., are recognized as the producers of many bioactive metabolites that are useful to humans in medicine, such as antibacterial, antifungal, antiviral, immunomodifiers, anti-tumor drugs and enzyme inhibitors; and in agriculture, including insecticides, herbicides, fungicides and growth promoting substances for plants and animals (Bressan, 2003). Actinobacteria also contributes to the biogeochemical cycling of soil and then to the crop production.

Until 2013, 228 Korean indigenous species belonging
to the phylum Actinobacteria were isolated, described and validated according to the List of Prokaryotic name with Standing in Nomenclature (LPSN; http://www.bacterio.net/). As a part of results obtained from the research program supported by NIBR, the present report focuses on the description of bacterial species belonging to the phylum Actinobacteria except suborder Streptomycineae, which have not been previously isolated in Korea. Here we report 38 unrecorded Actinobacterial strains in Korea.

**Materials and Methods**

A total of 45 bacterial strains assigned to the phylum Actinobacteria were isolated from various environmental samples collected from plant root, ginseng cultivating soil, mud flat, freshwater and seawater (Table 1). Each environmental sample was processed separately, spread onto diverse culture media (Becton Dickinson) including R2A, glucose yeast extract (Gordon and Mihm, 1962), marine, tryptic soy and nutrient agars and incubated at 25-30°C for 2-5 days. All strains were purified as single colonies and stored as 10-20% glycerol suspension at −80°C as well as lyophilized ampoules. Among them 7 strains produced aerial mycelium, belonged to the suborder Streptomycineae and were excluded in this work.

Colonial morphology of the strains was observed on agar plates with a magnifying glass after cells grew up to stationary phase. Cellular morphology and cell size were examined by either transmission electron microscopy or scanning electron microscopy (Fig. 1). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer’s instructions.

Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using the standard procedures described elsewhere. The 16S rRNA gene sequences of the strains assigned to the phylum Actinobacteria were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzTaxon-e server (Kim et al., 2012). For phylogenetic analyses, multiple alignments were performed using the Clustal_W program (Thompson et al., 1994) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Jukes-Cantor model (Jukes and Cantor, 1969). The phylogenetic trees were constructed by using the neighbour-joining (Saitou and Nei, 1987), the maximum-likelihood (Felsenstein, 1981) and the maximum-parsimony (Fitch, 1971) methods with the MEGA 6.0 Program (Tamura et al., 2013) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

**Results and Discussion**

All the 38 strains belonged to order Actinomycetales and affiliated to 12 families: 1 strain for Corynecbacteriaceae, 7 strains for Mycobacteriaceae, 2 strains for Nocardiaceae, 1 strain for Cellulomonadaceae, 4 strains for Intrasporangiaceae, 12 strains for Microbacteriaceae, 2 strains for Micrococccaeae, 2 strains for Promicromonosporaceae, 1 strain for Sanguibacteraceae, 1 strain for Micromonosporaceae, 4 strains for Nocardioidaceae and 1 strain for Geodermatophilaceae (Table 1).

All the strains were isolated from diverse environments and regions: 17 strains from fresh water, 6 strains from rhizosphere, 6 strains from ginseng cultivated soil, 4 strains from brackish water or lagoon, 3 strains from tidal flat sediment, each one strain from seawater dust; 13 strains from Gyeongnam, 9 strains from Daejeon, 6 strains from Gyeonggi, 3 strains from Jeonnam, each 2 strains from Jeonbuk, Ganfwon and Jeju, and one strain from Gyeongbuk.

These strains were Gram-staining-positive and chemoheterotrophic. Fig. 2 shows phylogenetic assignment of the strains based on 16S rRNA gene sequences.

Here we report 38 unrecorded bacterial species in Korea belonging to the phylum Actinobacteria.

**Description of Corynecbacterium doosanense W2-3-5**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex and entire after 2 days of incubation on R2A at 25°C. Positive for nitrate reduction and oxidase in API 20NE but negative for esculin hydrolysis, urease, gelatinase, β-galactosidase, indole production, glucose fermentation and arginine dihydrolase. D-Glucose, L-arabinose, D-mannose, D-mannitol, β-galactosidase, indole production, glucose fermentation and arginine dihydrolase. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain W2-3-5 (= NIBRBA0000114209) has been isolated from mineral water, Daejeon, Korea.

**Description of Mycobacterium aurum WM31**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are entire, opaque and orange colored after 7 days on R2A at 25°C. Negatives for nitrate reduction, Indole production, glucose fermentation, gelatin hydrolysis, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate and malic acid are not utilized. Strain WM31 (= NIBRBA0000114426) has been isolated from freshwater, Woopo wetland, Gyeongnam Province, Korea.
Table 1. Taxonomic affiliation and isolation information of the isolates belonging to the phylum Actinobacteria.

| Suborder                  | Family         | Genus             | Strain ID | NIBR NO.     | Most closely related species | Similarity (%) | Isolation Source | Medium | Incubation condition |
|--------------------------|----------------|-------------------|-----------|--------------|------------------------------|----------------|------------------|--------|----------------------|
| Corynebacterineae        | Corynebacteriaceae | Corynebacterium   | W2-3-5    | NIBRBA0000114209 | Corynebacterium doosanense CAU 212 | 99.3           | Mineral water     | R2A    | 25°C, 2 d           |
| Mycobacterineae          | Mycobacterium  | WM31              | NIBRBA000011426 | Mycobacterium aurum ATCC 23366 | 99.9 | Freshwater | R2A | 25°C, 7 d |
|                          |                | WS30              | NIBRBA0000114431 | Mycobacterium chubuense ATCC 27278 | 99.6 | Freshwater | R2A | 25°C, 3 d |
|                          |                | WM81              | NIBRBA0000114428 | Mycobacterium flavescens ATCC | 99.2 | Freshwater | R2A | 25°C, 7 d |
|                          |                | WM69              | NIBRBA0000114427 | Mycobacterium gilvum ATCC 43009 | 99.3 | Freshwater | R2A | 25°C, 7 d |
|                          |                | ST5-11            | NIBRBA0000114183 | Mycobacterium lutzerense MG15 | 99.2 | Plant root | TSA | 30°C, 2 d |
|                          |                | W2-2-3            | NIBRBA0000114211 | Mycobacterium obumens ATCC 27023 | 99.8 | Mineral water | R2A | 25°C, 2 d |
|                          |                | 01SU3             | NIBRBA0000114438 | Mycobacterium salaminis YIM | 99.6 | Freshwater | R2A | 25°C, 3 d |
| Nocardiae                | Nocardia       | W4-2-4            | NIBRBA0000114210 | Nocardia abscess NRC 100374 | 99.9 | Mineral water | R2A | 25°C, 2 d |
| Williamsia               |                | BM14              | NIBRBA0000114305 | Williamsia faeni N1390 | 99.2 | Mud flat | MA | 30°C, 3 d |
| Micrococineae            | Cellulosonaeae | Cellulosomona     | DT5-10    | NIBRBA0000114179 | Cellulosomona paksitanensis NCCP-11 | 99.6 | Plant root | TSA | 30°C, 2 d |
|                         | Arsenicoccus   | WM79              | NIBRBA0000114441 | Arsenicoccus boldiensis CCUG | 99.9 | Freshwater | R2A | 25°C, 3 d |
| Terrabacter              |                | WM26              | NIBRBA0000114440 | Terrabacter ginsenosidomutans Gsoil 3082 | 99.9 | Freshwater | R2A | 25°C, 7 d |
|                         |                | MS4Y-2-4          | NIBRBA0000114281 | Terrabacter korensis THG-e54 | 99.4 | Ginseng cultivating | MA | 30°C, 3 d |
| Microbacterineae         | Agreia         | JW4-5             | NIBRBA0000114437 | Terrabacter tumescens DSM 20308 | 99.3 | Fresh water | R2A | 25°C, 5 d |
|                         | Agroccus       | J0903             | NIBRBA0000114154 | Agrostis pratensis F229/10 | 99.8 | Freshwater | R2A | 25°C, 2 d |
|                         | Agromyces      | MM3               | NIBRBA0000114446 | Agromyces venetus DSM 9580 | 100 | Dust | MA | 25°C, 2 d |
| Frondihabitans           |                | MMD-3Y-10-4       | NIBRBA0000114276 | Agromyces cerinus subsp. cerinus DSM 8995 | 99.9 | Ginseng cultivating | MA | 30°C, 3 d |
| Hericobacter             |                | TMIL-2            | NIBRBA0000114323 | Frondihabitans cladoniphilus Caft 13 | 99.9 | Freshwater | R2A | 25°C, 3 d |
| Leifsonia                |                | CRM-11            | NIBRBA0000114200 | Hericobacter ginseng wged1 | 99.6 | Plant root | R2A | 25°C, 3 d |
| Microbacterium           |                | WM113             | NIBRBA0000114429 | Microbacterium deuranadyicum DSM 8607 | 99.0 | Freshwater | R2A | 25°C, 3 d |
| Rathayibacter            |                | 03SU14            | NIBRBA0000114439 | Rathayibacter tritici DSM 7486 | 99.1 | Freshwater | R2A | 25°C, 3 d |

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Description of *Mycobacterium chubuense* WS50

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are entire, convex, opaque and yellow colored after 3 days on R2A at 25°C. Positive for nitrate production in API 20NE, but negative for glucose fermentation, urease, esculin hydrolysis, β-galactosidase, indole production, arginine dihydrolase and gelatinase. D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain WS50 (NIBRBA0000114431) has been isolated from freshwater, Woopó wetland, Gyeongnam Province, Korea.

Description of *Mycobacterium flavescens* WM81

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are entire, convex, opaque and yellow colored after 7 days on R2A at 25°C. In API 20NE gallery, negative for urease, esculin hydrolysis, glucose fermentation and β-galactosidase, nitrate reduction, indole production, arginine dihydrolase and gelatinase. Does not utilize L-arabinose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid but utilize D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose and potassium gluconate. Strain WM81 (NIBRBA0000114428) has been isolated from freshwater, Woopó wetland, Gyeongnam Province, Korea.

Description of *Mycobacterium gilvum* WM69

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are entire, convex, opaque and yellow colored after 7 days on R2A at 25°C. In API 20NE gallery, negative for urease, esculin hydrolysis, glucose fermentation and β-galactosidase, nitrate reduction, indole production, arginine dihydrolase and gelatinase. D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, D-mannitol, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain WM69 (NIBRBA0000114427) has been isolated from freshwater, Woopó wetland, Gyeongnam Province, Korea.

Description of *Mycobacterium llatzerense* ST5-11

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are entire, glistening and orange colored after 2 days on TSA at 30°C. Positive for urease and esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. Utilize D-glucose, L-arabinose, D-mannitol, D-maltose, but not utilize D-mannose, N-acetyl-glucosamine, suborder, family, genus, strain ID, NIBR NO, most closely related species, similarity (%), isolation source, medium, incubation condition.

| Suborder | Family | Genus | Strain ID | NIBR NO. | Most closely related species | Similarity (%) | Isolation |
|----------|--------|-------|-----------|----------|-----------------------------|----------------|----------|
| Micrococceae | Micrococcaceae | Arthrobacter | CDR5 | NIBRBA0000114311 | Arthrobacter aribii DSM 20550 | 99.6 | Brackish water R2A | 30°C, 3 d |
| Micrococceae | Micrococcaceae | Arthrobacter | SR4-01 | NIBRBA0000114197 | Arthrobacter aribii DSM 20550 | 99.6 | Plant root R2A | 30°C, 3 d |
| Promicromonosporaceae | Isoptericola | Isopetiercola | BM28 | NIBRBA0000114308 | Isopetiercola manyanensis H17T | 99.3 | Mud flat MA | 30°C, 3 d |
| Promicromonosporaceae | Promicromonosporaceae | Promicromonosporaceae | MMD3Y-15-4 | NIBRBA0000114278 | Promicromonosporaceae subline IFO 14650 | 99.6 | Ginseng cultivating field Lagoon R2A | 30°C, 3 d |
| Sanguibacteraceae | Sanguibacter | Sanguibacter | HME9278 | NIBRBA0000114396 | Sanguibacter kiedleri DSM 10542 | 99.8 | Plant root R2A | 30°C, 3 d |
| Micromonosporaceae | Micromonosporaceae | Micromonosporaceae | DR7-01 | NIBRBA0000114191 | Micromonosporaceae sedimentsal SH2-13T | 99.8 | Plant root R2A | 30°C, 3 d |
| Propionibacterineae | Propionibacteriaceae | Propionibacteriaceae | Good012 | NIBRBA0000114219 | Propionibacteriaceae alaminosa HKI 0478 | 99.4 | Ginseng cultivating field R2A | 25°C, 2 d |
| | | Propionibacteriaceae | WW39 | NIBRBA0000114436 | Marmoricola korecan Sc0-A36 | 99.1 | Freshwater R2A | 25°C, 3 d |
| | | Propionibacteriaceae | WW41 | NIBRBA0000114442 | Nocardioides atummus Cr7-14T | 99.1 | Freshwater R2A | 25°C, 3 d |
| | | Propionibacteriaceae | GDM10 | NIBRBA0000114313 | Nocardioides psychotolerans RH7-14T | 99.6 | Seawater R2A | 30°C, 3 d |
| Frankineae | Geodermatophilaceae | Geodermatophilaceae | CR9 | NIBRBA0000114312 | Geodermatophilaceae rosea KLBMP 1279 | 99.2 | Brackish water R2A | 30°C, 3 d |
Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, W2-3-5; 2, WM31; 3, WS50; 4, WMB1; 5, WM69; 6, ST5-11; 7, W2-2-3; 8, 01SU3; 9, W4-2-4; 10, BM14; 11, DT5-10; 12, WM79; 13, WM26; 14, MS4Y-2-4; 15, WW45; 16, JJ9003; 17, MM3; 18, MMD3Y-10-4; 19, TMIL-2; 20, SR5-11; 21, BM24; 22, WM113; 23, HME9262; 24, DR7-12; 25, MMD3Y-15-3; 26, MK6Y-6-1; 27, O3SU14; 28, CDR3; 29, SR4-01; 30, BM28; 31, MMD3Y-15-4; 32, HME9278; 33, DR7-01; 34, Gsoil012; 35, WW39; 36, WW41; 37, GDM10; 38, CR9.
Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains isolated in this study and their relatives of the phylum *Actinobacteria*. Bootstrap values (> 70%) are shown above nodes. Filled circles indicate the nodes recovered by three other treeing methods including maximum likelihood, maximum parsimony, and neighbor joining. Bar, 0.01 substitutions per nucleotide position. Suborder: A, *Micromonosporineae*; B, *Frankineae*; C, *Propionibactierineae*. 

Suborder: *Micromonosporineae*
capric acid, adipic acid, potassium gluconate, malic acid, trisodium citrate and phenylacetic acid. Strain ST5-11 (= NIBRBA0000114183) has been isolated from plant root, Daejeon, Korea.

**Description of Mycobacterium obuense W2-2-3**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are round, convex and yellow colored after 2 days on R2A at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase, esculin hydrolysis and β-galactosidase but positive for urease. L-arabinose, D-mannitol, potassium gluconate and D-maltose are utilized. Does not utilize D-glucose, D-mannose, capric acid, adipic acid and N-acetyl-glucosamine. Strain W2-2-3 (= NIBRBA0000114211) has been isolated from mineral water, Daejeon, Korea.

**Description of Mycobacterium sediminis 01SU3**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex, opaque and orange colored after 3 days of incubation on R2A at 25°C. Negatives for nitrate reduction, Indole production, glucose fermentation, gelatin hydrolysis, arginine dihydrolase, esculin hydrolysis and β-galactosidase in API 20NE, but positive for urease. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate and malic acid are not utilized. Strain 01SU3 (= NIBRBA0000114438) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

**Description of Nocardia abscessus W4-2-4**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex and entire after 2 days on R2A at 25°C. In API 20NE gallery, negative for nitrate reduction, arginine dihydrolase, urease, indole production, glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase. D-glucose, D-mannitol, potassium gluconate and malic acid are utilized, but L-arabinose, D-mannose, trisodium citrate, N-acetyl-glucosamine, D-maltose, capric acid, adipic acid and phenylacetic acid are not utilized. Strain W4-2-4 (= NIBRBA0000114210) has been isolated from a mineral water sample, Daejeon, Korea.

**Description of Williamsia faeni BM14**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire, glistening and yellow colored after 3 days on MA at 30°C. Positive for nitrate reduction, urease, esculin hydrolysis and β-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain BM14 (= NIBRBA0000114305) has been isolated from mud flat, Wando, Jeonnam Province, Korea.

**Description of Cellulomonas pakistanensis DT5-10**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are round, glistening, convex and yellow-colored after 2 days incubation on TSA at 30°C. Positive for glucose fermentation, esculin hydrolysis, β-galactosidase and gelatinase in API 20NE. Negative for nitrate reduction, indole production, glucose dihydrolase and urease. Utilize D-glucose, L-arabinose, N-acetyl-glucosamine, D-maltose, potassium gluconate and D-mannitol, but not utilize D-mannose, capric acid, adipic acid, phenylacetic acid, malic acid and trisodium citrate. Strain DT5-10 (= NIBRBA0000114779) has been isolated from a plant root, Daejeon, Korea.

**Description of Arsenicicoccus bolidensis WM79**

Cells are Gram-staining-positive, flagellated, non-pigmented and cocccoid. Colonies are opaque, round, smooth, umbonate and yellow-colored after 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis and gelatinase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. Does not utilize D-glucose, D-mannose, potassium gluconate, capric acid, adipic acid, trisodium citrate, malic acid and phenylacetic acid, but utilize D-mannitol, N-acetyl-glucosamine and D-maltose. Strain WM79 (= NIBRBA0000114441) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

**Description of Terrabacter ginsenosidimutans WM26**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are glossy, entire, smooth and pale yellow colored after 7 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE, but negative for indole production, glucose dihydrolase fermentation, urease and gelatinase. Utilizes D-glucose, D-mannose, D-mannitol, D-maltose, potassium gluconate and malic acid, but not L-arabinose, N-acetyl-glucosamine, adipic acid, capric acid, trisodium citrate and phenylacetic acid. Strain WM26 (= NIBRBA0000114440) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.
Description of *Terrabacter koreensis* MS4Y-2-4

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are glossy, entire, smooth and pale yellow colored after 3 days on MA at 30°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20 NE, but negative for glucose fermentation, urease, indole production, arginine dihydrolase and gelatinase. Positive for utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate and adipic acid, but negative for capric acid and phenylacetic acid. Strain MS4Y-2-4 (= NIBRBA0000114281) has been isolated from a freshwater sample, Wanju, Jeonbuk Province, Korea.

Description of *Terrabacter tumescens* WW45

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are glossy, entire, smooth and pale yellow colored after 5 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase in API 20 NE, but negative for indole production, glucose fermentation, urease and arginine dihydrolase. Utilizes L-arabinose and potassium gluconate, but not capric acid, malic acid, trisodium citrate, D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain WW45 (= NIBRBA0000114437) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

Description of *Agreia pratensis* JJ9003

Cells are Gram-staining-positive, non-flagellated and rod to coccus-shaped. Colonies are glossy, entire, smooth and pale yellow colored after 2 days on R2A at 25°C. Diffusible pigment is not produced. Positive for esculin hydrolysis, β-galactosidase and oxidase, but negative for nitrate reduction glucose fermentation, arginine dihydrolase, urease, gelatinase and indole production. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain JJ9003 (= NIBRBA0000114154) has been isolated from a freshwater sample, Anseong, Gyeonggi Province, Korea.

Description of *Frondihabitans cladoniiphilus* TMIL-2

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are irregular, smooth, glistening and yellow-colored after 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20 NE, but negative for indole production, urease, glucose fermentation, arginine dihydrolase and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose and potassium gluconate, but not utilize capric acid, malic acid, trisodium citrate, phenylacetic acid and adipic acid. Strain TMIL-2 (= NIBRBA0000114323) has been isolated from a freshwater sample, Milyang, Gyeongnam Province, Korea.

Description of *Herbiconiux ginsengi* SR5-11

Cells are Gram-staining-positive, non-flagellated, non-pigmented and short rod-shaped. Colonies are circular, convex and entire after 3 days on R2A at 30°C. Positive for arginine dihydrolase, esculin hydrolysis, β-galactosidase, urease and gelatinase, but negative for nitrate reduction, glucose fermentation and indole production in API 20 NE. Positive for utilization of D-glucose, D-mannose, D-mannitol and L-arabinose, weakly positive for N-acetyl-glucosamine, malic acid and D-maltose, but negative for potassium gluconate, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain SR5-11 (= NIBRBA0000114200) has been isolated from a freshwater sample, Gyeongnam Province, Korea.
plant root, Daejeon, Korea.

**Description of Leifsonia poae BM24**

Cells are Gram-staining-positive and rod-shaped. Colonies are circular, smooth, convex, glistening and yellow colored after 3 days on MA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase, but positive for esculin hydrolysis and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-maltose and N-acetyl-glucosamine are utilized. Does not utilize D-mannitol, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain BM24 (NIBRBA0000114307) has been isolated from mud flat, Wando, Jeonnam Province, Korea.

**Description of Microbacterium dextranolyticum WM113**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex, opaque and yellow colored after 3 days on R2A at 25°C. Positive for glucose fermentation, esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, arginine dihydrolase, indole production, urease and gelatinase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, D-maltose, malic acid and trisodium citrate are utilized. Does not utilize phenylacetic acid, capric acid and adipic acid. Strain WM113 (NIBRBA0000114429) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

**Description of Microbacterium hatanonis HME9262**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and yellow colored after 2 days on R2A at 30°C. Negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production, urease and gelatinase, but positive for esculin hydrolysis and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-maltose D-mannitol, N-acetyl-glucosamine, potassium gluconate and malic acid are utilized. Does not utilize D-glucose, L-arabinose, malic acid, trisodium citrate, potassium gluconate, phenylacetic acid, capric acid and adipic acid. Strain HME9262 (NIBRBA0000114394) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

**Description of Microbacterium lacus DR7-12**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and yellow colored after 3 days on R2A at 30°C. Positive for esculin hydrolysis, β-galactosidase and urease, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production and gelatinase in API 20NE. D-maltose, D-mannose and D-mannitol are utilized. Does not utilize D-glucose, L-arabinose, N-acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain DR7-12 (NIBRBA0000114192) has been isolated from plant root, Daejeon, Korea.

**Description of Microbacterium paraoxydans MMD3Y-15-3**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and golden yellow colored after 3 days on MA at 30°C. Positive for esculin hydrolysis, β-galactosidase and gelatinase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production, urease and gelatinase in API 20NE. D-Maltose, D-mannose, D-mannitol, N-acetyl-glucosamine and potassium gluconate are utilized. Does not utilize D-glucose, L-arabinose, malic acid, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain MMD3Y-15-3 (NIBRBA0000114277) has been isolated from Ginseng cultivated soil, Anseong, Gyeonggi Province, Korea.

**Description of Microbacterium phyllosphaerae MK6Y-6-1**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and golden yellow colored after 3 days on MA at 30°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production, urease and gelatinase in API 20NE. D-Glucose, D-mannose, D-mannitol, potassium gluconate, phenylacetic acid and malic acid are utilized. Does not utilize L-arabinose, N-acetyl-glucosamine, D-maltose, trisodium citrate, capric acid and adipic acid. Strain MK6Y-6-1 (NIBRBA0000114274) has been isolated from Ginseng cultivated soil, Anseong, Gyeonggi Province, Korea.

**Description of Rathayibacter tritici 03SU14**

Cells are Gram-staining-positive, non-flagellated and short rod shaped. Colonies are entire, convex, opaque and yellow colored after 3 days of incubation on R2A at 25 °C. In API 20NE, positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, gelatinase and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and potassium gluconate are utilized. Does not utilize N-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenyl-
acetic acid. Strain 03SU14 (=NIBRBA0000114439) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

**Description of Arthrobacter agilis CDR5**

Cells are Gram-staining-positive, flagellated and cocoid. Colonies are circular, entire, smooth, glistening, convex and deep yellowish pink-colored after 3 days on R2A at 30°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. Utilize D-glucose, D-mannose, D-mannitol, D-maltose and potassium gluconate, but not capric acid, but L-arabinose, N-acetyl-glucosamine, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CDR5 (= NIBRBA0000114311) has been isolated from brackish water sample, Jeju, Korea.

**Description of Micrococcus yunnanensis SR4-01**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and yellow colored after 3 days on R2A at 30°C. Positive for gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease and β-galactosidase in API 20NE. D-Glucose and D-maltose are utilized. Weakly utilize the capric acid and malic acid. Does not utilize L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain SR4-01 (= NIBRBA0000114197) has been isolated from plant root, Daejeon, Korea.

**Description of Isoptericola nanjingensis BM28**

Cells are Gram-staining-positive, non-flagellated and oval-shaped. Colonies are circular, smooth, convex, glistening and yellow colored after 3 days on MA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase in API 20NE but positive for oxidase, esculin hydrolysis urease, gelatinase and β-galactosidase. Positive for utilization of D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose and potassium gluconate; weakly positive for adipic acid. Does not utilize D-mannitol, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain BM28 (= NIBRBA0000114308) has been isolated from a mud flat sample, Wando, Jeonnam Province, Korea.

**Description of Promicromonospora sukumoe MMD3Y-15-4**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and pale yellow colored after 3 days on MA at 30°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production, urease and gelatinase in API 20NE. D-Glucose, L-arabinose, D-mannose, potassium gluconate and malic acid are utilized. Does not utilize D-maltose, D-mannitol, N-acetyl-glucosamine, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain MMD3Y-15-4 (= NIBRBA0000114278) has been isolated from Ginseng cultivated soil, Anseong, Gyeonggi Province, Korea.

**Description of Sanguibacter keddieii HME9278**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire, convex and yellow colored after 3 days on R2A at 30°C. Positive for glucose fermentation, esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and gelatinase. Utilizes D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine and D-maltose, but not capric acid, malic acid, trisodium citrate, D-mannitol, potassium gluconate, adipic acid and phenylacetic acid. Strain HME9278 (= NIBRBA0000114396) has been isolated from lagoon, Gangneung, Gangwon Province, Korea.

**Description of Micromonospora sediminicola DR7-01**

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are entire and rough after 3 days on R2A at 30°C. Orange colored colony darkened when aged. Positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE, but negative for glucose fermentation, urease, indole production, arginine dihydrolase and. Weakly utilizes D-glucose, L-arabinose, N-acetyl-glucosamine and D-maltose, but not caprylic acid, malic acid, trisodium citrate, D-mannose, D-mannitol, potassium gluconate, adipic acid and phenylacetic acid. Strain DR7-01 (= NIBRBA0000114191) has been isolated from plant root, Daejeon, Korea.

**Description of Kribbella aluminosa Gsoil012**

Cells are Gram-staining-positive and non-flagellated. White aerial mycelium and yellow substrate mycelium is produced after 2 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, glucose fermentation, urease indole production, arginine dihydrolase and gelatinase. D-Glucose, D-mannose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid are utilized. Does not utilize adipic acid, capric acid and trisodium citrate. Strain Gsoil012 (= NIBRBA0000114219)
Description of *Marmorica korecus* WW39

Cells are Gram-staining-positive, non-flagellated and coccolid shaped. Colonies are opaque, entire, drop-like and yellow colored after 3 days incubation on R2A at 25°C. Positive for glucose fermentation, esculin hydrolysis and gelatinase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase urease and β-galactosidase. Does not utilize capric acid, malic acid, trisodium citrate, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and phenylacetic acid. Strain WW39 (= NIBRBA0000114442) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

Description of *Nocardioides alpinus* WW41

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, smooth pale yellow colored after 3 days incubation on R2A at 25°C. Positive for nitrate reduction, β-galactosidase and gelatinase in API 20NE, but negative for glucose fermentation, urease, esculin hydrolysis and for indole production, arginine dihydrolase. Utilizes D-glucose, L-arabinose, D-mannitol, D-maltose and potassium gluconate, but not utilizes capric acid, malic acid, trisodium citrate, D-mannose, N-acetyl-glucosamine, adipic acid and phenylacetic acid. Strain WW41 (= NIBRBA0000114442) has been isolated from a freshwater sample, Woopo wetland, Gyeongnam Province, Korea.

Description of *Nocardioides psychrotolerans* GDM10

Cells are Gram-staining-positive, non-flagellated and rod shaped. Colonies are circular, entire, smooth pale yellow colored after 3-4 days incubation on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis and gelatinase in API 20NE, but negative for indole production, arginine dihydrolase glucose fermentation, urease and β-galactosidase. Does not utilize capric acid, malic acid, trisodium citrate, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and phenylacetic acid. Strain GDM10 (= NIBRBA0000114313) has been isolated from a seawater sample collected from seashore, Pohang, Gyeongbuk Province, Korea.

Description of *Modestobacter roseus* CR9

Cells are Gram-staining-positive, non-flagellated, non-pigmented and long rod-shaped. Colonies are circular, convex and light yellowish pink colored after 3 days of incubation on R2A medium at 30°C. Positive for esculin hydrolysis, oxidase and gelatinase, weakly positive for glucose fermentation, but negative for nitrate reduction, Indole production, urease, arginine dihydrolase and β-galactosidase. In API 20NE, positive assimilates for L-arabinose and N-acetyl-glucosamine, weakly positive for D-glucose, adipic acid and malic acid, but negative for D-mannose, D-mannitol, D-malose, D-maltose, potassium gluconate, trisodium citrate capric acid and phenyl acetic acid. Strain CR9 (= NIBRBA0000114312) has been isolated from a brackish water sample, Jeju, Korea.

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