

## Report on 24 unrecorded bacterial species of Korea belonging to the phylum *Firmicutes*

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As an outcome of the study on the bacterial species diversity in Korea, we report 24 unrecorded bacterial species of Korea belonging to the phylum Firmicutes. The unrecorded species excavated through this study were assigned to 12 different genera of 7 families, namely *Bacillus*, *Halobacillus*, *Lysinibacillus* and *Thalassobacillus* of *Bacillaceae*, *Brevibacillus* and *Paenibacillus* of *Paenibacillaceae*, *Viridibacillus* of *Planococcaceae*, *Salinicoccus* and *Staphylococcus* of *Staphylococcaceae*, *Enterococcus* of *Enterococcaceae*, *Lactobacillus* of *Lactobacillaceae*, and *Lactococcus* of *Streptococcaceae*, respectively. The bacterial isolates were obtained from various ecosystems in Korea. The isolates were identified based on 16S rRNA gene sequences, and those exhibiting at least 99% sequence similarity with known bacterial species but never reported in Korea were selected as unrecorded species. The selected isolates were subjected to further taxonomic characterization including the analysis of cell shape and fine structure using electron microscope, colony color and shapes, enzyme activities and carbon source utilization. The descriptive information on the 24 unrecorded species are provided.

Keywords: *Bacillales*, *Firmicutes*, *Lactobacillales*, unrecorded bacterial species

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### INTRODUCTION

The phylum Firmicutes encompasses broad bacterial taxa that have Gram positive cell walls and low DNA G + C contents (Dworkin *et al.*, 2006; De Vos *et al.*, 2009). Firmicutes can be isolated from virtually everywhere, i.e. soil, fresh or marine aquatic environment, air, and plant or animal hosts. Recent studies indicate that Firmicutes constitutes a major portion of human and mouse gut microbiome (Ley *et al.*, 2006). The phylum current-

ly contains three classes, namely Bacilli, Clostridia and Erysipelotrichia (De Vos *et al.*, 2009). The class Bacilli includes endospore-forming groups and lactic acid producing groups, Clostridia includes endospore forming or non-forming anaerobic groups and anoxygenic phototrophic groups, and Erysipelotrichia includes non-motile, non-spore-forming and aerobic groups. *Bacillus*, *Clostridium*, *Lactobacillus* and *Paenibacillus* are large membered genera, each containing more than 100 validly described species (List of Prokaryotic Names with Standing in Nomenclature, <http://www.bacterio.net>).

Firmicutes includes industrially important groups as well as causative agents of various diseases. Lactic acid bacteria are the representative probiotic bacteria, and thus one of the most industrially important bacterial groups (Tannock, 2005). *Bacillus* and *Paenibacillus* are known as two important taxa that exhibit plant growth promoting potential, and thus can be used as agricultural agents (McSpadden Gardner, 2004). In contrast, species of *Bacillus* (anthrax, food poisoning), *Clostridium* (tetanus, food poisoning, gas gangrene), *Enterococcus* (urinary tract infection), *Listeria* (listeriosis), *Streptococcus* (pneumonia, meningitis, dental caries) and *Staphylococcus* (scalded skin syndrome) are some examples of medically significant bacteria of Firmicutes (Dworkin *et al.*, 2006).

In this study, bacteria belonging to Firmicutes were isolated from various sources such as ginseng cultivation soil, mud, salted fish and shrimp, guts of insects, plant root, freshwater and seawater. Through the phylogenetic analysis using 16S rRNA gene sequences, we recovered a total of 23 species that could be recognized as unrecorded species in Korea.

## MATERIALS AND METHODS

Bacterial strains were isolated as pure cultures from diverse environmental sources including soil, mud, freshwater, seawater, salted fish and shrimp, plant root, guts of insects. Diverse culture media including R2A, marine agar 2216, nutrient agar, Luria agar and tryptic soy agar were used to isolate diverse groups of bacteria belonging to Firmicutes, and the inoculated plates were incubated at 25-30°C for 2-5 days. The designation of strains, source of isolation and culture media are summarized in Table 1. All strains were purified as single colonies and stored as 10-20% glycerol suspension –80°C as well as lyophilized ampoules.

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 Firmicutes were selected for subsequent analysis. The selected strains were identified using the EzTaxon-e server (Kim *et al.*, 2012). The cutoff value of 98.7% sequence similarity was employed for identification. Phylogenetic trees were generated by using neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge and Farris, 1969) algorithms that are programmed in MEGA 6.0 (Tamura *et al.*, 2013). The robustness of the phylogenetic trees was confirmed by bootstrap analyses based on 1000 random replicates.

Colony 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). Gram staining was performed using a Gram-staining kit or the standard procedures. Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

## RESULTS AND DISCUSSION

From diverse sources, a total of 26 bacterial strains belonging to Firmicutes were obtained. All isolates could be assigned to 24 different species based on 16S rRNA gene sequence similarity. The taxonomic composition and identification results of the isolates are summarized in Table 1. The isolates could be assigned to 11 different genera of 7 families, namely *Bacillus* (5 species), *Halobacillus* (1 species), *Lysinibacillus* (1 species) and *Thalassobacillus* (1 species) of *Bacillaceae*, *Brevibacillus* (1 species) and *Paenibacillus* (5 species) of *Paenibacillaceae*, *Viridibacillus* (1 species) of *Planococcaceae*, *Salinicoccus* (1 species) and *Staphylococcus* (2 species) of *Staphylococcaceae*, *Enterococcus* (3 species) of *Enterococcaceae*, *Lactobacillus* (2 species) of *Lactobacillaceae*, and *Lactococcus* (1 species) of *Streptococcaceae*, respectively. The families *Bacillaceae*, *Paenibacillaceae*, *Planococcaceae* and *Staphylococcaceae* belong to the Order *Bacillales*, whereas the remaining three families belong to the order *Lactobacillales* (Figs. 2-4). Both orders belong to the class Bacilli.

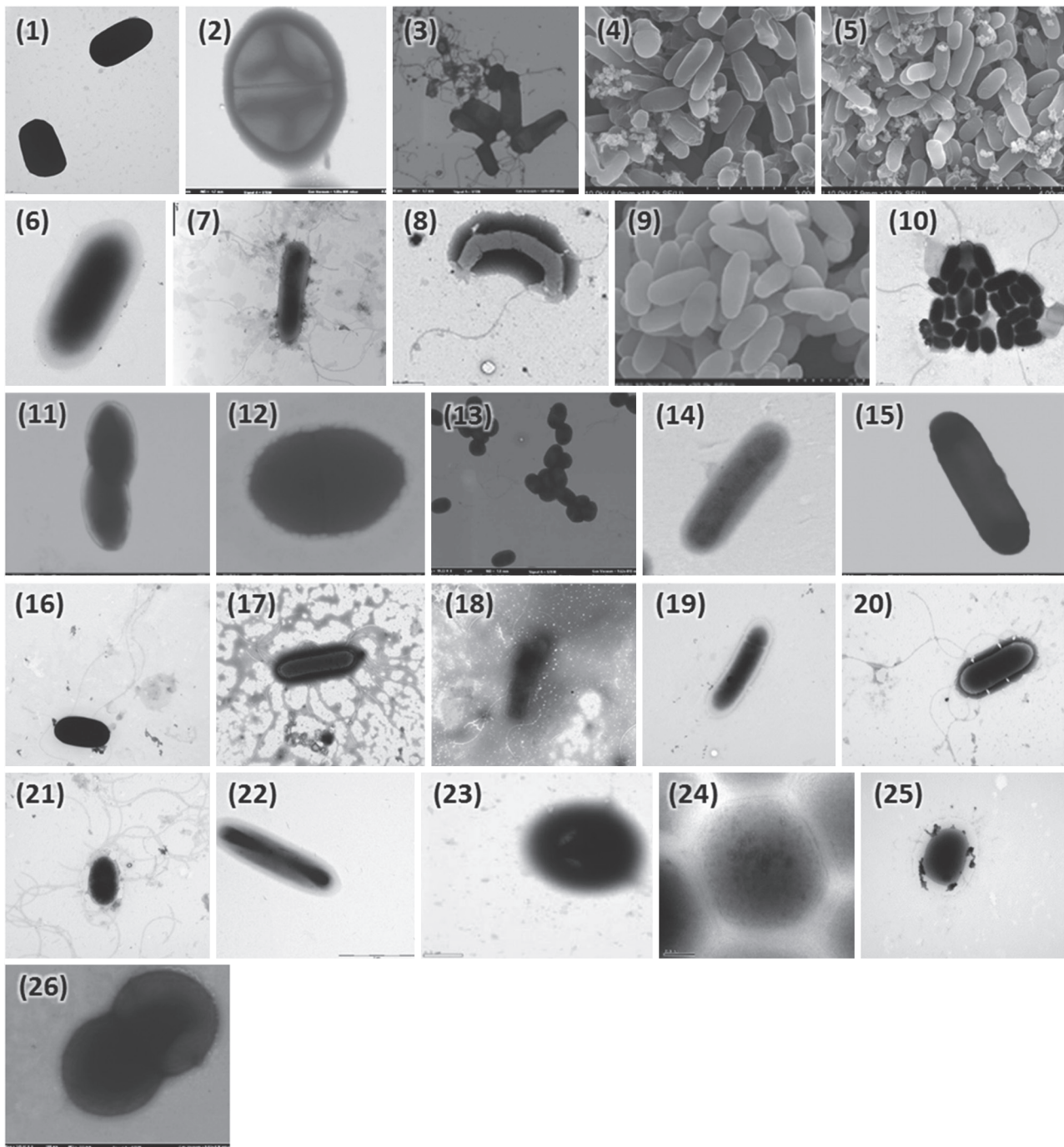
Soil and water are common habitats for the members of Firmicutes (Dworkin *et al.*, 2006; De Vos *et al.*, 2009). In contrast, not much is known on the other habitats. In this study, five strains belonging to *Bacillus*, *Enterococcus* and *Lactobacillus* were obtained from insect guts. Although there have been only a handful of reports on new species of those genera from insect gut, for example *Bacillus oleronius* (Kuhnik *et al.*, 1996), *Bacillus tryplexicola* (Aizawa *et al.*, 2010), *Enterococcus diestramenae* (Kim *et al.*, 2013) and *Lactobacillus apis* (Killer *et al.*, 2014), this study confirms that members of Firmicutes can be a major constituent in the gut microflora of insects. This finding is in line with a report that members of Firmicutes, *Bacillus* in particular, are known to form a significant portion of gut microbial community of soil invertebrates (König, 2006).

Two strains were recovered from salted marine animals in this study, namely one identified as *Halobacillus kuroshimensis*, and the other as *Salinicoccus siamensis*. This can be considered natural since the members of both genera are inhabitants of marine environment (Spring, 2010; Ventosa, 2010), although it is not clear whether the

**Table 1.** List of strains belonging to the phylum *Firmicutes* and their taxonomic assignment based on 16S rRNA gene analysis.

| Family                   | Genus                   | Strain code  | NIBR ID <sup>a</sup> | Identification                                | Sequence similarity (%) | Isolation source             | Medium <sup>b</sup> | Growth temperature |
|--------------------------|-------------------------|--------------|----------------------|---|-------------------------|------------------------------|---------------------|--------------------|
| <i>Bacillaceae</i>       | <i>Bacillus</i>         | RS3-5 B      | NIBRBA0000113942     | <i>B. aciditoler</i> CBD 119 <sup>T</sup>     | 99.9                    | Soil                         | R2A                 | 30°C               |
|                          |                         | DE L 1 4     | NIBRBA0000114025     | <i>B. aryabhatai</i> B8W22 <sup>T</sup>       | 99.7                    | Insect gut                   | LA                  | 25°C               |
|                          |                         | DE L 2 2     | NIBRBA0000114028     | <i>B. aerophilus</i> 28K <sup>T</sup>         | 99.9                    | Insect gut                   | LA                  | 25°C               |
|                          |                         | J27          | NIBRBA0000114104     | <i>B. idriensis</i> SMC 4352-2 <sup>T</sup>   | 100                     | Seawater                     | MA                  | 25°C               |
|                          |                         | J29          | NIBRBA0000114105     | <i>B. infantis</i> SMC 4352-1 <sup>T</sup>    | 100                     | Seawater                     | MA                  | 25°C               |
| <i>Bacillaceae</i>       | <i>Lysinibacillus</i>   | WR-M2W       | NIBRBA0000113998     | <i>L. fusiformis</i> AMNH732 <sup>T</sup>     | 99.7                    | Soil                         | MA                  | 25°C               |
|                          |                         | MA19         | NIBRBA0000113967     | <i>L. fusiformis</i> AMNH732 <sup>T</sup>     | 100                     | Soil                         | MA                  | 25°C               |
| <i>Thalassobacillus</i>  | <i>Thalassobacillus</i> | HME8790      | NIBRBA0000114093     | <i>T. devorans</i> DSM16966 <sup>T</sup>      | 99.4                    | Mud                          | MA                  | 30°C               |
|                          |                         | KYW872       | NIBRBA0000114114     | <i>T. devorans</i> DSM16966 <sup>T</sup>      | 99.9                    | Seawater                     | MA                  | 25°C               |
| <i>Halobacillus</i>      | <i>Halobacillus</i>     | PM1          | NIBRBA0000114056     | <i>H. kuroshimensis</i> IS-Hb7 <sup>T</sup>   | 99.4                    | Salted anchovy               | MA                  | 25°C               |
|                          |                         | OR L 1 6     | NIBRBA0000114027     | <i>E. plantarum</i> CCM 7889 <sup>T</sup>     | 99.7                    | Insect gut                   | LA                  | 25°C               |
| <i>Enterococcaceae</i>   | <i>Enterococcus</i>     | OR L 2 3     | NIBRBA0000114026     | <i>E. quebecensis</i> CCRI-16985 <sup>T</sup> | 99.7                    | Insect gut                   | LA                  | 25°C               |
|                          |                         | MR1          | NIBRBA0000114031     | <i>E. asini</i> ATCC 700915 <sup>T</sup>      | 99.8                    | Insect gut                   | R2A                 | 25°C               |
|                          |                         | IK 36        | NIBRBA0000113962     | <i>L. aquaticus</i> IMCC 1736 <sup>T</sup>    | 100                     | Freshwater                   | PCA                 | 25°C               |
| <i>Lactobacillaceae</i>  | <i>Lactobacillus</i>    | HY M 2 2     | NIBRBA0000114033     | <i>L. kuunkeei</i> YH-15 <sup>T</sup>         | 99.7                    | Insect gut                   | MRSA                | 25°C               |
|                          |                         | H4-2-1 H     | NIBRBA0000113934     | <i>P. glycanilyticus</i> DS-1 <sup>T</sup>    | 99.9                    | Soil                         | R2A                 | 30°C               |
| <i>Paenibacillaceae</i>  | <i>Paenibacillus</i>    | RS5-1        | NIBRBA0000113946     | <i>P. massiliensis</i> 2301065 <sup>T</sup>   | 99.8                    | Soil                         | R2A                 | 30°C               |
|                          |                         | Rk5-7 B      | NIBRBA0000113947     | <i>P. peoriae</i> DSM8320 <sup>T</sup>        | 99.7                    | Soil                         | R2A                 | 30°C               |
|                          |                         | MS5-14       | NIBRBA0000113948     | <i>P. timonensis</i> 230 1032 <sup>T</sup>    | 99.6                    | Soil                         | MA                  | 30°C               |
|                          |                         | MK5-2        | NIBRBA0000113949     | <i>P. xylanexedens</i> B22a <sup>T</sup>      | 99.7                    | Soil                         | MA                  | 30°C               |
|                          |                         | UEJ4-1 D     | NIBRBA0000113940     | <i>B. laterosporus</i> DSM25 <sup>T</sup>     | 99.5                    | Soil                         | R2A                 | 30°C               |
| <i>Planococcaceae</i>    | <i>Viridibacillus</i>   | CT1-1        | NIBRBA0000113860     | <i>V. arenosi</i> LMG 22166 <sup>T</sup>      | 99.8                    | Roof of <i>Pteridium</i> sp. | TSA                 | 30°C               |
|                          |                         | SJ2-6        | NIBRBA0000114061     | <i>S. siamensis</i> PNI-2 <sup>T</sup>        | 99.5                    | Salted shrimp                | MA                  | 25°C               |
| <i>Staphylococcaceae</i> | <i>Staphylococcus</i>   | ES05-9M-1-MA | NIBRBA0000113917     | <i>S. warneri</i> ATCC 27836 <sup>T</sup>     | 100                     | Seawater                     | MA                  | 25°C               |
|                          |                         | CNS5-1       | NIBRBA0000113957     | <i>S. nepalensis</i> CW1 <sup>T</sup>         | 99.9                    | Soil                         | NA                  | 30°C               |
| <i>Streptococcaceae</i>  | <i>Lactococcus</i>      | OR Y 1 1     | NIBRBA0000114037     | <i>L. garvieae</i> ATCC 29156 <sup>T</sup>    | 99.7                    | Insect gut                   | TSYA                | 25°C               |

<sup>a</sup> Identification number by the National Institute of Biological Resources (NIBR).<sup>b</sup> L-A, Luria agar; MA, mannitol agar; PCA, plate count agar; MRSA, tryptic soy agar; TSA, tryptic soy agar; TSYA, tryptic soya agar.



**Fig. 1.** Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1. RS3-5 B; 2. DE L 1 4; 3. DE L 2 2; 4. J27; 5. J29; 6. WR-M2W; 7. MA19; 8. HME8790; 9. KYW872; 10. PM1; 11. OR L 1 6; 12. OR L 2 3; 13. MR1; 14. IK36; 15. HY M 2 2; 16. H4-2-1 H; 17. RS5-1; 18. Rk5-7 B; 19. MS5-14; 20. MK5-2; 21. UEJ4-1 D; 22. CT1-1; 23. SJ2-6; 24. ES05-9M-1-MA; 25. CNS5-1; 26. OR Y 1 1.

strains were originated from applied salt or the animal bodies.

Through this study, the diversity of bacterial species belonging to Firmicutes whose presence in Korean peninsula has not been previously reported was unveiled.

Accordingly, the following 24 species are reported as unrecorded species in Korea.

#### **Description of *Bacillus acidiceler* RS3-5 B**

Cells are Gram-staining-positive, non-flagellated, non-



pigmented and rod shaped. Colonies are irregular, lobate, smooth and white colored after 2 days on R2A at 30°C. Based on API 20NE, positive for urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation and arginine dihydrolase. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and adipic acid are utilized. Does not utilize capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain RS3-5 B (=NIBRBA0000113942) was isolated from soil of ginseng cultivation field, Anseong, Korea.

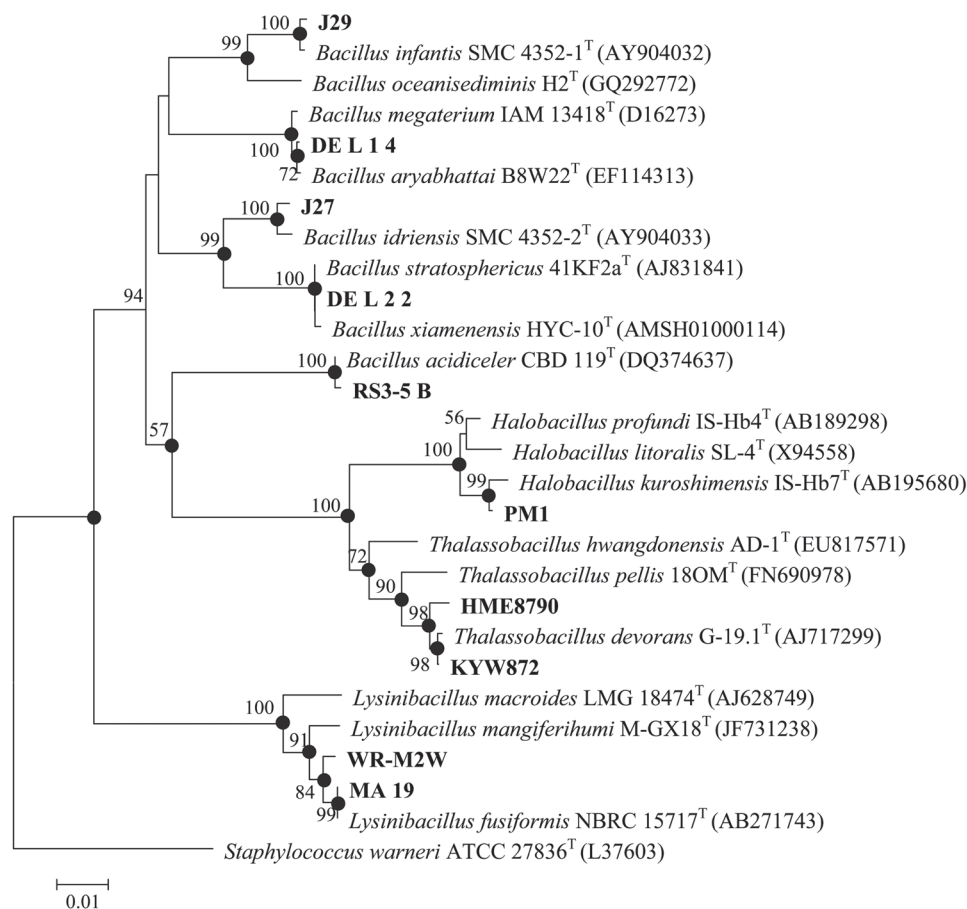
#### Description of *Bacillus aryabhatai* DE L 1 4

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and oval shaped. Colonies are circular, raised, entire and cream colored after 2 days on LA at 25°C. Based on API 20NE, positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase, but negative for nitrate re-

duction, indole production, glucose fermentation, arginine dihydrolase and urease. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DE L 1 4 (=NIBRBA0000) was isolated from gut of insect, Seoul, Korea.

#### Description of *Bacillus aerophilus* DE L 2 2

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and rod shaped. Colonies are circular, raised, entire and white colored after 2 days on LA at 25°C. Based on API 20NE, positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine, dihydrolase and urease. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic



**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 family *Bacillaceae*. Bootstrap values (>50%) are shown at 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.

acid. Strain DE L 2 2 (=NIBRBA0000) was isolated from gut of insect, Soeul, Korea.

#### **Description of *Bacillus idriensis* J27**

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and coccus shaped. Colonies are opaque, round, smooth, convex and cream colored after 3 days on MA at 25°C. Based on API 20NE, positive for glucose fermentation, esculin hydrolysis and gelatinase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Oxidase activity is positive. Strain J27 (=NIBRBA0000) was isolated from seawater, Gwangyang, Korea.

#### **Description of *Bacillus infantis* J29**

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod shaped. Colonies are opaque, round, smooth, convex and shell pink colored after 3 days on MA at 25°C. Based on API 20NE, positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid are utilized. Does not utilize L-arabinose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Oxidase activity is positive. Strain J29 (=NIBRBA0000) was isolated from seawater, Gwangyang, Korea.

#### **Description of *Lysinibacillus fusiformis* WR-M2W**

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are irregular, smooth, glistening and pale-yellow colored after 3 days of incubation on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase in API 20NE. Capric acid, malic acid, phenylacetic acid are utilized. Strain WR-M2W (=NIBRBA0000113998) was isolated from soil, Wando, Korea.

#### **Description of *Lysinibacillus fusiformis* MA19**

Cells are Gram-staining-positive, flagellated, non-pigmented, and rod-shaped. Colonies are circular, and gray-colored after 2 days of incubation on MA at 25°C. Based on API 20NE, positive for urease and gelatinase and, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and  $\beta$ -galactosidase. *N*-acetyl-glucosamine, gluconate

and malic acid are utilized. Strain MA19 (=NIBRBA0000113967) was isolated from soil, Gwanak Mountain, Seoul, Korea.

#### **Description of *Thalassobacillus devorans* HME8790**

Cells are Gram-staining-positive, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire and white-colored after 2 days of incubation on MA at 30°C. Based on API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase, but negative for glucose fermentation, indole production, arginine dihydrolase, and urease. Strain HME8790 (=NIBRBA0000114093) was isolated from mud, Shinan, Korea.

#### **Description of *Thalassobacillus devorans* KYW872**

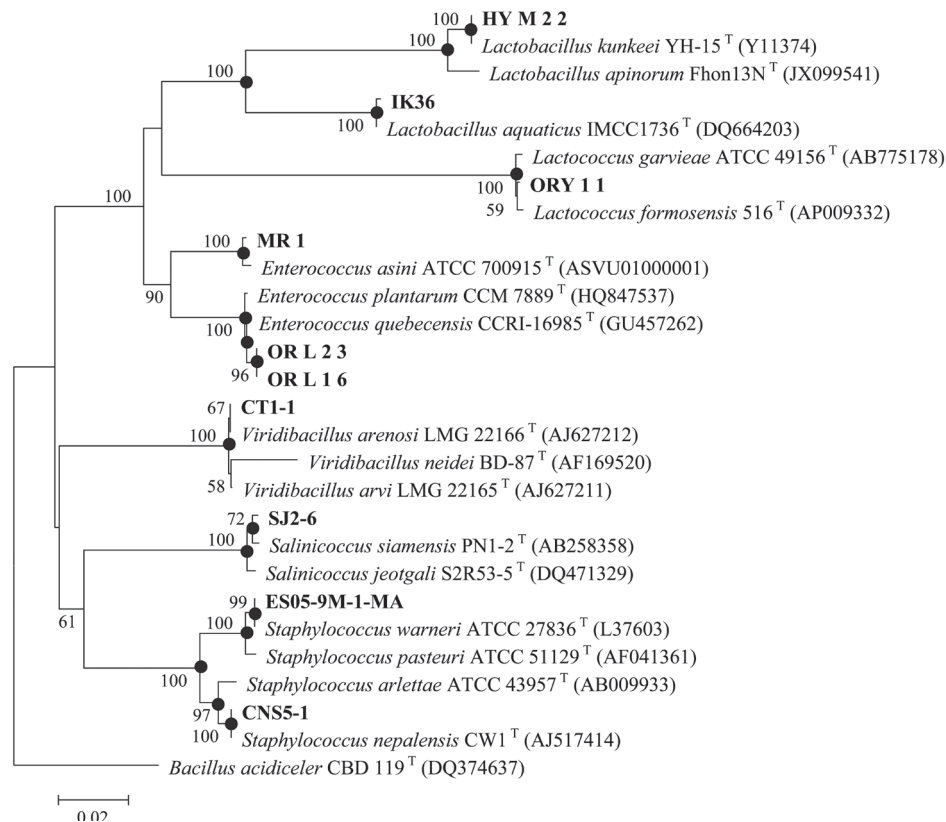
Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are opaque, round, smooth, convex, and cream-colored after 2 days of incubation on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase in API 20NE. Strain KYW872 (=NIBRBA0000114114) was isolated from seawater, Gwangyang, Korea.

#### **Description of *Halobacillus kuroshimensis* PM1**

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and pale yellow colored after 2 days on MA at 25°C. Based on API 20NE, positive for esculin hydrolysis, gelatin hydrolysis, and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. Potassium gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain PM1 (=NIBRBA0000114056) was isolated from salted anchovies, Korean fermented food.

#### **Description of *Enterococcus plantarum* OR L 1 6**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and oval-shaped. Colonies are circular, raised, entire and translucent after 2 days of incubation on LA at 25°C. Based on API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric



**Fig. 3.** 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 family *Enterococcaceae*, *Lactobacillaceae*, *Planococcaceae*, *Staphylococcaceae*, and *Streptococcaceae*. Bootstrap values (>50%) are shown at nodes. Filled circles indicate the nodes recovered by three other treeing methods including maximum likelihood, maximum parsimony, and neighbor joining. Bar, 0.02 substitutions per nucleotide position.

acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain OR L 1 6 (= NIBRBA0000114027) was isolated from gut of insect, Seoul, Korea.

#### Description of *Enterococcus quebecensis* OR L 2 3

Cells are Gram-staining-positive, non-flagellated, non-pigmented and oval-shaped. Colonies are circular, raised, entire and translucent after 2 days of incubation on LA at 25°C. Based on API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain OR L 2 3 (= NIBRBA0000114026) was isolated from gut of insect, Seoul, Korea.

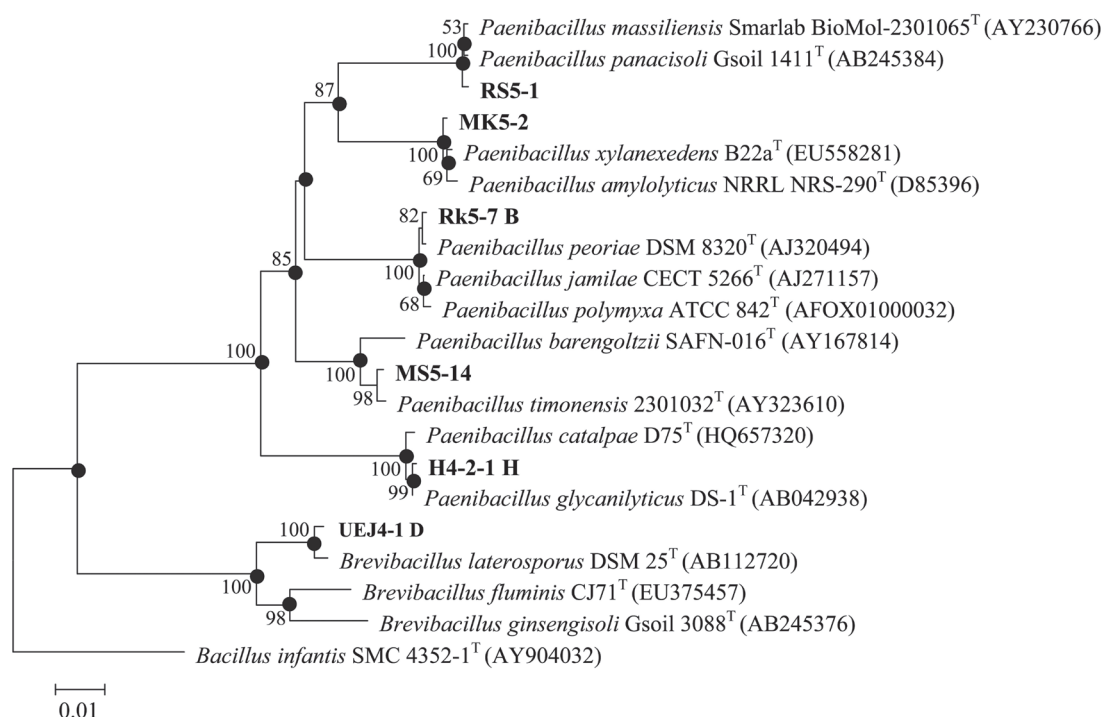
#### Description of *Enterococcus asini* MR1

Cells are Gram-staining-positive, non-flagellated, non-pigmented and oval-shaped. Colonies are circular, raised,

entire and translucent after 2 days of incubation on R2A at 25°C. Based on API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis and  $\beta$ -galactosidase, but negative for indole production, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MR1 (= NIBRBA0000114031) was isolated from gut of insect, Seoul, Korea.

#### Description of *Lactobacillus aquaticus* IK36

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod shaped. Colonies are circular, smooth and white colored after 5 days of incubation on PCA at 25°C. Based on API 20NE, positive for arginine dihydrolase, urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, gelatinase and  $\beta$ -galactosidase. D-Mannose is utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and



**Fig. 4.** 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 family *Paenibacillaceae*. Bootstrap values (>50%) are shown at 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.

phenylacetic acid. Strain IK36 (= NIBRBA0000113962) was isolated from freshwater, Incheon, Korea.

#### Description of *Lactobacillus kuunkeei* HY M 2 2

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod shaped. Colonies are circular, raised, entire and white colored after 2 days of incubation on MRSA at 25°C. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, glucose fermentation, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HY M 2 2 (= NIBRBA0000114033) was isolated from gut of insect, Seoul, Korea.

#### Description of *Paenibacillus glycanilyticus* H4-2-1 H

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, smooth, and pink colored after 2 days of incubation on R2A at 30°C. Based on API 20NE, positive for esculin hydrolysis and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose,

L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate are utilized. Does not utilize capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain H4-2-1\_H (= NIBRBA0000113934) was isolated from ginseng cultivation soil, Geumsan, Korea.

#### Description of *Paenibacillus massiliensis* RS5-1

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are rhizoid, undulate, smooth and white colored after 2 days of incubation on R2A at 30°C. Based on API 20NE, positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase. D-Mannose and D-mannitol are utilized. Does not utilize D-Glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain RS5-1 (= NIBRBA0000113946) was isolated from ginseng cultivation soil, Anseong, Korea.

#### Description of *Paenibacillus peoriae* Rk5-7 B

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, con-



vex, and yellow-colored after 3 days of incubation on VVG at 25°C. Based on API 20NE, positive for glucose fermentation, esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol and D-maltose are utilized. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain Rk5-7\_B (= NIBRBA0000113947) was isolated from ginseng cultivation soil, Anseong, Korea.

#### Description of *Paenibacillus timonensis* MS5-14

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are punctiform, entire, smooth and white-colored after 2 days of incubation on MA at 30°C. Based on API 20NE, positive for esculin hydrolysis and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and potassium gluconate are utilized. Does not utilize D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MS5-14 (= NIBRBA0000113948) was isolated from ginseng cultivation soil, Anseong, Korea.

#### Description of *Paenibacillus xylanexedens* MK5-2

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, smooth and light yellow-colored after 2 days of incubation on MA at 30°C. Based on API 20NE, positive for nitrate reduction, esculin hydrolysis and  $\beta$ -galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-mannose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MK5-2 (= NIBRBA0000113949) was isolated from ginseng cultivation soil, Anseong, Korea.

#### Description of *Brevibacillus laterosporus* UEJ4-1 D

Cells are Gram-staining-negative, flagellated, non-pigmented, and short rod-shaped. Colonies are circular, entire, smooth, and white-colored after 2 days of incubation on R2A at 30°C. Based on API 20NE, positive for nitrate reduction, esculin hydrolysis and gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and  $\beta$ -galactosidase. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize L-arabinose, potassium gluconate, capric acid, adipic acid, malic acid,

trisodium citrate and phenylacetic acid. Strain UEJ4-1\_D (= NIBRBA0000113940) was isolated from ginseng cultivation soil, Anseong, Korea.

#### Description of *Viridibacillus arenosi* CT1-1

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod shaped. Colonies are irregular and ivory colored after 2 days of incubation on TSA at 30°C. Based on API 20NE, positive for nitrate reduction and urease, but negative for indole production, arginine dihydrolase, esculin hydrolysis, glucose fermentation, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CT1-1 (= NIBRBA0000113860) was isolated from root of *Pteridium*.

#### Description of *Salinicoccus siamensis* SJ2-6

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and coccus-shaped. Colonies are circular, raised, entire and orange colored after 2 days on MA at 25°C. Based on API 20NE, positive for nitrate reduction and glucose fermentation, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and  $\beta$ -galactosidase. D-Glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannose, adipic acid, and phenylacetic acid. Strain SJ2-6 (= NIBRBA0000114061) was isolated from salted shrimp, Korean fermented food.

#### Description of *Staphylococcus warneri* ES05-9M-1-MA

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and coccoid-shaped. Colonies are circular, entire, smooth and beige colored after 4 days of incubation on MA at 25°C. Based on API 20NE, positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate are not utilized. Strain ES05-9M-1-MA (= NIBRBA0000113917) was isolated from seawater, Pohang, Korea.

#### Description of *Staphylococcus nepalensis* CNS5-1

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and coccoid-shaped. Colonies are circular, entire, smooth and beige colored after 2 days of incubation on NA at 30°C. Based on API 20NE, positive for nitrate reduction, urease, esculin hydrolysis, and  $\beta$ -ga-

lactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose and potassium gluconate are utilized. Strain CNS5-1 (=NIBRBA0000113957) was isolated from a soil sample, Anseong, Korea.

### Description of *Lactococcus garvieae* OR Y 1 1

Cells are Gram-staining-positive, non-flagellated, non-pigmented and coccus shaped. Colonies are circular, raised, entire and white colored after 2 days of incubation on TSYA at 25°C. Based on API 20NE, positive for glucose fermentation, arginine dihydrolase, esculin hydrolysis, and  $\beta$ -galactosidase, but negative for nitrate reduction, indole production, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain OR Y 1 1 (=NIBRBA0000114037) was isolated from gut of insect, Seoul, Korea.

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