Journal of Species Research 12(2):158-164, 2023

A report of seven unrecorded bacterial species in Korea, isolated from marine sediment

Chi Young Hwang[1], Eui-Sang Cho[1], Dong-Hyun Jung[2,3], Ki-Eun Lee[2], In-Tae Cha[2], Won-Jae Chi[2] and Myung-Ji Seo[1,4,5,*]

[1]*Department of Bioengineering and Nano-Bioengineering, Incheon National University, Incheon 22012, Republic of Korea*
[2]*Microorganism Resources Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea*
[3]*Division of Food and Nutrition, Chonnam National University, Gwangju 61186, Republic of Korea*
[4]*Division of Bioengineering, Incheon National University, Incheon 22012, Republic of Korea*
[5]*Research Center for Bio Materials & Process Development, Incheon National University, Incheon 22012, Republic of Korea*

**Correspondent: mjseo@inu.ac.kr*

In March 2021, marine sediment from East Sea samples were suspended in a 2% NaCl solution, and serial dilution was performed in fresh marine and Reasoner's 2A agar. Isolated bacterial strains were identified based on 16S rRNA gene sequences, and showed at least 98.7% sequence similarity with previously reported bacterial species. Finally, seven bacterial strains which were validly published but not reported in Korea, were obtained. These isolates were allocated to the orders *Bacillales* and *Flavobacteriales*. The three *Flavobacteriales* strains are classified into the family *Flavobacteriaceae*. The other four *Bacillales* belong to the families *Bacillaceae* and *Paenibacillaceae*. The seven unrecorded bacterial strains in this study are classified into seven different genera, which are assigned to *Mesobacillus*, *Paenibacillus*, *Gramella*, *Gillisia*, *Arenibacter*, *Fictibacillus*, and *Brevibacillus*. During the investigation, the possibility of excavation of various unrecorded species in domestic marine sediment was confirmed. Gram-staining, cell morphology, physiological and basic biochemical characteristics, and phylogenetic analysis were performed in this study and provided in the description of each strain.

Keywords: 16S rRNA gene sequences, bacteria, domestic marine sediment, unrecorded bacterial species

DOI:10.12651/JSR.2023.12.2.158

Introduction

Interest in the biology of marine microorganisms has grown since the Challenger expedition of 1872−1876 (Jannasch and Taylor, 1984). As low temperatures and high hydraulic pressure characterize the environment at the bottom of the ocean, microbes isolated from marine sediment may have special characteristics that enable them to survive in harsh conditions (Kato *et al*., 1995; Takami *et al*., 1999). In the marine environment, many bacterial species classified as alkaliphiles, thermophiles, psychrophiles, and halophiles have been isolated (Jahromi and Narzkar, 2018; Cheng *et al*., 2020). Consequently, there is growing interest in using microorganisms that have been isolated from marine habitats for industrial use according to their unusual properties (Porras *et al*., 2017).

There are innumerable prokaryotic cells in all marine environments (Whitman *et al*., 1998). Novel microorganisms have been discovered, and their potential applications in several applied fields have been discussed, particularly in marine biodiversity. Geographically, Korea is largely surrounded by the sea, so the bacterial biodiversity in the marine environment could be an important biological resource in the future.

The present study was part of the investigation performed to expand the current knowledge of domestic biodiversity and focuses mainly on unrecorded bacterial species that have not been previously reported in Korea. In the current study, we briefly describe seven bacterial species in the orders *Bacillales* and *Flavobacteriales* belonging to seven genera (*Mesobacillus*, *Paenibacillus*, *Gramella*, *Gillisia*, *Arenibacter*, *Fictibacillus*, and *Brevibacillus*) of three families (*Flavobacteriaceae*, *Bacillaceae*, and *Paenibacillaceae*) based on the 16S rRNA gene

sequences. The phenotypic characteristics of the species were reported and they were identified as unrecorded bacterial species in Korea.

Materials and Methods

Samples were collected from marine sediment in the East Sea in March 2021. The obtained marine sediment samples (1.0 g) were suspended in 10 mL of 2% NaCl (v/v) and vortexed. The sample solution was serially diluted and an aliquot (100 μL) of the marine sediment samples was directly spread onto Marine (BD Difco) and Reasoner's 2A (R2A) agar (MB cell), respectively. Each plate was incubated at 30°C for two weeks, and different colonies were selected and repeatedly subcultured to the new culture media at the interval of two days. After more than three times, the pure colonies were obtained and stored at −80°C in a 20% (w/v) glycerol stock solution for preservation.

Phenotypic experiments were performed on cultures grown at 30°C in Marine and R2A media, respectively. The cell morphology of isolated strains was examined by light microscopy (model CX 23; Olympus, Tokyo, Japan) and transmission electron microscopy (LIBRA 120; Carl Zeiss, Oberkochen, Germany). Gram-staining of isolated strains was performed using a Gram stain kit (BioWORLD) according to the manufacturer's instructions. Each strain was cultured in liquid media with NaCl ranging from 0% to 10% (w/v) at increments of 1% for 2 days at 30°C and 180 rpm to evaluate the cell growth at varied salinities. The pH range for cell growth of isolated strains was assessed by growing the cells for 2 days with increments of 1.0 pH unit from pH 4.0 to 12.0 at 30°C and 180 rpm using the following buffer solutions: 100 mM CH_3COOH/CH_3COONa buffer (pH 4.0−6.0), 100 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0−8.0), 100 mM $NaHCO_3/Na_2CO_3$ buffer (pH 9.0−10.0), and 100 mM Na_2CO_3/NaOH buffer (pH 11.0−12.0). To ascertain the temperature range for growth, each strain was cultivated on solid culture and incubated at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, and 55°C. Catalase and oxidase tests were performed using 3% (v/v) H_2O_2 and 1% tetramethyl-p-phenylenediamine. Other phenotypic and biochemical properties were examined using API 20NE, and API 32GN test kits according to the manufacturer's instructions (bioMérieux, Marcy-l'Étoile, France).

Genomic DNA was extracted using the HiYield™ Genomic DNA Mini Kit (RBC Bioscience). The partial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers (Lane, 1991). The obtained PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). The purified PCR products were sent to Macrogen Co., Ltd. (Seoul, Korea) for sequencing. The products were assembled using the SeqMan™ II expert sequence analysis software (Thombre *et al*., 2016). Assembled sequences were identified based on the EzBioCloud 16S-based ID and the closely related taxa were obtained by the EzTaxon-e server (http://www.ezbiocloud.net/) (Yoon *et al*., 2017). Multiple sequence alignments were conducted using the Clustal W multiple sequence alignment program in the BioEdit 7.2.6.1 software (Thompson *et al*., 1994; Hall, 1999). Phylogenetic trees were constructed in MEGA 7.0 (Kumar *et al*., 2016), based on the 16S rRNA gene sequence. Using the neighbor-joining (NJ) algorithm, the sequence relatedness was calculated (Saitou and Nei, 1987). The phylogenetic tree was conducted using the bootstrap method, and the bootstrap values were set to 1000 replications. The Kimura two-parameter model was applied to calculate evolutionary distances (Kimura, 1980).

Results and Discussion

The 16S rRNA gene sequence analyses obtained herein revealed that a total of seven strains belonged to previously unreported species in Korea. The strain information, identification, taxonomic assignment from species to classes, isolation source, and sequence accession numbers including the HNIBR and GenBank are listed in Table 1. The seven strains were distributed in two orders of *Bacillales* and *Flavobacteriales*, four strains in the order *Bacillales*, and 3 strains in the order *Flavobacteriales*. All these strains in this study were rod and short-rod-shaped bacteria (Fig. 1). The strains in the order *Bacillales* were Gram-staining-positive and the strains in the order *Flavobacteriales* were Gran-staining-negative.

The four strains in the order *Bacillales* belong to two families and four genera: *Mesobacillus* (one species), *Paenibacillus* (one species), *Fictibacillus* (one species), and *Brevibacillus* (one species). The three strains in the order *Flavobacteriales* belong to one family and three genera: *Gramella* (one species), *Gillisia* (one species), and *Arenibacter* (one species). The identification of the seven strains corresponding to two different orders based on sequence similarity was supported by the phylogenetic tree using the NJ algorithm, respectively (Figs. 2 and 3). Detailed morphological, physiological, and basic biochemical characteristics of the seven unrecorded bacterial species are elucidated in the following strain descriptions.

Description of *Mesobacillus thioparans* MBLB1969

Cells are Gram-stain-positive, flagellated, and rod-shaped cells with 1.0−1.1 μm in width by 1.9−2.2 μm in length. Colonies are pink color, circular, and raised after two days of incubation on MA at 30°C. Catalase and oxi-

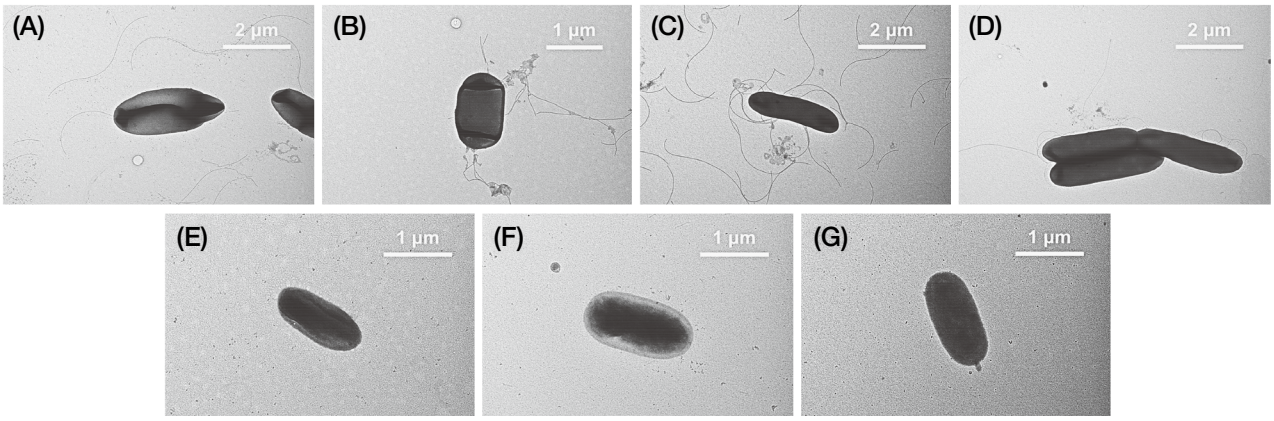

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: A. MBLB1969; B. MBLB2441; C. MBLB1992; D. MBLB2443; E. MBLB2135; F. MBLB2428; G. MBLB2431.

Table 1. Summary of all strains isolated in this study and their taxonomic affiliations.

Order	Family	Strain ID	Accession/NIBRBAC number	Closest species	Similarity (%)	Isolation source	Medium
Bacillales	*Bacillaceae*	MBLB1969	OP070049 NIBRBAC000509757	*Mesobacillus thioparans*	99.3	Marine sediment	MA at 30°C, 2 days
		MBLB2441	OP080646 NIBRBAC000509759	*Fictibacillus solisalsi*	99.9		R2A at 30°C, 2 days
	Paenibacillaceae	MBLB1992	OP077213 NIBRBAC000509758	*Paenibacillus contaminans*	98.7		R2A at 30°C, 2 days
		MBLB2443	OP080647 NIBRBAC000509760	*Brevibacillus fortis*	99.0		
Flavobacteriales	*Flavobacteriaceae*	MBLB2135	OP077214 NIBRBAC000509761	*Gramella oceani*	99.8		MA at 30°C, 2 days
		MBLB2428	OP080645 NIBRBAC000509762	*Gillisia hiemivivida*	99.2		
		MBLB2431	OP080644 NIBRBAC000509763	*Arenibacter catalasegens*	98.8		

dase activities are negative. Growth occurs in the 20−45°C (optimum, 30°C) at 0−6% NaCl (optimum, 3%) at pH 6.0−8.0 (optimum, pH 7.0). In API 20NE test, positive for the reduction of nitrates (NO_3) to nitrite (NO_2^-), esculin hydrolysis, and β-galactosidase activity; but negative for indole production, D-glucose fermentation, arginine dihydrolase, urease activity, and gelatin hydrolysis. In API 32GN test, D-mannitol, D-glucose, D-melibiose, D-sorbitol, 3-hydroxy-butyrate, L-rhamnose, D-sucrose, D-maltose, lactate, L-alanine, and glycogen are utilized; but salicin, L-fucose, L-arabinose, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, *N*-acetyl-D-glucosamine, D-ribose, inositol, itaconate, suberate, malonate, acetate, 5-ketogluconate, 3-hydroxybenzoate, and L-serine are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP070049. Strain MBLB1969 (=NIBRBAC000509757) was isolated from a marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36°42′21.8″N, 129°35′0.7″E).

Description of *Fictibacillus solisalsi* MBLB2441

Cells are Gram-stain-positive, flagellated, and short rod-shaped cells with 0.9−1.1 μm in width by 1.4−1.9 μm in length. Colonies are white-cream, circular, and convex

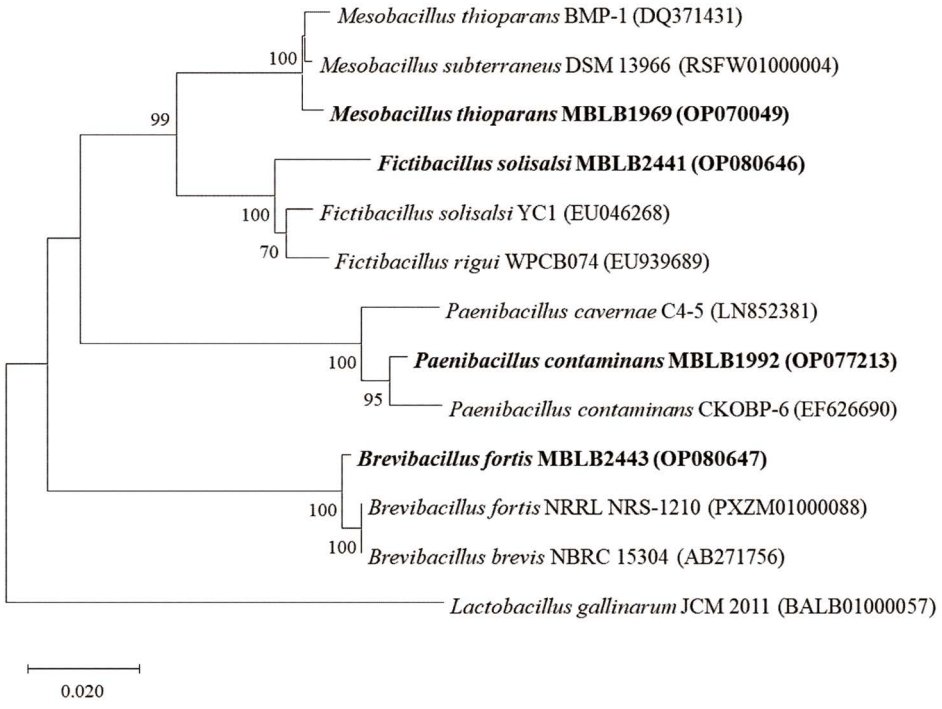

Fig. 2. Neighbor-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences between the strains isolated in this study belonging to the order *Bacillales*. A phylogenetic tree was constructed with their relatives of the genera *Mesobacillus*, *Paenibacillus*, *Fictibacillus*, and *Brevibacillus*. The numbers on the nodes indicate the bootstrap values (>70%). Bar, 0.02 accumulated changes per nucleotide, respectively.

after two days of incubation on R2A at 30°C. Catalase and oxidase activities are negative. Growth occurs in the 20−40°C (optimum, 30°C) at 0−4% NaCl (optimum, 2%) at pH 5.0−12.0 (optimum, pH 7.0). In API 20NE test, positive for esculin hydrolysis and β-galactosidase activity; but negative for reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), indole production, D-glucose fermentation, arginine dihydrolase, urease activity, and gelatin hydrolysis. In API 32GN test, D-mannitol, D-glucose, D-melibiose, L-fucose, L-arabinose, propionate, valerate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, L-proline, *N*-acetyl-D-glucosamine, D-ribose, D-sucrose, D-maltose, acetate, L-alanine, glycogen, and L-serine are utilized; but salicin, D-sorbitol, caprate, citrate, 4-hydroxy-benzoate, L-rhamnose, inositol, itaconate, suberate, malonate, lactate, 5-ketogluconate, and 3-hydroxy-benzoate are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP080646. Strain MBLB2441 (=NIBR BAC000509759) was isolated from a marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36° 42′21.8″N, 129°35′0.7″E).

Description of *Paenibacillus contaminans* MBLB1992

Cells are Gram-stain-positive, flagellated, and rod-shaped cells with 0.7−0.9 μm in width by 2.1−2.8 μm in length. Colonies are colorless, semi-transparent, circular, and convex after two days of incubation on R2A at 30°C. Catalase activity is negative, but oxidase activity is positive. Growth occurs in the 10−40°C (optimum, 30°C) at 0−2% NaCl (optimum, 1%) at pH 6.0−8.0 (optimum, pH 7.0). In API 20NE test, positive for esculin hydrolysis and β-galactosidase activity; but negative for reduction of nitrates (NO_3) to nitrite (NO_2^-), reduction of nitrates (NO_3) to nitrogen (N_2), indole production, D-glucose fermentation, arginine dihydrolase, urease activity, and gelatin hydrolysis. In API 32GN test, D-glucose, salicin, D-melibiose, *N*-acetyl-D-glucosamine, D-sucrose, and D-maltose are utilized; but D-mannitol, L-fucose, D-sorbitol, L-arabinose, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, D-ribose, inositol, itaconate, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP077213. Strain MBLB1992 (=NIBRBAC000509758) was isolated from marine sediment from the East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36°42′21.8″N, 129°35′0.7″E).

Description of *Brevibacillus fortis* MBLB2443

Cells are Gram-stain-positive, flagellated, and rod-

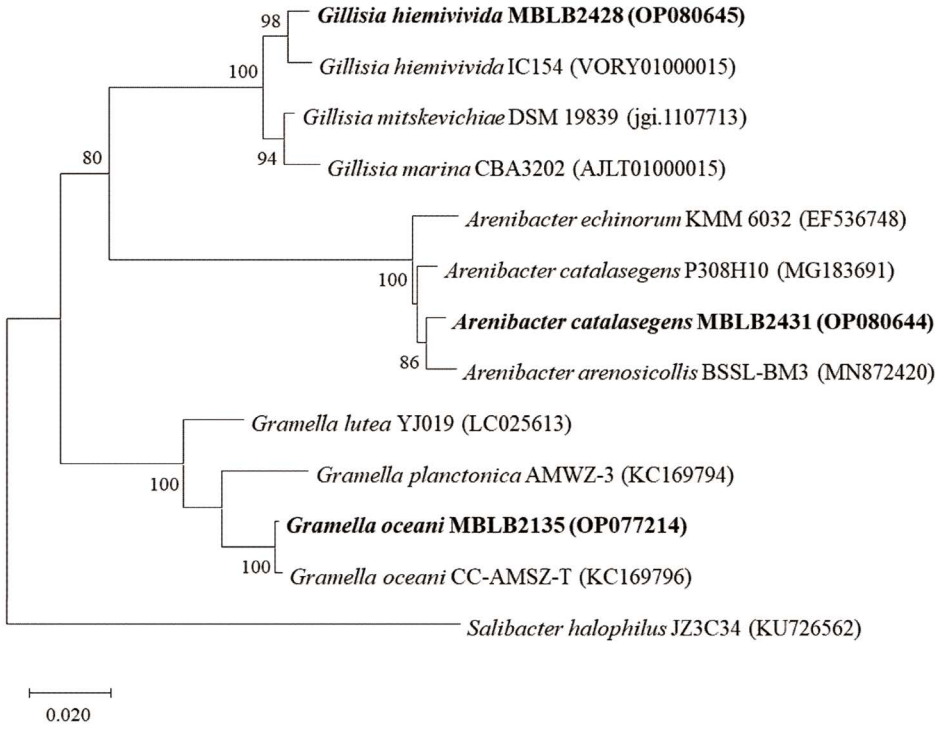

Fig. 3. Neighbor-joining (NJ) phylogenetic tree based on the 16S rRNA gene sequences between the strains isolated in this study belonging to the order *Flavobacteriales*. A phylogenetic tree was constructed with their relatives of the genera *Gramella*, *Gillisia*, and *Arenibacter*. The numbers on the nodes indicate the bootstrap values (>70%). Bar, 0.02 accumulated changes per nucleotide, respectively.

shaped cells with 0.7−0.9 μm in width by 3.0−3.2 μm in length. Colonies are white-cream color, circular, and convex after two days of incubation on R2A at 30°C. Catalase activity is negative, but oxidase activity is positive. Growth occurs in the 10−35°C (optimum, 30°C) at 0−3% NaCl (optimum, 1%) at pH 5.0−8.0 (optimum, pH 7.0). In API 20NE test, positive for the reduction of nitrates (NO_3) to nitrite (NO_2^-); but negative for indole production, D-glucose fermentation, arginine dihydrolase, urease activity, esculin and gelatin hydrolysis, and β-galactosidase activity. In API 32GN test, D-glucose, L-arabinose, L-histidine, 3-hydroxy-butyrate, *N*-acetyl-D-glucosamine, D-ribose, D-sucrose, acetate, L-alanine, and L-serine are utilized; but D-mannitol, salicin, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, L-rhamnose, inositol, D-maltose, itaconate, suberate, malonate, lactate, 5-ketogluconate, glycogen, and 3-hydroxy-benzoate are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP080647. Strain MBLB2443 (=NIBRBAC000509760) was isolated from a marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36°42′21.8″N, 129°35′0.7″E).

Description of *Gramella oceani* MBLB2135

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped cells with 0.6−0.7 μm in width by 1.2−1.4 μm in length. Colonies are yellow color, circular, and raised after two days of incubation on MA at 30°C. Catalase and oxidase activities are negative. Growth occurs in the 10−30°C (optimum, 25°C) at 0−6% NaCl (optimum, 3%) at pH 6.0−8.0 (optimum, pH 7.0). In API 20NE test, positive for esculin and gelatin hydrolysis; but negative for reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production, D-glucose fermentation, arginine dihydrolase, urease activity, esculin and gelatin hydrolysis, and β-galactosidase activity. In API 32GN test, D-mannitol, D-glucose, citrate, L-rhamnose, *N*-acetyl-D-glucosamine, 5-ketogluconate, and glycogen are utilized; but salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, propionate, caprate, valerate, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, acetate, lactate, L-alanine, 3-hydroxy-benzoate, and L-serine are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP077214. Strain MBLB2135 (=NIBRBAC000509761) was isolated from marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36°42′21.8″N, 129°35′0.7″E).

Description of *Gillisia hiemivivida* MBLB2428

Cells are Gram-stain-negative, non-flagellated, and short

rod-shaped cells with 0.7−0.8 μm in width by 1.5−1.7 μm in length. Colonies are yellow color, circular, and raised after two days of incubation on MA at 30°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs in the 20−40°C (optimum, 30°C) at 0−6% NaCl (optimum, 3%) at pH 6.0−9.0 (optimum, pH 7.0). In the API 20NE test, negative for reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production, D-glucose fermentation, arginine dihydrolase, urease activity, esculin and gelatin hydrolysis, and β-galactosidase activity. In API 32GN test, D-glucose, L-arabinose, propionate, L-proline, *N*-acetyl-D-glucosamine, D-sucrose, L-alanine, 5-ketogluconate, and glycogen are utilized; but D-mannitol, salicin, D-melibiose, L-fucose, D-sorbitol, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-rhamnose, D-ribose, inositol, D-maltose, itaconate, suberate, malonate, acetate, lactate, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP080645. Strain MBLB2428 (=NIBRBAC000509762) was isolated from a marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36°42′21.8″N, 129°35′0.7″E).

Description of *Arenibacter catalasegens* MBLB2431

Cells are Gram-stain-negative, non-flagellated, and short rod-shaped cells with 0.7−0.9 μm in width by 1.5−1.9 μm in length. Colonies are orange color, circular, and raised after two days of incubation on MA at 30°C. Catalase and oxidase activities are negative. Growth occurs in the 10−40°C (optimum, 30°C) at 0−6% NaCl (optimum, 3%) at pH 5.0−9.0 (optimum, pH 7.0). In API 20NE test, positive for esculin hydrolysis and β-galactosidase activity; but negative for reduction of nitrates (NO_3) to nitrite (NO_2^-), indole production, D-glucose fermentation, arginine dihydrolase, urease activity, and gelatin hydrolysis. In API 32GN test, D-mannitol, D-glucose, salicin, D-melibiose, D-sorbitol, L-arabinose, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, acetate, lactate, 5-ketogluconate, glycogen, and 3-hydroxy-benzoate are utilized; but L-fucose, propionate, caprate, valerate, citrate, L-histidine, L-proline, itaconate, suberate, malonate, L-alanine, and L-serine are not utilized as a sole energy source. The NCBI accession number for the 16S rRNA gene sequence is OP080644. Strain MBLB2431 (=NIBR BAC000509763) was isolated from a marine sediment from East Sea, Uljin-gun, Gyeongsangbuk-do, Korea (36° 42′21.8″N, 129°35′0.7″E).

Acknowledgements

This work was supported by grants from the National Institute of Biological Resources funded by the Ministry of Environment (No. NIBR202203112), Republic of Korea.

References

Cheng, T.H., N. Ismail, N. Kamaruding, J. Saidin and M. Danish-Daniel. 2020. Industrial enzymes-producing marine bacteria from marine resources. Biotechnology Reports 27:e00482.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Serries 41:95-98.

Jahromi, S.T. and N. Barzkar. 2018. Future direction in marine bacterial agarases for industrial applications. Applied Microbiology and Biotechnology 102:6847-6863.

Jannasch, H.W. and C.D. Taylor. 1984. Deep-sea microbiology. Annual Review of Microbiology 38:487-514.

Kato, C., T. Sato and K. Horikoshi. 1995. Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. Biodiversity and Conservation 4:1-9.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular and Evolution 16:111-120.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Lane, D.J. 1991. 16S/23S rRNA sequencing. In: E.G. Stackebrandt (ed.), Nucleic Acid Techniques in Bacterial Systematics, John Wiley and Sons, New York, NY, USA. pp. 115-148.

Porras, M.A., C. Witale, M.A. Villar and M.A. Cubitto. 2017. Bioconversion of glycerol to poly (HB-co-HV) copolymer in an inexpensive medium by a *Bacillus megaterium* strain isolated from marine sediments. Journal of Environmental Chemical Engineering 5:1-9.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Takami, H., K. Kobata, T. Nagahama, H. Kobayashi, A. Inoue and K. Horikoshi. 1999. Biodiversity in deep-sea sites located near the south part of Japan. Extremophiles 3:97-102.

Thombre, R.S., V.D. Shinde, R.S. Oke, S.K. Dhar and Y.S. Shouche. 2016. Biology and survival of extremely halophilic archaeon *Haloarcula marismortui* RR12 isolated from Mumbai salterns, India in response to salinity stress. Scientific Report 6:25642.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Whitman, W.B., D.C. Coleman and W.J. Wiebe. 1998. Prokaryotes: The unseen majority. PNAS 95:6578-6583.

Yoon, S.H., S.M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology 67:1613-1617.

Submitted: December 30, 2022
Revised: February 7, 2023
Accepted: February 9, 2023