Marivivens aquimaris sp. nov., isolated from seawater

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Abstract
A Gram-stain-negative, strictly aerobic, non-flagellated, rod-shaped bacterium, designated GSB7T, was isolated from seawater collected at the Yellow Sea coast of South Korea. Catalase and oxidase activities were positive. Growth occurred at pH 6.0–9.0 (optimum pH 7.0), 10–40 °C (optimum 30 °C) and with 0–8% NaCl (optimum 1–2%). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain GSB7T belonged to the genus Marivivens, showing the sequence similarities of 96.3, 96.1, and 96.0% with Marivivens niveibacter HSLHS2T, Limimaricola hongkongensis DSM17492T, and Marivivens donghaensis AM-4T, respectively. The respiratory quinone was ubiquinone-10 and the major fatty acids were summed feature 8 (C18:1 ω7c and/or C18:1 ω6c), C18:1 ω7c 11-methyl, C16:0 and C10:0 3-OH. The polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, one unidentified aminolipid, and five unidentified lipids. The DNA G + C content calculated from the whole-genome sequence was 60.6 mol%. On the basis of phenotypic, chemotaxonomic and genotypic characteristics presented in this study, strain GSB7T is suggested to represent a novel species of the genus Marivivens, for which the name Marivivens aquimaris sp. nov. is proposed. The type strain is GSB7T (= KCTC 82026T = JCM 34042T).

Keywords Marivivens aquimaris · Novel species · 16S rRNA · Genome · Taxonomy

Introduction
The genus Marivivens, belonging to the family Rhodobactraceae within the order Rhodobacterales, was first described by Park et al. (2016). Currently, the genus Marivivens comprises 2 species with validly published names: Marivivens donghaensis and Marivivens niveibacter. Two species have been isolated from seawater (Park et al. 2016; Hu et al. 2018). In this study, a bacterial strain GSB7T was isolated from the Yellow Sea, South Korea. To determine the taxonomic position of strain GSB7T, we carried out that included chemotaxonomic, phenotypic, phylogenetic investigations based on 16S rRNA gene sequences, and genomic analysis.

Materials and methods
Bacterial isolation and maintenance

Strain GSB7T was isolated from seawater collected at the Yellow Sea coast (located in Buan-gun), South Korea. For isolation, the seawater sample was serially diluted in sterile seawater and suspensions were spread-plated on marine agar 2216 (MA, Difco) and incubated at 25 °C for 5 days. Strain GSB7T was isolated and streaked on MA several times to
obtain pure single colony. The novel strain was routinely cultured on marine broth (MB, Difco) and maintained at –80 °C with 20% (v/v) glycerol. This isolate was deposited into the Korean Collection for Type Cultures (KCTC) as KCTC 82026T and the Japan Collection of Microorganisms (JCM) as JCM 34042T. *M. donghaensis* KCTC 42776T (= AM-4T) and *M. niveibacter* KCTC 52588T (= HSLHS2T) were obtained from KCTC and used as reference strains in this study.

### 16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of strain GSB7T was extracted using a genomic DNA purification kit (Invitrogen), according to the method provided by the manufacturer. The 16S rRNA gene was amplified by PCR using forward primer 27F (5′-AGA GTT TGA TCC TGGCT-3′) and reverse primer 1492R (5′-GGTTACCTTGTAGGACATT-3′), then cloned and sequenced using primers 518F (5′-CCAGCAGCGCGTA ATAC-3′) and 805R (5′-GACTACGGGATCTAATC-3′) by Biofact Co., Ltd. (Korea). The 16S rRNA gene pairwise similarity was determined using the EzBioCloud database (Yoon et al. 2017a). Sequence alignment and phylogenetic analysis were performed using MEGA X software (Kumar et al. 2018) and phylogenetic trees were reconstructed using the neighbor-joining (NJ) (Saitou and Nei 1987), the maximum-likelihood (ML) (Felsenstein 1981) and the maximum-parsimony (MP) (Fitch 1971). The topology of phylogenetic trees was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein 1985). Evolutionary distance was calculated using the Kimura 2-parameter model (Kimura 1980).

### Whole genome sequencing and analysis

The whole-genome sequence of strain GSB7T was obtained by PacBio RS II (Pacific Biosciences) sequencing at Macrogen Co., Ltd. (Republic of Korea) and the sequencing reads were assembled with Hierarchical Genome Assembly Process (HGAP) v3.0 (McCarthy 2010; Chin et al. 2013). The complete genome of GSB7T was deposited in the GenBank database and annotated using the eggNOG 4.5 database (Huerta-Cepas et al. 2016). The genomic DNA G+C content was calculated from whole-genome sequences. The average nucleotide identity (ANI) values between strain GSB7T and reference strains were calculated using Chun-Lab’s online ANI calculator (www.ezbiocloud.net/tools/orthoani). The digital DNA-DNA hybridization (dDDH) values were calculated using Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/ggdc.php). Phylogenomic analysis was performed with the up-to-date bacterial core gene set (UBCG) by following the pipelines suggested by Na et al. (2018).

### Morphology and physiology and biochemical analysis

The cell morphology of strain GSB7T was observed by transmission electron microscopy (Libra120, Zeiss) using cells grown on MA at 25 °C for 2 days. Gram reaction was performed using Gram Stain Kit (Difco) according to the manufacturer’s instructions. The gliding-motility test was performed as wet mount method using the phase-contrast optical microscope (DM500, Leica). The growth temperature range was determined under various temperatures (4, 10, 15, 20, 25, 30, 37, 42, 45, 50 and 55 °C) by incubating the MA plate for 5 days. The pH range for growth was tested in MB for 5 days at pH 3.0–11.0 using the appropriate biological buffers: citrate–phosphate (pH 3.0–6.0), Tris-HCl (pH 7.0–9.0) and sodium carbonate/sodium bicarbonate (pH 10.0–11.0) (Gomori 1955). The NaCl tolerance range was determined using the modified MB medium without NaCl, supplemented with 0–15% NaCl (w/v). Growth on various media such as R2A agar (Difco), nutrient agar (NA, Difco) and tryptic soy agar (TSA, Difco) and tryptic soy agar (TSA, Difco) and MA was evaluated. R2A, NA and TSA were supplemented with 2% (w/v) NaCl. Anaerobic growth on MA was assessed at 25 °C for 10 days using the Oxoid Anaerogen system (Oxoid, UK). Catalase activity was examined for bubble production after the addition of 3% (v/v) hydrogen peroxide and oxidase activity was determined using oxidase strip saturated with N,N,N′,N′′-tetramethyl-1,4-phenylenediamine (Oxidase strip, Milipore). Hydrolysis of casein (5%, w/v), starch (1%, w/v), α-cellulose (1%, w/v) and L-tyrosine (1%, w/v) were examined after 5 days of incubation (Barrow and Feltham 2004). Physiological and biochemical characterizations were carried out using API ZYM, 20NE and 50CH kits according to the manufacturer’s instructions (bioMérieux).

### Chemotaxonomic characterization

For fatty acid analysis, cells of strain GSB7T, *M. donghaensis* KCTC 42776T and *M. niveibacter* KCTC 52588T were grown on MB at 30 °C for 3 days. Then, cells were harvested and lyophilized. Fatty acid methyl esters (FAME) were analyzed using the Sherlock Microbial Identification System (MIDI) version 6.3 and the RTSBA6 version of the database according to the standard protocol (Sasser 1990). The respiratory quinone was extracted from the freeze-dried cells with hexane and analyzed by HPLC (Collins 1985). Polar lipids were extracted using a chloroform/methanol method and analyzed by separation using two-dimensional TLC (silica gel 60 F254 plates, art. 5554; Merck) (Minnikin et al. 1984). Total lipids were detected using molybdophosphoric
acid (Merck) and specific functional groups detected using specific reagents for defined functional groups (Collins and Jones 1980).

Results and discussion

Morphology, physiology and biochemical analysis

Colonies of strain GSB7T on MA were beige-colored, smooth, circular, convex and 1.0–1.5 mm in diameter after growing at 30 °C for 2 days. Cells of GSB7T were Gram-stain-negative, strictly aerobic, oxidase- and catalase-positive and rods (Fig. S1). Growth occurs on MA but not on R2A, NA and TSA medium in the presence of 2% NaCl. The detailed physiological and biochemical characteristics of the novel strain were summarized in the species description and compared to those of closely related strains in Table 1. Some negative reactions from API ZYM, API 20NE and API 50CH strip tests were shown in Table S1.

Phylogenetic and whole-genome analysis

The nearly complete 16S rRNA gene sequence of strain GSB7T (1402 bp) was obtained. Sequence comparison showed that strain GSB7T shared the highest 16S rRNA gene sequence similarity to M. niveibacter HSLHS2T (KY020996, 96.3%), followed by Limicaricola hongkongensis UST950701-009P T (AY600300, 96.1%) and M. donghaensis AM-4T (KT282004, 96.0%). This indicated that strain GSB7T represented a novel species based on 16S rRNA gene sequence similarity of 98.7% defined as the threshold value for bacterial species delineation (Stackebrandt and Ebers 2006). Phylogenetic tree based on 16S rRNA gene sequences, the GSB7T strain clustered with two species of the genus Marivivens in NJ (Fig. 1), ML and MP trees (Fig. S2, S3). The phylogenomic tree reconstructed by

Table 1

| Characteristic                                      | Strain 1 | Strain 2 | Strain 3 |
|-----------------------------------------------------|----------|----------|----------|
| Temperature range (optimum) (°C)                    | 10–40    | 10–40    | 5–40     |
| NaCl range (optimum) (%. w/v)                       | 0–8      | 0–6      | 0–10     |
| pH range (optimum)                                  | 6–9      | NA       | 6–10     |
| Reduction of nitrate                                | −        | +        | −        |
| Enzyme activity (ZYM)                               | +        | −        | +        |
| Alkaline phosphatase                                | +        | −        | +        |
| Esterase (C4)                                       | +        | −        | −        |
| Esterase Lipase (C8)                                | +        | −        | −        |
| N-Acetyl-β-glucosaminase                            | +        | −        | −        |
| Assimilation of (API 20NE, 50CH)                    |          |          |          |
| Mannose, D-Xylose                                   | +        | +        | −        |
| D-Fucose, Potassium 2-ketogluconate, Potassium 5-ketogluconate | +        | −        | +        |
| N-Acetyl-glucosamine, Potassium gluconate, D-Turanose, D-Lylose, L-Fucose, L-Arabitol, D-Tagatose | +        | −        | −        |
| D-Galactose, D-Fructose                              | −        | +        | +        |
| D-Sorbitol, D-Cellobiose, D-Lactose, D-Melibiose, D-Saccharose, D-Trehalose | −        | −        | +        |
| DNA G+C content                                     | 60.6     | 60.8     | 54.6     |
| Genome size (Mb)                                     | 4.05     | 3.34     | 3.07     |

Strains: 1, GSB7T; 2, M. donghaensis KCTC 42776T; 3, M. niveibacter KCTC 52588T. All data were obtained in this study unless otherwise indicated. +: positive, −: negative, NA: not available. All strains positive for: Leucine arylamidase, Naphotl-AS-BI-phosphohydrolase, β-glucosidase, β-galactosidase, malate assimilation, trisodium citrate assimilation, D-arabitol assimilation. All strains negative for: Lipase (C14), Crystine arylamidase, Trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, indole production, glucose fermentation, arginine dihydrolase, urease, protease (gelatin hydrolysis), capric acid assimilation

aData from Park et al. (2016)
bData from NCBI database (GCA 011806565)
cData from Hu et al. (2018)
the UBCG pipeline also showed that the type strains of the genus *Marivivens* were in the same cluster as the GSB7<sup>T</sup> strain (Fig. S4). The de novo assembly of the whole-genome sequence data of GSB7<sup>T</sup> resulted in 7 contigs, with an N50 of 3,292,022 bp and average genome coverage of 238 x. The total length of the draft genome of GSB7<sup>T</sup> was 4,051,696 bp with a DNA G+C content of 60.0 mol%, which contained 4015 gene, 3944 CDS, 58 tRNA, and 12 rRNA. The GenBank accession number of the genome of strain GSB7<sup>T</sup> is JADBGB000000000. The ANI values of strain GSB7<sup>T</sup> to *M. donghaensis* AM-4<sup>T</sup> and *M. niveibacter* HSLHS2<sup>T</sup> were 92.9% and 73.3%, which were lower than the 95–96% cut-off value for the bacterial species boundary (Chun et al. 2018; Yoon et al. 2017b). The dDDH values of GSB7<sup>T</sup> to *M. donghaensis* AM-4<sup>T</sup> and *M. niveibacter* HSLHS2<sup>T</sup> were 49.8% and 18.8%, which were also below the 70% similarity for the bacterial species boundary (Meier-Kolthoff et al. 2013). According to the results of phylogenetic and genome analysis, the strain GSB7<sup>T</sup> should represent a novel species of the genus *Marivivens*.

**Chemotaxonomic characterization**

The fatty acid compositions of the GSB7<sup>T</sup> and two reference strains are shown in Table 2. Major cellular fatty acids of strain GSB7<sup>T</sup> were summed feature 8 (C<sub>18:1</sub> ω7c and/or C<sub>18:1</sub> ω6c, 55.9%), C<sub>18:1</sub> ω7c 11-methyl (16.2%), C<sub>16:0</sub> (12.9%) and C<sub>10:0</sub> 3-OH (7.5%) which was similar to those
11-methyl contained 16.2% in the strain GSB7T but related strain dylethanolamine, which is differentiated between the closely Marivivens. The polar lipids profile of strain GSB7T consisted of phosphatidylglycerol, but not phosphatidylglycerol, diphosphatidylglycerol, one unidentified aminolipid and five unidentified lipids. Strain GSB7T contained phosphatidylglycerol, diphasphatidylglycerol, one unidentified aminolipid and five unidentified lipids. The DNA G+C content is 60.6 mol%.

The type strain, GSB7T (= KCTC 82026T = JCM 34042T), was isolated from seawater collected at Yellow Sea coast of South Korea.

The GenBank accession numbers of the 16S rRNA gene and genome sequence of strain GSB7T are MW035312 and JADBGB000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02305-7.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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**Table 2** Cellular fatty acid profiles of strain GSB7T and closely related strains

| Fatty acid          | 1   | 2   | 3   |
|---------------------|-----|-----|-----|
| C_{10.0} 3-OH       | 7.46| 7.94| 6.95|
| C_{12.0} 3-OH       | ND  | ND  | 4.55|
| C_{16.0}            | 12.86| 13.28| 14.18|
| C_{17.0}            | TR  | TR  | 1.72|
| C_{17.0} iso 3-OH   | 1.01| ND  | ND  |
| C_{18.0}            | 4.13| 2.08| 3.93|
| C_{18:1} ω7c 11-methyl | 16.19| 5.38| 3.09|
| Summed feature 3    | 1.27| 9.03| TR  |
| Summed feature 7    | TR  | TR  | 1.39|
| Summed feature 8    | 55.85| 60.57| 61.44|

Strains: 1, GSB7T; 2, *M. donghaensis* KCTC 42776T; 3, *M. niveibacter* KCTC 52588T. TR less than 1%, ND not detected. All data were obtained in this study. Fatty acids that represented >1.0% in all strains were shown.

Summed features represent groups of two or three fatty acids that cannot be separated using MIDI system. Summed feature 3, C_{16:1} ω7c and/or C_{16:1} ω6c; summed feature 7, C_{19:1} ω7c and/or C_{19:1} ω6c; summed feature 8, C_{18:1} ω7c or C_{18:1} ω6c.

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The taxonomic conclusion

Based on the phylogenetic, genomic, phenotypic, physiological, biochemical and chemotaxonomic analysis results, strain GSB7T represents a novel species of the genus *Marivivens*, for which the name *Marivivens aquimaris* sp. nov. is proposed.

**Description of Marivivens aquimaris** sp. nov.

*Marivivens aquimaris* (a.qui.ma’ris. L. fem. n. *aqua* water; L. gen. n. *maris* of the sea, marine; N.L. gen. n. *aquimaris* of seawater).

Cells are Gram-stain-negative, strictly aerobic, rods, approximately 0.8–1.0 μm wide and 1.5–4.0 μm long in size. After incubation for 2 days on MA plate, colonies are 1.0–1.5 mm in diameter, beige-colored, convex, smooth and circular. Growth occurs at pH 6.0–9.0 (optimum pH 7.0) and 10–40 °C (optimum 30 °C). The NaCl concentration range for growth is 0–8% (w/v) and optimal growth occurs at 1–2% (w/v). Catalase and oxidase are positive. H2S is not produced. Negative for the hydrolysis of casein, starch, α-cellulose and L-tyrosine. In the API ZYM strip test, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-glucosidase and N-acetyl-β-glucosaminidase; weak positive for valine arylamidase, acid phosphatase and α-glucosidase. In the API 20NE strip test, positive for β-glucosidase (aesculin hydrolysis), β-galactosidase. According to API 20NE and 50CH tests, positive for assimilation of D-glucose, D-mannose, D-mannitol, maltose, D-xylene, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, N-acetyl-glucosamine, potassium glconate, potassium 2-ketogluconate, potassium 5-ketogluconate, malic acid and trisodium citric acid; weak positive for assimilation of L-arabinose, adic acid and phenylacetic acid. The respiratory quinone is Q-10. Major fatty acids are summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c), C_{18:1} ω7c 11-methyl, C_{16:0} and C_{19:0} 3-OH. The polar lipids consist of phosphatidylglycerol, diphasphatidylglycerol, one unidentified aminolipid and five unidentified lipids. The DNA G+C content is 60.6 mol%.

The type strain, GSB7T (= KCTC 82026T = JCM 34042T), was isolated from seawater collected at Yellow Sea coast of South Korea.

The GenBank accession numbers of the 16S rRNA gene and genome sequence of strain GSB7T are MW035312 and JADBGB000000000, respectively.

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