Marivivens Aquimaris Sp. Nov., Isolated from Seawater

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Abstract

A Gram-stain-negative, strictly aerobic, non-flagellated, rod-shaped bacterium, designated GSB7^T, 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 GSB7^T belonged to the genus *Marivivens*, showing the sequence similarities of 96.3, 96.1, and 96.0% with *Marivivens niveibacter* HSLHS2^T, *Limimaricola hongkongensis* DSM17492^T, *Marivivens donghaensis* AM-4^T, respectively. The respiratory quinone was ubiquinone-10 and the major fatty acids were summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c), C_{18:1} ω7c 11-methyl, C_{16:0} and C_{10:0} 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 GSB7^T 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 GSB7^T (=KCTC 82026^T=JCM 34042^T).

Introduction

The genus *Marivivens*, belonging to the family *Rhodobacteraceae* 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. The common characteristics of the genus *Marivivens* include gram-stain-negative, aerobic, non-motile, catalase- and oxidase positive cells that contain Q-10 as a predominant respiratory quinone (Park et al. 2016; Hu et al. 2018). In this study, a bacterial strain GSB7^T was isolated from the Yellow Sea, South Korea. To determine the exact taxonomic position of strain GSB7^T 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 GSB7^T 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 GSB7^T 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 82026^T and the Japan Collection of Microorganisms (JCM) as JCM 34042^T. *M. donghaensis* KCTC 42776^T (= AM-4^T) and *M. niveibacter* KCTC 52588^T (= HSLHS2^T) were obtained from KCTC and used as reference strains in this study.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of strain GSB7^T 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'-AGAGTTTGATCCTGGCT-3') and reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3'), then cloned and sequenced using primers 518F (5'-CCAGCAGCCGCGGTAATAC-3') and 805R (5'-GACTACCAGGGTATCTAATC-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 1,000 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 GSB7^T 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 GSB7^T 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 GSB7^T and reference strains were calculated using ChunLab'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 GSB7^T 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 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, Millipore). 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 GSB7^T, *M. donghaensis* KCTC 42776^T and *M. niveibacter* KCTC 52588^T were grown on MB at 30°C for 3 days. Then cells were harvested and lyophilized. Fatty acid methyl esters (FAME) were analyzed by using the Sherlock Microbial Identification System (MIDI) version 6.3 and the RTSBA6 version of the database according to the standard protocol (Sasser 2001). 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 GSB7^T 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 GSB7^T were Gram-stain-negative, strictly aerobic, oxidase- and catalase-positive and rods (Fig. S1). The strain could grow at 10–40°C (optimum, 30°C), at pH 6–9 (optimum, pH 7) and with 0–8% NaCl concentrations (optimum, 1–2%). 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.

Table 1

Differential characteristics between strain GSB7^T and closely related species of the genus *Marivivens*

Characteristic	1	2	3
Temperature range (optimum) (°C)	10–40 (30)	10–40 ^a (30–35) ^a	5–40 ^c (35) ^c
NaCl range (optimum) (% w/v)	0–8 (1–2)	0–6 ^a (1–3) ^a	0–10 ^c (2) ^c
pH range (optimum)	6–9 (7)	NA ^a (7–8) ^a	6–10 ^c (6–8) ^c
Reduction of nitrate	–	+	–
Enzyme activity (API ZYM):			
Alkaline phosphatase	+	–	+
Esterase (C4)	+	–	–
Esterase Lipase (C8)	+	–	–
<i>N</i> -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-Lyxose, L-Fucose, L-Arabitol, D-Tagatose	+	–	–
D-Galactose, D-Fructose	–	+	+

Strains: 1, GSB7^T; 2, *M. donghaensis* KCTC 42776^T; 3, *M. niveibacter* KCTC 52588^T. All data were obtained in this study unless otherwise indicated. + positive, – negative, NA not available. All strains positive for: Leucine arylamidase, Naphtol-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)

Characteristic	1	2	3
D-Sorbitol, D-Cellobiose, D-Lactose, D-Melibiose, D-Saccharose, D-Trehalose	–	+	–
DNA G + C content	60.6	60.8 ^b	54.6 ^c
Genome size (Mb)	4.05	3.34 ^b	3.07 ^c
Strains: 1, GSB7 ^T ; 2, <i>M. donghaensis</i> KCTC 42776 ^T ; 3, <i>M. niveibacter</i> KCTC 52588 ^T . All data were obtained in this study unless otherwise indicated. + positive, – negative, NA not available. All strains positive for: Leucine arylamidase, Naphtol-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			
^a Data from Park et al. (2016)			
^b Data from NCBI database (GCA 011806565)			
^c Data from Hu et al. (2018)			

Phylogenetic and whole genome analysis

The nearly complete 16S rRNA gene sequence of strain GSB7^T (1,402 bp) was obtained. Sequence comparison showed that strain GSB7^T shared the highest 16S rRNA gene sequence similarity to *M. niveibacter* HSLHS2^T (KY020996, 96.3%), followed by *Limimanicola hongkongensis* UST950701-009P^T (AY600300, 96.1%) and *M. donghaensis* AM-4^T (KT282004, 96.0%). This indicated that strain GSB7^T represented a novel species based on 16S rRNA gene sequence similarity of 98.7% defined as the threshold value for bacterial species delineation (Kim et al. 2014). Phylogenetic tree based on 16S rRNA gene sequences, the GSB7^T 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 the UBCG pipeline also showed that the type strains of the genus *Marivivens* were in the same cluster as the GSB7^T strain (Fig. 2). The *de novo* assembly of the whole genome sequence data of GSB7^T resulted in 7 contigs, with an N50 of 3,292,022 bp and average genome coverage of 238 $\times$. The total length of the draft genome of GSB7^T was 4,051,696 bp with a DNA G + C content of 60.0 mol%, which contained 4,015 gene, 3,944 CDS, 58 tRNA, and 12 rRNA. The GenBank accession number of the genome of strain GSB7^T is JADBGB000000000. The ANI values of strain GSB7^T to *M. donghaensis* AM-4^T and *M. niveibacter* HSLHS2^T 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^T to *M. donghaensis* AM-4^T and *M. niveibacter* HSLHS2^T 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^T should represent a novel species of the genus *Marivivens*.

Chemotaxonomic characterization

The fatty acid compositions of the GSB7^T and two reference strains are shown in Table 2. Major cellular fatty acids of strain GSB7^T were summed feature 8 (C_{18:1} ω 7c and/or C_{18:1} ω 6c, 55.9%), C_{18:1} ω 7c 11-methyl (16.2%), C_{16:0} (12.9%) and C_{10:0} 3-OH (7.5%) which was similar to those the reference strains, but there were also some differences in the proportions of some fatty acids. For examples, C_{18:1} ω 7c 11-methyl contained 16.2% in the strain GSB7^T but relatively lower (5.4 and 3.1%) in the reference strains (KCTC 42776^T and KCTC 52588^T). Also, C_{17:0} iso 3OH contained in the strain GSB7^T but not detected in the reference strains. The quinone of strain GSB7^T was ubiquinone-10 (Q-10), which was in accordance with the member of the genus *Marivivens*. The polar lipids profile of strain GSB7^T consisted of phosphatidylglycerol, diphosphatidylglycerol, one unidentified aminolipid and five unidentified lipids. Strain GSB7^T contain diphosphatidylglycerol, but not phosphatidylethanolamine, which is differentiated between the closely related strain *M. donghaensis* KCTC 42776^T (Fig. S4).

Table 2
Cellular fatty acid profiles of strain GSB7^T and closely related strains

Fatty acid	1	2	3
C _{10:0} 3OH	7.46	7.94	6.95
C _{12:1} 3OH	ND	ND	4.55
C _{16:00}	12.86	13.28	14.18
C _{17:00}	TR	TR	1.72
C _{17:0} iso 3OH	1.01	ND	ND
C _{18:00}	4.13	2.08	3.93
C _{18:1} ω 7c 11-methyl	16.19	5.38	3.09
Summed Feature 3 ^a	1.27	9.03	TR
Summed Feature 7 ^a	TR	TR	1.39
Summed Feature 8 ^a	55.85	60.57	61.44
Strains: 1, GSB7 ^T ; 2, <i>M. donghaensis</i> KCTC 42776 ^T ; 3, <i>M. niveibacter</i> KCTC 52588 ^T . 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			
^a Summed features represent groups of two or three fatty acids that cannot be separated using MIDI system. Summed feature 3, C _{16:1} ω 7c and/or C _{16:1} ω 6c; summed feature 7, C _{19:1} ω 7c and/or C _{19:1} ω 6c; summed feature 8, C _{18:1} ω 7c or C _{18:1} ω 6c			

Taxonomic conclusion

Based on the phylogenetic, genomic, phenotypic, physiologic, biochemical and chemotaxonomic analysis results, strain GSB7^T 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, naphtol-AS-BI-phosphohydrolase, β-glucosidase and *N*-acetyl-β-glucosaminidase; weak positive for valine arylamidase, acid phosphatase and α-glucosidase; negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. In the API 20NE strip test, positive for β-glucosidase (aesculin hydrolysis), β-galactosidase; negative for reduction of nitrate, indole production, D-glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis. According to API 20NE and 50CH tests, positive for assimilation of D-glucose, D-mannose, D-mannitol, maltose, D-xylose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, *N*-acetyl-glucosamine, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, malic acid and trisodium citric acid; weak positive for assimilation of L-arabinose, adipic 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_{10:0} 3-OH. The polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, one unidentified aminolipid and five unidentified lipids. The DNA G + C content is 60.6 mol%.

The type strain, GSB7^T (= KCTC 82026^T = JCM 34042^T), 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 GSB7^T are MW035312 and JADBGB000000000, respectively.

Abbreviations

MA marine agar, MB marine broth, KCTC Korean Collection for Type Cultures, JCM Japan Collection of Microorganisms, NJ neighbor-joining, ML maximum-likelihood, MP maximum-parsimony, ANI Average nucleotide identity, dDDH digital DNA-DNA hybridization, UBCG up-to-date bacterial core gene set, NA nutrient agar, TSA tryptic soy agar

Declarations

Electronic supplementary material The online version of this article contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- Barrow GI, Feltham RKA (2004) Cowan and steel's manual for the identification of medical bacteria, 3rd edn. Cambridge university press
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569
- Chun JS, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu X-W, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 68:461–466
- Collins MD, Jones D (1980) Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* 48:459–470
- Collins MD (1985) Isoprenoid quinone analysis in classification and identification. In: Goodfellow M, Minnikin DE (editors). *Chemical Methods in Bacterial Systematics*. London: Academic Press, pp 267–287
- Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *J Mol Evol* 17:368–376
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791

- Fitch WM (1971) Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst Biol* 20:406–416
- Gomori G (1955) Preparation of buffers for use in enzyme studies. *Methods Enzymol* 1:138–146
- Hu D, Wang L, Lai Q, Sun F, Shao Z (2018) *Marivivens niveibacter* sp. nov., isolated from the seawater of tropical mangrove. *Int J Syst Evol Microbiol* 68:570–574
- Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P (2016) eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res* 44:D286–D293
- Kim M, Oh H-S, Park S-C, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 64:346–351
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) Mega x: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549
- McCarthy A (2010) Third generation DNA sequencing: Pacific biosciences' single molecule real time technology. *Chem Biol* 17:675–676
- Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60
- Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 2:233–241
- Na S-I, Kim YO, Yoon S-H, Ha S-M, Baek I, Chun J (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J Microbiol* 56:280–285
- Park S, Kim S, Jung YT, Yoon JH (2016) *Marivivens donghaensis* gen. nov., sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 66:666–672
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. MIDI Inc, Newark, DE

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017a) Introducing ezbiocloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017b) A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 110:1281–1286

Figures

Fig. 1

Figure 1

Neighbour-joining phylogenetic tree showing the position of strain GSB7T among related taxa based on 16S rRNA gene sequences. *Hyphomonas polymorpha* DSM 2665T (KF863144) was used as the out-group. Filled circles indicate nodes supported by all three phylogenetic methods (NJ, ML and MP). Bootstrap percentages are based on 1,000 replicates; only values greater than 50% are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.

Fig. 2

Figure 2

Maximum-likelihood phylogenomic tree showing the position of strain GSB7T among related taxa based on 92 bacterial core gene sequences as determined by UBCG. *Hyphomonas polymorpha* DSM 2665T (GCA 000685315) was used as the out-group. Bootstrap percentages are based on 1,000 replicates. Bar, 0.05 substitutions per nucleotide position.

Supplementary Files

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