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ORIGINAL PAPER

Mesobacterium hydrothermale sp. nov., isolated from shallow-sea hydrothermal systems off Kueishantao Island

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Abstract A Gram-negative, rod-shaped, non-motile, aerobic bacterium, designated as strain TK19101^T, was isolated from the intermediate seawater of yellow vent in the shallow-sea hydrothermal system located near Kueishantao Island. The strain was found to grow at 10–40 °C (optimum, 35 °C), at pH 6.0–8.0 (optimum, 7.0), and in 0–5% (w/v) NaCl (optimum, 1%). Strain TK19101^T was catalase-positive and oxidase-positive. The predominant fatty acids (> 10%) in strain TK19101^T cells were $C_{16:0}$, summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*), and $C_{18:0}$. The predominant isoprenoid quinone of strain TK19101^T was ubiquinone-10. The polar lipids of strain TK19101^T comprised phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phospholipid, and unknown polar lipid. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain TK19101^T belonged to the genus *Mesobacterium*. Strain TK19101^T exhibited highest 16S rRNA gene sequence similarity value to *Mesobacterium pallidum* MCCC M24557^T (97.48%). The estimated average nucleotide identity and digital DNA-DNA hybridization values between strain TK19101^T and the closest related species *Mesobacterium pallidum* MCCC M24557^T were 74.88% and 20.30%, respectively. The DNA G + C content was 63.49 mol%. On the basis of the analysis of 16S rRNA gene sequences, genotypic and phylogenetic data, strain TK19101^T has a unique phylogenetic status and represents a novel species of genus *Mesobacterium*, for which the name *Mesobacterium hydrothermale* sp. nov. is proposed. The type strain is TK19101^T (= MCCC 1K08936^T = KCTC 8354^T).

Keywords *Mesobacterium hydrothermale* · *Roseobacteraceae* · Polyphasic taxonomy · Shallow-sea hydrothermal system

Introduction

The family *Rhodobacteraceae* within the class *Alphaproteobacteria*, a diverse and widespread group of marine bacteria, comprises up to 30% of marine bacterial communities in pelagic environments (Simon et al. 2017), which is particularly known for their ecological versatility and significant role in marine ecosystems. The genus *Roseobacter* was first described in 1991

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(Shiba 1991) and classified into the family *Rhodobacteraceae*. It developed into the *Roseobacter* clade rapidly, which is an important bacterial assemblage within *Rhodobacteraceae*. Bacteria in the *Roseobacter* clade share similarities in environmental adaptability, genomes, and phylogenetic analysis, and also differ markedly from other bacteria in the family *Rhodobacteraceae*. Therefore, in 2021, the *Roseobacter* clade was officially separated from the family *Rhodobacteraceae* based on the Core-Genome Phylogeny and named as the *Roseobacteraceae* family (Liang et al. 2021).

The family *Roseobacteraceae*, a young clade in evolutionary terms, includes > 180 validly named genera. Organisms of this family have been detected in a large variety of habitats, including seawaters (Sung et al. 2015), marine organisms (Yang et al. 2018), coastal environments (Ivanova et al. 2010), sediments (Chang et al. 2019), polar sea ice (Gosink et al. 1997). In the open ocean surface waters, the family *Roseobacteraceae* accounts for 10% of marine microbial communities. This rises to 20% in coastal waters (Moran et al. 2007; Luo et al. 2014). The family *Roseobacteraceae* possesses abundant metabolic mechanisms, making it one of the most easily cultivable types of marine bacteria. The members of this group are mostly heterotrophic and capable of degrading a variety of organic materials, including aromatic compounds and dimethylsulfoniopropionate (Buchan et al. 2005; Wagner-Döbler et al. 2006). Many have the capability for aerobic anoxygenic photosynthesis and carbon monoxide oxidation (Allgaier et al. 2003; Wagner-Döbler et al. 2006). They have been identified as important players in global biogeochemical cycles, including those of sulfur and carbon. The genus *Mesobacterium*, belonging to the family *Roseobacteraceae*, was originally proposed by Kong et al. (2022). At the time of writing, the genus *Mesobacterium* only contains one validly named species, *Mesobacterium pallidum*, isolated from the deep-sea water in the southwest Indian ridge (Kong et al. 2022).

Materials and methods

Isolation and cultivation

Strain TK19101^T was obtained from a seawater sample collected from the intermediate seawater of a yellow vent in the shallow-sea hydrothermal system located near Kueishantao Island (24.83560° N 121.96339° E), Taiwan, China, in June 2019. The in situ temperature measured 25.9 °C, the salinity was 34.4‰ and pH was 6.3. The seawater sample was mixed with 15% (v/v) glycerol and stored at 4 °C before the laboratory experiment. After being diluted tenfold, the seawater sample was plated onto marine agar 2216 (MA; Hopebio, China). At ambient temperature (28 °C), the sample was cultivated for 5 days in aerobic environment. A light white colony was picked out. After being purified through multiple passages, strain TK19101^T showed relatively low 16S rRNA gene sequence similarity to other species. Then the culture was regularly sustained on MA and was preserved as glycerol suspensions (15%, v/v) at − 80 °C. *Mesobacterium pallidum* MCCC M24557^T (Kong et al. 2022), *Ponticoccus alexandrii* CCTCC AB 2017228^T (Yang et al. 2018), *Marinovum algicola* JCM 21266^T (Lafay et al. 1995), *Pseudoprimorskyibacter insulae* KCTC 42602^T (Park et al. 2015) and *Salipiger thiooxidans* DSM 10146^T (Sorokin et al. 2005) were selected as type strains and subjected to identical conditions for subsequent experiments.

Phenotypic, physiological, and biochemical characteristics

To determine cell size and morphology, strain TK19101^T was observed by transmission electron microscopy (TEM; Tecnai G2 Spirit BioTwin) using bacteria in the exponential growth phase. The following growth tests for salt, temperature, and pH tolerance were all conducted for a period of 6 days and detected by photometric measurement at 600 nm (OD_{600}). The temperature range (4, 10, 15, 20, 25, 30, 35, 40, 45 °C) for growth was assessed in 2216E Liquid Medium (LM; Hopebio, China). Salt tolerance was investigated in rich organic (RO) medium at 28 °C with NaCl concentrations ranging from 0 to 12% (intervals of 1%, w/v). The pH range for growth was examined in RO under 28 °C, by adjusting the pH values from 3 to 12 (intervals of 1 pH unit) with specific buffers (Na_2HPO_4/citric acid for pH 3.0–8.0, NaOH/glycine for pH 9.0–10.0, Na_2HPO_4/NaOH for pH 11.0–12.0).

The Gram staining test was performed using the Gram stain kit (HB8278, Hopebio). Semi-solid puncture inoculation method was employed to assess cell

motility, using an agar concentration of 0.5% (w/v) at 28 °C for 5 days. Oxidase activity was assessed using an oxidase reagent (Huankai Microbial). Catalase activity was examined observing bubbles produced by colonies under 3% H_2O_2. According to the manufacturer's instructions, API 20 NE and API ZYM kits (bioMérieux) were performed to assess other physiological and biochemical characteristics and enzyme activities of strain TK19101^T.

Chemotaxonomic characteristics

Strain TK19101^T and its close reference strains were grown aerobically in modified marine agar (BD) including CH_3COONa (1.0 g/L) and trisodium citrate (0.5 g/L) with pH 7.5 at 28 °C for 96 h. Subsequently cellular fatty acids were saponified, methylated and extracted, and the method was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sherlock Version 6.0B; TSBA6 Method) (Sasser 1990). Respiratory isoprenoid quinones were extracted from freeze-dried cells and analyzed by high-performance liquid chromatography (HPLC). Polar lipids were extracted using a chloroform/methanol system and analysed using one- & two-dimensional thin-layer chromatography (TLC), as described by Kates (1986).

Phylogenic analysis

The 16S rRNA gene of strain TK19101^T was amplified by PCR utilizing the universal primers 27F (5′-AGA GTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGG TTACCTTGTTACGACTT-3′) (Frank et al. 2008). The full-length 16S rRNA gene sequence was obtained by the tool ContEst16S (https://www.ezbiocloud.net/tools/contest16s) (Lee et al. 2017). To select closely related sequences of strain TK19101^T, the sequence of the 16S rRNA gene was uploaded and compared with sequences available in the EzBioCloud database (https://www.ezbiocloud.net/) (Yoon et al. 2017a) and NCBI database (https://www.ncbi.nlm.nih.gov/) (Johnson et al. 2008). Then the sequences were downloaded from the NCBI database. 16S rRNA phylogenetic trees were reconstructed using mega version 7.0 (Kumar et al. 2016). The neighbor-joining (NJ) algorithm with Kimura's two-parameter method (Saitou et al. 1987), the maximum-likelihood (ML) algorithm with the K2+G+I model (Felsenstein 1981) and the minimum-evolution (ME) algorithm (Rzhetsky et al. 1992) were employed. All trees constructions were subjected to bootstrapping with 1000 replications (Felsenstein 1985) to assess the reliability of the phylogenetic tree. *Neomegalonema perideroedes* DSM 15528^T was used as the outgroup.

Genomic analysis

The genomic DNA of strain TK19101^T was sequenced using the Illumina NovaSeq platform by Personalbio Company (Shanghai, China), and the raw data from sequencing was assembled de novo using A5-MiSeq (Coil et al. 2014) and SPAdes (Bankevich et al. 2012) software. A base correction was carried out using Pilon software (Walker et al. 2014). GeneMarkS-2 (http://exon.gatech.edu/GeneMark/genemarks2.cgi) (Lomsadze et al. 2018) was used to predict more accurate protein-coding genes. EggNOG 5.0 database (http://eggnog5.embl.de/#/app/home) (Huerta-Cepas et al. 2019) and eggNOG-mapper v2 (http://eggnog-mapper.embl.de/) (Cantalapiedra et al. 2021) were used for gene functional annotation. TBtools (Chen et al. 2023) was used to extract KEGG pathway information from eggNOG annotations.

The ANI calculator (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017b) was utilized to compute the average nucleotide identity (ANI) values between strain TK19101^T and its closely related strains. The estimates for digital DNA-DNA hybridization (dDDH) were obtained by utilizing the Genome-to-Genome Distance Calculator (GGDC 3.0) online service (https://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al. 2022). The percentage of conserved proteins (POCP) values between strain TK19101^T and closely related species were calculated using a R script tagged computePOCP+AAI v2.1 (https://github.com/2015qyliang/POCP) with software prodigal v2.6.3 and diamond v0.9.28. Based on universally present 120 conserved bacterial single-copy genes, the Genome Taxonomy Database Toolkit, GTDB-Tk v2.1.1 (Chaumeil et al. 2022), was utilized to construct a phylogenetic tree.

Results and discussion

Phenotypic, physiological, and biochemical characteristics

Detailed phenotypic properties of strain TK19101^T are shown in Table 1. After cultivation for 4 days, strain TK19101^T was observed to form little white, round, smooth, convex colonies on MA at 28 °C under air atmosphere. Under the electron microscope, the cells appeared rod-shaped (3–4.5 μm in length × 0.8–1.3 μm in width, Fig. 1). Strain TK19101^T could grow at 10–40 °C (optimum, 35 °C), pH 6.0–8.0 (optimum, 7.0), and in the presence of 0–5% (w/v) NaCl (optimally, 1% (w/v) NaCl). Cells were Gram-stain-negative, aerobic, non-motile, and positive for catalase and oxidase. In assays with the API 20NE system, nitrate reduction, hydrolysis (*β*-glucosidase), hydrolysis (protease), and the utilization of glucose, mannose, maltose, and gluconate were positive. With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, *α*-Glucosidase were positive, acid phosphatase was weakly positive (Table 1). The positive result of nitrate reduction is one of general characterizations within genus *Mesobacterium*.

Chemotaxonomic characteristics

The predominant fatty acids (> 10%) in TK19101^T cells were $C_{16:0}$ (16.81%), summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*, 48.78%), and $C_{18:0}$ (11.74%) (Table S1). The major polar lipids of strain TK19101^T comprised phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phospholipid (PL), and unknown polar lipid (L) (Fig. S1). The isoprenoid quinone found in strain TK19101^T was ubiquinone-10 (Q-10). Strain TK19101^T shared many chemotaxonomic characteristics with *Mesobacterium pallidum* MCCC M24557^T. They both had Q-10 as the dominant isoprenoid quinone. The content of $C_{16:0}$ in strain TK19101^T and *Mesobacterium pallidum* MCCC M24557^T (> 10%) was much higher than other closely related strains. PE, PC, PG were also found in the polar lipids profile of *Mesobacterium pallidum* MCCC M24557^T (Kong et al. 2022). Based on the results of chemotaxonomic characteristics, strain TK19101^T may represent a novel species of genus *Mesobacterium*.

Phylogenetic analysis

The 16S rRNA gene sequence of TK19101^T (1466 bp) was submitted to the GenBank database with the accession number OP508452. Comparison of the 16S rRNA gene sequences showed that TK19101^T shared the highest similarities with *Mesobacterium pallidum* MCCC M24557^T (97.48%), obviously lower than the species' threshold limits (98.65%) (Kim et al. 2014), followed by *Marinovum algicola* JCM 21266^T (96.53%), *Ponticoccus alexandrii* CCTCC AB 2017228^T (96.32%), *Salipiger thiooxidans* DSM 10146^T (96.29%) and *Pseudoprimorskyibacter insulae* KCTC 42602^T (95.46%). Phylogenetic analyses based on the NJ method clearly showed that TK19101^T clustered with *Mesobacterium pallidum* MCCC M24557^T which had the highest 16S gene sequence similarities (Fig. 2). Phylogenetic tree reconstructing by ML (Fig. S2) and ME methods (Fig. S3) showed the same results, which all supported that strain TK19101^T was a novel species of genus *Mesobacterium*.

Genomic analysis

The draft genome sequence of strain TK19101^T was 4,051,847 bp in length, resulting in 76 scaffolds. Scaffolds varied in length from 522 to 281,582 bp, with an N50 size of 119,573 bp. The DNA G + C content of strain TK19101^T calculated from the draft genome was 63.49%. In the phylogenomic tree (Fig. S4), strain TK19101^T was found to cluster with *Pseudoprimorskyibacter insulae* KCTC 42602^T and still close to *Mesobacterium pallidum* MCCC M24557^T.

The genome was found to contain a total of 3865 protein-coding genes, 3 rRNA genes, 44 tRNA genes, and 17 other non-coding RNA genes. A total of 3,321 genes were functionally annotated within the COG (Cluster of Orthologous Groups) database. The predicted genes are distributed among 19 COG categories. Number of genes involved in function unknown (S, 20.48%) showed the highest count, followed by amino acid transport and metabolism (E, 10.32%), transcription (K, 7.44%), energy production and conversion (C, 6.97%), replication, recombination and repair (P, 5.84%), inorganic ion transport and metabolism (M, 5.64%) (Fig. 3). COG categories distribution

Table 1 Comparison of characteristics of strain TK19101^T with the type strains of its closest phylogenetic relatives

Characteristic	1	2	3	4	5	6
Isolation source	Seawater	Deep-sea water[a]	The Phycosphere of the Toxin-Producing Dinoflagellate Prorocentrum lima[b]	Marine toxigenic dinoflagellate[c]	Junction between the ocean and a freshwater spring[d]	Seawater[e]
Cell size (μm)	0.8–1.3 × 3–4.5	1.1–1.5 × 2.1–2.7[a]	0.8–1.5 × 2–3[b]	0.5–0.8 × 0.7–1.2[c]	0.2–1.0 × 0.4–10.0[d]	1–1.5 × 0.6–0.8[e]
Optimum growth						
Temperature (°C)	35	28[a]	25–30[b]	28[c]	30[d]	25[e]
NaCl (%, w/v)	1	2[a]	0.1–2[b]	3–6[c]	2[d]	20[e]
pH	7.0	7.5[a]	7.5[b]	8[c]	7.0–8.0[d]	7.8[e]
API ZYM						
Alkaline phosphatase	+	+	+	+	w	+
Leucine arylamidase	−	+	+	+	+	+
Valine arylamidase	−	−	−	−	w	−
Acid phosphatase	w	−	w	w	w	+
Naphthol-AS-BI-phosphohydrolase	+	w	w	+	+	+
β-Galactosidase	−	−	w	−	w	−
α-Glucosidase	+	−	+	−	−	+
β-Glucosidase	−	w	−	−	+	w
API 20NE						
Nitrate reduction	+	+	−	−	+	−
d-glucose fermentation	−	−	+	−	−	−
Arginase	−	−	−	−	−	+
Urease	−	−	−	+	−	+
Hydrolysis (protease)	+	−	−	−	−	−
4-nitrophenyl-β-d-galactopyranoside	−	−	+	−	+	w
Glucose	+	−	−	−	−	−
Mannose	+	−	−	−	−	−
Maltose	+	−	−	−	−	−
Gluconate	+	−	−	−	−	−
DNA G + C content (mol%)	63.49	66.5[a]	65.05	68.8[c]	60.6[d]	67.5–69.2[e]

Strains: 1, Strain TK19101^T; 2, *Mesobacterium pallidum* MCCC M24557^T; 3, *Marinovum algicola* JCM 21266^T; 4, *Ponticoccus alexandrii* CCTCC AB 2017228^T; 5, *Pseudoprimorskyibacter insulae* KCTC 42602^T; 6, *Salipiger thiooxidans* DSM 10146^T. Data except where indicated, all obtained from this study

*Data obtained from: [a]Kong et al. (2022); [b]Lafay et al. (1995); [c]Yang et al. (2018); [d]Park et al. (2015); [e]Sorokin et al. (2005)

patterns of strain TK19101^T and its phylogenetically related species were compared and exhibited a general consistency (Fig. S5).

A total of 4436 genes were detected to participate in six categories of pathways in the KEGG (Kyoto Encyclopedia of Genes and Genomes) database.



Fig. 1 Transmission electron micrograph of strain TK19101^T from cultures grown on marine agar 2216 for 4 days at 28 °C. Bars 1.0 µm

Among them, Metabolism (52.52%) had the largest proportion, including amino acid metabolism, carbohydrate metabolism, energy metabolism, and metabolism of cofactors and vitamins (Fig. 4). In the KEGG database, a total of 35 genes related to carbon fixation pathways in prokaryotes (ko00720) and 37 genes related to sulfur metabolism (ko00920) had been identified. Two carbon fixation pathways have been proven to exist. Gene acsA and acsB encoding carbon monoxide dehydrogenase (CODH) and acetyl-CoA synthetase (ACS) respectively, enabled the fixation of CO into Acetyl-CoA. Then genes *accA*, *accB*, *accC*, and *accD* encoded acetyl-CoA carboxylase, which catalyzed its conversion to Malonyl-CoA. Gene *icd* encoding isocitrate dehydrogenase (ICD) collectively functions to fix CO_2 in the tricarboxylic acid cycle (TCA), together with the gene *acnA*. Gene *sdhA*, involved in the 3-hydroxypropionate cycle (3-HP) (Liu et al. 2022), was also detected in strain TK19101^T. In addition, genomic analysis showed that strain TK19101^T contained a complete Sox gene cluster and some genes related to sulfur metabolism. The Sox gene cluster consists of seven genes *soxXYZABCD*, coding for proteins required to oxidize sulfides to sulfate (Friedrich et al. 2001). We could speculate that strain TK19101^T may have the ability to oxidize sulfide to sulfate. The gene involved in DMSP transport and cleavage (*dddP*), and a gene involved in acrylate catabolism (*prpE*) were found in strain TK19101^T. DddP is a DMSP lyase and could cleave DMSP to generate DMS and acrylate (Todd et al. 2009). The acrylate can be catabolized by propionate-CoA ligase PrpE, and finally enter the TCA cycle (Reisch et al. 2013). This suggested that strain TK19101^T has the potential to utilize DMSP in the environment as a carbon source available to cells. More genes involved in other sulfur metabolic pathways, including assimilatory sulfate reduction (*cysC*, *cysQ*), sulfur reduction (*mccA*, *psrA*, *ttrB*), sulfide oxidation (*fccB*, *sqr*), linkages between inorganic and organic sulfur transformation (*cysE*, *mccB*, *metYZ*, *ssuD*, *xsc*), also found in strain TK19101^T (Yu et al. 2020). The abundance of sulfur metabolism genes

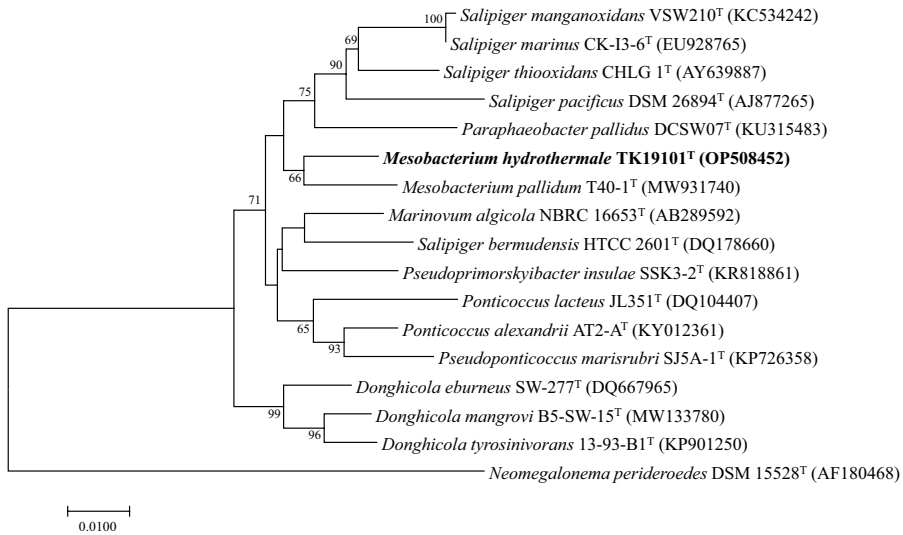


Fig. 2 A neighbor-joining tree based on 16S rRNA gene sequences of strain TK19101^T and related taxa. Bootstrap percentages were based on 1000 replications, *Neomegalonema perideroedes* DSM 15528^T was used as an outgroup. Bootstrap values (> 50%) are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position

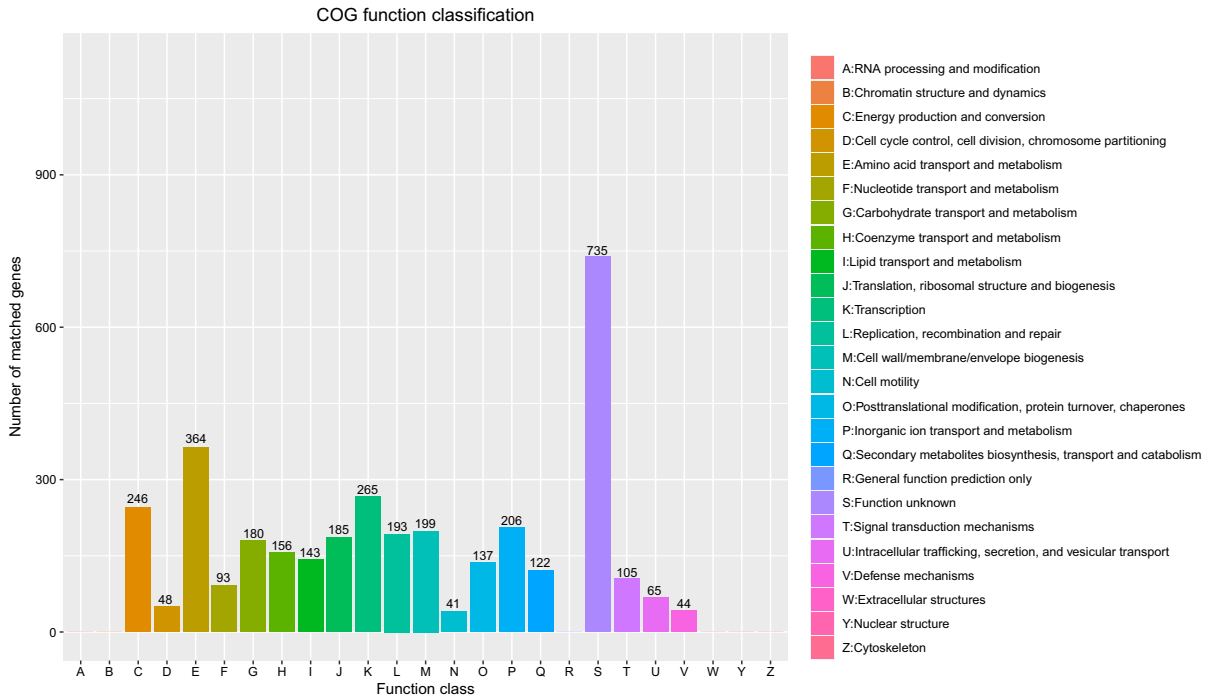


Fig. 3 Analysis of the COG function classification based on the genomes of strain TK19101^T

indicated that strain TK19101^T may deeply participate in the sulfur cycle.

Furthermore, the estimated ANI, dDDH, and POCP values between strain TK19101^T and five reference species were 74.88%–75.26%, 19.90%–20.30%, 55.02%–63.32%, respectively (Table S2), and compared to the closest species *Mesobacterium pallidum* MCCC M24557^T were 74.88%, 20.30%, 55.51%, respectively, which were far lower than the threshold values for species delimitation (95%–96% ANI, 70% dDDH) and higher than the recommended mean threshold for genus definition (73.34%–74.62% ANI, 50% POCP) (Meier-Kolthoff et al. 2013; Chun et al. 2018; Barco et al. 2020; Qin et al. 2014). These results support that strain TK19101^T represents a novel species of genus *Mesobacterium*.

Description of *Mesobacterium hydrothermale* sp. nov.

Hydrothermale (hy.dro.ther.ma'le. N.L. neut. adj. *hydrothermale*, to indicate that the organism obtained from a hydrothermal area).

Cells are Gram-stain-negative, rod-shaped, non-motile, aerobic, about 0.8–1.3 μm wide and 3–4.5 μm long. Cells are positive for catalase and oxidase. Colonies are little white, round, smooth, convex on marine agar 2216 incubated for 4–5 days at 28 °C under air atmosphere. Growth occurs at 10–40 °C (optimum, 35 °C), pH 6.0–8.0 (optimum, 7.0), and in the presence of 0–5% (w/v) NaCl (optimally, 1%). Nitrate reduction, *β*-glucosidase, protease, and the utilization of glucose, mannose, maltose, and gluconate were positive. Alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, *α*-glucosidase were positive, and acid phosphatase was weakly positive. The predominant fatty acids (> 10%) in cells are $C_{16:0}$, summed feature 8 ($C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*), and $C_{18:0}$. The polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phospholipid, and unknown polar lipid. Q-10 is the major isoprenoid quinone. The DNA G + C content of strain TK19101^T is 63.49 mol%.

The type strain, TK19101^T (= MCCC 1K08936^T = KCTC 8354^T), was isolated from seawater in shallow-sea hydrothermal systems of

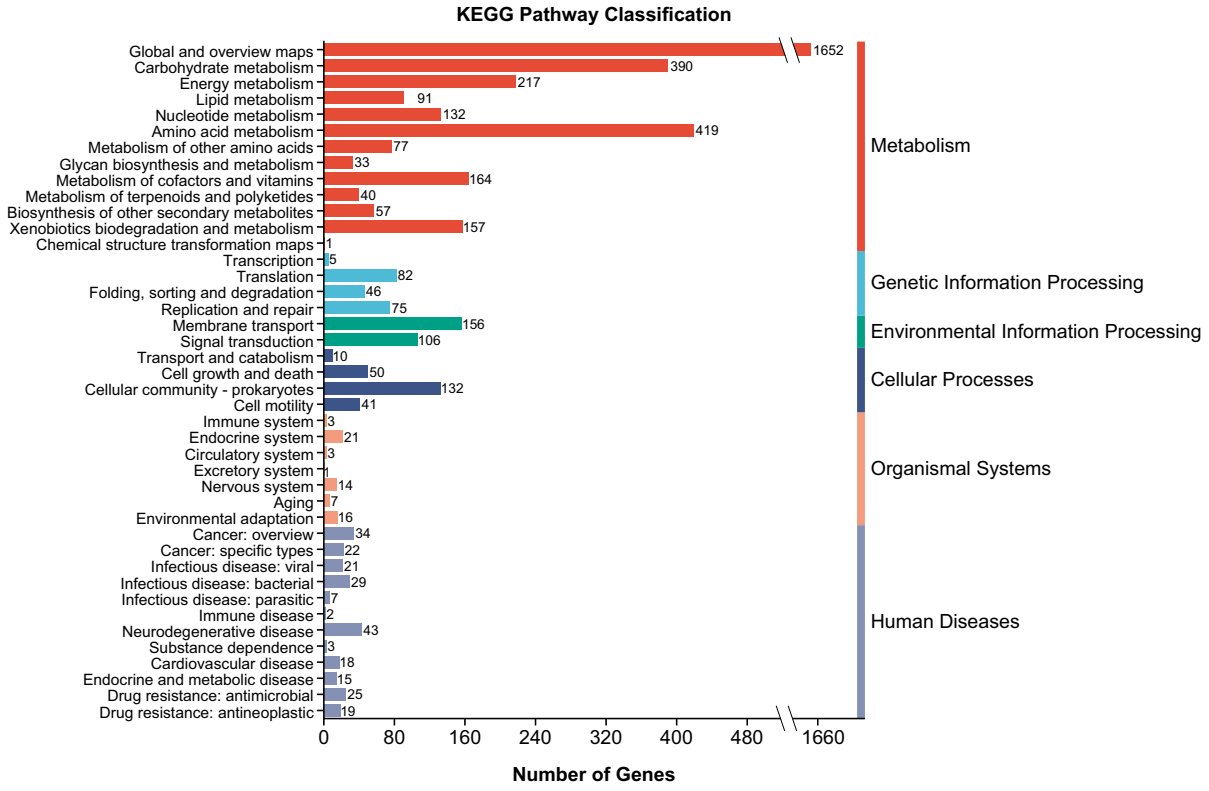


Fig. 4 Analysis of the KEGG pathway classification based on the genomes of strain TK19101^T

Kueishantao Island (24.83560°N 121.96339°E), Taiwan, China. The GenBank/ENA/DDBJ accession numbers for the 16S rRNA gene sequence and the complete genome sequence of strain TK19101^T are OP508452 and PRJNA1060878, respectively.

Author contributions ZS, YX, YX, BC, XQ and JY designed and performed the experiments. ZS, YX, YX, BC and KT analyzed the data. ZS and KT wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials The 16S rRNA gene sequence and the genome sequence of strain TK19101^T have been deposited in GenBank with accession numbers OP508452 and PRJNA1060878, respectively.

Declarations

Competing interests There are no conflicts of interest, according to the authors.

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