

# Thiosulfate oxidation and autotrophy potential by marine prevalent heterotrophic bacteria of genus *Marinobacter*

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## Abstract

The genus *Marinobacter* is very broadly distributed in global environments and is considered as aerobic heterotroph. In this study, six *Marinobacter* strains were identified with autotrophic thiosulfate oxidation capacity. These strains, namely *Marinobacter guineae* M3B<sup>T</sup>, *Marinobacter aromaticivorans* D15-8P<sup>T</sup>, *Marinobacter vulgaris* F01<sup>T</sup>, *Marinobacter profundus* PWS21<sup>T</sup>, *Marinobacter denitrificans* JB02H27<sup>T</sup>, and *Marinobacter* sp. ST-1M (with a 99.93% similarity to the 16S rDNA sequences of *Marinobacter salsuginis* SD-14B<sup>T</sup>), were screened out of 32 *Marinobacter* strains by autotrophic thiosulfate oxidization medium. The population of cells grew in a chemolithotrophic medium, increasing from 10<sup>5</sup> cells/mL to 10<sup>7</sup> cells/mL within 5 d. This growth was accompanied by the consumption of thiosulfate 3.59 mmol/L to 9.64 mmol/L and the accumulation of sulfate up to 0.96 mmol/L, and occasionally produced sulfur containing complex particles. Among these *Marinobacter* strains, it was also found their capability of oxidizing thiosulfate to sulfate in a heterotrophic medium. Notably, *M. vulgaris* F01<sup>T</sup> and *M. antarcticus* ZS2-30<sup>T</sup> showed highly significant production of sulfate at 9.45 mmol/L and 3.10 mmol/L. Genome annotation indicated that these *Marinobacter* strains possess a complete Sox cluster for thiosulfate oxidation. Further phylogenetic analysis of the *soxB* gene revealed that six *Marinobacter* strains formed a separate lineage within Gammaproteobacteria and close to obligate chemolithoautotroph *Thiomicrothrix arctica*. The results indicated that thiosulfate oxidizing and chemolithoautotrophic potential in *Marinobacter* genus, which may contribute to the widespread of *Marinobacter* in the global ocean.

**Key words:** *Marinobacter*, thiosulfate oxidation, autotrophic, mixotroph

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## 1 Introduction

Bacteria of the genus *Marinobacter* are broadly distributed in a wide variety of environments, having been isolated from offshore oil-well heads, coastal thermal springs, Antarctic seawater, saline soils, and associations with diatoms and dinoflagellates (Cooper et al., 2022). The genus *Marinobacter* was created by Gauthier (Gauthier et al., 1992) to accommodate Gram-negative bacteria which belongs to the family Alteromonadaceae in the class Gammaproteobacteria. Further physiological studies showed that this heterotrophic group is metabolically diverse, capable of consuming a wide variety of organic compounds, including hydrocarbons, carbohydrates, and amino acids, and is broadly characterized as facultatively anaerobic and facultatively halophilic (Handley and Lloyd, 2013). Few *Marinobacter* strains were reported to possess sulfur-oxidizing capabilities. *Marinobacter* sp. BR13 from marine sediments and *Marinobacter maroccanus* SDSWS8 from the shrimp mariculture ponds can oxidize thiosulfate and sulfide with the production of sulfate for autotrophic growth (Choi et al., 2009; Dou et al., 2022). *Marinobacter orientalis* W62<sup>T</sup> showed thiosulfate-oxidizing ability with hetero-

trophic growth (Lian et al., 2021). He et al. also isolated some *Marinobacter* strains from hydrothermal fields and showed autotrophic growth with thiosulfate and iron as electron donors (He et al., 2023).

Sulfur oxidation is widespread as an essential component of the earth's sulfur cycle. Sulfur-oxidizing bacteria are known as groups with the capability to produce sulfuric acid through the oxidation of hydrogen sulfide, elemental sulfur, and thiosulfates, which are widespread in different environments (Rana et al., 2020). Two different kinds of metabolically active groups exist: one is obligate chemolithotrophic bacteria, which belongs to genus like *Thiobacillus*, *Beggiatoa*, *Thiothrix*, *Thiomicrospira*, *Desulphuromonas* and *Achromatium*, can utilize the oxidizable S compounds with CO<sub>2</sub> as the source of carbon; and the other is heterotrophic, including genera *Pseudomonas*, *Escherichia*, *Alcaligenes* and *Xanthobacter* (Chaudhary et al., 2019). There are four major microbial sulfur oxidizing pathways reported in the previous studies: (1) the oxidation of HS<sup>-</sup>/S<sup>2-</sup> to elemental sulfur by Flavocytochrome c sulfide dehydrogenase (FCC) and sulfide quinone reductase (SQR); (2) the oxidation of elemental

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sulfur to sulfite by reverse dissimilatory sulfite reduction (rDsr) including dsrAB, aprAB and sat and heterodisulfide reductases-like (Hdr); (3) the oxidation of thiosulfate to sulfate by sulfur-oxidizing (Sox) system and TsdA; (4) the oxidation of elemental sulfite to sulfate by sulfur oxygenase/reductase (SOR) or adenosine 5'-phosphosulfate (APS) (Dahl, 2015, 2017; Watanabe et al., 2019).

Till now, 57 type species in the *Marinobacter* have been described (<https://lpsn.dsmz.de/genus/marinobacter>). Nonetheless, the previous reports hint that they may be of potential in sulfur oxidation and carbon fixation to support a chemolithoautotrophic lifestyle, as a versatile mixotroph. To evaluate the extent of this potential within the whole genus, this study conducted genomic and physiological analyses based on 53 genomes of the *Marinobacter* genus, and 32 strains (27 type species and 5 new isolates) in the marine culture collection center of China (MCCC). Their metabolic diversity will gain insights into the mechanisms underlying their wide distribution.

## 2 Material and methods

### 2.1 Bacterial strains

Twenty-seven type strains of *Marinobacter* and 5 strains isolated from deep sea hydrothermal vents in our lab were selected

for analysis (Table 1), which all are deposited in the MCCC (<https://mccc.org.cn/>).

### 2.2 Autotrophic thiosulfate oxidization test

Growth in the autotrophic sulfur-oxidizing medium was observed, which was modified with sodium thiosulfate as the sole electron donor and sodium bicarbonate as the sole carbon source according to He et al. (He et al., 2023). The artificial seawater in the autotrophic thiosulfate oxidization medium included 30.00 g NaCl, 0.25 g NH<sub>4</sub>Cl, 0.33 g KCl, 0.14 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 4.18 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.14 g K<sub>2</sub>HPO<sub>4</sub>, 0.50 mg NiCl<sub>2</sub>·6H<sub>2</sub>O, 0.50 mg Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O and 1 000 mL distilled water. After autoclaving, the medium was supplemented by sterilized 10 mL trace mineral solution (<https://www.atcc.org/products/md-tms>), 1 mL vitamin solution (<https://www.atcc.org/products/md-vs>), 5 mmol/L NaHCO<sub>3</sub>, and 10 mmol/L Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Before inoculation, the cells grown in marine 2 216 medium (BD Difco, San Diego, CA, USA) were washed three times with sterilized seawater as inoculum to avoid interference from organic carbon sources and then transferred to the autotrophic media five times at a ratio of 1:20 within 0.5 mL of the inoculum to 10 mL autotrophic thiosulfate oxidization medium. The strains were inoculated into the aerobic medium and cultured at 28°C Biochemical Incubator (ZXSD-1270) for 15 d and the cells number, thiosulfate, and sulfate concentra-

**Table 1.** Growth substrate tests of *Marinobacter* strains in this study

Strain	MCCC Deposition No.	Sulfur oxidation/ autotrophic liquid	Sulfur oxidation/ autotrophic agar plates	Sulfur oxidation/ heterotrophic liquid
<i>M. algicola</i> DG893	1A03261	–	+	+
<i>M. antarcticus</i> ZS2-30	1A14218	–	–	++
<i>M. aromaticivorans</i> D15-8P	1A14217	+	+	–
<i>M. bohaisensis</i> T17	1K03282	–	+	–
<i>M. bryozoorum</i> 50-11	1A03280	–	–	–
<i>M. confluentis</i> HJM-18	1A12745	–	–	–
<i>M. daepoensis</i> SW-156	1A03299	–	+	+
<i>M. denitrificans</i> JB02H27	1K05758	+	+	–
<i>M. flavimaris</i> SW-145	1A03282	–	–	–
<i>M. fuscus</i> NH169-3	1K03455	–	+	–
<i>M. gudaonensis</i> SL014B61A	1K05757	–	+	–
<i>M. guineae</i> M3B	1A00540	+	+	–
<i>M. halotolerans</i> CP12	1K05760	–	–	–
<i>M. koreensis</i> DD-M3	1A12340	–	–	–
<i>M. lacisalsi</i> FP2.5	1A06427	–	+	–
<i>M. lipolyticus</i> SM19	1A03253	–	+	–
<i>M. mangrovi</i> CHFG3-1-5	1A18306	–	+	–
<i>M. maritimus</i> CK47	1A00530	–	+	–
<i>M. maroccanus</i> N4	1A15864	–	+	–
<i>M. mobilis</i> CN46	1A16703	–	–	–
<i>M. nitratreducens</i> AK21	1A16708	–	+	–
<i>M. profundus</i> PWS21	1K03345	+	–	–
<i>M. salarius</i> R9SW1	1A12752	–	+	–
<i>M. salexigens</i> HJR7	1H00176	–	+	–
<i>M. sediminum</i> R65	1A03275	–	–	–
<i>M. vulgaris</i> F01	1H00290	+	+	++
<i>M. xestospongiae</i> UST090418-1 611	1A16705	–	+	–
<i>Marinobacter</i> sp. CuT1-2	M25615	–	–	–
<i>Marinobacter</i> sp. IOP-29	1A14007	–	+	–
<i>Marinobacter</i> sp. ST-1M	M23265	+	+	+
<i>Marinobacter</i> sp. ST-41	M23288	–	–	–
<i>Marinobacter</i> sp. ST-43	M23266	–	–	+

Note: + indicates growth; - indicates no growth.

tions were measured (refer to Section 2.4). In the autotrophic thiosulfate oxidization solid medium, 1.5% agar and 0.5 ppm phenol red were added (Ruby et al., 1981). pH change was used as an indicator of thiosulfate oxidation, the values of pH were detected by adding phenol red in the agar plates. During autotrophic sulfur-oxidizing incubation, 10  $\mu$ L of the culture was sampled on days 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 7 for cell counting by under microscopy (Nikon 80i, Japan). Each sample was counted in five grids (containing 80 subgrids) of a hemocytometer under microscopy and subsequently, the results were extrapolated to calculate the cell concentration (Randolph, 1944). All the counts and tests below were done in duplicates.

### 2.3 Heterotrophic thiosulfate oxidization tests

The heterotrophic thiosulfate oxidization medium was detected and investigated in the following medium (0.1% yeast extract, 0.5% peptone, 10 mmol/L  $\text{Na}_2\text{S}_2\text{O}_3$ ), prepared with artificial seawater. Before inoculation, the cells were washed three times with sterilized seawater and then transferred to the heterotrophic media at a ratio of 1:20. The strains were inoculated into the medium and cultured at 28°C for 15 d and the cells' number, pH, and sulfate concentrations were measured. In heterotrophic growth, 200  $\mu$ L of the culture was determined the absorbance at OD600 nm.

### 2.4 Thiosulfate and sulfate quantification

Thiosulfate and sulfate concentrations in the supernatant from autotrophic thiosulfate oxidization tests were measured by an ICS-2100 ion chromatography (Dionex, USA). The amount of sulfate ( $\text{SO}_4^{2-}$ ) from heterotrophic thiosulfate oxidization tests was also determined spectrophotometrically by barium chloride colorimetric assay. Sulfate was measured by adding 1:1 barium chloride solution (10% w/v) with bacterial culture supernatant followed by mixing the suspensions vigorously. The white turbidity due to barium sulfate formation was measured at 450 nm with a Varioskan LUX (Thermo Scientific, Waltham, MA, USA). The amount of turbidity formed is proportional to the sulfate concentration standard sulfate solutions were made by dissolving  $\text{Na}_2\text{SO}_4$  in deionized water to known concentrations in the range 0 mmol/L to 3 mmol/L (Behera et al., 2014).

### 2.5 Phylogeny and genome analysis

In this study, 53 genomes of *Marinobacter* genus were downloaded from National Center for Biotechnology Information database, NCBI (<https://www.ncbi.nlm.nih.gov/>). Up-to-date bacterial core gene (UBCG) was used to construct a phylogenomic tree and infer the phylogenomic relationship of *Marinobacter* (Na et al., 2018). The phylogenomic tree was visualized, modified, and annotated by One Table (tvBOT) (Xie et al., 2023). Genome annotation was performed using the software BLASTP 2.13.0+, the e value was set to  $10^{-5}$  (Camacho et al., 2009), and using the Rapid Annotation Subsystems Technology (RAST) server (Aziz et al., 2008). The e value indicates the probability that other sequences are more similar to the target sequence than this displayed sequence in a random situation. In general, if  $e < 10^{-50}$ , the database match should be the result of a homology relationship with a very high confidence level; and an e value less than  $10^{-5}$  is the result of a more sexually acceptable S-value.

The proteins for sulfur oxidation were searched in the 53 genomes of *Marinobacter* by locally blastP with reference sequences, including soxB form *Allochrochromatium vinosum* DSM 180 (accessions: Q1W3E6) (Hensen et al., 2006), Tsd from *Allochrochromatium vinosum* DSM 180 (accessions: ADC61061) (Denkmann et al.,

2012), Sqr from *Aquifex aeolicus* VF5 (accessions: O67931) (Griesbeck et al., 2002), AprA from *Megalodesulfobivrio gigas* DSM 1382 (accessions: T2G6Z9) (Fritz et al., 2002), DsrA from *Archaeoglobus fulgidus* DSM 4304 (accessions: Q59109) (Pott and Dahl, 1998), and Fcc from *Allochrochromatium vinosum* DSM 180 (accessions: Q06530) (Chen et al., 1994).

### 2.6 Phylogenetic analyses of Sox genes

Sox gene clusters including key gene *soxB* is essential for thiosulfate oxidation. The phylogenetic relationship of the *soxB* protein of *Marinobacter* with other 90 sulfur oxidizer *soxB* proteins of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Chlorobi, and Campylobacteria were aligned with MUSCLE (Edgar, 2004) in the phylogenetic software MEGAX (Kumar et al., 2018), and constructed the maximum-likelihood tree with 1 000 bootstraps using the "LG + G + I" model (Felsenstein, 1981). Then the Linux version of IQ-tree 2.0 (Trifinopoulos et al., 2016) was used to construct a phylogenetic tree, using the One Table (tvBOT) (Xie et al., 2023) to visualize the phylogenetic tree. The complete Sox multi-enzyme complex encoding genes including SoxRSVWXYZABCDEFGHIJ were mapped for *sox* gene clusters by online tool chipLOT (<https://www.chipLOT.online/tvbot.html>).

## 3 Results

### 3.1 Autotrophic thiosulfate oxidization of *Marinobacter* spp.

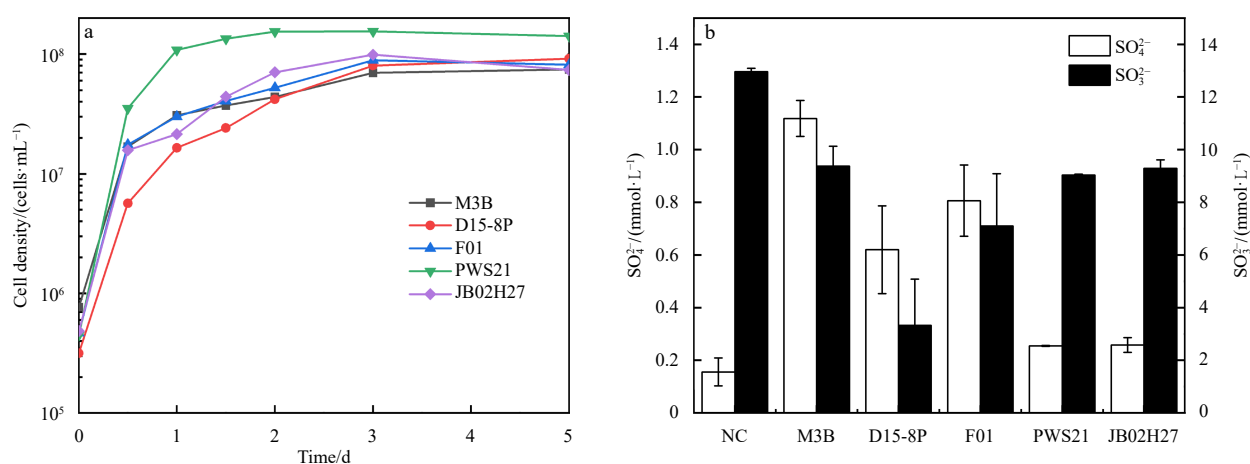
In the study, 27 type strains and 5 deep sea hydrothermal vents strains were chosen for physiologic characterization (Table 1). Among them, only 5 type strains and 1 deep sea hydrothermal vent isolate showed autotrophic sulfur oxidizing ability with thiosulfate as electron donor, including type strains *M. guineae* M3B<sup>T</sup>, *M. aromaticivorans* D15-8P<sup>T</sup>, *M. vulgaris* F01<sup>T</sup>, *M. profundus* PWS21<sup>T</sup>, *M. denitrificans* JB02H27<sup>T</sup> and deep sea hydrothermal vents strain *Marinobacter* sp. ST-1M (99.93% similarity to *Marinobacter* *salsuginis* SD-14B<sup>T</sup>) (Table 1, Fig. S1). Five type strains had growth in the autotrophic thiosulfate oxidization liquid medium from initial  $1 \times 10^5$  cells/mL to  $8.88 \times 10^7$ – $9.72 \times 10^7$  cells/mL in 5 d (Fig. 1a). These strains grew fast in the first 24 h, and reached a peak of cell density  $10^7$  cells/mL. Among the five positive type strains, *M. profundus* PWS21<sup>T</sup> had the fastest growth rate and the highest number of cells in the stable phase.

Ion chromatography was used to detect the concentration changes of thiosulfate and sulfate during autotrophic sulfur oxidation growth. These 5 type strains produced sulfate ranging from 0.25 mmol/L to 1.44 mmol/L (non-bacterial abiotic control was 0.17 mmol/L) with the consumption of thiosulfate from 3.60 mmol/L to 9.10 mmol/L (the beginning was 12.97 mmol/L) in 5 d, but without  $\text{SO}_3^{2-}$  detected, under autotrophic growth conditions (Fig. 1b). *Marinobacter guineae* M3B<sup>T</sup> had the highest ability with the production of sulfate to 138.23 mg/L.

On the solid medium of autotrophic thiosulfate oxidization containing phenol red, five *Marinobacter* strains turned the color of the media from purple to yellow (Fig. S2), indicating the pH was reduced by sulfur oxidation. They were *M. guineae* M3B<sup>T</sup>, *M. aromaticivorans* D15-8P<sup>T</sup>, *M. vulgaris* F01<sup>T</sup>, *M. denitrificans* JB02H27<sup>T</sup> and *Marinobacter* sp. ST-1M. *Marinobacter profundus* PWS21<sup>T</sup> couldn't grow on the autotrophic thiosulfate oxidization agar medium.

### 3.2 Heterotrophic thiosulfate oxidization of *Marinobacter* spp.

$\text{BaCl}_2$  colorimetric assay was used to test sulfate production in heterotrophic thiosulfate oxidization medium of 27 type strains and 5 strains from deep sea hydrothermal vents (Table 1, Fig. 2).



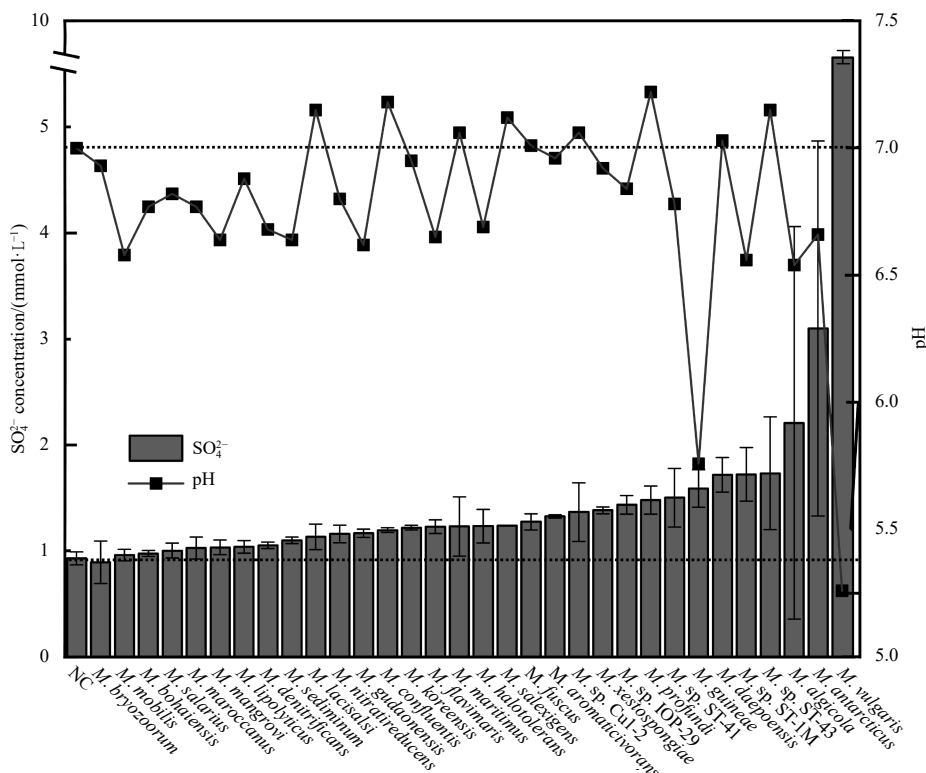
**Fig. 1.** Profile of cell density, thiosulfate, sulfate concentrations during autotrophic thiosulfate oxidizing growth over 5 d of *Marinobacter* type strains including *Marinobacter guineae* M3B<sup>T</sup>, *Marinobacter aromaticivorans* D15-8P<sup>T</sup>, *Marinobacter vulgaris* F01<sup>T</sup>, *Marinobacter profundus* PWS21<sup>T</sup> and *Marinobacter denitrificans* JB02H27<sup>T</sup>. a. Cell density. b. Thiosulfate and sulfate concentrations on the 5th day. NC: negative control without inoculums.

The *t*-test results revealed that 6 *Marinobacter* strains showed significant sulfate producing ability ( $p < 0.05$ ), especially *M. antarcticus* ZS2-30<sup>T</sup> and *M. vulgaris* F01<sup>T</sup> with highly significant differences ( $p = 0$ ) of 3.10 mmol/L and 9.45 mmol/L sulfate production. In addition, the other four were *M. algicola* DG893<sup>T</sup>, *M. dae-poensis* SW-156<sup>T</sup>, *Marinobacter* sp. ST-1M and *Marinobacter* sp. ST-43, which produced sulfate by 2.20 mmol/L, 1.72 mmol/L, 1.72 mmol/L and 1.73 mmol/L, heterotrophic thiosulfate oxidation growth. The pH obviously decreased from 7.0 to below 6.0 in the late phase growth culture of *M. guineae* M3B<sup>T</sup> and *M. vulgaris* F01<sup>T</sup> due to acid production (Fig. 2).

### 3.3 Mixotrophic growth of *M. guineae* M3B<sup>T</sup> and *M. vulgaris* F01<sup>T</sup>

As representative strains, *M. guineae* M3B<sup>T</sup> and *M. vulgaris* F01<sup>T</sup> were chosen for further mixotrophic ability identification. *Marinobacter guineae* M3B<sup>T</sup> was isolated from marine sediment collected from Antarctica (Montes et al., 2008), and *M. vulgaris* F01<sup>T</sup> was isolated from the solar saltern of Weihai, China (Zhang et al., 2020).

Under autotrophic growth conditions with  $\text{HCO}_3^-/\text{CO}_2$  as the sole carbon source, *M. guineae* M3B<sup>T</sup> oxidized thiosulfate from 12.97 mmol/L to 8.62 mmol/L with the sulfate production up to 1.44 mmol/L in 7 d (Fig. 3a); and under heterotrophic growth



**Fig. 2.** Sulfate concentrations and pH values in heterotrophic sulfur oxidation medium of 27 *Marinobacter* type strains and 5 deep sea hydrothermal vents *Marinobacter* strains over 5 d. NC: negative control without inoculum.

conditions, it also oxidized thiosulfate by reducing the concentration from 11.53 mmol/L to 4.71 mmol/L with the production of sulfate to 0.47 mmol/L in 3 d (Fig. 3b).

Under autotrophic growth conditions, *M. vulgaris* F01<sup>T</sup> could oxidize thiosulfate from 12.96 mmol/L to 7.10 mmol/L, with the production of sulfate to 0.81 mmol/L in 7 d; and under heterotrophic growth conditions, it oxidized thiosulfate from 11.53 mmol/L to 4.55 mmol/L with the production of sulfate to 1.78 mmol/L. In the late growth phase of *M. vulgaris* F01<sup>T</sup> culture, elemental sulfur, as well as sulfur-containing complexes were observed. Morphological observations under scanning electron microscope (SEM) showed that elemental sulfur was mainly shaped as spherical (Fig. 4a) and the sulfur-containing complexes were irregular or elliptical in morphology (Fig. 4b), and energy dispersive spectrometer (EDS) analyses revealed that the atomic percentage of sulfur in the particles ranged from –2.76% to 22.84%.

### 3.4 Genes for sulfur oxidation and autotrophy in *Marinobacter*

Thiosulfate can be oxidized to sulfate by the Sox system (Sox-ABCDXY) in the periplasm. Four protein components, *soxYZ*, *soxXA*, *soxB*, and *soxCD* are required for the complete oxidation

of thiosulfate to sulfate (Wasmund and Mussmann, 2017). Six *Marinobacter* strains encode a complete core set of Sox genes (*soxC-soxZ-soxY-soxZ-soxA-soxB*) (Fig. 5), including *M. antarcticus* ZS2-30<sup>T</sup>, *M. guineae* M3B<sup>T</sup>, *M. orientalis* W62<sup>T</sup>, *M. pelagius* HS225<sup>T</sup>, *M. salinus* Hb8<sup>T</sup>, and *M. vulgaris* F01<sup>T</sup>, which are similar with *Amphritea japonica* and *Neptunomonas marina*. Fourteen *Marinobacter* genomes contain SoxB (six strains with an e value of 0), which may involve in converting thiosulfate to sulfate.

The phylogenetic tree based on 90 SoxB protein sequences (Fig. 6) can be divided into seven clusters. From bottom to top in the tree : Cluster I , *Alphaproteobacteria* containing genera *Rhodovulum* and *Roseinatronobacter*; Cluster II , the genera *Acidihalobacter* and *Thiohalorhabdus* in *Gammaproteobacteria*; Cluster III, *Gammaproteobacteria*, mainly containing the genera *Thioalkalivibrio*, *Ectothiorhodospira*, *Leucothrix* and *Thiothrix*; Cluster IV, mainly *Thiobacillus*, *Sulfuriferula*, *Thiomonas*, *Pandora*, *Advenella* in *Betaproteobacteria*, *Azospirillum* in *Alphaproteobacteria*, and *Acidiferrobacter*, *Sulfuricaulis* in *Gammaproteobacteria*; Cluster V , *Gammaproteobacteria*; Cluster VI, *Marinobacter* in *Gammaproteobacteria* and *Chlorobaculum* belonged to *Chlorobi*, and Cluster VII, *Sulfurimonas*, *Sulfurovum*, and *Nitratiruptor* in *Campylobacteria*.

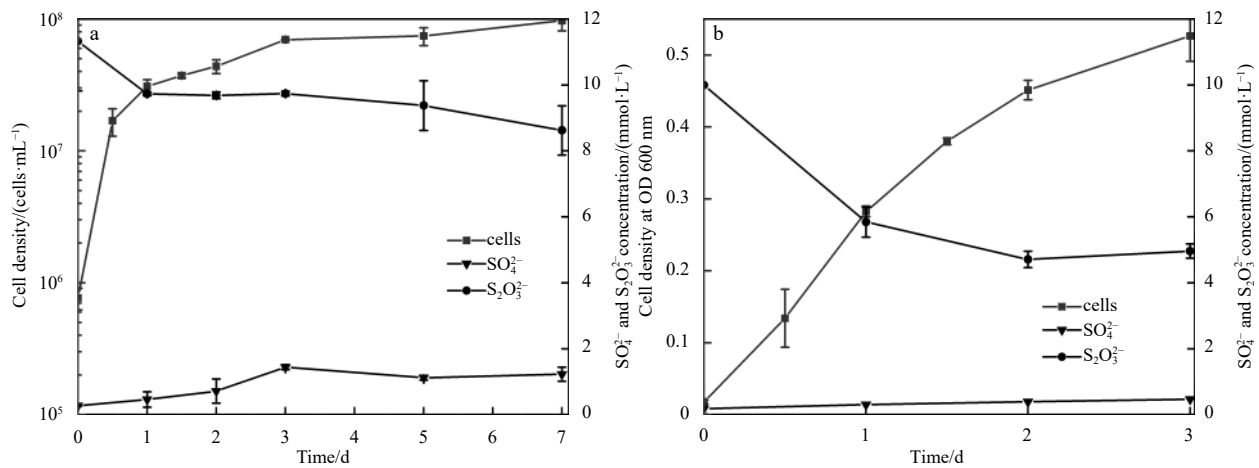


Fig. 3. Profiles in the cells number, thiosulfate and sulfate concentrations of *Marinobacter guineae* M3B<sup>T</sup> in autotrophic thiosulfate oxidization liquid medium (a) and in heterotrophic thiosulfate oxidization liquid medium (b).

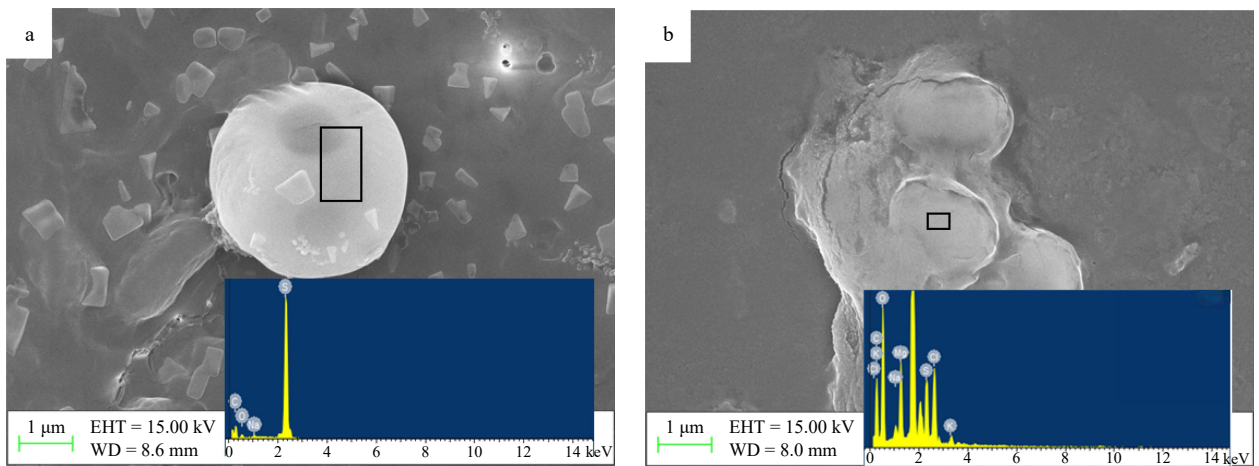
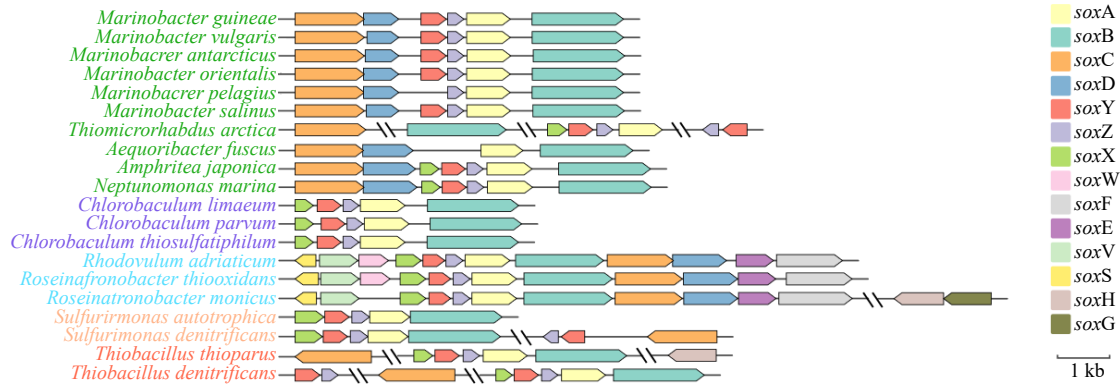
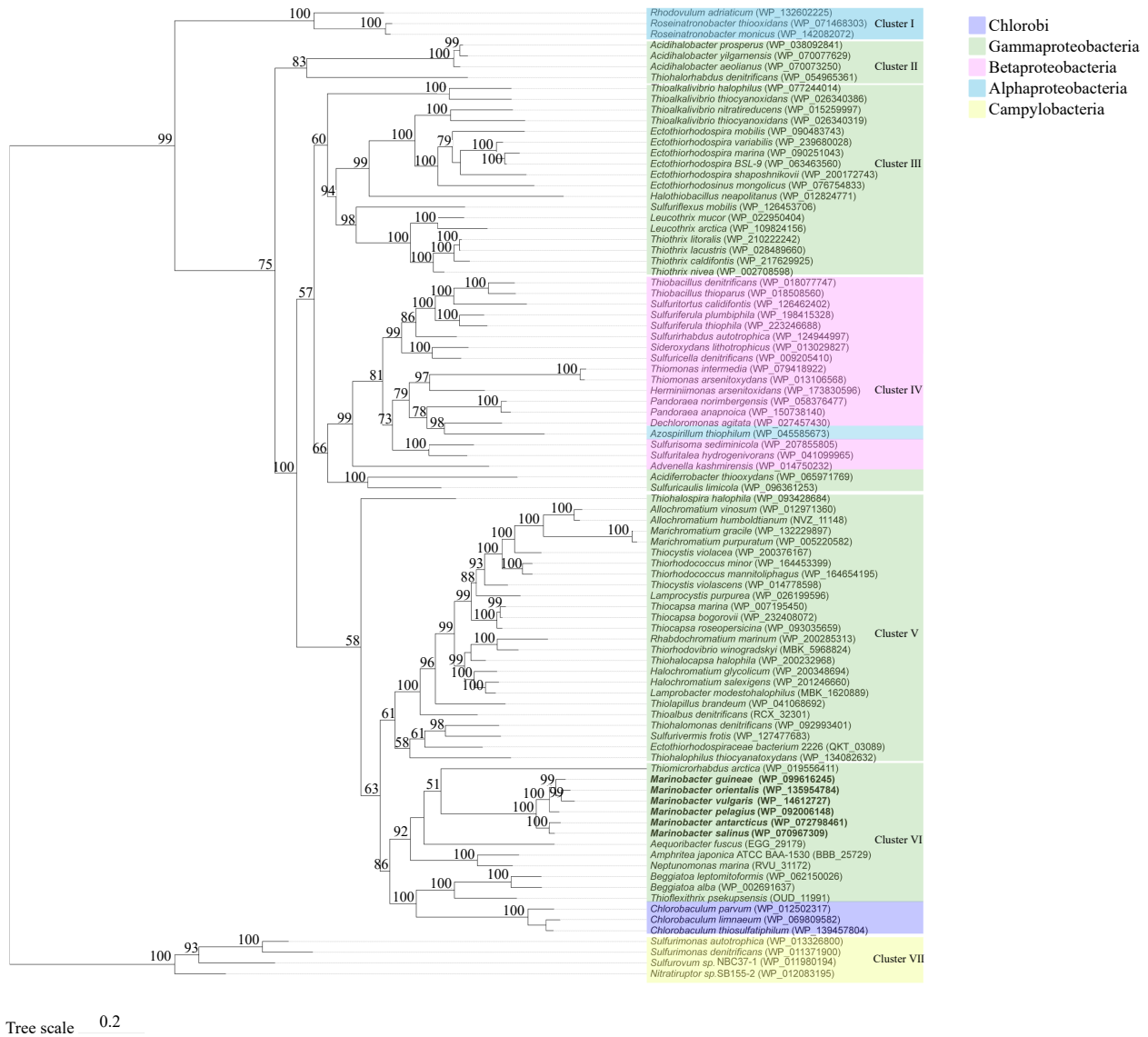


Fig. 4. Scanning electron microscope (SEM) and energy dispersive spectrometer (EDS) images of sulfur-containing complexes particles of strain *Marinobacter vulgaris* F01<sup>T</sup> in the autotrophic thiosulfate oxidation growth. a. Spherical sulfur-containing complexes; b. irregular sulfur-containing complexes. EHT, Extra High Tension; WD, Working Distance.



**Fig. 5.** Map of the *sox* gene cluster of six *Marinobacter* strains and other sulfur oxidizing bacteria.



**Fig. 6.** Maximum likelihood phylogenetic tree of full-length protein sequences of *soxB* protein derived from *Marinobacter* strains and other representative species. Bootstrap values indicated at each node are based on a total of 1 000 bootstrap replicates. Branch node values below 50% are not shown. Genera of the same class are in the same color and the genus *Marinobacter* is indicated in black bold.

*Marinobacter* formed a separate lineage within Gammaproteobacteria and close to *Thiomicrohabdus arctica* and *Aequoribacter fuscus*. *Marinobacter* spp. might obtain the *soxB* gene through

lateral gene transfer from sulfur oxidizing Gammaproteobacteria in the environment.

Other sulfur oxidizing related proteins including Tsd, Sqr,

AprA, DsrA, and Fcc were also searched in all 53 *Marinobacter* genomes with reference proteins in this study. Thirty strains contain Tsd (3 strains with e value less than  $10^{-50}$ , *M. aromaticivorans* D15-8P<sup>T</sup>, *M. changyiensis* CLL7-20<sup>T</sup>, *M. salicampi* ISL-40<sup>T</sup>), which catalyzes thiosulfate to tetrathionate; 30 strains encode Sqr (2 strains with e value less than  $10^{-50}$ , *M. oulmenensis* Set74<sup>T</sup>, *M. persicus* M9B<sup>T</sup>) and 29 strains encode Fcc (no strain with e value less than  $10^{-50}$ ), which oxidize sulfide (HS<sup>-</sup>/S<sup>2-</sup>) to ZVS; 6 strains were annotated AprA (no strain with e value less than  $10^{-50}$ ) for sulfite oxidation; no *Marinobacter* strain contain DsrA, which oxidizes sulfide to sulfite (Fig. 7). It indicated that sulfur oxidizing potential exists in *Marinobacter* spp. According to their physiologic tests, the results showed that sulfur oxidizing *Marinobacter* strains probably oxidize thiosulfate by Sox pathway.

Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analysis revealed that the presence of the two essential enzymes of the Calvin cycle (CBB cycle), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) in autotrophic thiosulphate-oxidizing *Marinobacter* strains, including 5 type *Marinobacter*, *M. guineae* M3B<sup>T</sup>, *M. aromaticivorans* D15-8P<sup>T</sup>, *M. vulgaris* F01<sup>T</sup>, *M. profundus* PWS21<sup>T</sup>, and *M. denitrificans* JB02H27<sup>T</sup>, suggesting their autotrophic potential.

#### 4 Discussion

*Marinobacter* spp. is widely distributed in a variety of marine environments, including the deep ocean, coastal seawater and sediment, hydrothermal fields, oceanic basalt, sea-ice, solar salt-erns, and oil fields (Cooper et al., 2022) (Fig. S2). This bacterial group has also been reported in marine hypoxic zones, for example, the oxygen-deficient zone of the Zhujiang River Estuary and the adjacent northern South China Sea (Wu et al., 2021). There was only one type strain from the deep-sea hydrothermal environment (Handley et al., 2009). Recently, we isolated several pure isolates of *Marinobacter* from hydrothermal vent sediment,

chimney sulfide, and plume. The frequent isolation of this genus in vent niches and other saline environments reminds us to detect the potential adaptation mechanisms that facilitate their global distribution.

Till now, 53 type strains in *Marinobacter* genus were sequenced (listed in Table S1), which genome sizes varying from 3.16 Mbp (*M. persicus* M9B<sup>T</sup>) to 5.27 Mbp (*M. halodurans* YJ-S3-2<sup>T</sup>) with an average of 4.11 Mbp. The number of predicted coding sequences (CDSs) vary from 2 867 (*M. persicus* M9B<sup>T</sup>) to 4 746 (*M. halodurans* YJ-S3-2<sup>T</sup>), with an average of 3 670. Genomic Guanine-Cytosine (GC) content varies from 53.7 % (*M. salexigens* HJR7<sup>T</sup>) to 63.2 % (*M. lutaensis* T5054<sup>T</sup>), with an average of 57.8 %. A maximum-likelihood phylogeny of the 53 type strains based on 92 single-copy orthologous genes was analyzed (Fig. 7). It revealed six clusters of *Marinobacter*, composed of four to eighteen species each, in the clusters I – III, most *Marinobacter* are sediment-derived; in the cluster VI, seawater-derived *Marinobacter* are most; and in the other three clusters, their origins are more diverse. It showed that *Marinobacter* genus had the genetic diversity, but their phylogeny is not related to environmental factors. The possible reason is that their genes acquired via horizontal gene transfer (HGT) from distantly related species in the environments. In this study, we found that more than half (43 of 53 species) possess metabolic potential with SoxB, Tsd, Sqr, AprA, or Fcc proteins to get energy by sulfur oxidation of thiosulfate and sulfide based on genome annotation. Further physiologic tests indicated that 6 of 32 *Marinobacter* strains (18.75%) were indeed capable of sulfur oxidizing. They might get the ability by HGT.

Bacteria of *Thiomicrospira*, *Halothiobacillus*, *Hydrogenovibrio* and *Thioclava* have been frequently recognized as marine sulfur-oxidizing bacteria (SOB). For example, *Thiomicrospira thermophile* EPR85, was reported of the maximum thiosulfate consumption of about 9.35 mmol/L, with the maximum sulfate

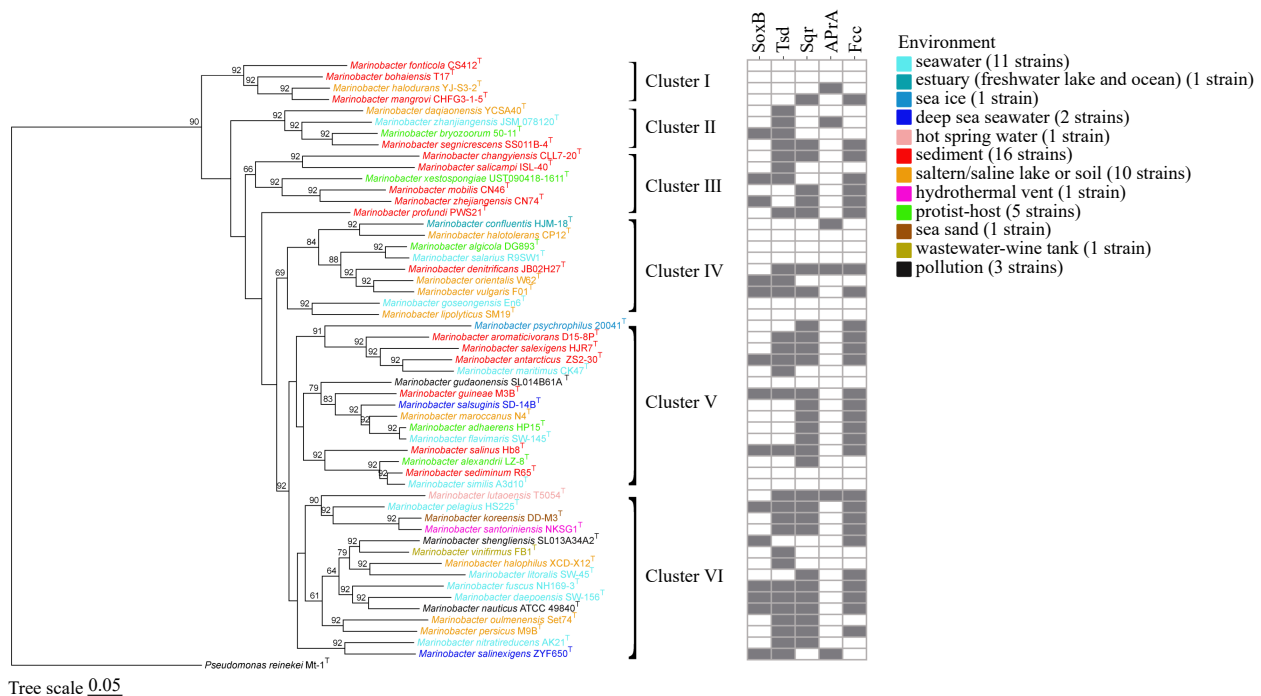


Fig. 7. Phylogenomic tree of *Marinobacter* species (53 strains) based on 92 core proteins. The color of leaves indicated the source of these strains. The tree was generated with UBCG 3.0. Bootstrap values indicated at each node are based on a total of 1 000 bootstrap replicates. Branch node values below 50% are not shown. The heatmaps represent distribution of genes for sulfur metabolism.

production of about 11.15 mmol/L in 8 h under no buffer conditions (Houghton et al., 2016). Similarly, *Halothiobacillus neapolitanus* strain I19 was reported of the highest sulfate concentration 17.3 mmol/L (Boroujeni et al., 2021). Additionally, other marine heterotrophic bacteria such as *Rhodobacter* (Ding et al., 2023), *Pseudomonas* (He et al., 2023), *Halomonas* (Du et al., 2022; He et al., 2023), and *Marinobacter* (Choi et al., 2009; He et al., 2023; Lian et al., 2021) have also been reported of sulfate formation. Moreover, *Halomonas* has been reported of oxidizing thiosulfate to tetrathionate (Du et al., 2022). *Pseudomonas* sp. IOP\_13 and *Halomonas* sp. IOP\_14 from hydrothermal vents could produce  $\text{SO}_4^{2-}$  at concentrations of 3.0 mmol/L and 4.1 mmol/L with consumption of 6.47 mmol/L and 6.69 mmol/L thiosulfate during 8 d (He et al., 2023). In our study, *M. guineae* M3B<sup>T</sup> produced the highest concentration of sulfate at 1.40 mmol/L in the autotrophic medium in 5 d and *M. algicola* DG893<sup>T</sup> produced sulfate by 2.20 mmol/L in the heterotrophic medium in 7 d. *Marinobacter arinobacter guineae* M3B<sup>T</sup> and *M. vulgaris* F01<sup>T</sup> both showed mixotrophic growth, in addition to utilizing organic carbon; they also could use bicarbonate as carbon source, thiosulfate as electron donor and oxygen as electron acceptor. Compared with chemolithoautotrophic SOB, mixotrophic SOB, such as *Marinobacter*, *Halomonas*, *Pseudomonas* etc, show lower sulfur-oxidizing ability. It is worth to note that *M. guineae* M3B<sup>T</sup> and *M. vulgaris* F01<sup>T</sup> lowered the pH more significantly during thiosulfate oxidation than the other strains. This may be owing to their efficient, Paracoccus sulfur oxidation (PSO) pathway, in which thiosulfate is oxidized directly to sulfate by Sox cluster (Teske et al., 2000). Different with *Marinobacter*, some facultative chemolithotrophic S-oxidizing bacteria such as *Halomonas* and *Pseudomonas*, they use S4 intermediate pathway (S4I), which includes the formation and oxidation of polythionate and/or S, sulfite from thiosulfate (Ghosh and Dam, 2009).

*Marinobacter* spp. are considered to be typical heterotrophic bacteria, with few exceptions as autotrophic (Choi et al., 2009; Dou et al., 2022). Together, our study provides a hint for further understanding of the metabolic diversity of *Marinobacter*, including mixotrophic growth style, sulfur oxidation pathways, etc. Their physiological versatility and environment adaptations may contribute to their wide distribution in marine and other environments.

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## Supplementary information

**Table S1:** Genomes used in this study, their size, GC content, protein, gene, isolation environment (brief), 16S rDNA GenBank Accession, genomes RefSeq accession, MCCC deposition No., isolation environment (detailed) references.

**Figure S1:** Strains cultured in autotrophic SOB medium (a) and SOB solid medium with phenol red (b).

**Figure S2:** Distribution world map with isolation locations of each species of *Marinobacter*. Each symbol and color represent the isolation environment of the species.

The supplementary information is available online at <https://doi.org/10.1007/s13131-023-2263-x> and <http://www.aosocean.com/>. The supplementary information is published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.