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Characterization of two novel chemolithoautotrophic bacteria of *Sulfurovum* from marine coastal environments and further comparative genomic analyses revealed species differentiation among deep-sea hydrothermal vent and non-vent origins

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Bacteria of the genus *Sulfurovum* within the class *Campylobacteria* are widespread in global oceans and are predominant in sulfide-rich environments. However, little is known about their adaptation to such harsh environments owing to their resistance to cultivation. In this study, we obtained three pure cultures of this genus from marine coastal environments and compared them with those obtained from the deep sea. Phylogenetic analysis of 16S rRNA gene sequences indicated that they represent two novel species of the genus, sharing 95.9%–96.1% sequence similarities to *Sulfurovum aggregans* Monchim33^T. Based on the polyphasic classification results, the type strains XTW-4^T and zt1-1^T were proposed to represent two new species: *Sulfurovum xiamenensis* sp. nov. and *Sulfurovum zhangzhouensis* sp. nov., respectively. These coastal isolates were also obligate chemoautotrophs featuring molecular hydrogen as an electron donor and molecular oxygen, thiosulfate, or elemental sulfur as the sole electron acceptor. Comparative genomic analyses based on 11 *Sulfurovum* species further revealed a clear differentiation between hydrothermal vent and non-vent origins. The non-vent *Sulfurovum* can use thiosulfate as an electron acceptor but lacks denitrification pathways, whereas the vent bacteria can respire nitrate through complete denitrification pathways. Moreover, the non-vent *Sulfurovum* contained a nitrogen fixation pathway, implying their adaptation to nitrogen source-deficit niches. In addition, non-

vent *Sulfurovum* species adapted to a higher oxygen concentration *via* multiple antioxidative defense mechanisms. These phenotypic and genotypic features help us to understand the ecological role of *Sulfurovum* bacteria in marine ecosystems.

KEYWORDS

***Sulfurovum xiamenensis*, *Sulfurovum zhangzhouensis*, nitrogen fixation, coastal environment, hydrothermal vent, environmental adaptation**

Introduction

The genus *Sulfurovum* is an important taxon of sulfur-oxidizing bacteria belonging to the family *Sulfurovaceae* of the phylum *Campylobacterota* (Waite et al., 2018) and can be found in various habitats, including mangroves (Lin et al., 2019), nearshore sediments (Marziah et al., 2016), shallow hydrothermal zones (Wang et al., 2017), glaciers (Wright et al., 2013), seagrass beds (Sun Y. Y. et al., 2020), and deep-sea hydrothermal vents (Nakagawa et al., 2006; Dahle et al., 2013; Meier et al., 2017). *Sulfurovum* bacteria are especially abundant in deep-sea hydrothermal vent environments and are widely distributed in various hydrothermal habitats, including chimneys, sediments, plumes, diffuse-flow vent fluids, and symbionts with animals (Akerman et al., 2013; Sheik et al., 2015; Motoki et al., 2020; Moulana et al., 2020; Lee et al., 2021; Chiu et al., 2022). *Sulfurovum* species are strict chemolithoautotrophs that have diverse energy metabolic pathways (Meier et al., 2017; Wang et al., 2023). They usually use hydrogen and reduced sulfur compounds as electron donors and oxygen, nitrate, and elemental sulfur as electron acceptors to fix CO_2, thus contributing to primary productivity in chemosynthetic ecosystems (Wright et al., 2013; Sheik et al., 2015). Recently, *Sulfurovum* was found to have potential applications in environmental remediation by removing hydrogen sulfide and nitrite from industrial wastewater (Li W. et al., 2022; Wang K. Q. et al., 2022; Zheng et al., 2022).

Currently, the genus *Sulfurovum* comprises only five species with validly published names and one provisional species. Five species, *Sulfurovum lithotrophicum* 42BKT^T, *Sulfurovum aggregans* Monchim33^T, *Sulfurovum riftiae* 1812E^T, *Sulfurovum denitrificans* eps51^T, and *Sulfurovum indicum* ST-419^T, were isolated from deep-sea hydrothermal vent environments, including vent sediments, plumes, chimneys, and outer biofilms of the polychaete *Riftia pachyptila* (Inagaki et al., 2004; Mino et al., 2014; Giovannelli et al., 2016; Mori et al., 2018; Xie et al., 2021). One provisional species, "*Candidatus Sulfurovum sediminum*", strain AR, was isolated from a non-vent environment. It was an enrichment culture from 78-m deep marine sediment collected near Svalbard Island in the Arctic Circle (Park et al., 2012). Recently, two strains *Sulfurovum* sp. TSL1 and TSL 6 were isolated from a tsunami-launched marine sediment based only on genomic analysis (Guo et al., 2022). Thus, in view of their widespread distribution and potentially high diversity, more bacteria of this genus are being isolated from different environments.

Although sequences affiliated with genus *Sulfurovum* have been detected from worldwide marine systems (Teske et al., 2002; Takai et al., 2004; Sylvan et al., 2012; Takai et al., 2015), physiological diversity and the ecological role of this genus have not yet been fully elucidated due to the lack of cultivated strains. Compared to vent *Sulfurovum* species, which has been studied extensively, there has been less exploration of non-vent *Sulfurovum* bacteria owing to fewer pure cultures being available from non-vent marine environments. In this study, three strains of the genus *Sulfurovum* were isolated from intertidal sediments, mangrove sediments, and mariculture pond sediments in subtropical estuary areas along the west bank of the Taiwan Strait. To elucidate their ecological roles and environmental adaptations in coastal marine ecosystems, we characterized strains belonging to two novel species using a polyphasic taxonomic approach. In addition, we performed comparative genomic analyses of all members of the genus *Sulfurovum*, including those with vent and non-vent origins, to gain insights into the environmental adaptation mechanisms underlying their widespread distribution.

Materials and methods

Bacterial enrichment and isolation

The marine sediments were sampled from intertidal zone of Xiamen island on April 12, 2021 (11812′25′′E 2429′52′′N), the mangrove garden of Xia-Tan-Wei at the estuary of Xixi River of Xiamen on June 27, 2022 (11812′4′′E 2439′14′′N), and an abandoned pond nearby the mangrove conservation area at the estuary of Zhangjiang River, Zhangzhou on September 14, 2022 (11725′55′′E 2357′18′′N) (Supplementary Figure S1). These samples are black and smell like rotten eggs, implying these sediments are enriched in sulfide. The samples were kept cool and returned to the laboratory. Each of 2 g (wet mass) was suspended with artificial seawater, and then 1 ml was transferred into 50 ml anaerobic bottles respectively containing 10 ml MMJHS medium supplied with 76% H_2/20% CO_2/4% O_2 (200kPa) with hydrogen and thiosulfate as energy sources, or 10 ml of MMJS medium, which contains 76% N_2/20% CO_2/4% O_2 (200 kPa) with thiosulfate as the sole energy source, or 10 ml MMJH medium which contains 76% H_2/20% CO_2/4% O_2 (200 kPa) with hydrogen as the energy source (See supplementary material for medium

composition). All of them were incubated at 32°C according to a method described previously (Jiang et al., 2017). After 3-round enrichment, the cell growth in MMJH medium was observed. Subsequently, cells were purified three times with the dilution-to-extinction technique. The culture in the serum bottle showing growth at the highest dilution was designated as strains XGS-02, XTW-4, and zt1-1. The purity of these cultures was confirmed by microscopic examination and 16S rRNA gene sequencing.

Phenotypic and chemotaxonomic characterization

Cultures were grown in MMJH medium at 30°C for 2 days, cell morphology was observed under transmission electron microscopy (HT7800, Hitachi, Japan). The physiological characterization of three strains was performed in MMJHS medium and growth was measured by direct cell counting using a phase contrast microscope (Eclipse 80i, Nikon) according to the method previously described (Jiang et al., 2017). The temperature range for growth was determined at 4, 10, 15, 20, 25, 28, 30,32, 35, 37, 40, 45, 50 and 60°C. The salinity range for growth was examined by adjusting the concentrations of NaCl between 0 and 9.0% (w/v) at 0.5 (w/v) intervals. Oxygen sensitivity was examined using MMJHS medium with different O_2 concentrations (0%, 1%, 2%, 4%, 6%, 8%, 10%, 15% and 20%) in the headspace gas. In the case of oxygen absence, 10 mM nitrate was added as a potential electron acceptor. The pH range was investigated from 3.5 to 9.0 with a 0.5 pH unit interval by altering pH with several buffers, including 30 mM acetate/acetic acid buffer (pH 3.0–5.0), MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), HEPES (pH 7.5–8.0), Tris and CAPSO (pH 8.0 and above).

To determine the electron donors and acceptors, MMJ synthetic seawater containing 0.1% (w/v) $NaHCO_3$ was used as a basal medium, and H_2 was tested as the electron donor for the growth of three strains, nitrate (0.1%, w/v), nitrite (0.1 and 0.01%, w/v), thiosulfate (0.1%, w/v), sulfite (0.01%, w/v), elemental sulfur (1%, w/v), or molecular oxygen (1%), were tested as the only electron acceptor. The sulfur oxidation was tested with MMJS medium, using sodium thiosulfate (0.1%, w/v) or elemental sulfur (S) (1%, w/v) as sole electron donor, nitrate (0.1%, w/v), nitrite (0.1 and 0.01%, w/v), or molecular oxygen (0.1 and 1%, v/v) were tested as the only electron acceptor. Heterotrophic growth was tested in MMJ medium in the absence of $NaHCO_3$ and in a 94% N_2/6% O_2 (200 kPa) gas phase. The following potential organic carbon compounds were tested: 0.02% (w/v) glucose, galactose and fructose, 0.1% (w/v) peptone, starch and casein, 5 mM formate, acetate, citrate, fumarate and pyruvate.

For fatty acids analysis, cells grown on MMJHS medium at 30C for 24h were saponified, methylated, and extracted following the standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analyzed by gas chromatography (Agilent Technologies 6850), and then the result was identified using the TSBA6.0 database of the Microbial Identification System.

Phylogenetic analysis and genome sequencing

The total DNA of the three strains was extracted and the 16S rRNA gene was amplified as described previously (Jiang et al., 2009). The similarity of 16S rRNA gene sequences was determined using the EzBioCloud server (http://www.ezbiocloud.net/) and NCBI database, and sequences of close strains were downloaded for the phylogenetic analysis. Sequence comparison was performed using CLUSTAL_W (Larkin et al., 2007) and phylogenetic trees were constructed using MEGA X (Kumar et al., 2018) according to the Neighbor-Joining (Tamura et al., 2004), Maximum likelihood and Maximum Parsimony methods (Felsenstein, 1981). The online website TVBOT was used to embellish the phylogenetic tree (Xie et al., 2023). Genetic distance was calculated using Kimura two-parameter model. Bootstrap analysis was calculated based on 1000 replications. The complete genome of strain XGS-02 was sequenced by Tianjin Biochip Corporation (Tianjin, PR China) using the single molecule real-time (SMRT) technology on the Pacific Biosciences (PacBio) sequencing platform. *De novo* assembly was performed using the Hierarchical Genome Assembly Process (Version 4) workflow, and the complete circular genome was derived. Draft genome sequences of strains XTW-4 and zt1-1 were sequenced using Illumina Hiseq 2000 platform (Major Bio-Pharm Technology Co., Ltd, China). Raw reads were clipped and trimmed using Trimmomatic version 0.32 (Bolger et al., 2014). Trimmed reads were assembled using SOAPdenovo (Luo et al., 2015). The G+C content of the genomic DNA was determined from the whole genome sequence. Gene prediction and annotation were performed using NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (Tatusova et al., 2016) and the Rapid Annotation using Subsystem Technology (RAST) pipeline (Overbeek et al., 2014)

Genomic properties and comparative genomic analysis

We downloaded all culturable *Sulfurovum* genomes available from NCBI database, including four strains *Sulfurovum indicum* ST-419^T, *Sulfurovum riftiae* 1812E^T, *Sulfurovum lithotrophicum* ATCC BAA-797^T, and *Sulfurovum* sp. NBC37-1 from deep-sea hydrothermal vent environments, four strains, *Sulfurovum* sp. TSL1, *Sulfurovum* sp. TSL6, *Candidatus Sulfurovum* sp. AR, and *Sulfurovum* sp. HSL1-3 from coastal sediments. A total of eleven genomic sequences, including three genomes from our newly isolated strains XGS-02, XTW-4, and zt1-1, were checked for completeness and contamination using CheckM (Parks et al., 2015). The phylogenomic tree was constructed based on an up-to-date 92 bacterial core gene sets by UBCG version 3.0 (Na et al., 2018), the ANI was compared using the pyani tool (Pritchard et al., 2016), Pan-genomic analysis was performed with BPGA software (Chaudhari et al., 2016). Protein sequences were identified using PowerBLAST (Zhang and Madden, 1997). Sequence comparison

was performed using MEGA X (Kumar et al., 2018), and phylogenetic trees based on amino acid sequences were constructed using the Maximum Likelihood method with bootstrap values based on 1000 replicates.

Data availability

The GenBank accession numbers for 16S rRNA gene sequence of strains XGS-02, XTW-4 and zt1-1 are OM960633, OP810811 and OP810810, respectively. The genome sequences have been deposited at GenBank under the accession numbers of CP093312, JAQIBC000000000 and JAQIBD000000000, respectively. The three *Sulfurovum* strains were deposited in the Marine Culture Collection of China (MCCC) under collection numbers MCCC 1A18820, MCCC 1A19406, MCCC 1A19490.

Results

Phylogenetic analysis based on 16S rRNA gene and core genome

With enrichments with H_2 as the sole energy source, three strains of *Sulfurovum* were isolated from coastal sediments and designated as strains XGS-02, XTW-4, and zt1-1. In other enriched media, the isolates belonged to genus *Thiomicrorhabdus* and *Sulfurimonas*, which were not the focus of this study. The full-length 16S rRNA gene sequences of strains XGS-02 and zt1-1 retrieved from genomes were 1,503 bp and 1,513 bp, respectively. The 16S rRNA gene sequences of XTW-4 was 1,419 bp. BLAST analysis of the 16S rRNA gene sequences indicated that strains XGS-02, XTW-4, and zt1-1 shared the highest sequence similarity with *S. aggregans* Monchim33^T, which was isolated from a deep-sea hydrothermal vent chimney with similarities of 96.4%, 96.1%, and 95.9%, respectively. They were also closely related to *S. indicum* ST-419^T from the deep-sea hydrothermal plume with 95.6%, 94.6%, and 95.2% similarity, respectively. Furthermore, strains XGS-02 and XTW-4 shared 99.6% sequence similarity with each other and 95.9% and 96.0% similarity with strain zt1-1, respectively. The results indicate that strains XTW-4 and zt1-1 represent two novel species of the genus *Sulfurovum*.

Based on 16S rRNA gene sequences, the phylogenetic tree indicated that strains XGS-02 and XTW-4 clustered with *Sulfurovum* species from non-vent marine habitats, whereas those from vent environments formed separate clusters. Notably, strains zt1-1 and HSL1-3 represented a new branch located differently from other *Sulfurovum* species (Figure 1, Supplementary Figures S2, S3). The phylogeny was confirmed by whole-genome phylogenetic analysis based on 92 core gene sequences (Figure 2), which was consistent with the phylogenetic tree based on the 16S rRNA gene sequences. Combining the phylogenetic tree based on 16S rRNA gene sequences and whole genomes, three different evolutionary branches of the genus *Sulfurovum* were revealed. Correspondingly, three groups were named: Group I, bacteria from intertidal sediments; Group II, bacteria from hydrothermal vent areas; and Group III, bacteria from intertidal sediments (Figure 2).

Phenotypic and chemotaxonomic characterization

Morphological observation by TEM showed that strains XTW-4 and zt1-1 had no flagella. The result was confirmed by a genomic analysis, without genes encoding flagella in their genomes. The cells of strain XTW-4 was short rods, 0.2–0.4 μm wide and 0.4–1.2 μm long (Supplementary Figure S4). The morphology of strain zt1-1 was short rod-shaped, 0.2–0.5 μm wide, and 0.4–1.2 μm long, which was similar to strain XTW-4 (Supplementary Figure S4).

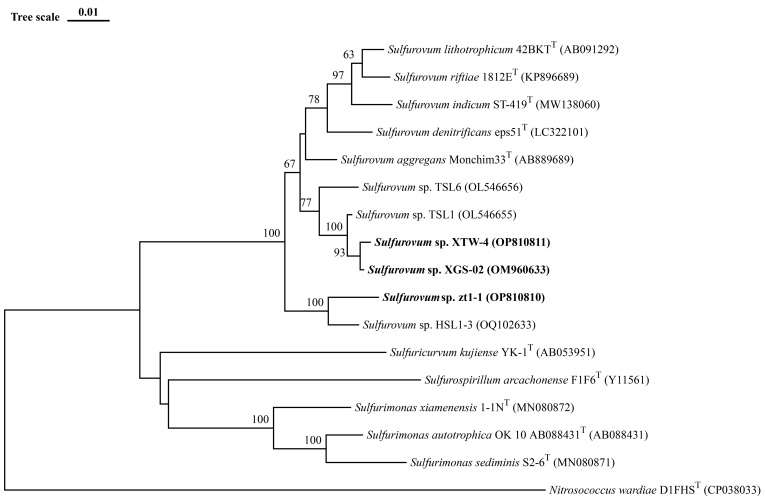

FIGURE 1
Neighbor-Joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of strains XGS-02, XTW-4, zt1-1 and other members of the genus *Sulfurovum*. The tree was rooted using *Nitrosococcus wardiae* as an outgroup. Bootstrap values based on 1000 replicates are shown at branch node. Higher than 50% bootstrap values are shown. Bar, 0.01 substitutions per nucleotide position.

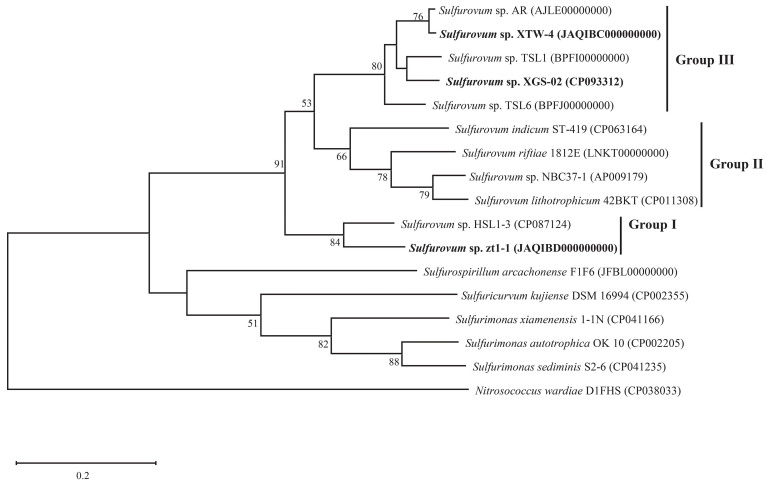

FIGURE 2
Whole genome phylogenetic tree based on 92 core gene sequences showing the position of XGS-02, XTW-4, zt1-1 and closely related taxa within the genus *Sulfurovum* using the maximum-likelihood algorithm. The node is labeled with Gene Support Index (GSI) values. Branch node values below 50% are not shown. The accession numbers of the genomes are shown in parentheses. Bar, 0.2 substitutions per position.

The growth experiment showed that strain XGS-02 could grow at a temperature range of 10–45°C, salinity range of 0.5–4.0% (w/v) NaCl, pH range of 5.5–8.5, and oxygen concentration range of 1–20%. Strain XTW-4 could grow at temperature (15–45°C), salinity (0.5–5.0% (w/v) NaCl), pH (5.0–8.5), and oxygen (1–15%) ranges. Strain zt1-1 could grow at 10–45°C, 0%–7.0% (w/v) NaCl, 4.5–8.5 pH, and 1%–20% O_2. The optimal temperature for the three newly isolated strains was approximately 30°C and the optimum pH was 6.0–6.5. The optimal salinity was 2.5% (w/v) NaCl for strains XGS-02 and XTW-4, and 1.5% (w/v) NaCl for strain zt1-1. The optimum oxygen concentrations were 8% for XGS-02 and XTW-4 and 15% for zt1-1. Chemoautotrophic growth showed that the three strains could utilize hydrogen and thiosulfate as electron donors but fail to oxidize elemental sulfur. They had the highest biomass with hydrogen as the electron donor. Thus, hydrogen may be the preferred energy source for these bacteria. All oxygen, thiosulfate and elemental sulfur can be used as electron acceptors. It is worth mentioning that the strains grew weakly when thiosulfate acted as the electron donor and oxygen acted as electron acceptor. All strains could not use nitrate and nitrite as electron acceptors.

We further compared the physiological characteristics of the three new non-vent strains with those of the vent-type strains, *S. indicum* ST-419^T (Xie et al., 2021), *S. riftiae* 1812E^T (Giovannelli et al., 2016), *S. lithotrophicum* ATCC BAA-797^T (Inagaki et al., 2004), *S. denitrificans* eps51^T (Mori et al., 2018), and *S. aggregans* Monchim33^T (Mino et al., 2014) (Table 1). The results revealed an evident divergence between the vent and non-vent strains. The non-vent strains had an optimal oxygen range of 8–15%, showing higher oxygen adaptation than the vent strains. Moreover, the three non-vent strains could not use nitrate as an electron acceptor, whereas all the vent strains could. All the non-vent *Sulfurovum*-type strains could reduce thiosulfate, but all those of the vent species could not, except for *S. aggregans*. In the case of electron donor utilization, all non-vent strains can utilize hydrogen as an electron donor, while most (3/5) vent strains cannot. In contrast, none of the non-vent strains can grow with elemental sulfur as the sole energy source, whereas most (4/5) vent strains can grow.

The fatty acid profiles of strains XTW-4 and zt1-1 and related type strains are listed in Table 1. Strain XTW-4 contained the predominant cellular fatty acids $C_{18:1}\omega7c$ (35.0%), $C_{16:1}\omega7c$ (24.5%), and $C_{16:0}$ (16.4%), and strain zt1-1 mainly contained $C_{16:1}\omega7c$ (35.9%), $C_{18:1}\omega7c$ (18.1%), and $C_{16:0}$ (17.6%). These profiles are similar to those of *S. indicum* ST-419^T, *S. riftiae* 1812^T, and *S. denitrificans* eps51^T.

Genomic features of the two novel species of *Sulfurovum*

The genome size of strain XTW-4 was 2.20 Mb with a GC content of 39.1%, and the genome size of zt1-1 was 2.18 Mb with a GC content of 39.3%. A total of 2,268 genes were predicted in the genome of strain XTW-4, including 2,203 protein-coding and 65 RNA genes. The genome of strain zt1-1 contains 2,267 genes, including 2,209 protein-coding genes and 58 RNA genes. The genomic similarity of strains XTW-4 and zt1-1 with other species of the genus *Sulfurovum* was determined using ANI values. The paired ANI values between strain XTW-4 and its close relatives, *S. indicum* and *S. riftiae*, were 86.0% and 85.7%, respectively. The paired ANI values between strain zt1-1 and its close relatives, *S. indicum* and *S. riftiae*, were 84.0% and 84.4%, respectively. These findings support the classification of strains XTW-4 and zt1-1 as two distinct species based on the cut-off threshold of ANI (95–96%) for the delineation of prokaryotic species. Strains XTW-4, zt1-1, and others of *Sulfurovum*

TABLE 1 Comparison of physiological characteristics within the genus *Sulfurovum*. .

		Sulfurovum sp. XTW-4	*Sulfurovum* sp. zt1-1	*Sulfurovum* sp. XGS-02	*S.aggregans* Monchim33^T	*S.indicum* ST-419^T	*S.riftiae* 1812^T	*S.lithotrophicum* ATCC BAA-797^T	*S.denitrificans* eps51^T
Source of Isolation		Non-vent	Non-vent	Non-vent	Vent	Vent	Vent	Vent	Vent
Temperature ranges (°C)		15–45	10–45	10–45	15–37	4–45	25–40	10–40	10–35
(Optimum)		(32)	(32)	(30)	(33)	(37)	(35)	(28–30)	(30)
Salinity ranges (%)		0.5–5.0	0–7.0	0.5–4.0	2.0–4.0	1.0–5.0	1.5–4.0	2.0–4.0	2.0–5.0
(Optimum)		(2.5)	(1.50)	(2.5)	(2.5)	(3.0)	(3.0)	(2.5)	(3.0)
Oxygen conditions (%)		1–15	1–20	1–20	3	1–20	–	1–7.5	20
(Optimum)		(8–10)	(15)	(8–10)	ND	(5)	–	ND	ND
pH ranges		5.0–8.5	4.5–8.5	5.5–8.5	5.5–8.6	5.0–8.6	5.0–8.0	5.0–9.0	6.5–7.5
(Optimum)		(6.0–6.5)	(6.0–6.5)	(6.0–6.5)	(6.0)	(6.0)	(6.0)	(6.5–7.0)	(7.0)
Electron donor	H_2	+	+	+	+	+	–	–	–
	$S_2O_3^{2-}$	+	+	+	–	+	+	+	+
	S^0	–	–	–	–	+	+	+	+
Electron acceptor	O_2	+	+	+	ND	+	–	+	+
	NO_3^-	–	–	–	+	+	+	+	+
	$S_2O_3^{2-}$	+	+	+	+	–	–	–	–
	S^0	+	+	+	+	+	–	–	–
Major fatty acids %		$C_{16:1}\omega7c$ (24.47)	$C_{16:1}\omega7c$ (35.9)	ND	$C_{16:1}\omega7c$ (18.1)	$C_{16:1}\omega7c$ (50.3)	$C_{16:1}\omega7c$ (34.3)	$C_{16:1}\omega7c$ (53.7)	$C_{16:1}\omega7c$ (61.1)
		$C_{18:1}\omega7c$ (35.0)	$C_{18:1}\omega7c$ (18.1)		$C_{18:0}$ (18.9)	$C_{18:1}\omega7c$ (19.5)	$C_{18:1}\omega7c$ (28.4)	$C_{16:0}$ (31.3)	$C_{18:1}\omega7c$ (17.4)
		$C_{16:0}$ (16.4)	$C_{16:0}$ (17.6)		$C_{16:0}$ (40.0)	$C_{16:0}$ (11.3)	$C_{16:0}$ (15.4)	$C_{18:0}$ (15.0)	$C_{16:0}$ (13.2)

Data of S.riftiae 1812^T from Donato Giovannelli (Giovannelli et al., 2016), S. lithotrophicum ATCC BAA-797^T from Fumio Inagaki (Inagaki et al., 2004), S.denitrificans eps51^T from Koji Mori (Mori et al., 2018), and S.aggregans Monchim33^T from Sayaka Mino (Mino et al., 2014). +, present; –, absent; ND, no data.

species shared less than 40.0 % DDH values (Table S2). The low DDH values (< 70%) confirmed that strains XTW-4 and zt1-1 represented two novel species within the genus *Sulfurovum* (Stackebrandt and Goebel, 1994; Auch et al., 2010; Meier-Kolthoff et al., 2014).

Comparative genomic analysis within the genus

A total of 11 *Sulfurovum* genomes from hydrothermal vents and intertidal environments were used for comparative analysis in this study. The genome size range of the non-vent *Sulfurovum* species was 2.1–2.6 Mb, and the G+C content ranged from 38.4 to 43.6%, while the genome size range of the vent strain was 2.2–2.6 Mb and the range of G+C content was 42.5–45.7%. The results showed that *Sulfurovum* species from deep-sea hydrothermal vent environments had relatively higher G+C values (Table 2). In addition, comparative genomic analysis revealed 1051 core genes in all *Sulfurovum* genomes (Supplementary Figure S5). The percentage of core genes per genome ranged from 42.8% to 50.9%, and the percentage of unique genes per genome ranged from 5.4% to 19.6%. *Sulfurovum* genomes belonging to Group I had 12.2–19.3% of unique genes, and those genomes from Group II had 11.9–17.9%. Group III from non-vent genomes had the lowest percentage of unique genes (< 9%) (Table S1).

Central metabolisms of *Sulfurovum*

Nitrogen metabolism and its differentiation among vent and non-vent origins

Comparative genomic analysis showed significant differences in nitrogen metabolism between the vent and non-vent *Sulfurovum* species (Table 3). *Sulfurovum* species from hydrothermal vent habitats contain genes required for the complete denitrification pathway, including the *napAB* gene encoding outer membrane nitrate reductase, the *nirS* gene encoding nitrite reductase, the *norBC* gene encoding nitric oxide reductase, and the *nosZ* gene encoding nitric oxide reduction enzyme. In addition, the genes *nrfA* and *napA*, performing a heterotrimeric nitrate reduction pathway, were present in all vent *Sulfurovum* genomes. Intriguingly, the denitrification and dissimilatory nitrate reduction pathways were completely absent in all non-vent *Sulfurovum* genomes, except for strain TSL1, which only has a denitrification-related *nirk* gene.

TABLE 2 Genomic information of all *Sulfurovum* strains available in NCBI database and obtained in this study.

Strains	completeness (%)	contamination (%)	Genome size (Mb)	G + C (%)	CDS	Source of Isolation	NCBI accession No.
S.xiamenensis XGS-02	99.6	2.3	2.2	40.2	2131	coastal sediments	CP093312
S.xiamenensis XTW-4	99.6	2.5	2.2	39.1	2203	mangrove sediments	JAQIBC000000000
S.zhangzhouensis zt1-1	99.0	2.1	2.2	39.4	2209	coastal sediments	JAQIBD000000000
Sulfurovum sp. HSL1-3	99.6	2.7	2.6	43.6	2533	mangrove sediments	CP087124
Candidatus Sulfurovum sp. AR	99.0	1.6	2.1	39.2	2122	marine sediment	NZ_AJLE00000000
Sulfurovum sp. TSL1	99.6	4.7	2.4	40.7	2292	marine sediment	BPFI00000000
Sulfurovum sp. TSL6	99.6	2.7	2.3	38.4	2205	marine sediment	BPFJ00000000
S.indicum ST-419^T	99.2	0.4	2.2	42.5	2155	hydrothermal vent field	CP063164
Sulfurovum sp. NBC37-1	99.6	0.6	2.6	43.9	2546	deep-sea hydrothermal vent	AP009179
S.lithotrophicum ATCC BAA-797^T	98.0	1.6	2.2	44.3	2184	hydrothermal vent sediments	CP011308
S.riftiae 1812E^T	98.6	1	2.4	45.7	2343	hydrothermal polychaete	NZ_LNKT00000000

Interestingly, key genes encoding nitrogen-fixing enzymes were observed for the first time in *Sulfurovum* genomes, including *nifH, nifD*, and *nifK*, but only in the non-vent strains zt1-1 and HSL1-3. In the phylogenetic tree, we found that the *nifH* of *Sulfurovum* represents a different branch from that of *Betaproteobacteria* and *Alphaproteobacteria* (Supplementary Figure S6).

Hydrogen metabolism and the differentiation within the genus

Sulfurovum species contain three types of hydrogenases (Table 3): Type I, Type II, and Type IV. However, *S. lithotrophicum* only contains Type IV hydrogenase. Type I hydrogenases are the most widely distributed among members of the genus *Sulfurovum*. They usually have multiple copies of Type I hydrogenases, and *S. indicum* contains four copies of Type I hydrogenases (Figure 3). Type II was not conserved in *Sulfurovum* species, as in Type I, which was absent in some non-vent isolates, such as *Sulfurovum* sp. AR, TSL1, TSL6, and the vent-type *S. lithotrophicum* strains. Intriguingly, Type IV was only detected in the vent isolates, which belong to the hydrogenase Hyc type, except for *S. indicum* with a Coo type. In addition, phylogenetic analysis based on the large subunit of hydrogenase showed that hydrogenases from vent genomes and non-vent genomes of the genus *Sulfurovum* clustered together.

Differentiation in sulfur metabolism among vent and non-vent origins

For sulfide oxidation, *Sulfurovum* species contain four sulfoquinone oxidoreductase (Sqr) types, including Type II, Type III, Type IV, and Type VI (Supplementary Figure S7). Type II Sqr was present in all strains (Table 3), implying that it is essential for sulfide oxidation in *Sulfurovum* species. Notably, the vent strains contained more copies of Type II Sqr, up to four copies in strain NBC37-1 and *S. riftiae*, in contrast to the single copy in the non-vent strains. Type III Sqr was present only in the non-vent strain HSL1-3, whereas Type VI Sqr was present in all strains in a single copy. Phylogenetic analysis showed that the vent and non-vent branches of Sqr were located in different evolutionary clusters, and Sqr from different groups of *Sulfurovum* clustered together.

In addition, all *Sulfurovum* species encode the complete sox gene cluster (*soxABXYZ* and *soxCDYZ*) for thiosulfate oxidation. The phylogenetic tree based on the SoxB protein showed a topology similar to that of the phylogenomic analysis of *Sulfurovum*, suggesting that the SoxB gene in *Sulfurovum* species is acquired by vertical inheritance within the lineage (Supplementary Figure S8). For elemental sulfur respiration, all *Sulfurovum* strains have polysulfide reductase (Psr) of the three groups, which can reduce elemental sulfur to sulfide (Table 3). Phylogenetic analysis showed that polysulfide reductase Group I was detected in all *Sulfurovum* species from non-vent environments, encoded by five genes, *psrA1B1CDE*. Groups II and III were also present in some non-vent strains, such as TSL6, XGS-02, and HSL1-3 (Supplementary Figure S9).

TABLE 3 Comparison of key enzymes for nitrogen, hydrogen, and sulfur metabolisms in *Sulfurovum* species based on RAST annotations in this study.

		S.xiamenensis XGS-02	*S.xiamenensis* XTW-4	*S.zhangzhouensis* zt1-1	*Sulfurovum* sp. HSL1-3	*Candidatus Sulfurovum* sp. AR	*Sulfurovum* sp. TSL1	*Sulfurovum* sp. TSL6	*S.indicum* ST-419^T	*Sulfurovum* sp. NBC37-1	*S.lithotrophicum* ATCC BAA-797^T	*S.riftiae* 1812E^T
Nitrogen metabolism	Denitrification	-	-	-	-	-	*nirK*	-	*napA,napB,nirS, norB,norC,nosZ*	*napA,napB,nirS, norB,norC,nosZ*	*napA,napB,nirS, norB,norC,nosZ*	*napA,napB,nirS, norB,norC,nosZ*
	Dissimilatory nitrate reduction to ammonium (DNRA)	-	-	-	-	-	-	-	*napA*	*napA,nrfA*	*napA*	*napA,nrfA*
	Nitrogen fixation	-	-	*nifH,nifD,nifK*	*nifH,nifD, nifK*	-	-	-	-	-	-	-
Hydrogen metabolism	Hydrogenases Type I	3	3	3	2	2	2	2	4	2	-	2
	Hydrogenases Type II	+	+	+	+	-	-	-	+	+	-	+
	Hydrogenases Type IV	-	-	-	-	-	-	-	+	+	+	+
Sulfur metabolism	Sulfur oxidation protein (Sox)	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ	SoxABXYZ SoxCDYZ
	Sulfide:quinone oxidoreductase (Sqr)	II(1), IV(1), VI(1)	II(1), IV(1), VI(1)	II(1), IV(1),VI(1)	II(1), III(1), IV(1),VI(1)	II(1), IV(1), VI(1)	II(1), IV(1), VI(1)	II(1), VI(1)	II(1), IV(1),VI (1)	II(4), IV(1),VI (1)	II(3), IV(1),VI(1)	II(4), IV(1),VI (1)
	Polysulfide reductase (PsrA)	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$*	*psrA$_1$B$_1$CDE*	*psrA$_1$B$_1$CDE*	*psrA$_1$B$_1$CDE, psrA$_3$B$_3$*	*psrA$_1$B$_1$CDE*	*psrA$_1$B$_1$CDE*	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$*	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$, psrA$_3$B$_3$, psrA$_4$B$_4$*	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$*	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$*	*psrA$_1$B$_1$CDE, psrA$_2$B$_2$*
Oxidative stress	Superoxide dismutase	+	+	+	+	+	+	+	2	-	-	-
	Catalase	+	+	+	+	+	+	+	-	+	+	-
	Cytochrome c peroxidase	+	+	+	2	+	+	+	3	3	3	3
	Thiol peroxidase	+	+	+	+	+	+	+	+	+	+	+
Respiratory metabolism	heme-copper oxidase, caa3-type	*coxA,coxB, coxC*	*coxA,coxB, coxC*	*coxA,coxB,coxC*	-	*coxA,coxB, coxC*	*2(coxA, coxB,coxC)*	*coxA,coxB, coxC*	-	-	-	-
	heme-copper oxidase cbb3-type	*ccoN,ccoO, cooP,ccoQ*	*ccoN,ccoO, cooP,ccoQ*	*ccoN,ccoO, cooP, ccoQ*	*ccoN,ccoO, cooP,ccoQ*	*ccoN,ccoO, cooP,ccoQ*	*ccoN,ccoO, cooP,ccoQ*	*ccoN,ccoO, cooP,ccoQ*	*ccoN(2),ccoO(2), cooP,ccoQ*	*ccoN(2),ccoO(2), cooP,ccoQ*	*ccoN,ccoO, cooP, ccoQ*	*ccoN(2),ccoO(2), cooP,ccoQ*
	bd oxidases Superfamily											
	bd-type quinol oxidase	-	-	*cydA,cydB*	*cydA,cydB*	-	*cydA,cydB*	-	*cydA,cydB*	-		

"+" indicated present; "-" indicated absent.

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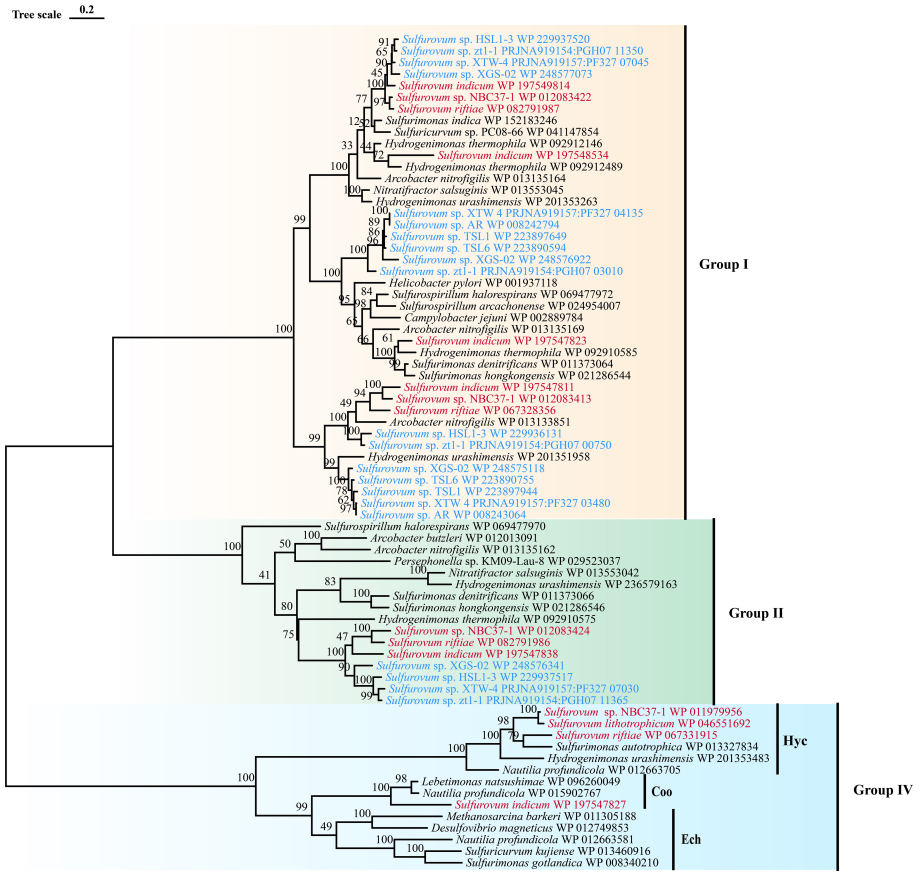

FIGURE 3
Maximum-likelihood tree based on large subunit sequences of hydrogenase from *Sulfurovum*. Bootstrap values shown on each node are based on a total of 1000 bootstrap replicates. Branch node values below 50% are not shown. The isolation source of the *Sulfurovum* species were indicated by different font colors: red, deep-sea hydrothermal vent environments; blue, marine non-vent system.

Differentiation in antioxidation and terminal oxidases

Genes encoding superoxide dismutase and catalase, which are involved in oxidative stress, were found in non-vent strains (Table 3), while superoxide dismutase was absent in vent strains NBC37-1, *S. lithotrophicum* and *S. riftiae*, and catalase was absent in *S. indicum* and *S. riftiae*. In addition, all *Sulfurovum* strains contain genes encoding cytochrome c peroxidase and thiol peroxidase, which scavenge peroxides and provide defense against oxidative damage (Missall et al., 2004). Vent *Sulfurovum* genomes usually have more copies of cytochrome c peroxidase than those from non-vent environments. Multiple terminal oxidases are present in the *Sulfurovum* genomes, all containing cbb3-type cytochrome c oxidase (Table 3). Phylogenetic analysis showed that cbb3 type cytochrome c oxidase clustered into two groups: CcoN I and CcoN II (Supplementary Figure S10). All strains containing CcoN I and CcoN II were of vent origin. The *coxA*-encoded caa3 type terminal oxidase was present in non-vent strains and absent in vent strains (Table 3). In addition, some *Sulfurovum* strains, such as zt1-1, HSL1-3, TSL1, and *S. indicum*, contained genes encoding cytochrome bd ubiquinone oxidase.

Discussion

Sulfurovum spp. in the *Campylobacteria* class are important chemolithoautotrophic bacteria with wide distribution in sulfidic habitats in the ocean, and they play an essential role in marine carbon, nitrogen, and sulfur biogeochemical cycling (Patwardhan et al., 2018; Sun Q. L. et al., 2020; Huang L. B. et al., 2021; Wang Y. et al., 2022). However, knowledge about this important bacterial taxon has been limited to deep-sea hydrothermal vent environments, and little is known about its diversity and ecological role in non-vent environments. In the present study, we isolated these species from coastal sediments. Further comparative analyses revealed the presence of unique metabolic features. Evident diversification was confirmed by phylogenetic analysis of vent and non-vent origins.

Two novel species of non-vent origin of the genus

To our knowledge, this is the first report of a novel species of non-vent origin in *Sulfurovum*. The combined phenotypic, chemotaxonomic, and phylogenetic characteristics demonstrated

that strains XTW-4 and zt1-1 represent two new species in the genus *Sulfurovum*, for which the names *Sulfurovum xiamenensis* sp. nov. and *Sulfurovum zhangzhouensis* sp. nov. are proposed, both of which showed the closest 16S rRNA gene sequence similarity to *S. aggregans* Monchim33^T (95.8% and 95.4%, respectively). They differed from other type strains in phenotypical features, such as optimum salinity and oxygen concentrations and the utilization of electron acceptors. Intriguingly, the two coastal species both respired for thiosulfate, while the vent strains could not; however, while the vent strains could respire nitrate, the two coastal strains could not (Table 1).

Environmental adaptations of Sulfurovum revealed by comparative genomic analyses

To understand their environmental adaptation, we performed comparative genomic analyses between the vent and non-vent *Sulfurovum* strains. Significant differences were observed in key metabolic mechanisms and stress responses between the vent and non-vent strains, implying that their adaptive evolution and species diversification were related to inhabitance.

Correspondingly, we found that all members of the genus *Sulfurovum* from deep-sea hydrothermal vents contained a complete denitrification pathway in addition to some genes involved in dissimilatory nitrate reduction to ammonia (DNRA) in part vent strains. Denitrification and DNRA processes are currently considered to be two competing pathways for nitrate reduction (Bu et al., 2017; Jia et al., 2020; Pandey et al., 2020; Li S. et al., 2022), and few studies have reported their coexistence except for *Pseudomonas* and *Shewanella* (Kuypers et al., 2018; Huang X. J. et al., 2021; Liu et al., 2021). The nitrate reduction pathway (NAP) is considered conserved and widespread in the phylum *Campylobacterota* based on bacteria from deep-sea hydrothermal vent habitats (Vetriani et al., 2014). However, these two processes were absent in our coastal strains. Sulfur oxidation coupled with nitrate reduction is considered the primary source of energy in vent ecosystems (Wang et al., 2009), whereas this process does not occur in coastal *Sulfurovum* species. The genome revealed that the denitrification and DNRA pathways were completely absent, and experimental validation showed that they could not use nitrate as an electron acceptor. To compensate, our two *Sulfurovum* species adapted to higher oxygen concentrations and preferred to use oxygen as an electron acceptor, which is in agreement with the loss of denitrification and DNRA-related genes, as well as other non-vent *Sulfurovum* strains (unpublished data).

Intriguingly, members of the genus *Sulfurovum* from coastal sediments possess genes for nitrogen fixation, which are absent in deep-sea vent *Sulfurovum*. Coastal mangrove sediments are recognized to be nitrogen-limited due to the unbalance of carbon and nitrogen caused by burying of carbon-rich plant litter (Lin et al., 2019; Luo et al., 2021). Nitrogen-fixing bacteria are considered to play a key role in nitrogen amendment (Alfaro-Espinoza and Ullrich, 2015; Tang et al., 2020; Luo et al., 2021). Therefore, *Sulfurovum* containing nitrogenase genes are of competitive advantage in coastal environments. Recently, the expression of Nif genes associated with *Sulfurovum* spp., as well as the presence of the *nifH* gene in a *Sulfurovum* metagenome-assembled genome (MAG) was demonstrated at a coastal gas vent site (Patwardhan et al., 2021). Certainly, not all coastal *Sulfurovum* have nitrogenase genes. This is probably due to highly variable environmental parameters in coastal sediments. In contrast, ammonia is relatively abundant in the vent fluid, which can reach micromolar level *in situ* (Xu et al., 2014), and can be oxidized to nitrate by ammonia-oxidizing archaea (Crepeau et al., 2011; Zhang et al., 2016; Ding et al., 2017). Thus, genes for nitrogen fixation in deep-sea vent may be redundant and lost in all vent *Sulfurovum*. The differentiation of *Sulfurovum* in accordance with unique habitats expands our understanding of the genus in terms of environmental adaptation (Figure 4).

Reduced sulfur compounds, such as sulfides, are important energy sources for chemolithoautotrophs in deep-sea vents. Unexpectedly, we found that coastal isolates of *Sulfurovum* grew best with hydrogen as an electron donor and weakly with thiosulfate and sulfide. Moreover, all members of this genus contain an extensive suite of hydrogenases, including [NiFe]-hydrogenase groups 1b, 2d, and 4 (Hyc and Coo). The most frequently occurring hydrogenases belonged to Group I. Almost all *Sulfurovum* members contained multiple copies of this group, probably in response to different hydrogen concentrations, suggesting that hydrogenase in Group I plays an important role in energy draining to support bacterial growth (Grote et al., 2012; Berney et al., 2014).

Regarding sulfide oxidation, *Sulfurovum* species contain genes encoding different types of Sqr, classified as types II, III, IV, and VI. The diversification of these Sqr genes is thought to play important roles in sulfide oxidation, sulfide assimilation, energy production, heavy metal tolerance, detoxification, and sulfide signaling (Marcia et al., 2010). Additionally, *Sulfurovum* bacteria have one or more copies of Type II Sqr (Table 3), which may compensate for other Sqrs under specific environmental conditions (Wang et al., 2021). Thiosulfate (Figure 4) can commonly be oxidized by *Sulfurovum* of any origin, but as an electron acceptor, it can be used by non-vent *Sulfurovum*, as confirmed by our coastal pure cultures (Table 1).

Sulfurovum usually grows in a mixed zone with unsaturated oxygen concentration (Meier et al., 2017). Antioxidation systems may be essential for traveling across redox gradients to oxic areas. Reactive oxygen species (ROS) are formed by the intermediate products of oxygen reduction and can cause damage to cellular macromolecules (Cabiscol et al., 2000). Superoxide dismutase and catalase are the most well-characterized ROS defense mechanisms (Johnson and Hug, 2019). Interestingly, we found that the genes encoding both superoxide dismutase and catalase were present in the non-vent strains but were partially or completely absent in the vent strains (Table 3). In addition, some terminal oxidases function in the defense against ROS (Kaminski et al., 1996; Hassani et al., 2010; Borisov and Siletsky, 2019), allowing certain strictly anaerobic bacteria to grow in microaerobic environments, such as cbb3-type hemocopper oxidase and cytochrome bd ubiquinone oxidase. All pure culture strains of *Sulfurovum* contain the CcoNOQP operon, which encodes a cbb3-type cytochrome c oxidase that supports bacterial growth under anaerobic and microaerobic conditions

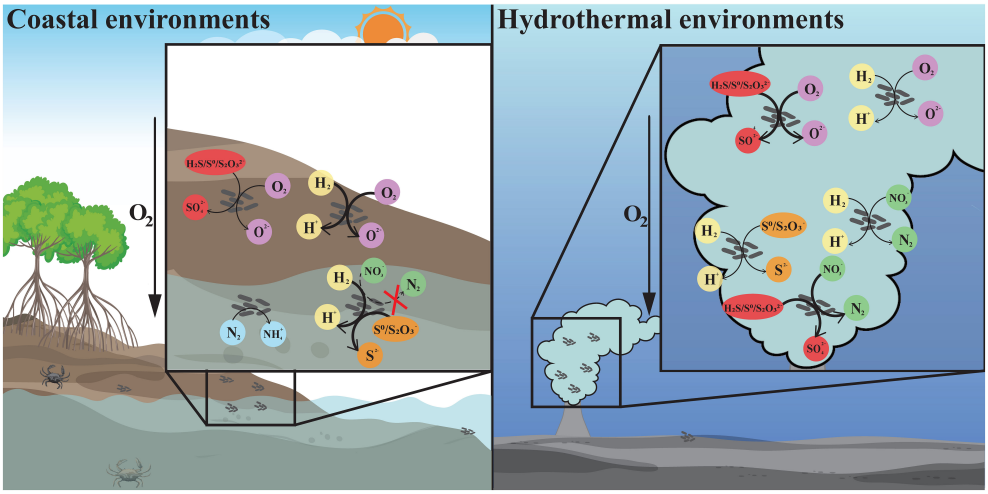

FIGURE 4
The differentiation and adaptation of *Sulfurovum* bacteria in coastal sediment and deep-sea hydrothermal vent environments. Metabolic features of *Sulfurovum* with different origins, including hydrogen oxidation (yellow), sulfur oxidation (red), oxygen reduction (purple), sulfur reduction (orange), denitrification (green), and nitrogen fixation (blue). The arrows with different styles indicate the presence or absence of the related metabolic pathway, and whether it is the major energy-acquiring pathway. Thick solid line: the process is present and probably the main process; thin solid line: the relevant gene is present; thin dashed line: the relevant gene is absent. Left: coastal environments represented by a mangrove ecosystem; right: deep-sea hydrothermal vent environment.

owing to its high affinity for oxygen. Some strains also encode cytochrome bd ubiquinone oxidase, which is an alternative oxidase found in many bacteria that oxidizes ubiquinone and reduces oxygen as part of the electron transport chain. In addition, unlike vent strains, non-vent strains have a caa3-type hemocopper oxidase that catalyzes the reduction of oxygen to water and may play an important role in oxygen adaptation. Hence, it is possible that the ROS defense mechanisms enable coastal *Sulfurovum* bacteria to deal with oxidative stress caused by increased O_2 concentrations in coastal environments. This is consistent with their wider growth ranges with regard to oxygen concentrations (1–15%) and higher optimum oxygen concentration compared the vent *Sulfurovum* bacteria (Table 1).

Conclusions

Two novel *Sulfurovum* species were characterized from coastal marine habitats for the first time, *Sulfurovum xiamenensis* and *Sulfurovum zhangzhouensis*, contributing to the understanding of the ecological role of *Sulfurovum* in marine environments. As summarized in Figure 4, the coastal isolates significantly differed from the vent species of this genus in energy metabolism and environmental adaptation. Non-vent *Sulfurovum* bacteria cannot respire nitrate because of the lack of nitrate reduction pathways but can reduce thiosulfate coupling hydrogen oxidation in addition to high oxygen concentrations. Moreover, non-vent members can perform nitrogen fixation, which might facilitate their survival in niches with limited N sources. These results reveal the diversification among members inhabiting deep-sea vent and non-vent ecosystems and highlight the unique roles of *Sulfurovum* in coastal marine environments.

Description of *Sulfurovum xiamenensis* sp. nov.

Sulfurovum xiamenensis (xia.men.en'sis. N.L. masc. adj. *xiamenensis* of Xiamen, a city in Fujian, China, where the type strain was isolated).

Cells are Gram-negative short rods (0.4-1.2 × 0.2-0.4 μm) without flagella. Optimum oxygen concentration 8%-10%. Growth occurs at 15-45°C (optimum 32°C), pH 5.0-8.5 (optimum pH 6.0-6.5), and 0.5-5.0% (w/v) NaCl (optimum 2.5%). Obligate chemolithoautotrophic growth occurs with H_2 as electron donor, and oxygen, thiosulfate, and S^0 can be utilized as electron acceptors. It also growth occurs with thiosulfate as an electron donor and oxygen as an electron acceptor. Organic substrates are not utilized as carbon sources and energy sources. Major cellular fatty acids are $C_{18:1}\omega7c$, $C_{16:1}\omega7c$ and $C_{16:0}$.

The type strain, XTW-4^T (=MCCC 1A19406), was isolated from mangrove sediments in, Xiamen, Fujian Province, PR China. The genomic G+C content of the type strain is 39.1 mol%.

Description of *Sulfurovum zhangzhouensis* sp. nov.

Sulfurovum zhangzhouensis (zhang.zhou.en'sis. N.L. masc. adj. *zhangzhouensis* of Zhangzhou, a city in Fujian, China, where the type strain was isolated).

Cells are Gram-negative short rods (0.4-1.2 × 0.2-0.5 μm) without flagella. Optimum oxygen concentration 15%. Growth occurs at 10-45°C (optimum 32°C), pH 4.5-8.5 (optimum pH 6.0-6.5), and 0.5-7.0% (w/v) NaCl (optimum 1.5%). Obligate chemolithoautotrophic growth occurs with H_2 as electron donor, and oxygen, thiosulfate, and S^0 can be utilized as electron acceptors. It also growth occurs with thiosulfate

as an electron donor and oxygen as an electron acceptor. Organic substrates are not utilized as carbon sources and energy sources. Major cellular fatty acids are $C_{16:1}\omega7c$, $C_{18:1}\omega7c$ and $C_{16:0}$.

The type strain, zt1-1^T (=MCCC 1A19490), was isolated from the sediment of retirement ponds in Yunxiao County, Zhangzhou City, Fujian Province, PR China. The genomic G+C content of the type strain is 39.4 mol%.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

ZS and LJ conceived the study. JW, LJ, QZ, and ZS designed the experiments. JW, YZ, and QY participated in the sample collection. JW performed the experiments and analyzed the data. JW and JZ drafted the manuscript. SW, QZ, LJ, and ZS revised the manuscript, which was finally read and corrected by ZS. All authors read and approved the final manuscript. All authors contributed to the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1222526/full#supplementary-material

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