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Exploring environmental adaptation mechanisms of symbiotic microorganisms in marine reducing ecosystems: harnessing genomic comparison to unveil the underlying mechanisms

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Various invertebrates, with microorganisms as their symbionts, inhabit diverse and
dynamically changing environments such as hydrothermal vents (HVs) and cold
seeps (CSs). The ongoing \dispersal of these symbionts is crucial for their
biogeographic distribution and connectivity, which in turn facilitates the
persistence of mutualistic relationships. To gain insights into the mechanisms
underlying the adaptation of symbionts in response to environmental changes,
this perspective analyzed two genes related to sulfur metabolism in the symbionts,
based on their genome annotations. Our findings revealed that the gene encoding
cysteine desulfurase (CSD) is ubiquitous among these symbionts, regardless of their
geographic locations, hosts, or genome sizes. This suggests that these symbionts
possess the ability to utilize sulfur from cysteine. Similarly, genes encoding sulfate
adenylyltransferase (SAT), which is essential for sulfate assimilation, are also widely
present in the genomes of the symbionts, with notable exceptions being some
isolates from sponges. Notably, most of the investigated symbionts possess both *sat*
and *csd* genes, hinting at their capability to utilize both organic and inorganic sulfur
resources. The presence of both *sat* and *csd* genes may confer an advantage to the
symbionts while cessation of hydrothermal and cold seep activity or during their
dispersal among isolated locales. Further comparative genomic studies, particularly
those focusing on the versatile adaptation strategies of symbionts across different life
stages, can enhance our understanding of their ecological fitness and broaden our
knowledge about how these symbiotic microorganisms successfully dwell in the
dynamic marine environments.

Introduction

Globally, the emissions of fluids and gases from the seafloor, such as those from widespread hydrothermal vents (HVs) and cold seeps (CSs), serve as resources to chemoautotrophic microorganisms. These microorganisms harness energy from oxidization of reducing chemicals (e.g., H_2S, S^0, H_2, CH_4) to fix inorganic carbon into biomass (Dick, 2019). A variety of invertebrate species host chemoautotrophic microorganisms as symbionts, often within gills or trophosomes, forming close nutritional relationship with them (Dubilier et al., 2008). Since the discovery of chemosynthetic symbioses between bacteria and invertebrates at marine hydrothermal vents on the Galapagos Rift, it has been realized that chemosynthetic symbioses occur worldwide in a diverse range of habitats. These include cold seeps, whale and wood falls, and shallow-water coastal sediments (Dubilier et al., 2008). To gain a deeper understanding of these featured mutualistic associations, numerous genomes of marine chemoautotrophic symbiotic microorganisms have been sequenced. The genomic data reveal the genetic blueprint of their metabolic capabilities, and the symbionts' metabolic roles within the symbiosis and their adaptations to intracellular conditions have been studied (Kuwahara et al., 2007; Newton et al., 2007). For instance, in the genome of the symbiont Candidatus *Vesicomyosocius okutanii*, which is approximately 1.0 million base pairs (Mbps) in size (Table 1), genes that are unnecessary for an intracellular life stage, as well as some essential genes, appear to have been lost (Kuwahara et al., 2007). Another example is the genome of an endosymbiont associated with armored snails (approximately 2.6 Mbps in size, Table 1). Compared to three other free-living relatives, this genome is smaller and displays features that are consistent with ongoing genome reduction (Nakagawa et al., 2014). Moreover, a recent genomic analysis of the ectosymbiont of *Catillopecten margaritatus* revealed that the symbiont genome is significantly smaller than its free-living relatives and has lost cellular components required for free-living (Lin et al., 2024). The accumulating genome sequences of symbionts provide an opportunity for studying the mechanisms of the adaptation by genomic comparisons. Recently, extensive genomic analyses have unveiled a widespread conservation of metabolic pathways for sulfur oxidation across sulfur-oxidizing symbionts derived from diverse host species and habitats (Sudo et al., 2024). It is hypothesized that the expansion and diversification of the SoxY gene family, which encodes a pivotal sulfur anion carrier protein integral to the sulfur-oxidizing multi-enzyme complex, may enhance the metabolic versatility of sulfur-oxidizing symbionts (Sudo et al., 2024).

Environmental adaptations

Numerous genome-based studies have explored the partnerships between the hosts and symbionts and their adaptations to the reducing marine environments. However, there are few analyses conducted regarding their adaptation to the chemical changes while cessation of hydrothermal and cold seep activity or dispersal. Since symbionts play critical roles in supporting their hosts, the transmission of these symbionts between generations of the hosts is of paramount importance. Previous studies have provided evidences for the horizontal transmissions of microbial symbionts and its importance in maintaining mutualisms (Cary et al., 1993; Kádár et al., 2005; Nussbaumer et al., 2006; Wentrup et al., 2014; Breusing et al., 2022; Hauer et al., 2023).

Building upon observations of faunal overlap across diverse chemosynthetic communities, Smith et al. postulated that whale carcasses might function as stepping stones for fauna dispersal, potentially facilitating the colonization of new habitats situated hundreds of kilometers apart, such as hydrothermal vents (Smith et al., 1989; Sumida et al., 2016). As illustrated in Figure 1, the dispersal of marine fauna serves as a crucial link in the chain of symbiont transmission. The discontinuous distribution of hydrothermal vents and cold seeps suggest they themselves can serve as 'stepping stones' for faunal dispersion. Considering that sporadic volcanic and tectonic events destroy existing vent fields and create new ones, HVs are dynamically changing habitats accompanied by extirpation (local extinction) and novel colonization of species (Vrijenhoek, 2010). The organic falls, such as whale falls and sunken wood, are also isolated habitats on the seafloor that undergo dynamic changes. Therefore, ongoing dispersal of symbionts, whether in host-associated or free-living lifestyle, may facilitate persistence of mutualistic relationship and enable the spread and colonize a wide range of habitable sites (Figure 1). However, the symbiotic microorganisms from reducing marine environments (e.g. HVs and CSs), may have to encounter situations with significant chemical changes, such as cessation of hydrothermal and cold seep activity. Moreover, during dispersal in seawater, leaving HVs and CSs, the symbionts face crucial chemical changes, such as the form of sulfur changing from reducing sulfur compounds to sulfate. The intriguing question that remains is how these symbionts adapt to these environmental changes. Given that sulfur is a vital element for cells (Zhou et al., 2024), to adapt to the changes, symbionts may modulate their intracellular sulfur sources, e.g. cysteine (an organic sulfur source), or sulfate from the environment. Therefore, it is highly meaningful to study the plasticity of sulfur metabolism. The genes *csd* (encoding cysteine desulfurase) and *sat* (encoding sulfate adenylyltransferase) are involved in the initiation of pathways utilizing cysteine and sulfate as substrates, respectively. From this perspective, the presence of these two genes is examined in the sequenced genomes of symbionts (Table 1) to study their potential for sulfur metabolic plasticity. This perspective aims to promote further research into the adaptive mechanisms employed by symbionts.

Presence of genes encoding cysteine desulfurase in symbiont genomes

The sulfur-containing amino acid cysteine, regardless of whether derived from the hosts or the symbionts themselves, serves as a sulfur

TABLE 1 Presence of *csd* and *sat* gene in symbiont genomes.

BioProject in GenBank	Symbiont	Strain/ Isolate	Genome size (Mbp)	*csd* gene (GenBank Locus tag)	*sat* gene (GenBank Locus tag)	Habitat	Depth (m)	Host	Reference
PRJNA454446	endosymbiont of *Escarpia* spicata	A1462	4.1	DIZ78_04490	DIZ78_08635	Seep locality in the Mississippi Canyon	754	Annelida: *Escarpia* spicata	(Li et al., 2018)
PRJNA283904	endosymbiont of *Ridgeia piscesae*	ind11	3.4	Ga0074115_12725; Ga0074115_10756	Ga0074115_106100	Hulk hydrothermal vent in the Main Endeavour Field	2190	Annelida: *Ridgeia piscesae*	(Perez and Juniper, 2016)
PRJNA532304	Gammaproteobacteria bacterium	ARCO_sym	4.3	FE835_14165	FE835_03060	hydrothermal vent at Manus Basin	1693	Annelida: *Arcovestia* sp.	(Li et al., 2020)
PRJNA454446	endosymbiont of *Galathealinum brachiosum*	A1464	3.8	DIZ80_02640; DIZ80_16555; DIZ80_16560	DIZ80_03400	Seep locality in the Mississippi Canyon	754	Annelida: *Galathealinum brachiosum*	(Li et al., 2018)
PRJNA454446	endosymbiont of *Lamellibrachia luymesi*	A1422	3.5	DIZ79_04040	DIZ79_01875	Seep locality in the Mississippi Canyon	754	Annelida: *Lamellibrachia luymesi*	(Li et al., 2018)
PRJNA191058	Osedax symbiont	Rs1	4.5	OFPII_23700; OFPII_03360	OFPII_31760	whale fall	1891	Annelida: *Osedax frankpressi*	(Goffredi et al., 2014)
PRJNA191057	Osedax symbiont	Rs2	4.6	OFPI_24090	OFPI_00520; OFPI_00530	whale fall	1891	Annelida: *Osedax frankpressi*	(Goffredi et al., 2014)
PRJNA762254	Candidatus *Endoriftia persephone*	Tica-EPR-9o50.N	3.6	L0Y14_RS04430	L0Y14_RS06615	Tica hydrothermal vent	2514	Annelida: *Riftia pachyptila*	(De Oliveira et al., 2022)
PRJNA454446	endosymbiont of *Seepiophila jonesi*	A1423	3.5	DIZ77_01020	DIZ77_15380	Seep locality in the Mississippi Canyon	754	Annelida: *Seepiophila jonesi*	(Li et al., 2018)
PRJNA291958	*Endozoicomonas ascidiicola*	AVMART05	6.1	AKL11_RS02805; AKL11_RS07840; AKL11_RS12280	AKL11_RS01380; AKL11_RS01385	Gullmarsfjorden	22-30	Chordata: *Ascidiella* sp.	(Schreiber et al., 2016)
PRJNA422318	*Endozoicomonas acroporae*	Acr-14	6.0	CX664_RS08195; CX664_RS09875; CX664_RS22715	CX664_RS02065; CX664_RS02070	off the coast	No data	Cnidaria: *Acropora* Coral	(Tandon et al., 2018)
PRJNA252578	*Endozoicomonas montiporae*	LMG 24815	5.6	GZ77_RS03910; GZ77_RS10330	GZ77_RS07005; GZ77_RS07010; GZ77_RS19045; GZ77_RS19050	Coastal water	10–15	Cnidaria: *Montipora aequituberculata*	(Neave et al., 2014)
PRJNA641445	Candidatus *Ruthia* sp.	Apha_13_S6	1.5	Rpha_0131	Rpha_2134	hydrocarbon seep	3550	Mollusca: *Abyssogena phaseoliformis*	(Perez et al., 2022)

(Continued)

TABLE 1 Continued

BioProject in GenBank	Symbiont	Strain/ Isolate	Genome size (Mbp)	*csd* gene (GenBank Locus tag)	*sat* gene (GenBank Locus tag)	Habitat	Depth (m)	Host	Reference
PRJNA641445	Candidatus *Ruthia* sp.	Asou_11_S2	1.6	Rsou_0801	Rsou_1665	hydrothermal vents	3038	Mollusca: *Abyssogena southwardae*	(Perez et al., 2022)
PRJNA532304	Campylobacterota bacterium	ALBOsym3	1.4	FAF05_06770	FAF05_02150	hydrothermal vent at Manus Basin	1693	Mollusca: *Alviniconcha boucheti*	(Li et al., 2020)
PRJNA532304	Campylobacterota bacterium	ALBOsym2	1.5	FAF04_04075	FAF04_05255	hydrothermal vent at Manus Basin	1693	Mollusca: *Alviniconcha boucheti*	(Li et al., 2020)
PRJNA532304	Campylobacterota bacterium	ALBOsym1	2.3	FAF03_01205	FAF03_08645	hydrothermal vent at Manus Basin	1693	Mollusca: *Alviniconcha boucheti*	(Li et al., 2020)
PRJNA532304	Gammaproteobacteria bacterium	BAMA_sym	2.5	FE834_09310	FE834_03630	hydrothermal vent at Manus Basin	1693	Mollusca: *Bathymodiolus manusensis*	(Li et al., 2020)
PRJNA339702	*Bathymodiolus thermophilus* thioautotrophic gill symbiont	BAT/ CrabSpa'14	3.1	BGC33_RS00940	BGC33_RS13095	Crab Spa hydrothermal vent on East Pacific Rise	2500	Mollusca: *Bathymodiolus thermophilus*	(Ponnudurai et al., 2017).
PRJNA16841	Candidatus *Ruthia magnifica*	Cm	1.2	RMAG_RS02885	RMAG_RS00465	the East PacificRise	No data	Mollusca: *Calyptogena magnifica*	(Newton et al., 2007)
PRJDA18267	Candidatus *Vesicomyosocius okutanii*	HA	1.0	COSY_RS02655	COSY_RS00440	Sagami Bay	No data	Mollusca: *Calyptogena okutanii*	(Kuwahara et al., 2007)
PRJNA1029732	*Catillopecten margaritatus* gill symbiont	Gill1	1.5	Ctma_0056	Ctma_0847	Cold seep	1433–1441	Mollusca: *Catillopecten margaritatus*	(Lin et al., 2024)
PRJNA252578	*Endozoicomonas elysicola*	DSM 22380	5.6	GV64_RS12330; GV64_RS21190	GV64_RS20855; GV64_RS20860	Coastal water	15	Mollusca: *Elysia ornata*	(Neave et al., 2014)
PRJDB5337	methanotrophic gill symbiont	HPD1508-B01-01	4.0	CDZ78_RS00435; CDZ78_RS08675; CDZ78_RS15845; CDZ78_RS18105	CDZ78_RS05865; CDZ78_RS05870	cold seep in Sagami Bay	857	Mollusca: *Gigantidas platifrons*	(Takishita et al., 2017)
PRJNA612619	endosymbiont of *Gigantopelta aegis*	Gae_MOB	2.9	JEU20_RS05385; JEU20_RS11140	JEU20_RS00630; JEU20_RS00625	hydrothermal vent field	2761	Mollusca: *Gigantopelta aegis*	(Lan et al., 2021)

TABLE 1 Continued

BioProject in GenBank	Symbiont	Strain/ Isolate	Genome size (Mbp)	csd gene (GenBank Locus tag)	sat gene (GenBank Locus tag)	Habitat	Depth (m)	Host	Reference
PRJNA612619	endosymbiont of *Gigantopelta aegis*	Gae_SOB	4.9	JEU79_RS18530	JEU79_RS00950; JEU79_RS14300	hydrothermal vent field	2761	Mollusca: *Gigantopelta aegis*	(Lan et al., 2021)
PRJNA376164	*Thiosocius teredinicola*	DSM 108030	4.8	B1781_RS07495; B1781_RS08665	B1781_RS08635; B1781_RS09960	Coastal Water	No data	Mollusca: *Kuphus polythalamius*	(Altamia et al., 2019)
PRJNA258543	*Solemya velum* gill symbiont	WH	2.7	JV46_RS07600	JV46_RS00240	sediment of shallow eelgrass beds	No data	Mollusca: *Solemya velum*	(Dmytrenko et al., 2014)
PRJNA30839	*Teredinibacter turnerae*	T7901	5.2	TERTU_RS07040; TERTU_RS11675; TERTU_RS11695	TERTU_RS06135; TERTU_RS06140; TERTU_RS19175; TERTU_RS19180	Estuary	No data	Mollusca: Teredinidae (shipworms)	(Yang et al., 2009)
PRJDB691	endosymbiont of scaly-foot gastropod	Monju	2.6	EBS_RS04995	EBS_RS05635	Kairei Hydrothermal Field	2420	Mollusca: unidentified scaly snail	(Nakagawa et al., 2014)
PRJEB19464	*Parendozoicomonas haliclonae*	S-B4-1U	5.5	EHSB41UT_RS01135; EHSB41UT_RS02135	EHSB41UT_RS03455; EHSB41UT_RS03460	No data	No data	Porifera: a sponge of *Haliclona*	(Bartz et al., 2018)
PRJNA274222	Candidatus *Synechococcus spongiarum*	15L	2.2	TQ37_06065; TQ37_06075	No data	Mediterranean Sea	No data	Porifera: *Aplysina aerophoba*	(Burgsdorf et al., 2015)
PRJNA279233	*Endozoicomonas arenosclerae*	ab112	6.5	WG89_RS03880; WG89_RS20035; WG89_RS27875	WG89_RS00030; WG89_RS00035; WG89_RS07425; WG89_RS07430	Coastal water	10	Porifera: *Arenosclera brasiliensis*	(Rua et al., 2014)
PRJNA746413	*Mycetocola spongiae*	MSC19	3.2	KXZ72_RS00450; KXZ72_RS09020; KXZ72_RS13920	No data	Junction of the Mariana Trench and the Yap Trench	2681	Porifera: *Cacospongia mycofijiensis*	(Chen et al., 2022)
PRJNA273429	Candidatus *Synechococcus spongiarum*	142	2.3	TH68_03095; TH68_05300; TH68_05315	No data	Mediterranean Sea	No data	Porifera: *Ircinia variabilis*	(Burgsdorf et al., 2015)
PRJNA19345	*Pseudovibrio* sp.	JE062	5.7	PJE062_RS03300; PJE062_RS03320	PJE062_RS02640; PJE062_RS02645	sandy patch reef	22	Porifera: *Mycale laxissima*	(Enticknap et al., 2006)
PRJNA238381	Candidatus *Synechococcus spongiarum*	SH4	1.7	BG35_RS0101305; BG35_RS0108680	BG35_RS0106780	Red Sea	No data	Porifera: *Phyllospongia foliascens*	(Gao et al., 2014)
PRJNA318572	*Cycloclasticus* sp. symbiont of *Poecilosclerida* sp. N	specimen N	1.9	A6F72_06230	A6F72_05040; A6F72_05045	Bahia de Campeche	No data	Porifera: *Poecilosclerida* sp.	(Rubin-Blum et al., 2017)

(Continued)

TABLE 1 Continued

BioProject in GenBank	Symbiont	Strain/ Isolate	Genome size (Mbp)	csd gene (GenBank Locus tag)	sat gene (GenBank Locus tag)	Habitat	Depth (m)	Host	Reference
PRJNA318573	*Cycloclasticus* sp. symbiont of *Poecilosclerida* sp. M	specimen M	2.3	A6F71_06660	A6F71_04090; A6F71_04095	Bahia de Campeche	No data	Porifera: *Poecilosclerida* sp.	(Rubin-Blum et al., 2017)
PRJNA252578	*Endozoicomonas numazuensis*	DSM 25634	6.3	GZ78_RS05155; GZ78_RS08595; GZ78_RS25915	GZ78_RS02825; GZ78_RS02830	Coastal water	No data	Porifera: Sponge, cf. *Haliclona* spp.	(Neave et al., 2014)

source for symbiotic microorganisms. Cysteine desulfurase (CSD) catalyzes the conversion of L-cysteine to L-alanine and sulfane sulfur via the formation of a protein-bound cysteine persulfide intermediate on a conserved cysteine residue (Hidese et al., 2011). The persulfide sulfur atoms could be utilized in various biosynthetic pathways to produce sulfur-containing biofactors, such as iron–sulfur clusters, molybdopterin, transfer RNA thionucleosides, biotin, thiamin, and lipoic acid. The biofactors play pivotal roles in numerous essential and diverse cellular processes, including DNA repair, respiration, intermediary metabolism, gene regulation, and redox sensing (Das et al., 2021). Given the essential function of CSDs in the biosynthesis of these sulfur-containing biofactors, we investigated the presence of the gene encoding CSD in the genomes of symbiotic microorganisms.

Despite thriving in markedly different geochemical conditions, all investigated symbiotic microorganisms exhibited the presence of the *csd* gene in their genome annotations (Gene Locus tag shown in Table 1). The investigation covered symbionts from a variety of habitat types, including hydrothermal vents, cold seeps, whale remains, sunken wood, and others, as listed in Table 1. The habitats are distributed across a wide range of geographic locations and depths, spanning from shallow to deep sea environments (Table 1). The seep located at Aleutian Trench in Pacific Ocean, reaching a depth of 3550, is inhibited by Candidatus *Ruthia* sp. Apha_13_S6 and its host *Abyssogena phaseoliformis* (Perez et al., 2022). As shown in Table 1, the genomes of two dominant endosymbionts Rs1 and Rs2 living in deep-sea worm *Osedax frankpressi*, collected from a whale fall, contained genes belonging to the CSD family. Additionally, the chemolithoautotrophic sulfur-oxidizing endosymbiotic bacterium strain *Thiosocius teredinicola* DSM 108030[T], isolated from the giant shipworm *Kuphus polythalamius* in sunken wood (Distel et al., 2017), also possesses the *csd* gene (Table 1).

The *csd* genes were present in microorganisms belonging to distinct clades. For example, the mussel *B. manusensis* and tubeworm *Arcovestia ivanovi*, both sampled from PACManus hydrothermal area, are colonized by Gammaproteobacteria from different clades, specifically isolate BAMA_sym and ARCO_sym, respectively (Li et al., 2020). The *csd* genes were detected in the genome annotations of both isolates (see Table 1). Additionally, the *csd* genes were also present in genome annotations of all three Epsilonproteobacteria symbionts harbored in vent-mouth-dwelling snail *Alviniconcha boucheti*, suggesting that CSD is crucial to their survival. Furthermore, these *csd* genes are not limited to sulfur-oxidizing endosymbionts but are also found in methane-oxidizing endosymbiont. For instance, the genes were detected in both the genome of sulfur-oxidizing endosymbiont isolate Gae_SOB and methane-oxidizing endosymbiont isolate Gae_MOB in the deep-sea snail *Gigantopelta aegis* (Table 1). Notably, regardless of how small the genome is, this gene is always present (Table 1). Larger genomes frequently harbor multiple *csd* genes, with many genomes possessing three, and in the case of the symbiont isolate HPD1508-B01-01, the genome even contains up to four (Table 1).

As shown in the Table 1, the gene encoding CSD is present in the annotations of all symbiont genomes, regardless of whether the host belongs to Annelida, Mollusca, Porifera, Cnidaria, or
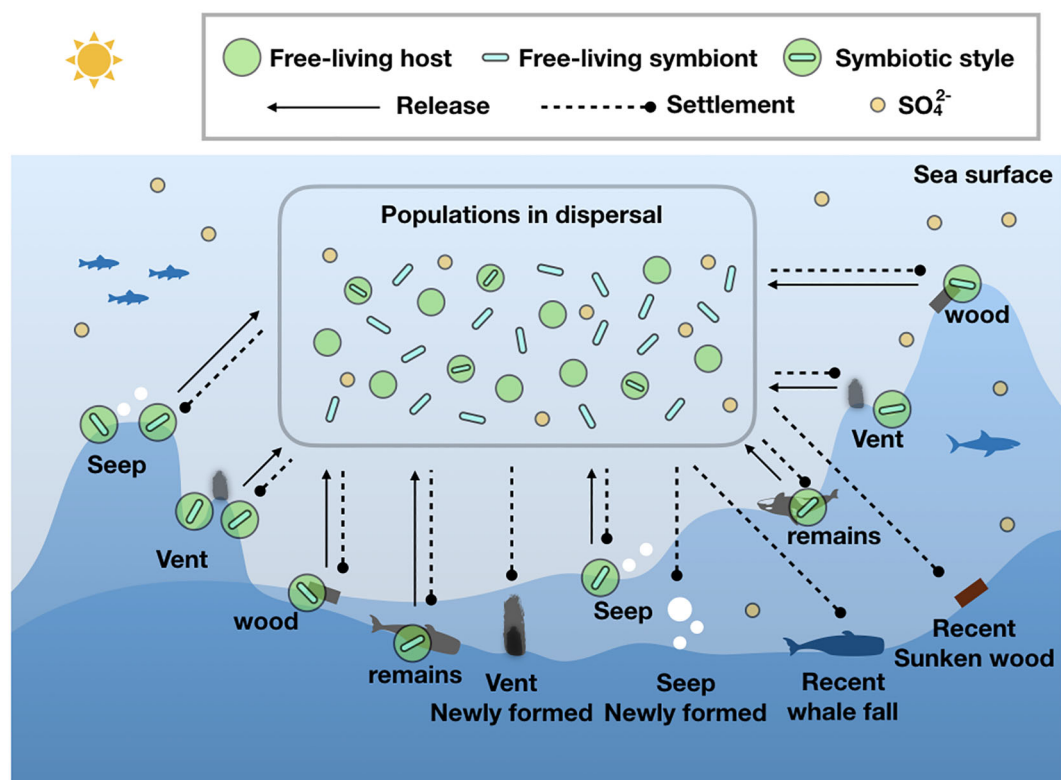
FIGURE 1
Schematic diagram of dispersal and settlement of symbionts and hosts.

Chordata. In summary, all symbiotic genomes investigated in this perspective possess genes annotated as encoding CSD, highlighting the significance of cysteine and CSD to the symbionts.

Presence of genes encoding sulfate adenylyltransferase in symbiont genomes

In addition to organic sulfur sources, sulfate is abundant in modern ocean, with concentrations reaching approximately 28 mM, making it the second most prevalent anion in seawater (Fakhraee et al., 2024). Consequently, seawater serves as a potential sulfur resource for cells. While assimilatory sulfate reduction is increasingly being documented in marine microorganisms (e.g., *Methanothermococcus thermolithotrophicus* DSM 2095 and *Phototrophicus methaneseepsis* ZRK33) (Jespersen and Wagner, 2023; Zheng et al., 2024), the potential for symbionts to assimilate sulfate as a sulfur source is also worth further investigation. Bacterial sulfate assimilation pathways involve the activation of inorganic sulfur through intermediates such as adenosine 5′-phosphosulfate (APS) or 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (Williams et al., 2002). PAPS has been recognized as a universal sulfuryl donor in cells, such as the substrate for producing sulfolipids. The biosynthetic pathway of PAPS in bacteria initiates with the formation of adenosine 5′-phosphosulfate from ATP and inorganic

sulfate, a reaction catalyzed by sulfate adenylyltransferase (SAT) (Duffel, 2023). Therefore, we also investigated the presence of the *sat* gene encoding SAT in the genomes of symbiotic microorganisms.

Similarly, all investigated symbiotic microorganisms exhibit the presence of genes encoding SAT in their genome annotations, except for some isolates from sponges, such as *Mycetocola spongiae* MSC19[T] and Candidatus *Synechococcus spongiarum* isolates 15L and 142 (Table 1). *M. spongiae* MSC19[T], belonging to the Actinobacteria phylum, was isolated from the deep-sea sponge *Cacospongia mycofijiensis*. Its genome comprises a single circular chromosome of 3.2 Mbps (Chen et al., 2022). However, no *sat* gene was annotated in its genome. Given that the genome coverage has reached 800x (see its Assembly in BioProject), it is unlikely that the absence of the *sat* gene is due to insufficient sequencing depth. Intriguingly, gene annotation revealed that *M. spongiae* MSC19[T] possesses three genes encoding CSD family proteins (Table 1), suggesting that cysteine could serve as an important sulfur source. Isolates 15L and 142 of the Candidatus *S. spongiarum* were symbionts of the sponge *Aplysina aerophoba* and *Ircinia variabilis*, respectively (Burgsdorf et al., 2015). Neither *sat* gene was annotated in their genomes (Table 1). Considering that sponges can reproduce vegetatively by fission or budding (Fields and Levin, 2020), one of the possible explanations for this phenomenon is that the symbionts, such as *M. spongiae* MSC19[T], may stick to its host through the vegetative reproduction and utilize organic sulfur instead of acquiring the sulfate from environment, which could be result of adaptive evolution, but this requires

further in-depth research. As uptake of environmental symbionts bears a risk of infection to the host by cheaters (Douglas, 2008), maintaining the host and symbiont together (without separation) may help prevent infection and maintain the persistence of mutualistic relationship. Notably, not all symbionts in sponges lack the gene for SAT. For instance, *Pseudovibrio* sp. strain JE062, isolated from sponge *Mycale laxissima*, possesses *sat* gene (Table 1). Previous study has provided evidence for vertical transmission of bacterium *Pseudovibrio* sp. strain JE062 via the larvae of sponge *Mycale laxissima* (Enticknap et al., 2006). Further research in connection with other genes in the sulfate assimilation pathway is required.

Discussion

Cysteine serves as a common sulfur source in cells. The *csd* gene is ubiquitous among symbionts, regardless of their geographic location, hosts, or genome sizes. The conserved presence of genes encoding CSD in symbiotic microorganisms suggest their ability to utilize sulfur from cysteine. Cysteine may originate from the host or from the microorganism. This capability may assist symbionts in harnessing cysteine as a sulfur source during dispersal. Similarly, genes encoding SAT, which is crucial for the assimilation of inorganic sulfur, have also been broadly detected in the genomes of symbiotic microorganisms. Most of the symbiotic microorganisms investigated in this context possess both genes encoding SAT and CSD. The presence of *sat* and *csd* gene may benefit the dispersal of symbionts among isolated locales. There are variations in copy number of genes from the CSD and SAT families per genome, which may be shaped by interaction with hosts and local environmental conditions, conferring ecological advantages. In contrast, symbionts from sponges harboring only *csd* gene may be the result of evolution due to the formation of intimate symbiosis with their hosts.

Notably, even the symbiotic microorganisms with small genomes possess gene from both the SAT and CSD families (Table 1), highlighting their importance for the survival of these microorganisms. For example, the genome of Candidatus *V. okutanii* HA is approximately 1.0 Mbps in size, and the genes that are unnecessary for an intracellular lifestyle, as well as some essential genes (e.g., *ftsZ* for cytokinesis), appear to be absent (Kuwahara et al., 2007). Reductive evolution of the genome might be ongoing in the vertically transmitted *Calyptogena* symbionts (Kuwahara et al., 2007). Despite this reduction, the *sat* gene still exists in this small symbiont genome. Similarly, the *sat* gene is also present in the small genome of Candidatus *R. magnifica* Cm, which is 1.2 Mbps in size (see Table 1). The *sat* gene is present in the genome of gill symbiont isolate Gill1 from *Catillopecten margaritatus* as well (Table 1). Genomic analysis of this symbiont reveals that its genome is substantially smaller than those of its free-living relatives and has lost cellular components required for free-living (Lin et al., 2024). The presence of *sat* genes in the compact genomes of these symbionts indicates that *sat* may play a pivotal role in the functioning of these symbionts, despite the overall reduction in their genome size. It is crucial to acknowledge that these analyses are based solely on gene annotation, and their metabolic functions require further experimental validation.

Perspective

With advances in large-scale, high-throughput sequencing and assembly technologies, the high-quality genome sequences generated from projects like the Aquatic Symbiosis Genomics Project, which covers a wide range of aquatic host organisms and their microbial symbionts (McKenna et al., 2024), will help us better understand how these organisms interact with each other and their environment. In this perspective, we attempt to attract broader attention by linking genomic traits of symbionts to their adaptation. By a preliminary genomic analysis, this perspective highlights the conservation of the two genes, *csd* and *sat*, across diverse marine symbiotic microorganisms from varied hosts and reducing marine habitats, indicating their importance. Further studies on the metabolic flexibility in utilizing both organic and inorganic sulfur sources are needed, such as functional demonstration and ecological relevance. Besides, HV and CS ecosystems are chemically complex and exhibit distinct chemical profiles relative to seawater. Moreover, the marine symbiotic microorganisms experience various environmental transitions, such as changes in temperature, hydrostatic pressure, pH, nutrient availability, and osmotic stress, and have evolved traits to overcome many of these stressors (Apprill, 2020). Starting from the studies on how these symbionts might acquire and utilize sulfur during the dispersal, further explorations on metabolic plasticity in response to environmental changes will advance our understanding of the complex adaptation strategies. Dispersal of symbionts and their hosts is crucial for their biogeographic distribution and connectivity. Metabolic plasticity may prolong the survival of symbionts under adverse conditions. The dispersal capacity of symbionts may aid the mutualism persistence across habitats in the oceans. Understanding the dispersal patterns of the symbionts has implications for spatio-temporal dynamics and biodiversity conservation. The studies on metabolic plasticity will also improve our interpretation of their ecological roles in connection with biogeochemical conditions. The genomic traits that symbionts evolve to adapt to these conditions may enhance their tolerance and adaptations to environmental stressors. Therefore, this perspective concludes with a call for further research on metabolic plasticity to deepen our understanding of the connections between the genomic traits of symbionts from reducing habitats and environmental adaptation in the oceans. By combining comparative genomic analysis with consideration of lifestyles (such as symbiotic and free-living life stages) and animal behaviors (e.g. reproductive modes), we can better comprehend their versatile adaptation strategies and ecological fitness. Specifically, studying conserved genes and their functions in the core genome will aid in understanding the shared mechanisms of adaptation.

Additionally, by analyzing the accessory genomes with considerations of geographical differences and animal behaviors, we can gain insights into their specificity, such as unique adaptive features. Furthermore, differences in gene expression, translation or post-translational modifications may play roles in adapting to free-living or host-associated lifestyles. Comparisons of transcriptomes and proteomes between free-living and host-associated symbiont populations may yield additional clues about how symbiont adapt to different lifestyles. An integrative investigation would help us comprehensively elucidate the adaptability of these symbionts to their respective hosts and free-living lifestyle.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

PZ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. X-QH: Writing – original draft, Writing – review & editing. PX: Funding acquisition, Resources, Writing – review & editing. D-SZ: Funding acquisition, Resources, Writing – review & editing. C-SW: Funding acquisition, Resources, Writing – review & editing.

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.

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