Synechococcus sp. Strain PCC7002 Uses Sulfide:Quinone Oxidoreductase To Detoxify Exogenous Sulfide and To Convert Endogenous Sulfide to Cellular Sulfane Sulfur

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ABSTRACT Eutrophication and deoxygenation possibly occur in coastal waters due to excessive nutrients from agricultural and aquacultural activities, leading to sulfide accumulation. Cyanobacteria, as photosynthetic prokaryotes, play significant roles in carbon fixation in the ocean. Although some cyanobacteria can use sulfide as the electron donor for photosynthesis under anaerobic conditions, little is known on how they interact with sulfide under aerobic conditions. In this study, we report that Synechococcus sp. strain PCC7002 (PCC7002), harboring an sqr gene encoding sulfide:quinone oxidoreductase (SQR), oxidized self-produced sulfide to S0, present as persulfide and polysulfide in the cell. The Δsqr mutant contained less cellular S0 and had increased expression of key genes involved in photosynthesis, but it was less competitive than the wild type in cocultures. Further, PCC7002 with SQR and persulfide dioxygenase (PDO) oxidized exogenous sulfide to tolerate high sulfide levels. Thus, SQR offers some benefits to cyanobacteria even under aerobic conditions, explaining the common presence of SQR in cyanobacteria.

IMPORTANCE Cyanobacteria are a major force for primary production via oxygenic photosynthesis in the ocean. A marine cyanobacterium, PCC7002, is actively involved in sulfide metabolism. It uses SQR to detoxify exogenous sulfide, enabling it to survive better than its Δsqr mutant in sulfide-rich environments. PCC7002 also uses SQR to oxidize endogenously generated sulfide to S0, which is required for the proper expression of key genes involved in photosynthesis. Thus, SQR has at least two physiological functions in PCC7002. The observation provides a new perspective for the interplays of C and S cycles.

KEYWORDS sulfide:quinone oxidoreductase, persulfide dioxygenase, cyanobacteria, H2S detoxification, sulfane sulfur

Oxygen contents in the ocean have been declining during the past decades (1, 2), and the oxygen minimum zones (OMZs) where oxygen level is low or zero, are spreading (3, 4). With ocean warming, decreased solubility of O2 and increased biological activities, including photosynthesis and respiration, lead to the expansion of OMZs (5). Eutrophication induced by excessive nutrient input coming from agriculture and aquaculture is a common phenomenon in coastal waters (6), contributing to the expansion of OMZs. Nutrient enrichment via natural processes such as coastal upwelling may also be associated with deoxygenation (7). Hypoxia has serious impacts on marine ecosystems (8, 9). The sporadic accumulation of sulfide (H2S, HS−, and S2−) in OMZs is also a noticeable aspect of hypoxia hazards (10, 11).
The toxic effect of sulfide is well known, inhibiting respiration by acting on cytochrome c oxidase in heterotrophic bacteria (12–15) and photosynthesis by binding to metalloproteins of photosynthesis system II (PSII) (16–19). Sulfide can reach high concentrations in specific habitats, such as hydrothermal vents and seeps (10) and coastal mudflats (11). Sulfide is mainly derived from sulfate as the terminal electron acceptor for organic mineralization by sulfur-reducing bacteria in OMZs (10).

Sulfide is easily oxidized under both aerobic and anaerobic conditions. Chemolithotrophic bacteria oxidize it under aerobic conditions, while sulfur-dependent photosynthetic bacteria oxidize it under anaerobic conditions, using the generated electrons for anaerobic photosynthesis (20–22). Recently, mammalian mitochondria and heterotrophic bacteria were reported to oxidize sulfide via a pathway involving two key enzymes, sulfide:quinone oxidoreductase (SQR) and persulfide dioxygenase (PDO) (23, 24). SQR oxidizes sulfide to polysulfide, which spontaneously reacts with glutathione (GSH) to produce glutathione persulfide (GSSH); PDO oxidizes GSSH to sulfite, which spontaneously reacts with polysulfide to produce thiosulfate (25). This pathway is common in heterotrophic bacteria (21).

SQRs are widely distributed in microorganisms as well as in animal mitochondria (21, 26, 27). SQR oxidizes sulfide to polysulfide and transfers electrons into the electron transport chain in mitochondria (28), heterotrophic bacteria (21), chemolithotrophic bacteria (29, 30), and photolithotrophic bacteria (27, 31, 32). SQRs are grouped into six types based on sequence and structural analyses (33, 34). Besides being further oxidized, polysulfide (H₂Sn) can be converted into other forms of cellular sulfane sulfur, including protein persulfidation at Cys residues in bacteria (35). Sulfane sulfur is zero valence sulfur in various forms, such as persulfide (RSSH), polysulfide (RSSnH and RSSnR, n ≥ 2), and elemental sulfur. Sulfane sulfur can act as either an electrophile or a nucleophile (36). The nucleophilic property allows cells to resist reactive oxygen species, and the electrophilic property causes protein persulfidation, affecting enzyme activities or signaling (37, 38).

The cyanobacteria play a vital role in global primary production as the most ancient and abundant phytoplankton in the ocean (39, 40). Cyanobacteria possess both photosynthesis system I (PSI) and photosynthesis system II (PSII), and they usually perform oxygenic photosynthesis but can do anoxygenic photosynthesis (41, 42). They are effective participants in marine carbon cycles (43). Cyanobacteria are found to inhabit multiple marine habitats, and Synechococcus spp. dominate the picocyanobacterial communities in eutrophic coastal and mesotrophic open ocean waters (44, 45). In some habitats, they will encounter sulfide. The sulfur cycle is generally coupled with the carbon cycle (46–48); however, it is still unclear how sulfide affects Synechococcus spp. and how they deal with it.

Here, we report that Synechococcus sp. strain PCC7002 (PCC7002) used SQR to oxidize self-produced sulfide to polysulfide, maintaining a relatively high level of cellular sulfane sulfur, which offers growth advantages to the wild type over the Δsqr mutant. Further, PCC7002 also survived better in sulfide-rich environments via the detoxification role of SQR and PDO. The two enzymes collectively oxidize sulfide to sulfite and thiosulfate, resembling the newly reported pathway in heterotrophic bacteria (25).

RESULTS

**PCC7002 uses SQR to oxidize endogenous sulfide.** PCC7002 contains one sqr gene (CyanoBase: SYNPPCC7002_G0075), encoding a type I SQR. To generate the Δsqr mutant (see Table S1 in the supplemental material), the gene was replaced with a kanamycin resistance gene via homologous recombination (Fig. S1). The mutant was complemented with the sqr gene inserted on the chromosome of the mutant at a neutral site (CyanoBase: SYNPPCC7002_A0933) (Table S1). The wild type and the complementation strain PCC7002 Δsqr:sqr did not accumulate sulfide, but the mutant did (Fig. 1A). We also checked cellular sulfane sulfur contents by using sulfane sulfur probe 4 (SSP4), which releases a fluorescent compound after reacting with sulfane sulfur (49).
The Δsqr mutant contained a sharply decreased level of cellular sulfane sulfur in comparison with the wild type and PCC7002 Δsqr::sqr (Fig. 1B). The results indicate that PCC7002 uses SQR to oxidize endogenously produced sulfide, allowing the bacterium to keep a relatively high level of cellular sulfane sulfur.

PCC7002 has a competitive advantage over the Δsqr mutant in coculture. To investigate the effect of SQR, we monitored the growth and photosynthesis of the wild type and the Δsqr mutant. The deletion of sqr had no effect on the growth of PCC7002 (Fig. S2). We then cocultured PCC7002 and PCC7002Δsqr to access their competitive capacity. The relative abundance of PCC7002 and PCC7002Δsqr in the coculturing system was detected by real-time quantitative reverse transcription-PCR (qRT-PCR) with specific primer pairs (Table S2). Different ratios of PCC7002 and PCC7002Δsqr were mixed, and the qRT-PCR results correlated with the ratios accordingly (Fig. S3), confirming the approach. PCC7002 and PCC7002Δsqr showed an apparent advantage over PCC7002Δsqr (red bars) after transfer 4 times. The mixing of two strains at equal cell numbers was defined as the first transfer. The time interval between each transfer was 48 h, and real-time quantitative PCR (qPCR) was done at 48 h. All data are averages from three samples with standard deviation (error bar). The experiment was repeated at least three times. ****, P < 0.0001 (paired t test).

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**The effects of sqr inactivation on the photosynthesis process.** The photosynthesis parameters of the wild type and the Δsqr mutant were tested. The Δsqr mutant had a higher oxygen evolution rate than that of the wild type (Fig. 2A). The increased oxygen evolution rate upon the Δsqr mutant was consistent with an acceleration of H₂O oxidation to make up for the loss of H₂S oxidation. The maximal PSII quantum yield
was calculated as Fv/Fm, a measure of the conversion efficiency of the light energy of the PSII reaction center. Here, the Fv/Fm value showed little difference between the wild type and the mutant (Fig. 2B). The relative electron transport rate (rETR) increased in the mutant (Fig. 2C). Thus, photosynthesis efficiency was increased in the Δsqr mutant. However, the apparently increased efficiency in photosynthesis did not affect the growth (Fig. S2). We then checked the expression levels of major genes involved in photosynthesis in the wild type and the Δsqr mutant. PCC7002 had three psbA genes, encoding the isoforms of D1 protein, a key protein of the PSII core, and the isoforms allowed cyanobacteria to adapt to different light intensities. The expression levels of psbA1, psbA2, and psbA3 were all upregulated (1.5- to 2-fold) in the Δsqr mutant (Fig. S5). The rbcX, rbcL, and rbcS genes encoding RuBisCO and the tkt gene encoding transketolase of the Calvin-Benson-Bassham (CBB) cycle all showed apparent transcriptional increases (>2-fold) in the Δsqr mutant in comparison with the wild type (Fig. 2D). The mRNA level of petA encoding apocytochrome f precursor increased by 1.8-fold, and the transcripts of petC encoding a Rieske-FeS protein increased by 1.5-fold in the Δsqr mutant. However, the transcripts of the petB gene encoding cytochrome b6 showed little change (Fig. S5). Overall, the qRT-PCR results indicated that the inactivation of sqr in PCC7002 affected many physiological processes, including PSII, the CBB cycle, and the photosynthetic electron transport chain (Fig. 2D and Fig. S5).

**SQR plays a detoxification role in PCC7002.** We treated the wild type, the Δsqr mutant, and the complementation strain with different concentrations of NaHS for 6 h and then washed, diluted, and inoculated cells onto the A+ medium for incubation under 50 μmol photons m⁻² s⁻¹ at 30°C. Without sulfide, all the three strains grew well.
on the A+ agar plate. After treatment with 3 mM NaHS, the Δsqr mutant grew worse than the wild type and the complementation strain (Fig. 3A). The growth of the Δsqr mutant was fully inhibited while the wild type and the complementation strain were partly inhibited after treatment with 4 mM and 5 mM NaHS.

Whether PCC7002 oxidized exogenous sulfide was also tested. Cells at log phase of growth (optical density at 730 nm [OD730] = 1) were harvested and resuspended in 50 mM Tris buffer (pH 8.0) at an OD730 of 8. Without induction, all the three strains (PCC7002, PCC7002Δsqr, and PCC7002Δsqr::sqr) showed no oxidation activity (data not shown). After induction with 2 mM NaHS for 6 h, cell suspensions of the wild type and the complementation strain showed apparent activity with about 500 μM NaHS being fully oxidized in 8 h (Fig. 3B). However, the Δsqr mutant showed a similar oxidation rate as the buffer control (Fig. 3B). Thus, SQR plays a detoxification role in PCC7002 by oxidizing excessive sulfide.

Recombinant Escherichia coli with SQR and PDO from PCC7002 oxidized sulfide to sulfite and thiosulfate. PCC7002 also has a persulfide dioxygenase (PDO). We constructed recombinant strains E. coli(pBBR5-sqr), containing only sqr, and E. coli(pBBR5-sqr-pdo), containing both sqr and pdo. The cell suspensions of E. coli(pBBR5-sqr) rapidly oxidized 500 μM sulfide to about 250 μM polysulfide in 20 min with no apparent production of sulfite and thiosulfate (Fig. 4A to D). E. coli(pBBR5-sqr-pdo) oxidized 500 μM sulfide to about 22 μM sulfite and 205 μM thiosulfate with elevated levels of polysulfide (>50 μM) (Fig. 4A to D). The results were similar to recombinant E. coli with sqr and pdo from heterotrophic bacteria (25).
The presence of *sqr* and *pdo* in cyanobacteria. The previously reported SQRs and PDOs were used to search for potential SQRs and PDOs from 130 sequenced cyanobacterial genomes (NCBI database, updated to 17 June 2019) (21). Sixty-seven SQRs from 59 cyanobacterial genomes and 101 PDOs from 85 cyanobacterial genomes were identified after checking via phylogenetic analysis; 54 genomes possessed both SQRs and PDOs (Fig. 5). All the cyanobacterial SQRs belonged to the type I and type II SQRs (Fig. S6).

**DISCUSSION**

Our data indicate two physiological roles of SQR in PCC7002 (Fig. 6). First, SQR oxidizes endogenous H$_2$S to polysulfide, which makes PCC7002 more competitive in coculture of the wild type and the Δ*sqr* mutant (Fig. 1). It is likely that polysulfide is

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**FIG 4** Sulfide oxidation by recombinant *E. coli*. Sulfide (A), polysulfide (B), sulfite (C), and thiosulfate (D) were consumed or produced by *E. coli* (pBBR5-*sqr*) (black circles), *E. coli* (pBBR5-*sqr-pdo*) (red squares), and *E. coli* (pBBR1MCS5) (blue triangles, control). All data are averages from at least three samples with standard deviation (error bar). The experiment was repeated at least three times.

**FIG 5** The distribution of SQRs and PDOs in sequenced cyanobacterial genomes. Fifty-nine of 130 sequenced cyanobacterial genomes possessed SQR, and 85 of 130 possessed PDO. Fifty-four cyanobacterial genomes possessed both SQR and PDO.

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converted to other species of cellular sulfane sulfur, participating in the regulation of key genes of photosynthesis as a signaling molecular. H$_2$S has been proposed as a signaling molecule in bacteria since 2006 (50). Recently, it was shown that the signaling role of H$_2$S is via sulfane sulfur, which is sensed by gene regulators that activate the genes involved in H$_2$S oxidation in bacteria (51–53). OxyR also senses high levels of cellular sulfane sulfur to produce enzymes for its removal (54). Our evidence suggests that sulfane sulfur is likely involved in regulating photosynthetic genes (Fig. 2D; see also Fig. S5 in the supplemental material).

Second, SQR plays a detoxification role. Sulfide may accumulate at high concentrations in OMZs (10). With SQR, PCC7002 survives better than the Δsqr mutant in the presence of high concentrations of sulfide (Fig. 3A). The detoxification also requires PDO that prevents the excessive accumulation of polysulfide, and the sulfide oxidation pathway is the same as in heterotrophic bacteria (25). Bioinformatics analysis shows that about 45% of the sequenced cyanobacterial genomes contain SQR (Fig. 5), while only 21% of the sequenced bacterial genomes contain SQR (21), indicating the wide distribution and importance of SQR in cyanobacteria. Thus, SQR may facilitate PCC7002 and possibly other cyanobacteria to adapt to changing environments and increase their growth fitness.

SQR may have a third role in cyanobacteria, allowing them to use H$_2$S as the electron donor for photosynthesis. In the sulfur photosynthetic bacteria, SQR oxidizes H$_2$S and passes the electrons to drive anaerobic photosynthesis (52). Some cyanobacteria also perform anoxygenic photosynthesis in sulfide-rich environments (18, 31, 41), partly

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**FIG 6** SQR helps PCC7002 deal with high sulfide and improves its growth fitness. On the one hand, SQR oxidizes exogenous H$_2$S to sulfane sulfur, which spontaneously reacts with GSH and generates GSSH. Then, PDO oxidizes GSSH to sulfite and generates a new GSH. At last, sulfite spontaneously reacts with sulfane sulfur to generate thiosulfate, which is transported out of the cell. Hence, SQR coupling with PDO plays a detoxification role by oxidizing H$_2$S to sulfite and thiosulfate. On the other hand, SQR oxidizes endogenous H$_2$S to sulfane sulfur, which may act as a signal molecule and participates in the regulation of photosynthesis, especially in the CBB cycle (the proposed signaling function of sulfane sulfur is indicated with a dashed black line). Meanwhile, H$_2$S oxidation may donate to the photosynthetic electron transport (proposed electron transfer from SQR to plastoquinone [PQ] is indicated with a dashed gray line). As a result, SQR enables PCC7002 to increase its growth fitness as shown when culturing the wild type and the mutant in a mixture.
because sulfide is a better electron donor and partly because sulfide at high concentrations inhibits photosystem II (16). We observed that the Δsqr mutant had a higher oxygen evolution rate than that of the wild type (Fig. 2A). The observation may be a result of increased expression of photosynthetic genes or the oxidation of endogenously produced sulfide by SQR. Further investigation is needed to clarify whether sulfide provides electrons for photosynthesis under aerobic conditions. The physiological roles of SQR reported here may guide the investigation of cyanobacteria in ecological niches with varied O₂ and sulfide contents, as many sequenced cyanobacterial genomes contain SQR (Fig. 5).

The source of endogenous H₂S in PCC7002 is likely from the metabolism of cysteine or homocysteine, similar to H₂S production by heterotrophic bacteria during normal growth (21), or from assimilatory sulfate reduction (55). The endogenous H₂S has been shown to act as a signal molecule at low concentrations (12). Cellular sulfane sulfur, including polysulfide and GSSH, is believed to be the actual mediator of H₂S signaling (56–59); sulfane sulfur can also protect Escherichia coli cells from oxidative stress (35).

When SQR is inactivated in PCC7002, an interesting phenomenon is that the growth advantage of PCC7002 occurs only in the coculture process (Fig. 1C and Fig. S2). This may be caused by the intracellular polysulfide coming from endogenous H₂S oxidation by SQR. The mutant contains less sulfane sulfur (Fig. 1B) but has increased oxygen evolution rates and rETRs with elevated expression of photosynthesis genes (Fig. 2). The gene expression variation between the mutant and the wild type may also be a result of the lowered sulfane sulfur in the mutant, as sulfane sulfur is a known signaling molecule participating in many physiological processes in eukaryotes (58). And from our results, we infer that carbon and sulfur metabolisms can be linked via sulfane sulfur just like carbon and nitrogen metabolisms in cyanobacteria (60, 61). As we know, sulfane sulfur can perform as a reductant to help relieve oxidative stress (35, 37). Here, increased photosynthesis efficiency with lower sulfane sulfur content in the Δsqr mutant may cause oxidative stress (62), thus making it less competitive in coculture. However, it is unclear how polysulfide regulates key genes involved in photosynthesis processes, such as PSII, the CBB cycle, and photosynthesis electron transport.

In summary, SQR helps PCC7002 to convert endogenous H₂S to polysulfide, which is used to maintain a relatively high level of cellular sulfane sulfur, and to detoxify exogenous H₂S. The relatively high cellular sulfane sulfur offers the wild type a growth advantage over the Δsqr mutant in a mixed culture. The detoxification role of SQR may enable PCC7002 to survive better in OMZs, thus helping relieve deoxygenation by generating more O₂. Thus, sulfide oxidation plays a vital role in the adaption process of PCC7002 and possibly other cyanobacteria. We present a new perspective for understanding the coupling of carbon and sulfur cycles, which are key components of the marine biogeochemical cycle.

MATERIALS AND METHODS

Strains and culture conditions. PCC7002 cultures were grown in conical flasks containing medium A, supplemented with 1 mg of NaNO₃ ml⁻¹ (designated medium A+1) (63) under continuous illumination of 50 μmol photons m⁻² s⁻¹ at 30°C. Glycerol (10 mM) was added as a supplement in the A+ medium to serve as the carbon and energy source. For mutant strains, the concentrations of appropriate antibiotics required by the mutant strains were as following: 100 g/ml kanamycin and 20 g/ml chloramphenicol. E. coli was cultured in LB medium at 37°C. The strains and plasmids used in this paper are all listed in Table S1 in the supplemental material.

Strain construction. The Δsqr mutant of PCC7002 was constructed by homologous recombination. Two gene segments immediately upstream and downstream of the sqr gene (CyanoBase: SYNPCCC7002_G0075), about 1,000 bp long, were amplified by PCR from genomic DNA of the wild-type strain with the primer sets sqr-del-1/sqr-del-2 and sqr-del-5/sqr-del-6 (Table S2). A kanamycin resistance cartridge was excised from the plasmid pET30a with the primers sqr-del-3/sqr-del-4. All the above three fragments were joined via fusion PCR, with the kanamycin resistance cartridge in the middle. Then, the fusion fragment was cloned into pJET1.2 blunt vector (Thermo, Beijing, China). The sqr gene was inserted at neutral site 1 (CyanoBase: SYNPCCC7002_A09933) of PCC7002 to form the complementation strain (64). Wild-type cells were transformed with these constructs, and transfectants were selected using appropriate antibiotics. The primers used in this paper are all listed in Table S2.

The sqr gene and pdo gene (CyanoBase: SYNPCCC7002_A2866) of PCC7002 were also cloned and expressed in E. coli BL21(DE3). The fragment of sqr was amplified from the PCC7002 genome using
primers pbr-sqr-F and pbr-sqr-R (listed in Table S1) containing 20-bp extensions overlapping the adjacent fragment. The sqr fragment was cloned into the pAEX expression plasmid pAAEX10 (pAAEX) by using the TEEA assembly (65). The plasmid pAAEX-sqr-pdo containing both sqr and pdo was constructed using the same protocol with primers listed in Table S1. The two plasmids pAAEX-sqr and pAAEX-sqr-pdo were transformed to E. coli BL21(DE3).

**Sulfide accumulation detection.** The method used for the detection of sulfide accumulation was the same as described previously (65, 66). PCC7002 cultures were transferred into 3 ml of A medium in a 15-ml glass tube and incubated with shaking for 96 h with lead acetate (Pb(Ac)₂) paper strips at the top of the tube, which would turn black in the presence of sulfide as the production of PbS black precipitates.

**Toxicity analysis of sulfide.** PCC7002 and its mutants at log phase at an OD₇₃₀ of 0.6 to 0.7 were treated with 3 mM, 4 mM, or 5 mM NaHS for 6 h in the sealed tubes. After incubation, cells were washed and resuspended in fresh A medium. The cells were diluted with A medium at an OD₇₃₀ of 0.05, and 10 μl was plated on the A agar plate. The differences between PCC7002 and its mutants appeared after cultivation at 30°C under continuous illumination of 50 μmol photons·m⁻²·s⁻¹ for 7 days.

**Sulfide oxidation and product analysis.** Sulfide oxidation by PCC7002 and its mutants was assayed. Cells were harvested after induction with 2 mM NaHS for 6 h at log phase. They were resuspended in 50 mM Tris buffer (pH 8.0) at an OD₇₃₀ of 8 after being washed twice with the same buffer. The reaction was performed in 30-ml serum bottles sealed with a rubber stopper to minimize the loss of sulfide. NaHS (0.5 mM) was added to the bottle to initiate the reaction.

Sulfide oxidation by recombiant E.coli was similar, but cells were harvested and resuspended to an OD₇₃₀ of 2. The concentrations of sulfide, polysulfide, sulfite, and thiosulfate were analyzed as described previously (65, 66). Briefly, sulfide was detected by a colorimetric method; polysulfide, sulfite, and thiosulfate were derivatized with monobromobimane and then measured by high-performance liquid chromatography (HPLC) with a fluorescence detector.

**Competition experiment.** PCC7002 and PCC7002Δsqr at log phase with an OD₇₃₀ of 0.6 to 0.7 were transferred to fresh A medium to a final concentration of an OD₇₃₀ of 0.05 each as a mixture. The total genomic DNA of the mixed culture was extracted and used as the template for real-time quantitative PCR (qPCR) analysis after culturing for 48 h. The abundance of the sqr gene was used as the marker for the relative abundance of PCC7002, and the abundance of the kan gene was used for PCC7002Δsqr, in which the sqr gene was replaced by the kan gene. Then, the mixed culture was transferred to fresh A medium at an OD₇₃₀ of 0.05 for another 48 h and analysis. The mixing of two strains was defined as the first transfer. The mixture was transferred 4 times.

**Endogenous sulfane sulfur analysis.** Sulfane sulfur probe 4 (SSP4) was used to detect endogenous sulfane sulfur as previously described (35, 49). Cells were harvested, washed, and resuspended in 50 mM Tris buffer (pH 8.0). Cells were disrupted by ultrasonication, and debris was removed by centrifugation at 12,500 × g for 10 min. SSP4 (10 μM) was added to the supernatant and incubated in the dark at 30°C for 15 min. The fluorescence resulting from the SSP4 reaction with sulfane sulfur was detected with excitation of 482 nm and emission of 515 nm by using a Synergy H1 microplate reader. The fluorescence values were expressed as the intensity per milligram of protein.

**Oxygen evolution and chlorophyll fluorescence assay.** Cells at log phase with an OD₇₃₀ of 0.6 to 0.7 were collected for oxygen evolution measurement via a Clark oxygen electrode (Chlorolab 2). The samples were illuminated under continuous stirring (900 rpm) at 30°C with increasing light intensities (from 0 to 1,000 μmol photons·m⁻²·s⁻¹). The rate of oxygen evolution was recorded continuously for 2 min at each light level. The oxygen evolution rate was then normalized to OD₇₃₀.

Chlorophyll fluorescence was measured by a lightweight, hand-held fluorometer (AP-C 100; AquaPen, Drasov, Czech Republic). Cells at log phase with an OD₇₃₀ of 0.6 to 0.7 were collected and then adapted to darkness for half an hour. Fv/Fm and rETR were all automatically detected by AP-C 100.

**RNA extraction and qRT-PCR analysis.** Cells at log phase with an OD₇₃₀ of 0.6 to 0.7 were harvested by centrifugation at 10,000 × g, 4°C, for 10 min. Total RNA was isolated by using the TaKaRa MiniBEST universal RNA extraction kit, and the concentration of RNA was verified by Qubit 4 (Thermo Fisher). The cDNA was synthesized by using the Prime Script RT reagent kit with genomic DNA (gDNA) eraser (TaKaRa, Beijing, China). The SYBR Premix Ex Taq II kit (TaKaRa) was used for qRT-PCR, and the reactions were run in a Light Cycler 480 II sequence detection system (Roche, Shanghai, China). Primers for target genes are all shown in Table S1, and mpA (SYNPCC7002_A0989) was used as the reference gene (67). The results were analyzed according to the threshold cycle (2⁻ΔΔCT) method (68).

**Bioinformatics analysis.** One hundred thirty cyanobacterial genomes were downloaded from the NCBI database (update to 17 June 2019). The query sequences of SQR and PDO were based on our previous work (21). The SQR and PDO candidates were obtained by searching the database with the standalone BLASTP algorithm, using conventional criteria (E value of ≤e⁻⁸, coverage of ≥45%, and identity of ≥30%). The candidates were analyzed by using ClustalW for alignment and MEGA 7.0 for neighbor-joining tree building with the following parameters: pairwise deletion, p-distance distribution, and bootstrap analysis of 1,000 repeats (69). The candidates in the same clade as the seeds were selected. The published flavocytochrome c sulfide dehydrogenase (FCSĐ) sequences were used as the outgroup of SQR, and the GloB (GloB hydroxyacylglutathione hydrolase) sequences were used as the outgroup of PDO.

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1, TIF file, 1.3 MB.**

**FIG S2, TIF file, 0.1 MB.**
FIG S3, TIF file, 0.4 MB.

FIG S4, TIF file, 0.3 MB.

FIG S5, TIF file, 1 MB.

FIG S6, TIF file, 1.5 MB.

TABLE S1, DOCX file, 0.03 MB.

TABLE S2, DOCX file, 0.03 MB.

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REFERENCES

1. Schmidtko S, Stramma L, Visbeck M. 2017. Decline in global oceanic oxygen content during the past five decades. Nature 542:335–339. https://doi.org/10.1038/nature21399.

2. Breitburg D, Levin LA, Oschlies A, Gregoire M, Chavez FP, Conley DJ, Montes I, Naqvi SWA, Lifrich K, Gobler CJ, Barriocanal-Casado E, Santos-Fandila Á, Romero M, Sayed RK, Duarte J, Barollier-Casado J, Lopez LC. 2017. CoQ deficiency causes disruption of mitochondrial sulfide metabolism. Mol Med 9:78–95. https://doi.org/10.15252/emmm.201606345.

3. Shepherd JG, Brewer PG, Oschlies A, Watson AJ. 2017. Ocean ventilation and deoxygenation in a warming world: introduction and overview. Philos Trans R Soc A 375:20170242. https://doi.org/10.1098/rsta.2017.0240.

4. Delong EF, Revsbech NP, Ulloa O. 2010. A cryptic sulfur cycle in oxygenating marine ecosystems. Science 321:926–929. https://doi.org/10.1126/science.1156401.

5. Klatt JM, Haas S, Yilmaz P, De Beer D, Polerecky L. 2015. Hydrogen sulfide can inhibit and enhance oxygenic photosynthesis in a cyanobacterium from sulfidic springs. Environ Microbiol 17:3301–3313. https://doi.org/10.1111/1462-2920.12791.

6. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/aem.70.2.736-744.2004.

7. Oren A, Padan E, Malkin S. 1979. Sulfide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplasts. Biochin Biophys Acta 546:270–279. https://doi.org/10.1016/0005-2788(79)90045-8.

8. Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R. 1986. Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407. https://doi.org/10.1128/AEM.51.2.398-407.1986.

9. Klatt JM, Haas S, Yilmaz P, De Beer D, Polerecky L. 2015. Hydrogen sulfide can inhibit and enhance oxygenic photosynthesis in a cyanobacterium from sulfidic springs. Environ Microbiol 17:3301–3313. https://doi.org/10.1111/1462-2920.12791.

10. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/aem.70.2.736-744.2004.

11. Breitburg D, Levin LA, Oschlies A, Gregoire M, Chavez FP, Conley DJ, Montes I, Naqvi SWA, Lifrich K, Gobler CJ, Barriocanal-Casado E, Santos-Fandila Á, Romero M, Sayed RK, Duarte J, Barollier-Casado J, Lopez LC. 2017. CoQ deficiency causes disruption of mitochondrial sulfide metabolism. Mol Med 9:78–95. https://doi.org/10.15252/emmm.201606345.

12. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/aem.70.2.736-744.2004.

13. Oren A, Padan E, Malkin S. 1979. Sulfide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplasts. Biochin Biophys Acta 546:270–279. https://doi.org/10.1016/0005-2788(79)90045-8.

14. Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R. 1986. Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407. https://doi.org/10.1128/AEM.51.2.398-407.1986.

15. Grieshaber MK, Volkel S. 1998. Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53. https://doi.org/10.1146/annurev.physiol.60.1.333.

16. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/aem.70.2.736-744.2004.

17. Oren A, Padan E, Malkin S. 1979. Sulfide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplasts. Biochin Biophys Acta 546:270–279. https://doi.org/10.1016/0005-2788(79)90045-8.

18. Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R. 1986. Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407. https://doi.org/10.1128/AEM.51.2.398-407.1986.

19. Miller SR, Bebout BM. 2004. Variation in sulfide tolerance of photosystem II phylogenetically diverse cyanobacteria from sulfidic habitats. Appl Environ Microbiol 70:736–744. https://doi.org/10.1128/aem.70.2.736-744.2004.

20. Canfield DE, Stewart FJ, Thamdrup B, Dalsgaard T, Delong EF, Revsbech NP, Ulloa O. 2010. A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast. Science 330:1375–1378. https://doi.org/10.1126/science.1196889.

21. Xia Y, Lu C, Hou N, Xin Y, Liu J, Liu H, Xun L. 2017. Sulfide production and oxidation by heterotrophic bacteria under aerobic conditions. ISME J 11:2754–2766. https://doi.org/10.1038/ismej.2017.125.

22. Mehta-Kolte MG, Loutey D, Wang O, Youngblut MD, Hubbard CG, Wetmore KM, Conrad ME, Coates JD. 2017. Mechanism of H2S oxidation by the dissimilatory perchlorate-reducing microorganism Azospira sulfurredunder. ISME J https://doi.org/10.1128/AEM.03281-13.

23. Grim SL, Dick GJ. 2016. Photosynthetic versatility in the genome of the dissimilatory perchlorate-reducing microorganism Azospira suillum. ISME J https://doi.org/10.1038/ismej.2017.125.

24. Liu H, Xin Y, Xun L. 2014. Distribution, diversity, and activities of sulfur dioxygenases in heterotrophic bacteria. Appl Environ Microbiol 80:1799–1806. https://doi.org/10.1128/AEM.03281-13.

25. Xin Y, Liu H, Cui F, Liu H, Xun L. 2016. Recombinant Escherichia coli with sulfide:quinone oxidoreductase and persulfide dioxygenase rapidly oxidises disulphide to sulfite and thiosulfate via a new pathway. Nat Microbiol 18:5123–5136. https://doi.org/10.1111/1462-2920.13511.

26. Cherney MM, Zhang Y, Solomonson M, Weiner HJ, James MN. 2010. Crystal structure of sulfide:quinone oxidoreductase from Acidithiobacillus ferrooxidans: insights into sulfidotrophic respiration and detoxification. J Mol Biol 398:292–305. https://doi.org/10.1016/j.jmb.2010.03.018.

27. Grim SL, Dick GJ. 2016. Photosynthetic versatility in the genome of Geitlerinema sp. PCC 9228 (formerly Oscillatoria limnetica ‘Solar Lake’), a model anoxygenic photosynthetic cyanobacterium. Front Microbiol 7:1546. https://doi.org/10.3389/fmicb.2016.01546.

28. Theissen U, Hoffmeister M, Grieshaber M, Martin W. 2003. Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during nuclear and mitochondrial times. Mol Biol Evol 20:1564–1574. https://doi.org/10.1093/molbev/msg174.

29. Dahl C, Franz B, Hensen D, Kesselheim A, Zigann R. 2013. Sulfite oxidation in the purple sulfur bacterium Allochromatium vinosum: identifica-
Function of SQR in Cyanobacteria

33. Marcia M, Ermler U, Peng G, Michel H. 2010. A new structure-based classification of sulfidequinone oxidoreductases. Proteins 78:1073–1083. https://doi.org/10.1002/prot.22665.

34. Shen J, Peng H, Zhang Y, Trinidad JC, Giedroc DP. 2016. Staphylococcus aureus sfr encodes a type II sulfidequinone oxidoreductase and impacts reactive sulfur speciation in cells. Biochemistry 55:6524–6534. https://doi.org/10.1021/acs.biochem.6b00714.

35. Li K, Xin Y, Xuan G, Zhao R, Liu H, Xia Y, Xun L. 2019. Escherichia coli uses Sqr functions as a master regulator of sulfide-dependent photosynthesis. Proc Natl Acad Sci U S A 114:2355–2360. https://doi.org/10.1073/pnas.1901293114.

36. Greiner R, Palinkas Z, Basell K, Becher D, Antelmann H, Nagy P, Dick TP. 2014. The CsoR-like sulfurtransferase repressor (CstR) is a persulfide sensor in Staphylococcus aureus. Mol Microbiol 94:1343–1360. https://doi.org/10.1111/mmi.12835.

37. Koch T, Dahl C. 2018. A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. ISME J 12:2479–2491. https://doi.org/10.1038/s41396-018-0209-7.

38. Shah V, Zhao X, Lundeen RA, Inglis AE, Nicastro D, Morris RM. 2019. Morphological plasticity in a sulfide-oxidizing marine bacterium from the SUP05 clade enhances dark carbon fixation. mBio 10:e00216-19. https://doi.org/10.1128/mBio.00216-19.

39. Golub B, Jahnke K, Kerschbaumer R. 2015. The SUP05 clade enhances dark carbon fixation. ISME J 11:1017–1027. https://doi.org/10.1038/ismej.2015.106.

40. Li Y, Tang K, Zhang L, Zhao Z, Xie X, Chen CA, Wang D, Jiao N, Zhang Y. 2018. Coupled carbon, sulfur, and nitrogen cycles mediated by microorganisms in the water column of a shallow-water hydrothermal ecosystem. Front Microbiol 9:2718. https://doi.org/10.3389/fmicb.2018.02718.

41. Koch T, Dahl C. 2018. CsoR-like sulfurtransferase repressor (CstR) is a persulfide sensor in Staphylococcus aureus. Mol Microbiol 94:1343–1360. https://doi.org/10.1111/mmi.12835.

42. Greiner R, Palinkas Z, Basell K, Becher D, Antelmann H, Nagy P, Dick TP. 2014. The CsoR-like sulfurtransferase repressor (CstR) is a persulfide sensor in Staphylococcus aureus. Mol Microbiol 94:1343–1360. https://doi.org/10.1111/mmi.12835.

43. Koch T, Dahl C. 2018. A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. ISME J 12:2479–2491. https://doi.org/10.1038/s41396-018-0209-7.

44. Shah V, Zhao X, Lundeen RA, Inglis AE, Nicastro D, Morris RM. 2019. Morphological plasticity in a sulfide-oxidizing marine bacterium from the SUP05 clade enhances dark carbon fixation. mBio 10:e00216-19. https://doi.org/10.1128/mBio.00216-19.

45. Golub B, Jahnke K, Kerschbaumer R. 2015. The SUP05 clade enhances dark carbon fixation. ISME J 11:1017–1027. https://doi.org/10.1038/ismej.2015.106.

46. Li Y, Tang K, Zhang L, Zhao Z, Xie X, Chen CA, Wang D, Jiao N, Zhang Y. 2018. Coupled carbon, sulfur, and nitrogen cycles mediated by microorganisms in the water column of a shallow-water hydrothermal ecosystem. Front Microbiol 9:2718. https://doi.org/10.3389/fmicb.2018.02718.

47. Koch T, Dahl C. 2018. A novel bacterial sulfur oxidation pathway provides a new link between the cycles of organic and inorganic sulfur compounds. ISME J 12:2479–2491. https://doi.org/10.1038/s41396-018-0209-7.

48. Shah V, Zhao X, Lundeen RA, Inglis AE, Nicastro D, Morris RM. 2019. Morphological plasticity in a sulfide-oxidizing marine bacterium from the SUP05 clade enhances dark carbon fixation. mBio 10:e00216-19. https://doi.org/10.1128/mBio.00216-19.