Synechococcus sp. PCC7002 Uses Peroxiredoxin to Cope with Reactive Sulfur Species Stress

Daixi Liu, a,c Jinyu Chen, a,c Yafei Wang, a,c Yue Meng, a,c Yuanning Li, a,c Ranran Huang, a,c Yongzhen Xia, b Huaiwei Liu, b Nianzhi Jiao, a,c,d Luying Xun, b,e Jihua Liu a,c,d

a Institute of Marine Science and Technology, Shandong University, Qingdao, People’s Republic of China
b State Key Laboratory of Microbial Technology, Shandong University, Qingdao, People’s Republic of China
c Joint Lab for Ocean Research and Education at Dalhousie University, Shandong University and Xiamen University
d Institute of Marine Microbes and Ecosystems, Xiamen University, Xiamen, China
e School of Molecular Biosciences, Washington State University, Pullman, Washington, USA

ABSTRACT
Cyanobacteria are widely distributed groups of microorganisms in the ocean, and they often need to cope with the stress of reactive sulfur species, such as sulfide and sulfane sulfur. Sulfane sulfur refers to the various forms of zero-valent sulfur, including persulfide, polysulfide, and element sulfur (S₈). Although sulfane sulfur participates in signaling transduction and resistance to reactive oxygen species in cyanobacteria, it is toxic at high concentrations and induces sulfur stress, which has similar effects to oxidative stress. In this study, we report that Synechococcus sp. PCC7002 uses peroxiredoxin to cope with the stress of cellular sulfane sulfur. Synechococcus sp. PCC7002 contains six peroxiredoxins, and all were induced by S₈. Peroxiredoxin I (PrxI) reduced S₈ by forming a disulfide bond between residues Cys⁵₃ and Cys¹⁵₃ of the enzyme. A partial deletion strain of Synechococcus sp. PCC7002 with decreased copy numbers of the prxI gene was more sensitive to S₈ than the wild type. Thus, peroxiredoxin is involved in maintaining the homeostasis of cellular sulfane sulfur in cyanobacteria. Given that peroxiredoxin evolved before the occurrence of O₂ on Earth, its original function could have been to cope with reactive sulfur species stress, and that function has been preserved.

IMPORTANCE
Cyanobacteria are the earliest microorganisms that perform oxygenic photosynthesis, which has played a key role in the evolution of life on Earth, and they are the most important primary producers in the modern oceans. The cyanobacterium Synechococcus sp. PCC7002 uses peroxiredoxin to reduce high levels of sulfane sulfur. That function is possibly the original role of peroxiredoxin, as the enzyme evolved before the appearance of O₂ on Earth. The preservation of the reduction of sulfane sulfur by peroxiredoxin5-type peroxiredoxins may offer cyanobacteria an advantage in the complex environment of the modern oceans.

KEYWORDS
peroxiredoxin, reactive sulfur species, cyanobacteria, sulfane sulfur reduction

Cyanobacteria are one of the most important microbial groups; they provided the first source of O₂ on Earth via oxygenic photosynthesis (1, 2). However, some environments that cyanobacteria inhabit periodically experience decreased oxygen levels. Cyanobacterial mats are one environment with periodically anoxic conditions, in which cyanobacteria perform oxygenic photosynthesis in the daytime and turn to respiration in the dark. The insufficient diffusion of O₂ into the mat makes the mat turn anoxic. As a result, heterotrophic bacteria in the mat perform sulfate respiration and produce hydrogen sulfide (H₂S). Cyanobacteria can use H₂S as an electron donor to perform anoxygenic photosynthesis when oxygenic photosynthesis is inhibited by high concentrations of H₂S. Tons of sulfane...
sulfur may be produced by the oxidation of \( \text{H}_2\text{S} \) via sulfide:quinone oxidoreductase (SQR) in the mats (3, 4). Sulfane sulfur refers to the various forms of zero-valent sulfur, including persulfide, polysulfide, and elemental sulfur (\( \text{S}_8 \)). Cyanobacteria inhabiting oxygen minimum zones (OMZs), where \( \text{H}_2\text{S} \) is sporadically accumulated, also face low-\( \text{O}_2 \) and sulfidic conditions. Moreover, cyanobacteria encounter sulfur in the photic zones above OMZs, which are even visible as “clouds” on satellite images. Sulfane sulfur is also likely to be abundant in the benthic realm (5, 6). \( \text{H}_2\text{S} \) and sulfane sulfur are two of the most important reactive sulfur species (RSS) that tend to be present in sulfidic conditions. RSS are a diverse class of sulfur-containing compounds and functional groups with important roles in chemical biology and bioinorganic chemistry (7–10). Therefore, cyanobacteria need to cope with the RSS stress caused by high concentrations of \( \text{H}_2\text{S} \) and the accumulation of sulfane sulfur in the environments discussed above (11, 12).

Sulfane sulfur, including persulfide forms (RSSH and HSSH), polysulfide forms (RSSnH, RSSnR, and H2Sn, \( n \geq 2 \)), and elemental sulfur (\( \text{S}_8 \)), is commonly present in the cytoplasm of living organisms and plays important roles in maintaining intracellular redox homeostasis and metabolic regulation (7, 13–15). However, a high concentration of sulfane sulfur is toxic to cells and causes protein persulfidation and disulfide bond formation (16, 17). Inorganic polysulfides generated from organosulfur compounds inhibit several types of pathogenic and drug-resistant bacteria (18). Elemental sulfur is used as a potential antifungal agent (19). Consequently, cells have various enzymes and regulatory systems to protect against excessive levels of sulfane sulfur (20).

In some sulfidic environments, cyanobacteria can perform anoxygenic photosynthesis by using \( \text{H}_2\text{S} \) as an electron donor, and the key enzyme in this process is SQR (1, 21–23). Cyanobacteria SQR and peroxidase dioxygenase (PDO) can work together to detoxify \( \text{H}_2\text{S} \) (24). PDO is normally involved in the oxidation of the sulfane sulfur that is produced by SQR, but it functions at high levels of cellular sulfane sulfur. Other pathways that help to maintain the homeostasis of sulfane sulfur in cyanobacteria remain to be explored, considering the important role of sulfane sulfur in cellular signaling (25).

Microorganisms have evolved a series of mechanisms by which to maintain the homeostasis of intracellular sulfane sulfur. Besides PDO (26, 27), thioredoxins (Trx) and glutaredoxins (Grx) also reduce sulfane sulfur to \( \text{H}_2\text{S} \) (28–31). Furthermore, some cyanobacteria are capable of sulfur respiration, using elemental sulfur as an electron acceptor in dark and anoxic conditions (3, 32, 33). Moreover, catalase, which typically catalyzes the disproportionation of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) and \( \text{O}_2 \) (34, 35), also has the ability to oxidize inorganic persulfide (\( \text{H}_2\text{S}_2 \)), which is structurally similar to \( \text{H}_2\text{O}_2 \) (36). Because peroxiredoxin (Prx) also uses \( \text{H}_2\text{O}_2 \) as a substrate (37–39), an immediate question is whether Prx can metabolize sulfane sulfur.

Prxs are ubiquitous in plants, animals, and bacteria (38, 40–43). Their active Cys residue is oxidized to sulfonic acid by \( \text{H}_2\text{O}_2 \) and organic peroxides. Depending on whether one or two Cys residues are involved in the process of recycling sulfonic acid back to the thiol form, they can be divided into three categories: typical 2-Cys Prxs, atypical 2-Cys Prxs, and 1-Cys Prxs (40, 44, 45). However, this classification was not unequivocally accepted. Kimberly et al. developed a method that used the Deacon Active Site Profiler tool to extract functional site \( \text{PXXTXTXXCP} \) profiles from structurally characterized Prxs and classify the Prxs into six distinct subclasses (46, 47): alkyl hydroperoxide reductase subunit C (AhpC-Prx), bacterioferritin comigratory protein (BCP-PrxQ), alkyl hydroperoxide reductase subunit E (AhpE), peroxiredoxin 5 (Prx5), peroxiredoxin 6 (Prx6), and thiol peroxidase (Tpx). Because inorganic polysulfide (\( \text{H}_2\text{S}_n \)) and \( \text{H}_2\text{O}_2 \) are structural analogs, we hypothesize that Prx can also metabolize \( \text{H}_2\text{S}_n \). From the perspective of evolution, the origin of Prxs precedes the appearance of \( \text{O}_2 \) on Earth (48). Therefore, Prx might have been used to manage intracellular RSS before the appearance of \( \text{O}_2 \) and may offer cyanobacteria the advantage of being able to move in and out of hypoxic areas in the modern ocean (49–51).

Synechococcus sp. PCC7002 (PCC7002) contains six hypothetical Prxs: PrxI (ACA98797.1), PrxII (ACA98565.1), PrxIII (ACA99108.1), PrxIV (ACA98330.1), PrxV (ACA98124.1), and PrxVI (ACA98565.1), PrxIII (ACA99108.1), PrxIV (ACA98330.1), PrxV (ACA98124.1), and PrxVI
Among these, the role of PrxI in H2O2 metabolism has been confirmed (52). Here, we report that the Prxs in PCC7002 are all induced by S8. However, only PrxI was able to reduce S8 to H2S. The Cys53 and Cys153 residues of PrxI play critical roles in the reduction of sulfane sulfur to H2S via the formation of a disulfide bond. PrxI, which belongs to the Prx5 subfamily, was distinct from the other Prxs of PCC7002 in a phylogenetic analysis. These results improve our understanding of the sulfane sulfur metabolic pathway of cyanobacteria, provide some explanation for the widespread distribution of cyanobacteria in the modern ocean, and provide a new perspective from which to explore the important role of cyanobacteria in the early evolution of life on Earth.

RESULTS
Sulfane sulfur upregulates the expression of prxs in PCC7002. Sulfane sulfur plays an important role in the regulation of the gene expression associated with photosynthesis in PCC7002, but it is toxic at high concentrations (24). S8 at the concentrations of 500 μM and 1 mM were fatal to PCC7002 (Fig. S1). Then, 100 and 250 μM S8 were used to induce PCC7002, and the expression of prxs were detected. All six prxs in PCC7002 were upregulated after induction by S8, as determined by a quantitative polymerase chain reaction (qPCR) analysis at all tested concentrations (Fig. 1A). At the concentration of 100 μM, the expression levels of prxIV and prxVI were upregulated by approximately 5-fold. The expression of prxl was increased notably (>10-fold). Furthermore, the expressions of prxl, prxII, and prxV levels were also increased notably (from 20-fold to 30-fold). All prxs were also significantly upregulated at the concentration of 200 μM, although the amplitudes were not as high as those observed at 100 μM. Under reactive oxygen species (ROS) pressure, the expression of prxs changed slightly, by a maximum of about 2-fold after H2O2 induction (Fig. 1B) or incubation under 2%, 10%, or 20% O2 (Fig. 1C).

PrxI metabolized S8 and produced H2S. To compare the functions of Prxs, H2S production by recombinant Prxs fused to the C-terminus of MBP was detected (Fig. 2). Prxs with His-tags were found to be insoluble. First, 100 μg/mL purified Prx–MBP fusion protein (Fig. S2A) were incubated with 200 μM elemental sulfur (S8) and 100 μM dithiothreitol (DTT) for 5 min at 30°C in 50 mM HEPES buffer (pH 7.0). About 90 μM H2S was released by Prx–MBP, while the H2S production by PrxII through PrxVI was not significantly different from that of a control that contained only 200 μM S8 and 100 μM DTT in HEPES buffer (Fig. 2A). Second, 200 μM S8 were added to cell lysates of recombinant E. coli BL21 expressing Prx–MBP fusion (10 mg of protein mL−1). An SDS-PAGE analysis showed a similar amount of the fused proteins in each sample (Fig. S2B). The lysate of recombinant E. coli BL21 expressing the Prxl fusion protein released about

(ACA99379.1). Among these, the role of Prxl in H2O2 metabolism has been confirmed (52). Here, we report that the Prxs in PCC7002 are all induced by S8. However, only Prxl was able to reduce S8 to H2S. The Cys53 and Cys153 residues of Prxl play critical roles in the reduction of sulfane sulfur to H2S via the formation of a disulfide bond. Prxl, which belongs to the Prxs subfamily, was distinct from the other Prxs of PCC7002 in a phylogenetic analysis. These results improve our understanding of the sulfane sulfur metabolic pathway of cyanobacteria, provide some explanation for the widespread distribution of cyanobacteria in the modern ocean, and provide a new perspective from which to explore the important role of cyanobacteria in the early evolution of life on Earth.

FIG 1 The effects of S8, H2O2, and O2 on the expression of prxs in PCC7002. The expression levels of prxl, prxII, prxIII, prxIV, prxV, and prxVI were measured using RT-qPCR after induction by S8 (A) and H2O2 (B) for 3 h. (C) The expression of prxs in PCC7002 incubated under 2%, 10%, and 20% O2 in the gas phase. To determine the expression levels of prx, relative quantitative PCR was used. The relative gene expression represented the prx expression levels, standardized by the reference gene rpaA. All data are averages from three samples with standard deviations shown (error bars). The experiment was repeated at least three times. *, P value < 0.1; **, P value < 0.01; ***, P value < 0.001; ****, P value < 0.0001; ns, not significant (paired t-test).
The metabolism of S₈ by Prxs. (A) The production of H₂S by purified Prxl-MBP, Prxl-MBP, Prxl-IV-MBP, Prxl-V-MBP, and Prxl-VI-MBP. 100 μg/mL purified Prxl-MBP was incubated with 200 μM elemental sulfur (S₈) and 100 μM DTT for 15 min at 30°C in 50 mM HEPES buffer (pH 7.0). CKA represented the HEPES buffer with 200 μM elemental sulfur (S₈) and 100 μM DTT. (B) The production of H₂S by the lysates of the recombinant E.coli BL21 (DE3) (pMal-C2X) (CKB), E.coli BL21 (DE3) (pMal-prxl) (Prxl I), E.coli BL21 (DE3) (pMal-prxlII) (Prxl II), E.coli BL21 (DE3) (pMal-prxlIII) (Prxl III), E.coli BL21 (DE3) (pMal-prxlIV) (Prxl IV), E.coli BL21 (DE3) (pMal-prxlV) (Prxl V), and E.coli BL21 (DE3) (pMal-prxlVI) (Prxl VI), with a total protein concentration of 10 mg/mL. 200 μM S₈ was used as the source of sulfane sulfur, and the treatment time was 5 min. (C) The production of H₂S by recombinant E.coli BL21 (DE3) (pMal-C2X) (CKC) and E.coli BL21 (DE3) (pMal-prxl-VI) cells. The recombinant E.coli BL21 strains were harvested and resuspended to an OD₆₀₀ nm of 10, and then 200 μM S₈ was added to initiate the reaction. The treatment time was 15 min. All data are averages from three samples with standard deviations shown (error bars). The experiment was repeated at least three times. *, P value < 0.1; **, P value < 0.01; ***, P value < 0.001; ****, P value < 0.0001; ns, not significant (paired t-test).

130 μM H₂S in 5 min of incubation. The control E. coli BL21 with empty vector pMal-C2X released only 90 μM H₂S. Compared to the control, no more H₂S was released by the lysates containing Prxl through PrxlVI (Fig. 2B). Third, the ability of resting cells expressing Prxl-MBP fusion protein to metabolize S₈ and produce H₂S was also measured, and only the resting cells with the Prxl-MBP fusion protein produced more H₂S than did the control cells with the empty vector (Fig. 2C). Thus, Prxl clearly reduced sulfane sulfur to H₂S.

A previously reported CstR-mKate reporter (53) was adapted to analyze the function of PCC7002 Prxl and its Cys residues. The reporter system included CstR and mKate, in which CstR inhibits the expression of mkate. Sulfane sulfur could relieve the inhibitory effect of CstR. Thus, the fluorescence intensity of mKate in the E. coli host cells could serve as an indicator of the levels of intracellular sulfane sulfur (Fig. 3A1), reaching a maximum when E. coli cells entered the early stationary phase (25). When prxl was cloned behind mkate, the mkate fluorescence was decreased because of the metabolism of sulfane sulfur by Prxl (Fig. 3A2). Prxl contains three cysteine residues (Cys⁵³, Cys⁷⁸ and Cys¹⁵³), and they were individually mutated to serine (Ser). The mkate fluorescence intensity in the modified reporter system with Prxl C78S was slightly higher than that in the system with wild-type Prxl. However, the mkate fluorescence intensities with Prxl C53S and Prxl C153S were significantly enhanced compared with those for the construct containing Prxl, and the control without Prxl had the highest mkate fluorescence (Fig. 3A2). Thus, the mutation of Cys⁵³ and Cys¹⁵³ destroyed the ability of Prxl to reduce sulfane sulfur.

Furthermore, the Cys residues of Prxl in pMal-C2X were also individually mutated to Ser. Purified Prxl-MBP C53S and Prxl-MBP C153S produced less H₂S than did wild-type Prxl-MBP and Prxl-MBP C78S, indicating the importance of Cys⁵³ and Cys¹⁵³ (Fig. 3B and Fig. S3A). Meanwhile, the lysate of E. coli BL21 cells expressing Prxl-MBP C53S or Prxl-MBP C153S also produced less H₂S from added S₈ than did cells expressing wild-type Prxl-MBP or Prxl-MBP C78S (Fig. 3C). The cell lysates of the E. coli BL21 expressing Prxl-MBP and its mutants were standardized by protein concentration and were confirmed to contain similar amounts of proteins by an SDS-PAGE analysis (Fig. S3B).

We tested whether the Cys⁵³ and Cys¹⁵³ of Prxl formed a disulfide bond. The MBP fusion proteins were purified and cleaved by Factor Xa to release Prxl, Prxl C53S, Prxl C78S, and Prxl C153S. The released Prxl proteins were analyzed by non-reducing SDS-PAGE.
In the SDS-PAGE, untreated PrxI and PrxI C78S showed two bands, with the upper band being dominant. The upper band was converted to the lower band upon treatment with 250 μM S₈. PrxI C53S and PrxI C153S showed only the upper band, and S₈ treatment did not affect it (Fig. 3D). The upper band represented the PrxI protein without an intramolecular disulfide bond, while the lower band represented the protein with an intramolecular disulfide bond. All modifications were converted back to thiols by treatment with DTT. Hence, the Cys53 and Cys153 of PrxI are involved in reducing S₈ to H₂S, and they form an intramolecular disulfide bond.

PrxI enhanced the survival of PCC7002 after sulfane sulfur exposure. The deletion of prxI affected the survival of PCC7002 after sulfane sulfur exposure. We tried to construct a single deletion strain by homologous recombination. However, prxI could only be partially knocked out (to give strain PCC7002ΔprxI-p), as the kanamycin-resistant S₈, could relieve the repression of mkate by CstR. The mkate fluorescence was used to characterize the abilities of Prxs to metabolize sulfane sulfur. (A2) The fluorescence intensities of mkate in the CstR-reporter system coupled with PrxI and its cysteine mutants. (B) The production of H₂S by purified PrxI, PrxI C53S, PrxI C78S, and PrxI C153S. (C) The production of H₂S by lysates of recombinant E.coli BL21 (DE3) cells expressing prxI and its cysteine mutants. (D) Nonreducing SDS-PAGE of PrxI, PrxI C53S, PrxI C78S, and PrxI C153S after S₈ and DTT treatment. The proteins were cleaved from the MBP-fusion proteins by Factor Xa at room temperature for 24 h, and 6 μg of PrxI proteins were loaded. All data are averages from three samples with standard deviations shown (error bars). The experiment was repeated at least three times. *, P value < 0.1; **, P value < 0.01; ns, not significant (paired t-test).
resistant mutant contained both the intact \textit{prxI} gene and the kanamycin resistance gene when checked by PCR (Fig. S4). Cyanobacteria often have multiple chromosomes per cell (54), and many critical genes cannot be completely deleted from all chromosomes, as that would be fatal to the cell. Because \textit{prxI} could not be completely deleted, \textit{PrxI} is likely to play an essential physiological role in PCC7002. Even though not all copies of \textit{prxI} were knocked out, the mutant showed a distinct response to S8 exposure compared to that of the wild type. PCC7002 and PCC7002\textit{ΔprxI-p} cells were treated with various amounts of S8 for 6 h and then placed on A\textsubscript{1} agar plate for a further cultivation of 7 days under 30°C and 50 \textmu mol photons m\textsuperscript{-2} s\textsuperscript{-1} illumination. (C) The growth curve of PCC7002 and PCC7002\textit{ΔprxI-p} in the presence of S\textsubscript{8}. PCC7002 showed a higher resistance to S\textsubscript{8} treatment than did PCC7002\textit{ΔprxI-p}. All data are averages from three samples with standard deviations shown (error bars). The experiment was repeated at least three times.

**Phylogenetic analysis of Prxs in PCC7002.** We conducted a phylogenetic analysis of the six Prxs in PCC7002 (Fig. 5A). Based on an analysis using the Deacon Active Site Profiler tool, Prxs are classified into six subfamilies (47). Here, representative sequences from each subfamily were selected to analyze the classification of the Prxs in PCC7002 (Table S3). The Prxs in PCC7002 belonged to five subfamilies: PrxI belonged to the Prx5 subfamily, PrxII belonged to the AhpC-Prx1 subfamily, PrxIII and PrxV belonged to the...
The genetic diversity of Prxs in PCC7002. The six Prxs in PCC7002 were divided into five subclasses: PrxI PCC7002 belonged to the Prx5 family, PrxII PCC7002 belonged to the

A

AhpC-Prx1

Prx6

AhpE

BCP-PrxQ

Tpx

Prx5

B

With Prx5-type Prx
Without Prx5-type Prx

FIG 5 Phylogenetic analysis of Prxs in Cyanobacteria. (A) The genetic diversity of Prxs in PCC7002. The six Prxs in PCC7002 were divided into five subclasses: Prxl PCC7002 belonged to the Prx5 family, PrxII PCC7002 belonged to the (Continued on next page)
BCP-PrxQ subfamily, PrxVI belonged to the AhpE subfamily, and PrxIV belonged to the Prx6 subfamily. There is no Prx in PCC7002 belonging to the Tpx subfamily. Although the Prx5 subfamily was significantly different from the other subfamilies, as evidenced by it occupying a separate branch in the phylogenetic tree (Fig. 5A), the sequence around the active site of Prx5 subfamily members (PXXXTXXCP, where CP is the Cys53 of PrxI) is highly conserved (Fig. S5A). Based on the above findings, we deduced that the sequence specificity of PrxI determined its activity.

Currently, 198 genomes of cyanobacteria have been sequenced, and we searched them for Prxs, using the queries in Table S3. There were 1,272 probable Prxs in these cyanobacteria, of which 194 belonged to the AhpC-Prx subfamily, 148 to the Prx6 subfamily, 189 to the AhpE subfamily, 612 to the BCP-PrxQ subfamily, and 129 to the Prx5 subfamily (Table S4). No Tpx family members were found in these cyanobacteria. The 129 Prx5 subfamily members were distributed across 127 cyanobacteria, and 65.5% of the sequenced cyanobacteria encoded at least one Prx5 (Fig. 5B and Fig. S5 and Table S5).

**DISCUSSION**

Here, we report the participation of PrxI in sulfane sulfur metabolism in cyanobacteria (Fig. 6). S₈ significantly induced the expression of all six prxs in PCC7002 (Fig. 1). Among them, we demonstrated that Prxl reduces S₈ to H₂S by donating an electron, thereby generating a disulfide bond between Cys⁵³ and Cys¹⁵³. Thioredoxin (Trx) then reduces the disulfide bond. As a result, Prxl helps PCC7002 to cope with the reactive sulfur species stress in the living environments.

Three experiments were designed to prove the function of Prxs in PCC7002: one using the purified Prxs-MBP (Fig. 2A), one using the cell lysate of E. coli expressing Prxs-MBP (Fig. 2B), and one using the resting cells of recombinant E. coli expressing Prxs-MBP (Fig. 2C). All three experiments indicated that only Prxl had the ability to reduce S₈. DTT was used as a reductant in the purified protein experiment. Even though DTT can directly react with S₈ to produce H₂S, the existence of Prxl in the reac-
tion produced more H$_2$S (Fig. 2A). The maximum production of H$_2$S by purified Prxl was at 15 min, while that of the cell lysate was at 5 min. This may be due to the fact that DTT was a chemical reductant which could be much lower than the physiological reductant in the cell lysate (55). The maximum production of H$_2$S by the recombinant _E. coli_ with Prxl-MBP was also at 15 min, which may be due to the slow transformation of S$_8$ to cells. Furthermore, the lysates of _E. coli_ expressing PrxII-MBP and PrxIV-MBP, as well as the resting cells expressing PrxIV-MBP and Prxn-MBP, had lower H$_2$S production. That may be due to the interaction of Prx with cellular components, as H$_2$S production was not decreased in the experiment with using purified proteins (Fig. 2A).

Prxs may have the ability to metabolize H$_2$S in the presence of cellular components. It has been reported that Cu/Zn superoxide dismutase (SOD) catalyzed H$_2$S oxidation to form polysulfide (56). We deduced that Prx may also have that ability, and this needs to be explored in a further study.

Prxs are antioxidant enzymes that play an important role in redox homeostasis and in redox regulation (28, 29). The mechanism of H$_2$O$_2$ metabolism by Prxs has been well-studied (38, 43). The “peroxidative” cysteine of the catalytic site (C$_p$) attacks H$_2$O$_2$ and is oxidized to sulfenic acid (C$_p$–SOH) in the first step of the catalytic cycle. Then, the resolving cysteine (C$_r$) attacks the (C$_p$–SOH) to release an H$_2$O molecule and form a disulfide bond (C$_p$–C$_r$). Prxs are divided into three classes based on the way the sulfenic acid (C$_p$–SOH) is recycled back to a thiol (C$_p$–SH): typical 2-Cys Prxs, atypical 2-Cys Prxs, and 1-Cys Prxs. In the typical 2-Cys Prxs, the C$_p$–SOH from one subunit is attacked by the C$_r$ from the other subunit, resulting in the formation of an inter-subunit disulfide bond. In the atypical 2-Cys Prxs, both the C$_p$ and the C$_r$ are contained in the same subunit, and the condensation reaction results in the formation of an intramolecular disulfide bond. The 1-Cys Prxs contain only C$_r$ and are without C$_p$. The C$_r$ and C$_p$ residues of PCC7002 Prxl are Cys$^{53}$ and Cys$^{153}$, and they formed an intramolecular disulfide bond.

A probable mechanism of sulfane sulfur reduction by PCC7002 Prxl is also proposed (Fig. 6) based on that of H$_2$O$_2$ metabolism, in which Cys$^{53}$ reacts with sulfane sulfur, such as S$_8$, to produce a persulfide (Cys$^{53}$–SSH), and Cys$^{153}$ attacks Cys$^{53}$–SSH to form an intramolecular disulfide bond (Cys$^{53}$–Cys$^{153}$) and release H$_2$S. In summary, we deduced that PCC7002 Prxl belongs to the atypical 2-Cys Prx family based on its mechanism (37, 40, 43), while it belongs to the Prx5 subfamily based on the analysis by the Deacon Active Site Profiler tool (Fig. 5A).

All six Prxs in PCC7002 were induced by S$_8$ (Fig. 1). However, in this study, only Prxl had the S$_8$ reduction activity. According to a phylogenetic analysis (Fig. 5), Prxl belongs to a separate branch from the other Prxs in PCC7002, the Tpx subfamily, while the sequences near the C$_r$ of the Tpx family are highly conserved (Fig. S5B), suggesting that this region may be the key site for sulfane sulfur reduction, which is also vital for H$_2$O$_2$ reduction. It remains to be investigated whether other Prxs, especially the Prx5-type Prxs, reduce sulfane sulfur. Our analysis showed that 65% of cyanobacteria encode Prx5-type Prx (Fig. 5B). These results indicate the widespread and important roles of Prx5-type Prx in cyanobacteria.

Prxs are most likely the primary enzymes responsible for maintaining intracellular sulfane sulfur homeostasis in cyanobacteria in anoxic or hypoxic conditions (21, 57). In aerobic conditions, PDO oxidizes sulfane sulfur to sulfitc (24). Because the RSS stress is more severe in hypoxic conditions (5, 12), Prxs may play important roles in sulfane sulfur metabolism in anoxic or hypoxic conditions. The action of Prxs against RSS may have been preserved through evolution, as Prxs existed long before oxygen became abundant on Earth (48).

Prxs are known to participate in ROS metabolism (58). Here, we report that they are also involved in RSS metabolism. Cyanobacteria are the oldest surviving microorganisms, and they have experienced the transformation from an anaerobic environment on Earth to an aerobic environment (59, 60). Given the long history of cyanobacterial sulfur exposure, Prxs were most likely first used to resist RSS (5, 60). Interestingly, many of the strategies used against ROS stress, such as catalase, superoxide dismutase, and
OxyR, have also been shown to be involved in coping with RSS stress (20, 36, 56). Here, the expression levels of prxs in PCC7002 were not as sensitive to H₂O₂ induction as they were to induction by S₈ (Fig. 1C), which might be due to of the activity of other H₂O₂ mitigating enzymes, such as catalase and superoxide dismutase. Furthermore, sulfane sulfur might also disturb H₂O₂ homeostasis by downregulating catalase, thereby affecting the expression pattern of prx (Fig. 1). In summary, there is a close relationship between the strategies for coping with ROS and RSS (15, 61).

The maintenance of sulfane sulfur homeostasis in cyanobacteria is of great importance. In aerobic conditions, sulfane sulfur is an important intracellular signaling molecule that is involved in the regulation of critical photosynthesis genes in cyanobacteria. Sulfane sulfur reduction by Prx would help maintain the normal physiology and photosynthesis of cyanobacteria (24). In hypoxia and darkness, elemental sulfur can be used as an electron receptor for sulfur-dependent respiration, which enables cyanobacteria to yield ATP via the fermentation of endogenous stored glycogen (3, 33). However, high concentrations of sulfane sulfur in this environment can also be toxic to cells, so Prx-mediated sulfane sulfur reduction is a key pathway for detoxification, as photosynthesis ceases and oxidation by PDO is excluded in hypoxia and darkness.

In summary, here, a Prx is shown for the first time to act as a sulfur reductase that reduces S₈ to H₂S. Also, cyanobacteria may use Prxs to deal with RSS stress. S₈ induced prxI in PCC7002, and Prxl worked effectively to reduce S₈ to H₂S, thereby improving the tolerance of PCC7002 to S₈. The conserved sequence of Prxl near residue C₈ appears to be important for the activity of Prxl. Sulfane sulfur metabolism by Prxs could be the main strategy by which ancient cyanobacteria coped with RSS stress, which facilitated the survival of cyanobacteria in complex environments, especially oxygen-limited areas in the modern oceans.

MATERIALS AND METHODS

Strains and culture conditions. PCC7002 and its mutants were grown in conical flasks containing medium A (62), supplemented with 1 mg of NaN₃ mL⁻¹ (designed as medium A⁻) under continuous illumination by 50 μmol photons m⁻² s⁻¹ at 30°C. Kanamycin (50 μg/mL) was used to select the prxl mutant. To explore the effect of O₂ concentrations, we cultured PCC7002 by bubbling with a mixture of O₂ and N₂, with O₂ contents of 2%, 10%, and 20%. Escherichia coli (E. coli) was cultured in Luria-Bertani (LB) medium at 37°C. The strains and plasmids used in this paper are listed in Table S1.

Induction, RNA extraction, and qRT-PCR analysis. PCC7002 cells in logarithmic growth with an OD₆₀₀ value of 0.6 to 0.7 were induced by S₈, H₂O₂, and O₂ under continuous illumination by 50 μmol photons m⁻² s⁻¹ at 30°C for 3 h. The cells were then harvested by centrifugation at 10,000 × g at 4°C for 10 min. Total RNA was isolated using the TaKaRa MiniBEST Universal RNA Extraction Kit, and the concentration of RNA was verified by a Qubit 4 instrument (Thermo Fisher). cDNA was produced using the Prime Script RT Reagent Kit with gDNA Eraser (TaKaRa, Beijing, China). The SYBR Premix Ex Taq II Kit (TaKaRa) was used for a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and the reactions were run in a Light Cycler 480 II sequence detection system (Roche, Shanghai, China). Primers for target genes are given in Table S2. The results were analyzed according to the 2⁻ΔΔCT method (64).

Overexpression of Prxs and enzyme activity determination. Recombinant Prxs were fused to the C-terminus of maltose binding protein (MBP) and were overexpressed using the vector pMal-C2X (65, 66). Whole fragments encoding prxl-VI were amplified from PCC7002 genomic DNA using primers pMal-prxl-VI-F/R. Then, the fragments were ligated with pMal-C2X and transformed into E. coli DH5α. The resulting plasmids were transformed into E. coli BL21 (DE3) to overexpress the recombinant Prx fusion proteins. E. coli BL21 (pMal-C2X) and E. coli BL21 (pMal-prxs) were cultured in LB at 37°C to an OD₆₀₀ of 0.6. Next, 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added, and the cells were further cultivated at 30°C for 6 h. For resting cell analysis, cells were collected and resuspended in phosphate-buffered saline (PBS; 50 mM, pH 7.4) at an OD₆₀₀ of 10. Then, S₈ (200 μM) was added to initiate the reaction, and the release of H₂S was determined by the methylene blue method (67). Here, the S₈ was made by dissolving sulfur powder in acetone, in which it was soluble in the range of concentrations we used. For the analysis of cell lysates, the collected cells in PBS were disrupted using a pressure cell homogenizer (SPECH-18; Stansted Fluid Power Ltd., United Kingdom). The total protein content in the cell lysates was adjusted to 20 mg mL⁻¹, and SDS-PAGE was used to verify whether the lysates contained similar amounts of recombinant Prxs. Again, 200 μM S₈ was added to start the reaction, and the release of H₂S was determined by the methylene blue method. For the analysis of the purified protein, induced cells were harvested and resuspended in binding buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA). Then, the cells were disrupted using a pressure cell homogenizer, and the mixture was centrifuged at 20,000 × g for 20 min to acquire crude cell extract. The crude extract was loaded onto amylose resin, and the target protein was eluted using the binding buffer containing 10 mM maltose. The eluted

July/August 2022 Volume 13 Issue 4 10.1128/mbio.01039-22 10
protein solution was then loaded onto a PD-10 desalting column (GE) for buffer exchange to desalting buffer (20 mM NaH2PO4, 10% glycerol, pH 7.6). The purified proteins were then resolved by SDS-PAGE. The reaction mixtures contained 100 μg/mL Prx, 100 μM DTT, 200 μM Sα, and 50 mM HEPES-NaOH (pH 7.0). The control containing DTT and Sα but no Prx was included. The cysteine to serine mutants of PrxI were generated using the primer pairs prxl-C53S-F/R, prxl-C78S-F/R, and prxl-C153S-F/R with a modified QuickChange site-directed mutagenesis method (68, 69). The reduction of Sα by cell lysates of E. coli BL21 (pMal-prxl C53S), E. coli BL21 (pMal-prxl C78S), and E. coli BL21 (pMal-prxl C153S) was detected as described above.

Non-reducing SDS-PAGE. Prxl, Prxl C53S, Prxl C78S, and Prxl C153S with the MBP tag were purified in the same way as described above. Prxl, Prxl C53S, Prxl C78S, and Prxl C153S were released from the fusion with MBP by using Factor Xa at room temperature for 24 h. The released proteins were treated with 250 μM Sα at 25°C for 30 min. After the Sα treatment, 1 mM DTT was added to convert the modified thiol back to reduced thiol. No treatment and treatment with only 1 mM DTT were used as controls. The samples were then resolved by nondenaturing SDS-PAGE, in which the loading buffer contained no DTT or other reducing agents.

Construction of the CstR reporter system. A CstR-based reporter plasmid was constructed by following a reported protocol to assess the ability of Prxl to metabolize sulfane sulfur (53). In Staphylococcus aureus, CstR (Copper-sensing operon repressor [CsoR]-like sulfurtransferase repressor) is a transcriptional repressor that represses the expression of the cst operon, which encodes a putative sulfide oxidation system, by binding to the OP1 and OP2 sites of the cst promoter (70). Here, CstR and the cst promoter with OP1 and OP2 sites were used to regulate the expression of mKate (encoding a red fluorescent protein, mKate). CstR represses the expression of mKate, but sulfane sulfur can act on CstR and depress the repression. In this way, the fluorescence intensity of mKate could be used to characterize the concentration of intracellular sulfane sulfur. We constructed plasmids with the prxl gene expressed, coupled behind the mKate-encoding gene. The prxl gene was cloned using primers cstr-mKate-prxl-F/R that contained 20-bp extensions overlapping the vector fragment. Then, the segments were connected with the CstR-OP1-mKate vector by using a TEDA assembly. The three cysteines in Prxl were all individually mutated to serine, using primer pairs prxl-C53S-F/R, prxl-C78S-F/R, and prxl-C153S-F/R, by a QuickChange site-directed mutagenesis to assess their roles. Correct CstR reporter plasmids were transformed into E. coli BL21 for experiments.

Construction of PCC7002 mutants. A prxl mutant of PCC7002 (PCC7002Δprxl-p) was constructed by homologous recombination as previously reported (24). Briefly, the primer sets prxl-del-1/prxl-del-2 and prxl-del-5/prxl-del-6 (Table S2) were used to acquire the upstream and downstream segments of the prxl gene by PCR from the genomic DNA of PCC7002. The lengths of the segments were about 1,000 bp. The kanamycin resistance cartridge was amplified from pET30a using primers prxl-del-3/prxl-del-4. Long fragments coupling the upstream segment, the kanamycin resistance cartridge, and the downstream segment were obtained by fusion PCR. The fused fragment was connected with the pJET1.2 blunt vector by using the TEDA method (71), and then the resulting vector was transformed into E. coli DH5α by electroporation. Correct transformants were verified by PCR and by sequencing. The correct plasmid was then transformed into PCC7002 by natural transformation. The final transformants were selected using kanamycin (50 μg/mL) and confirmed by PCR.

Toxicity analysis of sulfane sulfur. PCC7002 and PCC7002Δprxl-p in the logarithmic growth phase with an OD730 of 0.6 to 0.7 were treated with Sα for 6 h in sealed centrifugation tubes. After incubation, the cells were washed and resuspended in fresh A1 medium. The cells were diluted with A2 medium to an OD730 of 0.05, and 10 μL were spread on an A3-agar plate. Differences between strains PCC7002 and PCC7002Δprxl-p were observed after cultivation at 30°C under continuous illumination by 50 photons m⁻² s⁻¹ for about 7 days. Furthermore, the growth curves of PCC7002 and PCC7002Δprxl-p were also monitored. Sα was added to the medium at the beginning of the cultivating.

Phylogenetic analysis. 198 cyanobacterial genomes were downloaded from the NCBI database (updated 17 December 2021). The query sequences of the Prxs were based on reported data (Table S3) (46). The Prx candidates in PCC7002 were analyzed by using ClustalW software for sequence alignment and MEGA 7.0 to build neighbor-joining phylogenetic trees. The parameters were: pairwise deletion, p distance distribution, and bootstrap analysis with 1,000 repeats.
REFERENCES

1. de Beer D, Weber M, Chennu A, Hamilton T, Lott C, Macalady J, Klatt JM. 2017. Oxidic and anoxydic photosynthesis in a microbial mat from an anoxic and sulfidic spring. Environ Microbiol 19:1251–1265. https://doi.org/10.1111/1462-2920.13654.

2. Huang S, Wilhelm SW, Harvey HR, Taylor K, Jiao N, Chen F. 2012. Novel lin- eages of Prochlorococcus and Synechococcus in the global oceans. ISME J 6:285–297. https://doi.org/10.1038/ismej.2011.106.

3. Stal LJ, Moezelar R. 1997. Fermentation in cyanobacteria. FEMS Microbiol- ogy Rev 21:179–211. https://doi.org/10.1007/s10464-97000056-9.

4. Meier E, Dick GJ, de Beer D, Grum S, Hubener T, Littmann S, Olsen K, Stuart D, Lavik G, Marchant HK, Klatt JM. 2021. Nitrate respiration and diele migration patterns of diatoms are linked in sediments underneath a microbial mat. Environ Microbiol 23:1442–1453. https://doi.org/10.1111/1462-2920.15345.

5. Schunck H, Lavik G, Desai DK, Großkopf T, Kalvelage T, Löscher CR, Calbeck CM, Lavi G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Callbeck CM, Lavik G, Ferdelman TG, Bus JS, Popa JA, Boreiko CJ, Andjelkovich DA. 1984. A critical review of the literature on hydrogen sul- fur compounds to inorganic polysulfides against resistant bacterial infections. Nature Communication 9:3713. https://doi.org/10.1038/s41467-018-04041-x.

6. Barton LL, Fardeau ML, Fauque GD. 2014. Hydrogen sul- fur compounds to inorganic polysulfides against resistant bacterial infections. Nature Communication 9:3713. https://doi.org/10.1038/s41467-018-04041-x.

7. Kimura H. 2015. Signaling molecules: hydrogen sul- fur. Current Opinion in Biotechnology 34:207–212. https://doi.org/10.1016/j.copbio.2015.10.006.

8. Beauchamp RO, Jr., Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. 1984. A critical review of the literature on hydrogen sul- fur compounds to inorganic polysulfides against resistant bacterial infections. Nature Communication 9:3713. https://doi.org/10.1038/s41467-018-04041-x.

9. Callbeck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

10. Barton LL, Fardeau ML, Fauque GD. 2014. Hydrogen sul- fur: a toxic gas produced by dissimilatory sulfate and sulfur reduction and consumed by microbial oxidation. Met Ions Life Sci 14:237–277. https://doi.org/10.1007/987-94-017-9269-9.

11. Breitburg D, Levin LA, Oschlies A, Greigore M, Chavez FP, Conley DJ, Garcon V, Gilbert D, Gutierrez D, ISensek K, Jacinto GS, Limburg KE, Montes I, Navez SWA, Petcher GC, Rabalais NN, Roman MR, Rose KA, Seibel BA, Telszewski M, Yin I, Górny M, Gutter D, Gutiérrez D, Iseensee K, Jacinto GS, Limburske K, Nakov I, Pastor-Flores D, Dick TP, Schmidt EE, Arnér ES, Nagy P. 2016. A novel per- sul-Script PMID:277. https://doi.org/10.1111/1462-2920.13654.

12. Callbeck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

13. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

14. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

15. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

16. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

17. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.

18. Beck CM, Lavik G, Ferdelman TG, Fuchs B, Gruber-Vodicica HR, Hache PF, Littmann S, Schoffelen NJ, Kalvelage T, Thomsen S, Schunck H, LÖsch CR, Schunck RA, Kuyper MMM, Laroche J. 2013. Giant hydrogen sul- fur plume in the oxygen minimum zone off Peru supports chemolithoautotrophy. PLoS One 8:e68661. https://doi.org/10.1371/journal.pone.0068661.
33. Moozelaar R, Bijvank SM, Stal LJ. 1996. Fermentation and sulfur reduction in the nit-building cyanobacterium Microcystis chthonoplastes. Appl Environ Microbiol 62:1752–1758. https://doi.org/10.1128/aem.62.5.1752-1758.1996.

34. Mueller S, Riedel HD, Stemmele W. 1997. Direct evidence for catalase as the predominant H2O2-removing enzyme in human erythrocytes. Blood 90:4973–4978. https://doi.org/10.1182/blood.V90.12.4973.

35. Nandl A, Yan LJ, Jana CK, Das N. 2019. Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxid Med Cell Longev 2019: 9613090. https://doi.org/10.1155/2019/9613090.

36. Olson KR, Gao Y, DeLeon ER, Arif M, Arif F, Arora N, Straub KD. 2017. Cata- lase as a sulfide-sulfur oxido-reductase: an ancient (and modern?) regulator of reactive sulfur species (RSS). Redox Biol 12:325–339. https://doi.org/10.1016/j.redox.2017.02.021.

37. Dietz KJ. 2011. Peroxiredoxins in plants and cyanobacteria. Antioxid Redox Signal 15:1129–1159. https://doi.org/10.1089/ars.2010.3657.

38. Rhee SG, Chae HZ, Kim K. 2005. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med 38:1543–1552. https://doi.org/10.1016/j.freeradbiomed.2005.02.026.

39. Rocha S, Gomes D, Lima M, Bronze-da-Rocha E, Santos-Silva A. 2015. Peroxiredoxins are thioredoxin dependent. J Bacteriol 191:7477–7488. https://doi.org/10.1128/JB.00831-09.

40. Takahashi T, Oishi M, Nishitani K, Takeda Y, Nakamura-Yamamoto S. 2018. Peroxiredoxin functions in cyanobacteria. mBio 9(5). https://doi.org/10.1128/mbio.01039-22.

41. Pérez-Pérez ME, Mata-Cabana A, Sánchez-Riego AM, Lindahl M, Florencio FJ. 2015. Peroxiredoxin functions of Peroxiredoxin in Cyanobacteria mBio July/August 2022 Volume 13 Issue 4 10.1128/mbio.01039-22

42. Xia Y, Li K, Li J, Wang T, Gu L, Xun L. 2019. Synthetic gene circuits enable Escherichia coli to use endogenous H2S as a signaling molecule for quorum sensing. ACS Synth Biol 8:2113–2120. https://doi.org/10.1021/acssynbio.9b00310.

43. Watanebe S, Ohbayashi R, Shiva Y, Noda A, Kanesaki Y, Chibazakura T, Yoshikawa H. 2012. Light-dependent and asynchronous replication of cyanobacterial multi-copy chromosomes. Mol Microbiol 83:856–865. https://doi.org/10.1111/j.1365-2958.2012.07971.x.

44. Horta BB, de Oliveira MA, Discola KF, Cussioli JR, Netto LE. 2010. Structural and biochemical characterization of peroxiredoxin Qbeta from Xyella fastidiosa: catalytic mechanism and high reactivity. J Biol Chem 285: 16051–16065. https://doi.org/10.1074/jbc.M109.094839.

45. Olson KR, Gao Y, Arif F, Arora N, Patel S, DeLeon ER, Sutton TR, Feelsch M, Cortese-Krott MM, Straub KD. 2018. Metabolism of hydrogen sulfide (H2S) and production of reactive sulfur species (RSS) by superoxide dismutase. Redox Biol 15:74–85. https://doi.org/10.1016/j.redox.2017.11.009.

46. Vaquer-Sunyer R, Duarte CM. 2008. Thresholds of hypoxia for marine biodiversity. Proc Natl Acad Sci U S A 105:15452–15457. https://doi.org/10.1073/pnas.0803831105.

47. Storz G, Jacobson FS, Tartaglia LA, Morgan RW, Silveira LA, Ames BN. 1989. An alkyd hydroperoxide reductase induced by oxidative stress in Salmonella typhimurium and Escherichia coli: genetic characterization and cloning of ahp. J Bacteriol 171:2049–2055. https://doi.org/10.1128/jb.171.4.2049-2055.1989.

48. Dorr M, Kassbohrer J, Grunert C, Brand WA, Werner RA, Gellmann H, Apfel C, Robl C, Weigand W. 2003. A possible prebiotic formation of ammonia from dinitrogen on iron sulfide surfaces. Angew Chem Int Ed Engl 42: 1540–1543. https://doi.org/10.1002/anie.200205031.

49. Hancock JR, Lamp H, Parks DH, Fischer WW, Hugenholtz P. 2017. On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacte- ria. Science 355:1436–1440. https://doi.org/10.1126/science.aal3794.

50. Olson KR. 2019. Hydrogen sulfide, reactive sulfur species and coping with reactive oxygen species. Free Radic Biol Med 140:74–83. https://doi.org/10.1016/j.freeradbiomed.2019.01.020.

51. Stevens SE, Porter RD. 1980. Transformation in Agmenellum quadruplicatum. Proc Natl Acad Sci U S A 77:6052–6056. https://doi.org/10.1073/pnas.77.10.6052.

52. Szekeres E, Sicora C, Dragos N, Druga B. 2014. Selection of proper reference genes for the cyanobacterium Synechococcus PCC 7002 using real-time quantitative PCR. FEMS Microbiol Lett 359:102–109. https://doi.org/10.1111/1574-6968.12574.

53. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-Delta Delta CT method. Methods 25:402–408. https://doi.org/10.1016/S1046-2023(01)00499-X.

54. Waugh DS. 2016. The remarkable solubility-enhancing power of Escherichia coli maltose-binding protein. Postepy Biochem 62:377–382. https://doi.org/10.18388/pb.2016.41.

55. Zhao X, Li G, Liang S. 2013. Several affinity tags commonly used in chromatographic purification. J Anal Methods Chem 2013:581093. https://doi.org/10.1155/2013/581093.

56. Hughes MN, Centenels MN, Moore KP. 2009. Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. Free Radic Biol Med 47: 1346–1453. https://doi.org/10.1016/j.freeradbiomed.2009.09.018.

57. Xia Y, Chu W, Qi O, Xun L. 2015. New insights into the QuikChange pro- tocol: the use of Phusion DNA Polymerase for site-directed mutagenesis. Nucleic Acids Res 43:e12. https://doi.org/10.1093/nar/gku1189.

58. Xia Y, Xun L. 2017. Revised mechanism and improved efficiency of the QuikChange site-directed mutagenesis method. Methods Mol Biol 1469: 367–374. https://doi.org/10.1007/978-1-4939-6472-7_25.

59. Luebke JL, Shen J, Bruce KE, Kehl-Fie TE, Peng H, Skaar EP, Giedroc DP. 2014. The CsoI-like sulfurtransferase repressor (CsrI) is a persulfide sen- sor in Staphylococcus aureus. Mol Microbiol 94:1343–1360. https://doi.org/10.1111/mmi.12835.

60. Xia Y, Li K, Li J, Wang T, Gu L, Xun L. 2019. TX exonuclease-dependent as- sembly offers a low-cost method for efficient cloning and site-directed mutagenesis. Nucleic Acids Res 47:e15–e15. https://doi.org/10.1093/nar/gky1169.