



# Regulation of *fadR* on the ROS defense mechanism in *Shewanella oneidensis*

Qiu Meng · Yinming Xu · Liming Dai ·  
Xuzhe Ge · Pei Qiao

Received: 4 February 2024 / Revised: 11 March 2024 / Accepted: 25 March 2024 / Published online: 6 May 2024  
© The Author(s), under exclusive licence to Springer Nature B.V. 2024

**Abstract** Protein FadR is known as a fatty acid metabolism global regulator that sustains cell envelope integrity by changing the profile of fatty acid. Here, we present its unique participation in the defense against reactive oxygen species (ROS) in the bacterium. FadR contributes to defending extracellular ROS by maintaining the permeability of the cell membrane. It also facilitates the ROS detoxification process by increasing the expression of ROS neutralizers (KatB, KatG, and AhpCF). FadR also represses the leakage of ROS by alleviating the respiratory action conducted by terminal cytochrome *cbb3*-type heme-copper oxidases (*ccoNOQP*). These findings suggest that FadR plays a comprehensive role in modulating the bacterial oxidative stress response, instead of merely strengthening the cellular barrier against the environment. This study sheds light on the complex mechanisms of bacterial ROS defense and offers FadR as a novel target for ROS control research.

**Keywords** FadR · Cell membrane · ROS detoxification · Respiratory action · Terminal oxidase

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10529-024-03487-y>.

Q. Meng · Y. Xu · L. Dai · X. Ge · P. Qiao (✉)  
College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China  
e-mail: peiq@zjut.edu.cn

## Introduction

Reactive oxygen species (ROS) are oxygen-containing molecules that can exist independently in the cytoplasm. This group of molecules includes oxygen free radicals, such as peroxide (-O-O-), superoxide ( $O_2^-$ ), hydroxyl radical ( $\cdot OH$ ), singlet oxygen ( $^1O_2$ ), and ozone ( $O_3$ ) (Jakubczyk et al. 2020). ROS are by-products of oxygen metabolism and play important roles in cell metabolism, such as signaling and tissue homeostasis (Ray et al. 2012). However, under the duress of certain circumstances (for example, UV or heat exposure), cells usually produce excess ROS, leading to oxidative stress (Schieber and Chandel 2014). Oxidative stress can adversely affect cell modification and cause damage to virtually all biomolecules such as DNA, RNA, lipids, and proteins. For example, ROS would cause lipid peroxidation (LPO), the oxidation of unsaturated fatty acids (UFA). The LPO process produces malignant molecules that contribute to many diseases and pathologies (Nam 2011). Under normal circumstances, the generation and peroxidation of lipids is in dynamic equilibrium. However, when cells encounter excess ROS, the balance of LPO is disrupted and cell components that constitute high contents of UFA are damaged by ROS, such as cell membranes and lipoproteins (Pizzino et al. 2017). Therefore, the consumption of unsaturated fatty acids and the corresponding electron transfer process in the respiratory chain is closely related to the generation and detoxification of reactive oxygen

species (ROS). However, conclusive evidence endorsing this hypothesis is still missing and awaits experimental validation.

Cells have evolved many strategies to remove excessive ROS, such as the expression of scavenging enzymes like hydroperoxidase, superoxide dismutase (SOD), catalase (KAT), ascorbate peroxidase (APX) (Fath et al. 2001). These enzymes convert free ROS to water and oxygen independently or cooperatively. For example, the alkyl hydroperoxidase reductase system *ahpCF* that contains *ahpC* and *ahpF* detoxifies H<sub>2</sub>O<sub>2</sub> in many species. The residue Cys<sup>46</sup> in AhpC is oxidized by H<sub>2</sub>O<sub>2</sub> to form a disulfide bond (Cha et al. 2015). As is generally accepted, when the cellular concentration of H<sub>2</sub>O<sub>2</sub> surpasses 20 nM, the scavenging ability of AhpCF meets its limit, and the OxyR system is activated. OxyR is a regulator that directly senses H<sub>2</sub>O<sub>2</sub> by oxidizing two conserved cysteine residues and forming an intramolecular disulfide bond (Cha et al. 2015). In *E. coli* or *Salmonella enterica* serovar, OxyR also acts as an activation regulator to induce downstream catalase expression (Hahn et al. 2002). Not only the scavenging enzymes but also the bacterial terminal oxidases are reported to participate in the control of cellular ROS levels, like quinol oxidase and heme-copper oxidase (Borisov et al. 2021).

To elucidate the possible correlation between fatty acid metabolism and ROS defense in bacteria, we investigated these processes in the *Shewanella oneidensis* MR-1. This bacterium belongs to the phylum Gammaproteobacteria, a facultative anaerobe widely distributed in marine and freshwater environments. MR-1 is an ideal model organism for this study as it thrives in redox-stratified environments with excess ROS (Ikeda et al. 2021) and it contains multiple respiratory pathways for a variety of organic and inorganic substances to generate excessive ROS and electron leakages. The other advantage of using *S. oneidensis* MR-1 as the research subject is its thoroughly studied fatty acid metabolism. This advantage provides a valuable foundation for modifying fatty acid synthesis and understanding its implications in the ROS defense mechanism.

A key factor for modifying the bacterial fatty acids metabolism system is the regulator FadR. It binds to specific DNA sequences and controls the expression of the genes involved in the synthesis, degradation, and transport of fatty acids (Cronan et al. 1998; Zhang et al. 2015). For instance, FadR

down-regulates several genes in the fatty acid degradation pathway, including *fadE*, *fadBA*, *fadH*, and *fadIJ* (Feng and Cronan 2009) in *E. coli*. It also down-regulates *fadD* that involved in the conversion of fatty acids to acyl-CoAs, and *fadL* which transports fatty acids across the cell membrane (Cronan 2021). FadR up-regulates *fabA* and *fabB* which count for the biosynthesis of UFA (Campbell and Cronan 2001). The deletion of *fadR* represses the UFA biosynthesis and enhances the fatty acid degradation, thus reducing the total UFA concentrations (Nunn et al. 1983). Correspondingly, the over-expression of FadR could slow down the fatty acid degradation and enhance UFA biosynthesis, thus increasing the accumulation of cellular UFA (Luo 2014).

As shown above, the modification of *fadR* is a feasible approach for changing the cellular UFA concentration, and this study will be focused on the physiological consequences of the ROS-related metabolism processes brought by the *fadR* modifications. The results showed that the *fadR* knockout MR-1 mutant ( $\Delta fadR$ ) is more sensitive to H<sub>2</sub>O<sub>2</sub>, and this sensitivity was related to the compromised membrane permeability of MR-1, the enhanced respiration intensity, and the down-regulated ROS-scavenging enzymes (AhpCF, KatB, and KatG). This is the first evidence of FadR's impact on the overall bacterial ROS defense system.

## Materials and methods

### Strains, plasmids, and chemicals

The strain *Shewanella oneidensis* MR-1, the plasmid pHGEI01-*lacZ*, and the plasmid pHGEptac were kind gifts given by Dr. Haichun Gao from Zhejiang University. The strains DH5 $\alpha$  and WM3064 were commercial cells purchased from manufacturer TsingkeBiotechnology Co., Ltd. The rest of the strains and plasmids used in this study were constructed in-house. The abbreviations of the strains with genetic modifications are explained in detail in Table 1. All the chemicals used in this study were purchased from Sinopharma Co., Ltd.

**Table 1** Plasmids and strains used in this study

Strain or plasmid	Description
pHGEI01- <i>lacZ</i>	Integrative plasmid pHGEI01 with <i>lacZ</i> reporter
pHGEI01-P <sub>katB</sub> - <i>lacZ</i>	pHGEI01- <i>lacZ</i> complemented with promoter P <sub>katB</sub>
pHGEI01-P <sub>ccoNOQP</sub> - <i>lacZ</i>	pHGEI01- <i>lacZ</i> complemented with promoter P <sub>ccoNOQP</sub>
pHGEptac- <i>fadR</i>	IPTG-inducible plasmid pHGEptac expressing <i>fadR</i>
pHGEptac- <i>fabB</i>	IPTG-inducible plasmid pHGEptac expressing <i>fabB</i>
DH5 $\alpha$	Host strain for plasmids construction
WM3064	Donor strain for conjugation with <i>dapA</i> knockout
WT	Wild type <i>S. oneidensis</i> MR-1 strain
$\Delta$ <i>fadR</i>	<i>S. oneidensis</i> MR-1 strain with <i>fadR</i> knockout
$\Delta$ <i>fabB</i>	<i>S. oneidensis</i> MR-1 strain with <i>fabB</i> knockout
$\Delta$ <i>amiB</i>	<i>S. oneidensis</i> MR-1 strain with <i>amiB</i> knockout
$\Delta$ <i>fadR</i> /P <sub>tac</sub> - <i>fadR</i>	$\Delta$ <i>fabB</i> complemented by plasmid pHGEptac- <i>fadR</i>
$\Delta$ <i>fabB</i> /P <sub>tac</sub> - <i>fabB</i>	$\Delta$ <i>fadR</i> complemented by plasmid pHGEptac- <i>fabB</i>
WT/P <sub>katB</sub> - <i>lacZ</i>	WT containing pHGEI01-P <sub>katB</sub> - <i>lacZ</i>
$\Delta$ <i>fadR</i> /P <sub>katB</sub> - <i>lacZ</i>	$\Delta$ <i>fadR</i> containing pHGEI01-P <sub>katB</sub> - <i>lacZ</i>
WT/P <sub>ccoNOQP</sub> - <i>lacZ</i>	WT containing pHGEI01-P <sub>ccoNOQP</sub> - <i>lacZ</i>
$\Delta$ <i>fadR</i> /P <sub>ccoNOQP</sub> - <i>lacZ</i>	$\Delta$ <i>fadR</i> containing pHGEI01-P <sub>ccoNOQP</sub> - <i>lacZ</i>

### Disk diffusion assay

The disk diffusion assay was used to test the sensitivity to hydrogen peroxide of *S. oneidensis* MR-1 strains with different genetic modifications. The cells in the log phase were collected at 4000 rpm for 2 min and adjusted to 10<sup>9</sup> cells/ml. The cell mixture was diluted 5 times with fresh LB medium before being spread on the LB plates containing IPTG (0.1 mM) at 30 °C for 24 h. Two hundred microliters of cell culture in the mid-exponential phase were spread on LB plates. After 6 h inoculation, a circular paper disk of 6 mm diameter soaked with 10  $\mu$ l H<sub>2</sub>O<sub>2</sub> was placed on the bacterial lawn at 30 °C for 16 h.

### Droplet assay

The droplet assay was used to evaluate the growth-inhibition effects of SDS on the *S. oneidensis* MR-1 strains with different genetic modifications. *S. oneidensis* MR-1 cells in the log phase were collected by centrifugation (4000 rpm) and adjusted to 10<sup>9</sup> cells/ml. Then, a serial dilution by tenfold was performed by fresh LB medium. Diluted cell culture (5  $\mu$ l) was dropped onto the LB plates supplemented with IPTG (0.1 mM) and SDS of different concentrations and inoculated at 30 °C for 24 h.

### $\beta$ -Galactosidase activity assay

The  $\beta$ -galactosidase activity was determined with the *E. coli lacZ* integrated reporter gene pHGEI01 (Meng et al. 2018). Briefly, we amplified a sequence approximately 500 bp upstream of the DNA sequence that might contain a promoter for the gene of interest on the 5'-end of the *lacZ* gene. The vector was constructed in *E. coli* DH5 $\alpha$  and transferred into *S. oneidensis* MR-1 through the conjugation of *E. coli* WM3064. *S. oneidensis* MR-1 cells in log-phase (optical density 600 nm OD<sub>600</sub> ~ 0.4) were collected by centrifugation, washed with PBS, and then subjected to o-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) -based assays as described previously. The  $\beta$ -galactosidase activity was determined by monitoring the color development at 420 nm using a TECAN microplate reader, and the results are presented as Miller Units.

### Minimum inhibitory concentration (MIC) assay

MR-1 was cultured overnight, and 30  $\mu$ l of the bacterial solution was transferred to a 3 ml LB liquid medium and incubated at the corresponding temperature with a shaking machine to OD<sub>600</sub> ~ 0.5 to ensure that the strain was in the logarithmic growth phase. Take a certain amount of bacterial liquid in the test

tube, add LB liquid medium, and dilute it 5000 times for use. The H<sub>2</sub>O<sub>2</sub> was diluted in a new 96-well plate according to a two-fold concentration gradient, and then the bacteria solution was added and mixed to set up a control group. The 96-well plates were placed in the corresponding temperature incubator for 16 to 24 h, and then the absorbance value was measured and recorded by a microplate reader.

### Microscopy

MR-1 was cultivated to the mid-logarithmic (OD<sub>600</sub>~0.4), mixed 1:1 with 0.2 M H<sub>2</sub>O<sub>2</sub>, and spotted onto a glass slide containing LB medium. LW300LHT phase contrast microscope was employed to observe cell morphology. Micrographs were captured with a camera.

### Cytochrome oxidase activity assay

The Nadi test was used for visual analysis of cytochrome *cbb3* oxidase activity (Wan et al. 2017). Three microliters of each culture at the mid-log phase under test were dropped onto LB plates, and the plates were incubated for 24 h. A solution of 0.5%  $\alpha$ -naphthol in 95% ethanol and 0.5% N, N-dimethyl- $p$ -phenyleneidiamine monohydrochloride (DMPD) was applied to cover the droplets developed. The formation of indophenols blue was timed as an indicator of cytochrome *cbb3* activity.

### FOX assay

The ferrous oxidation-xylenol orange (FOX) assay was employed to test the residual H<sub>2</sub>O<sub>2</sub> on the outside of *S. oneidensis* MR-1 cells after H<sub>2</sub>O<sub>2</sub> treatment (Feng et al. 2020). The reagent I contained 100 mM mannitol and 125  $\mu$ M dimethylthiophenol. The reagent II contained 25 mM ferrous ammonium sulfate and 2.5 M sulfuric acid. The FOX working reagent was made by mixing reagent I and reagent II at a ratio of 100:1. The standard curve was made by mixing 20  $\mu$ l H<sub>2</sub>O<sub>2</sub> (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5 mM) with 180  $\mu$ l FOX reagent. Cultivate different strains until OD<sub>600</sub> reaches 0.5 and lyse the cells with ultrasonication. Then, clarify 50  $\mu$ l samples with a 25  $\mu$ m filter tip at 0, 1, 5, and 10 min. Mix 20  $\mu$ l filtered sample with 180  $\mu$ l FOX reagent in a 96-well plate and incubate at 37 °C for 30 min. Afterward,

take the absorbance of the mixture at a wavelength of 560 nm. The H<sub>2</sub>O<sub>2</sub> concentration can be calculated according to the standard curve.

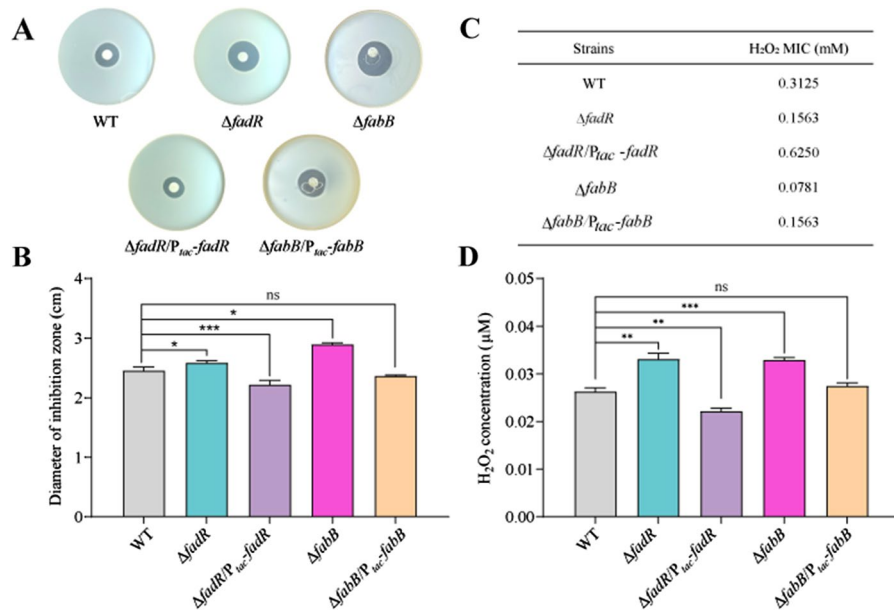
## Results and discussion

FadR-mediated fatty acids synthesis is directly related to the defense against ROS by MR-1

As is shown in Fig. 1A, with the treatment of H<sub>2</sub>O<sub>2</sub>, a larger bacteriostatic zone appeared on the cell lawn of mutant MR-1 strain  $\Delta$ *fadR* (with the *fadR* knockout) than the WT (wild-type MR-1 strain). When the  $\Delta$ *fadR* was complemented by a plasmid pHGE*P*<sub>*tac*</sub>-*fadR* that expresses FadR ( $\Delta$ *fadR*/*P*<sub>*tac*</sub>-*fadR*), its H<sub>2</sub>O<sub>2</sub> resistance was recovered, if not enhanced (Fig. 1A, B, C). This means that the defense mechanism in *S. oneidensis* MR-1 against extracellular ROS is enhanced by *fadR*. A similar observation was made during the investigation on *fabB* (3-oxoacyl-(acyl-carrier-protein) synthase) that catalyzes a key reaction in UFA synthesis, the elongation of the cis-3-decenoyl-ACP (Feng and Cronan 2011). Like *fadR* knockout,  $\Delta$ *fabB* also manifested deteriorated tolerance against H<sub>2</sub>O<sub>2</sub>, and this defect was remedied by overexpressing the *fabB* gene on pHGE*P*<sub>*tac*</sub>-*fabB* (Fig. 1A, B, C). These observations manifest that the synthesis of fatty acids controlled by *fadR* helps MR-1 cells defend ROS from the outside.

FadR participates in ROS defense by maintaining the permeability of cell membrane

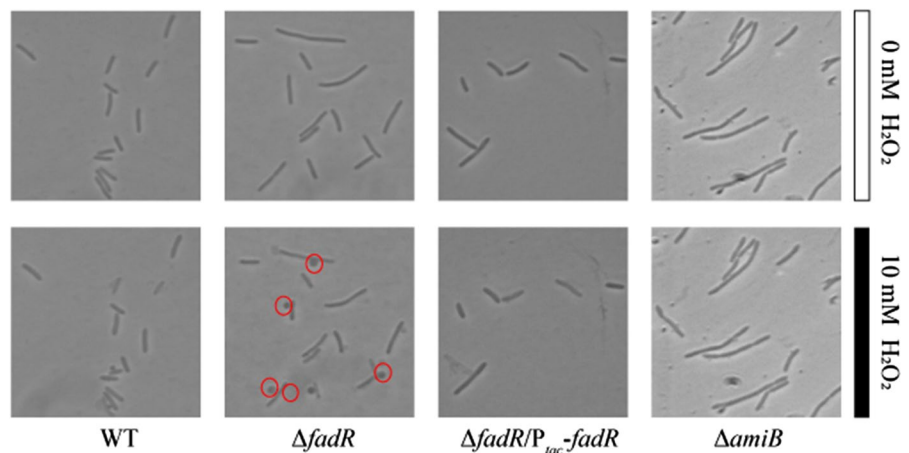
As *fadR* functions to increase intracellular fatty acid concentration and maintain the cell membrane integrity, the cell permeability towards ROS should also be subjected to *fadR* regulation. Phase-contrast microscopy images revealed no noticeable abnormality in cell morphology for wild-type MR-1 cells in the presence of 10 mM H<sub>2</sub>O<sub>2</sub> (Fig. 2). However, when the *fadR* gene was knocked out, the MR-1 cell became longer and an increased rate of cell envelope rupture (from 22.8% to 39.3%) was observed in the presence of 10 mM H<sub>2</sub>O<sub>2</sub> (Fig. 2). When *fadR* was complemented back to  $\Delta$ *fadR*, the  $\Delta$ *fadR*/*P*<sub>*tac*</sub>-*fadR* cell manifested reduced rupture rate than the  $\Delta$ *fadR* (4.8%). Since the  $\Delta$ *fadR* cells were longer than the WT, to rule out the possibility that the compromised



**Fig. 1** Genes *fadR* and *fabB* facilitate the bacterial resistance against ROS. **A** The inhibition effects of H<sub>2</sub>O<sub>2</sub> on the cell lawn of *Shewanella oneidensis* MR-1 with different genetic modifications. The abbreviations of the strains with genetic modifications are explained in detail in Table 1. **B** The diameter of

the inhibition zone formed by H<sub>2</sub>O<sub>2</sub> treatment to these strains. **C** The H<sub>2</sub>O<sub>2</sub> minimum inhibitory concentration (MIC) of these strains. **D** The residual concentration of H<sub>2</sub>O<sub>2</sub> generated by these strains. Biological triplicates were performed with the data presented as means ± SEM

**Fig. 2** Gene *fadR* mediates cell permeability by changing the contents of the cell membrane. The upper/lower panels show the cell morphology of the genetically modified MR-1 strains without/with the treatment of hydroperoxide. The strains are named in Fig. 1



ROS resistance was due to the changed cell morphology or cell wall components, we knocked out the *amiB* (N-acetylmuramoyl-L-alanine amidase) gene from MR-1. AmiB belongs to the hydrolase’s protein family, it specifically hydrolyzes the carbon–nitrogen bonds and cleaves the link between N-acetyl muramyl residues and amino acid residues in cell wall glycopeptides, it participates in the synthesis of bacterial

cell wall (Yakhnina et al. 2015). The mutant  $\Delta amiB$  also manifests longer cell length, but the  $\Delta amiB$  was not more vulnerable to ROS than the WT with no obvious cell rupture observed under the microscope (Fig. 2). These results suggest it is the cell membrane permeability that plays a central role in the ROS defense mechanism, instead of cell morphology or cell wall. This theory is also validated in the defense

mechanism of MR-1 against sodium dodecyl sulfate (SDS). Like the  $H_2O_2$  treatment, treatment of SDS at different concentrations has also manifested stronger harm to  $\Delta fadR$  than the WT or  $\Delta fadR/P_{tac-fadR}$  (Fig. 3). These results showed that *fadR* contributes to the ROS defense mechanism in M-1 by maintaining the integrity and permeability of cell membrane.

#### FadR represses the expression of terminal oxidases CcoNOQP to reduce ROS generation

Not only the defense efficiency against extracellular ROS was enhanced under *fadR* regulation, but the cellular ROS generation was also reduced. The FOX assay was used to measure the concentration of hydroperoxide generated by MR-1 (Banerjee et al. 2003). The results showed that the concentration of  $H_2O_2$  generated by MR-1 increased with the knockout of *fadR* or *fabB*, and decreased when *fadR* or *fabB* was complemented back (Fig. 1D). This means that the function of *fadR* in the ROS defense is not only controlling the cell membrane permeability, but it also impacts the cellular ROS metabolism.

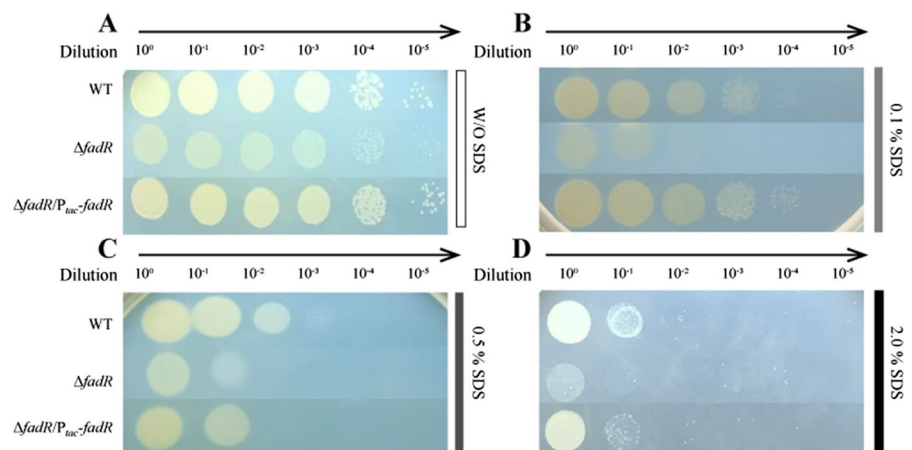
*S. oneidensis* MR-1 has three terminal oxidases for respiration: *bd*-type quinol oxidase (*cydABX*), *caa3*-type heme-copper oxidases (*SO4606-9*), and *cbb3*-type heme-copper oxidases (*ccoNOQP*) (Laz et al. 2014; Kouzuma et al. 2012). The electron transfer process is closely related to the activity of terminal oxidase. Electron leakage occurs during the electron transfer process, and higher terminal oxidase activity could generate more leaked electrons, resulting in more accumulation of ROS (Schönfeld

and Wojtczak 2008). Under microaerobic and aerobic conditions, the predominant oxidase on the cell membrane of MR-1 is CcoNOQP (Laz et al. 2014). The Nadi reaction was performed to test the terminal oxidase activity in WT and mutant MR-1 strains (Yu et al. 2021). After five minutes of Nadi reaction, the cell lawn of  $\Delta fadR$  formed a distinct dark blue circle, while the color of WT or  $\Delta fadR/P_{tac-fadR}$  was shallower, indicating a stronger respiration in  $\Delta fadR$  (Fig. 4A). Since the activity of the promoter of *ccoNOQP* ( $P_{ccoNOQP}$ ) was higher in  $\Delta fadR$  than the WT, the enhanced respiration activity was possibly due to the increased expression level of *ccoNOQP* (Fig. 4A). As a result, the *fadR* knockout induced an enhanced respiration pathway that generated more ROS, which has been observed in Fig. 1D. Thus, in the WT MR-1, *fadR* represses respiration activity by reducing the expression of *ccoNOQP* to control the overall ROS generation.

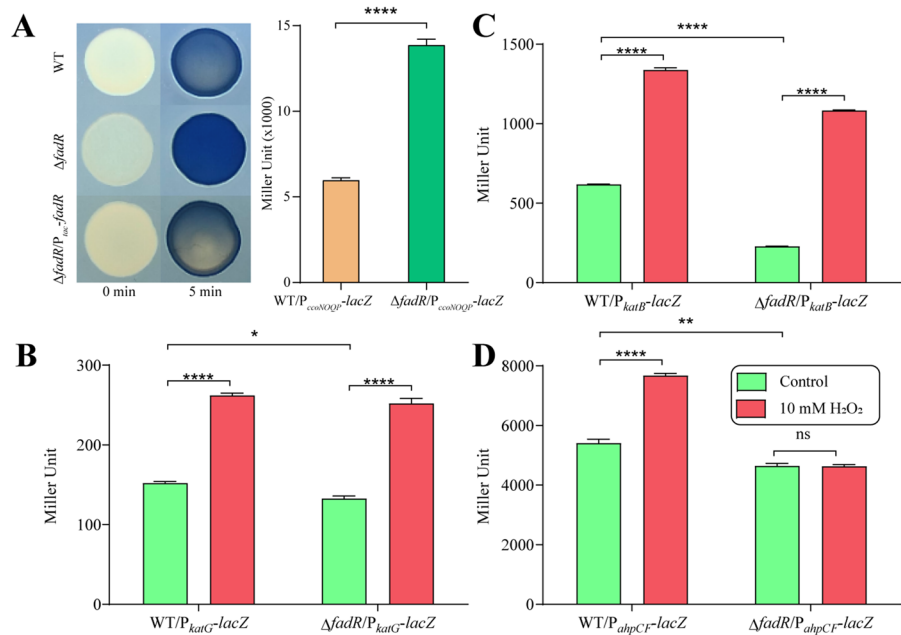
#### FadR enhances ROS detoxification by promoting the expression of ROS-scavenging enzymes

We next investigated the regulation of FadR on the ROS scavenging enzymes KatB, KatG, and AhpCF (Feng et al. 2020; Toporek, et al. 2023). As is shown in Fig. 4B–D, the promoter activity of these three genes was significantly enhanced in the WT in the presence of 10 mM  $H_2O_2$ , endorsing the participation of these genes in ROS metabolism. When *fadR* was knocked out, the activity of these promoters was lower than the WT in the absence of  $H_2O_2$ , suggesting the potentially positive regulatory effects of *fadR*

**Fig. 3** Gene *fadR* helps sustain the cellular permeability by sustaining the cell membrane integrity. **A–D** The cell lawn of *Shewanella oneidensis* MR-1 at the presence of SDS of concentration of 0%, 0%, 0.5%, and 2.0%



**Fig. 4** Gene *fadR* mediates the ROS defense mechanism by repressing the generation and enhancing the detoxification of ROS. **A** The activity of the ROS-generating cytochrome *cbb3* type oxidase and its promoter  $P_{cbb3}$  was increased in  $\Delta fadR$ . **B–D** The promoters of the detoxification genes *kcatB*, *kcatG*, and *ahpCF* were repressed in  $\Delta fadR$ , and inductive effects of  $H_2O_2$  toward  $P_{ahpCF}$  were eliminated. Biological triplicates were performed with the data presented as means  $\pm$  SEM



on these genes under normal conditions (Fig. 4B–D). Interestingly, the  $H_2O_2$  stress did not increase the promoter activity of *ahpCF* in  $\Delta fadR$  while the other two promoters were upregulated (Fig. 4D). This result implied that the regulatory effects of *fadR* on the ROS scavenging enzyme AhpCF were higher than that of KatB or KatG under ROS stress.

To sum up, this study revealed that fatty acid metabolism regulator FadR participates in the ROS defense mechanism in *S. oneidensis* MR-1 by preserving the cell membrane permeability, repressing the terminal cytochrome *cbb3*-type heme-copper oxidases, and activating the ROS scavenging enzymes *kcatB*, *kcatG*, and *ahpCF*.

**Author contributions** P. Q. and Q. M. contributed to the funding used in this work. Q.M. and P.Q. conceived the scope of this study and designed the experiments. Y.X., L.D., and X.G. performed the data collection. P.Q. drafted the first manuscript. P.Q., Q.M., and Y.X. revised, edited, and polished the manuscript. All authors approved the final version for publication and agreed to be accountable for all aspects of the work.

**Funding** This work was supported by the National Natural Science Foundation of China (no. 32300018), and the Basic Public Welfare Research Program of Zhejiang Province (LQ23C010003 and LQ22C010004).

**Declarations**

**Competing interests** The authors declare no competing interests.

## References

- Jakubczyk K et al (2020) Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski* 48(284):124–127
- Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* 24(5):981–990
- Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. *Curr Biol* 24(10):R453–R462
- Nam TG (2011) Lipid peroxidation and its toxicological implications. *Toxicol Res* 27(1):1–6
- Pizzino G et al (2017) Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev* 2017:8416763
- Fath A, Bethke PC, Jones RL (2001) Enzymes that scavenge reactive oxygen species are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. *Plant Physiol* 126(1):156–166
- Cha MK et al (2015) Characterization of two alkyl hydroperoxide reductase C homologs alkyl hydroperoxide reductase C\_H1 and alkyl hydroperoxide reductase C\_H2 in *Bacillus subtilis*. *World J Biol Chem* 6(3):249–264
- Hahn JS, Oh SY, Roe JH (2002) Role of OxyR as a peroxide-sensing positive regulator in *Streptomyces coelicolor* A3(2). *J Bacteriol* 184(19):5214–5222
- Borisov VB et al (2021) ROS Defense Systems and Terminal Oxidases in Bacteria. *Antioxidants* 10(6):839

- Ikeda S et al (2021) *Shewanella oneidensis* MR-1 as a bacterial platform for electro-biotechnology. *Essays Biochem* 65(2):355–364
- Cronan J, John E, Subrahmanyam S (1998) FadR, transcriptional co-ordination of metabolic expediency. *Mol Microbiol* 29(4):937–943
- Zhang H et al (2015) Binding of *Shewanella* FadR to the fabA fatty acid biosynthetic gene: implications for contraction of the fad regulon. *Protein Cell* 6(9):667–679
- Feng Y, Cronan JE (2009) *Escherichia coli* Unsaturated Fatty Acid Synthesis: complex transcription of the FabA gene and in vivo identification of the essential reaction catalyzed by FabB. *J Biol Chem* 284(43):29526–29535
- Cronan JE (2021) The *Escherichia coli* FadR transcription factor: Too much of a good thing? *Mol Microbiol* 115(6):1080–1085
- Campbell JW, Cronan JE (2001) *Escherichia coli* FadR positively regulates transcription of the fabB fatty acid biosynthetic gene. *J Bacteriol* 183(20):5982–5990
- Nunn WD et al (1983) Role for fadR in unsaturated fatty acid biosynthesis in *Escherichia coli*. *J Bacteriol* 154(2):554–560
- Luo Q et al (2014) Transcription factors FabR and FadR regulate both aerobic and anaerobic pathways for unsaturated fatty acid biosynthesis in *Shewanella oneidensis*. *Front Microbiol* 5:736
- Meng Q, Liang H, Gao H (2018) Roles of multiple KASIII homologues of *Shewanella oneidensis* in initiation of fatty acid synthesis and in cerulenin resistance. *Biochim Biophys Acta Mol Cell Biol Lipids* 1863(10):1153–1163
- Wan F, Shi M, Gao H (2017) Loss of OxyR reduces efficacy of oxygen respiration in *Shewanella oneidensis*. *Sci Rep* 7(1):42609
- Feng X, Guo K, Gao H (2020) Plasticity of the peroxidase AhpC links multiple substrates to diverse disulfide-reducing pathways in *Shewanella oneidensis*. *J Biol Chem* 295(32):11118–11130
- Feng Y, Cronan JE (2011) Complex binding of the FabR repressor of bacterial unsaturated fatty acid biosynthesis to its cognate promoters. *Mol Microbiol* 80(1):195–218
- Yakhnina AA, McManus HR, Bernhardt TG (2015) The cell wall amidase AmiB is essential for *Pseudomonas aeruginosa* cell division, drug resistance and viability. *Mol Microbiol* 97(5):957–973
- Banerjee D et al (2003) Measurement of plasma hydroperoxide concentration by FOX-1 assay in conjunction with triphenylphosphine. *Clin Chim Acta* 337(1–2):147–152
- Le Laz S et al (2014) A biochemical approach to study the role of the terminal oxidases in aerobic respiration in *Shewanella oneidensis* MR-1. *PLoS ONE* 9(1):e86343
- Kouzuma A, Hashimoto K, Watanabe K (2012) Influences of aerobic respiration on current generation by *Shewanella oneidensis* MR-1 in single-chamber microbial fuel cells. *Biosci Biotechnol Biochem* 76(2):270–275
- Schönfeld P, Wojtczak L (2008) Fatty acids as modulators of the cellular production of reactive oxygen species. *Free Radic Biol Med* 45(3):231–241
- Yu Q, Sun W, Gao H (2021) Thiosulfate oxidation in sulfur-reducing *Shewanella oneidensis* and its unexpected influences on the cytochrome c content. *Environ Microbiol* 23(11):7056–7072
- Toporek Y et al (2023) Probing cytoplasmic peroxide metabolism in *Shewanella oneidensis*. *FEMS Microbiol Lett* 370:fnad075

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.