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MsrR is a thiol-based oxidation-sensing regulator of the XRE family that modulates *C. glutamicum* oxidative stress resistance

Meiru Si^{1*†}, Can Chen^{2†}, Jingyi Zhong¹, Xiaona Li¹, Yang Liu¹, Tao Su¹ and Ge Yang^{1*}

Abstract

Background: *Corynebacterium glutamicum* thrives under oxidative stress caused by the inevitably extreme environment during fermentation as it harbors antioxidative stress genes. Antioxidant genes are controlled by pathway-specific sensors that act in response to growth conditions. Although many families of oxidation-sensing regulators in *C. glutamicum* have been well described, members of the xenobiotic-response element (XRE) family, involved in oxidative stress, remain elusive.

Results: In this study, we report a novel redox-sensitive member of the XER family, MsrR (multiple stress resistance regulator). MsrR is encoded as part of the *msrR-3-mst* (3-mercaptopyruvate sulfurtransferase) operon; *msrR-3-mst* is divergent from multidrug efflux protein *MFS*. MsrR was demonstrated to bind to the intergenic region between *msrR-3-mst* and *mfs*. This binding was prevented by an MsrR oxidation-mediated increase in MsrR dimerization. MsrR was shown to use Cys62 oxidation to sense oxidative stress, resulting in its dissociation from the promoter. Elevated expression of *msrR-3-mst* and *mfs* was observed under stress. Furthermore, a Δ *msrR* mutant strain displayed significantly enhanced growth, while the growth of strains lacking either *3-mst* or *mfs* was significantly inhibited under stress.

Conclusion: This report is the first to demonstrate the critical role of MsrR-3-MST-MFS in bacterial stress resistance.

Keywords: Oxidative stress, MsrR, Transcription regulation, *Corynebacterium glutamicum*

Background

Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•-}), hydroxyl radical (•OH), hydroperoxy radical (HO₂•), singlet oxygen (¹O₂), and organic hydroperoxides (OHPs), are inevitable byproducts of aerobic respiration that are also generated under environmental stress by perturbation of the electron transfer chain [1]. ROS can react with the membrane, free fatty acids, and other macromolecules via free radical chain reactions, resulting in the production

of a wide spectrum of detrimental carbonyl-containing compounds [2, 3]. The excessive production of ROS is harmful to living systems as it induces oxidative stress and causes subsequent cellular damage to molecules such as DNA, proteins, and lipids [4]. To ensure survival in a hostile environment, versatile resistance defense mechanisms, such as eliminating ROS, deterring the transformation of ROS into more toxic compounds and repairing damaged biomacromolecules, have been developed [5–7]. Low-molecular-weight (LMW) thiols and multiple antioxidant enzymes play crucial roles in defense mechanisms. When bacteria encounter oxidative stress due to a specific ROS, they modulate the expression of the corresponding resistance enzymes [8, 9]. To achieve this, bacteria use pathway-specific transcription factors that

*Correspondence: smr1016@126.com; yangge100@126.com

†Meiru Si and Can Chen contributed equally to this work

¹ College of Life Sciences, Qufu Normal University, Qufu 273165, Shandong, China

Full list of author information is available at the end of the article



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act in response to specific ROS and coordinate the appropriate oxidative stress-associated genetic response. Thus, the regulation of antioxidant expression is an important issue. The constant sensing of ROS can be mediated by oxidation of one or more thiols in regulators [10].

Many of the best characterized bacterial sensors of ROS, such as the LysR (DNA-binding transcriptional dual-lysine regulator) family regulator OxyR (the thiol-based redox sensor for peroxides) [11, 12], zinc-associated extracytoplasmic function (ECF)-type sigma factor H (SigH) [13, 14], the ferric uptake regulator (Fur) family regulator PerR (a peroxide regulon repressor) [15], the MarR (multiple antibiotics resistance regulators) family regulator OhrR (an organic hydroperoxide resistance regulator) [16], the TetR (a tetracycline repressor protein) family regulator NemR (a N-ethylmaleimide regulator) [17], and the AraC (cytosine β -D-arabinofuranoside) family regulator RclR (a regulator of hypochlorous acid (HOCl)-specific resistance) [18], have been shown to contribute to or to modulate antioxidant gene expression [11–18]. These sensors specifically sense ROS via a thiol-based mechanism [11–18]. Upon exposure to oxidative stress, these regulators are activated or inhibited by morphological changes caused by cysteine oxidation, after which they are released from or bind the promoters of target genes, leading to the upregulation of these target genes. Interestingly, more recently, Hu et al. found that the xenobiotic response element (XRE) family transcriptional regulator SrtR (stress response transcriptional regulator) in *Streptococcus suis* is also involved in oxidative stress tolerance, the only report of stress resistance in a member of the XRE family thus far [19]. Unfortunately, its exact molecular mechanism related to oxidant sensing, its target genes, and its interplay with other regulators have not yet been described. XREs, which are widely distributed in living organisms, control the expression of virulence factors, antibiotic synthesis and resistance genes, and stress response genes [20]. Although the XRE family is the second most common family of regulators in bacteria, XRE family members have been reported in only a limited number of bacteria, such as *Staphylococcus aureus* [21], *Rhizobium etli* [22], *S. suis* [19], and *Chloroflexus aurantiacus* [23]. Until now, research on XREs has mainly focused on XREs in eukaryotes. In eukaryotes, the regulatory mechanism of XREs is well known but different from that of ROS-sensing regulators; many xenobiotics acting as inducers, such as oxidants, heavy metals, antibiotics, and toxins, bind aromatic hydrocarbon (Ah) receptors in the cytoplasm to form an Ah receptor-ligand complex, which then interacts with XREs in the nucleus, finally stimulating

the transcription of the target genes [24, 25]. However, the functions of XREs in eukaryotes were not reported to be related to oxidative stress or other tolerance to other stresses. Thus, much research about XREs remains to be carried out, especially on the functions and mechanisms of XREs related to oxidative stress and tolerance to other stresses in bacteria.

Corynebacterium glutamicum, a nonpathogenic, GC-rich, and gram-positive bacterium, is not only an important industrial strain for the production of amino acids, nucleic acids, organic acids, alcohols, and biopolymers but also a key model organism for the study of the evolution of pathogens [26]. During the fermentation process, *C. glutamicum* inevitably encounters a series of unfavorable conditions [27, 28]. However, *C. glutamicum* thrives under the adverse stresses of the fermentation process using several antioxidant defenses, such as millimolar concentrations of mycothiol (MSH) and antioxidant enzymes [29–32]. Although many thiol-based redox-sensing regulators from different transcription factor families, including LysR (OxyR), MarR [RosR (regulator of oxidative stress response)/OhsR (organic hydroperoxides stress regulator)/CosR (*C. glutamicum* oxidant-sensing regulator)/QorR (quinone oxidoreductase regulator)], TetR [OsrR (Oxidative stress response regulator)], ArsR [CyeR (*Corynebacterium* yellow enzyme regulator)], and SigH, have been well studied [14, 29–31, 33–35], whether the XRE proteins of *C. glutamicum* play a role in protecting against oxidative stress by directly regulating antioxidant genes remains obscure. The putative XRE family transcriptional regulator NCgl2679, named MsrR (multiple stress resistance regulator) due to the results of this study, is not only located immediately downstream and in the opposite direction of the multidrug efflux protein NCgl2680 (MFS) but also organized in an operon with 3-Mercaptopyruvate sulfurtransferase (NCgl2678, 3-MST) and the putative protein NCgl2677. This genetic organization allowed us to investigate the function of *C. glutamicum* MsrR in response to environmental stresses. In the present study, MsrR was found to directly control expression of the *msrR-3-mst-ncgl2677* operon and the *mfs* gene as a thiol-based redox-sensing transcriptional repressor. The expression of *msrR*, *3-mst* and *mfs* was induced by oxidative stress. MsrR contains only one cysteine residue at position 62 (Cys62). Upon oxidative stress induced by various xenobiotics, MsrR underwent dimerization and lost its DNA-binding activity through the formation of an intermolecular disulfide bond between the Cys62 residue of each subunit. These findings suggest that MsrR is a redox-sensing transcriptional regulator involved in the oxidative stress response of *C. glutamicum* by its regulation of *3-mst* and *mfs* expression.

Methods

Strains and culture conditions

Bacterial strains and plasmids used in this study were listed in Additional file 1: Table S1. *Escherichia coli* and *C. glutamicum* were cultured in Luria–Bertani (LB) broth aerobically or on LB agar plates as previously reported [36]. $\Delta msrR$, $\Delta 3\text{-}mst$ and Δmfs in-frame deletion mutants were produced as described [37]. Briefly, the pK18*mobsacB*- $\Delta msrR$ plasmid was transformed into *C. glutamicum* wild type (WT) through electroporation to carry out single crossover. The transconjugants were selected on LB agar medium containing 40 $\mu\text{g/ml}$ nalidixic acid and 25 $\mu\text{g/ml}$ kanamycin. Counter-selection for markerless in-frame deletion was performed on LB agar plates with 40 $\mu\text{g/ml}$ nalidixic acid and 20% sucrose [37]. Strains growing on this plate were tested for kanamycin sensitivity (KAN^S) by parallel picking on 40 $\mu\text{g/ml}$ nalidixic acid-containing LB plate supplemented with either 25 $\mu\text{g/ml}$ kanamycin or 20% sucrose. Sucrose-resistant and kanamycin-sensitive strains were tested for deletion by PCR using the DMsrR-F1/DMsrR-R2 primer pair (Additional file 1: Table S2) and confirmed by DNA sequencing. The $\Delta 3\text{-}mst$ and Δmfs in-frame deletion mutants were constructed in similar manners by plasmid pK18*mobsacB*- $\Delta 3\text{-}mst$ and pK18*mobsacB*- Δmfs using primers listed in Additional file 1: Table S2. For performing sensitivity assays, bacteria growth in LB broth containing 0.3 mM cumene hydroperoxide (CHP), 0.9 mM menadione (MEN), 45 mM H₂O₂, 0.4 mM HOCl, 1.5 mM tert-butyl hydroperoxide (*t*-BHP), 5 mM iodoacetamide (IAM), 0.1 $\mu\text{g/ml}$ gentamicin, or 17 μM cadmium chloride (CdCl₂) was measured according to Helbig et al. [38].

Cloning, expression, and recombinant protein purification

The genes encoding *C. glutamicum* MsrR (NCgl2679), 3-MST (NCgl2678), MFS (NCgl2680) were amplified using primers listed in Additional file 1: Table S2 by PCR. The amplified DNA fragments were digested and subcloned into similar digested pET28a, pXMJ19, or pXMJ19-His₆ vectors, obtaining pET28a-*msrR*, pXMJ19-*msrR*, pXMJ19-His₆-*msrR*, pXMJ19-3-*mst*, and pXMJ19-*mfs*, respectively.

The plasmids pK18*mobsacB*- $\Delta msrR$, pK18*mobsacB*- $\Delta 3\text{-}mst$, and pK18*mobsacB*- Δmfs were constructed by overlap-PCR [39]. Briefly, primer pairs DMsrR-F1/DMsrR-R1 and DMsrR-F2/DMsrR-R2 listed in Additional file 1: Table S2 were used to amplify the 806-bp upstream fragment and the 820-bp downstream fragment of *msrR*, respectively. The primer pair DMsrR-F1/DMsrR-R2 was used to fuse the upstream and downstream fragments together by overlap extension PCR [39]. The obtained PCR products were digested with *Eco*RI and *Bam*HI, and cloned into similar digested pK18*mobsacB*

to produce pK18*mobsacB*- $\Delta msrR$. The knock-out plasmid pK18*mobsacB*- $\Delta 3\text{-}mst$ and pK18*mobsacB*- Δmfs were constructed in a similar manner by using the primers listed in Additional file 1: Table S2.

The *lacZY* fusion reporter vectors pK18*mobsacB*- P_{msrR} ::*lacZY* and pK18*mobsacB*- P_{mfs} ::*lacZY* were obtained by fusion of the *msrR* or *mfs* promoter to the *lacZY* reporter gene via overlap-PCR [40]. Firstly, the primers $P_{msrR}\text{-F}/P_{msrR}\text{-R}$ and *lacZY*-F/*lacZY*-R were used in the first round of PCR to amplify the 232-bp *msrR* promoter DNA fragments (corresponding to nucleotides +12 to -220 relative to the translational start codon (ATG) of *msrR* gene) and the *lacZY* DNA fragments, respectively. Secondly, $P_{msrR}\text{-F}/\text{lacZY}\text{-R}$ as primers and the first round PCR products as templates were used to perform the second round of PCR, and the resulting fragments were digested with *Sma*I and *Pst*I, and inserted into similar digested pK18*mobsacB* to obtain the pK18*mobsacB*- P_{msrR} ::*lacZY* fusion construct [29]. A similar process was used to construct pK18*mobsacB*- P_{mfs} ::*lacZY*. Briefly, the 235-bp *mfs* promoter DNA fragments (corresponding to nucleotides +15 to -220 relative to the translational start codon (ATG) of *mfs* gene) was amplified with the primers listed in Additional file 1: Table S2 and fused to the *lacZY* reporter genes. The resulting P_{mfs} ::*lacZY* was inserted into similar digested pK18*mobsacB*.

For obtaining pK18*mobsacB*- P_{msrRM} ::*lacZY*, 232-bp *msrR* promoter DNA containing mutagenesis sequence of the predicted MsrR binding site (P_{msrRM}) was first directly synthesized by Shanghai Biotechnology Co., Ltd.. Start and stop sites of P_{msrRM} were the same as those of P_{msrR} in P_{msrR} ::*lacZY*. Then, the resulting 232-bp P_{msrRM} was fused to a *lacZY* reporter gene. Finally, P_{msrRM} ::*lacZY* was inserted into similar digested pK18*mobsacB*. A similar process was used to construct pK18*mobsacB*- P_{mfsM} ::*lacZY*. Briefly, 235-bp *mfs* promoter DNA containing a mutagenesis sequence of the predicted MsrR binding site (P_{mfsM}) was directly synthesized and its start and stop sites were the same as those of P_{mfs} in P_{mfs} ::*lacZY*. Then, 235-bp P_{mfsM} was fused to a *lacZY* reporter gene to obtain P_{mfsM} ::*lacZY*. Finally, P_{mfsM} ::*lacZY* was inserted into similar digested pK18*mobsacB*.

For complementation or overexpression in *C. glutamicum* strains, pXMJ19 or pXMJ19-His₆ derivatives were transformed into the corresponding *C. glutamicum* strains by electroporation, and the transformants were selected on 10 $\mu\text{g/ml}$ chloramphenicol and 40 $\mu\text{g/ml}$ nalidixic acid-containing LB agar plates. The transformant's expression was induced by adding 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) into medium [40].

To make the cysteine residue at position 62 of MsrR into a serine residue (MsrR:C62S), site-directed mutagenesis was made by two rounds of PCR [41]. In brief, in the

first round of PCR, primer pairs DMsrR-F1/MsrR-C62S-R and MsrR-C62S-F/DMsrR-R2 were used to amplify segments 1 and 2, respectively. The second round of PCR was performed by using CMsrR-F/CMsrR-R or OMsrR-F/OMsrR-R as primers and fragment 1 and fragment 2 as templates to produce the *msrR:C62S* DNA segment. The *msrR:C62S* segment was digested and subcloned into digested pET28a, pXMJ19 or pXMJ19-His₆ plasmid, obtaining the corresponding plasmids. To express and purify His₆-tagged recombinant proteins, the pET28a derivatives were transformed into *E. coli* BL21(DE3). Recombinant proteins were purified according to previously described method [40]. Primers used in this study were listed in Additional file 1: Table S2.

The fidelity of all constructs was confirmed by DNA sequencing (Sangon Biotech, Shanghai, China).

Construction of chromosomal fusion reporter strains and β -galactosidase assay

The *lacZY* fusion reporter plasmids pK18*obsacB*-*P_{msrR}::lacZY*, pK18*obsacB*-*P_{mfs}::lacZY*, pK18*obsacB*-*P_{msrRM}::lacZY*, and pK18*obsacB*-*P_{mfsM}::lacZY* were transformed into *C. glutamicum* parental strain containing the high copy number of empty plasmid pXMJ19 (the strains were named WT), Δ *msrR* (strains lacking *msrR* gene containing empty pXMJ19) and Δ *msrR*⁺ (Δ *msrR* was complemented with pXMJ19 plasmids carrying the wild-type *msrR* gene) by electroporation, respectively. The introduced pK18*obsacB* derivatives were integrated into the chromosome using fusion promoter regions homologous to the genome of *C. glutamicum* by single crossover and then the chromosomal WT(*P_{msrR}::lacZY*), Δ *msrR*(*P_{msrR}::lacZY*), Δ *msrR*⁺(*P_{msrR}::lacZY*), WT(*P_{msrRM}::lacZY*), Δ *msrR*(*P_{msrRM}::lacZY*), Δ *msrR*⁺(*P_{msrRM}::lacZY*), WT(*P_{mfs}::lacZY*), Δ *msrR*(*P_{mfs}::lacZY*), Δ *msrR*⁺(*P_{mfs}::lacZY*), WT(*P_{mfsM}::lacZY*), Δ *msrR*(*P_{mfsM}::lacZY*), and Δ *msrR*⁺(*P_{mfsM}::lacZY*) fusion reporter strains were selected by plating on LB agar plates containing 40 μ g/ml⁻¹ nalidixic acid, 25 μ g/ml⁻¹ kanamycin, and 10 μ g/ml⁻¹ chloramphenicol [37]. The resulting strains were grown in LB medium to an optical density at 600 nm of 0.6–0.7 and then treated with different reagents of various concentrations at 30 °C for 30 min. β -galactosidase activities were assayed with *o*-Nitrophenyl- β -D-Galactopyranoside (ONPG) as the substrate [39]. The standard assay for quantitating the amount of β -galactosidase activity in cells, originally described by Miller for assay of bacterial cultures, involves spectrophotometric measurement of the formation of the yellow chromophore *o*-nitrophenol (ONP) as the hydrolytic product of the action of β -galactosidase on the colorless substrate *o*-Nitrophenyl β -D-galactopyranoside (ONPG)

[42]. All β -galactosidase experiments were performed with at least three independent biological replicates.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from exponentially growing WT, Δ *msrR* and Δ *msrR*⁺ strains exposed to different toxic agents of indicated concentrations for 30 min using the RNeasy Mini Kit (Qiagen, Hilden, Germany) along with the DNase I Kit (Sigma-Aldrich, Taufkirchen, Germany). Purified RNA was reverse-transcribed with random 9-mer primers and MLV reverse transcriptase (TaKaRa, Dalian, China). Quantitative RT-PCR analysis (7500 Fast Real-Time PCR; Applied Biosystems, Foster City, CA) was performed as described previously [40]. The primers used were listed in Additional file 1: Table S2. To obtain standardization of results, the relative abundance of 16S rRNA was used as the internal standard.

H₂O₂-dependent structural change of MsrR in vivo

The H₂O₂-dependent structural change of MsrR and its variant in vivo were determined by a previously reported method [39]. Δ *msrR* (pXMJ19-His₆-*msrR*) and Δ *msrR* (pXMJ19-His₆-*msrR:C62S*) strains were cultured in LB containing 0.5 mM IPTG, 10 μ g/ml chloramphenicol, and 40 μ g/ml nalidixic acid at 30 °C. Cells were grown to mid-exponential phase and split into 100 ml aliquots for H₂O₂ treatment (0–30 mM, 60 min). The treated samples were harvested immediately by centrifugation, broken through ultrasound on ice, and then crude cell lysates were centrifuged. Obtained supernatants were subjected to nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) or reducing SDS-PAGE, and the structural properties of MsrR and its variant were visualized by immunoblotting using the anti-His antibody.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed using the method of Si et al. [30]. Briefly, a 162-bp *msrR* promoter sequence [*P_{msrR}*; corresponding to nucleotides –154 to +8 relative to the translational start codon (GTG) of the *cssR* ORF] containing the predicted MsrR binding site was amplified using primer pair EMsrR-F/EMsrR-R (Additional file 1: Table S2). The binding reaction mixture (20 μ l) contained 10 mM Tris–HCl (pH 7.4), 5 mM MgCl₂, 50 mM KCl, 5% glycerol, 0.1% Nonidet P 40 (NP40), 1 μ g poly(dI:dC), 0–60 nM of MsrR, and 40 ng *P_{msrR}*. 162-bp DNA fragments amplified from MsrR ORF (40 ng) instead of *P_{msrR}* were used as a negative control. A 162-bp EMSA promoter DNA containing the mutated sequence of the predicted MsrR-binding site and having the same start and stop sites as *P_{msrR}* (*P_{msrRM}*) was directly synthesized

by Shanghai Biotechnology Co., Ltd.. After the binding reaction mixture was incubated at room temperature for 30 min, the mixture was subjected to electrophoresis on 8% nondenaturing polyacrylamide gel made with 10 mM Tris buffer containing 50 mM KCl, 5 mM MgCl₂ and 10% glycerol in 0.5× TBE electrophoresis buffer [50 mM Tris, 41.5 mM borate (pH 8.0), 10 mM Na₂EDTA.H₂O], and stained either with a 10,000-fold diluted Synergy Brand (SYBR) Gold nucleic acid staining solution (Molecular Probes) or GelRed™ and photographed. The DNA bands were visualized with UV light at 254 nm.

The reversibility of the loss of binding due to oxidation was tested as follows. H₂O₂ was added to MsrR solution to a final concentration of 10 mM, immediately aliquots were taken and incubated with 40 ng *P_{msrR}* for EMSA. In the next step, dithiothreitol (DTT) was added to the H₂O₂-treated MsrR solutions to a final concentration of 50 mM, and again aliquots were taken for EMSA. All aliquots were incubated in binding buffer with 40 ng *P_{msrR}* for 30 min at room temperature and separated on an 8% nondenaturing polyacrylamide gel and the gel was stained using SYBR Gold nucleic acid staining solution.

For the determination of apparent *K_D* values, increasing concentrations of the MsrR (0–100 nM) were incubated for 30 min at room temperature with 40 ng *P_{msrR}*. The samples were applied onto an 8% native polyacrylamide gel and separated at 180 V for 1 h on ice. The gels stained with GelRed™ and photographed were quantified using ImageQuant software (GE Healthcare), and the percentage of shifted DNA was calculated. These values were plotted against the MsrR concentration in log₁₀ scale, and a sigmoidal fit was performed using GraphPad Prism software (GraphPad Software, San Diego California USA), considering the error bars as well as 0 and 100% shifted DNA as asymptotes, the turning point of the curve was defined as the apparent *K_D* value. All determinations were performed in triplicate.

Western blot analysis

Western blot analysis was conducted as previously described [29]. The cytosolic RNA polymerase β (RNA

polβ) was used as a loading control as in our previous study [29].

Statistical analysis

Statistical analyses of survival rate, transcription level and protein level were determined with paired two-tailed Student's t-test. GraphPad Prism Software was used to carry out statistical analyses (GraphPad Software, San Diego California USA).

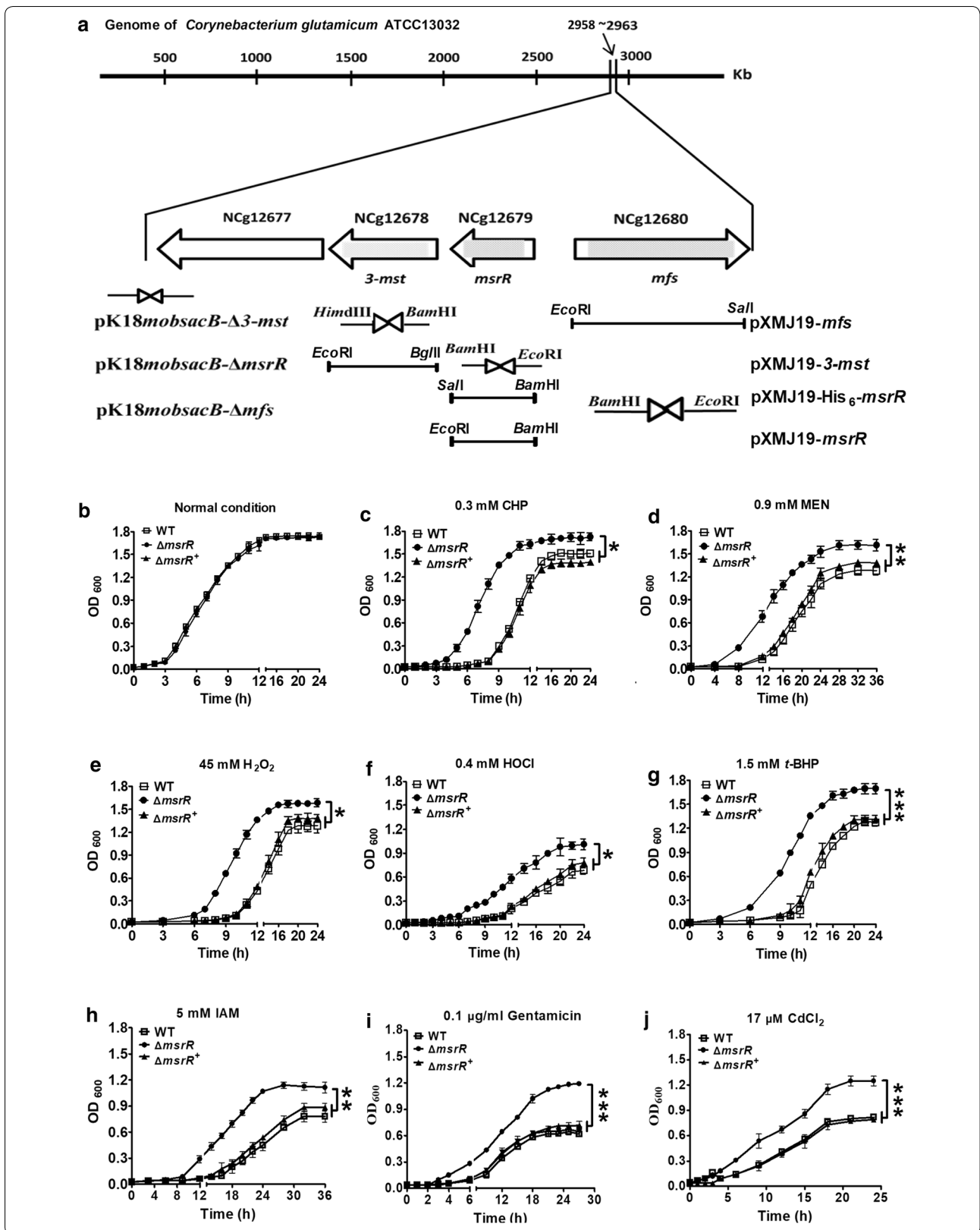
Results and discussion

The *ΔmsrR C. glutamicum* strain showed reduced sensitivity to challenge by oxidants, antibiotics, heavy metals, and alkylating agents

The 723-bp *C. glutamicum ncgl2679* gene is located from bp 2,960,466 to 2,961,188 (Fig. 1a, upper panel) and encodes a hypothetical transcriptional regulator consisting of 240 amino acid residues with a molecular mass of 26.2 kDa. The putative protein product, which contains a helix-turn-helix motif, shares similarity with XRE (xenobiotic response element) family transcription factors from *Corynebacterium crudilactis*, *Corynebacterium efficiens*, *Corynebacterium callunae*, *Corynebacterium epidermidicanis*, and *Corynebacterium minutissimum* (80%, 68%, 64%, 42%, and 40% amino acid sequence identity, respectively) (Additional file 1: Figure S1). A recent study showed that the transcriptional regulator SrtR, an XRE family member, is involved in oxidative and high temperature stress tolerance [19]. This finding prompted us to examine whether NCgl2679 plays a role in protecting the soil bacterium *C. glutamicum* from various stresses. The functions of NCgl2679 were identified by gene disruption and complementation (Fig. 1a, lower panel). Growth analysis of different *C. glutamicum* strains on LB medium in the absence of stress revealed that the wild-type *C. glutamicum* strain (WT, *C. glutamicum* transformed with the empty plasmid pXMJ19), the *Δncgl2679* mutant strain (the *ncgl2679* deletion mutant expressing pXMJ19) and the *Δncgl2679*⁺ strain (the *ncgl2679* deletion mutant expressing the wild-type *ncgl2679* gene in the shuttle vector pXMJ19) showed almost the same growth rates (Fig. 1b). However, the growth of the WT strain in LB

(See figure on next page.)

Fig. 1 MsrR was required for optimal growth under various stress. **a** Physical map of the *msrR-3-mst-ncgl2677* genetic cluster and *mfs* gene in *Corynebacterium glutamicum* (upper panel) and construction of plasmids for gene disruption (pK18*mobsacB* derivatives) and complementation (pXMJ19 derivatives) (lower panel). Open reading frames (ORFs) were marked by open arrows, and the deleted regions were in gray. The restriction sites were indicated. *msrR*, *3-mst*, and *mfs* represented *ncgl2679*, *ncgl2678*, and *ncgl2680* genes, encoding multiple stress resistance regulator, 3-Mercaptopyruvate sulfurtransferase, and a major facilitator superfamily protein, respectively. **b** Growth of the indicated three strains in LB broth without stress was used as control. **c–j** Growth of indicated strains in LB broth with 0.3 mM cumene hydroperoxide (CHP), 0.9 mM menadione (MEN), 45 mM hydrogen peroxide (H₂O₂), 0.4 mM hypochlorous acid (HOCl), 1.5 mM tert-butyl hydroperoxide (t-BHP), 5 mM iodoacetamide (IAM), 0.1 μg/ml gentamicin, or 17 μM cadmium chloride (CdCl₂), respectively. Data show the averages of three independent experiments, and error bars indicated the SDs from three independent experiments. ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05



medium containing oxidants, alkylating agents, antibiotics, or heavy metals was markedly inhibited relative to the growth of the $\Delta ncgl2679$ mutant strain (Fig. 1c–j). The complementary strain $\Delta ncgl2679^+$ exhibited a growth rate equivalent to that of the wild-type strain under various stresses, consistent with a previous evaluation of XREs under stress [19]. These results indicated that NCgl2679 is involved in the resistance of *C. glutamicum* to various stresses. Thus, we named NCgl2679 multiple stress response regulator (MsrR).

MsrR negatively regulates expression of the divergently oriented genes *mfs* and *msrR-3-mst*

In the *C. glutamicum* genome, *msrR* (*ncgl2679*) is organized in a putative operon with *ncgl2678* and *ncgl2677*, which were shown to be co-transcribed by reverse transcription PCR (Additional file 1: Figure S2). Further downstream from *ncgl2679* is the *ncgl2680* gene, which was annotated as the multidrug efflux protein MFS. The *mfs* and *msrR* genes are oriented in opposite directions. By bioinformatics molecular analysis, two putative overlapping and divergent promoter sequences in the intergenic region between the start codons of *mfs* and *msrR* were found (Additional file 1: Figure S3), and one of these promoter sequences was found to be located upstream of the *msrR* gene. Neighboring *mfs* is a putative –10 and –35 promoter sequence, which was found to be the *mfs* promoter.

On the basis of bioinformatics analysis, a putative MsrR-binding site in the putative overlapping, divergent promoters of the *msrR-ncgl2678* locus and *mfs* gene was found (Additional file 1: Figure S3). Thus, we speculated that MsrR negatively regulates the *msrR-ncgl2678-ncgl2677* locus and represses transcription of the adjacent, oppositely oriented *mfs* gene. To verify this speculation, *msrR*, *ncgl2678* and *mfs* transcription levels in the WT, $\Delta msrR$ mutant, and $\Delta msrR^+$ strains were analyzed by qRT-PCR and determination of the *lacZY* activity of the chromosomal promoter fusion reporter. Notably, to study the expression of *msrR* in the $\Delta msrR$ mutant strain by qRT-PCR, a 104-bp *msrR* transcript (corresponding to nucleotides +1 to +104 relative to the translational start codon (GTG) of the *msrR* gene) was amplified from the remaining *msrR* ORF in the $\Delta msrR$ mutant strain with the primers QmsrR-F and QmsrR-R (Additional file 1: Figure S4). As expected, *msrR*, *ncgl2678* and *mfs* transcription levels in the $\Delta msrR$ mutant strain were obviously higher than those in the WT and $\Delta msrR^+$ strains (Fig. 2 and Additional file 1: Figure S5). These results indicated that MsrR negatively controls the expression of NCgl2678, MFS, and its structural gene.

ncgl2678, which was annotated as 3-mercaptopyruvate sulfurtransferase (3-MST), is mainly responsible for

hydrogen sulfide (H₂S) production [43]. Previous studies found that H₂S made by nonsulfur bacteria alleviates oxidative stress imposed by diverse stresses through increasing levels of intracellular antioxidants, including glutathione (GSH); antioxidant enzymes; and glutamate uptake [44, 45]. This finding suggests that the absence of *3-mst* probably cause the decrease of H₂S content, which in turn reduction of the antioxidant capacity of *C. glutamicum* strains. In addition, many reports have revealed that cells expressing MFS can excrete various poisons [46, 47], suggesting that *C. glutamicum* MFS is also important for resistance to diverse stresses. Thus, the functions of *3-mst* and *mfs* were identified by gene disruption and complementation with *C. glutamicum* (Fig. 1a, lower panel). As shown in Fig. 3, while deletion of *3-mst* or *mfs* did not affect bacterial growth under normal conditions, compared to the WT strain, the $\Delta 3-mst$ and Δmfs mutant strains devoid of *3-mst* or *mfs*, respectively, exhibited obvious growth inhibition under challenge with various diverse stresses. The growth of *3-mst* or *mfs* deletion mutant strains under diverse stresses was restored to a level similar to that of the WT strain by transformation with the plasmid-encoded wild type *3-mst* or *mfs* gene ($\Delta 3-mst^+$ or Δmfs^+), in agreement with the results of Li et al. regarding MST [48] (Fig. 3).

Expression of *msrR*, *3-mst* and *mfs* was induced by oxidative stress via MsrR

Previous studies revealed that the transcriptional activation of target genes controlled by XREs is mediated by xenobiotics, which act as inducers [49, 50]. The mechanism by which various xenobiotics act as inducers and affect the conformation of XREs is a key feature for induction activity. Thus, these studies, combined with the above finding that MsrR is involved in tolerance to various stresses, led us to investigate whether MsrR participates in the induction of its own gene and the *3-mst* and *mfs* genes by xenobiotics. For simplicity, we used H₂O₂ and CdCl₂ as inducers in the following experiments. As shown in Fig. 2a and Additional file 1: Figure S5c, in the absence of H₂O₂, the $\Delta msrR$ strain had significantly higher *msrR* and *mfs* expression levels than the WT and $\Delta msrR^+$ strains, whereas the *lacZY* activities of *msrR* and *mfs* in the WT strain exposed to H₂O₂ were obviously higher than those in the untreated-H₂O₂ WT strain. The addition of H₂O₂ did not change the *lacZY* activities of *msrR* or *mfs* in the $\Delta msrR$ strain, which were maintained at the same levels observed in the $\Delta msrR$ strain without H₂O₂ treatment. Moreover, analysis of the *lacZY* activities showed a dose-dependent change in expression in the WT and $\Delta msrR^+$ strains in response to H₂O₂ (Fig. 2a and Additional file 1: Figure S5c). A similar regulatory pattern

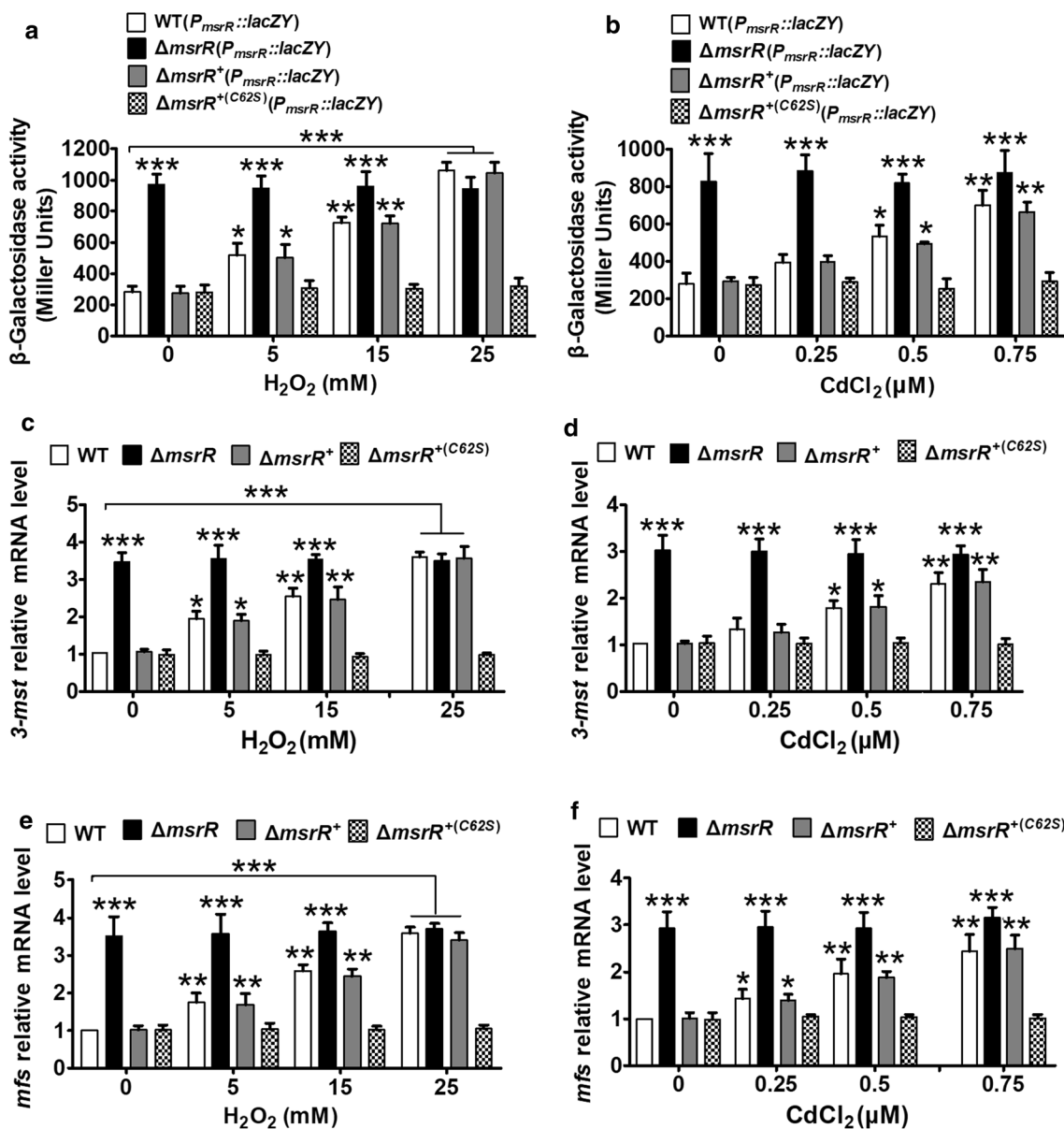
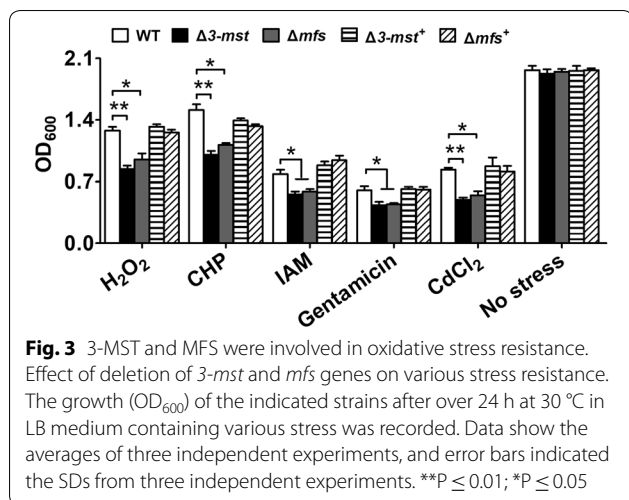


Fig. 2 Negative regulation of *msrR*-*3-mst* and *mfs* expressions by MsrR. **a, b** β -galactosidase analysis of the *msrR* promoter activity was performed using the transcriptional $P_{msrR}::lacZY$ chromosomal fusion reporter expressed in indicated strains exposed to H_2O_2 and $CdCl_2$. **c-f** qRT-PCR assay was performed to analyze the expression of *3-mst* and *mfs* in indicated strains exposed to H_2O_2 and $CdCl_2$. The mRNA levels were presented relative to the value obtained from WT cells without treatment. Relative transcript levels of WT strains without stress treatment were set at a value of 1.0. Data show the averages of three independent experiments, and error bars indicated the SDs from three independent experiments. *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

of *msrR*, *3-mst* or *mfs* by MsrR was also observed at the mRNA transcriptional level by qRT-PCR analysis (Fig. 2c, e and Additional file 1: Figure S5a). These results clearly demonstrated that *msrR*, *3-mst* and *mfs* were upregulated in response to increasing H_2O_2 concentration, indicating that oxidation inhibited the

DNA binding of MsrR, inducing the expression of its own gene and the *3-mst* and *mfs* genes. This derepression of *msrR*, *3-mst* and *mfs* transcription by $CdCl_2$ was mediated via MsrR in a matter similar to that of H_2O_2 (Fig. 2b, d, f and Additional file 1: Figure S5b, d).



The ability of MsrR to bind the intergenic region between *msrR* and *mfs* was reversibly inhibited by ROS

To determine whether MsrR directly regulates its own transcription and the transcription of 3-MST and MFS, we examined the interaction between purified MsrR and a DNA promoter fragment in the intergenic region between *msrR* and *mfs* (named P_{msrR}) using EMSA. Incubation of P_{msrR} with His₆-MsrR caused a clear delay in promoter DNA migration, and P_{msrR} migrated in a manner dependent on the concentration of His₆-MsrR (Fig. 4b and Additional file 1: Figure S6b). The apparent K_D value for P_{msrR} was about 17 nM MsrR (Additional file 1: Figure S7a), which is within the range found for other transcriptional regulators [33]. Moreover, this effect was specific because the combination of His₆-MsrR and DNA fragments amplified from the MsrR ORF did not delay migration (Fig. 4a and Additional file 1: Figure S6a). However, the binding of His₆-MsrR to P_{msrR} was prevented by the addition of 10 mM H₂O₂ (Fig. 4c and Additional file 1: Figure S6c). Importantly, the impaired DNA-binding activity of His₆-MsrR by H₂O₂ could be restored via the addition of an excess of the reducing agent DTT (50 mM), indicating that the effects of oxidation and reduction on the DNA-binding activity of MsrR were reversible. (Fig. 4c and Additional file 1: Figure S6c). Mutations in the predicted MsrR-binding site (a 162-bp EMSA promoter DNA contained the mutated sequence of the predicted MsrR-binding site (P_{msrRM}), which had the same start and stop sites as P_{msrR}) (Additional file 1: Figure S3) disrupted the formation of DNA–protein complexes (Fig. 4d and Additional file 1: Figure S6d), and promoter DNA mutations in the predicted MsrR-binding site (a 232-bp DNA fragment contained the mutated sequence of the predicted MsrR-binding site for *lacZY* activity, which had the same start and stop

sites as a 232-bp DNA fragment on $P_{msrR}::lacZY$; a 235-bp DNA fragment contained the mutated sequence of the predicted MsrR-binding site for *lacZY* activity, which had the same start and stop sites as a 235-bp DNA fragment on $P_{mfs}::lacZY$) caused extremely high $P_{msrRM}::lacZY$ and $P_{mfsM}::lacZY$ activities in the WT and $\Delta msrR^+$ strains, similar to those in the $\Delta msrR$ mutant strain (Additional file 1: Figure S8), further indicating the recognition of DNA elements by MsrR. Interestingly, the addition of CdCl₂ did not induce the dissociation of MsrR from P_{msrR} , inconsistent with the finding that derepression of *msrR* transcription by CdCl₂ was mediated via MsrR in vivo (Fig. 4e and Additional file 1: Figure S6e). Combined with the discovery that expression of *msrR* was affected by H₂O₂ (Fig. 2), we speculated that this was related to CdCl₂-mediated perturbation of the electron transfer chain, resulting in the formation of ROS in vivo, which inactivated XRE DNA-binding activity by the oxidation of cysteine residues [51, 52]. In fact, many studies have reported that the most potent xenobiotics, including oxidants, alkylating agents, antibiotics, and heavy metals, can generate ROS by redox cycling to produce oxidative stress inside bacteria [1, 51–56]. Thus, we speculated that MsrR does not directly sense ligands such as CdCl₂, gentamicin, MEN and IAM.

Together, these results show that MsrR specifically recognized operators and then directly bound the *msrR* and *mfs* intergenic region in a sequence-specific manner. Upon exposure to oxidative stress, MsrR was inhibited by changes in conformation caused by ROS and released from the promoter, leading to the upregulation of target genes.

Oxidation promoted MsrR dimerization and inaction

Many redox-sensitive regulators, such as RosR, CosR, and OhsR, exist as homodimers via intersubunit disulfide bonds upon oxidation [29, 30, 33]. The amino acid sequence of MsrR shows that it contains one cysteine residue at position 62 (Additional file 1: Figure S1). Thus, we thought it might share a similar oxidation-sensing mechanism and that MsrR is oxidized to form an intersubunit disulfide-containing dimer. As shown in Fig. 5a, nonreducing SDS-PAGE showed that the native MsrR protein was monomeric with an apparent MW of approximately 30 kDa, corresponding well to the molecular mass of MsrR deduced from its amino acid sequence, while MsrR incubated with H₂O₂ migrated as a band of approximately 60 kDa, as judged by its behavior on 15% nonreducing SDS-PAGE, which corresponded to MsrR in its dimeric form. This dimeric formation was reversed by an excess of DTT (Fig. 5b). Moreover, dimers of H₂O₂-treated MsrR:C62S were not observed. These results suggested

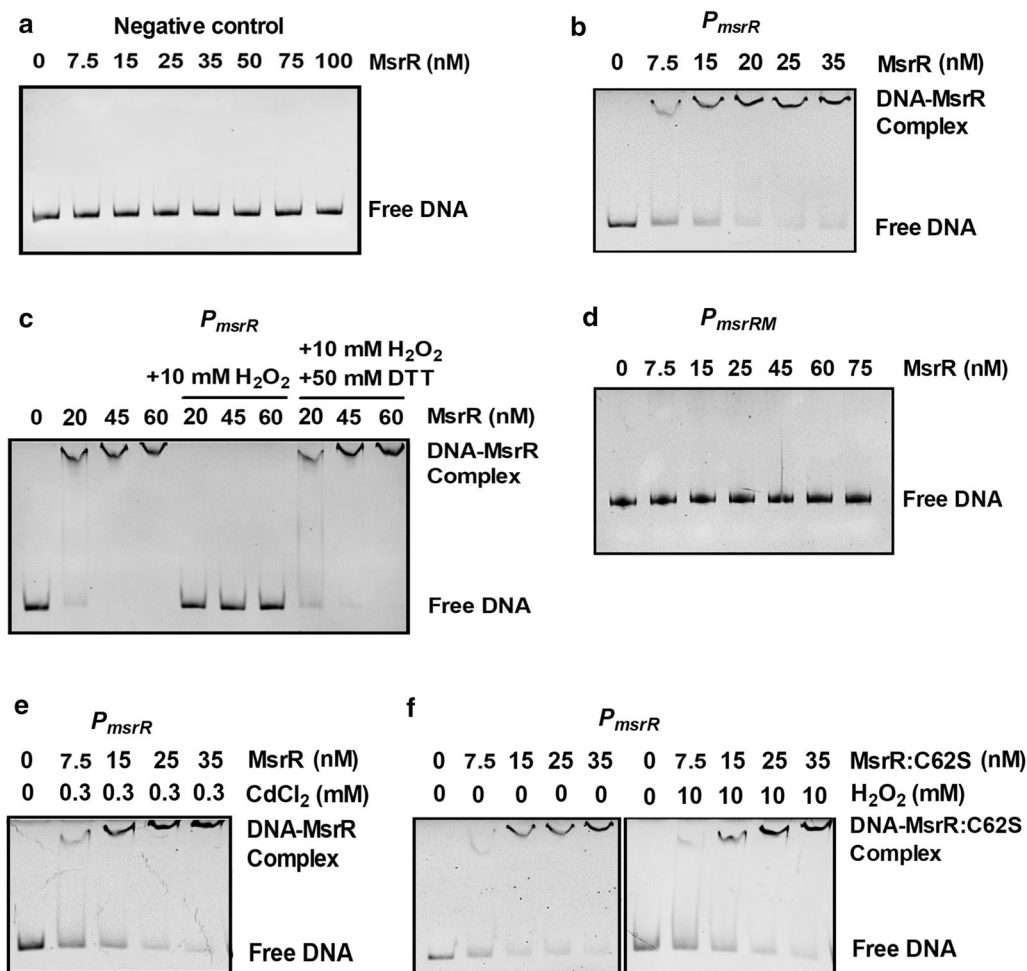
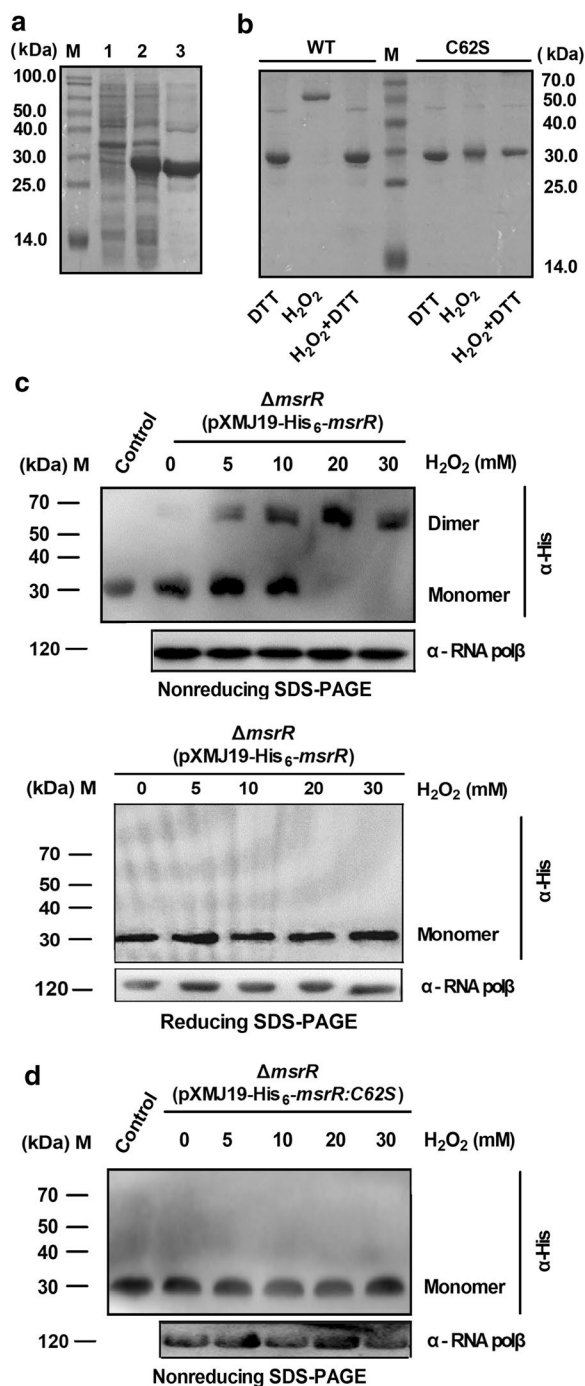


Fig. 4 Reversible inhibition of the DNA binding activity of MsrR by H_2O_2 and role of Cysteine residue. **a** The interaction between His₆-MsrR and DNA fragments amplified from MsrR's ORF. **b** The interaction between His₆-MsrR and the promoter fragment in the intergenic region between *msrR* and *msf* (named P_{msrR}). **c** Inhibition of the DNA binding activity of MsrR by H_2O_2 and reversal of the inhibition by DTT. MsrR was prepared in three different concentrations, and aliquots were taken for EMSAs (control). Then H_2O_2 was added to the binding reaction mixture to a final concentration of 10 mM, and aliquots were taken for EMSA. In the next step DTT (a final concentration of 50 mM) was added to 10 mM H_2O_2 -containing binding reaction mixture, and aliquots were taken for EMSAs. All aliquots were incubated in binding buffer, pH 8.0, with 40 ng P_{msrR} and then separated on an 8% native polyacrylamide gel. **d** The interaction between His₆-MsrR and the promoter mutating the predicted MsrR binding region (P_{msrRM}). **e** CdCl_2 was added to the binding reaction mixture to a final concentration of 0.3 mM, and the interaction between His₆-MsrR and P_{msrR} was performed. **f** The interaction between the mutated derivatives MsrR:C62S and P_{msrR} in the absence (left panel) or presence (right left) of 10 mM H_2O_2 . Results were obtained in three independent experiments, and data show one representative experiment done in triplicate

that the formation of dimeric MsrR occurs via a disulfide bond between MsrR proteins.

To further examine whether the formation of MsrR dimers can be induced under H_2O_2 treatment in vivo, we treated cells of the $\Delta msrR$ (pXMJ19-His₆-*msrR*) and $\Delta msrR$ (pXMJ19-His₆-*msrR*:C62S) strains with H_2O_2 at various concentrations and probed the forms of MsrR by immunoblotting with anti-His antibody after non-reducing SDS-PAGE separation (Fig. 5c, d; Additional file 1: Figure S9). Under normal conditions (no stress), MsrR in the $\Delta msrR$ (pXMJ19-His₆-*msrR*) strain existed

as monomers, but upon exposure to different concentration of H_2O_2 , the monomeric form changed into an intermolecular disulfide bond-containing dimeric form (Fig. 5c, upper panel and Additional file 1: Figure S9a, upper panel). The dimeric form completely disappeared on reducing SDS-PAGE, indicating that dimeric MsrR in vivo could be also reversed, which was consistent with the results in vitro (Fig. 5c, lower panel and Additional file 1: Figure S9a, lower panel). However, whether under H_2O_2 treatment or not, MsrR in the $\Delta msrR$ (pXMJ19-His₆-*msrR*:C62S) strain existed in a monomeric form



(Fig. 5d and Additional file 1: Figure S9b). These results indicated that H₂O₂ causes a structural change in MsrR and that Cys62 is responsible for the morphological changes in MsrR observed under H₂O₂ treatment.

Fig. 5 Redox response of MsrR in vitro and in vivo. **a** Nonreducing SDS-PAGE analysis of proteins expressed in *E. coli* containing pET28a-*msrR* plasmid. M, broad-range protein marker; lane 1, crude extract (5 μ g) without IPTG induction; lane 2, crude extract (5 μ g) with induction; lane 3 purified His₆-MsrR protein. **b** Redox response of MsrR and its variant detected by nonreducing SDS-PAGE. 15 μ M proteins treated with 50 mM DTT were further incubated with or without 50 μ M H₂O₂, or 50 μ M H₂O₂ and 50 mM DTT, and then samples were separated by 15% nonreducing SDS-PAGE. **c, d** Oxidative stress-dependent structural changes of relevant MsrR in vivo. Proteins extracted from cells exposed to different concentrations of H₂O₂ for 30 min were resolved on nonreducing or reducing SDS-PAGE, and analyzed with Western blotting by using the anti-His antibody. RNA polymerase β (RNA pol β) was used as a loading control. Similar results were obtained in three independent experiments, and data show one representative experiment done in triplicate

Inactivation of the DNA binding of MsrR by ROS is dependent on the oxidation state of Cys62

The reduction and oxidation of cysteine residues is involved in the control of ROS-sensing sensor activity [10]. It would be interesting to know whether Cys62 of MsrR plays an important role in the H₂O₂-sensing and transcription mechanisms of MsrR. Thus, the ability of the MsrR:C62S variant to suppress *msrR*, *3-mst* and *mfs* expression in response to H₂O₂ was evaluated in the Δ *msrR* strain using promoter *lacZY* activity and qRT-PCR analysis. Analysis of the transcriptional levels revealed that Δ *msrR*^{+(C62S)} (the Δ *msrR* strain containing the pXMJ19-*msrR*:C62S plasmid) inhibited *msrR*, *3-mst* and *mfs* expression under H₂O₂ treatment conditions to equal degrees, similar to that in the untreated-H₂O₂ WT strain, indicating that Cys62 plays a role in the dissociation of MsrR from the promoter under H₂O₂ treatment conditions (Fig. 2 and Additional file 1: Figure S5).

To further probe whether Cys62 is responsible for the observed dissociation of MsrR under oxidation, MsrR:C62S was used instead of WT MsrR to perform the EMSA experiment. As shown in Fig. 4f and Additional file 1: Figure S6f, in the presence or absence of 10 mM H₂O₂, MsrR:C62S still exhibited obviously retarded mobility. Although its affinity constant for *P*_{*msrR*} ($K_D=23.08$) was slightly high than that of MsrR, MsrR:C62S behaved high similarly to MsrR without H₂O₂ condition (Additional file 1: Figure S7b). These results mean that oxidation of Cys62 was important for inhibition of DNA binding by H₂O₂. The above results further showed that the inhibition of DNA binding by H₂O₂ was caused by the oxidation of cysteine residue.

Conclusions

Thiol-based redox-sensing regulators are recognized as an efficient way to combat diverse ROS-inducing stress conditions and enhance the survival of bacteria under oxidative stress. The XRE family is involved in the control of the response to environmental stress, but the functions of XREs related to oxidative stress tolerance, especially their antioxidative molecular mechanisms, are very rarely reported. In this study, we found a MsrR-binding site in the intergenic region between two divergent gene clusters, *msrR-3-mst* and *mfs*. β -galactosidase activity assay and qRT-PCR analysis showed that MsrR is indeed negatively autoregulated and also negatively controls the adjacent *3-mst* and *mfs*. In vivo, expression of *msrR* is induced by H_2O_2 and $CdCl_2$, and the *msrR*-deleted ($\Delta msrR$) mutant displays increased resistance to H_2O_2 and $CdCl_2$. However, EMSA experiment shows the ability of MsrR to bind the promoter DNA is inhibited by H_2O_2 but not $CdCl_2$. Many studies reported that the most potent xenobiotics, including oxidants, alkylating agents, antibiotics, or heavy metals, are capable of generating ROS by redox-cycling to produce oxidative stress inside bacteria [51–56]. Thus, $CdCl_2$ might contribute indirectly to ROS production, thereby leading to the derepression of the MsrR operon. Considering high gentamicin- and alkylating agents-resistant phenotype of $\Delta msrR$ strains, we speculated that antibiotics and alkylating agents might also mediate the DNA binding of MsrR with a mechanism similar to $CdCl_2$. We further verified that the XRE-type regulatory MsrR senses and responds to oxidative stress by a derepression of the *msrR*, *3-mst* and *mfs* genes via intermolecular disulfide formation. Mutational analysis of the sole cysteine in MsrR showed that Cys62 is critical for inactivation of the DNA binding of MsrR, distinguishing it from previously discovered stress response properties of XREs in eukaryotes. On the contrary, the regulatory mechanism of MsrR is similar to those of the ROS sensors OxyR, PerR, and OhrR, which are activated or inhibited by changes in conformation caused by cysteines oxidation.

The XRE family is the second most common family of regulators in bacteria, only four members of which have been reported in previous researches, including *S. suis* SrtR [19], *S. aureus* XdrA (XRE-like DNA-binding regulator, A) [21], *R. etli* RHE-CH00371 [22], and *C. aurantiacus* MltR (MmyB-like transcription regulator) [23]. Except for SrtR, no obvious effect on oxidative stress resistance for any of the previously studied examples has been reported so far. *S. aureus* XdrA is shown to play an important role in the β -lactam stress response. Expression of *R. etli* RHE-CH00371 is reported to be down-regulated in an H_2O_2 -sensitive *R.*

etli mutant. *C. aurantiacus* MltR is described as being involved in the regulation of antibiotic biosynthesis and thus represents an example for a rather specialized XRE-type regulator. Sequence analysis clearly indicates that the similarity between MsrR and the XREs of bacteria mentioned above is very low, and Cys62 of MsrR is not very conserved (Figure S1b–e), which only appears in position 66 of *S. suis* SrtR and 55 of *S. aureus* XdrA. The result is consistent with the previous report that the XRE family contains more than 35,000 proteins and more than 70 structures are available [23]. We suggested that differences in structure may cause versatile features and regulatory mechanisms. It is important to point out, despite their low sequence similarity to MsrR (about 30% identity), we thought *S. suis* SrtR and *S. aureus* XdrA might share an oxidation-sensing mechanism as they not only contain the cysteine presumed to serve for oxidation sensing in a relatively conserved position, but they confer resistance to oxidant and β -lactam, respectively, which is similar to MsrR. Combining a phenomenon that β -lactam antibiotics, such as penicillin, can also generate ROS by redox-cycling to produce oxidative stress inside bacteria [55], we speculate that *S. suis* SrtR and *S. aureus* XdrA act as a transcriptional sensor via cysteine oxidation-based thiol modifications. Thus, our results provided, for the first time, insight into a new regulatory mechanism adopted by an XRE protein in which DNA-binding ability is regulated by the oxidation of a cysteine residue in the MsrR protein in response to oxidants but not directly bound ligands, such as antibiotics, heavy metals, and alkylating agents. Our data further confirmed the results of Hu et al. showing a member of the XRE family of transcriptional regulators responsible for oxidant tolerance in bacteria [19], facilitating understanding of antioxidant mechanisms in bacteria and providing initial insight into the molecular mechanisms of XREs involved in oxidative stress tolerance. In addition, MsrR is found to be widely distributed in several species of the genera *Corynebacterium*, such as *C. crudilactis*, *C. efficiens*, *C. callunae*, *C. epidermidicis*, and *C. minutissimum*. Therefore, our study on the regulatory mechanism of MsrR may lead to a better understanding of the stress response mechanisms of these species. Together, our data show that *C. glutamicum* MsrR acts as a thiol-based redox sensor and, with 3-MST and MFS, comprises an important pathway for protection against oxidative stress.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12934-020-01444-8>.

Additional file 1: Table S1. Bacterial strains and plasmids used in this study. **Table S2.** Primers used in this study. **Figure S1.** Multiple sequence alignment of MsrR with XREs from other organisms. **Figure S2.** Assays for the *ncgl2679-ncgl2678-ncgl2677* co-transcription by reverse transcription PCR. **Figure S3.** Detailed genetic maps of the regulatory region of MsrR. **Figure S4.** 104-bp *msrR* transcript (from the translational start codon (GTG) of *msrR* gene to 104th nucleotide) was amplified from the remaining *msrR* ORF (Open Reading Frame) in Δ *msrR* mutant with primers QmsrR-F and QmsrR-R. **Figure S5.** Negative regulation of *msrR* and *mfs* by MsrR. **Figure S6.** Reversible inhibition of the DNA binding activity of MsrR by H₂O₂ and role of Cysteine residue. **Figure S7.** Determination of the apparent K_D values of MsrR and MsrR:C62S for *P_{msrR}*. **Figure S8.** Mutations in the predicted MsrR binding site derepressed the *msrR* expression. **Figure S9.** Oxidative stress-dependent structural changes of relevant MsrR in vivo.

Abbreviations

ROS: Reactive oxygen species; CHP: Cumene hydroperoxide; t-BHP: t-Butyl hydroperoxide; H₂O₂: Hydrogen peroxide; MSH: Mycothiol; GSH: Tripeptide glutathione; MEN: Menadione; DMPD: N,N-dimethyl-p-phenylenediamine; ONPG: o-Nitrophenyl β-D-galactopyranoside; DTT: Dithiothreitol; 3-MST: 3-Mercaptopyruvate sulfurtransferase; XRE: Xenobiotic response element; HOCl: Hypochlorous acid.

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Authors' contributions

MS and CC designed the research. MS, CC, JZ, XL, YL, and TS performed the research and analyzed the data. GY supervised the research. MS and GY wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

All the data generated or analyzed during this study are included in the manuscript and its additional file.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have agreed to submit this manuscript to microbial cell factories.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ College of Life Sciences, Qufu Normal University, Qufu 273165, Shandong, China. ² College of Life Science and Agronomy, Zhoukou Normal University, Zhoukou 466001, Henan, China.

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