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CzcR-dependent reduction of catalase gene expression and induction of catalase activity in *Pseudomonas aeruginosa* during zinc excess

Ting Li^{1†}, Zhifeng Mo^{1†}, Yuying Zhao¹, Huiluo Cao², Shuo Sheng^{3*} and Zeling Xu^{1*}

Abstract

Background *Pseudomonas aeruginosa* is able to survive, grow, and cause severe infections at different sites throughout the human body owing to its ability to sense diverse signals and precisely modulate target gene expression using its abundant signaling systems. Release of zinc (Zn) and hydrogen peroxide (H₂O₂) within the phagocyte are two major host strategies to defend against bacterial infections. It was previously shown that the response regulator CzcR controls global gene expression including catalase genes during Zn excess, but regulatory mechanisms of catalase gene expression and the role of CzcR in H₂O₂ tolerance remain unclear.

Results In the study, comparative transcriptome analysis comprehensively described the CzcR-dependent and -independent gene regulatory pattern in *P. aeruginosa* during Zn excess, which revealed the counteractive co-regulation of two key H₂O₂-detoxifying catalase genes *katA* and *katB* through CzcR-dependent and -independent pathways in response to Zn excess. Protein-DNA interaction assay demonstrated that CzcR negatively regulates the expression of catalase genes *katA* and *katB* by directly binding to their promoters. While interestingly, we further showed that CzcR positively regulates H₂O₂ tolerance by inducing the catalase activity during Zn excess.

Conclusion This study reported the opposite functions of CzcR in negatively regulating the expression of catalase genes *katA* and *katB* but in positively regulating the activity of catalase and H₂O₂ tolerance during Zn excess in *P. aeruginosa*.

Keywords *P. aeruginosa*, Two-component system, CzcS/CzcR, Catalase, Oxidative tolerance

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Background

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that can cause a variety of acute and chronic infections in patients with compromised immune defenses [1]. It is able to survive and grow under adverse environmental or host-related conditions owing that the pathogen is equipped with a wide range of mechanisms for adaptation [2]. For example, *P. aeruginosa* frequently encounters oxidative stress due to the emergence of toxic reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide, and hydroxyl free radicals during infections [3]. To cope with endogenously produced or exogenous ROS, *P. aeruginosa* encodes antioxidant enzymes including catalases KatA and KatB, which are



two critical enzymes for the adaptation of this pathogen to high levels of H₂O₂ [4, 5].

Adaptation to diverse environmental conditions is exquisitely regulated, which largely depends on the abundant signaling systems in *P. aeruginosa*. With regards to the major catalase genes *katA* and *katB*, their expression can be upregulated at both transcriptional and translational levels by a variety of regulatory systems or pathways when the pathogen is surrounded by oxidizing agents [3]. For example, OxyR is the key transcriptional activator of *katA* and *katB*, contributing significantly to H₂O₂ tolerance [6]. In addition, expression of catalase genes and activity of catalases are partly controlled by other regulators such as IscR, ANR and are also associated with metal availability, oxygen availability, quorum sensing activity, nitrate respiration, etc [7–10].

An important kind of regulatory system in *P. aeruginosa* is the two-component system (TCS) that typically employs a histidine kinase to sense specific intracellular or extracellular signals and a response regulator to control target gene expression [11, 12]. CzcS/CzcR, composed of the histidine kinase CzcS and the response regulator CzcR, is a well-studied TCS that was found for zinc (Zn) detoxification by upregulating the expression of Zn exporters during Zn excess [13]. It was also found to control global gene expression and demonstrated to regulate multiple physiological processes such as antibiotic resistance, quorum sensing, motility, copper (Cu) tolerance, iron (Fe) homeostasis [14–17].

Interestingly, when we were investigating the co-regulation of Cu tolerance and pyochelin biosynthesis by CzcR-dependent and -independent pathways during Zn excess [17], we also noticed that the expression of the major H₂O₂-detoxifying catalase genes *katA* and *katB* was significantly induced due to the loss of the response regulator CzcR during Zn excess, however, we observed a significant growth defect of the Δ *czcR* mutant in the presence of both Zn and H₂O₂ [17], implying the complicated and elusive regulation of *P. aeruginosa* susceptibility to H₂O₂ by CzcR and its inducing signal Zn. Zn is a traditional antimicrobial agent. Unlike Fe and Cu which toxify microbes by inducing oxidative stress through Fenton reaction [18], Zn is a non-redox transition metal and it mainly kills microbes by disrupting the homeostasis of other key trace metals or inhibiting enzymatic activity by competing with other metals due to its top position at the Irving-Williams series [19]. It is interesting that Zn was found to induce the sensitivity of *Streptococcus pneumoniae* to oxidative stress but also provide protection against the toxicity of H₂O₂ in *Burkholderia pseudomallei* [20, 21]. Regulatory mechanisms of oxidative tolerance in microbes including *P. aeruginosa* in response to Zn remain largely elusive. Therefore, this study aimed

to further explore the mechanisms underlying the regulation of catalase gene expression and catalase activity mediated by CzcR and its inducing signal Zn in *P. aeruginosa*.

Methods

Bacterial strains, plasmids, primers, and growth conditions

Bacterial strains, plasmids, and primers used in this study are listed in Table S1. 0.5 mM ZnSO₄ was supplemented in the medium which is prepared from Invitrogen LB Broth Base for Zn treatment. Antibiotics were supplemented in the medium when required: 15 µg/ml tetracycline (reporters of promoter activity) for *E. coli* DH5α; 50 µg/ml tetracycline (reporters of promoter activity), 50 µg/ml gentamycin (complementation of *czcR*) for *P. aeruginosa* PAO1.

Comparative transcriptome analysis

The transcriptome data was retrieved from NCBI (project accession number: PRJNA896733). Mapping of qualified reads was performed using BWA v0.7.17-r1188 [22], SAMtools v1.15 [23] and bamkeepgoodreads of Stampy v1.0.32 [24] with the genome of PAO1 (NC_002516.2) as the reference. Count matrix was generated by featureCounts of Subread v2.0.3 [25] and then used to identify differentially expressed genes (DEGs) by DEseq2 [26]. KEGG pathway enrichment analysis was performed using clusterProfile v4.0 [27].

Reverse transcription-quantitative PCR (RT-qPCR)

Overnight culture of *P. aeruginosa* strains was 1:50 diluted and then cultured with or without the supplementation of Zn for 6 h. 1 ml bacterial culture was harvested by centrifugation and total RNA was extracted using the Eastep[®] Super Total RNA Extraction Kit (Promega). cDNA was reverse transcribed using TransScript[®] OneStep gDNA Removal and cDNA Synthesis SuperMix (TransGen). qPCR was performed using the ChamQ Universal SYBR qPCR Master Mix (Vazyme). The house-keeping *recA* gene was used as the internal reference control.

Promoter activity assay

Promoter regions were amplified from the PAO1 genome and then inserted into the mini-CTX-*lux* plasmid to generate reporters (*PkatA-lux* and *PkatB-lux*) for evaluating promoter activities. Overnight cultures of *P. aeruginosa* strains containing *PkatA-lux* or *PkatB-lux* were 1:50 diluted and then cultured with or without the supplementation of 0.5 mM Zn for 6 h. Luminescence and OD₆₀₀ of the bacterial culture were measured in a microplate reader (BioTek).

Electrophoretic mobility shift assay (EMSA)

His₆-tagged CzcR protein was purified according to the previously reported procedures [17]. Promoters of the *kataA*, *katB*, and *pvdR* genes were amplified from the PAO1 genome by PCR and labeled by the Biotin 3' End DNA Labeling Kit (ThermoFisher Scientific). Biotin-labeled promoter fragments were incubated with CzcR at different concentrations and then separated by polyacrylamide gel electrophoresis. Interactions between CzcR and target promoters were examined using the LightShift[®] Chemiluminescent EMSA Kit (ThermoFisher Scientific). Unprocessed gel images of the EMSA results were shown in Figure S1.

H₂O₂ tolerance assays

Overnight culture of *P. aeruginosa* strains was 1:50 diluted and then cultured with or without the supplementation of Zn for 6 h. Cells were harvested by centrifugation and washed by fresh LB medium without the supplementation of Zn. Next, cells were resuspended in fresh LB medium and cell density of each sample was adjusted to OD₆₀₀ of 1.0. For survival rate assay, H₂O₂ was added into the medium to the concentrations of 1.5 mM, 2.5 mM, or 3.5 mM. After incubation for 30 min, cell culture was serially diluted and plated. Recovered colonies were counted and the survival rate was calculated. For growth curve measurement, bacterial suspension was 1:100 diluted and 0.5 mM H₂O₂ was added into the medium. OD₆₀₀ was measured every 1 h to plot the growth curve.

Catalase activity assay

Overnight culture of *P. aeruginosa* strains was 1:50 diluted and then cultured with or without the supplementation of Zn for 6 h. *P. aeruginosa* cells were collected from 0.5 ml cell culture by centrifugation and lysed by sonication. After centrifugation, suspension was collected for catalase activity measurement using the Catalase (CAT) Activity Assay Kit (Sangon Biotech) according to the manufacturer's instructions.

Results

Transcriptomic profile changes of PAO1 WT and Δ *czcR* strains in response to Zn excess

Considering the interesting co-regulation of Cu tolerance and PCH biosynthesis by CzcR-dependent and -independent pathways as we recently observed during Zn excess [17], we sought to first comprehensively explore CzcR-dependent and -independent gene regulatory patterns during Zn excess by re-analyzing the transcriptome data of the PAO1 WT and Δ *czcR* strains after they were cultured with or without the treatment of 0.5 mM

ZnSO₄. Firstly, it was shown that Zn treatment led to significant expression changes (P value < 0.05) of 279 genes in the PAO1 WT strain (Fig. 1A and Table S2). Among them, 100 genes were upregulated, and 179 genes were downregulated during Zn excess. In contrast, there were 1,055 genes differentially expressed with statistical significance (P value < 0.05) in the Δ *czcR* mutant after Zn treatment (Fig. 1B and Table S3), which contained 776 more genes than that was displayed in the WT strain. This result further indicated the importance of the TCS CzcS/CzcR in maintaining the normal expression levels of abundant genes and the physiological balance in *P. aeruginosa* during Zn excess.

CzcR-dependent and -independent target gene regulation during Zn excess

We next moved to classify the genes with different regulatory patterns involving Zn and/or CzcR by combining two datasets from the WT strain and the Δ *czcR* mutant. According to the gene expression changes in both strains, all the differentially expressed genes (DEGs) listed in Tables S2 and S3 could be generally classified into 8 groups (Fig. 2A and Table S4), displaying the complicated CzcR-dependent and -independent gene regulatory patterns in *P. aeruginosa* during Zn excess. Group No.1 included 54 genes such as *ptrA*, *PA2807*, *czcC*, *czcB*, and *czcD* that were exclusively found as up-regulated genes in the WT strain but not the Δ *czcR* mutant during Zn excess, meaning that their expression is induced by Zn through CzcR exclusively. Likewise, group No.2 included 112 genes such as *oprD* that were repressed by Zn through CzcR exclusively. Groups No.3 and No.4 contained 21 and 40 genes that are positively and negatively regulated by Zn, respectively, possibly through CzcR-independent pathways. Groups No.5 to 8 included genes that are regulated by Zn through a combination of CzcR-dependent and -independent pathways. For example, the *pchR* gene was found in the group No. 7 which indicated that its expression is unchanged in the WT strain during Zn excess. This result was consistent with the unchanged expression of *pchR* in the WT strain but not the Δ *czcR* mutant as we recently reported [17], suggesting the accuracy of the classification.

Interestingly, it was shown that the majority of DEGs (942 out of 1,221) were listed in groups No.7 and 8 (Fig. 2B). Genes found in these two groups are inversely regulated by CzcR-dependent and -independent pathways at comparable levels and, consequently, their expression did not show any responses to Zn excess. This result confirmed that CzcR plays a critical role in balancing the expression of about a thousand of genes that could be disturbed by Zn excess. Enrichment analysis based on the Kyoto Encyclopedia of Genes and Genomes

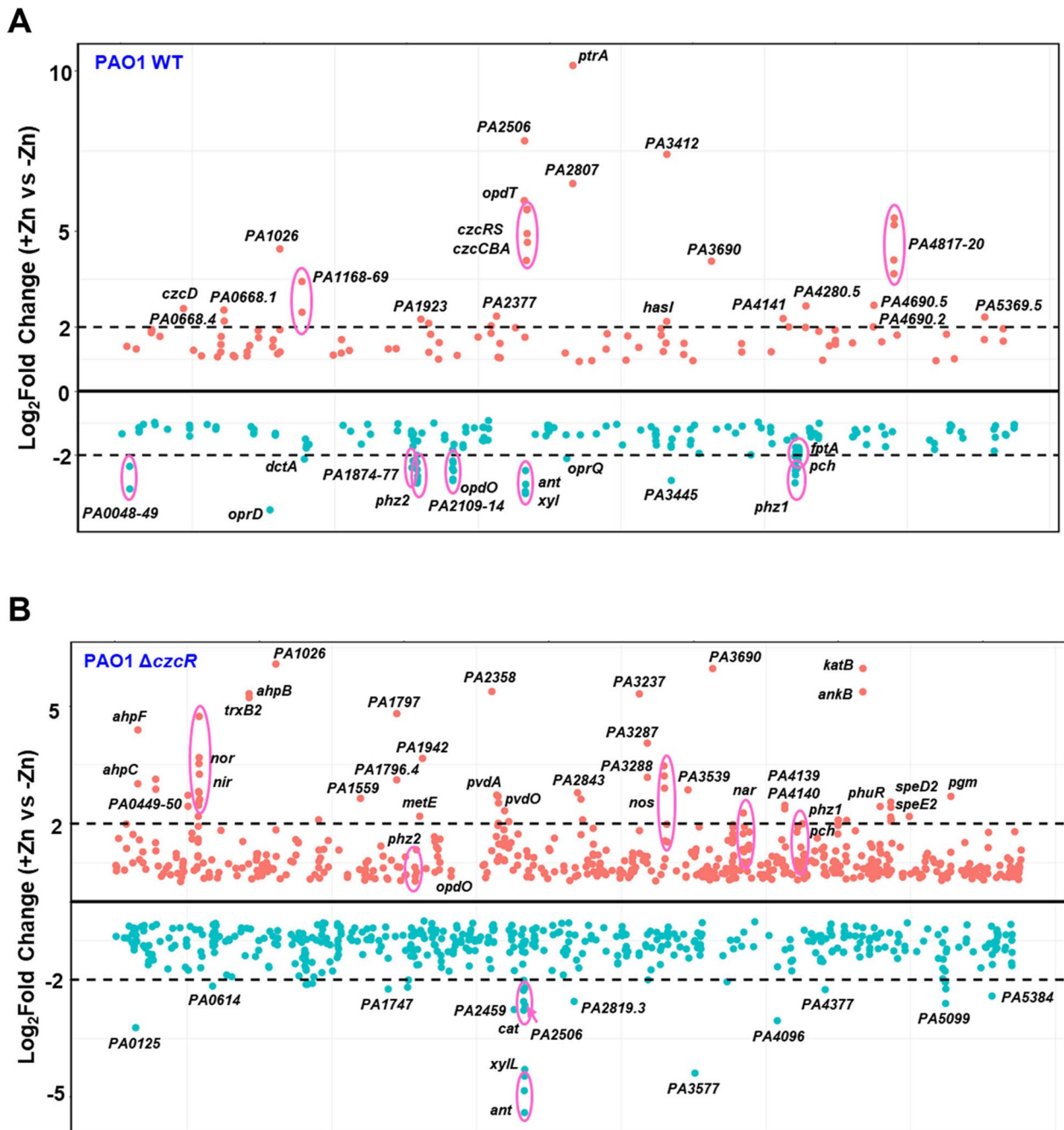


Fig. 1 Transcriptomic profile changes in PAO1 WT and $\Delta czcR$ strains during Zn excess. **A** Genome-wide transcriptomic profiles of the PAO1 WT strain when it was cultured with or without Zn treatment. **B** Genome-wide transcriptomic profiles of the $\Delta czcR$ mutant when it was cultured with or without Zn treatment. Orange dots were the genes upregulated during Zn excess and green dots were the genes downregulated during Zn excess. The black dot lines represented 2-Log₂Fold expression changes

(KEGG) pathways showed that genes in the group No.7 were mainly enriched in pathways of tyrosine metabolism, biosynthesis of secondary metabolites, nitrogen metabolism, protein export, and ribosome (Fig. 2C), while genes in the group No.8 were enriched in pathways

of glycine, serine and threonine metabolism, propanoate metabolism, benzoate degradation, glyoxylate and dicarboxylate metabolism, valine, leucine and isoleucine degradation, degradation of aromatic compounds, lipoic acid metabolism, ABC transporters (Fig. 2C).

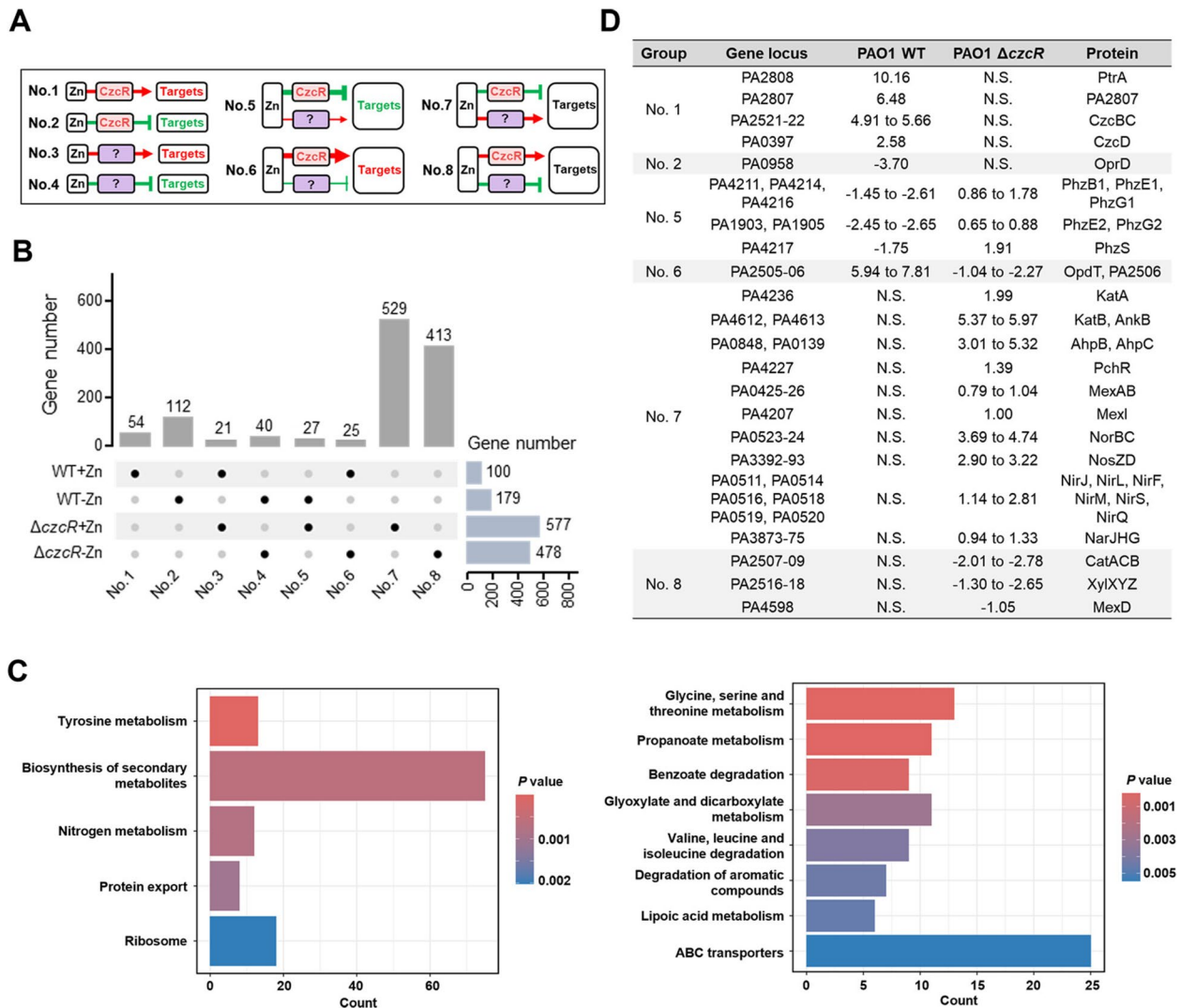


Fig. 2 Global gene regulation by CzcR-dependent and -independent pathways during Zn excess. **A** A diagram showing eight groups of target genes that were regulated by Zn in CzcR-dependent or/and CzcR-independent pathways. Question marks indicate unclear factors involved in the pathways. Red arrows and green T-shaped symbols indicate upregulation and repression, respectively. Targets highlighted in red indicate genes that are upregulated by Zn in the WT strain, targets highlighted in green indicate genes that are downregulated by Zn in the WT strain, targets in black indicate genes whose expression is not changed by Zn in the WT strain. **B** An UpSet plot showing the distribution of DEGs within eight groups as shown in (A). **C** KEGG pathway enrichment of genes that were present in the groups No.7 (left) and No.8 (right). **D** Representative target genes in groups No.1, 2, 5, 6, 7 and 8 were presented

Counteractive co-regulation of catalase genes *katA* and *katB* through CzcR-dependent and -independent pathways during Zn excess

Comparative transcriptome analysis between the WT and ΔczcR strains during Zn excess revealed the negative regulation of two key catalase genes *katA* and *katB* by CzcR [17]. Here, we showed that both genes were listed in group No.7 (Fig. 2D), meaning that these genes are co-regulated by CzcR-dependent and -independent pathways in a balanced manner during Zn excess. RT-qPCR verified that the expression of *katA* and *katB* genes

was not influenced in the WT strain during Zn excess but significantly upregulated by Zn in the ΔczcR mutant (Fig. 3A and B). To further confirm the regulatory patterns of *katA* and *katB* genes, we constructed two promoter activity reporters, *PkatA-lux* and *PkatB-lux*, and then used these two reporters to examine the transcriptional activities of both genes. As shown in Fig. 3C and D, the transcriptional activities of two genes were also not changed in the WT strain during Zn excess. However, the transcriptional activities of both genes were significantly induced by Zn in the ΔczcR mutant. This result not only

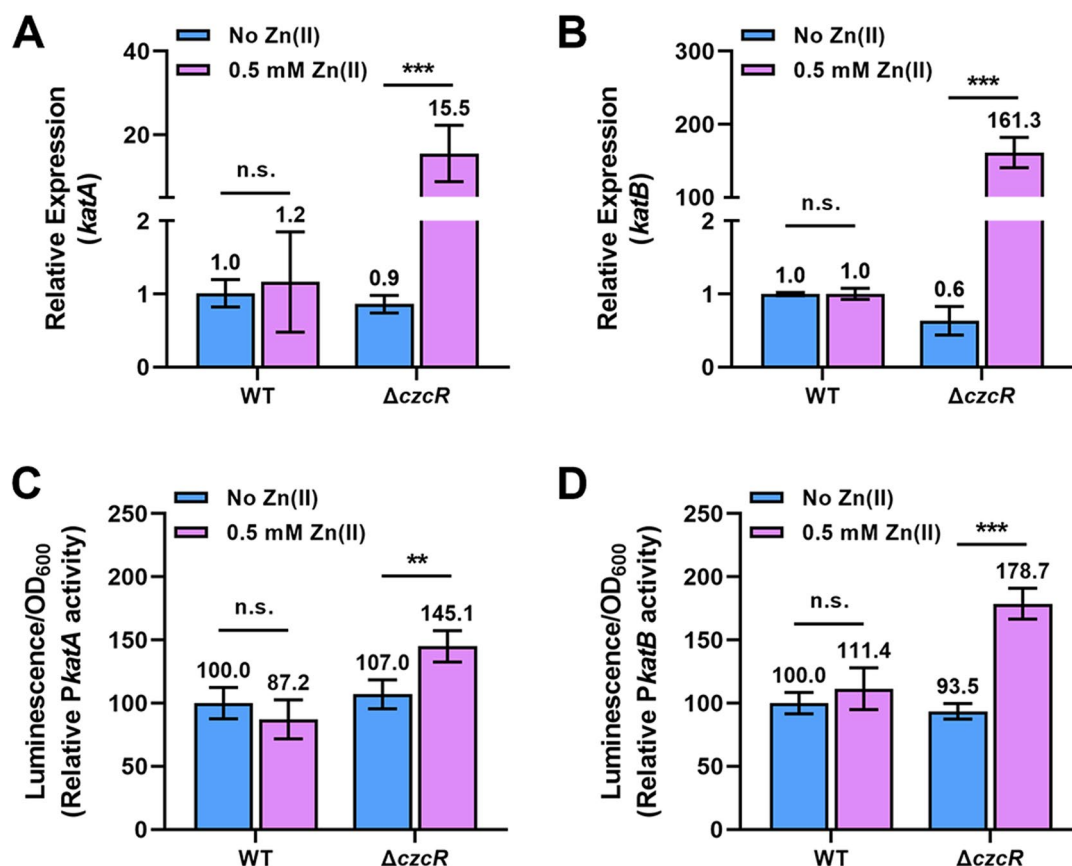


Fig. 3 CzcR represses the expression of *katA* and *katB* during Zn excess. **A** and **B** Relative expression of the *katA* (A) and *katB* (B) genes in the WT and $\Delta czcR$ strains when they were cultured with or without Zn treatment. **C** and **D** Activities of *PkatA-lux* (C) and *PkatB-lux* (D) were measured in the WT and $\Delta czcR$ strains when they were cultured with or without Zn treatment. Statistical significance was calculated based on two-way analysis of variance (ANOVA) (n.s., not significant; **, $P < 0.01$; ***, $P < 0.001$)

confirmed that CzcR negatively regulates the expression of *katA* and *katB* during Zn excess but also demonstrated the simultaneous presence of CzcR-independent pathways that are induced by Zn to upregulate the expression of two genes.

CzcR is a transcription factor that was demonstrated to regulate the expression of genes involved in quorum sensing, flagellar biosynthesis, antibiotic resistance, and metal homeostasis by directly binding to their promoters [14–17]. To understand how CzcR regulates the expression of *katA* and *katB*, we then searched for potential CzcR-binding sites in their promoters by aligning the promoter sequences with the 16-bp CzcR-binding motif (GAAACN₆GTAAT) [28]. Potential CzcR-binding sites were found in both promoters (Fig. 4A and B). This analysis suggested the possible interaction between CzcR and two promoters. Therefore, EMSA was performed, which showed that CzcR could bind to the promoters of both *katA* and *katB* genes (Fig. 4C). In contrast, CzcR did not bind to the promoter of *pvdR* which was selected as a negative control for EMSA (Fig. 4C) [17]. These results

revealed that the expression of *katA* and *katB* is repressed by CzcR through its direct binding at their promoters.

CzcR-mediated induction of catalase activity and H₂O₂ tolerance during Zn excess

KatA and KatB are two key catalases responsible for H₂O₂ detoxification [4, 5]. Considering their upregulation in the $\Delta czcR$ mutant during Zn excess, we speculated that the tolerance of the mutant to H₂O₂ might be enhanced. We pre-treated WT and $\Delta czcR$ strains with or without Zn and then incubated them in the presence of 1.5 mM, 2.5 mM, or 3.5 mM H₂O₂ to compare the survival rates. Unexpectedly, we found that the survival rate of the WT strain but not the $\Delta czcR$ mutant was significantly elevated after Zn treatment (Fig. 5A). Growth of WT and $\Delta czcR$ strains in the 0.5 mM H₂O₂ also showed that Zn could promote the growth of the WT strain and deletion of *czcR* led to an obvious growth defect (Fig. 5B), meaning that CzcR was essential for the increased H₂O₂ tolerance during Zn excess. We further examined the survival rate of the $\Delta czcR$ mutant with the complementation of

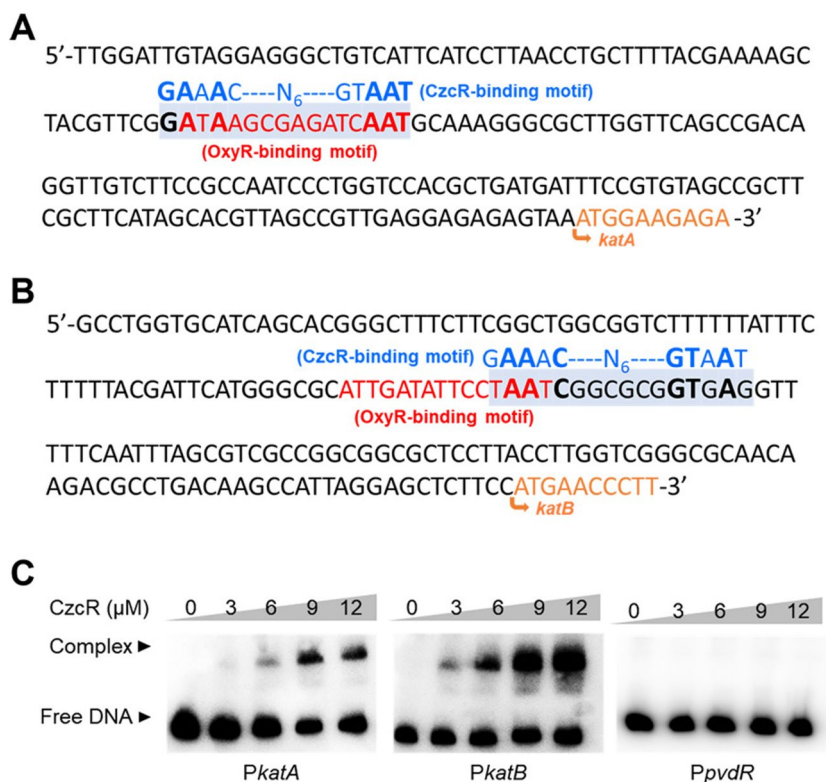


Fig. 4 CzcR directly targets the promoters of *katA* and *katB*. **A** and **B** Prediction of the CzcR-binding site in the promoters of *katA* (A) and *katB* (B). **C** EMSAs showed the binding ability of CzcR at the promoters of *katA* (*PkatA*) and *katB* (*PkatB*) but not the negative control promoter *PpvdR*

the *czcR* gene after the treatment of 3.5 mM H₂O₂. The result confirmed that the H₂O₂ tolerance induced by Zn is dependent on CzcR (Fig. 5C). These results displayed that Zn induced H₂O₂ tolerance in the WT strain instead of the $\Delta czcR$ mutant, which is not consistent with the induced expression of two catalase genes in the $\Delta czcR$ mutant but not the WT strain during Zn excess.

To explain the inconsistency, we next measured the catalase activity of the WT strain, $\Delta czcR$ mutant and the $\Delta czcR$ mutant with the complementation of *czcR*. It was found that the catalase activity was significantly increased by Zn treatment in the WT strain and the $\Delta czcR$ mutant with the complementation of *czcR*, while there was no change of the catalase activity in the $\Delta czcR$ mutant during Zn excess (Fig. 5D), supporting the enhanced H₂O₂ tolerance as observed in the WT strain instead of the $\Delta czcR$ mutant during Zn excess. Together, these results demonstrated that Zn induces CzcR-mediated catalase activity and H₂O₂ tolerance in *P. aeruginosa*.

Discussion

P. aeruginosa is equipped with abundant signal detection and transduction systems, which enable the pathogen to quickly adapt to the changing environment. TCS, typically composed of a histidine kinase to sense

environmental signals and a response regulator to control target genes expression, is one of the important signaling systems that connect bacterial behaviors including survival, metabolism, virulence, antibiotic resistance, interbacterial competition to the diverse environmental signals [29–31]. CzcS/CzcR is a versatile TCS that regulates global genes expression in response to the elevated Zn concentration in *P. aeruginosa*. By re-analyzing the effects of Zn on the transcriptomic profiles of the PAO1 WT strain and the $\Delta czcR$ mutant, in this study, we discovered a delicate and complex regulatory pattern of gene expression by Zn through CzcR-dependent and -independent pathways. Focusing on the key catalase genes *katA* and *katB*, we showed an interesting function of CzcR in negatively regulating the expression of both catalase genes but in positively regulating the activity of catalase and H₂O₂ tolerance during Zn excess (Fig. 6).

Bacterial gene expression and phenotypes are frequently co-regulated by multiple factors or pathways [32]. With the combined transcriptome analysis between WT and $\Delta czcR$ strains, this study comprehensively revealed the co-regulatory effects exerted by CzcR and its inducing signal Zn. Owing that most (~1,000) genes were inversely regulated by CzcR-dependent and -independent pathways during Zn excess, this study displayed the

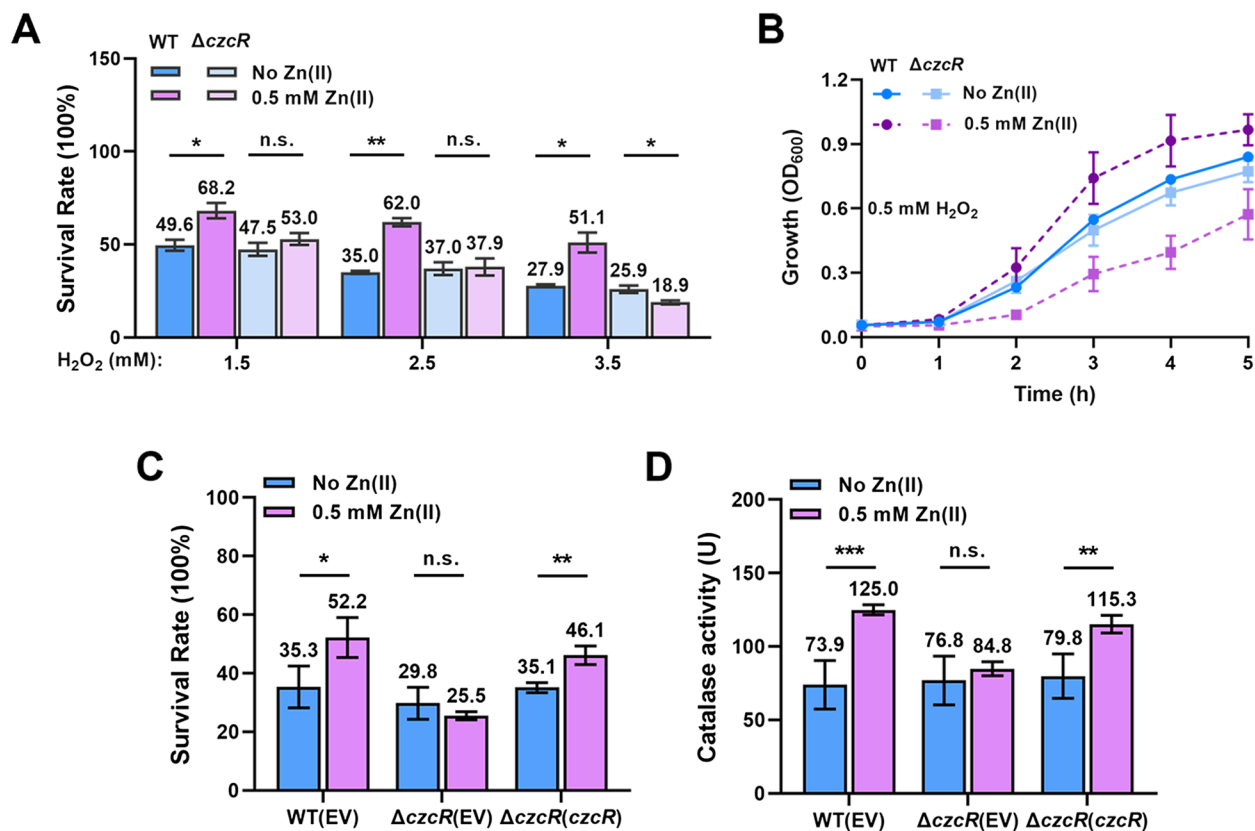


Fig. 5 CzcR-mediated induction of H₂O₂ tolerance and catalase activity during Zn excess. **A** Survival rate of the WT and $\Delta czcR$ strains when they were treated with 1.5 mM, 2.5 mM, and 3.5 mM H₂O₂ with the pre-treatment of Zn or not. **B** Growth curves of the WT and $\Delta czcR$ strains when they were cultured in the presence of 0.5 mM H₂O₂ with the pre-treatment of Zn or not. **C** Survival rate of the WT, $\Delta czcR$, and $\Delta czcR(czcR)$ strains when they were cultured in the presence of 3.5 mM H₂O₂ with the pre-treatment of Zn or not. **D** Catalase activity was measured in the WT, $\Delta czcR$, and $\Delta czcR(czcR)$ strains when they were cultured with or without Zn treatment. Statistical significance was calculated based on two-way ANOVA (n.s., not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

contribution of CzcR to maintain physiological balance by sustaining the expression of target genes that are disturbed by Zn excess, further highlighting the importance of TCS in stress adaptation. Possibly for this reason, a large number of physiological processes targeted by Zn are previously neglected owing that changes of their gene expression are neutralized by CzcR. For example, DNA microarray only identified 34 DEGs with significance in *P. aeruginosa* after the treatment of 1 mM ZnO nanoparticles and most of them are from groups No. 1 to 6 such as the *czc* genes, porin genes, *ptrA* and *phz* genes [33].

Although it has been notified that CzcR negatively regulates the expression of catalase genes *kata* and *katB* during Zn excess [17], molecular mechanisms underlying the regulation was not explored. By examining the promoter activities of two genes and interactions between CzcR and two promoters, we demonstrated that CzcR regulates the expression of *kata* and *katB* by directly and specifically recognizing and binding to their promoters. Moreover, by comparing the expression of *kata* and *katB*

in the WT strain when the strain was treated with Zn or not, we found that Zn did not influence their expression levels. Zn only induced their expression in the absence of CzcR, meaning that Zn simultaneously activates the expression of *kata* and *katB* independently of CzcR. However, the mechanism of activation is still unclear. Regulators such as OxyR, DksA that positively control catalase genes expression might act antagonistically with CzcR [34, 35]. Particularly, OxyR is a critical activator of both *kata* and *katB* and sequence analysis showed the potentially overlapped DNA targeting sites of OxyR and CzcR at the promoters of *kata* and *katB* (Fig. 4A and B) [35–37]. Thus, it is possible that the competitive binding of CzcR at the OxyR-binding site prevented the upregulation of *kata* and *katB*. When *czcR* was deleted, it could be OxyR that induced the expression of both genes in response to the elevated Zn concentration. However, expression changes of *oxyR* were not detected in both WT and $\Delta czcR$ strains after treatment of Zn from the transcriptome data. Whether Zn induces the activity of

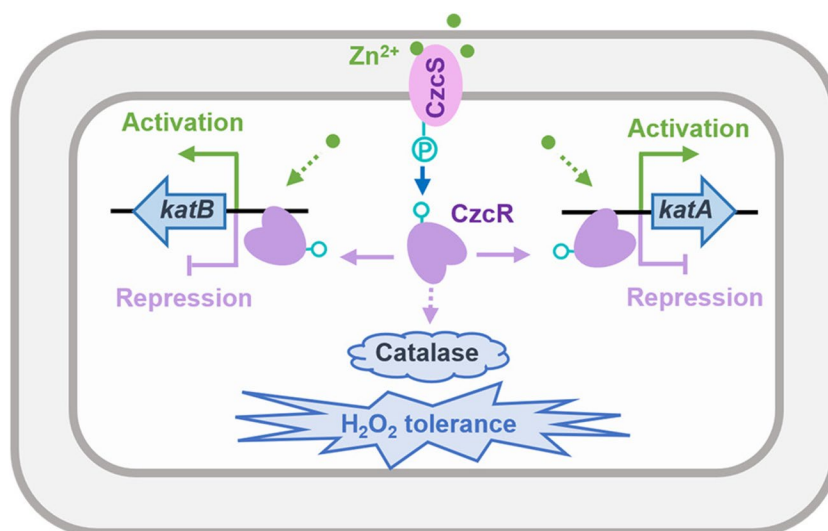


Fig. 6 A schematic diagram showing the regulation of catalase gene expression and catalase activity during Zn excess in *P. aeruginosa*. Zn positively regulates the expression of *katA* and *katB* with unclear mechanisms, and simultaneously, Zn activates CzcS/CzcR to negatively regulate the expression of *katA* and *katB* through the binding of CzcR at their promoters. As a result, the expression of the key catalase genes *katA* and *katB* is not influenced by Zn excess. In spite of the unchanged expression of *katA* and *katB*, Zn excess induces catalase activity and H₂O₂ tolerance of *P. aeruginosa* in a CzcR-dependent manner

OxyR and how Zn directly or indirectly induces catalase genes expression independently of CzcR require further investigations. Given that abundant DEGs including *katA* and *katB* were listed in groups No.7 and 8, another two possibilities may contribute to the unchanged expression of *katA* and *katB* in the WT strain during Zn treatment. One is the presence of additional Zn-responsive regulators that control global gene expression antagonistically with CzcR. The other is one or some DEGs such as genes involved in nitrate respiration are involved in the co-regulation of *katA* and *katB* with CzcR.

RNA-seq, RT-qPCR, and promoter activity assays showed that *katA* and *katB* were dramatically upregulated during Zn excess in the $\Delta czcR$ mutant. While interestingly, when evaluating the H₂O₂ tolerance of WT and $\Delta czcR$ strains, we did not observe increased catalase activity and H₂O₂ tolerance in the $\Delta czcR$ mutant during Zn excess in spite of the upregulation of two genes in this mutant. Instead, we found that catalase activity and H₂O₂ tolerance were significantly induced in the WT strain during Zn excess. These results not only answered why the $\Delta czcR$ mutant showed an obvious growth defect compared to the WT strain during Zn excess as reported in the previous study [17], but further demonstrated that Zn induces the catalase activity in the CzcR-dependent manner without changing the expression of catalase genes. However, mechanisms underlying the CzcR-dependent elevation of catalase activity during Zn excess are still unclear. Owing that expression of *katA* and *katB* genes were measured at the transcriptional level in this work,

whether their translation is activated by CzcR or members from the CzcR regulon is unknown. Because catalase activity and H₂O₂ tolerance are associated with the translational levels of *katA* and *katB* as well. For example, decreased protein levels of KatA and KatB were observed in mutants lacking the tRNA-modifying enzymes GidA or TrmB, which showed a decreased catalase activity and a H₂O₂-sensitive phenotype [38, 39]. Nonetheless, this finding provided new insights into the host adaptation of *P. aeruginosa*. Because release of both Zn and H₂O₂ in the phagocyte is a main strategy to eliminate engulfed pathogens [40, 41]. In addition to the activation of Zn export systems and repression of Zn import systems following the switch from Zn-deficient environment to Zn-rich environment [42], induced H₂O₂ tolerance in response to the elevation of Zn will further promote the survival of *P. aeruginosa* during infection. Therefore, the development of antimicrobial agents targeting the CzcS/CzcR signaling pathway may help to alleviate or prevent *P. aeruginosa* infections.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-024-03671-0>.

- Supplementary Material 1
- Supplementary Material 2
- Supplementary Material 3
- Supplementary Material 4
- Supplementary Material 5

Authors' contributions

T.L. and Z.X. designed the research; T.L., Z.M., and Y.Z. performed experiments; T.L., H.C., and Z.X. analyzed the data; T.L. drafted the manuscript; S.S. and Z.X. acquired fundings; H.C., S.S., and Z.X. revised the manuscript.

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Data availability

The transcriptome data was retrieved from NCBI (project accession number: PRJNA896733).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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