

RESEARCH ARTICLE

Open Access



OxyR-regulated catalase CatB promotes the virulence in rice via detoxifying hydrogen peroxide in *Xanthomonas oryzae* pv. *oryzae*

Chao Yu^{1†}, Nu Wang^{1†}, Maosen Wu¹, Fang Tian¹, Huamin Chen¹, Fenghuan Yang¹, Xiaochen Yuan², Ching-Hong Yang² and Chenyang He^{1*}

Abstract

Background: To facilitate infection, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the bacterial blight pathogen of rice, needs to degrade hydrogen peroxide (H₂O₂) generated by the host defense response via a mechanism that is mediated by the transcriptional regulator OxyR. The catalase (CAT) gene *catB* has previously been shown to belong to the OxyR regulon in *Xoo*. However, its expression patterns and function in H₂O₂ detoxification and bacterial pathogenicity on rice remain to be elucidated.

Results: The *catB* gene encodes a putative catalase and is highly conserved in the sequenced strains of *Xanthomonas* spp. β-galactosidase analysis and electrophoretic mobility shift assays (EMSA) showed that OxyR positively regulated the transcription of *catB* by directly binding to its promoter region. The quantitative real-time PCR (qRT-PCR) assays revealed that the expression levels of *catB* and *oxyR* were significantly induced by H₂O₂. Deletion of *catB* or *oxyR* drastically impaired bacterial viability in the presence of extracellular H₂O₂ and reduced CAT activity, demonstrating that CatB and OxyR contribute to H₂O₂ detoxification in *Xoo*. In addition, Δ *catB* and Δ *oxyR* displayed shorter bacterial blight lesions and reduced bacterial growth in rice compared to the wild-type strain, indicating that CatB and OxyR play essential roles in the virulence of *Xoo*.

Conclusions: Transcription of *catB* is enhanced by OxyR in response to exogenous H₂O₂. CatB functions as an active catalase that is required for the full virulence of *Xoo* in rice.

Keywords: *Xanthomonas oryzae* pv. *oryzae*, Catalase, Hydrogen peroxide, Virulence

Background

Plant innate immune responses to bacterial infection include an oxidative burst through elevating the levels of reactive oxygen species (ROS), which are toxic to bacterial cells and cause damages to proteins, nucleic acids, and cell membranes [1]. Hydrogen peroxide (H₂O₂), an important ROS, accumulates and diffuses widely between neighboring xylem elements [2]. Accordingly, pathogenic bacteria need to overcome the stress caused by H₂O₂ and gradually establish their infection in plants [3].

One of the mechanisms of bacterial resistance to H₂O₂ is through antioxidant enzymes including catalases [4]. Bacteria maintain basal oxidative stress resistance, but also possess a highly inducible oxidative stress response that is largely controlled by redox-sensing transcription factors which act as redox-operated genetic switches to activate genes involved in the oxidative stress response [5]. As one of the redox-sensing transcription factors, OxyR has been characterized in a number of pathogenic bacteria [6, 7]. OxyR is a DNA-binding transcription factor that is activated under oxidizing conditions by the formation of a disulfide bond between two cysteine residues [8]. When activated, OxyR regulates the expression of genes involved in detoxification by binding to their promoter regions, thus triggering cellular responses to H₂O₂ [5, 9].

* Correspondence: hechenyang@caas.cn

†Equal contributors

¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

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



Bacterial catalases (CATs) are central components of detoxification pathways, which prevent formation of highly reactive hydroxyl radical by catalyzing the conversion H_2O_2 to water and oxygen [10]. Based on their enzymatic properties, catalases fall into three classes including monofunctional heme-containing catalases, bifunctional heme-containing catalase-peroxidases, and nonheme or Mn-containing catalases [11]. Multiple catalase isozymes encoded by different genes have been identified in many bacterial species. Interestingly, these genes have different expression patterns in growth phases and in response to oxidative stress, suggesting that they may have different roles in physiological processes and bacteria-host interactions [12]. Moreover, the transcriptional levels and activities of bacterial catalases were largely induced by H_2O_2 [13]. For example, *Xanthomonas campestris* pv. *campestris* and *X. axonopodis* pv. *citri* displayed increased levels of catalases KatE and KatG and H_2O_2 resistance after H_2O_2 treatment [10, 14, 15]. In addition, several studies have reported that catalase activity was induced during the bacterial infection [3, 16], however, the regulatory mechanism of H_2O_2 detoxification and its relation to bacterial virulence remains to be demonstrated.

Bacterial leaf blight caused by *X. oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases of rice, which causes annual yield losses of 10–50 % in many rice growing countries [17–19]. Understanding the molecular mechanisms *Xoo* virulence is pivotal to develop effective disease control strategies [20]. *Xoo* produces several virulence-related factors including exopolysaccharide, extracellular enzymes, toxins, adhesins, and the type III secretion system and its effectors during infection [21, 22]. Furthermore, to facilitate its virulence, *Xoo* might have evolved a mechanism for suppression and evasion of basal defense response of rice, such as employing catalases to detoxify H_2O_2 elicited by host innate immunity [23–25]. Detoxification of endogenous H_2O_2 that is generated through normal metabolic processes, such as aerobic respiration, is mediated by OxyR via regulating the *ahpC* and *ahpF* genes encoding alkyl hydroperoxide reductase [26, 27]. Deletion of *ahpC* significantly affected H_2O_2 accumulation during the rice-*Xoo* interaction [28]. *catB* (*PXO_02830*), *katE* (*PXO_02109*) and *srpA* (*PXO_02864*), three putative catalase genes were revealed in the genome of *Xoo* wildtype strain PXO99^A by *in silico* analysis [29]. The transcription of these genes were strongly induced during the bacterial interaction with rice suspension-cultured cells [30]. In addition, in-frame deletion of the *katE* gene significantly attenuated bacterial pathogenesis in rice but not H_2O_2 resistance [31]. The regulatory mechanisms and involvement in H_2O_2 detoxification and pathogenesis of CatB remain unknown.

In this study, we characterized the regulatory mechanism and function of CatB in H_2O_2 resistance and its

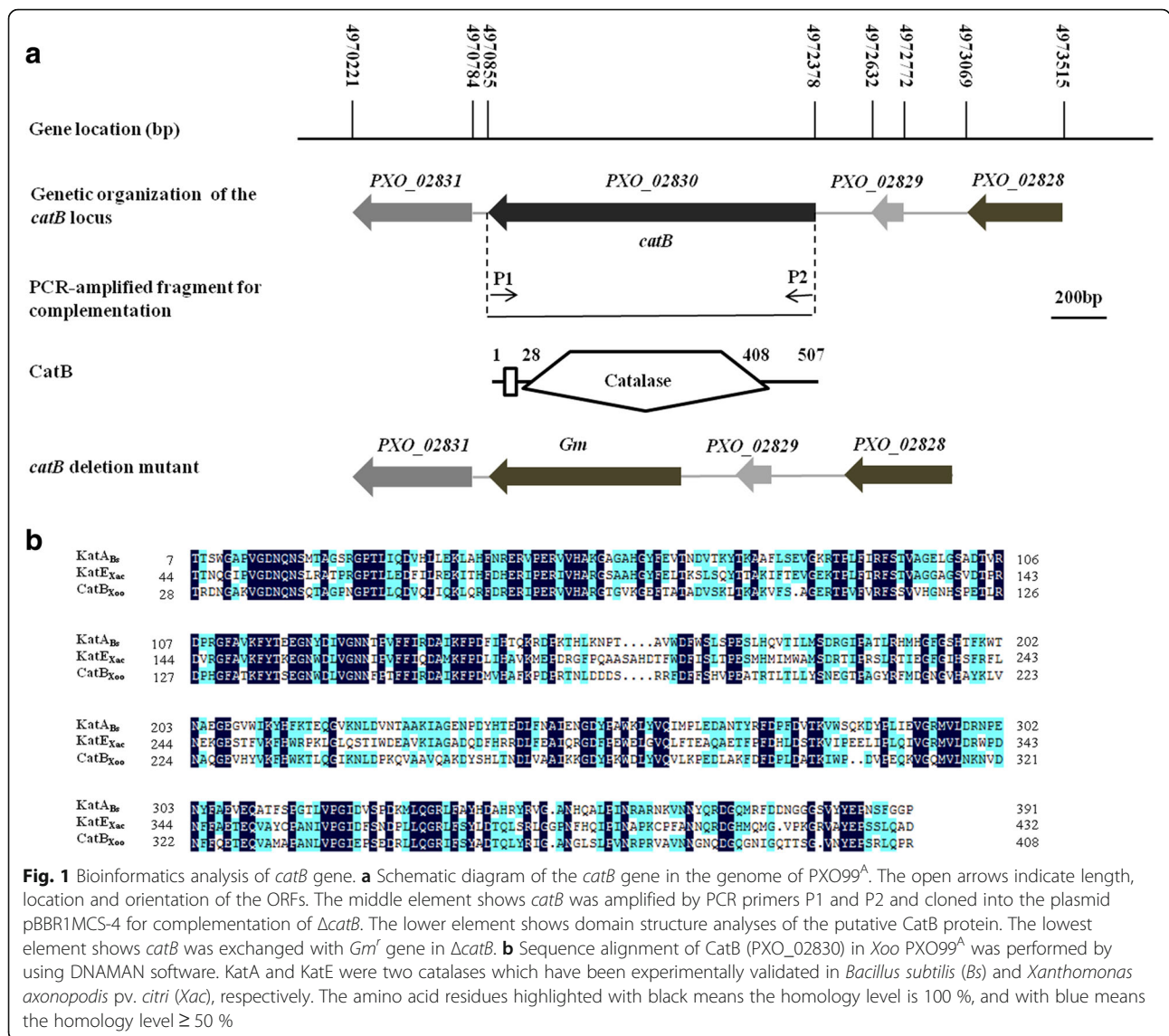
contribution to virulence in rice. Promoter activities and qRT-PCR assays demonstrated that *catB* gene was transcriptionally responsive to H_2O_2 and positively regulated by OxyR. Gene deletion and complementation analysis revealed that CatB greatly contributed to both H_2O_2 detoxification and full virulence. This study demonstrates that CatB is one of the key virulence factors to facilitate pathogenesis of *Xoo* in rice via H_2O_2 detoxification.

Results

Identification, deletion and complementation of the *catB* gene

It has been reported that *catB* is one of three putative catalases-encoding genes (*catB* (*PXO_02830*), *katE* (*PXO_02109*), and *srpA* (*PXO_02864*)) that are responsible for H_2O_2 degradation in the genome of *Xoo* wild-type strain PXO99^A [29]. The open reading frame (ORF) of *catB* is 1,524 bp in length and located in the genome at nucleotide position 4970855–4972378 (Fig. 1a). CatB contains one catalase domain (residues 28–408) with a predicted isoelectric point of 8.66 and molecular weight of 56.4 kD. CatB is also well conserved in other sequenced strains of *Xoo* (MAFF311018, KACC10331, and PXO86) (Additional file 1: Table S1). Additionally, CatB shared over 90 % amino acid identity with those in other important plant-pathogenic *Xanthomonas* species, including *X. campestris* pv. *campestris*, *X. campestris* pv. *vesicatoria*, *X. axonopodis*, *X. perforans*, *X. fragariae*, *X. arboricola*, *X. hortorum*, and *X. fuscans* (Additional file 1: Table S1). Sequence alignment analysis with experimentally validated active CATs, such as KatE from *X. axonopodis* pv. *citri* and KatA from *Bacillus subtilis* revealed that many critical residues in the catalase domain of CatB were probably conserved (Fig. 1b). These observations indicate that CatB might function as an active CAT.

To investigate the potential biological function of CatB in *Xoo*, a *catB* gene deletion mutant ($\Delta catB$) and complementary strain $\Delta catB$ (pBBR-*catB*) were constructed as described in the Methods. DNA sequencing analysis showed that the corresponding region of *catB* was replaced by the *Gm^r* gene (855 bp in length) in the $\Delta catB$ mutant. The growth for PXO99^A, $\Delta catB$ and $\Delta catB$ (pBBR-*catB*) were detected in M210 liquid medium. No significant differences were observed in growth rates and bacterial populations between the three strains (Additional file 2: Figure S1). In addition, our previous studies have shown that there was no difference between $\Delta oxyR$ and wild type in growth *in vitro* [26]. These results suggest that deletion of *catB* or *oxyR* does not affect the viability of *Xoo* under normal growth conditions.

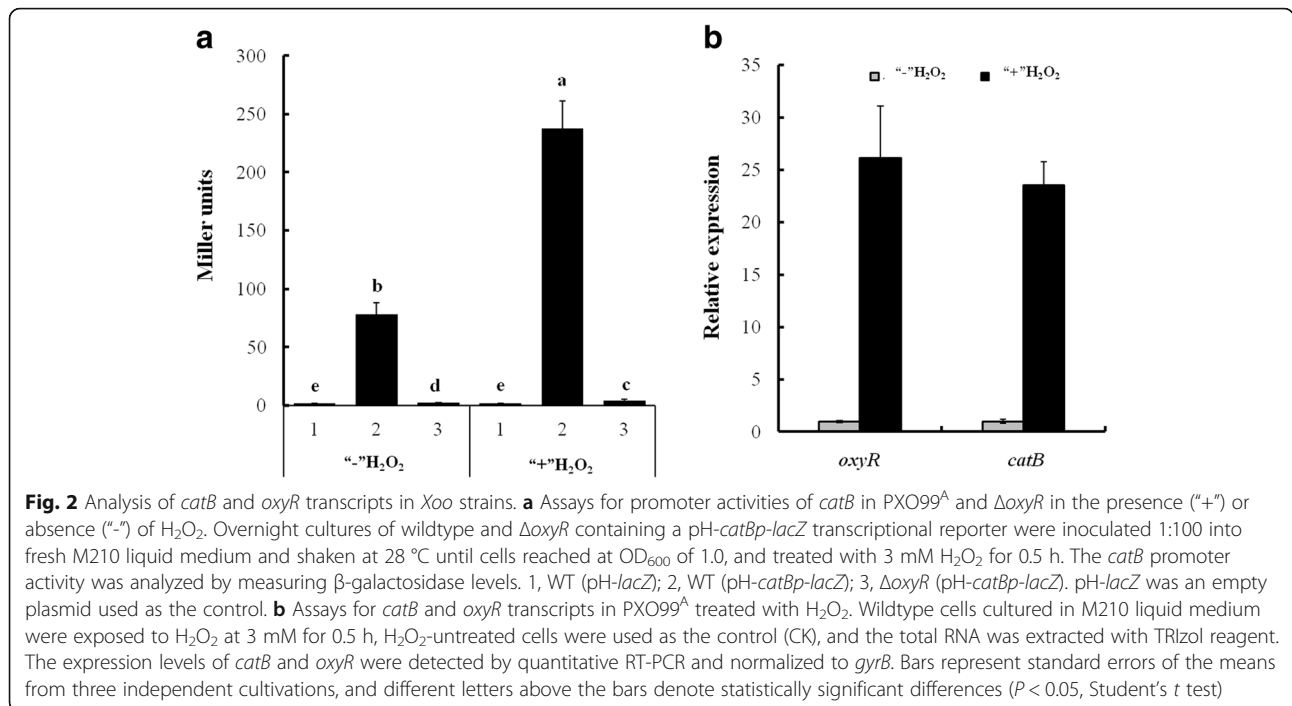


***catB* is transcriptionally regulated by OxyR and responsive to exogenous H₂O₂**

OxyR has been shown to function as a transcriptional regulator mediating H₂O₂ detoxification in *Xoo*. Our previous studies showed that the *catB* transcripts were significantly reduced in the *oxyR* gene deletion mutant ($\Delta oxyR$) [26, 30], implying expression of *catB* is regulated by OxyR. To examine whether *catB* is a direct target of OxyR, we first expressed the recombinant OxyR protein in *E. coli* BL21 strain and obtained purified protein (Additional file 3: Figure S2). We then examined the promoter activity of *catB* by the measuring β -galactosidase activities of *catBp-lacZ* fusion in various strains. Our results showed that the β -galactosidase activity was 17-fold higher in PXO99^A than in $\Delta oxyR$ (Fig. 2a). Then, electrophoretic mobility shift assay (EMSA) was performed to detect the binding between

OxyR protein and the *catB* promoter. The results indicated that the OxyR protein bound directly to the *catB* promoter region (Fig. 3). In contrast, the negative control BSA did not bind to the *catB* promoter region. Addition of unlabeled *catB* promoter DNA fragments as a competitive probes resulted in reduced binding of OxyR with the labeled DNA fragment, while mixture with the 16S rDNA as a non-specific probe did not affected the binding (Fig. 3). These data demonstrated that OxyR regulated the transcription of *catB* gene by directly binding to its promoter region.

To investigate whether their expressions respond to H₂O₂ stress, the transcripts of both *catB* and *oxyR* in wild-type PXO99^A in the presence or absence of exogenous H₂O₂ were assayed by qRT-PCR. The transcription levels of *catB* and *oxyR* were significantly elevated when exogenous H₂O₂ was applied (Fig. 2b). In addition, the activity of

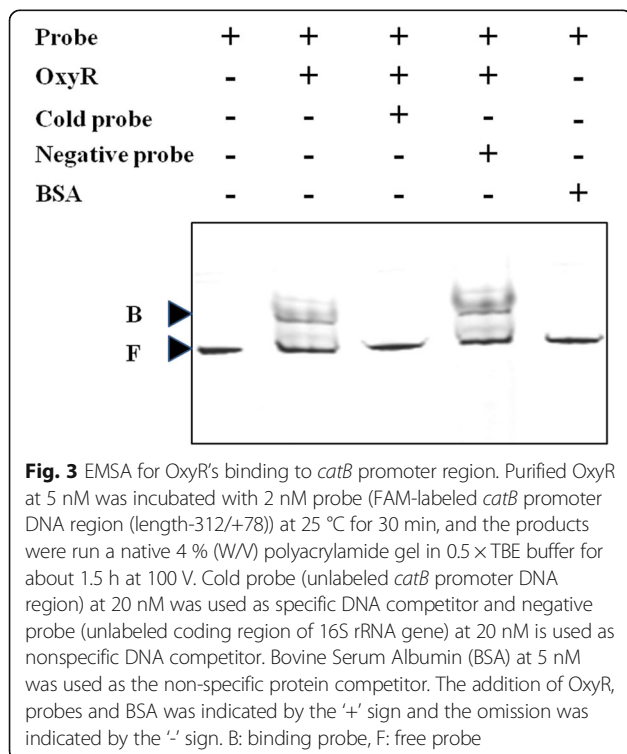


catB promoter in wildtype PXO99^A and Δ *oxyR* were measured under exogenous H₂O₂ conditions. As expected, the activity of *catB* promoter was dramatically induced in wildtype PXO99^A but only slightly increased in Δ *oxyR* in the presence of exogenous H₂O₂ (Fig. 2a). Therefore,

these results demonstrated that OxyR sensed the presence of H₂O₂ and then activated the transcription of *catB*.

CatB and OxyR enhance bacterial viability and CAT activity under H₂O₂ stress

To determine the role of *catB* and *oxyR* in H₂O₂ resistance, the halo assays of wildtype, Δ *catB*, Δ *oxyR* and their complementary strains Δ *catB* (pBBR-*catB*) and Δ *oxyR* (pBBR-*oxyR*) were performed with the presence of 0.25, 0.5 and 1 M of H₂O₂, respectively. The sensitivity of bacteria to H₂O₂ was indicated by the zone of inhibition. As shown in Fig. 4a and b, the diameters of inhibitory zone for Δ *catB* and Δ *oxyR* were significantly bigger than that of the wildtype at each concentration of H₂O₂, while no differences were observed between wildtype and the complementary strains. To further test the H₂O₂ sensitivity, the growth ability of PXO99^A, Δ *catB* and Δ *oxyR* and their complementary strains Δ *catB* (pBBR-*catB*) and Δ *oxyR* (pBBR-*oxyR*) in M210 with the presence of 0, 0.25, 0.5 and 1 mM of H₂O₂, respectively, were detected. In the absence of H₂O₂, there was no significant difference in growth rate and bacterial population at 12 and 24 h among these strains (Fig. 4c). Compared with the wild type, Δ *catB* and Δ *oxyR* showed more sensitivities at the concentration of 0.25 and 0.5 mM of H₂O₂ in M210, and their complementary strains were restored to the wild-type levels (Fig. 4c). Moreover, the growth rates of these strains were significantly inhibited and the bacterial numbers were dramatically decreased in the presence of 1 mM of H₂O₂ (Fig. 4c). This demonstrates the



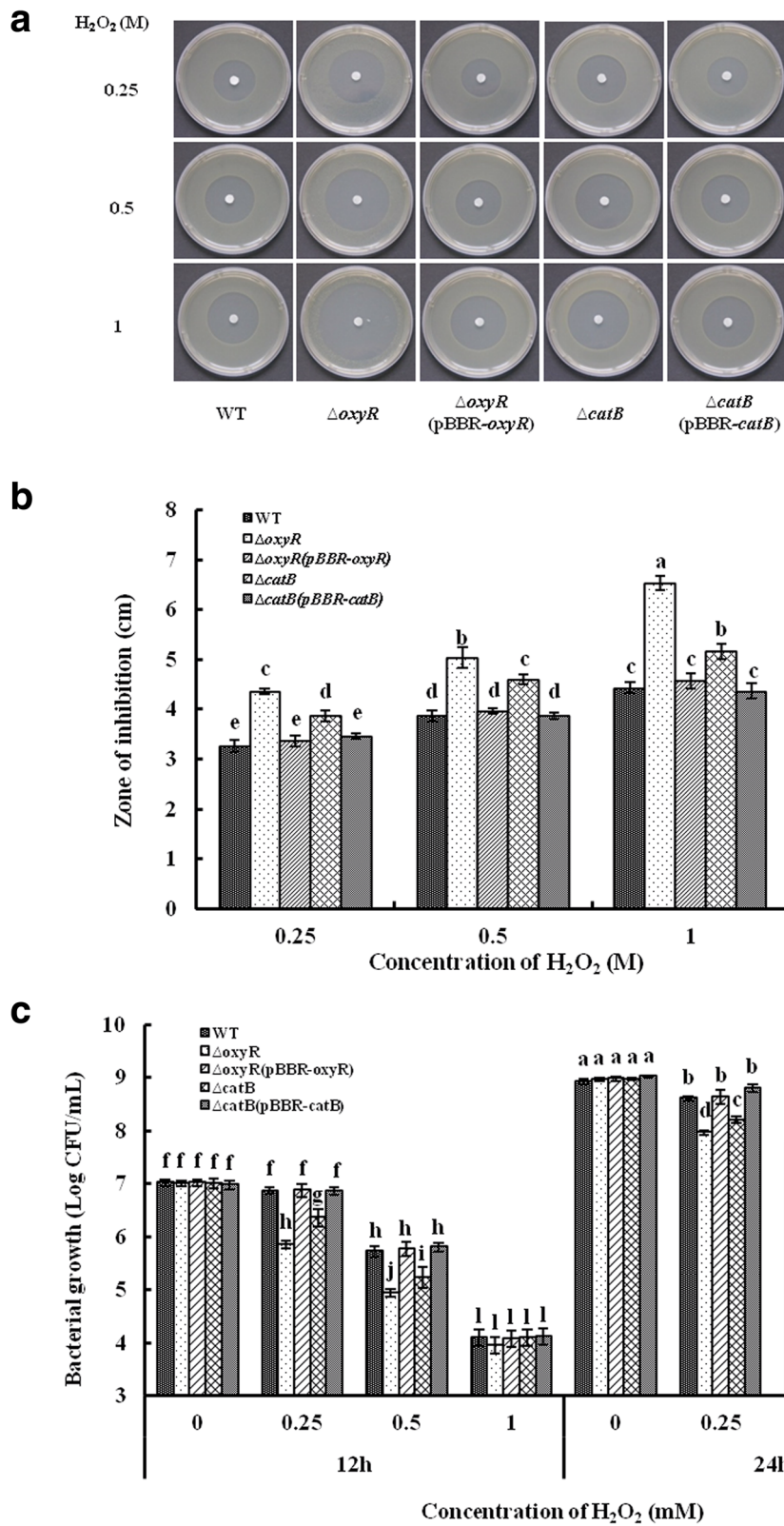


Fig. 4 (See legend on next page.)

(See figure on previous page.)

Fig. 4 Assays for H₂O₂ resistance of *Xanthomonas oryzae* pv. *oryzae* strains. **a** Disk diffusion assays. Wildtype, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ strains at OD₆₀₀ of 1.0 were mixed with PSA medium at 1 : 100 v/v and disks saturated with different concentrations (0.25, 0.5 and 1 M) of H₂O₂ were placed on the central of plates. These plates were incubated at 28 °C for 72 h and the H₂O₂ inhibition zones were observed. **b** Diameters of the H₂O₂ inhibition zones. **c** H₂O₂ sensitivity assays. Wildtype, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ strains at OD₆₀₀ of 1.0 were mixed with fresh M210 liquid medium at 1: 1000 v/v, and the H₂O₂ were added to the final concentration at 0, 0.25, 0.5 and 1 mM, respectively. The mixtures were incubated at 28 °C with 200 rpm, and the bacterial population were detected at 12 and 24 h. These experiments repeat three times, independently. Error bars represent standard derivations, and different letters above the bars denote statistically significant differences ($P < 0.05$, Student's *t* test)

essential role of CatB and OxyR in protecting bacterial viability under H₂O₂ stress in *Xoo*.

To further unveil the function of *catB* and *oxyR* in H₂O₂ degradation, we comparatively measured the CAT activities of PXO99^A, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$. The catalase activities of $\Delta catB$ and $\Delta oxyR$ were significantly decreased compared to that of PXO99^A, but were restored in their complementary strains $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ (Fig. 5a). In addition, the H₂O₂ accumulations of these

strains were measured. Compared with wildtype, the H₂O₂ concentrations were significantly increased in $\Delta catB$ and $\Delta oxyR$, but were restored in their complementary strains (Fig. 5b). Our results suggested that CatB and OxyR contributed to CAT activity for H₂O₂ detoxification in *Xoo*.

$\Delta catB$ and $\Delta oxyR$ elicit early H₂O₂ production in rice

In response to pathogenic infection, host plants produce ROS to defend themselves [32]. To evaluate their abilities in the induction of ROS, H₂O₂ production were detected in rice leaves at 12 and 24 h post-inoculation of wildtype, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$, and $\Delta oxyR(pBBR-oxyR)$ strains by using 3, 3'-diaminobenzidine (DAB) staining. Similar dark spots formed by DAB in the presence of H₂O₂ were observed in all bacterium-inoculated areas at 12 h, whereas no H₂O₂ accumulation was found in the H₂O control (Additional file 4: Figure S3). The same results were observed for all treatments at 24 h post-inoculation. Thus, these findings indicate that wildtype, $\Delta catB$ and $\Delta oxyR$ induce H₂O₂ accumulation in rice at early stage of infection.

$\Delta catB$ and $\Delta oxyR$ showed attenuated virulence and bacterial growth in rice

To determine the functions of CatB and OxyR in virulence, pathogenicity tests for wildtype, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ on susceptible rice plants were performed. The bacterial cells were inoculated onto the tip of rice leaves by leaf-clipping. The bacterial blight symptoms of rice were scored 14 days after bacterial inoculation. Compared with PXO99^A, $\Delta catB$ and $\Delta oxyR$ displayed reduced disease severity with shorter lesion lengths (Fig. 6a and b), and decreased bacterial growth in rice leaf tissues, while $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ showed disease phenotypes at near-wildtype levels (Fig. 6c). These observations demonstrated that the CatB and OxyR were required for the full virulence and *in planta* growth of *Xoo* in rice.

Discussion

Pathogenic bacteria successfully survive in the environment and infect plant tissues in part by depending on

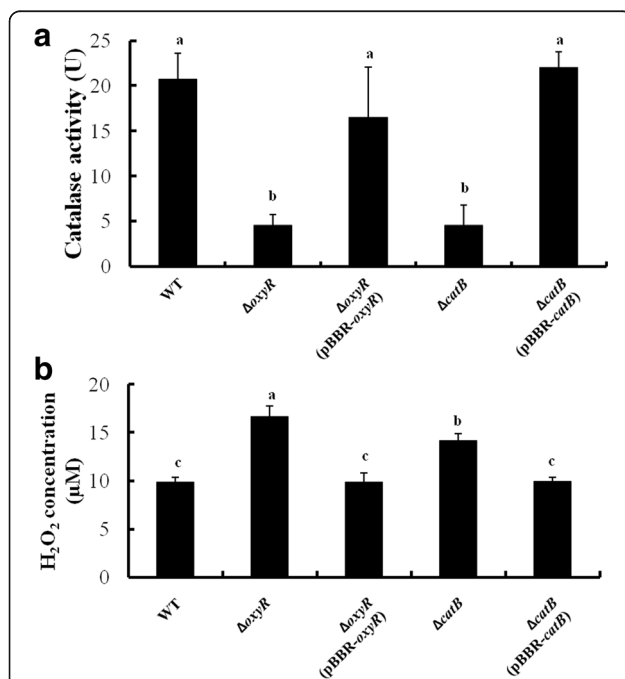
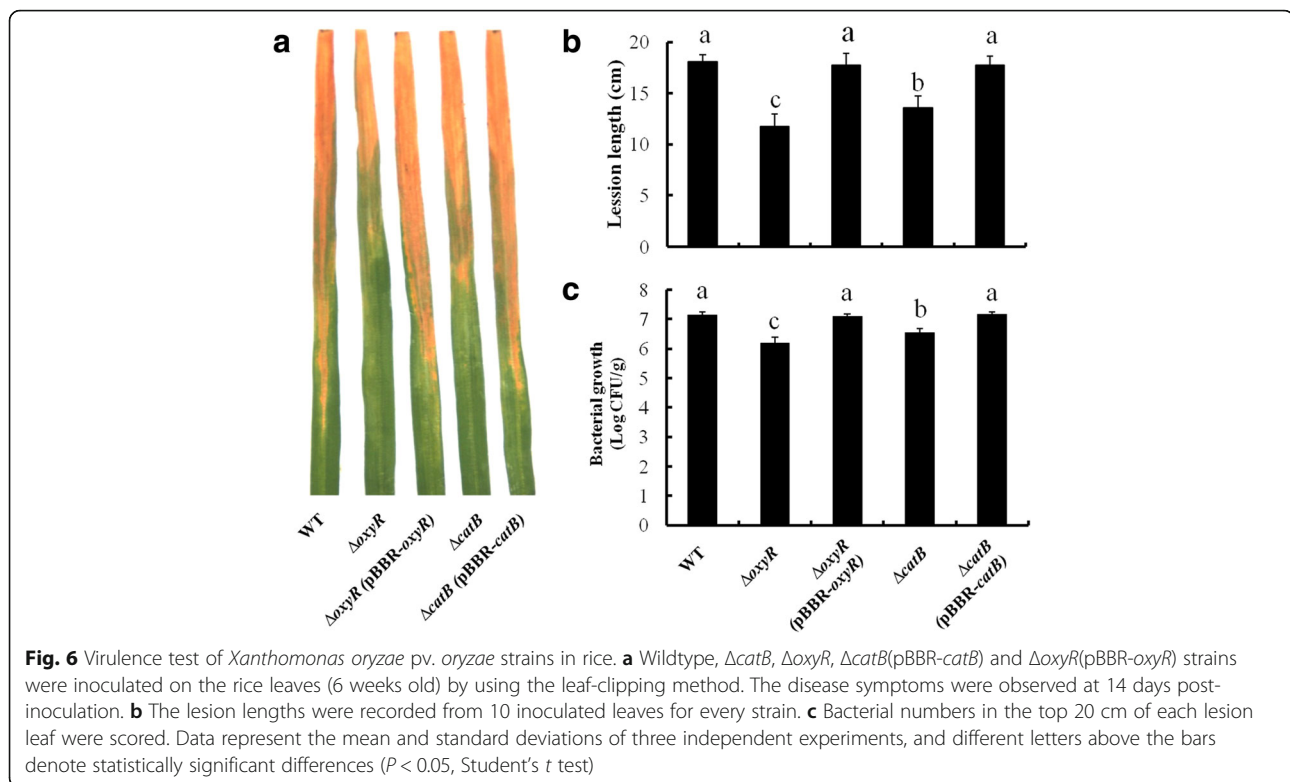


Fig. 5 Assays for catalase activity and H₂O₂ accumulation of *Xanthomonas oryzae* pv. *oryzae* strains. **a** Catalase activity assays. The cells of wildtype, $\Delta catB$, $\Delta oxyR$, $\Delta catB(pBBR-catB)$ and $\Delta oxyR(pBBR-oxyR)$ were disrupted by sonication, and the cell extracts were separated by centrifugation at 12,000 g. Catalase activities associated with cell extracts were assessed by spectrophotometric assay. One unit (U) is defined as the amount of activity required to decompose 1 μ mol of H₂O₂ in one minute. **b** H₂O₂ accumulation assays. The H₂O₂ concentrations of *Xoo* strains were measured as described in Methods, and the amount of H₂O₂ was accounted by the standard curve. Error bars represent standard derivations from three replicates, and different letters above the bars denote statistically significant differences ($P < 0.05$, Student's *t* test)



their abilities to counteract oxidative stresses including H_2O_2 , which can penetrate through bacterial membranes to affect a variety of cellular processes [1]. Since *Xoo* is constantly exposed to H_2O_2 , its catalases are very likely to be critical to the H_2O_2 detoxification process, whether it is produced endogenously through normal aerobic respiration, or the oxidative burst of rice plant cells during plant-pathogen interactions. In the current study, we revealed that CatB, working with OxyR, played a crucial role in H_2O_2 resistance and bacterial virulence in *Xoo* by using bioinformatics and genetic analysis approaches. To our immediate knowledge, CatB is the first catalase functionally characterized to facilitate pathogenesis via H_2O_2 detoxification in *Xoo*.

Bacterial catalases have been reported to be involved in the H_2O_2 -degradation pathway and increased tolerance to oxidative stress [13]. *Xoo* depends on a diverse repertoire of antioxidative enzymes to detoxify H_2O_2 for its in vitro growth and survival under different H_2O_2 stress conditions [26]. Transcription of three catalase genes *catB*, *katE* and *srpA* were strongly induced by exogenous H_2O_2 and during the bacterial interaction with rice suspension-cultured cells in *Xoo* [30]. Gene deletion of *katE* remarkably reduced bacterial growth in vitro and diseases leaf lesion of rice, but did not attenuate CAT activity and bacterial resistance to exogenous H_2O_2 [31]. The physiological role of *SrpA* in *Xoo* needs to be studied in the future. In this study, we identified a catalase

gene encoded CatB in *Xoo*, which shared high homology with two validated catalases *KatE* and *KatA* from *X. axonopodis* pv. *citri* and *Bacillus subtilis*, respectively (Fig. 1). Deletion of *catB* dramatically attenuated exogenous H_2O_2 resistance and CAT activity (Figs. 4 and 5), but did not affect the bacterial growth in vitro (Additional file 2: Figure S1). These results suggested that CatB was one of the key players participating in the H_2O_2 degradation pathway in *Xoo*. Furthermore, the homologues of CatB were widely found in other important plant-pathogenic *Xanthomonas* species and pathovars (Additional file 1: Table S1), suggesting that there might be a conserved and functional H_2O_2 resistance mechanism by CatB and its homologs in *Xanthomonas* species.

OxyR was identified as the primary H_2O_2 sensor responsible for H_2O_2 resistance in gram-negative bacteria [8, 33]. Previous studies showed that the cysteine residue (C199) of OxyR in *E. coli* was directly oxidized by H_2O_2 to a sulfenic acid, and generated a disulfide bond [34]. This oxidation process activated OxyR as a transcription factor. In *Pseudomonas aeruginosa*, the expression of OxyR was dramatically increased by H_2O_2 , and mutation of three cysteine sites (C199S, C208S and C296S) for OxyR displayed hypersensitivity to H_2O_2 [35]. In this study, the transcription level of *oxyR* was significantly induced by exogenous H_2O_2 (Fig. 2b), implying that OxyR might have similar function to sense H_2O_2 , thereby activating the downstream target gene expression in *Xoo*.

As a transcriptional activator in response to H₂O₂, OxyR has the ability to directly regulate the transcription of target genes through binding to the upstream DNA region of their promoter [36]. The binding sites of four OxyR regulated genes, *kata*, *dps*, *ftn* and *cydA*, were studied in *Corynebacterium glutamicum* [37]. A 50-bp region protected by the OxyR protein in the promoter of each gene was identified using DNase I footprinting analyses. However, no significant sequence similarity was found among the four OxyR-binding sites [37], suggesting the binding sites may vary in different promoters. In this study, we demonstrated that OxyR directly bound to the DNA fragment between -312 and +78 bp with respect to the translation start site of *catB* in *Xoo* using EMSA (Fig. 3). In addition, the promoter activity of *catB* was significantly reduced in *oxyR* deletion mutant (Fig. 2a). These results suggested that OxyR positively regulated the transcription of *catB* by binding to its promoter region. The OxyR binding sites in the *catB* promoter region will be further studied in the future.

OxyR plays a role during the peroxide stress response by regulating the transcription of catalase genes has been reported in various bacteria, but the regulatory mechanisms are different [5, 16]. For example, OxyR functioned as a positive regulator to activate the expression of catalase genes, and the *oxyR* deletion mutant was hypersensitive to hydrogen peroxide in *Escherichia coli* and *Salmonella typhimurium* [38]. In contrast, OxyR in *Corynebacterium diphtheria* repressed catalase production, and the *oxyR* deletion mutant displayed increased tolerance to H₂O₂ [39]. In this study, the promoter activity of *catB* in wildtype *Xoo* strain was significantly enhanced by H₂O₂, but dramatically decreased in $\Delta oxyR$ (Fig. 2a). In addition, deletion of *oxyR* or *catB* significantly reduced the tolerance to H₂O₂ and catalase activity (Figs. 4 and 5). These results showed that OxyR acts as a positive regulator to mediate H₂O₂ detoxification via controlling *catB* gene transcription in *Xoo*.

Our earlier studies have revealed that OxyR mediated endogenous H₂O₂-degradation by regulating the alkyl hydroperoxide reductase genes *ahpC/ahpF* in *Xoo* [26, 27]. Moreover, the transcriptional levels of catalase gene, *katE* and *srpA*, were down-regulated in $\Delta oxyR$ [26]. These results showed that several genes related with H₂O₂ detoxification were regulated by OxyR in *Xoo*. In this study, we showed that $\Delta oxyR$, in comparison with $\Delta catB$, was more hypersensitive to H₂O₂ (Fig. 4), and contained higher level of endogenous H₂O₂ (Fig. 5b), suggesting that there might be other genes besides *catB* that are regulated by OxyR in response to H₂O₂ in *Xoo*. Interestingly, no significant difference in catalase activity in $\Delta catB$ and $\Delta oxyR$ was found (Fig. 5a), implying that the CatB might be one of the major catalases in *Xoo*.

As an important host innate immune response, H₂O₂ is generated at the attempted invasion site in plant cells during interactions with potential pathogens and increased host disease resistance [2]. Previously, we have shown that H₂O₂ accumulation was induced in rice by *Xoo* infection. Deletion of an alkyl hydroperoxide reductase gene *ahpC* in *Xoo* significantly decreased the endogenous H₂O₂ accumulation. However, the H₂O₂ scavenging activity was increased with a unknown compensatory mechanism, which led to a lower level of H₂O₂ accumulation in the *ahpC* mutant than the wild type during their interactions with rice host plants [28]. These results indicated that AhpC plays the role in the detoxification of endogenous H₂O₂ in *Xoo*. In this study, compared with wildtype, $\Delta catB$ and $\Delta oxyR$ showed more hypersensitivity to exogenous H₂O₂ (Fig. 4) and higher concentration of endogenous H₂O₂ (Fig. 5a), indicating that CatB and OxyR in *Xoo* might be responsible for detoxification of both endogenous and exogenous H₂O₂. In addition, H₂O₂ accumulations were also observed in rice plants infected by *Xoo* strains (Additional file 4: Figure S3), suggesting that the ability of *Xoo* strains to induce and/or degrade H₂O₂ is a key determinant of outcome in the *Xoo*-rice interaction. To further study the differences in H₂O₂ accumulation induced by wildtype, $\Delta catB$ and $\Delta oxyR$, quantitative analysis of H₂O₂ production in rice and the ability of H₂O₂ detoxification in *Xoo* strains would be required.

During evolution, catalase activity has become inducible to help pathogen colonize its host and cause disease by detoxifying H₂O₂, implicating that there is a close relationship between bacterial ability to survive H₂O₂ stress and its virulence [3, 16]. In *X. campestris*, a catalase KatG is required for virulence in a host plant by providing protection against low levels of H₂O₂ [14]. Here, we showed the disease severity and bacterial population were dramatically reduced in rice leaves inoculated with $\Delta catB$ and $\Delta oxyR$ (Fig. 6), suggesting both $\Delta catB$ and $\Delta oxyR$ were significantly attenuated in detoxification of H₂O₂ in rice leaves, thereby resulting in the reduced pathogenicity. Based on these observations, combined with our earlier result that *catB* and *oxyR* were transcriptionally induced by H₂O₂ produced during interaction with rice suspension-cultured cells and also in real time of infection of rice [26, 30], we propose that both CatB and OxyR are required for full virulence and *in planta* growth of *Xoo* in rice by detoxification of H₂O₂. Moreover, shorter lesion lengths and fewer bacterial numbers in rice caused by $\Delta oxyR$ were observed than that by $\Delta catB$ (Fig. 6), indicating that there are other virulence factors regulated by OxyR in *Xoo*-rice interactions. This result is consistent with the previous studies that the OxyR regulon comprised of multiple genes involved in H₂O₂

detoxification, heme biosynthesis, reductant supply, thiol-disulfide isomerization, Fe-S center repair, iron binding and so on [5]. Accordingly, our identification of CatB regulated by OxyR in *Xoo* highlights the requirement of a functional H₂O₂-detoxification for bacterial pathogenesis in rice.

Conclusions

The *Xoo catB* gene is transcriptionally up-regulated by OxyR in response to H₂O₂ either exogenously-applied or generated in rice upon bacterial infection. CatB functions as an active catalase to detoxify H₂O₂ and is also required for the full virulence. Thus, OxyR-regulated catalase CatB promotes the bacterial pathogenesis in rice through H₂O₂ detoxification.

Methods

Bacterial strains and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were grown in Luria-

Bertani medium at 37 °C. *Xoo* wildtype strain PXO99^A and derived mutants were cultured at 28 °C on peptone sucrose agar (PSA) [40] medium or M210 [41] liquid medium with appropriate antibiotics. The antibiotics used were ampicillin (Ap), kanamycin (Km), spectinomycin (Sp) and gentamycin (Gm) at concentrations of 100, 50, 50, and 50 µg mL⁻¹, respectively.

Bioinformatics analysis of CatB

The domain organization of CatB was analyzed using online software available at the SMART Website (<http://smart.embl-heidelberg.de/>). The amino acid sequences of active CATs, which represent the conserved catalase-domain were obtained from the National Center for Biotechnology Information (NCBI) website. BLASTP was used for searching the homology in *Xanthomonas* species. Relevant sequence alignment was performed using the DNAMAN software (Lynnon Biosoft, San Ramon, USA).

Table 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics ^a	Source or Reference
<i>Escherichia coli</i>		
DH5a	supE44 ΔlacU169(Φ80lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1	Hanahan 1983 [47]
BL21	For protein expression	Novagen
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>		
PXO99 ^A	Wildtype strain, Philippine race 6	Lab collection
Δ <i>catB</i>	<i>catB</i> gene deletion mutant derived from PXO99 ^A , Gm ^r	This study
Δ <i>catB</i> (pBBR- <i>catB</i>)	Complementary bacterium strain of Δ <i>catB</i> , Ap ^r	This study
Δ <i>oxyR</i>	<i>oxyR</i> gene deletion mutant derived from PXO99 ^A , Gm ^r	Our lab
Δ <i>oxyR</i> (pBBR- <i>oxyR</i>)	Complementary bacterium strain of Δ <i>oxyR</i> , Ap ^r	Our lab
Plasmid		
pMD18-T	Cloning vector, Ap ^r	TaKaRa, Tokyo
pET-28a	Expression vector to generate a N-terminal His ₆ tag, Km ^r	Haigene
pK18mobsacB	Suicidal vector carrying <i>sacB</i> gene for mutagenesis, Gm ^r	Schafer et al., 1994 [44]
pBBR1MCS-4	Broad-host range expression vector, Ap ^r	Kovach et al., 1995 [48]
pHM1	Broad-host range expression vector, Sp ^r	Hopkins et al., 1992 [49]
pHT304BZ	Promoterless <i>lacZ</i> vector, Ap ^r	Lereclus et al., 1996 [50]
pMDCatB	pMD18-T derivative carrying the full length of <i>catB</i> , Ap ^r	This study
pMDCatBr	pMD18-T derivative carrying the right fragment of <i>catB</i> , Ap ^r	This study
pMDCatBl	pMD18-T derivative carrying the left fragment of <i>catB</i> , Ap ^r	This study
pMDoxyR	pMD18-T derivative carrying the full length of <i>oxyR</i> , Ap ^r	This study
pKcatB	pK18mobsacB derivative carrying the full length of <i>catB</i> , Gm ^r	This study
pEToxyR	pET-28a derivative carrying the full length of <i>oxyR</i> , Km ^r	This study
pBBR- <i>catB</i>	pBBR1MCS-4 derivative carrying the full length of <i>catB</i> , Ap ^r	This study
pHTpB	pHT304BZ derivative carrying the promoter region of <i>catB</i> , Ap ^r	This study
pH- <i>lacZ</i>	pHM1 derivative carrying the promoterless <i>lacZ</i> , Sp ^r	This study
pH- <i>catBp-lacZ</i>	pHM1 derivative carrying the promoter region of <i>catB</i> and promoterless <i>lacZ</i> , Sp ^r	This study

^aAp^r, Km^r, Sp^r, and Gm^r indicate resistant to ampicillin, kanamycin, spectinomycin and gentamicin, respectively

Expression and purification of OxyR

The full length (942 bp) of *oxyR* (gene ID: PXO_04591) was PCR-amplified using the primer pairs *oxyRF*/*oxyRR* (Additional file 5: Table S2). The PCR fragment was gel purified and cloned to the middle vector pMD18-T (Takara, Tokyo, Japan), resulting in construct pMD*oxyR*, which was verified by DNA sequencing (Beijing Genomics Institute, Beijing, China). The coding region of *oxyR* was digested from pMD*oxyR* using BamHI and HindIII, and then cloned into expression vector pET28a, resulting in pET*oxyR*. The recombinant plasmid was transformed into *E. coli* BL21 strains for protein expression. The OxyR purification was performed as previously described [42]. OxyR was induced by addition of isopropyl-thiogalactopyranoside at a final concentration of 0.1 mM and the bacterial culture was then incubated at 20 °C for 6 h. Bacterial cells were chilled at 4 °C and collected by centrifugation. The supernatant containing the soluble protein was collected and mixed with pre-equilibrated Ni₂_resin (GE Healthcare, Piscataway, NJ, USA) for 2 h at 4 °C, then placed into a column and extensively washed with buffer containing 1 × PBS and 20 mM imidazole. OxyR was subsequently eluted with buffer containing 100 mM imidazole. The purified OxyR protein was obtained through the gradient dialysis of 1×PBS buffer. The purified OxyR protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Electrophoretic mobility shift assay (EMSA)

The *catB* promoter DNA region (−312 to +78, the nucleotide site upstream or downstream of translation start (+1)) was amplified by PCR using 5' ends FAM labeled primers *catBpF*/*catBpR* (Additional file 5: Table S2). DNA binding was performed in a 10 μL reaction volume containing EMSA/Gel-Shift Binding Buffer (Beyotime, Shanghai, China), 2 nM labeled DNA fragment and 5 nM His-OxyR protein. Three controls were included in each EMSA experiment: (I) cold probe as specific DNA competitor (unlabeled *catB* promoter DNA region, 20 nM), (II) negative probe as nonspecific DNA competitor (unlabeled coding region of 16S rRNA gene, 20 nM), and (III) Bovine Serum Albumin (BSA, 5 nM) as nonspecific protein competitor. After incubation at 25 °C for 30 min, the products were loaded onto a native 4 % (W/V) polyacrylamide gel and electrophoresed in 0.5 × TBE buffer for about 1.5 h at 100 V. The fluorescence of samples was detected by Typhoon FLA-5100 (Fuji film, Tokyo, Japan) at 488 nm.

Construction of *catB* promoter fusion and assay for β-galactosidase

A promoter DNA region (−312 to +78) of *catB* was obtained by PCR with the PrimeSTAR[®] Max DNA Polymerase (Takara, Tokyo, Japan) and the primers *catBpF*

and *catBpR* (Additional file 5: Table S2), while the PXO99^A genome DNA as a template. The PCR fragment was cloned directionally into the HindIII and BamHI sites of plasmid pHT304BZ that harbors an ampicillin resistance gene (*Ap^r*) and a promoterless *lacZ* reporter gene. The resulting clone pHTpB was verified by DNA sequencing (Beijing Genomics Institute, Beijing, China). Next, pHTpB was treated with HindIII and KpnI, and the fragment containing *catB* promoter region and the promoterless *lacZ* reporter gene was purified with TIANGel Midi Purification Kit (Tiangen, Beijing, China), and then cloned into pHM1, resulting in plasmid pH-*catBp-lacZ*. Meanwhile, the fragment of promoterless *lacZ* was obtained from pHT304BZ using BamHI and KpnI and cloned into pHM1, resulting plasmid pH-*lacZ*. These recombinant plasmids were introduced into *Xoo* strains. The plasmid pH-*lacZ* was used as a negative control. *Xoo* strains transformed with the recombinant plasmid were grown in M210 at 28 °C till an optical density (OD₆₀₀) of 1.0 and exposed to 3 mM H₂O₂ or sterilized deionized and distilled water (ddH₂O) for 0.5 h, then harvested by centrifugation at 7,000 g for 5 min. The β-galactosidase activity in the cellular extracts was measured using the β-Galactosidase Enzyme Assay System (Promega, Wisconsin, USA). All assays were performed with three biological replicates and three repeats.

RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

The transcriptional levels of *catB* and *oxyR* at H₂O₂ treatment were detected as described previously with some modifications [42]. Bacterial cells were grown in M210 at 28 °C till an OD₆₀₀ of 0.8 and exposed to 3 mM H₂O₂ for 0.5 h, then harvested for analysis of gene expression. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase. First-stand cDNA was synthesized from total RNA using the Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). RT-qPCR was performed using SYBR Green detection reagents (Quanta Biosciences, Carlsbad, CA, USA) in Applied Biosystem's 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the primers (*catBqF*/*catBqR*, *oxyRqF*/*oxyRqR*), and *gyrB* was used as a reference gene (Additional file 5: Table S2). The relative expression ratio was calculated using 2^{−ΔΔCt} method [43]. All experiments were performed in three biological replicates and triplicate PCR.

Cloning, deletion and complementation of *catB*

The full length (1,524 bp) of *catB* including ribosome binding site (gene ID: PXO_02830) was amplified by polymerase chain reaction (PCR) using the primer pairs

P1/P2 (Additional file 5: Table S2). A right fragment (489 bp) and a left one (582 bp) were amplified by PCR using the primer pairs catBrF/catBrR and catBIF/catBIR (Additional file 5: Table S2), respectively. The PCR fragments were gel purified and cloned to the middle vector pMD18-T (Takara, Tokyo, Japan), resulting in constructs pMDcatB, pMDcatBr, and pMDcatBl, which were verified by DNA sequencing (Beijing Genomics Institute, Beijing, China).

The gene deletion mutant $\Delta catB$ derived from PXO99^A was constructed by the homologous recombination as described previously by using the suicide vector pK18mob-SacB [44]. The vector pMDcatBl with the left fragment and the vector pMDcatBr with the right fragment were digested with corresponding restriction enzymes and ligated to pK18-mobsacB. A gentamicin resistance gene (*Gm^r*) at 855 bp was then inserted into the intermediate region between left and right fragment carried by pK18mobsacB, resulting in plasmids pKcatB, and then introduced into PXO99^A by electroporation. The deletion mutants were screened on PSA plates containing gentamicin and 10 % sucrose. For the complementation experiment, the vector pMDcatB with the *catB* gene including ribosome binding site was digested by enzymes and cloned into pBBR1MCS-4, generating pBBR-*catB*, and then transferred into $\Delta catB$ by electroporation and screened on PSA plates containing ampicillin.

Growth curve assay

The bacterial growth assay was performed as previously described [45]. In brief, *Xoo* wildtype, *catB* deletion mutant and complementary strain were grown in M210 liquid medium overnight at 28 °C, then these strains were diluted in M210 medium to a final cell density ($OD_{600} = 0.01$). The diluted cells were cultured at 28 °C with 200 rpm, and bacterial population was measured after every 6 h. For bacterial population assay, the bacterial cells were spread onto PSA plates after optional diluted, and cultured at 28 °C for 3 days, the bacterial colonies then were counted. These experiments were repeated three times, independently.

H₂O₂ resistance assay

Bacterial strains were cultured in M210 liquid medium using a shaker (200 rpm, 28 °C) until an $OD_{600} = 1$. For H₂O₂ disc diffusion assays, 200 μ L of each culture was taken and mixed with 20 mL soft PSA medium containing 0.5 % sodium carboxymethyl cellulose (Sigma-Aldrich, Louis, MQ, USA), then poured out the mixture quickly to PSA medium containing 1 % carboxymethyl cellulose, and ensured the mixture cover the whole PSA plate fully and smoothly. After 5 min, when the mixture became dry, put a sterilized dry Whatman 3MM filter discs of 8 mm diameter on the central of PSA plate. 10 μ L of H₂O₂ at

different concentrations (1, 0.5 and 0.25 M) was spotted onto the discs, respectively. The treated PSA plates were cultured in incubator at 28 °C, and the diameters of H₂O₂ inhibition zone were measured after 72 h. For H₂O₂ sensitivity assays, 1 mL bacterial cultures were taken and mixed with 100 mL M210 liquid medium, and H₂O₂ was then added to the cell suspensions to different concentrations (0, 0.25, 0.5 and 1 mM). The mixtures were cultured at 28 °C on 200 rpm, and the bacterial population was measured at 12 and 24 h, respectively. These experiments were repeated three times with three replicates.

Catalase activity assay

The analysis of catalase activity was performed as described previously [14, 31]. The bacterial culture conditions were the same as described above. The bacterial concentration of each strain is $OD_{600} = 1$. The bacterial cells were chilled at 4 °C, collected by centrifugation at 6,000 g, and then re-suspended in ddH₂O. Sonication was followed until the bacterial liquid visible clearly. The cell extracts were separated by centrifuge with rate 12,000 g for 30 min, and the most upper layer liquid which contained the protein was transferred to a new tube. 100 μ L protein was taken and mixed with 1 mL ddH₂O, and the optical density of this mixture was measured at 240 nm both before and after adding H₂O₂ to the final concentration at 10 mM. The catalase activity was calculated by an extinction coefficient of 43.6 M⁻¹ cm⁻¹ at 240 nm. One unit of catalase activity was defined as the amount of activity required to decompose 1 μ mol of H₂O₂ per minute under the assay conditions. The experiments were repeated three times with three replicates.

H₂O₂ detection

The H₂O₂ accumulation in *Xoo* strains were detected as previously described. Briefly, *Xoo* strains were grown in M210 liquid medium using a shaker (200 rpm, 28 °C) until an OD_{600} of 1, and harvested by centrifugation at 7,000 g for 5 min. The bacterial cells were re-suspended with 50 mM of potassium phosphate (pH 7.8), and the supernatants were collected by centrifugation again. To measure H₂O₂, 0.45 mL of supernatant was mixed with 0.25 mL of 200 μ M Amplex red (Sigma-Aldrich, Louis, MQ, USA) and 0.25 mL of 0.02 mg/mL horseradish peroxidase (Sigma-Aldrich, Louis, MQ, USA). The amount of OD_{610} was then measured and converted to H₂O₂ concentration using a curve obtained from standard samples. The experiment was repeated three times.

Histochemical detection of H₂O₂ in rice leaves

The cultured bacteria re-suspended in sterilized ddH₂O at an OD_{600} of 1.0 were prepared as described above, and cells were infiltrated into rice cultivar (*Oryza sativa* L. *subsp. japonica*) leaves grown for two weeks using a

needleless syringe. The H₂O₂ was detected by DAB staining as previously reported [46]. Briefly, leaf sections (3–5 mm) at 12 and 24 h post-inoculation were cut and placed in water with 0.01 % Triton-X-100 and DAB at 1 mg mL⁻¹, then this solution was infiltrated with low vacuum pressure for 10 min and the leaves were incubated for 8 h at room temperature. Finally, leaves were boiled with 95 % ethanol for 10 min and then rinsed with water, and presence of H₂O₂ was visualized as reddish brown colored dark spots by a light microscope (Leica, Heidelberg, Germany).

Pathogenicity test

The bacterial cells were grown in M210 medium for 24 h at 28 °C as described above, and re-suspended in sterilized ddH₂O at an OD₆₀₀ of 1.0. The susceptible rice cultivar (*Oryza sativa* L. *subsp. japonica*) plants grown for 6 weeks were used for bacterial inoculation with the leaf-clipping method [42]. At least ten leaves were inoculated for each strain, and the experiment was repeated three times. The disease lesion length was recorded 14 d after bacterial inoculation. The top 20 cm of each lesion leaf was cut down and weighted, and then grinded by the sterilized pestle. The extract was gradually diluted by ddH₂O, and then poured out to PSA medium and cultured in incubator with 28 °C for 3 d after it dry. The bacterial numbers in each plate were accounted, and the bacterial amount in the lesion leaf was calculated.

Data analysis

All analysis were conducted using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). The hypothesis test of percentages (*t*-test, *P* = 0.05) was used to determine significant differences in the assays for bacterial H₂O₂ resistance, catalase activity, endogenous H₂O₂ concentration, pathogenicity, gene expression and in vivo growth.

Additional files

Additional file 1: Table S1. The homologs of *catB* in plant pathogenic *Xanthomonas* species. (DOCX 15 kb)

Additional file 2: Figure S1. In vitro growth of *Xanthomonas oryzae* pv. *oryzae* wildtype, Δ *catB* and Δ *catB*(pBBR-*catB*) strains. The bacterial strains were cultured in M210 liquid medium at 28 °C for 200 rpm, and bacterial population were determined by OD₆₀₀ density measurement at the time points indicated. Data represent the mean and standard deviations of three independent experiments. (TIF 74 kb)

Additional file 3: Figure S2. Coomassie blue staining of recombinant OxyR protein purified from *E. coli* BL21 strains. About 10 μ g of protein was loaded in the lane. (TIF 141 kb)

Additional file 4: Figure S3. Histochemical detection of H₂O₂ in situ by DAB staining in rice leaves. Rice plants were grown for two weeks and inoculated with ddH₂O control (A), wildtype (B), Δ *catB* (C), Δ *catB*(pBBR-*catB*) (D), Δ *oxyR* (E), and Δ *oxyR*(pBBR-*oxyR*) (F) by using a needleless syringe. Dark spots represent presence of H₂O₂. The experiment repeats three times, independently. (TIF 991 kb)

Additional file 5: Table S2. The information of primers in this study. (DOCX 15 kb)

Abbreviations

Ap: Ampicillin; *Bs*: *Bacillus subtilis*; BSA: Bovine Serum Albumin; CAT: Catalase; CFU: Colony forming units; DAB: 3, 3'-diaminobenzidine; EMSA: Electrophoretic mobility shift assay; Gm: Gentamycin; H₂O₂: Hydrogen peroxide; Km: Kanamycin; NCBI: National Center for Biotechnology Information; OD: Optical density; PCR: Polymerase chain reaction; PSA: Peptone sucrose agar; qRT-PCR: Quantitative real-time PCR; ROS: Reactive oxygen species; Sp: Spectinomycin; U: Unit; *Xac*: *Xanthomonas axonopodis* pv. *citri*; *Xoo*: *Xanthomonas oryzae* pv. *oryzae*

Acknowledgements

Not applicable.

Funding

This work is supported by the grants from the National Basic Research Program (2011CB100700) and the National High-Technology Research Program (2012AA101504).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its Additional files 1, 2, 3, 4 and 5.

Authors' contributions

CY, NW, MSW and CYH designed the experiments; CY and NW performed the experiments; CY, NW, MSW, FT, HMC, FHY and CYH analyzed the data; MSW, FT, HMC and FHY contributed reagents/materials/analysis tools; CY, NW, FT, XCY, CHY and CYH wrote the manuscript; All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Author details

¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China. ²Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA.

Received: 30 August 2016 Accepted: 3 November 2016

Published online: 08 November 2016

References

- Cabiscol E, Tamarit J, Ros J. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol*. 2000;3(1):3–8.
- Ros Barcelo A. Xylem parenchyma cells deliver the H₂O₂ necessary for lignification in differentiating xylem vessels. *Planta*. 2005;220(5):747–56.
- Miller RA, Britigan BE. Role of oxidants in microbial pathophysiology. *Clin Microbiol Rev*. 1997;10(1):1–18.
- Storz G, Tartaglia LA, Farr SB, Ames BN. Bacterial defenses against oxidative stress. *Trends Genet*. 1990;6(11):363–8.
- Dubbs JM, Mongkolsuk S. Peroxide-sensing transcriptional regulators in bacteria. *J Bacteriol*. 2012;194(20):5495–503.
- Kim SO, Merchant K, Nudelman R, Beyer Jr WF, Keng T, Deangelo J, Hausladen A, Stamler JS. OxyR: a molecular code for redox-related signaling. *Cell*. 2002;109(3):383–96.
- Burbank L, Roper MC. OxyR and SoxR modulate the inducible oxidative stress response and are implicated during different stages of infection for the bacterial phytopathogen *Pantoea stewartii* subsp. *stewartii*. *Mol Plant Microbe Interact*. 2014;27(5):479–90.
- Zheng M, Aslund F, Storz G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science*. 1998;279(5357):1718–21.
- Hahn JS, Oh SY, Roe JH. Role of OxyR as a peroxide-sensing positive regulator in *Streptomyces coelicolor* A3(2). *J Bacteriol*. 2002;184(19):5214–22.

10. Tondo ML, Petrocelli S, Ottado J, Orellano EG. The monofunctional catalase KatE of *Xanthomonas axonopodis* pv. *citri* is required for full virulence in citrus plants. *PLoS One*. 2010;5(5):e10803.
11. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. *Cell Mol Life Sci*. 2004;61(2):192–208.
12. Switala J, Triggs-Raine BL, Loewen PC. Homology among bacterial catalase genes. *Can J Microbiol*. 1990;36(10):728–31.
13. Loprasert S, Vattanaviboon P, Praituan W, Chamnongpol S, Mongkolsuk S. Regulation of the oxidative stress protective enzymes, catalase and superoxide dismutase in *Xanthomonas*-a review. *Gene*. 1996;179(1):33–7.
14. Jittawuttipoka T, Buranajitpakorn S, Vattanaviboon P, Mongkolsuk S. The catalase-peroxidase KatG is required for virulence of *Xanthomonas campestris* pv. *campestris* in a host plant by providing protection against low levels of H₂O₂. *J Bacteriol*. 2009;191(23):7372–7.
15. Tondo ML, Delprato ML, Kraiselburd I, Fernandez Zenoff MV, Farias ME, Orellano EG. KatG, the bifunctional catalase of *Xanthomonas citri* subsp. *citri*, responds to hydrogen peroxide and contributes to epiphytic survival on citrus leaves. *PLoS One*. 2016;11(3):e0151657.
16. Eason MM, Fan X. The role and regulation of catalase in respiratory tract opportunistic bacterial pathogens. *Microb Pathog*. 2014;74(1):50–58.
17. Mew TH. Current status and future prospects of research on bacterial blight of rice. *Annu Rev Phytopathol*. 1987;25:359–82.
18. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, Dow M, Verdier V, Beer SV, Machado MA, et al. Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol Plant Pathol*. 2012;13(6):614–29.
19. Liu W, Liu J, Triplett L, Leach JE, Wang GL. Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annu Rev Phytopathol*. 2014;52:213–41.
20. Nino-Liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. *Mol Plant Pathol*. 2006;7(5):303–24.
21. Das A, Rangaraj N, Sonti RV. Multiple adhesin-like functions of *Xanthomonas oryzae* pv. *oryzae* are involved in promoting leaf attachment, entry, and virulence on rice. *Mol Plant Microbe Interact*. 2009;22(1):73–85.
22. White FF, Yang B. Host and pathogen factors controlling the rice-*Xanthomonas oryzae* interaction. *Plant Physiol*. 2009;150(4):1677–86.
23. Mongkolsuk S, Loprasert S, Vattanaviboon P, Chanvanichayachai C, Chamnongpol S, Supsamran N. Heterologous growth phase- and temperature-dependent expression and H₂O₂ toxicity protection of a superoxide-inducible monofunctional catalase gene from *Xanthomonas oryzae* pv. *oryzae*. *J Bacteriol*. 1996;178(12):3578–84.
24. Mongkolsuk S, Whangsuk W, Vattanaviboon P, Loprasert S, Fuangthong M. A *Xanthomonas* alkyl hydroperoxide reductase subunit C (ahpC) mutant showed an altered peroxide stress response and complex regulation of the compensatory response of peroxide detoxification enzymes. *J Bacteriol*. 2000;182(23):6845–9.
25. Grewal RK, Gupta S, Das S. *Xanthomonas oryzae* pv. *oryzae* triggers immediate transcriptomic modulations in rice. *BMC Genomics*. 2012;13:49.
26. Zhang J, Gao J, Wu M, Chen H, He C. Roles of OxyR, a transcriptional regulator of *Xanthomonas oryzae* pv. *oryzae* in regulation of detoxification of hydrogen peroxide. *Acta Microbiol Sin*. 2009;49(7):874–9.
27. Wang Y, Wu M, Tian F, Chen H, He C. Transcriptional regulation analysis and prokaryotic expression of *ahpC* gene putatively-encoding alkyl hydroperoxide reductase in *Xanthomonas oryzae* pv. *oryzae*. *Biotechnol Bull*. 2012;12:114–9.
28. Li X, Qiao J, Yang L, Li X, Qiao S, Pang X, Tian F, Chen H, He C. Mutation of alkyl hydroperoxide reductase gene *ahpC* of *Xanthomonas oryzae* pv. *oryzae* affects hydrogen peroxide accumulation during the rice-pathogen interaction. *Res Microbiol*. 2014;165(8):605–11.
29. Salzberg SL, Sommer DD, Schatz MC, Phillippy AM, Rabinowicz PD, Tsuge S, Furutani A, Ochiai H, Delcher AL, Kelley D, et al. Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99^A. *BMC Genomics*. 2008;9:204.
30. Zhou JB, Wu MS, Hu J, He CY. Induction of bacterial catalase-related gene expression by H₂O₂ produced during interaction of rice suspension-cultured cells with *Xanthomonas oryzae* pv. *oryzae* or applied exogenously. *Acta Phytopathologica Sinica*. 2009;39(4):399–404.
31. Wang N, Wu M, Tian F, Chen H, He C. Effects of gene deletion of *katE* encoding putative catalase on resistance to hydrogen peroxide and pathogenicity of *Xanthomonas oryzae* pv. *oryzae*. *Acta Phytopathologica Sinica*. 2013;43(6):615–21.
32. Zhang J, Zhou JM. Plant immunity triggered by microbial molecular signatures. *Mol Plant*. 2010;3(5):783–93.
33. Jo I, Chung IY, Bae HW, Kim JS, Song S, Cho YH, Ha NC. Structural details of the OxyR peroxide-sensing mechanism. *Proc Natl Acad Sci U S A*. 2015; 112(20):6443–8.
34. Imlay JA. Transcription factors that defend bacteria against reactive oxygen species. *Annu Rev Microbiol*. 2015;15(69):93–108.
35. Bae HW, Cho YH. Mutational analysis of *Pseudomonas aeruginosa* OxyR to define the regions required for peroxide resistance and acute virulence. *Res Microbiol*. 2012;163(1):55–63.
36. Ni B, Zhang Y, Huang X, Yang R, Zhou D. Transcriptional regulation mechanism of ter operon by OxyR in *Yersinia pestis*. *Curr Microbiol*. 2014; 69(1):42–6.
37. Teramoto H, Inui M, Yukawa H. OxyR acts as a transcriptional repressor of hydrogen peroxide-inducible antioxidant genes in *Corynebacterium glutamicum* R. *FEBS J*. 2013;280(14):3298–312.
38. Christman MF, Storz G, Ames BN. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. *Proc Natl Acad Sci U S A*. 1989;86(10):3484–8.
39. Kim JS, Holmes RK. Characterization of OxyR as a negative transcriptional regulator that represses catalase production in *Corynebacterium diphtheriae*. *PLoS One*. 2012;7(3):e31709.
40. Tsuchiya K, Mew TW, Wakimoto S. Bacteriological and pathological characteristics of wild type and induced mutants of *Xanthomonas campestris* pv. *oryzae*. *Phytopathology*. 1982;72:43–6.
41. Fan S, Tian F, Li J, Hutchins W, Chen H, Yang F, Yuan X, Cui Z, Yang CH, He C. Identification of phenolic compounds that suppress the virulence of *Xanthomonas oryzae* on rice via the type III secretion system. *Mol Plant Pathol* 2016. DOI: 10.1111/mpp.12415
42. Yang F, Tian F, Sun L, Chen H, Wu M, Yang CH, He C. A novel two-component system PdeK/PdeR regulates c-di-GMP turnover and virulence of *Xanthomonas oryzae* pv. *oryzae*. *Mol Plant Microbe Interact*. 2012;25(10):1361–9.
43. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods*. 2001;25(4):402–8.
44. Schafer A, Tauch A, Jager W, Kalinowski J, Thierbach G, Puhler A. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene*. 1994;145(1):69–73.
45. Tian F, Yu C, Li H, Wu X, Li B, Chen H, Wu M, He C. Alternative sigma factor RpoN2 is required for flagellar motility and full virulence of *Xanthomonas oryzae* pv. *oryzae*. *Microbiol Res*. 2015;170:177–83.
46. Thordalchristensen H, Zhang ZG, Wei YD, Collinge DB. Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant J*. 1997;11(6):1187–94.
47. Hanahan D. Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol*. 1983;166(4):557–80.
48. Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop 2nd RM, Peterson KM. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene*. 1995; 166(1):175–6.
49. Hopkins CM, White FF, Choi SH, Guo A, Leach JE. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol Plant Microbe Interact*. 1992;5(6):451–9.
50. Lereclus D, Agaisse H, Gominet M, Salamitou S, Sanchis V. Identification of a *Bacillus thuringiensis* gene that positively regulates transcription of the phosphatidylinositol-specific phospholipase C gene at the onset of the stationary phase. *J Bacteriol*. 1996;178(10):2749–56.