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Heterologous Growth Phase- and Temperature-Dependent Expression and H_2O_2 Toxicity Protection of a Superoxide-Inducible Monofunctional Catalase Gene from *Xanthomonas oryzae* pv. oryzae

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Catalase is an important protective enzyme against H_2O_2 toxicity. Here, we report the characterization of a *Xanthomonas oryzae* pv. oryzae catalase gene (*katX*). The gene was localized and its nucleotide sequence was determined. The gene codes for a 77-kDa polypeptide. The deduced *katX* amino acid sequence shares regions of high identity with other monofunctional catalases in a range of organisms from bacteria to eukaryotes. The transcriptional regulation of *katX* was atypical of bacterial monofunctional *kat* genes. Northern (RNA) analysis showed that *katX* transcription was highly induced by treatments with low concentrations of menadione, a superoxide generator, and methyl methanesulfonate, a mutagen. It was only weakly induced by H_2O_2. Unlike in other bacteria, a high level of catalase in *Xanthomonas* spp. provided protection from the growth-inhibitory and killing effects of H_2O_2 but not from those of organic peroxides and superoxide generators. Unexpectedly, heterologous expression of *katX* in *Escherichia coli* was both growth phase and temperature dependent. Catalase activity in *E. coli kat* mutants harboring *katX* on an expression vector was detectable only when the cells entered the stationary phase of growth and at 28°C. The patterns of transcription regulation, heterologous expression, and physiological function of *katX* are different from previously studied bacterial *kat* genes.

Catalase is a heme-containing enzyme involved in dismutation of H_2O_2 to oxygen and water. The enzyme plays an important role in detoxifying H_2O_2 and minimizing oxidative stress caused by highly reactive oxygen species (ROS, i.e., OH·) which arise from H_2O_2 degradation in the Fenton reaction (13). Mutations in *kat* have always resulted in increased sensitivity to H_2O_2 stress, and this is an indication of the important physiological role of the enzyme (10, 13). In many bacteria, there are two types of catalase enzyme, namely a monofunctional catalase and a bifunctional catalase/peroxidase. Each enzyme is encoded by a different gene (e.g., in *Escherichia coli*, *katE* and *katG* code for monofunctional and bifunctional catalases, respectively) (35, 45). Monofunctional catalases share regions of an amino acid sequence that is highly conserved among microbial, plant, and mammalian enzymes (5, 26, 47). In many bacteria, the two *kat* genes are regulated differently in terms of growth phase and response to oxidative stress, suggesting that they may have different physiological functions (10, 23, 29).

Xanthomonas oryzae pv. oryzae (*Xoo*) is the causative agent for the most destructive bacterial disease (the bacterial leaf blight) in rice (37). Oxidative stress is an important component of normal aerobic life and in bacterial-plant interactions. Increasing production of ROS, including superoxide, H_2O_2, and OH·, is associated with an active plant defense response and with aerobic respiration (44). ROS are highly toxic to all cellular components, and their rapid detoxification is essential for microbial survival.

Xoo monofunctional catalase shows atypical regulation. The catalase level is highest during the exponential phase of growth and is under oxygen tension regulation (7). *Xoo* catalase is highly inducible by superoxide generators and by methyl methanesulfonate (MMS) but is weakly inducible by H_2O_2 (8). However, because the physiological role of *Xoo* catalase is not clear, its atypical regulation could play an important role in the survival of *X. oryzae* in the interaction with its host plant.

To facilitate elucidation of the physiological role of catalase in *Xoo*, we have previously isolated a putative *Xoo kat* (*katX*) from a *Xoo* genomic library (33). Here, we characterize *katX* and investigate its unusual expression in both homologous and heterologous hosts. The physiological effect of increased Kat levels on the *Xanthomonas* oxidative stress response was also examined.

MATERIALS AND METHODS

Bacteria and plasmids. In cloning experiments, *E. coli* DH5α (*recA*) and pBluescript KS (Stratagene) were used as the host and the vector, respectively. The relevant genotypes of the *E. coli kat* mutants UM255, UM2, and UM258 (35) are *katG2*, *katE12*::Tn*10*, *recA*; *katG15* and *katE2*; and *katG2* and *katF13*::Tn*10*, respectively. *Xanthomonas* strains were from the laboratory stock or from J. Leach. pRK415 (24) was a gift from N. Keen.

Growth chemicals and media. All chemicals were of the highest grade and were purchased from Sigma. All *Xanthomonas* strains were grown aerobically in Silva Buddenhagen (SB) (37) medium at 28°C while all *E. coli* was grown at 37°C in Luria-Bertani medium with inclusion of appropriate antibiotics. For *E. coli* growth phase experiments, an overnight culture was inoculated into fresh Luria-Bertani medium at the 0.5% level and grown at either 28°C or 37°C. Bacterial growth was monitored at an optical density at 600 nm (OD_{600}). At selected times, samples were withdrawn and used for catalase assays. *Xoo* induction experiments were performed by subculturing 15-h *Xoo* cultures (late log phase) into fresh SB

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medium (2% level). Growth was then continued for 1 h before addition of inducing agents (100 μM menadione, 150 μM H_2O_2, 0.001% MMS). Induced cultures were grown for an additional 2 h before they were harvested and total RNA was extracted.

Nucleic acid extraction, analysis, and molecular techniques. Plasmid extraction was performed by the alkaline lysis method (31). *Xanthomonas* genomic DNA was isolated as previously described (21). Cloning experiments were performed as described by Maniatis et al. (31). Total RNA was extracted by the hot phenol method (2). RNA samples were analyzed with denaturing formaldehyde gels. Both Southern and Northern (RNA) blots were made by vacuum blotting for at least 4 h and hybridization under standard conditions (2). Radiolabelled probes were made with a random prime kit according to the manufacturer's (Promega) instructions.

Bacterial transformation. Strains of electrocompetent *E. coli* and *Xanthomonas* were prepared and electroporated as previously described (21). For *Xanthomonas* strains, 1 ml of SB medium was added immediately after electroporation and the bacteria were allowed to grow for 4 h before being plated on selective media.

Preparation of bacterial lysates and enzyme assays. Bacterial lysates were prepared by resuspending cell pellets in 3 ml of ice-cold 50 mM phosphate buffer, pH 7.0, containing 1 mM phenylmethylsulfonyl fluoride and then disrupting them by sonication. Cell debris was removed by centrifugation at 10,000 × *g* for 15 min at 4°C (8), and cleared lysates were used immediately in the catalase assay. The catalase assay was carried out spectrophotometrically, according to the method of Beers and Sizer (3). One unit of catalase was defined as the amount of enzyme required to decompose 1.0 μmol of H_2O_2 at 25°C at pH 7.0. Catalase plate assays were performed by placing a drop of 3% H_2O_2 onto the edge of colonies. A positive reaction was indicated by formation of O_2 bubbles. Protein concentration was measured by the dye binding method described by Bradford (6).

Growth of *Xanthomonas* cultures and oxidative stress killing. The effects of various oxidants on *Xanthomonas* growth rates were investigated by measuring changes in optical density at OD_{600}. Essentially, overnight, early-stationary-phase *Xanthomonas* cultures were subcultured at 2% inoculum. The cultures were allowed to grow for an additional hour prior to the addition of oxidants at various concentrations. Thereafter, bacterial growth was monitored hourly for 8 h. Survival experiments were performed by subculturing *Xanthomonas* overnight cultures into fresh media at 2% inoculum, and this was followed by growth for an additional 3 h to give an OD_{600} of approximately 1.0 (mid log) (44). For comparison between different cultures, it was important to use bacteria of the same metabolic state and growth phase since large variations in oxidative stress resistance levels were linked to these parameters (46). Prior to the addition of killing concentrations of oxidants (50 mM H_2O_2, 100 mM *tert*-butyl hydroperoxide, and 250 mM menadione), culture densities were adjusted to give equal OD_{600} readings. After the addition of the oxidants, aliquots were withdrawn every 10 min and washed once with fresh media before being plated on SB plates. Colonies were counted after 36 h of incubation. Mid-log-phase *Xanthomonas* cultures harboring pRK415 or pRK-katX were used for Kat assays. The growth conditions were the same as those for the oxidative stress killing experiment except tetracycline (15 μg/ml) was added to all cultures.

Nucleotide sequence accession number. The *katX* nucleotide sequence has been deposited at EMBL under accession number X97673.

RESULTS

Localization of *katX*. We have shown that phages containing the putative *katX* isolated from a *Xoo* genomic library contained a common 7.2-kb *Bam*HI fragment, which hybridized to the PCR *Xoo kat* probe (33). This fragment was cloned in pBluescript in both orientations and resulted in pkatXA and pkatXB. Both recombinant plasmids were transformed into UM255 and screened for catalase activity. Under all the conditions tested, no catalase activity was detectable.

Restriction enzyme mapping, Southern blotting, and deletion analysis of pkatXA were performed to localize *katX*. *katX* was localized to a smaller 3.2-kb *Xho*I-*Bam*HI fragment. The fragment was cloned into pBluescript in both orientations. This resulted in the new recombinant plasmids pkatXC and pkat XD. These plasmids were sequenced and tested for catalase activity. The results are summarized in Fig. 1.

KatX amino acid sequence analysis. The 2.5-kb *Xho*I-*Sma*I fragment from pkatXF was completely sequenced in both orientations. The *katX* deduced amino acid sequence for a 77-kDa protein had regions of high homology with monofunctional catalases from mammals, plants, and bacteria. The results of a multiple alignment of catalase amino acid sequences by the Clustal V (20) program are shown in Fig. 2. Considering both

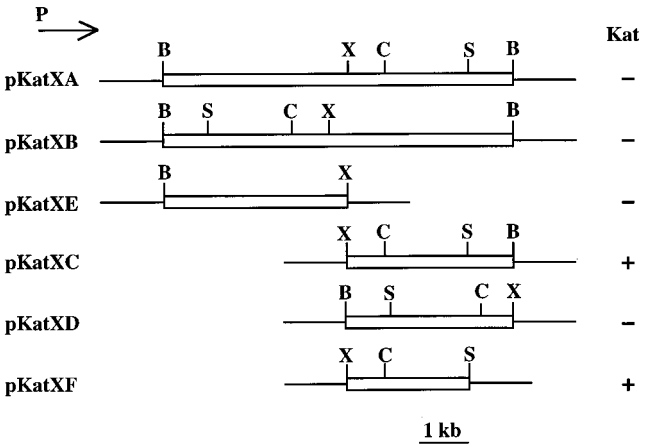

FIG. 1. Localization of *katX* coding region. The direction of transcription of the vector *lacZ* promoter is shown at upper left. Boxes represent cloned *Xoo* DNA. Abbreviations for restriction endonucleases: B, *Bam*HI; C, *Cla*I; S, *Sma*I; and X, *Xho*I. Catalase activity was determined by catalase plate assay as described in Materials and Methods. +, catalase activity (indicated by formation of O_2 bubbles).

identical and conservative replacement of amino acids, KatX showed the highest degree of total identity (67%) with *E. coli* HPII (KatE). Identities of greater than 50% were observed between KatX and catalases from bovine, maize, *Listeria seeligeri* (17), *Lactobacillus sake* (26), *Pseudomonas syringae*, and *Bacillus subtilis* (5). More importantly, all amino acid residues involved in the active site (i.e., His-128, Ser-167, and Asn-201 in KatE and His-89, Ser-128, and Asn-162 in KatX) and in heme binding (i.e., Val-127, Thr-168, Phe-206, and Phe-214 in KatE and Val-88, Thr-129, Phe-167, and Phe-175 in KatX on the distal side of the heme and Pro-393, Arg-411, and Tyr-415 in KatE and Pro-354, Arg-372, and Tyr-376 in KatX on the proximal side of the heme) were highly conserved (47).

Comparison of catalase amino acid sequences also suggested a possible mechanism of KatX. Bovine liver catalase has been extensively studied, and the enzyme is thought to bind NADPH and water molecules. The amino acid residues involved in binding these molecules (His-304 for binding the pyrophosphate group of NADPH and His-234, Lys-236, and Tyr-214 for binding water) have been established in bovine catalase. von Ossowski et al. (47) studied differences in amino acid residues at the binding sites of these molecules in *E. coli* HPII (KatE) and other proteins. They found that the presence of Glu-362 in KatE in an analogous location to His-304 in bovine catalase made binding of the negatively charged pyrophosphate groups of NADPH to the negatively charged Glu-362 unlikely. The presence of Phe-272 in KatE as opposed to Tyr-214 in bovine catalase also prevented binding of a water molecule, which requires an OH group. We also saw amino acid changes in KatX at these important positions, i.e., Glu-323 and Phe-233 of KatX. This favored the notions that KatX would behave similarly to KatE with respect to lack of binding to NADPH and water molecules and that it would be different from bovine catalase.

In many bacteria, multiple catalase isozymes have been detected (22, 23, 28, 45). *Xoo* genomic DNA was digested with several restriction enzymes and probed with *katX*. The results in Fig. 3 show one major positively hybridized band in *Xoo* genomic DNA digested with different restriction enzymes, except for the *Sal*I-digested sample, in which there was an additional band. This was due to an internal restriction enzyme site

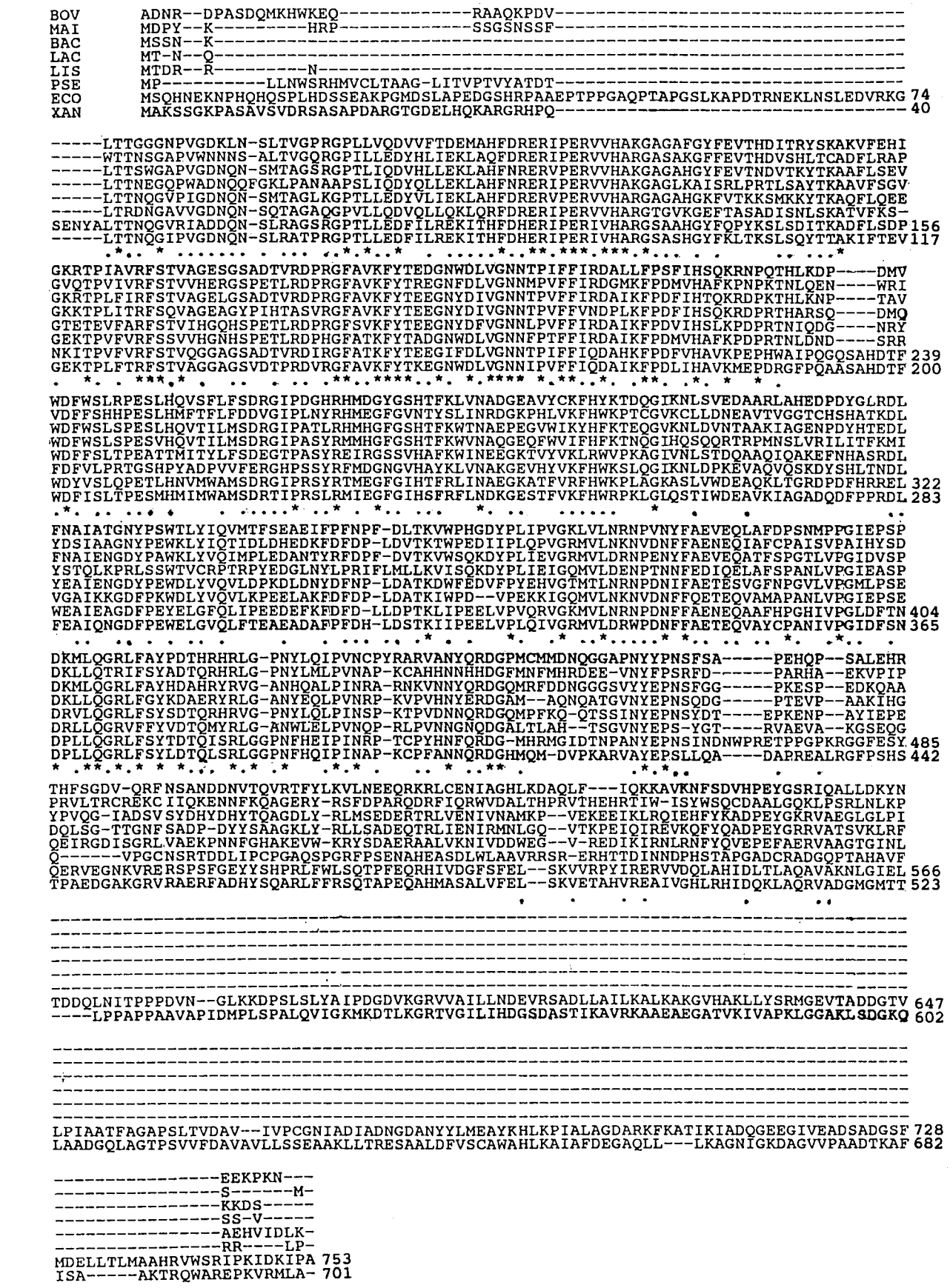

FIG. 2. Alignments of the deduced KatX amino acid sequence with various other catalases. Catalase amino acid sequences were aligned using the Clustal V program. *, identical amino acids residues in all eight catalase sequences; ·, four or more identical amino acids among eight catalase sequences. Abbreviations for the catalase amino acid sequences: BAC, *Bacillus subtilis*; BOV, bovine; ECO, *katE* (*E. coli*); LAC, *Lactobacillus sake*; LIS, *Listeria seeligeri*; MAI, maize; PSE, *Pseudomonas syringae*; XAN, *Xoo.*

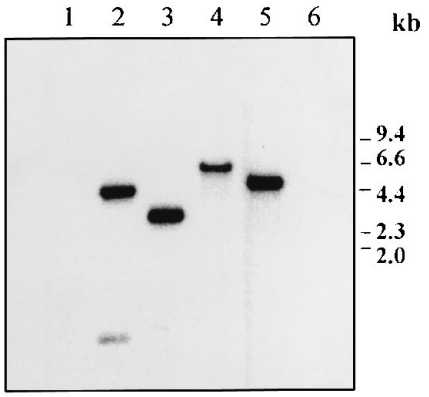

FIG. 3. Hybridization of *katX* probe against *Xoo* genomic DNA digested with various restriction enzymes. Five micrograms of *Xoo* genomic DNA were digested with *Sal*I (lane 2), *Pst*I (lane 3), *Bam*HI (lane 4), and *Xho*I (lane 5). Lanes 1 and 6 were λ *Hin*dIII-digested molecular weight markers. After electrophoresis, digested DNAs were transferred to a nylon membrane and hybridized to 750-bp *Xho*I-*Cla*I fragments containing *kat*. The filter was washed under high stringency conditions. The molecular sizes of λ *Hin*dIII-digested markers are at the right.

within the coding region. The hybridization patterns were consistent with *katX* being a single-copy gene.

Oxidative stress and mutagen induction of *katX* transcription in *Xoo*. We have shown that a monofunctional *Xoo kat* is highly induced by superoxide generators (i.e., paraquat and menadione) and by MMS (8- to 10-fold) but is only weakly induced by H_2O_2 (less than 2.5-fold) (8). Here, we investigated mRNA levels in response to various inducers. RNA samples were separated and then blotted and hybridized with a *katX* coding region probe. The results of a Northern analysis of *katX* expression are shown in Fig. 4. Full-length *kat* transcripts of approximately 2.4 kb and lower molecular weight transcripts of 1.2 kb, possibly *katX* mRNA degradative products, were detected. A significant increase in the amount of *kat* transcripts was observed in MMS- and menadione- but not in H_2O_2-treated samples compared with the uninduced level. MMS and menadione appeared to be equally potent in inducing *katX*. We have performed a H_2O_2-catalase dose-response analysis and found that neither high doses of H_2O_2 which inhibit bacteria growth (8) nor varying inducing protocols to double or triple H_2O_2 doses (data not shown) increased levels of catalase induction. This suggested that the lack of catalase induction by H_2O_2 was not due to insufficient H_2O_2 doses.

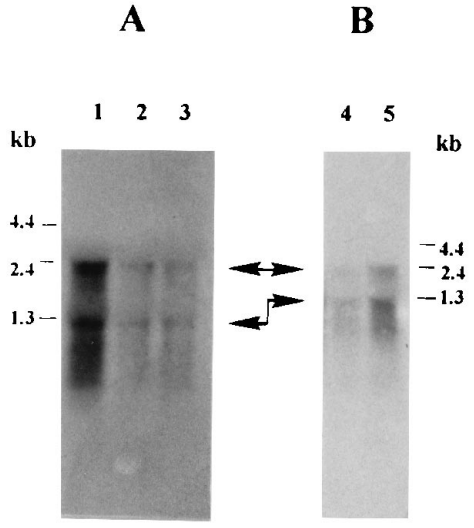

FIG. 4. Induction of *katX* mRNA by menadione and MMS. Total mRNA was isolated from *Xoo*. (A) Lane 1, induction by menadione; lane 2, no induction; lane 3, induction by H_2O_2. (B) Lane 4, no induction; lane 5, induction by MMS. RNA samples (10 μg) were separated on a formaldehyde gel, blotted to a nylon membrane, hybridized with a 750-bp *Xho*I-*Cla*I fragment containing a *katX* coding region, and washed, as described in Materials and Methods. The arrows indicate full-length *kat* (2.4 kb) and shorter (1.3 kb) transcripts. Molecular sizes of RNA markers (BRL) are indicated at the left and right.

TABLE 1. Increase of catalase activity in *Xanthomonas* strains harboring pRK-katX during log phase of growth

Strain	Catalase activity (U/mg of protein)[a]	
	pRK 415	pRK-katX
X. campestris pv. campestris	7.4	23.6
X. campestris pv. phaseoli	4.4	27.5
Xoo	20.1	70.4

[a] Values are averages from three experiments.

Increased catalase activity in *Xanthomonas* spp. conferred protection against H_2O_2-induced growth inhibition and killing. In many bacteria, *kat*-negative mutants are hypersensitive to H_2O_2 growth inhibition and killing effects (5, 10, 13). However, there is no correlation between catalase level and H_2O_2 resistance level in wild-type strains (1, 19). Active plant defense responses generate ROS (12, 27, 44), which could induce high levels of catalase expression. This may have important physiological consequences for *Xanthomonas* spp. Expression of cloned *katX* in *Xanthomonas* spp. (Table 1) permitted investigations into the effects of high catalase levels on (i) growth in the presence of low concentrations of oxidants and (ii) ability to survive killing concentrations of oxidants. We have also considered that increased catalase levels may have effects on the levels of other oxidative stress protective enzymes, i.e., superoxide dismutase and glutathione reductase. Thus, the levels of these enzymes were measured and found to be identical in strains harboring pRK-katX or pRK415 (data not shown). The effects of oxidants on the growth rates of *Xoo* harboring pRK415 or pRK-katX are shown in Fig. 5. *Xoo*-pRK415 growth was retarded by the addition of 300 μM H_2O_2. However, in *Xoo*-pRK-katX, the growth-inhibitory effect of H_2O_2 was partially overcome and a higher growth rate resulted. Growth rates in the presence of a superoxide generator (menadione) or an organic hydroperoxide (*tert*-butyl hydroperoxide) were not significantly different between the two strains (data not shown). Similar results were observed in *Xanthomonas campestris* pv. campestris and *Xanthomonas campestris* pv. phaseoli harboring pRK415 or pRK-katX (data not shown). The results indicate that the high catalase levels resulting from pRK-katX in *Xanthomonas* spp. (Table 1) conferred a selective growth advantage in the presence of H_2O_2. However, this does not necessarily mean that high catalase levels would protect bacteria from the killing effect of high doses of H_2O_2. To test whether high catalase levels would protect *X. oryzae* against the killing effect of H_2O_2, menadione, or *tert*-butyl hydroperoxide, log-phase *Xoo* harboring pRK-katX or pRK415 were exposed to lethal concentrations of these oxidants. Results of these oxidative stress killing experiments are shown in Fig. 6. *Xoo*-pRK-katX was 2.5 logs more resistant than similar strains harboring only pRK415 vector. The resistance levels to *tert*-butyl hydroperoxide and menadione killing were similar for the two strains. The data support the notion that there is a corre-

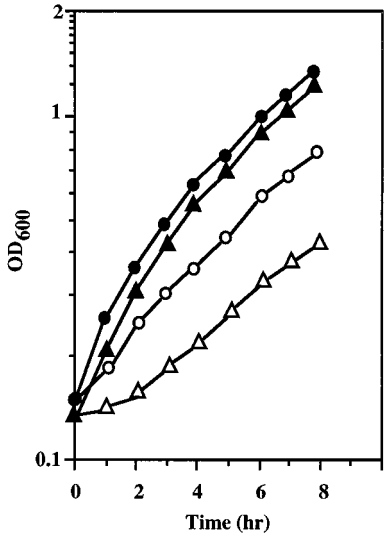

FIG. 5. Growth of *Xoo*-pRK415 and -pRK-katX in the presence of H_2O_2. *Xoo* harboring pRK415 or pRK-katX was grown in SB medium supplemented with 15 μg of tetracycline per ml. H_2O_2 (200 μM) was added to treated cultures. Bacterial growth was monitored spectrophotometrically at OD_{600}. ▲, untreated cultures of *Xoo*-pRK415; ●, untreated cultures of *Xoo*-pRK-katX; △, H_2O_2-treated cultures of *Xoo*-pRK415; ○, H_2O_2-treated cultures of *Xoo*-pRK-katX.

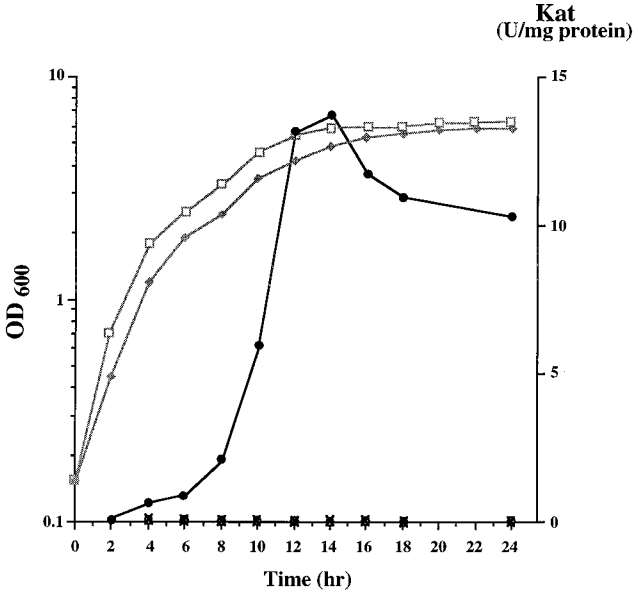

FIG. 7. Growth phase- and temperature-dependent expression of *katX* in an *E. coli kat* mutant. UM255 harboring pkatXC was grown in Luria-Bertani broth containing 100 μg of ampicillin per ml at 28°C (◆) and 37°C (□). Bacterial growth was monitored spectrophotometrically at OD_{600}. At selected times, samples were withdrawn and catalase activity was determined. Catalase activities specified by pkatXC grown at 28°C (●) or at 37°C (■) are plotted on the graph. No catalase activity was detected in UM255 harboring pKS vector at either growth temperature (data not shown). The results shown are typical values from three independently performed experiments.

lation between catalase level and level of H_2O_2 resistance. The protective effects of high catalase level only applied to H_2O_2 and not to other oxidants.

Heterologous temperature-sensitive and growth phase-dependent expression of *katX*. Both pkatXC and pkatXD were transformed into UM2, UM255, and UM258 and screened for catalase activity by catalase plate assay. Catalase was detectable only when the *kat* mutants harboring pkatXC and not pkatXD were grown at 28°C. To test if temperature-sensitive expression was due to the heat-labile nature of KatX, bacterial lysates prepared from *Xoo* and from UM255 harboring pkatXC grown at 28°C were heated at different temperatures. Samples were withdrawn every 10 min and catalase activity was assayed. The results showed that heating at 55°C for 60 min did not affect catalase activity, which suggested that once active catalase had formed, the enzyme was no longer temperature labile.

During screening for catalase activity of recombinant *katX* plasmids in *E. coli*, it was noticed that at 28°C, growing colonies of UM255-pkatXC produced much less catalase than overnight colonies. Thus, catalase activity in UM255-pkatXC was monitored through all growth phases at either 28°C or 37°C. The results are shown in Fig. 7. In UM255-pkatXC, no catalase

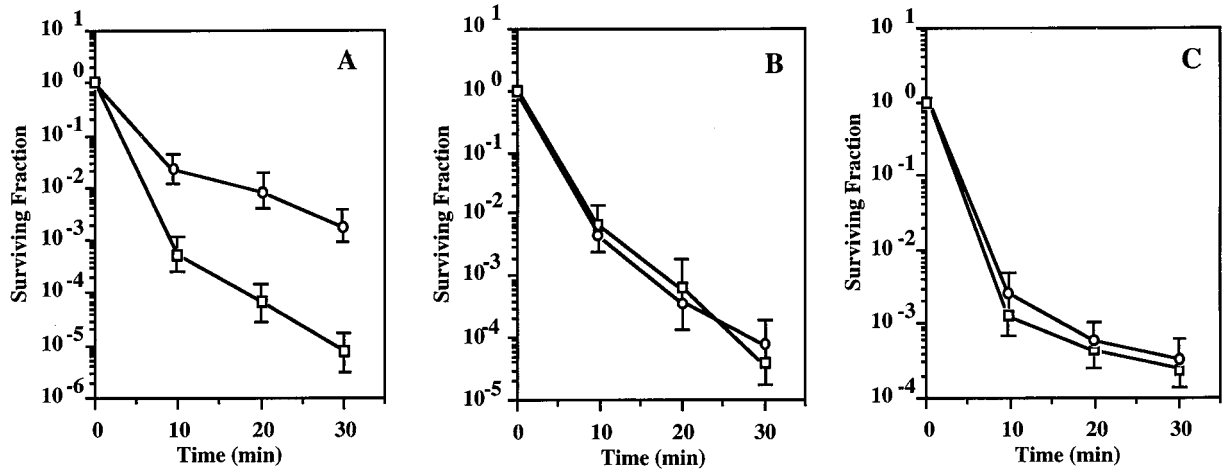

FIG. 6. Treatment of *Xoo* harboring pRK415 or pRK-katX with killing concentration of oxidants. *Xoo* harboring pRK415 (□) or pRK-katX (○) was treated with 100 mM H_2O_2 (A), 100 mM menadione (B), or 100 mM *tert*-butyl hydroperoxide (C), and at selected times (10, 20, and 30 min) samples were withdrawn, washed, and diluted prior to plating on SB plates. Colonies were counted after 36 h of incubation. The surviving fraction is defined as the number of cells after treatment divided by the number of cells prior to treatment.

activity was observed during the log phase of growth at either temperature. However, in the culture grown at 28°C, catalase activity was detectable as bacterial growth neared the end of log phase and reached peak levels during stationary phase. No catalase activity was detected at any growth phase in cultures grown at 37°C.

katX was cloned into the broad-host-range vector (pRK415) and resulted in pRK-katX. Both plasmids were electroporated into UM2 and various *Xanthomonas* spp. and catalase activities were determined. The results are shown in Table 1. In *E. coli*, UM2 harboring pRK-katX showed both growth phase- and temperature-dependent expression (data not shown). Catalase activities in various *Xanthomonas* spp. harboring pRK-katX were three- to fourfold higher than in the same strains harboring pRK415. High catalase activities were detected at mid-log phase in all *Xanthomonas* strains harboring pRK-katX, suggesting that lack of *katX* expression during log phase in *E. coli* was due to inefficient heterologous expression of *katX*. It was not possible to test the temperature-sensitive expression of cloned *katX* in *Xanthomonas* spp. because at 37°C, *Xanthomonas* growth was severely inhibited (data not shown).

DISCUSSION

Analysis of amino acid residues involved in catalytic activity and various cofactor binding sites suggests that KatX is likely to work by a mechanism similar to that of KatE. However, *katX* and *katE* regulation have both common and different aspects. Both genes are weakly induced by H_2O_2 (8, 36, 40), while ascorbate, superoxides, and MMS induce only *katX* (8). Expression analysis for *katX* at the mRNA level showed that the gene was highly induced by a superoxide generator and by MMS but weakly induced by H_2O_2. This pattern of regulation is different from other bacterial *kat*. In *E. coli*, *katE* is regulated by *rpoS*, while *katG* is H_2O_2 and ascorbate induced and is regulated by *oxyR* (9, 40, 43). *katX* appeared to be regulated by superoxides in a fashion similar to genes in the *soxRS* regulon of *E. coli* (10, 16). It can be argued that superoxide anions generated by redox-cycling agents would be converted to H_2O_2 by superoxide dismutase (18). The H_2O_2 would in turn induce *kat* expression (15). We do not favor such a hypothesis because, in comparison with a superoxide generator, H_2O_2 was a poor inducer of *katX* expression. The MMS induction of *katX* is highly unusual. We have shown that other mutagens (e.g., nitrosourea, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) (8) do not induce catalase. Thus, MMS induction of *katX* was not due to activation of the SOS response. The mechanism of MMS induction of *katX* is unknown and has not been observed in other bacteria. Monofunctional catalases in other microbes also show different regulation. For example, the monofunctional catalase of *Haemophilus influenzae* is highly induced by both H_2O_2 and ascorbate (4), and this differs from *katX*. Thus, monofunctional *kat* in each microbe may respond differently to various environmental factors. This implies that various bacteria that show the same enzyme activities may nonetheless show subtle differences in their physiological roles.

Resistance level to H_2O_2 killing is the result of complex interactions between proteins and various environmental conditions (19, 46). Mutations in oxidative stress protective genes (*kat*, *sod*) (10, 13), DNA repair genes (*recA*, exonuclease III genes) (10, 39), protein repair genes (i.e., methionine sulfoxide reductase gene [34]), and heat shock genes cause increased sensitivity to H_2O_2 killing. However, in wild-type cells an increased level of these enzymes does not necessarily confer additional H_2O_2 resistance (1, 19). Thus, in these bacteria an increased expression of catalase may not have any beneficial effects. In *Xoo*, a high catalase level appears to have physiological benefits in that it permits *Xoo* to grow better in the presence of H_2O_2 and to be protected from H_2O_2 killing (Fig. 6 and 7). *Xoo* catalase is highly induced by sublethal concentrations of ascorbate and superoxides (8). Such inducing conditions could take place during plant-microbe interactions, which could result in high levels of catalase. H_2O_2 is thought to function in both bacterial growth inhibition and killing and to be a signal molecule for further activation of plant defense responses. Thus, increased catalase levels may not only protect *Xanthomonas* spp. from growth inhibition and the killing effects of H_2O_2 but may also increase the rate of H_2O_2 degradation, which could dampen plant defense responses. Both conditions would favor *Xanthomonas* growth in the plant host. These possibilities are under investigation.

Menadione toxicity has been shown to require a conversion of superoxide to H_2O_2 (14). Superoxides intracellularly generated by redox-cycling agents can be converted to more reactive H_2O_2 by superoxide dismutase. H_2O_2 is subsequently detoxified by catalase. Thus, one might expect that high catalase levels would confer increased protection against superoxide generators because of the rapid breakdown of H_2O_2. This was not observed and thus implies that in *Xanthomonas* spp., menadione toxicity may not be mediated by increased H_2O_2 production. Monofunctional catalase cannot use organic hydroperoxides as substrates, and thus high catalase levels had no effect on organic hydroperoxide resistance.

What could be the possible benefits to *Xoo* of having atypical regulation for catalase? *Xoo* is sensitive to H_2O_2 and organic hydroperoxides but highly resistant to superoxide killing (reference 8 and unpublished observations). Also, we observed that low concentrations of menadione conferred cross-protection against killing concentrations of both H_2O_2 and organic hydroperoxides, whereas exposure to either peroxide did not confer cross-protection (unpublished observations). Increased production of superoxide radicals has been observed during the initial stages of plant-microbe interaction. For *Xoo*, this condition could induce protection against killing by both peroxides.

katX expression in *E. coli* was both temperature and growth phase dependent. *katX* temperature-sensitive expression is most likely to take place at protein folding or stability steps. Low temperature is known to affect levels of chaperonins (42) and reduce the rate of translation, which may help correct folding of heterologous protein. Protein stability may also increase at a low temperature since the level of Lon protease is also lower (42). These interpretations do not explain growth phase-dependent expression. Some possible explanations of the observation are (i) growth phase-dependent translation regulation and stability, similar to the regulation of *E. coli rpoS* (30, 32, 36); (ii) growth phase-specific modification of KatX; and (iii) appearance of cofactors. Alternatively, recent observations by Derman and Beckwith (11) suggest that inactive *E. coli* alkaline phosphatase can be converted to active enzyme by forming disulfide bonds in cytoplasm when the cells stop growing and are incubated at low temperature. This is similar to the pattern of *katX* expression in *E. coli*. In the case of well-characterized catalases, i.e., bovine and *E. coli*, disulfide bonds are not involved in the formation of active catalase enzyme. However, recent reports of periplasmic catalase in several bacteria (25, 41) suggest that at least some catalases may require disulfide bond formation. Nonetheless, we could not detect any catalase activity in *E. coli* harboring *katX* in periplasm (data not shown). Apparently, *katX* growth phase-dependent expression was an effect of heterologous expression of a foreign gene in *E. coli.*

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