Catalase has a novel protective role against electrophile killing of *Xanthomonas*

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The ability of *Xanthomonas campestris* pv. *phaseoli* to protect itself against lethal concentrations of man-made (*N*-ethylmaleimide, NEM) and endogenously produced (methylglyoxal, MG) electrophiles was investigated. Pretreatment of *X. c.* pv. *phaseoli* with a low concentration of NEM induced protection against lethal concentrations of NEM and MG. MG pretreatment weakly induced protection against NEM but not against MG itself. NEM-induced protection against electrophile killing required new protein synthesis and was abolished by the addition of a protein synthesis inhibitor. By contrast, MG-induced protection against NEM killing was independent of *de novo* protein synthesis. *X. c.* pv. *phaseoli* harbouring an expression vector carrying a catalase gene was over 100-fold more resistant to MG and NEM killing. High expression levels of genes for other peroxide-protective enzymes, such as those for alkyl hydroperoxide reductase (*ahpC* and *ahpF*) and *ohr*, failed to protect against electrophile killing. Thus, catalase appears to have a novel protective role(s) against electrophile toxicity. This finding suggests that in *X. c.* pv. *phaseoli* NEM and MG toxicity might involve accumulation and/or increased production of H₂O₂. This idea was supported by the observation that addition of 10 mM sodium pyruvate, a compound that can react chemically with peroxide or hydroxyl radical scavengers (DMSO and glycerol), was found to protect *Xanthomonas* from electrophile killing. The protective role of catalase and the role of H₂O₂ in electrophile toxicity are novel observations and could be generally important in other bacteria. In addition, unlike other bacteria, *Xanthomonas* in stationary phase was more susceptible to electrophile killing compared to cells in exponential phase.

Keywords: methylglyoxal, *N*-ethylmaleimide, resistance, catalase

INTRODUCTION

Xanthomonas spp. are important bacterial plant pathogens. In the environment and on plants, bacteria are exposed to a variety of chemicals, some of which could modulate bacterial physiological responses. Increased production and accumulation of reactive oxygen species, including H₂O₂, organic peroxide and superoxide anions, are important components of plant active defence responses to microbial invasion (Levine *et al.*, 1994; Sutherland, 1991). Exposure to low concen-

trations of peroxide and superoxide anions affects the stress responses of *Xanthomonas* spp. in complex ways (Loprasert *et al.*, 1996; Mongkolsuk *et al.*, 1997b). These alterations in physiological responses could affect disease progression and outcome. Understanding complex bacterial physiological processes not only contributes to our understanding of pathological processes but also might reveal new targets for drug development to control bacterial proliferation.

In nature, bacteria are exposed to toxic electrophiles both from within the cell and from their environment. Methylglyoxal (MG) is the major electrophile produced intracellularly by bacterial cells. Millimolar concentrations of MG are produced during growth under certain conditions (Ferguson *et al.*, 1998b, 1999). In addition, electrophiles are released into the environment

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Abbreviations: MG, methylglyoxal; NEM, *N*-ethylmaleimide; SB, Silva-Buddenhagen.

in the form of herbicides and chemicals used in the poultry industry (Stevens *et al.*, 1995; Zablotowicz *et al.*, 1995). The toxicities of these compounds are believed to stem from their interactions with nucleophilic centres of macromolecules. Electrophiles react with amino acid residues such as arginine, lysine and cysteine (Lo *et al.*, 1994). These compounds are known also to react with DNA and are highly mutagenic (Ferguson *et al.*, 1998a; Ferguson *et al.*, 2000). Electrophiles must be rapidly detoxified to prevent cellular and genetic damage. In bacteria, MG and NEM detoxification involves glutathione. MG is detoxified by glutathione-dependent glyoxalase systems (Ferguson *et al.*, 1995; MacLean *et al.*, 1998). In addition, both MG and NEM glutathione adducts activate the potassium transport system, KefB/KefC, resulting in acidification of the cytoplasm and protection against electrophile killing (Ferguson *et al.*, 1995).

Recently, we have shown that electrophiles such as NEM can modulate the oxidative stress responses in *Xanthomonas* spp. (Mongkolsuk *et al.*, 1997b; Vattanaviboon *et al.*, 1999). Exposure to low concentrations of NEM was shown to induce high levels of the peroxide-scavenging enzymes alkyl hydroperoxide reductase subunit C and catalase in an OxyR-dependent manner. This gives high-level protection against peroxide toxicity. Exposure of bacteria to low concentrations of some compounds can induce protection to subsequent exposure to lethal concentrations of the same compound (adaptive) or of other non-related compounds (cross-protection). These responses are important strategies for bacterial survival under stressful conditions. Thus, exposure to electrophiles will affect various physiological processes. Here, we determined the effects of pre-exposure to low concentrations of electrophiles on the response to subsequent exposure to lethal concentrations of electrophiles. A novel protective mechanism against electrophile toxicity was also investigated.

METHODS

Bacterial growth conditions. All *Xanthomonas campestris* pv. *phaseoli* strains were grown aerobically in SB (Silva-Buddenhagen) medium (Mongkolsuk *et al.*, 1996) at 28 °C. To ensure synchronous exponential growth, overnight cultures were subcultured into fresh SB medium to give OD₆₀₀ of 0.1. Bacterial growth was monitored spectrophotometrically at OD₆₀₀, using a Shimadzu UV2100 spectrophotometer. Exponential-phase cells (OD₆₀₀ of 0.5, after 4 h of growth) and stationary-phase cells (OD₆₀₀ of 5.5 after 24 h of growth) were used in experiments, as indicated.

Quantitative determination of resistance levels to oxidants. The induced adaptive and cross-protection response experiments were performed by adding either 0.01% (w/v) MG, 100 µM NEM or 100 µM iodoacetamide to exponential-phase *X. c.* pv. *phaseoli* cultures. The cultures were allowed to grow for 1 h before aliquots of cells were treated with lethal concentrations of MG (0.1% w/v) or NEM (1.0 mM). At the indicated times, samples were removed and washed twice with fresh SB medium before cells were plated on SB agar. Colonies were counted after 36 h incubation at 28 °C. Surviving

fractions are defined as the number of c.f.u. recovered after the treatment divided by the number of c.f.u. prior to the treatment. To test the effect of sodium pyruvate on electrophile killing, experiments were performed as described, except that cells were plated on SB agar with and without 10 mM sodium pyruvate. The effects of hydroxyl radical scavengers were determined as previously described (Vattanaviboon & Mongkolsuk, 1998) by exposing *X. c.* pv. *phaseoli* cultures to protecting concentrations of DMSO (0.4 M) and glycerol (1.0 M) for 30 min prior to exposure to lethal concentrations of NEM (1.0 mM). All experiments were repeated at least four times and means and standard deviations are shown.

Qualitative determination of levels of resistance to lethal concentration of NEM. The levels of resistance against a lethal concentration of NEM was qualitatively determined by using the inhibition zone method (Mongkolsuk *et al.*, 2000). Exponential-phase cells (10⁸ c.f.u. ml⁻¹) were mixed with SB top agar and overlaid onto SB agar plates. Then 6 mm diameter filter paper discs soaked with 5 µl of 10 mM NEM were placed on top of the *Xanthomonas* cell lawn. The zone of growth inhibition was measured after 24 h incubation.

Catalase activity gel and assays. Total catalase activity was assayed as previously described (Mongkolsuk *et al.*, 1996). Preparation of cell lysates for enzyme assays and catalase activity gels were prepared according to Mongkolsuk *et al.* (1996). Essentially, cells were pelleted and washed once with 50 mM sodium phosphate buffer pH 7.0 and lysed by brief sonication followed by centrifugation at 10000 g for 10 min. Cleared lysates were used for enzyme assay and catalase activity gels. Catalase isozymes were visualized on native PAGE gels as described by Vattanaviboon & Mongkolsuk (2000). After electrophoresis gels were immersed at room temperature for 45 min in 50 mM sodium phosphate buffer pH 7.0 containing 50 µg ml⁻¹ horseradish peroxidase (Sigma) and then in 50 mM sodium phosphate buffer pH 7.0 containing 5 mM H₂O₂ for 10 min. Subsequently, each gel was washed twice and finally treated with 0.5 mg ml⁻¹ diaminobenzidine. Catalase activity appeared as colourless bands against a dark brown background.

RESULTS AND DISCUSSION

Adaptive and cross-protection responses to electrophile killing

The effect of pretreatment of *X. c.* pv. *phaseoli* with low concentrations of electrophiles on survival to subsequent challenge with lethal concentrations of the chemicals was investigated. Two model electrophiles were used in the study, NEM, a man-made agent, and MG, an endogenously generated compound. Pretreatment of *X. c.* pv. *phaseoli* with 100 µM NEM induced a more than 100-fold increase in resistance when cells were subsequently exposed to a lethal concentration (1.0 mM) of the same compound (Fig. 1a). By contrast, pretreatment of the bacteria with 0.01% (w/v) MG did not induce protection against a lethal concentration of MG (Fig. 1c). Several other pretreatment concentrations of MG were also tested, none of which induced protection against MG killing (data not shown). The ability of the electrophiles to induce cross-protection against exposure to their lethal concentrations was also investigated. The results in Fig. 1(b) show that NEM pretreatment gave some protection to *X. c.* pv. *phaseoli*

Fig. 1. Electrophile-induced adaptive and cross-protection responses in *X. c. pv. phaseoli*. Effects of pretreatment with 100 μ M NEM alone [in (a) and (b); $\triangle$] and in the presence of 150 μ g ml⁻¹ chloramphenicol [in (a) and (b); ∇], 100 μ M DTT [in (a), $\blacktriangle$] or 0.01% w/v MG alone [in (c) and (d); $\square$] and in the presence of 150 μ g ml⁻¹ chloramphenicol [in (d); ∇] on subsequent exposure to 1.0 mM NEM [in (a) and (d)] and 0.1% w/v MG [in (b) and (c)] compared to uninduced cells [in (a), (b), (c) and (d); $\circ$]. Experiments were performed as described in Methods. Values are means of four replicates and error bars indicate sd.

against MG killing. Conversely, pretreatment of *X. c. pv. phaseoli* cultures with 0.01% w/v MG induced a small (approx. 10-fold) increase in resistance to NEM killing (Fig. 1d).

In *Escherichia coli*, cytoplasm acidification confers protection against electrophile killing and is independent of *de novo* protein synthesis (Ferguson *et al.*, 1995). Accordingly, we tested whether the electrophile-induced protection responses to MG and NEM killing required new protein synthesis. Adaptive and cross-protection experiments were repeated with addition of chloramphenicol at 150 μ g ml⁻¹ [a concentration of drug which has been shown to inhibit protein synthesis in *Xanthomonas* spp. (Mongkolsuk *et al.*, 1997b)]. NEM did not induce resistance to MG and NEM killing in chloramphenicol-treated cultures (Fig. 1a, b). This suggests that the NEM-induced adaptive and cross-protection responses require *de novo* protein synthesis and probably result from NEM-induced expression of stress-protective genes. By contrast, MG-induced protection against NEM killing was not affected by addition of chloramphenicol (Fig. 1d), implying that new gene expression was not required. The data clearly show that MG and NEM induce different protective responses to electrophile killing.

The different electrophile-induced adaptive responses were unexpected. They suggested that *X. c. pv. phaseoli*

has evolved the ability to mount an adaptive response against a man-made electrophile NEM but not against an endogenously produced one, MG. The ability of NEM to induce high-level protection against both MG and itself implied that it is a potent inducer of a pathway which can detoxify both NEM and MG. In contrast, MG is not an inducer of this detoxification pathway(s) and is unable to confer protection against itself. Since MG-induced protection against NEM was found to be independent of new protein synthesis, MG probably effects some modification of existing cellular components that results in an increase in resistance to NEM killing. A likely mechanism for MG-induced resistance is via the KefB/KefC system induced by MG/glutathione conjugates, resulting in cytoplasmic acidification and protection against NEM killing (Ferguson *et al.*, 1995; Ferguson, 1999). This process is independent of new protein synthesis (Ferguson *et al.*, 1995). In *Xanthomonas*, unlike *E. coli*, cytoplasm acidification is not sufficient to confer protection against MG toxicity, as shown by lack of MG-induced adaptation. In addition, the inability of MG to induce high-level protection against electrophile killing could be due to the ability of the bacteria to metabolize MG effectively. This could prevent the intracellular concentration of MG from reaching the critical level required to activate a protective pathway(s), while NEM is more slowly metabolized; so its concentration could increase rapidly

Fig. 2. Effects of high-level expression of various peroxide stress protective genes on NEM and MG killing. *X. c. pv. phaseoli* strains harbouring the expression vector pUFR047 alone (○) or containing genes involved in peroxide protection, catalase (pkat, □), alkyl hydroperoxide reductase C and F subunits (pahpCF, ■) and Ohr (pohr, ▲) were treated with lethal concentrations of either NEM (a) or MG (b).

to the level needed to induce the protective pathways. The data show that in *Xanthomonas*, NEM and MG toxicity arise from several routes and there are protective pathways against these compounds. Variation in the ability of each electrophile to induce a protective response(s) is likely to have significant physiological effects.

Catalase has important protective roles against electrophile killing

We were interested to determine the mechanisms responsible for induced adaptive and cross-protection responses to MG and NEM killing. We have shown that exposure of *Xanthomonas* spp. to low concentrations of NEM induces cross-protection to H_2O_2 killing (Vattanaviboon *et al.*, 1999). NEM induces a more than 10-fold increase in each of the peroxide-scavenging enzymes, catalase and alkyl hydroperoxide reductase subunit C (AhpC) in an OxyR (a peroxide sensor and transcription regulator)-dependent manner (Vattanaviboon *et al.*, 1999). This mechanism is likely to be responsible for NEM-induced cross-resistance to peroxide killing. We have observed that a *X. c. pv. phaseoli* H_2O_2 -resistant mutant has a more than 200-fold increase in both catalase and alkyl hydroperoxide reductase activities and is more resistant to NEM killing (Fuangthong & Mongkolsuk, 1997). *E. coli* strains with suppressors of *oxyR* mutants which have high levels of catalase and AhpC show reduced susceptibility to NEM killing (Greenberg & Demple, 1988). We suspect that peroxide-scavenging enzymes might help protect against electrophile killing. We tested this idea by determining the resistance levels to MG and NEM killing in *X. c. pv. phaseoli* harbouring recombinant plasmids containing genes for peroxide-scavenging enzymes. *X. c. pv.*

Fig. 3. NEM-induced Kat1. Catalase activity gel displaying the form of catalase induced by 100 μ M NEM (NEM) for 1 h in exponential-phase cells. Also shown are catalase profiles of extracts of uninduced cells from exponential phase (Uninduced) and from stationary phase (Stationary). Protein (80 μ g) was loaded into each lane and catalase activity was detected as described in Methods.

phaseoli harbouring pUFR047 (vector alone), pahpCF [containing genes for alkyl hydroperoxide reductase C and F subunits, *ahpC* and *ahpF* (Loprasert *et al.*, 1997; Mongkolsuk *et al.*, 1997a)], pkat [containing the monofunctional catalase gene, *katX* (Mongkolsuk *et al.*, 1996)], pohr [containing a gene conferring organic peroxide resistance (Mongkolsuk *et al.*, 1998a)] were treated with lethal concentrations of MG and NEM. The results (Fig. 2a, b) clearly showed that only cells expressing high levels of catalase were protected from MG and NEM killing. High-level expression of genes for all other peroxide-scavenging enzymes tested had no protective effect. The ability of catalase to protect bacterial cells against MG and NEM killing is a novel finding and could be generally important in other bacteria.

Our results show that low concentrations of NEM are potent inducers of protective pathways against electrophile killing and also of catalase enzyme (Figs 1a, b and 3). There appears to be a correlation between the abilities of electrophiles to induce catalase and to induce protection against electrophile killing. The idea was further tested by determining the effects of pretreatment of *X. c. pv. phaseoli* with MG on catalase activity. Several inducing concentrations of MG were tested. MG did not significantly induce catalase at any of the concentrations tested (data not shown). Thus, the ability of NEM to induce catalase correlates with its ability to induce resistance to MG and NEM. In contrast, MG is unable to induce catalase and does not induce protection against itself. MG-induced resistance against NEM

killing was independent of new protein synthesis and appeared to involve some other protective pathway(s).

There are at least two monofunctional catalases and a bifunctional catalase/oxidase in *X. c. pv. phaseoli* (Vattanaviboon & Mongkolsuk, 2000). We have determined that NEM did not induce bifunctional catalase/oxidase (data not shown). Thus, it was considered important to determine the form of monofunctional catalase induced by NEM. Analysis of catalase activity gels showed that Kat1, the major form of catalase in *X. c. pv. phaseoli*, was induced by NEM (Fig. 3). This induction was abolished in an *oxyR* mutant (Mongkolsuk *et al.*, 1998b). MG did not induce Kat1 (data not shown). Additional evidence for the correlation of catalase induction and induced resistance to electrophile killing came from the observation that a *Xanthomonas oxyR* mutant (Mongkolsuk *et al.*, 1998b) that could not induce catalase was more sensitive to NEM killing than was the parent strain. The parental strain had a zone of growth inhibition caused by NEM of 27.0 mm, compared to 30.5 mm for the *oxyR* mutant (zone of inhibition experiments were performed as described in Methods). These data strongly support the conclusion that Kat1 is responsible for resistance to electrophile killing. Nonetheless, we have not been able conclusively to establish the role of Kat1 in the mechanism of protection against electrophile killing due to the lack of a knock-out mutant in the gene. Experiments are in progress to isolate the gene and construct a *kat1* knock-out mutant.

These observations raised the question of how catalase protects cells from electrophile toxicity. H_2O_2 is the only substrate for monofunctional catalase. It is unlikely that the enzyme can react directly with either MG or NEM, suggesting that MG and NEM toxicity might reflect accumulation and/or increased production of H_2O_2 . Studies on MG and NEM toxicity in other bacteria have not conclusively shown that H_2O_2 is involved in electrophile-induced toxicity (Ferguson *et al.*, 1995, 1998a; Ferguson, 1999). Nevertheless, there is evidence that oxidative stress might be involved in electrophile toxicity. For example, expression of an oxidative stress protective gene (*dps*) has been shown to increase resistance to electrophile killing (Ferguson *et al.*, 1998a). Support for the role of H_2O_2 in electrophile toxicity came from two observations. First, addition of 10 mM sodium pyruvate [a compound that chemically inactivates peroxide (Nath *et al.*, 1995)] to the growth medium increased *X. c. pv. phaseoli* resistance levels more than 100-fold to MG and NEM killing (Fig. 4). Secondly, H_2O_2 killing of bacteria involves production of highly reactive hydroxyl radicals. In *Xanthomonas*, compounds which absorb hydroxyl radicals protect cells from H_2O_2 killing (Vattanaviboon & Mongkolsuk, 1998). The effects of hydroxyl radical scavengers (DMSO and glycerol) on NEM killing was investigated. The results in Fig. 5(a) show that both DMSO and glycerol produced around 10-fold protection against NEM killing. The question as to how exposure to NEM or MG leads to an increase in the level of H_2O_2 remains

obscure. Electrophiles are known to react with amino acids in proteins, especially with cysteine residues (Lo *et al.*, 1994). Many peroxide-scavenging enzymes, such as alkyl hydroperoxide reductase or the regulator of the peroxide stress response OxyR, have cysteine residues at their active sites (Loprasert *et al.*, 1997; Mongkolsuk *et al.*, 1997a). Thus, electrophiles could react directly with cysteine residues at active sites of these proteins and inactivate their biological functions. This, in turn, would reduce the cell's ability to detoxify toxic peroxides, leading to intracellular accumulation of these compounds. Glutathione is an important component of electrophile-detoxification systems. High levels of glutathione have protective effects against electrophile toxicity (Ferguson *et al.*, 1995). Electrophiles could lower the ratio of reduced versus oxidized glutathione. Depletion of glutathione would directly affect the rate of electrophile metabolism, electrophile resistance levels and the redox balance of the cell. The condition not only reduces the rate of electrophile metabolism and the associated resistance level but also the inducing oxidative stress condition(s). These factors might be expected to combine to increase intracellular concentrations of H_2O_2 . Thus, a thiol reagent such as iodoacetamide that causes depletion of glutathione (Kondo *et al.*, 1987) is expected to affect electrophile killing of the bacteria. *Xanthomonas* cultures were pretreated with 100 μ M iodoacetamide prior to exposure to lethal concentrations of NEM. The results clearly showed that iodoacetamide induced high-level (1000-fold) protection against NEM killing (Fig. 5b). Iodoacetamide caused over 10-fold induction of total catalase activity (data not shown). In addition, we observed that a reducing agent such as DTT partially reversed the effects of NEM-induced protection against itself (Fig. 1a). Thus, in cells that produce high levels of catalase, or when grown in media with high levels of pyruvate or hydroxyl radical scavengers (DMSO or glycerol), H_2O_2 accumulation is prevented. This results in the observed increase in resistance to electrophile killing.

Growth-phase-dependent resistance to electrophile killing

In the environment, bacteria have a short period of rapid exponential growth when nutrients are plentiful followed by a long stationary phase during nutrient limitation. The ability to survive stresses during the stationary phase is important for bacteria in the environment. Bacterial stress resistance varies greatly with growth phase (Vattanaviboon *et al.*, 1995; Ferguson *et al.*, 1998a). In general, stationary-phase cells are more resistant than exponential-phase cells to a variety of stresses. Moreover, the level of stationary-phase stress resistance often does not correlate with the levels of known stress-protective enzymes, suggesting that other factors such as alterations in membrane structure, altered metabolic activity and non-specific DNA-binding proteins are more important (Ferguson *et al.*, 1998a; Martinez & Kolter, 1997). Here, we investigated MG and NEM killing during exponential and stationary

Fig. 4. Effects of addition of 10 mM pyruvate on NEM and MG killing. *X. c. pv. phaseoli* were treated with 1.0 mM NEM (a) or 0.1% w/v MG (b). At the indicated times, samples were removed, washed twice and plated on SB agar with 10 mM sodium pyruvate (●, ■) or without addition (○, □). Values are means of four replicates and error bars indicate SD.

Fig. 5. Effects of hydroxyl radical absorbers and a thiol reagent on NEM killing. In (a), exponential-phase uninduced cells of *X. c. pv. phaseoli* were treated with 1.0 mM NEM in the absence (○), or in the presence of hydroxyl radical scavenger, 1.0 M glycerol (△) or 0.4 M DMSO (□). In (b), *X. c. pv. phaseoli* cultures were induced with 100 µM iodoacetamide for 1 h (▽) or grown uninduced (○) prior to exposure to 1.0 mM NEM. Growth and electrophile killing conditions were as described in Methods. Values are means of four replicates and error bars indicate SD.

Fig. 6. Growth phase variation in resistance levels to NEM and MG killing. Exponential-phase (○, □) or stationary phase (●, ■) cells were treated with 1.0 mM NEM (a) or 0.1% w/v MG (b). Growth and electrophile killing conditions were as described in Methods. Values are means of four replicates and error bars indicate SD.

phases. The results presented in Fig. 6 show that stationary-phase cells were over 100-fold more sensitive than exponential-phase cells to both NEM and MG

killing, a finding in contradiction to previous reports (Ferguson *et al.*, 1998a). In *E. coli*, resistance to electrophile killing was found to increase during station-

ary phase (Ferguson *et al.*, 1998a). The products of genes in the *rpoS* regulon and a non-specific DNA binding protein (Dps) have been shown to contribute to the stationary-phase electrophile resistance phenotype (Ferguson *et al.*, 1998a). In *Xanthomonas*, increased sensitivity to electrophile killing during stationary phase could be due to a decrease in the total catalase level. Unlike other bacteria, in *Xanthomonas* total catalase activity decreases in the stationary phase (Loprasert *et al.*, 1996). Analysis of catalase activity gels loaded with lysates prepared from a *X. c. pv. phaseoli* culture during exponential and stationary growth phases shows that the activity of the major form of monofunctional catalase (Kat1) decreased as cells entered stationary phase. Even though a minor form of growth-phase-regulated catalase KatE increases during stationary phase (Vattanaviboon & Mongkolsuk, 2000), the increase in KatE activity did not compensate for the decrease in Kat1 activity, resulting in a lower total catalase activity during the stationary phase (Vattanaviboon & Mongkolsuk, 2000). In addition, while in stationary phase, cells are starved of nutrients, resulting in alterations in flux through various biochemical pathways. This, in turn, could affect the ratio of glutathione to glutathione disulphide. Lowering the availability of glutathione not only reduces the capacity to detoxify electrophiles but also reduces the resistance to electrophiles.

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REFERENCES

- Ferguson, G. P. (1999). Protective mechanisms against toxic electrophiles in *Escherichia coli*. *Trends Microbiol* 7, 242–247.
- Ferguson, G. P., McLaggan, D. & Booth, I. R. (1995). Potassium channel activation by glutathione-S-conjugates in *Escherichia coli*: protection against methylglyoxal is mediated by cytoplasmic acidification. *Mol Microbiol* 17, 1025–1033.
- Ferguson, G. P., Creighton, R. I., Nikolaev, Y. & Booth, I. R. (1998a). Importance of RpoS and Dps in survival of exposure of both exponential- and stationary-phase *Escherichia coli* cells to the electrophile N-ethylmaleimide. *J Bacteriol* 180, 1030–1036.
- Ferguson, G. P., Totemeyer, S., MacLean, M. J. & Booth, I. R. (1998b). Methylglyoxal production in bacteria: suicide or survival? *Arch Microbiol* 170, 209–218.
- Ferguson, G. P., Battista, J. R., Lee, A. T. & Booth, I. R. (2000). Protection of the DNA during the exposure of *Escherichia coli* cells to a toxic metabolite: the role of the KefB and KefC potassium channels. *Mol Microbiol* 35, 113–122.
- Fuangthong, M. & Mongkolsuk, S. (1997). Isolation and characterization of a multiple peroxide resistant mutant from *Xanthomonas campestris* pv. *phaseoli*. *FEMS Microbiol Lett* 152, 189–194.
- Greenberg, J. T. & Demple, B. (1988). Overproduction of peroxide-scavenging enzymes in *Escherichia coli* suppresses spontaneous mutagenesis and sensitivity to redox-cycling agents in *oxyR*[−] mutants. *EMBO J* 7, 2611–2617.
- Kondo, T., Kawakami, Y., Taniguchi, N. & Beutler, E. (1987). Glutathione disulfide-stimulated Mg²⁺-ATPase of human erythrocyte membranes. *Proc Natl Acad Sci USA* 84, 7373–7377.
- Levine, A., Tenhaken, R., Dixon, R. & Lamb, C. (1994). H₂O₂ from oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79, 583–593.
- Lo, T. W., Westwood, M. E., McLellan, A. C., Selwood, T. & Thornalley, P. J. (1994). Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N- α -acetylarginine, N- α -acetylcysteine, and N- α -acetyllysine, and bovine serum albumin. *J Biol Chem* 269, 32299–32305.
- Loprasert, S., Vattanaviboon, P., Praituan, W., Chamnongpol, S. & Mongkolsuk, S. (1996). Regulation of the oxidative stress protective enzymes, catalase and superoxide dismutase in *Xanthomonas* – a review. *Gene* 179, 33–37.
- Loprasert, S., Atichartpongkum, S., Whangsuk, W. & Mongkolsuk, S. (1997). Isolation and analysis of the *Xanthomonas* alkyl hydroperoxide reductase gene and the peroxide sensor regulator genes *ahpC* and *ahpF-oxyR-orfX*. *J Bacteriol* 179, 3944–3949.
- MacLean, M. J., Ness, L. S., Ferguson, G. P. & Booth, I. R. (1998). The role of glyoxalase I in the detoxification of methylglyoxal and in the activation of the KefB K⁺ efflux system in *Escherichia coli*. *Mol Microbiol* 27, 563–571.
- Martinez, A. & Kolter, R. (1997). Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. *J Bacteriol* 179, 5188–5194.
- Mongkolsuk, S., Loprasert, S., Vattanaviboon, P., Chanvanichayachai, C., Chamnongpol, S. & Supsamran, N. (1996). 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* 178, 3578–3584.
- Mongkolsuk, S., Loprasert, S., Whangsuk, W., Fuangthong, M. & Atichartpongkum, S. (1997a). Characterization of transcription organization and analysis of unique expression patterns of an alkyl hydroperoxide reductase C gene (*ahpC*) and the peroxide regulator operon *ahpF-oxyR-orfX* from *Xanthomonas campestris* pv. *phaseoli*. *J Bacteriol* 179, 3950–3955.
- Mongkolsuk, S., Vattanaviboon, P. & Praituan, W. (1997b). Induced adaptive and cross-protection responses against oxidative stress killing in a bacterial phytopathogen, *Xanthomonas oryzae* pv. *oryzae*. *FEMS Microbiol Lett* 146, 217–222.
- Mongkolsuk, S., Praituan, W., Loprasert, S., Fuangthong, M. & Chamnongpol, S. (1998a). Identification and characterization of a new organic hydroperoxide resistance (*ohr*) gene with a novel pattern of oxidative stress regulation from *Xanthomonas campestris* pv. *phaseoli*. *J Bacteriol* 180, 2636–2643.
- Mongkolsuk, S., Sukchawalit, R., Loprasert, S., Praituan, W. & Upaichit, A. (1998b). Construction and physiological analysis of a *Xanthomonas* mutant to examine the role of the *oxyR* gene in oxidant-induced protection against peroxide killing. *J Bacteriol* 180, 3988–3991.
- Mongkolsuk, S., Whangsuk, W., Fuangthong, M. & Loprasert, S. (2000). Mutations in *oxyR* resulting in peroxide resistance in *Xanthomonas campestris*. *J Bacteriol* 182, 3846–3849.
- Nath, K. A., Ngo, E. O., Hebbel, R. P., Croatt, A. J., Zhou, B. & Nutter, L. M. (1995). α -Ketoacids scavenge H₂O₂ *in vitro* and *in vivo* and reduced menadione-induced DNA injury and cytotoxicity. *Am J Physiol* 268, C227–C236.

- Stevens, K. L., Wilson, R. F. & Friedman, M. (1995).** Inactivation of a tetrachloride mutagen from stimulated processing water. *J Agric Food Chem* **43**, 2424–2427.
- Sutherland, M. W. (1991).** The generation of oxygen radicals during host plant response to infection. *Physiol Mol Plant Pathol* **39**, 79–93.
- Vattanaviboon, P. & Mongkolsuk, S. (1998).** Evaluation of the role hydroxyl radicals and iron play in hydrogen peroxide killing of *Xanthomonas campestris* pv. *phaseoli*. *FEMS Microbiol Lett* **169**, 255–260.
- Vattanaviboon, P. & Mongkolsuk, S. (2000).** Expression analysis and characterization of the mutant of a growth phase- and starvation-regulated monofunctional catalase gene from *Xanthomonas campestris* pv. *phaseoli*. *Gene* **241**, 259–265.
- Vattanaviboon, P., Praituan, W. & Mongkolsuk, S. (1995).** Growth phase dependent resistance to oxidative stress in a phytopathogen *Xanthomonas oryzae* pv. *oryzae*. *Can J Microbiol* **41**, 1043–1047.
- Vattanaviboon, P., Varaluksit, T. & Mongkolsuk, S. (1999).** Modulation of peroxide stress response by thiol reagents and the role of a redox sensor-transcription regulator, OxyR in mediating the response in *Xanthomonas*. *FEMS Microbiol Lett* **176**, 471–476.
- Zablotowicz, R. M., Hoagland, R. E., Locke, M. A. & Hickey, W. J. (1995).** Glutathione S-transferase and metabolism of glutathione conjugates by rhizosphere bacteria. *Appl Environ Microbiol* **61**, 1054–1060.

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