S10-1

Chemicals Modulation of Physiological Adaptation and Cross Protection Responses Against Oxidative Stress in Soil Bacteria and Phytopathogen, *Xanthomonas*

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An oxidative stress condition arises from excess reactive oxygen species (ROS) including superoxide anions, H_2O_2, and organic hydroperoxides, which are highly toxic. The condition could arise from aerobic respiration, plant defense response to microbial infection and exposure to chemicals in soil (1, 8). Bacteria have evolved both enzymatic and non-enzymatic mechanisms to remove these ROS. Thus, any agents that affect the bacterial oxidative stress response could also alter the organism's ability to survive in the soil environment, as well as affect its pathogenicity.

Inducible adaptive response

In addition to growth phase dependent stress resistance mechanisms, most bacteria have inducible stress protective systems. With regards to oxidative stress protection, in *Xanthomonas* as well as other bacteria exposure to low concentrations of H_2O_2 induces high-level resistance to subsequent challenge with killing concentrations of H_2O_2 (7). The genetics basis and regulatory genes involved in the response have been elucidated. In *Xanthomonas*, OxyR is the major peroxide sensor and transcription regulator. In normal cells OxyR exists in the reduced form that repressed expression of genes in its regulon. Upon exposure to peroxides, OxyR becomes oxidized at the reactive sensing Cystein C199 and a subsequent disulphide bond formation (2). The oxidized OxyR activates transcription of genes in its regulon (2). The role of OxyR in *Xanthomonas* adaptive response to oxidative stress has been investigated (5). The H_2O_2 adaptive protection against H_2O_2 killing is depended on a functional *oxyR* . *oxyR* is the regulator of genes involved in peroxide metabolism. As expected peroxide scanvenging enzymes, catalase and alkyl hydroperoxide reductase (AhpC) are highly induced (at least 5-10 fold over the uninduced levels) by pre-treatments with peroxides in an OxyR dependent manner. The levels of these enzymes show direct correlation with resistance H_2O_2 levels in exponential phase cells.

Inducible cross protection response.

Superoxide generators such as paraquat, menadione are strong inducers of cross protective response to H_2O_2 and organic peroxide killing. The genetics basis of the regulation of the induced cross protection is complex. By contrast to the H_2O_2 adaptive response, a superoxide generator (menadione) induced protection against H_2O_2 and organic peroxide is OxyR independent (5). The induction mechanism is depended on oxidation of OxyR possibly by dismutation of superoxide anions to H_2O_2 that in turn oxidized OxyR. Menadione treatment has no effect on *ohr* expression. Nonetheless, in the absence of OxyR, menadione could induce high levels of resistance to H_2O_2 and organic peroxides. Ohr, a thiol peroxide belongs to a new class of gene involved in organic peroxide metabolism first discovered in *Xanthomonas* (4). The enzyme metabolizes organic peroxide much more efficiently than H_2O_2. *ohr* is regulated by OhrR, an organic peroxide sensor and transcription repressor (3). Menadione induced physiological cross protection against H_2O_2 and organic peroxide involved activation of genes other than catalase, *ahpCF* and *ohr* that could efficiently metabolize or repair peroxides induced cellular damages. A induce cross protection between different type of peroxides has been observed in *Xanthomnas*. An organic peroxide (such as tert butyl hydroperoxide, tBOOH) induced protection to H_2O_2 is depended on functional *oxyR* (6).

A metal modulation of peroxide stress response.

Heavy metals are recognized as environmental pollutants and are released from both industrial and agricultural sources. Cadmium is an important heavy metal pollutant. Exposure of *X. campestris* to low concentrations of cadmium induces cross-protection against subsequent killing treatments with either H_2O_2 or tBOOH, but not menadione. The cadmium-induced resistance to peroxides is due to the metal ability to induce increased levels of peroxide stress protective enzymes such as alkyl hydroperoxide reductase (AhpC), monofunctional catalase (KatA) and Ohr. Cadmium induced resistance to H_2O_2 is dependent on functional OxyR. Cadmium induced resistance to organic hydroperoxide shows a more complex regulatory pattern. Inactivation of sensors/regulators of organic hydroperoxide, OxyR and OhrR, only partially inhibits cadmium-induced protection to tBOOH suggesting that these genes do have some role in the process. However, other, as yet unknown, mechanisms are involved in inducible organic hydroperoxide protection.

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