Thesis Title	Characterization of a Gene Specified Organic Hydroperoxide Resistance in *Xanthomonas*
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ABSTRACT

A gene encoding organic hydroperoxide resistance was isolated from *Xanthomonas campestris* pv. *phaseoli* chromosomal DNA using complementation of an *ahp* mutant *E. coli* (TA4315) by selected for *tert*-butyl hydroperoxide resistant phenotype. The gene was designated *oprX* (organic hydroperoxides resistance). Subcloning and complementation analysis of the gene showed that it was localized on a 1.2 kb of *Kpn*I fragment. Southern blot analysis confirmed that the *oprX* gene was from *Xanthomonas campestris* pv. *phaseoli* chromosomal DNA. The gene was highly conserved among different *Xanthomonas* spp. but not in other non related bacteria. An insertion inactivation of *oprX* was carried out by inserting a *tet* gene into the *Not*I site. The 1.2 kb *Kpn*I fragment was sequenced in both orientations. DNA

sequencing data showed two open reading frames, one of which covered *Not*I site. ORF B was a likely candidate for the *oprX* gene because of its location, which encompassed the *Not*I site and predicted size of this ORF was equaled to size of detected *oprX* mRNA from Northern blot experiments. ORF B was located between position 199 to 627 of complementary strand of the 1.2 *Kpn*I fragment, which encoded for a 143 amino acids protein of M.W. 14.48 kDa. Comparison of the predicted amino acid sequence of OprX with those of conventional antioxidant enzymes including catalase, peroxidase, superoxide dismutase, alkyl hydroperoxide reductase and other known proteins revealed no significant sequence homology except with an *E.coli* protein OsmC (osmotic inducible protein). The inactivated *oprX::tet* was inserted into *Xanthomonas campestris* pv. *phaseoli* chromosome by a gene replacement technique. The resultant mutants were designated XP18 and XP19. Both mutants showed increase sensitivity towards organic hydroperoxides by disc assay and peroxides killing experiments. Moreover, the functional *oprX* can complement the organic hydroperoxide sensitive phenotype of the mutants to wild-type level. This results suggest that OPRX plays an important role in the defense against organic hydroperoxides toxicity.