

Thesis Title Cloning of Catalase Gene (*kat*) from
Xanthomonas oryzae pv. *oryzae* Isolate 8736

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ABSTRACT

Xanthomonas oryzae pv. *oryzae* isolate 8736, a rice pathogen, is exposed to oxidative stress during interactions with plants. The stress has to be detoxified to prevent damages to cellular and genetics components. Catalase is the most important defense against H₂O₂ toxicity. Genes isolation are the first step towards elucidation of the physiological role of the enzymes in oxidative stress response. The *Xanthomonas kat* gene was isolated using the *Xoo kat* specific PCR product as a probe to screen the lambda genomic library of *Xanthomonas oryzae* pv. *oryzae* isolate 8736. The *Xoo kat* specific PCR product was made by *Xanthomonas oryzae* pv. *oryzae* isolate 8736 genomic DNA and a set of primers corresponding to the KAT conserved amino acid sequences derived from the alignment of *kat* coding sequences of bacteria, plant, animals and human at the positions 117 to 126 and 372 to 381. The isolated *Xoo kat* gene was characterized by restriction mapping and expression in a

heterologous host. The location of the gene was determined by sequential deletion technique and restriction endonuclease mapping. The presence of a functional *Xoo kat* gene was confirmed by the detection of KAT activity in *E. coli kat* mutants (devoids of endogenous KAT) harboring the isolated *Xoo kat* cloned in expression vector (pKSKA3.4). The expression of *Xoo kat* in *E. coli* was temperature sensitive, the KAT activity was detectable only when *E. coli* harboring pKSKA3.4 plasmid was grown at 28°C. At 37°C no KAT activity was detected. Furthermore, we have shown that the *Xoo kat* expression was also growth phase dependent, the KAT activity was detectable only during late log growth phase. Deletion analysis of pKSKA3.4 suggests that the 2.9 kb of cloned *kat* fragment is essential for the KAT activity. As expected, *Xoo kat* gene is closely related to other *Xanthomonas* spp. but not to unrelated bacteria and other organisms. The gene would provide useful initial steps toward studying regulation of *Xoo kat*.