

Thesis Title Regulation of *Xanthomonas* Catalase Gene in
Escherichia coli and *Xanthomonas oryzae*

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ABSTRACT

Regulation of *Xanthomonas catalase* gene was studied in *Escherichai coli* and *Xanthomonas oryzae* pv. *oryzae*. pKSkatX contained a functional *katX* could restore catalase production in *E.coli* catalase mutants. (UM255, UM2, UM258) only at 28°C. At 37°C, no Kat activity was observed. At 28°C, UM255 harbouring *katX*, Kat activities were highest during late logarithmic phase and declined as growth progressed. Temperature dependent expression of *katX* gene at 37°C was not due to temperature sensitive enzyme but likely to be due to the effect

of temperature on Kat synthesis. For *katX* expression in *Xanthomonas* spp., increase expression was observed in *Xanthomonas* harbouring the cloned *katX* gene. This increase expression conferred protection to *Xanthomonas* from the killing effect of H₂O₂. No protection against *tert*-butylhydroperoxide or menodione was observed. Transcription regulation analysis by chloramphenicol acetyltransferase gene (*cat*) fusion was studied. At all stages of growth phase and at 28°C or 37°C, Cat activity revealed no difference in level of expression which indicated that the *katX* regulation in *E. coli* was at posttranscription level. The transcription terminator in the coding portion between the *Cla*I-*Pst*II region of *katX* gene was observed and studied. The terminator activity was monitored by using *cat* or *gus* gene fusions. *katX* gene did not have a functional promoter but instead used the *lacZ* promoter for expression both in *E. coli* and *Xoo*. Analysis of regulation of *katX* expression using Tn5-*gusA* transposons were performed. In *E. coli*, transcriptional and translational *gusA* fusions were studied. Transcriptional *gusA* fusion transposon supported previous results that there was no transcription regulation of *katX* and the regulation is likely to be at posttranscriptional level. Also, the quantity of blue colour of GusA activity was used to analysed transcription terminator activity. For translational *gusA* fusion transposon, 136 kDa GusA fusion protein was observed at 28°C and at late logarithmic phase. This confirmed the previous results that *katX* expressed only at 28°C and at late

logarithmic phase. This suggested that translational regulation may be involved in Kat synthesis.

In *Xoo*, the 136 kDa GusA fusion protein from Kat translational fusion was also detected at mid logarithmic phase. Indicating that the growth phase regulation was only observed in *E. coli* and not in *Xanthomonas*.