4036471 SCBT/M : MAJOR : BIOTECNOLOGY ; M.Sc. (BIOTECHNOLOGY)
KEY WORDS : *XANTHOMONAS* / TRANSALDOLASE / REGULATOR OF NUCLEOTIDE DIPHOSPHATE KINASE / OXIDATIVE STRESS
TANUTRA VARALUKSIT MOLECULAR CLONING AND CHARACTERIZATION OF TRANSALDOLASE GENE IN *XANTHOMONAS* SP. THESIS ADVISORS : SKORN MONGKOLSUK Ph.D., SAOVANEE DHARMSTHITI Ph.D., JARUNYA NARANGAJAVANA Dr.Agr.Sc.150 p. ISBN 974-664-724-5

A gene encoded transaldolase (talX), an enzyme in the non-oxidative branch of pentose phosphate pathway, was cloned from the *Xanthomonas campestris* pv. *phaseoli* (*Xp*) genomic library. One of the positive clones named pTal3 was further characterized and the nucleotide sequence was determined. Analysis of the nucleotide sequence revealed a putative open reading frame of 1.0 kb encoding a peptide with 322-amino acid and had a molecular weight of 34.6 kDa. This open reading frame had a high homology to *tal* genes. Analysis pattern of Southern experiments of *Xanthomonas* genomic DNA digested with various restrictive enzymes showed that *talX* was a single copy gene and existed in all *Xanthomonas* strains tested. Northern analysis showed that *talX* hybridized to 1.5 kb mRNA. This length of mRNA suggested that *talX* was in an operon with *rnk* (regulator of nucleotide diphosphate kinase). This assumption was confirmed by RT-PCR. The *rnk-talX* operon arrangement was observed in all *Xanthomonas* strains tested. Using Primer extension technique, a strong promoter was found at 5'end of *rnk* gene. Pre-exposure of the log-phase culture of *Xp* to sub-lethal concentrations of oxidants had no effects on *talX* expression. A *Xp talX* mutant strain was constructed by insertion and inactivation technique. The mutant did not show any adverse effects on aerobic growth or significant changes in resistance levels to oxidants. However, a small increase in glucose-6-phosphate dehydrogenase was observed in the mutant. This could be a compensatory response to the lack of *talX*.