Thesis Title Cloning of an *esterase* gene from *Xanthomonas campestris* pv. *glycines*

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ABSTRACT

Esterase are a group of enzymes that hydrolyze the ester bond in ester compounds, especially those with short chain fatty acid. Studied from cell lysates of *Xanthomonas campestris* pv. *glycines* showed that the intracellular esterase was a monomeric protein with MW of 59 kDa and expressed under control of growth-phase-dependent process. The study on factors affecting the production of the enzyme found that neither divalent cations nor oxidants agents have any intluence on the enzyme activity.

Esterase gene was screened from *X. campestris* pv. *glycines* DNA library in a ZipLox vector circularized into plasmids in a DH10BZIP. Seven colonies out of 10,000 colonies forming clear halo around the colonies were selected on TBA plates and further checked on rhodamine B plates. Due to inability to produce a fluorescent agent on Rhodamine B agar medium under UV light, all of 7 clones were proved to be true esterases. By restriction enzyme and Southern blot analysis, they shared a homologous band with MW of 0.9 kb, thus the smallest cloned with insert DNA MW of 1.6 kb was chosen for further study.

Results from Southern blot confirmed that the insert portion of this clone derived from *X. campestris* pv. *glycines* and showed high homology to a genomic DNA fragment from each of other *Xanthomonas* species. The entire coding region was found to be 1.3 kb and the expression was under the *lacZ* vector promoter. Adddition evidence from esterase assay in cell-free supernatant suggested that the cloned *esterase* gene produced a secreted enzyme. The overexpression study of the enzyme in *Xanthomonas* showed that the esterase levels had no effect on oxidative stress response. Currently, the biological role of esterase in *X. campestris* pv. *glycines* is not understood.