

Karan Lohmaneeratana 2011: Analysis of Enzyme and Gene Involved in Poly (L-Lactic Acid)-Degrading Activity from *Micromonospora*. Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Associate Professor Arinthip Thamchaipenet, Ph.D. 107 pages.

Three out of 164 endophytic actinomycete strains from Genetic-Microbiology Kasetsart University culture collection namely, GMKU 353, GMKU 358 and GMKU 362, showed degradation of poly(L-lactic acid) (PLA) on emulsified PLA agar medium and PLA film in basal medium. Deteriorated structure of PLA films after incubation with the cell culture were observed under scanning electron microscope (SEM). Degradation of PLA with crude enzyme from GMKU 358 was observed by surface plasmon resonance (SPR) with the highest activity at pH 8-10 and ionic strength at zero concentration of sodium chloride, with the specific activity of $0.22 \text{ mg hr}^{-1} \text{ mg}^{-1}$ crude enzyme. 16S rRNA gene sequencing of those three strains revealed that they belong to genus *Micromonospora*. A gene encoding serine protease which predicted to involve in degradation of PLA was amplified from *Micromonospora* sp. GMKU 358 by PCR. Nucleotide sequences revealed its similarity to peptidase S8 and S53 subtilisin kexin sedolisin from *Micromonospora* sp. L5 (84 % identity, 92 % similarity) and serine protease from *Micromonospora* sp. ATCC 39149 (66 % identity 79 % similarity). Subsequently the PCR product was cloned into conjugative vector pIJ8671 to obtain pIJ8671-SER. Gene disruption was performed by intergeneric conjugation between *E. coli* ET12567/pUZ8002/pIJ8671-SER and *Micromonospora* sp. GMKU 358. However, the mutant still maintain PLA-degrading activity. Therefore, this putative serine protease gene from *Micromonospora* sp. GMKU 358 is not involved in degradation of PLA or there are more than one gene involved

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