Srisuda Hanphakphoom 2014: PLLA-degrading Enzyme Production by *Laceyella sacchari* LP175 using Agricultural Products as Substrates and Enzyme Characterization. Doctor of Philosophy (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Vichien Kitpreechavanich, D.Eng. 119 pages.

Eleven strains of poly(L-lactide)(PLLA)-degrading thermophilic bacteria isolated from forest soils were selected based on their ability on PLLA-degrading enzyme production at 50 °C. Among the isolates, strain LP175 showed the highest PLLA-degrading ability. Strain LP175 was identified to *Laceyella sacchari* based on similarity of 99.9% similarity on 16S rRNA gene sequence and its morphological, cultural and physiological characteristics. Factors affecting PLLA-degrading enzyme production by *L. sacchari* LP175 indicated that cassava chip and soybean meal as carbon source and proteinaceous substance, respectivly yielded the highest PLLA-degrading enzyme production. The production was stimulated by phosphate and Mg^{+2}, but repressed by ammonium sulfate. The optimal concentration of cassava chip, soybean meal and PLLA powder were 0.464%, 0.153%, and 0.031%, respectively by using central composite design (CCD) in the basal medium consisting of 0.2 % K_2HPO_4, 0.1% KH_2PO_4 and 0.02% $MgSO_4.7H_2O$ The statistical model predicted PLLA-degrading activity of 68.5 U/ml and the observed value was 65.5 U/ml. The optimal physical conditions were 0.5 vvm aeration rate, temperature 50 °C and pH 7.0 for PLLA-degrading enzyme production in the 3-L airlift fermenter that increased up to 94.4 U/ml within 18 h cultivation. To our knowledge, this is the first report of a low cost medium for PLLA-degrading enzyme production by *L. sacchari* LP175.

The PLLA-degrading enzyme produced by the strain was purified to homogeneity by 48.1% yield and specific activity of 328 U/mg protein with a 15.3-fold purity increase. The purified enzyme was strongly active against specific substrates such as casein and gelatin, and weakly active against Suc-$(Ala)_3$-*p*NA. Optimum enzyme activity was exhibited at a temperature of 60 ^oC with thermal stability up to 50 ^oC and a pH of 9.0 with pH stability in a range of 8.5–10.5. Molecular weight of the enzyme was approximately 28.0 kDa, as determined by gelfiltration and SDS-PAGE. The inhibitors PMSF, EDTA, and EGTA strongly inhibited enzyme activity, but the activity was not inhibited by 1 mM 1,10-phen. The N-terminal amino acid sequences had 100% homology with thermostable serine protease (thermitase) from *Thermoactinomyces vulgaris.* The results obtained suggest that the PLLA-degrading enzyme produced by *L. sacchari* strain LP175 is serine protease.

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