

Thesis Title	Production, Purification and Characterization of Lipase from <i>Aeromonas sobria</i> LP004.
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### ABSTRACT

From raw milk sample, the bacterium strain LP004 which produced relative thermostable lipase was isolated by screening on tributyrin and calcium-triolein agar plate. The strain LP004 was identified as *Aeromonas sobria*. *A. sobria* LP004 could grow and produce lipase well in NB at 37°C with shaking. Lipase production by *A. sobria* LP004 in NB could be induced by addition of 0.1% (W/V) gum arabic and 0.01% (W/V) CaCl<sub>2</sub>. The lipase production level could be elevated upto approximate 10 folds higher than that in NB when *A. sobria* LP004 was cultivated in the developed medium containing minimum medium P salts, 0.5% (W/V) yeast extract, 25% (V/V) whey, 0.1% (W/V) glucose and 1.0% (W/V) soybean meal. The *A. sobria* LP004 crude lipase prepared from culture cultivated in NB supplemented with gum arabic was purified to 10.29 fold homogenous state by ultrafiltration and column chromatographies on Phenyl Sepharose and Sephadex G-200. The molecular weight of the lipase determined by SDS PAGE was 97 kDa. The purified *A. sobria* LP004 lipase exhibited maximum activity at pH 6.0 and 45°C. The lipase was stable at alkaline condition and temperature lower than 40°C. The *A. sobria* LP004 lipase was a

metalloenzyme with dependence on divalent cations, especially  $\text{Ca}^{2+}$ . Addition of  $\text{Ca}^{2+}$  to the crude lipase could increase its stability significantly. The *A. sobria* LP004 lipase was 1,3-specific and exhibited a wide range of specificity to commercial and purified triglycerides.