CHAPTER 4

CONCLUSION

Immobilized lipase B from *Candida antarctica* (Novozym 435) was the most suitable immobilized lipase for PFAD glucose ester synthesis. The optimal conditions for PFAD glucose esters synthesis were equi-molar of 0.5 mmol glucose and PFAD, initial water activity (a_w) of 0.07, 1.0 g molecular sieves (4°A), 75 mg Novozym 435 (750 U). The highest conversion of 76.3% or 31.8 mg/mL solvent of PFAD glucose esters was obtained in acetone at 40°C for 72 h.

Eight isolates of bacteria PSU-AH55, PSU-AH56, PSU-AH130, PSU-AH191, PSU-AH192, LS, ME168 and ME177 produced lipase with the hydrolytic activity of 0.06, 0.10, 3.32, 0.35, 0.37, 0.11, 0.30 and 0.65 U/mL, respectively. The immobilized lipases obtained from these strains on celite could synthesize sugar esters (SE) and the immobilized lipase from the strain ME168 produced glucose esters using vinyl acetate, vinyl butyrate and vinyl caproate as acyl donors in *tert*-butanol/pyridine (55:45 v/v) at 45°C with the highest conversion of 93.4, 66.7 and 56.2%, respectively. Among the acyl donors and acyl acceptors used, glucose and vinyl caproate were suitable for SE synthesis. The highest conversion yield of 82.0% or 25.3 mg/mL solvent was obtained using equi-molar of 0.3 mmol glucose and vinyl caproate, initial water activity (a_w) of 0.33, 0.5 g molecular sieves (4°A), immobilized lipase from the strain ME168 on celite (100 U) in *tert*-butanol/pyridine (55:45 v/v) at 50°C for 72 h.

Strain ME168 was identified as *Streptomyces thermocarboxydus* ME168 and produced the highest lipase activity (3.01 U/mL) with total cell protein (0.98 g/L) when it was cultivated in the modified M65 medium (molasses (8 g/L), malt extract (10 g/L), yeast extract (4 g/L), palm oil (10 g/L) and gum arabic as emulsifier (1.0 g/L)), the initial pH 7.5 at 40°C for 120 h. The extracellular lipase from *Streptomyces thermocarboxydus* ME168 was purified to 9.6 folds with 20.3% yield and had the apparent molecular mass of 21 kDa by SDS-PAGE. The purified lipase showed maximum activity at 50°C with the half-life of 180 min at 65°C. The optimal pH of the purified enzyme was pH 8.5 and it showed high stability at broad pH range of 5-9 and was thermostable at the temperature range of 35-60°C. The K_m and V_{max} were 0.28 mM and 1,428 U/mg with *p*-nitrophenyl palmitate. It was active toward *p*-nitrophenyl esters with medium to long acyl chain (C_8-C_{16}). Lipase activity was inhibited by Zn^{2+}, dithiothreitol, EDTA and ethanol, *tert*-butanol and pyridine.