

Screening, Selection and Optimization of Extracellular Methanol and Ethanol Tolerant Lipase from *Acinetobacter* sp. K5B4

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Abstract : An extracellular methanol and ethanol tolerant lipase producing bacterial strain K5b4 was isolated from soil samples contaminated with hydrocarbon residues. It was identified by using morphological and biochemical characteristics and 16srRNA technique as *Acinetobacter* species. The immobilized lipase from *Acinetobacter* sp. K5b4 retained more than 98% of its residual activity after incubation with pure methanol and ethanol for 24 hours. The highest hydrolytic activity of the immobilized enzyme was obtained in the presence of 75% (v/v) methanol in the assay solution. In contrary, the enzyme was able to maintain its original activity up to only 25% (v/v) ethanol whereas at elevated concentrations of 50 and 75% (v/v) the enzyme activity was reduced to 10 and 40%, respectively. Maximum lipase activity of 31.5 mU/mL was achieved after 48 hr cultivation when the optimized medium (pH 7.0) that composed of 1.0% (w/v) olive oil, 0.2% (w/v) glycerol, 0.15% (w/v) yeast extract, and 0.05% (w/v) NaCl was inoculated with 0.4% (v/v) seed culture and incubated at 30°C and 150 rpm agitation speed. However, the presence of CaCl₂ in the growth media did not show any inhibitory or stimulatory effect on the enzyme production as it compared to the control experiment. Meanwhile, the other mineral salts MgCl₂, MnCl₂, KCl and CoCl₂ were negatively affected the production of lipase enzyme. The inhibition of lipase production from *Acinetobacter* sp. K5b4 in presence of glucose suggesting that lipase gene expression is prone to catabolic repression.

Keywords : K5B4, methanol and ethanol, *acinetobacter*, morphological

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