Molecular weight determination of local isolate lipase from *Thermus aquaticus* (KHA-P12)

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Abstract

KHA-P12 was thermophilic microorganism, which derived from local hot spring Kawah Hujan-A in Garut, West Java. Based on 16S rRNA sequence, this isolate were closely related to *Thermus aquaticus*. Crude extract of thermostable lipase produced trough incubation of local isolate KHA-P12 bacterial culture at 70°C for 18 hours under high aeration. Isolation of lipase from crude extract was collected using acetone precipitation. A thermostable lipase Crude extract lipase were purified using acetone fractionation and obtain 3 fractions, i.e. 0-30%, 30-50%, and 50-70% fraction. The 30-50% acetone fraction had the best specific activity in the amount of 0.0103 unit/mg protein with the yield of 82.6%. Zymogram and Native Electrophoresis assay toward 30-50% fraction gave a positive esterase activity. The protein remains show esterase activity after separation using electroelution method. Molecular weight determination by SDS-PAGE method discovers that the lipase was composed of variety molecular sub unit with range of 30kDa- 50kDa.

Keywords: Lipase, KHA-P12 Isolate, Thermostable, Acetone Fractionation, Esterase Activity

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyse the hydrolysis of ester bonds of triacylglycerols at the interface between an insoluble substrate and water. This feature distinguishes lipases from esterases (EC 3.1.1.1), which act only on water-soluble substrates, such as short chain fatty acid esters, although also lipases show some activity on watersoluble esters (Soliman, 2007). Lipase can be obtain from thermophilic and mesophilic organism. As most of the industrial processes operate at a temperature exceeding 45 °C, lipase should be active and stable at a temperature around 50 °C. Hence, alkalinophilic and thermophilic microorganisms have been the focus of a number of investigations into the sources of lipases that are stable and function optimally at extreme alkaline pH values and high temperature (Sharma et al., 2002).

Protein precipitation can be carried using ammonium sulfate or organic solvent as the precipitant agents. The aim of the present work was to determine the molecular weight of lipase using several purification techniques.

Materials and Methods

Bacterial Cultivation

The bacterial culture (KHA-P12) were cultivated in media containing bacto agar, peptone, yeast extract, NaCl, and CaCl₂, and incubated for 18 hours at 70°C.

Crude Extract Production

The bacterial culture of KHA-P12, were inoculated to 100mL of medium with composition of 0.5% peptone, 0.5% yeast extract, 0.05% NaCl, and 0.05% CaCl₂, and dissolved using 0.05M of glycine-NaOH buffer pH 9.0. The culture was incubated for 15 hours at 70°C, with the speed of aeration around 150 rpm. The extracellular lipase was cultivated using high speed centrifugation with speed of 10000 rpm for 20 minutes.

Lipase Activity Assay

Lipase activity was measured by spectrophotometric assay using *p*-nitrophenyl palmitate as the substrate (Dong-Woo et al., 1999). The enzyme activity was measured by monitoring the change in absorbance at 405 nm that represents the amount of released *p*-nitrophenol (PNP). One unit of lipase is defined as the amounts of enzyme