Identification of thermostable lipase from *Geobacillus lituanicus* YTae-13 isolated from Gedongsongo hot spring

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Abstract

Lipase is widely used among industries. In this research, lipase was isolated from *Geobacillus lituanicus* YTae-13, a thermopile microorganism isolated from Gedongsongo hot spring. Result from growth curve and lipase production curve in production media showed three times expression of lipases, which were in the middle of exponential phase, in the end of exponential phase, and in the end of stationary phase. Lipase isolated in this research was the ones that produced in the end of stationary phase. The crude extract was precipitated with ammonium sulfate followed by dialysis. Two fractions of protein were obtained, that were saturated 60-80% and 80-100% fractions whose activities was detected as 17.1 and 16.5 unit/mg protein respectively. An activity unit was defined as 1 nmol product deliberated by enzyme per minute at measurement condition. The specific activities were increased by 1.26 and 1.21 times higher for 60-80% and 80-100% fraction respectively compare to those of crude extract (13.6 unit/mg protein). Protein yield after fractionation were 10.4% and 13.7% for 60-80% and 80-100% fraction respectively. After fractionation, the enzymes were concentrated with filtration using polyethersulfone membrane. The specific activities and the yield of saturated 60-80% and 80-100% fraction after concentration treatment were 51.8, 40.6 unit/mg protein and 9.08%, 11.3% respectively. The specific activities were increased by 3.81 and 2.99 times higher for 60-80% and 80-100% fraction respectively compare to those of crude extract. Both fractions consist of several lipases activity with optimum temperature at 60°C and 80°C, that confirmed by zymogram data.

Keywords: *Geobacillus lituanicus*, thermopile microorganism, thermostable lipase, zymogram

Introduction

Lipase (EC 3.1.1.3 Triacylglycerol hydrolase) is an enzyme which is able to hydrolyze lipids. Lipase is widely used among industries, such as a biocatalyst to produce emulsifier in food and beverage industries, biocatalyst in transesterification reaction of biodiesel, as well as a biocatalyst in the detergent and the pharmaceutical industries (Sharma, 2001). In addition to high catalytic activity, biocatalyst required in industrial processes also must have high resilience and stability during the catalysis process takes place.

Research on thermostable enzymes, including thermostable lipase is essential to obtain a superior biocatalyst for industrial purposes. Thermostable enzymes were reported to have a more stable structure for biotechnological application (Jaeger, 1999). Another advantage of using thermostable enzyme is the possibility to carry out the reaction at high temperature. This condition leads to high solubility of the substrate and other reactants, thus increasing the effectiveness of reaction. The process that takes place at high temperatures also reduces the risk of microbiological contamination (Hasan et al., 2005).

Potential sources of thermostable enzymes are thermopile microorganisms. Thermopile microorganisms used to live at high temperatures so that their metabolites will be adaptive to their habitat. Thermopile microorganism that was studied in this research is *Geobacillus lituanicus* YTae-13. This YTae-13 was isolated from Gedongsongo Hot Spring (Aminin, 2008). This isolate was chosen to be studied due to its uniqueness that showed three times expression of lipases during its lifetime.

Materials and Methods

Growth Curve of Microorganism

Growth curve of YTae-13 isolate was plotted using production media, which consist of 1.5 w/v peptone, 0.5% w/v NaCl, and 0.1% CaCl₂. YTae-13 which has been adapted in solid production media, was cultivated in starter and then incubated (55°C, pH 8.3) until its optical density (OD₆₀₀) reached 0.8. Subsequently, 2% v/v of starter was added to 100 mL production media. Measurement of growth curve was conducted by monitoring optical density of production media at 600 nm.

Beside of growth curve, lipase production curve was also plotted using the very same production media. An amount of production media was centrifuged at 8000 g. The cell-free supernatant...