EFFECT OF TEMPERATURE AND INCUBATION TIME ON THERMOSTABLE ALKALINE LIPASE FROM LOCAL HOT SPRINGS

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INTRODUCTION

Lipases is one of important enzymes and used in industries, such as food, detergent, pharmaceutical, textile, waste treatment industries. Lipase or triacylglycerol acylhydrolases (E.C.3.1.1.3) are enzyme that catalyze the hydrolysis of relatively long chain triacylglycerides (with acyl chain lengths of>10 carbon atoms) with the formation of diacylglyceride, monoacylglyceride, glycerol and free fatty acids at the interface between the aqueous and organic phases. It is well known that the reaction is reversible. Lipases can catalyze ester synthesis and transesterification in the reaction containing low water concentrations [1,2]. Lipase can be produced by mesophilic and thermophilic microorganisme. Lipase from thermophilic microorganisms were reported to be highly stable under several operational conditions. At present, the use of alkaline lipase has increased remarkably with large proportion of commercially available alkaline lipases derived from thermophilic bacteria strains [3].

We screened and isolated a thermophilic bacteria producing thermostable lipase enzyme from local hot spring. The aim of the present work was to determine the culture condition for maximum lipase production by a eight local thermophilic isolates. A bacterial strains were isolated from some of hot springs from around West Java such as Domas, Papandayan and Kawah Hujan hot springs. The **t**emperature and incubation time were examined for the optimization of lipase production. The result should give useful information about thermostable alkaline lipase produced from local isolates.

MATERIAL AND METHODS

MATERIAL AND CHEMICAL REAGENTS

Thermophilic bacteria from hot spring around West Java, such as, Domas, Papandayan and Kawah Hujan (Kamojang) Hot Springs. Bacto peptone, yeast extract, NaCl, $CaCl_2$, Olive oil, Tween 80, Rhodhamine B, and chemicals were of reagent grade chemical or better.

METHODS

ENZYME PRODUCTION

The inoculum bacteria taken was 0,1 % of the production media stater. The media was transferred onto a 100 mL alkaline lipases production medium containing (bacto peptone 0,5 % g, yeast extract 0,5 %, NaCl 0,05 %, $CaCl_2$ 0,05 %). The inoculated media was incubated at 65 and 70 ^oC. The Flasks were kept on rotary shaker for 2, 4, 6, 8,1,12, 13,1 4, 15, 16 h of medium with shaking at 150 rpm. The supernatant of the culture after centrifugation (10000 g, 10 min) at 4 ^oC was used to determine extracellular alkaline lipase activity. The lipase activity was measured by spectrophotometric assay.

DETERMINATION OF LIPASE ASSAY

Lipase activity was estimated using a spectrophotometric assay as described by Lee *et al* with p-Nitropheny Palmitate as a substrate.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 amount of enzyme releasing 1 umol PNP per min under the assay condition assay [4].

RESULTS AND DISCUSSION

The effects of different incubation temperatures on lipase production have been evaluated. The effect of temperature on growth and production of thermophilic local isolate of lipase was done in temperature 65 (Figure 1 a and 1 b) and 70 ^oC (data not show) after incubation periods (2, 4, 6, 8,1,12, 13,1 4, 15, 16 h). Eight isolate which grew optimally, as determined by a series of batch cultures. Maximum growth rate was observed between 10 and 15 h of fermentation. Isolat DMS-3, KHA-T6, PPD2, DMS-1, KHN, KHA-P12 have growth rate better than PPD-1, KHA-T25 isolate. The rate of lipase secretion into fermentation broth was the highest between 12 to 15 h. Four of the best producing alkaline lipases, namely DMS-3, PPD-2, KHA-P12, KHA-T6, KHN. Maximum activity of lipase from the isolate were 21,835 U/min, 15,14 U/minute, 14,783, 12,29 U/minute, 12,06 U/minute, respectively. Isolate DMS 3 from Domas hot spring, produced the highest lipase activity at pH 9.0 and 65^o C. The growth and alkaline lipase activity is showed eight local isolate during fermentation at pH 9.0 and 70^o C. The result indicated six of the best growth namely DMS-3, PPD-2, KHA-P12, KHA-T6, DMS-1, KHA-T25. The results of producing alkaline lipase showed that DMS-3 and KHA-P12 produced lipolytic activity at 70 ^oC. The above results showed that DMS-3 and KHA-P12 produced lipolytic activity at 65 and

70 ^{o}C. Maximum activity of lipase from the both isolates at 65 and 70 ^{0}C temperature was 22, 321 U/minute, 14,95 obtained at incubation time15 hours and 21,272 and 11,982 obtained at incubation time 15 and 14 hours respectively. Isolated KHA-T6, KHN, PPD-2 showed lipolytic activity similar to that KHA P12 isolate at 65 ^{0}C and almost no lipase activity that produced 70 ^{o}C. The result referred to a positive relationship between lipase production and incubation temperature 65 and 70°C from eight local isolate.

CONCLUSION

In this study suggests that eight local thermophilic isolates have been identified as thermostable alkaline lipase producing isolates. DMS-3 and KHA-P12 produced lipolytic activity at 65 and 70 ^{o}C.

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