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ENHANCEMENT THE PRODUCTION OF LIPASE DERIVED FROM *BUKHODERIA CENOPACIA* ST8 BY OPTIMIZATION OF THE FERMENTATION OPERATION

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ABSTRACT

A lipase derived from *Burkhoderia cenocepacia* ST8 was optimized in this research. The experimental design based on 'change-one-factor-at-a-time' approach was applied to optimize the production of lipase. Parameters such as volume ratio of medium volume to container volume, concentration of carbon source, nutrient broth and metal ions of medium on lipase production were extensively studied. The optimum conditions in cultivation medium containing 1% (w/v) Tween 80, 0.3% (w/v) of nutrient broth and 0.1% (w/v) of $CaCl_2$ with volume ratio 20:80 of medium volume to container volume, a high average production of 43.8 U/mL was obtained. Therefore, the optimum formulations and conditions would produce high yield of lipase were potential utilized in the commercial industrial.

Keywords: *Volume ratio, Metal ion, Nutrient broth, Concentration, Enzyme*

INTRODUCTION

Lipases are produced by many microorganisms and higher eukaryotes and most useful commercial lipases are of microbial origin. Lipase-producing microorganisms have been found in diverse habitats, for example, industrial waste, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, decaying food, compost heaps, coal tip sand and hot springs.

Microbial lipases are produced mostly by a submerged culture, but solid state fermentation can also be used. Basically, the lipase production is organism specific and it is released during the late logarithmic or stationary phase. The cultivation period varies with the type of microorganism and fast growing bacteria have been found to secrete lipase within 24 hours. The lipase production mostly needs an inducer, and in many cases, oil acts as good inducer to lipase. There are other certain inducers that also have a profound effect on the stimulation of lipase production. These include triglycerides, free fatty acids, hydrolysable esters, bile salts and glycerol. The organisms are normally grown in a complex nutrient medium containing carbon (oil, sugars, mixed carbon sources), nitrogen and metal ions.

At present, there is no information available regarding improvement of *Burkholderia* sp. ST8 lipase production by optimization of the fermentation condition. Therefore, a detailed study was made with the aim of optimizing the production of lipase by altering the fermentation mediums and conditions. This chapter was initiated by screening and optimizing the production of *Burkholderia* sp. ST8 lipase under various nutrient and culture conditions.

MATERIALS AND METHODS

Materials

p-nitrophenyl laurate (*p*NPL) was obtained from Fluka (USA). Trichloroacetic acid (TCA) was purchased from Merck (Darmstadt, Germany). *p*-nitrophenyl (*p*NP), nutrient broth, gum arabic, Tween 80, calcium chloride ($CaCl_2$), Triton X-100, and a bicinchoninic acid (BCA) protein assay kit were obtained from Sigma-Aldrich (St. Louis, USA).

Cultivation of Burkholderia sp. ST8 cells

The *Burkholderia* sp. ST8 strain was provided by Dr. Hii Siew Ling (Universiti Tunku Abdul Rahman, Malaysia) and stored in a nutrient broth containing 15 % (v/v) glycerol at -80 °C. The strain was isolated from the forest soil in the Setapak area, Kuala Lumpur, Malaysia. The inoculum was prepared by inoculating a loop of subcultured *Burkholderia* sp. ST8 cells from a tributyrin agar plate into 10 mL nutrient broth. After a 16 h incubation period at 37 °C, 5 % (v/v) of inoculum was introduced into a 500 mL shake flask containing 100 mL fermentation medium: nutrient broth, 0.33 % (w/v); $CaCl_2$, 0.10 % (w/v); Tween 80, 1 % (v/v); and gum arabic, 1 % (w/v) at pH 9. The culture was then incubated at 37 °C with an agitation speed of 250 rpm. Later, the culture was harvested together with the biomass after 72 h of incubation and no further clarification step was performed on the culture.

Lipase activity assay

A lipase activity assay was performed in a microtiter plate by using methods that have been described previously (Gupta et al., 2002; Ishimoto et al., 2006) with some modifications. The reaction mixture consists of 25 µL enzyme extract, 200 µL 0.05 M phosphate buffer (pH 6.5), 5 µL Triton X-100 and 25 µL 0.02 M *p*NPL (dissolved in ethanol). It was then incubated for 30 min and the absorbance was taken at 405 nm using a microplate reader (Tecan Sunrise, Tecan, Switzerland). The results of the enzyme assay were expressed as a mean of triplicate readings. After that, the amount of *p*NP that was released from the hydrolysis of *p*NPL was calculated by using a standard curve of *p*NP. The enzymatic activity was expressed as a lipase unit, which can be defined as the amount of lipase required to catalyze the formation of 1 µM *p*NP per min.

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RESULTS AND DISCUSSION

Effect of pH

In this study, the initial pH had a prominent effect on the cell growth and lipase production by *Burkholderia* sp. ST8. The pH of the culture medium in the range of 4-11 promoted healthy growth and a pH of 9 was chosen as the optimal pH for lipase production in this research (Figure 3.5). In contrast, an extremely low or high culture pH such as 3 or 12 repressed the growth and lipase production in the fermentation process. Some reports mention that maximum lipase production occurs under alkali pH conditions (Rathi et al., 2000). This statement was proved by researchers Rathi and his colleagues who found that *Burkholderia* sp. ST8 and *bacillus* AB-1 prefer a pH of 9 for optimal growth and lipase production (Rathi et al., 2001). Therefore, the culture pH is an important factor which can affect the lipase production and it varies with different types of microorganisms. After reviewing the results shown in Figure 1, pH 9 was selected for further optimization.

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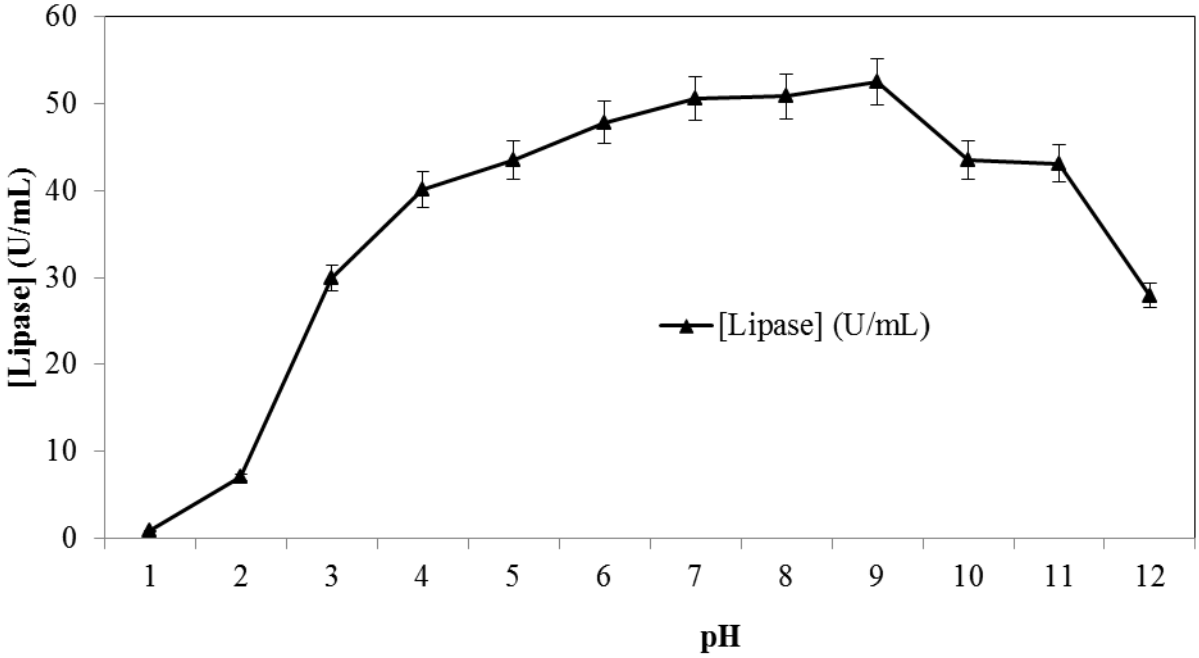

Fig. 1: The influence of culture pH on lipase production. The results are expressed as the means of triplicate readings with an estimated error of ±5 %.

Effect of fermentation temperature

Since lipase derived from *Burkholderia* sp. ST8 has the special characteristic of thermal stability, high temperatures do not cause a reduction in enzyme production. However, extremely high temperatures will result in protein denature as it is a basic characteristic of protein. On the other hand, at an extremely low temperature, the lipase production is reduced owing to the fact that enzyme activity becomes inactive and it needs a longer time to produce the same amount of lipase as compared to the optimum temperature in the fermentation process. According to the results displayed in Figure 2, a temperature of 37 °C showed the highest lipase activity among the temperatures in the range 20 °C to 80 °C. Hence, 37 °C was selected as the optimum temperature in the production of lipase derived from *Burkholderia* sp. ST8.

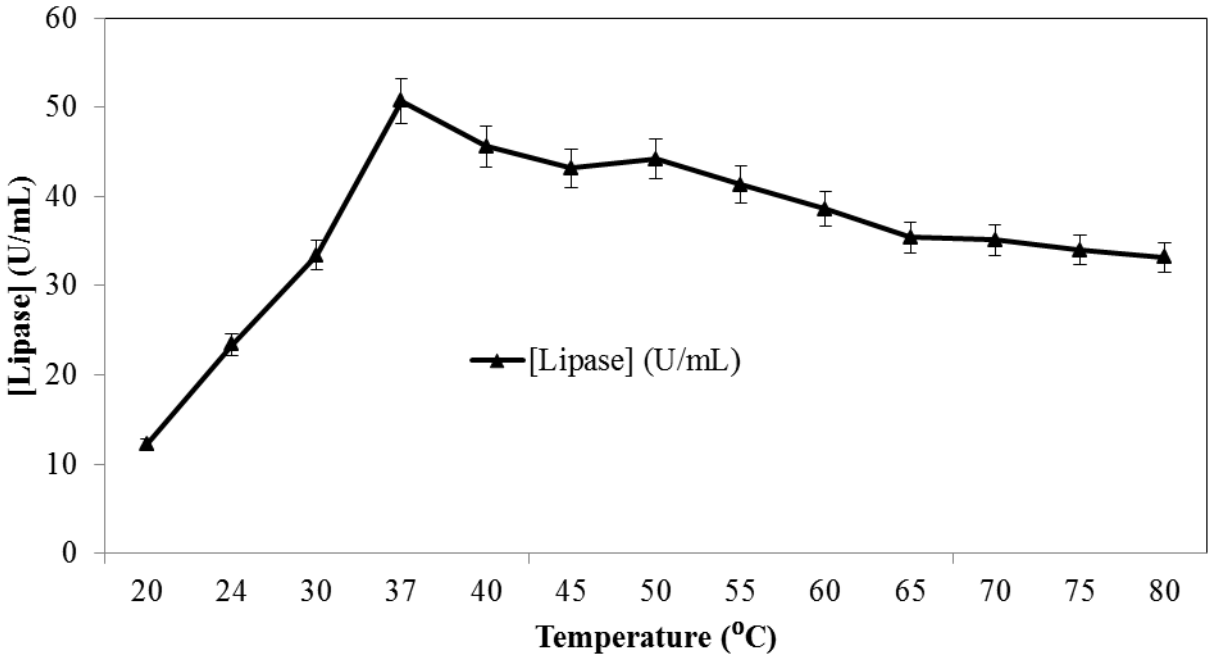

Fig. 2:The influence of temperature on lipase production. The results are expressed as the means of triplicate readings with an estimated error of ±5 %.

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Effect of agitation speed

It has been reported that the agitation speed of an incubation shaker can affect the fermentation efficiency. This is because the agitation speed can affect the respiration rate of the cells during the fermentation process (Sharma et al., 2001). Figure 3 illustrates the effect of agitation speed (50-400 rpm) upon the lipase concentration of the fermentation process. The data shows that a higher agitation speed results in a higher production of lipase in the fermentation. This phenomenon can be explained by the high agitation speed reducing the production of inhibitor during fermentation. The high agitation speed also increases the oxygen mass transfer rate and cell growth, which results in a higher lipase production. According to these results, the highest lipase activity (48.3 U/mL) was achieved during the fermentation process at a 250 rpm agitation speed. Thus, an agitation speed of 250 rpm was chosen as the optimum agitation speed in this study.

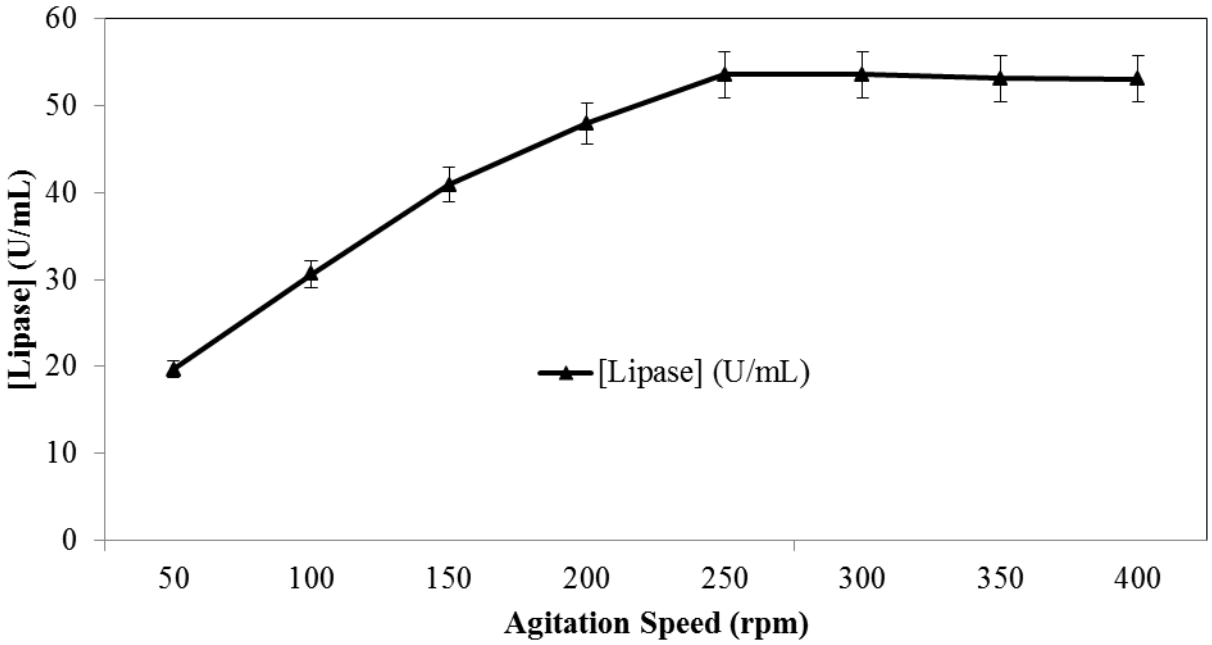

Fig. 3: The influence of agitation speed on lipase production. The results are expressed as the means of triplicate readings with an estimated error of ±5 %.

Effect of fermentation duration

Generally, lipase production is organism specific and it is released during the late logarithmic or stationary phase. The cultivation period varies with the microorganism and fast growing bacteria whereby they have been found to secrete lipase within a certain amount of hours. Ashipala and his colleagues (Ashipala et al.,2008) showed that the production of lipase from thermophilic *Bacilus sp*. continued during the stationary phase for over 24 h and the optimum time required for the maximum production of lipase was 36 h. All these facts stress the importance of the fermentation time that affects lipase production and its lipolytic activity. From the results shown in Figure 4, a fermentation time of 36 h was selected for further optimization in the fermentation process.

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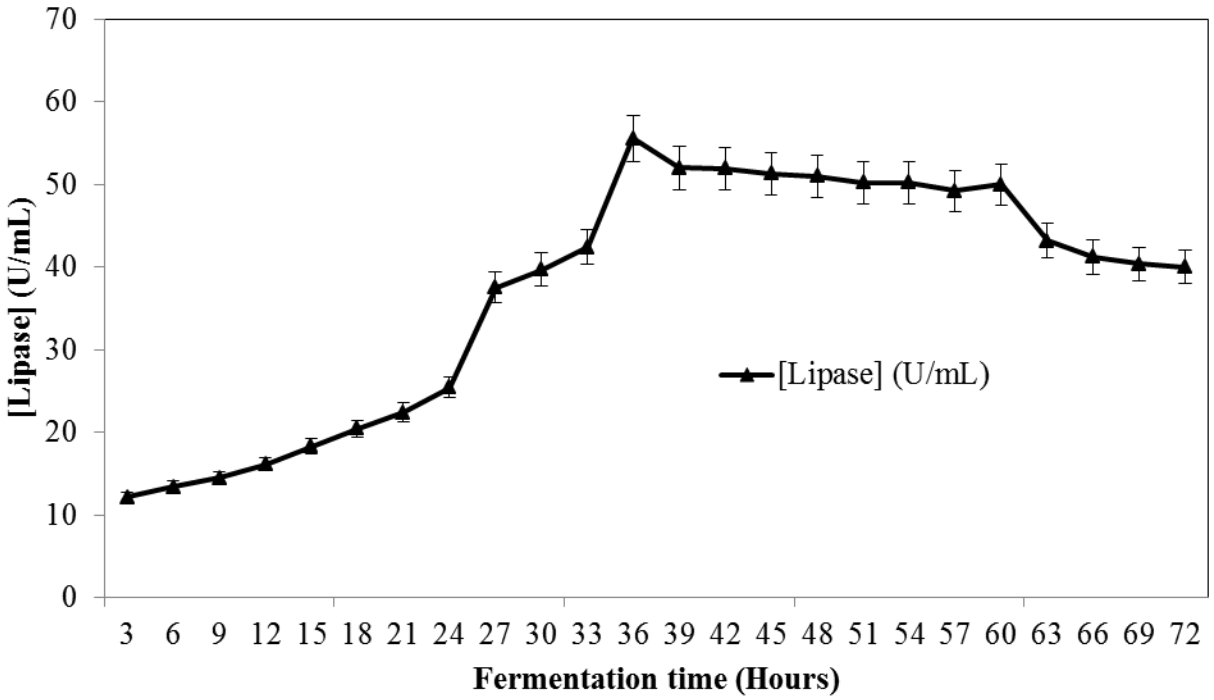

Fig. 4: The influence of fermentation time on lipase production. The results are expressed as the means of triplicate readings with an estimated error of ±5 %.

CONCLUSION

An indigenous lipase was successfully produced and optimized in a single fermentation process. The optimized operating process was determined under the following conditions: an agitation speed of 250 rpm at 36 h of fermentation time at a pH of 9 with 37 °C fermentation temperature. Under these optimised conditions, a high average lipase production of 48.3 U/mL was achieved.

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