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The Study of Biosurfactant Stability and The Effect on Lipase Activity

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Abstract

Lipase is one of hydrolase enzyme that catalyzed hydrolysis reaction of triacylglycerols into free fatty acids and monoglycerides or glycerol. These biocatalysts are widely used in several industries, namely food and pharmaceutical industry. The activity of lipase can increase significantly if the substrate forms an emulsion. Since biosurfactant has been known to have emulsification characteristic, the effect of biosurfactant addition into lipase is necessary to be investigated. It is the first report that evaluate the effect of microbial surfactant on lipase activity. The purpose of this research is to analyze the stability of biosurfactant emulsion under various conditions, such as salinity and pH as well as their potential to enhance lipase activity. Biosurfactant used was *Halomonas elongata BK-AG18* from the collection of Biochemistry Research Group ITB, Bandung, Indonesia. It was found that after the addition of NaCl, there was no significant decrease in the emulsification activity of the biosurfactant. The emulsification index (IE24) of biosurfactant with several NaCl concentrations (2, 5, 10, 15, 20, and 25%) was obtained around 50%. Stability test of biosurfactant at pH range 4-10 showed the highest IE24 of biosurfactant was obtained at pH 6. The effect of biosurfactants on lipase hydrolysis activity is also discussed in this article. Lipase hydrolysis activity was tested using p-nitrophenyl palmitate substrate. The highest lipase activity was obtained after the addition of 70% biosurfactant (v/v) at 0.026 units. This study shows that biosurfactant from H. elongata BK-AG18 has the potential to increase lipase activity.

Keywords: Biosurfactans; Bledug Kuwu; *H. elongata*; *Lipase* activity; Stability.

INTRODUCTION

Lipase is a type of water-soluble hydrolase enzyme that has the ability to hydrolyse triacylglycerols into free fatty acids and monoglycerides or glycerol (Yadav, *et al.*, 2017). This ability is utilised in the food and pharmaceutical industry, biodiesel, and biomederation (Melani, Tambourgi and Silveira, 2020). Guerrand (2017) reported that the dairy industry used lipase to break down milk fat and give cheese its unique flavour. Lipase can also modify fats and oils to produce biodiesel (Silveira, Tardioli and Farinas, 2016), while in the pharmaceutical industry lipase can synthesise chiral intermediate compounds to produce polixatel as an anticancer drugs (Fukaya, *et al.*, 2016). Lipase can be isolated from plants, animals, fungi and bacteria. When bacteria produce lipase, intermediates (by-products) are also produced, namely surfactants and known as biosurfactants.

There are two types of surfactants, synthetic (artificial) and derived from living things called biosurfactants. Biosurfactants are surface-active amphiphilic compounds that have surfactant properties, consisting of hydrophilic and hydrophobic parts (Xi, Ping and Alikhani, 2021). The two substances function to reduce surface tension and interference between two immiscible substances (Sharma and Oberoi, 2017) by forming interfacial micelles (Roy, 2017). In addition, biosurfactants can assist lipase to bind to the oil so that lipase can easily degrade the long chains of TAG. Biosurfactants are preferred over synthetic surfactants because of their environmental friendliness, low toxicity, and biodegradability (Santos, *et al.*, 2016), whereas synthetic surfactants have properties that are opposite to surfactants derived from living things. Markande, *et al.* (2021) explained that biosurfactants are widely used environmentally (biomederation, wastewater treatment, soil health, biofilm); industrial sector (food, textile, chemical, petroleum); agricultural sector as antifitogenic, animal manure management; health as antitumor, antimicrobial, antioxidant, antiinflammatory.

Many studies on biosurfactants isolated from microbes have been done in the past including *Ochrobactrum intermedium* (Zarinviarsagh, Ebrahimipour and Sadeghi, 2017), *Serratia* (Oliveira, *et al.*, 2021), *Burkholderia sp.* (Carvalho-Gonçalves and

Gorlach-Lira, 2018). It was reported by Spreb, *et al*. (2017) that the correlation of emulsification activity of *Aspergillus* strains with the biosurfactants produced was very high. Rahayu, *et al*. (2019) also reported linear lipase activity with biosurfactant activity isolated from *keratinolytic* bacteria. Lipase activity can increase significantly if the substrate forms an emulsion, so lipase activity depends on hydrophobic and hydrophilic regions. Therefore, lipase is defined as a carboxylesterase that reacts with the substrate to form an emulsion. It is known that biosurfactants have emulsification activity, therefore this study aims to determine whether lipase activity decreases or increases due to the addition of biosurfactants.

MATERIALS AND METHODS

Study area

Bledug Kuwu is a crater mud located in Semarang, Central Java Province, Indonesia. Sampling was conducted in sample point 7°07'04"S 111°07'17"E.

Procedures

Biosurfactant Stability and Emulsification Index (IE24)

The stability of biosurfactant was conducted by performing biosurfactant emulsification test on various concentrations of NaCl and buffer with pH range 4-10. NaCl solution was made with various concentrations (2, 5, 10, 15, 20, and 25% (b/v)) Then 2 mL of NaCl solution was put into a test tube, added to each tube 2 mL oil and 2 mL biosurfactant. Homogenised using a vortex for 2 minutes. After 24 hours, the IE24 value was determined.

Glycine buffer was prepared with a pH range of 4-10. The IE24 value in the pH range of 4-10 was carried out in the same way as the emulsification test at various NaCl concentrations.

Lipase Activity Test

The standard curve of p-nitrophenol was prepared with a concentration variation of (0, 10, 20, 30, 40, 50, 60, 70) 120 µg/mL. Afterwards, biosurfactant solution (CMC = 275 mg/L) with concentrations of 0%, 10%, 30%, 50%, 70%, and 100% of the total volume of glycine buffer was prepared.

Lipase activity test was carried out by preparing substrate solution (pH = 7.0) for blank, sample, and control made in a centrifuge tube by: 960 µL buffer and 100 µL ethanol (blank); 950 µL buffer, 100 µL ethanol, 10 µL pNPP (sample); 950 µL buffer, 100 µL ethanol, 10 µL pNPP (control).

To determine the occurrence of the enzymatic reaction, each substrate solution was mixed and added with the mixture as follows: 900 µL of substrate solution and 300 µL of soluble buffer (blank); 900 µL of substrate solution, 300 µL of crude porcine extract, biosurfactant solution of various concentrations (0, 10, 30, 50, 70, 100%) (Sample); 900 µL of substrate solution and 300 µL of crude porcine extract that had been boiled at 80°C for 30 minutes. If it was homogeneous, the incubation process was continued for 15 minutes in a 70°C incubator. Afterwards, centrifuged at 12000 rpm for 10 minutes. The absorbance of blank, sample, and control was measured at a wavelength of 405 nm in duplo.

Data analysis

IE24 value was determined by the following equation. he stands for height of emulsion layer and hs for total height of the solution and emulsion mixture

$$IE24 = he/hs \times 100\%$$

RESULTS AND DISCUSSION

Results

Stability of Biosurfactant in NaCl Solution

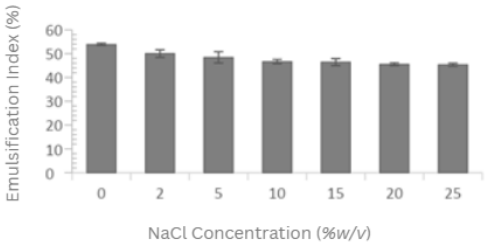

Figure 1. Emulsification activity of biosurfactants at various NaCl concentrations.

Stability of Biosurfactant at pH Change

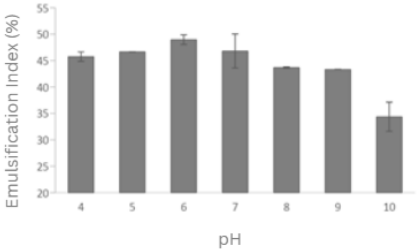

Figure 2. Emulsification activity of biosurfactants at various pH.

Effect of Biosurfactants on Lipase Activity

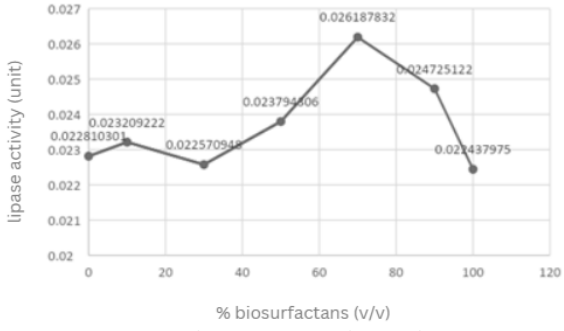

Figure 3. Lipase activity after the addition of Biosurfactant (v/v)

Discussion

The stability of biosurfactant in certain temperature, pH, and salinity range determines the application of the biosurfactant. In this study, only the influence of salinity and pH conditions on the stability of biosurfactant was conducted. The stability of biosurfactant produced by *H. elongata BK-AG18* in this study was measured by looking at its emulsification activity (IE24) in various NaCl levels, specifically 2, 5, 10, 15, 20 and 25%. Biosurfactant is said to have low emulsification activity if it has a low IE24 value, and vice versa. The IE24 value of biosurfactant at several salt concentrations is shown in **Figure 1**. The emulsification index of biosurfactant without NaCl addition has a percentage of 53.9%. Interestingly, the higher concentration of NaCl added did not cause a significant decrease in biosurfactant activity. This is shown from the IE24 value of 45.3% at 25% NaCl concentration. The stability of biosurfactants at various salt concentrations was proven by several researchers. The findings by Purwasena, *et al.* (2019) stated that salinity does not significantly affect the stability of biosurfactants. In addition, the emulsification activity of biosurfactants also did not change drastically when adding NaCl up to 10% (Saikia, *et al*. 2012). According to Bognolo, G. (1999) biosurfactants have a better ionic strength tolerance than synthetic surfactants that deactivate at 2-3% salt concentration. This ability of biosurfactants is needed especially for *Microbial Enhanced Oil Recovery (MEOR)* and bioremediation of oil spills in oceans that have extreme salinity conditions (Prieto, *et al.*, 2008).

Figure 2 shows the effect of pH value between 4-10 on the emulsification activity of biosurfactant from *H. elongata BK-AG18*. Starting from pH 4-6, there was a slight increase in biosurfactant emulsification activity until it reached its peak where almost 50% emulsification activity at pH 6. At pH 7-9, there was a slight decrease, followed by a very significant decrease at pH 10 where only 34.4% emulsification activity occurred. According to Ahmad, *et al.*, (2021), the emulsification activity of biosurfactants decreases at high pH levels. Different from the study conducted by Zambir, *et al*. (2017), where biosurfactant from *Streptomyces sp.* R1 bacteria showed relatively the same stability over a wide pH range (2-12) as proven by the relatively constant surface tension value at that pH.

This study investigates the effect of biosurfactant addition on lipase activity using the p-NPP method. So far, there have been many studies on the effect of surfactant addition on lipase activity. However, this study is the first to use crude biosurfactant from *H. elongata BK-AG18* to test its effect on lipase activity from *Burkholderia sp.* Surfactants and lipase can interact with each other, either in solution or at the interface. Several types of surfactant interactions with lipase enzymes can form micelle aggregates in solution or compete with lipase in the adsorption process. At low concentrations, surfactants can form complexes with enzymes, yet at high concentrations surfactants can also cause unfolding of the enzyme structure. The type and concentration of surfactant significantly determines the consequences of its interaction with lipase (Delorme, 2011). This shows that surface active agents can have both positive and negative effects on the lipase itself. Therefore, the effect of biosurfactants on lipase is very interesting to study.

In this study, it has been discovered that biosurfactants have the ability to emulsify palm oil up to 50%. The activity of emulsifying insoluble substrates such as oil or fat may affect the interaction between the substrate and lipase. Based on **Figure 3**, lipase activity without the addition of biosurfactant showed a value of 0.023 units. After the addition of 10% biosurfactant, there was a slight increase in lipase until it reached its peak (0.026 units) as a result of the addition of 70% biosurfactant. A decrease in lipase activity began to occur after the addition of 90% biosurfactant and the lowest activity (0.022 units) was shown in the addition of 100% biosurfactant. This shows that crude biosurfactant at certain concentrations can increase lipase activity despite the increase is not very significant. Some previous studies proved that surfactant can have a positive effect on lipase activity. A study conducted by Oliveira, *et al.* (2017) proved that the addition of Triton X-100 surfactant slightly increased the catalytic activity of lipase from *Rhizomucor miehei (RML)*.

CONCLUSIONS

Therefore, from this study can be conclude that the emulsification activity of biosurfactant from *H. elongata BK-AG18* was not significantly decreased by the addition of NaCl concentration up to 25%. This indicates that the biosurfactant has good stability in high salinity conditions. In contrast, pH has a more significant effect on emulsification activity than salt concentration. In this study, the biosurfactant was most stable at pH 6. In addition, this study also showed that the addition of biosurfactant at different concentrations showed different effects on lipase activity. The best lipase activity was shown by the addition of 70% biosurfactant.

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