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Biosurfactants production using bacterial isolate from Cangar hot spring, East Java, Indonesia

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ABSTRACT

Biosurfactants, a group of surface-active agents mainly produced by microorganisms, are one of the most sought after high-value bioproducts due to their multifunctionality in different industrial processes. Furthermore, biosurfactants are regarded as more economical to produce, sustainable, and environment friendly than petroleum-based synthetic surfactants. At the industrial level, thermostability of biosurfactants would give an advantage in many of their applications. In this research, we explore the microorganism biodiversity of Cangar Hot Spring in East Java, Indonesia to isolate biosurfactant-producing bacteria. One bacteria which identified as *Bacillus subtilis* subsp. *Inaquosorum* CGR-1 strain produced biosurfactants at 40-50 °C. Furthermore, crude biosurfactants produced by this strain could reduce surface tension and showed emulsification activity. The biosurfactant will be further characterized and utilized for different processes which occur in high temperature.

Key words : *Biosurfactants, Thermophilic bacteria, Bacillus subtilis subsp. Inaquosorum, Bioindustry, Hot springs*

Introduction

Biosurfactants are amphiphilic compounds, mainly produced by microorganisms on cell surfaces or can be secreted extracellularly (Unás *et al*., 2018). Biosurfactants are able to reduce the surface tension and the interfacial tension of growth medium which are valuable features for various applications such as oil recovery, bioremediation, as well as in antimicrobial agents, lubricants, cosmetic, food and pharmaceutical industries (Akbari *et al*., 2018). The compounds are promising alternative to synthetic surfactants due to their lower toxicity, higher biodegradability, effectiveness and efficiency in a wide range of pH and salinity and their sustainability and economic value since biosurfactants can be produced from renewable resources (Alves *et al*., 2019; De Almeida *et al*., 2016).

As one of the mega biodiversity countries with various unique ecosystems, Indonesia is rich with microorganisms which can produce biomaterials with industrial materials including biosurfactants. In previous study we have isolated one thermotolerant bacterial strain from Cangar hot springs in East java province which identified as *Bacillus subtilis* Subs. *Inaquosorum* CGR-1 (CGR-1 strain). Members of genus *Bacillus* are considered as a suitable group for industrial synthesis of biosurfactants because they are well known producers of surface-active metabolites such as Surfactin. Thus, CGR-1 strain is expected to produce biosurfactants at high temperature.

Here, we screened the potency of CGR-1 strain on producing biosurfactants at high temperature (40-50 °C). The ability of the strain on producing biosurfactant using low-cost and widely-available molasses (Mouafo *et al*., 2018; Rane *et al*., 2017) as sole carbon source was also be explored. Finally, the produced biosurfactant was also tested for surface tension reduction and emulsification activity.

Materials and Methods

Screening of Biosurfactants Production – The ability of *Bacillus subtilis* subsp. *Inaquosorum* CGR-1 to produce biosurfactants was screened using hemolytic activity assay (Ibrahim, 2018). In this assay Nutrient Agar (NA) supplemented with 5% sheep blood was used. Single colony of CGR-1 strain was streaked onto blood agar plates and incubated at 40 °C for 48h. Hemolytic type was confirmed according to hemolysis result and clear zone color.

Biosurfactant Production – One colony of *Bacillus subtilis* subs. *Inaquosorum* CGR-1 was inoculated into 3 mL Nutrient Broth media and incubated at room temperature in rotary shaker (120 rpm) for 1 day. Four hundred µL of the culture (2%) was inoculated into 20 mL AMS media (Ni'matuzahroh *et al*., 2019) supplemented with different concentration of molasses. The inoculated AMS media + 1% / 2% molasses were incubated at room temperature in rotary shaker (120 rpm) for 2 days. Cell free supernatant was obtained by centrifugation (6000 rpm, 15 minutes) and used as the source for crude biosurfactant.

Surface-Activity Determination – Surface tension measurements of cell-free supernatant were performed according to the De-Noüy-Ring method (Ni'matuzahroh *et al*., 2019). All the measurements were performed in triplicate at room temperature (25°C).

Emulsification Activity Determination – Emulsification activity determination was conducted using 1 mL of cell-free supernatant (crude biosurfactant). The cell-free supernatants were mixed with 1 mL of kerosene. The mixture was homogenized using vortex mixer for 2 minutes in high speed, then incubated for 24 hours at room temperature (25 °C). The emulsification index (E_{24}) was calculated as the percentage of the height of the emulsified layer (mm) divided by the total height of the liquid column (mm). All the emulsification indexes were performed in triplicate.

Results and Discussion

The ability of CGR-1 strain to produce biosurfactant was screened using hemolysis assay. In this assay biosurfactants produced by tested microorganisms could lyse the red blood cells *via* colloidosmosis mechanism which result in transparent or yellow/ green halos around colonies in blood agar. As shown in figure 1, transparent green halo was observed around the colony of CGR-1 strain after 24h incubation at 40° and 50 °C, respectively (Figure 1a and 1b). These results emphasize the potency of CGR-1 strain to be used in industrial process that involve high temperature such as oil recovery and waste removal (Cai *et al*., 2017; Restaino *et al*., 2018). To further confirm the biosurfactant production by CGR-1, surface tension reduction and emulsification activity assays were conducted.

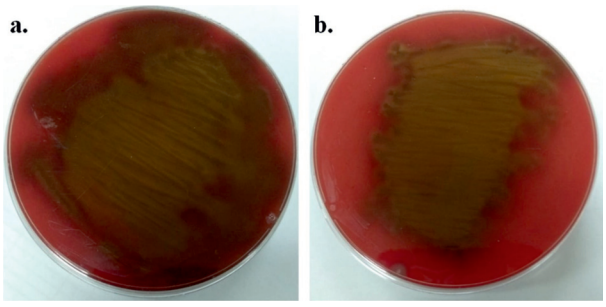

Fig. 1. (a) Hemolysis activity of CGR-1 strain after 24 hours incubation at 40 °C and (b) 50 °C.

One of the most straight forward and appropriate methods to confirm biosurfactant production is the direct measurement of the interfacial or surface activity of culture supernatant via Du-Noüy-Ring method. The cell free supernatant of CGR-1 strain showed a remarkably lower value (47.38 with 1% molasse and 43.66% with 2% molasse as sole carbon source, respectively) in surface tension than that of control strain of *E. coli* BL21(DE3) (60.64 with 1% molasse as sole carbon source). While the control culture broth with 1% molasse gave surface tension value of 65.91. The use of 2% molasse as carbon source improved the surface tension reduction capacity, this might be due to increased biosurfactant production by CGR-1 strain. In general, the surface tension reduction capacity value ≥ 20% by the cell-

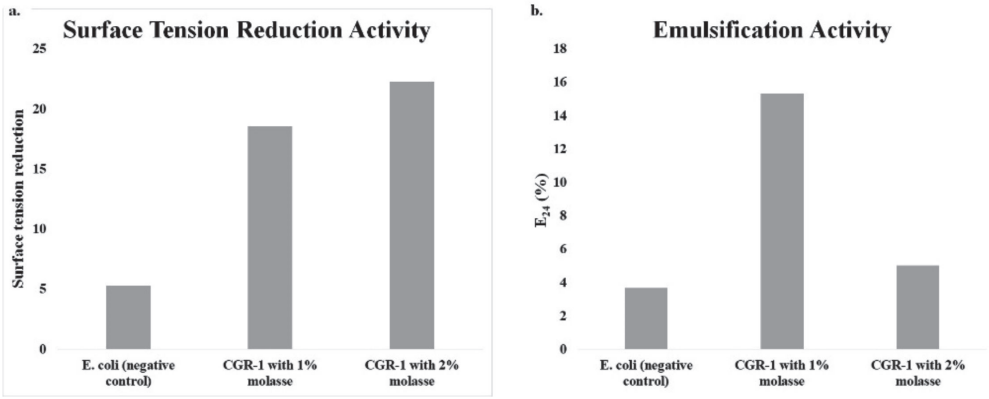

Fig. 2. (a) Surface reduction activity of E. coli BL21(DE3) (negative control), CGR-1 strain grown in media with 1% and 2% molasse as sole carbon source, respectively. (b) (a) Surface reduction activity of E. coli BL21(DE3) (negative control), CGR-1 strain grown in media with 1% and 2% molasse as sole carbon source, respectively.

free supernatant in this study, strongly confirm the ability of CGR-1 strain to produce biosurfactants and secrete them in the culture medium (Syahriansyah and Hamzah, 2016; Walter *et al*., 2010).

Emulsifying activity of crude biosurfactants produced by CGR-1 strain was also tested. Emulsification index (E_{24}) of was employed to investigate the emulsifying properties of biosurfactant using kerosene as a substrate. Although the E_{24} value in the culture with 1% molasse (15.33%) was significantly higher than that of the *E. coli* control strain (3.67%), however the value is considered as low. Other *Bacillus subtilis* strains were reported to show E_{24} value of 87% and 94%, respectively (Maia *et al*., 2018; Nayarisseri *et al*., 2018).

Conclusion

Thermotolerant *Bacillus subtilis* subs. *Inaquosorum* CGR-1 isolated from Cangar hot springs Indonesia showed biosurfactant production at 50 °C. The strain could produce and secrete biosurfactant to the culture media using molasse as sole carbon source. The produced biosurfactants showed significant surface tension reduction value especially using 2% molasse as sole carbon source. However, the produced biosurfactants did not show significant emulsification activity. In the future, optimum production condition and the thermostability of produced biosurfactant by CGR-1 would be studied to obtained valuable biosurfactant which is suitable for application in industrial scale.

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