The Study of Nutritional Factor on The Production of Biosurfactant from Locally Isolated Strain *Pseudomonas sp.* DSB7

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Abstract. This study was aimed to investigate different nutritional factors including carbon source, nitrogen source and hydrocarbon source towards the production of biosurfactant by *Pseudomonas sp* DSB7. The strain was isolated from soil sediment of Sungai Dungun estuary and 16S rRNA analysis revealed that the isolate was closely related to *Pseudomonas aeruginosa*. To study the effect of nutritional factor on the production of biosurfactant, the strain was supplemented with different carbon and nitrogen sources and the production of biosurfactant was evaluated by emulsification index. Highest emulsification index was recorded when the strain was grown in minimal salt media (MSM) formulated with sucrose and yeast extract. To study the effect of hydrocarbon as the inducer for biosurfactant production by *Pseudomonas sp* DSB7, engine oil and kerosene was added to the formulated MSM media. Emulsification index showed that engine oil was a better inducer for biosurfactant production by isolate DSB7.

INTRODUCTION

Surfactants are amphiphilic organic compounds comprising of non-polar and polar moieties which confer its ability to reduce surface tension of liquid-air interface and interfacial tension of aqueous and non-aqueous phases [1, 2]. These fascinating features allow surfactants to be used as wetting agents, foaming agents, stabilizer, cleaning agents, antimicrobial agents, emulsifier as well as de-emulsifier and have been applied in various industrial applications such as food and beverages, cosmetics and personal care products, laundry detergents, agricultures, pharmaceuticals, medical and health care products, petroleum and petrochemical sectors as well as bioremediation [3, 4]. Surfactants can be chemically synthesized (petrochemical and/or oleochemical) or derived naturally from plants and microorganisms such as bacteria and fungi. For decades, chemical surfactants were widely used for industrial applications and environmental clean-up due to low cost of production. For example, alkylbenzene sulfonate (ABS), the earlier synthetic surfactant used as cleaning agent was synthesized from secondary olein sulphates derived from petroleum fractions which can be produced in large quantities [5]. Despite their important roles in many applications, chemical surfactants also have some disbenefits. Chemical surfactants are reported to be less biodegradable by natural microorganisms and more toxic to microbiota and aquatic life. Some household cleaning formulations and personal care products also contain significant surfactant solution which can caused dermatological problem such as skin irritation [6].

Currently, microbial derived-surfactant or biosurfactant had gained significant interest from researchers and industrial practitioner as potential substitution for chemical surfactant. Biosurfactants have higher biodegradability, better environmental compatibility, and specificity in the detoxification of specific pollutants which more suitable to be used in environmental clean-up. Meanwhile, high tolerance to temperature, pH and ionic strength renders the

International Conference on Bioengineering and Technology (IConBET2021)
AIP Conf. Proc. 2454, 030003-1–030003-6; https://doi.org/10.1063/5.0079099
Published by AIP Publishing. 978-0-7354-4193-4/$30.00

biosurfactants to be a better option to replace chemical surfactants in industrial applications [7, 8]. Like chemical surfactant, biosurfactant also consist of two portions of hydrophobic and hydrophilic moieties. The nonpolar hydrophobic portion is usually based on saturated or unsaturated of hydrocarbon chains or fatty acids. Meanwhile, carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol form the polar part of hydrophilic portion [9, 10]. Biosurfactants that are produced by microorganisms can be either extracellular biological compounds or intracellular compounds [11, 12].

Major issue in biosurfactant production mainly arises from costly substrates, low end-product titer, and formation of a variety of by-products as opposed to a singular desired biosurfactant leading to a higher price of crude biosurfactant. Researchers were looking into cheaper alternative substrates, optimization of the environmental conditions, and improvement on the downstream recovery processes to overcome these problems [13]. Optimization of nutritional factors is considered as one of crucial factors in the production of biosurfactant since they constitute about 30–40% of the total production cost at industrial scale [14]. Formulation of nutritional factor for optimum biosurfactant production consist of selection of proper nutrients at correct levels to give an ideal microenvironment for supporting growth and metabolite production [15].

MATERIALS AND METHOD

Bacterial Strain

Pseudomonas sp DSB7 was isolated from soil sediment of Sungai Dungun estuary. The isolation was conducted using mineral salt media (MSM) supplemented with kerosene. The strain was maintained on the same media throughout the experiment. Sequencing analysis of 16S rRNA gene amplified from the bacterium revealed highest similarity to *Pseudomonas aeruginosa* (manuscript in preparation).

Preparation of MSM Media

Bacterial strain was maintained on minimal Salt Media (MSM) containing (g/L): Na_2HPO_4 (6.78), KH_2PO_4 (3.0), K_2HPO_4 (3.0), NH_4Cl (1.0), NaCl (0.5), $NaNO_3$ (0.5) yeast extract (1.0) and trace elements of 1.0 M $CaCl_2$ (0.015), 0.1 M $MgSO_4$ (0.05). Production of biosurfactant from the strain was also conducted using the same media supplemented with appropriate carbon, nitrogen and hydrocarbon source.

Evaluation of Biosurfactant Production by Different Carbon, Nitrogen and Hydrocarbon Source

The production of biosurfactant from *Pseudomonas sp* DSB7 was evaluated using different carbon, nitrogen and hydrocarbon sources. Production of biosurfactant was first evaluated using three different carbon sources namely glucose (30g/L), sucrose (30g/L) and glycerol (30ml/L). For negative control, *Pseudomonas sp* DSB7 was grown on MSM media without addition of any carbon source. Carbon source yielded highest biosurfactant activity was used in the formulation of MSM media with different nitrogen source which are yeast extract (3g/L), peptone (3g/L) and sodium nitrate (3g/L). The production of biosurfactant was evaluated to determine the optimum nitrogen source. For negative control, *Pseudomonas sp* DSB7 was grown using the same formulated MSM media minus the addition of nitrogen source. Finally, the production of biosurfactant was evaluated using two different hydrocarbon sources: engine oil (2%) and kerosene (2%). The hydrocarbon was added to MSM media formulated with the most optimum carbon and nitrogen source. *Pseudomonas sp* DSB7 grown on MSM media formulated with the most optimum carbon and nitrogen sources without the addition of hydrocarbon was used as negative control.

Production of Biosurfactant

1 loopful of bacterial colonies from MSM media were inoculated in 10 mL nutrient broth and incubated at 37°C and 150rpm until it reaches optical density (OD_{600}) approximately 0.5. Then 2% of overnight culture was transferred to MSM media formulated with different carbon, nitrogen and hydrocarbon source for the evaluation of biosurfactant production. The culture was then, incubated for 5 days at 37°C, 150 rpm.

Emulsification Assay

The production of biosurfactant by *Pseudomonas sp* DSB7 was evaluated quantitatively based on the emulsification activity or emulsification index known as E_{24}. Emulsification assay was carried out by adding 2mL kerosene oil into 1mL of cell free supernatant which was obtained after centrifugation. The mixture was then vortexed for about 5 minutes to ensure the mixing of both liquids. The emulsification activity was calculated by observing the height of the emulsion formed before and after 24 hours with the use of formula below:

$$E24 = \frac{Height\ of\ emulsion\ layer}{Height\ of\ total\ solution} \text{ x } 100$$

RESULTS AND DISCUSSION

Evaluation of Biosurfactant with Different Carbon Source

Production of biosurfactant using different carbon source was evaluated using glucose (30g/L), sucrose (30g/L) and glycerol (30ml/L) respectively. Based on the emulsification assay conducted using cell free supernatant from *Pseudomonas sp.* DSB7 culture grown on the MSM formulated with the selected carbon source, culture grown in MSM and sucrose has the highest emulsification index (45%) compared to glucose (38.7%) and glycerol (35%) as shown Figure 1. The observed result is opposite to other reports on production of biosurfactant by *Pseudomonas* species. Glucose was reported as good carbon source for the production of biosurfactant by *Pseudomonas nitroducens* and *Pseudomonas aeruginosa* TMN [16, 17].

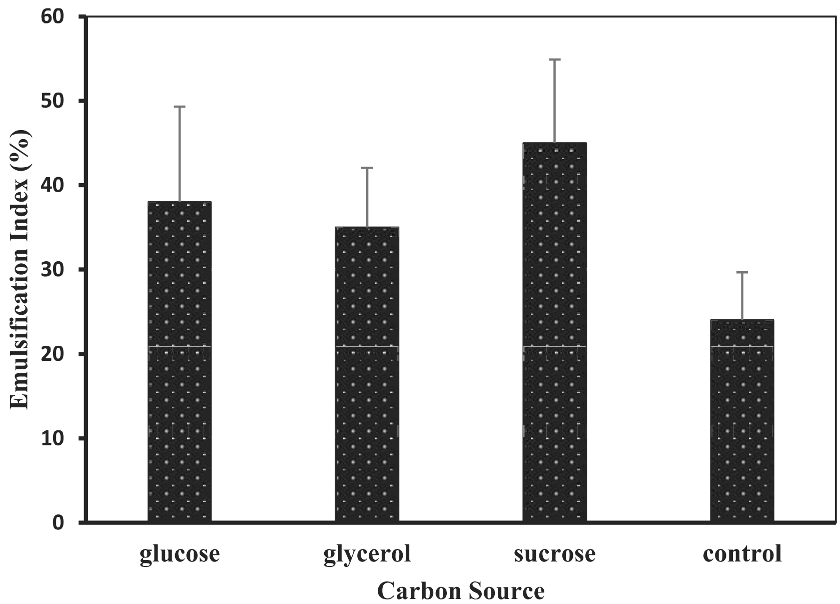

FIGURE 1. Effect of carbon source on the production of biosurfactant based on the emulsification index

Evaluation of Biosurfactant with Different Nitrogen Source

Nitrogen source is one of the key elements in the production of biosurfactant [18]. Biosurfactant, especially rhamnolipid was produced as secondary metabolite by *Pseudomonas sp* when cell growth reached a steady state because of exhaustion of the nitrogen source [19, 20]. Thus, an addition of small amount of nitrogen source can induce the production of biosurfactant [18, 21]. In this study, 3 types of nitrogen source were used to evaluate their effect on biosurfactant production by *Pseudomonas sp* DSB7. As the result shown in Figure 2, it was observed that the highest emulsification activity was recorded when the strain was cultivated in MSM formulated with yeast extract (39%) followed by sodium nitrate (17%) and peptone (13%).

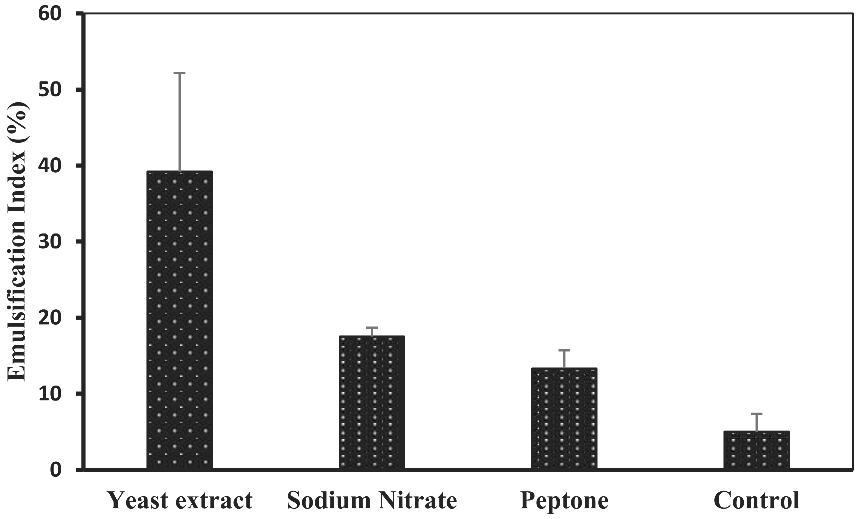

FIGURE 2. Effect of nitrogen source on the production of biosurfactant based on the emulsification index

Evaluation of Biosurfactant Production Using Different Hydrocarbons as Inducer

Numbers of biosurfactant producers were also reported to have the ability to degrade policylic aromatic hydrocarbon [22, 23]. The amphipilic nature of biosurfactant contribute to the reduction of surface tension of an interface. In water/oil interface, biosurfactant molecules secreted by hydrocarbon degrader will generate a new surface area by forming a surfactant oriented monolayer around the hydrocarbon particle with hydrophobic tail of the surfactant pointing out the liquid phase [24]. The inrease surface area will facilitate emulsification thus enhances the bioavailability and solubility of hydrocarbons for microbial degradation [25]. The fermentative biosurfactant production was carried out using hydrocarbon as sole carbon source to induce the production of biosurfactant. In this study, it was observed that the biosurfactant production was dramatically increased when 2% (v/v) engine oil was added to the fermentation media (Figure 3). However, lower emulsification activity of *Pseudomonas sp* DSB7 was recorded when 2% (v/v) kerosene was added to the media.

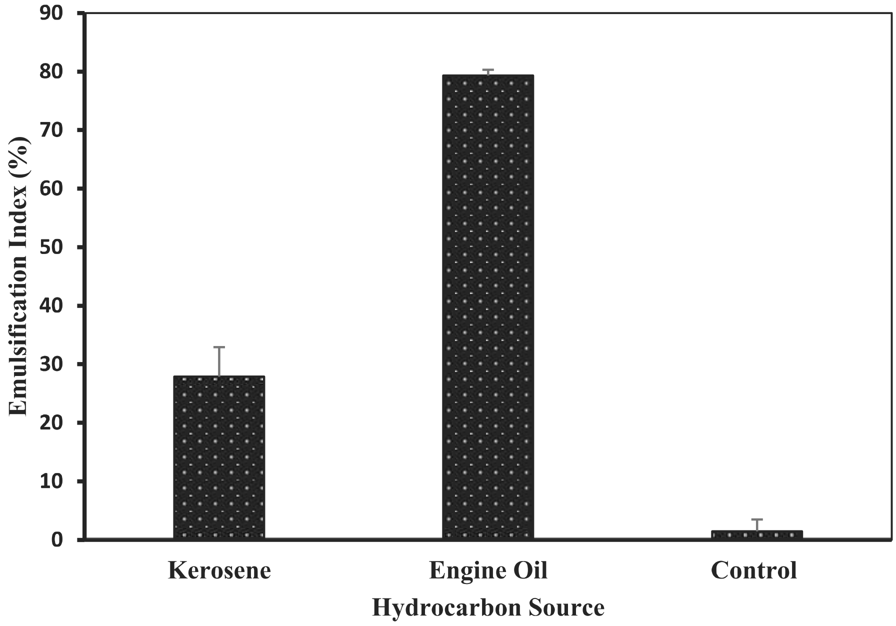

FIGURE 3. Effect of hydrocarbon on the production of biosurfactant based on the emulsification index

CONCLUSION

In this study, three types of carbon source; glucose, sucrose and glycerol and three types of nitrogen source; yeast extract, peptone and sodium nitrate were tested to study the effect of different carbon and nitrogen source on the production of biosurfactant by locally isolated bacterium, *Pseudomonas sp.* DSB7. Based on emulsification activity, it was found that *Pseudomonas sp* DSB7 grown on MSM formulated with sucrose and yeast extract yielded the highest emulsification index (E_{24}). The formulated media was then added with 2% (v/v) engine oil and 2% kerosene (v/v) respectively to study the effect of different hydrocarbon as an inducer for biosurfactant production. *Pseudomonas sp.* DSB7 grown on MSM media supplemented with 2% (v/v) engine oil showed higher E_{24}, suggesting engine oil as a better inducer for biosurfactant compared to kerosene. This preliminary finding is very important for the optimization of biosurfactant production for industrial applications.

ACKNOWLEDGEMENT

This work is supported by Research Acculturation Research Scheme (RAGS) R/RAGS/A07.00/01106A/001/2015/000192 and Fundamental Research Grant Scheme (FRGS) R/FRGS/A1300/01106A/005/2019/00703 awarded by the Ministry of Higher Education (MOHE). Authors would like to thank the Faculty of Bioengineering and Technology and Universiti Malaysia Kelantan for their support in this research work.