MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

INTERFACIAL ACTIVITY AND EMULSIFICATION CAPABILITY OF BACTERIA FROM SUNGAI DUNGUN ESTUARY, TERENGGANU, MALAYSIA TOWARDS HYDROCARBONS

Nurazeerah Khamis[1], Ainihayati Abdul Rahim[1,2,*], Noor Azlina Ibrahim[1,2] and Khomaizon Abdul Kadir Pahirul Zaman[3]

[1]*Department of Bio and Natural Resources Technology, Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan*
[2]*Advanced Industrial Biotechnology Cluster, Universiti Malaysia Kelantan*
[3]*Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia*

*Corresponding Author: ainihayati@umk.edu.my

International E-Conference of Science and Biosphere Reserve 2021

Keywords:

Emulsification; Hydrocarbon; Biosurfactant; Bioemulsifier

Abstract

Emulsifying agents or emulsifiers are low molecular weight surface-active agents (surfactants) responsible for initiating and stabilizing the emulsions. The long-established chemical surfactants which are derived from the petroleum literally could give some disbenefits such as less degradable by environmental microorganisms due to their complex structures, and the harsh chemicals such as Sodium Laureth Sulphate, Ammonium Laureth Sulphate, or Alkyl Ether Phosphates can irritate the sensitive skin hence contribute to dermatological problems. Many studies have switched on the investigation of surfactants from natural resources as a replacement for chemical surfactants as they are more environmentally friendly, more biodegradable, and lower in toxicity. Considering the importance of natural-derived compounds in many applications, this study was conducted to evaluate the potential of eleven indigenous bacteria which were successfully isolated from Sungai Dungun estuary to reduce interfacial tension and emulsify hydrocarbons. Two different types of hydrocarbons i.e., kerosene (fuel oil) and used motor oil (lubricant oil) were tested for emulsification activity by the extracellular bioemulsifiers produced by the eleven bacterial isolates through Emulsification Index (EI_{24}) test. From the eleven bacterial isolates, two noteworthy strains named DSB7 and DWC1 were noticed to produce bioemulsifiers that showed the highest emulsification index towards kerosene and used motor oil with the index values of 53.57% and 85.67% respectively. The 16S rRNA gene identification and the phylogenetic tree revealed the isolate DSB7 as 99% homologous to *Pseudomonas aeruginosa* and isolate DWC1 as 98% homologous to *Aeromonas taiwanensis*.

INTRODUCTION

Emulsification is a mixing process of two immiscible liquid phases, where one liquid system is dispersed into the microscopic droplets (dispersed phase) in another liquid system (continuous phase) to form a mixture called emulsion [1–3]. The emulsion formation is a dynamic process as the water-oil interfaces are continuously changing, therefore, emulsion stabilization is a vital factor to be constant for the quality and excellent performance in applications. Emulsifiers are low molecular weight surface-active agents (surfactants) which have a great significance to be adsorbed at intermediate of water-oil phases in a relatively short time to stabilize the emulsion [4].

Being the important agents to initiate and stabilize the emulsions, emulsifiers play vital roles in various industrial applications such as in food processing, agriculture, cosmetic and personal care, medicals, laundries as well as

chemical-based and petroleum-based industries [5–8]. The emulsions in industrial or research fields can be generated by several techniques involving either low or high energy, mechanical devices such as shear mixers, high-pressure valve or membrane homogenizers, micro-fluidizers, and sonicators [4], or by spontaneous emulsification process which is more practical to reduce the cost and as an alternative to the current high energy method [9]. Meanwhile, in the environment, the emulsification process is one of the essential stages of hydrocarbon pollutants degradation that is naturally activated by microorganisms. Microorganisms in the environment interact with hydrocarbons by releasing biosurfactant to reduce the interfacial tension between organic and aqueous phases allowing the interfacial mass exchange in the surface, thus leading to the solubilization by increasing the permeation of organic compounds into the aqueous solution through the formation [10,11]. Therefore, the bioavailability of hydrophobic compounds for biodegradation by environment microorganisms is enhanced.

Recent studies have diverted to "green" sources of biosurfactants as an alternative to substitute the long-established synthetic counterparts since synthetic surfactants can contribute to some disbenefits such as less degradable by environmental microorganisms due to their complex structures, and the harsh chemical compositions such as Sodium Laureth Sulphate, Ammonium Laureth Sulphate, or Alkyl Ether Phosphates that can irritate the sensitive skin [8]. On the contrary, bioemulsifiers have better environment compatibility, more biodegradable, and lower toxicity [8]. Therefore, the objective of this study was to evaluate the emulsifying activity of indigenous bacteria of Sungai Dungun estuary by screening their interfacial tension reduction and emulsification ability towards two types of hydrocarbon oils with different carbon-chain length; kerosene (C13 to C17), and used motor oil (C16 to C36) [10,12] through oil displacement test and Emulsification Index (EI).

MATERIALS AND METHODS

Materials

All chemical reagents were purchased from Teraslab Saintifik Sdn. Bhd. and Dira Resources. The commercial motor oil (Shell Advance SAE40- AX3) as an additional hydrocarbon in the growth media was obtained from the local mobile workshop. The G-Spin™ Genomic DNA Extraction Kit for bacterial genomic DNA extraction, 2x PCR Master mix and i-Taq™ DNA Polymerase used for PCR process, and the MEGAquick-spin™ Total Fragment DNA Purification kit to purify the PCR products were from brand iNtRON Biotechnology, Inc. The DNA marker used was VC 1kb DNA Ladder from Vivantis Technologies Sdn Bhd. Meanwhile, both 68F and 1392R primers used in the DNA amplification were from Integrated DNA Technologies (IDT) supplied by Apical Scientific Sdn. Bhd.

Methods

Isolation of Biosurfactant Producers

In this study, Sungai Dungun estuary was selected as a source of isolation of bioemulsifier producers since it is one of the main rivers in Terengganu, Malaysia that is located near to the local wet market, food stores and become a port to dock the fishing boats. Hence, this estuarine region is assumed to be contaminated by hydrocarbons whether from the fishing boats or the wastewater drainage of food stores and the wet market. The hydrocarbon-contaminated soils and water were collected from three different areas of Sungai Dungun estuary and labelled accordingly with respective location coordinates as follows; Area A: near the local wet market (4°46’44”N 103°25’20”E), Area B: around the fuel tanks (4°46’44”N 103°25’32”E), and Area C: fishing boat harbour (4°46’44”N 103°25’25”E). Meanwhile, the water samples were collected close to the river cliff in all sampling areas. All samples were stored in clean containers before they were brought to laboratory and processed within a week.

The Minimal Salt Media (MSM) with the formulation of (g/L): Na2HPO4 (6.78), KH2PO4 (3.0), K2HPO4 (3.0), NH4Cl (1.0), NaCl (0.5), NaNO3 (0.5), yeast extract (1.0) and trace elements of 1.0 M CaCl2 (0.015), 0.1 M MgSO4 (0.05), supplemented with 1 % (v/v) of 50 % glycerol and 1 % (v/v) of commercial motor oil (Shell Advance SAE40-AX3) was used as an enrichment media for the bacterial growth. For the soil samples, 10 % (g/L) of each soil was inoculated in the MSM broth and incubated at 30 °C, 150 rpm for 48 hours. Then, the broth cultures were serially diluted with sterile distilled water before spread on the fresh MSM agar and re-incubated under the same condition. Meanwhile, the water samples were direct to serially dilution and spread plate with the same procedures as the soil samples. Then, the single colonies were subcultured several times and kept maintained on the Nutrient agar at 4 °C.

Screening Tests of Interfacial Tension Reduction and Emulsification

For screening tests, the cell-free supernatant was used. An inoculum (OD_{600} approximately 0.5) with concentration of 1% (v/v) was inoculated in 100 mL of the MSM added with 1% (v/v) motor oil and incubated for 48 hours at 30 °C,150 rpm. Then, the broth culture was centrifuged at 10,000 rpm for 10 minutes to get the supernatant. The 1% (v/v) Sodium Lauryl Sulfate (SLS) was used as the positive control. Meanwhile, the MSM broth without inoculum and distilled water were assigned as the negative control and the blank respectively.

Oil Displacement Test

This test was done to evaluate the interfacial tension reduction of oil by biosurfactants of each bacterial isolate according to Bhat et al., (2015) [13] but with some modification. Eleven petri dishes containing 25 mL of distilled water represented each biosurfactant was prepared. Then, 100 µL of used motor oil was dropped onto the surface of each petri dish containing distilled water followed by 10 µL drops of supernatant containing biosurfactant from each bacterial isolate onto the centre of the oil layer. The diameter of the clear zone produced by each biosurfactant was measured in triplicates after 30 seconds in unit centimetre (cm).

Emulsification Index

In the emulsification index, two different types of oil (used motor oil and kerosene) were used as the tested hydrocarbons. To eleven sterile test tubes (18 x 150 mm), 3 mL of used motor oil was added to each tube and left to equilibrate for several minutes. Then, 3 mL of each supernatant containing biosurfactant from each bacterial isolate was added into the respective test tube with the ratio of 1:1. The mixture in the test tubes were vortexed for two minutes and left at room temperature for 24 hours. The same steps were followed for kerosene. Then, the emulsification index of each test tube was calculated by dividing the height of the emulsion layer (cm) by the total layer of liquid (cm) and multiplying by 100 [14].

Analyses

16S rRNA Gene Identification and Phylogenetic Tree Construction

Two strains, DSB7 and DWC1 were selected as the potent bioemulsifier producers based on the highest Emulsification Index (EI_{24}) towards kerosene and used motor oil, respectively in this study. The bacterial genomic DNAs of isolates DSB7 and DWC1 were isolated according to the procedures of G-Spin™ Genomic DNA Extraction Kit by iNtRON Biotechnology, Inc. Then, the 16S rRNA gene of the isolated DNA was amplified by the Polymerase Chain reaction (PCR) using the primers 68F (5'- TNA NAC ATG CAA GTC GAR -3') and 1392R (5'-ACG GGC GGT GTG TRC -3') with the thermal conditions as follows: (a) initial denaturation (95 °C, 5 minutes), (b) denaturation (95 °C, 90 seconds), (c) annealing (54 °C, 30 seconds), and (d) extension (72 °C, 30 seconds), with 30 times of reaction cycles throughout the process. The purification of the PCR products was according to the procedures by the MEGAquick-spinTM Total Fragment DNA Purification kit (iNtRON Biotechnology, Inc.). The purified PCR products were sent to the First Base Laboratories Sdn. Bhd. for 16S rRNA gene sequencing service. The obtained sequencing results were further analysed through the Basic Local Alignment Search Tool (BLAST) to find the highest similarity sequences that matched with the Genbank databases in the National Center for Biotechnology Information (NCBI). The multiple sequences retrieved from the BLAST analysis were aligned by the ClustalW which is incorporated in the Molecular Evolutionary Genetics Analysis (MEGA X) software version 10.1.8 before the construction of the phylogenetic trees by using the neighbour-joining method.

Extraction of Crude Biosurfactant and FTIR Analysis

The crude biosurfactant was extracted by using acid precipitation and solvent extraction method [14,15]. The broth cultures were prepared by inoculating 3% (v/v) of bacterial inoculums with OD_{600} approximately 0.5 into 100 mL of MSM containing 1 % (v/v) motor oil and incubated at 30 °C, 150 rpm for five days before the centrifugation at 10,000 rpm for 10 minutes to get the supernatant. Then, the supernatant was acidified by 6N HCl to pH 2.0 and left for 30 minutes to equilibrate at room temperature. An equal volume of chloroform-methanol (2:1) which is equivalent to the concentration of 66.67:33.33 (mL) was added into the acidic supernatant and the mixture was shaken at 200 rpm for 30 minutes before stored at 4 °C for two days. The next step was centrifugation at 10,000 rpm at 4 °C for 10 minutes and the white precipitate was collected. The precipitate was dried at 50 °C until all the liquid residue was evaporated. The FT-IR analyses of extracted crude were performed by Perkin–Elmer Spectrophotometer (PerkinElmer). The IR spectra were collected over the range of 400 to 4,000 cm^{-1} with a resolution of 4 cm^{-1} and were analyzed using OMNIC Software from Thermofisher Scientific.

RESULTS AND DISCUSSIONS

Isolation of Biosurfactant Producers

Eleven bacterial isolates were successfully isolated from Sungai Dungun estuarine soil and water by using the MSM amended with 1% (v/v) motor oil, whereby 8 isolates were from the water samples and 3 isolates were from the soil samples. Most of the bacteria were happened to present in the area which is presumed to be highly contaminated with hydrocarbons such as near the fishing boat harbour (Area C) as shown in Table 1. This is because biosurfactant producers are well known to play a major role in the degradation of insoluble pollutants in the environment [16].

Screening Tests of Interfacial Tension Reduction and Emulsification

Oil Displacement Test

The production of biosurfactants by the bacterial isolates was preliminary screened based on the interfacial activity. According to Syahriansyah et al., (2016) [17], biosurfacants that can spread the oil layer with diameters from 0.9 to 2.0 cm were considered as good biosurfactant, while the oil displacement area with diameters of 0.1 to 0.2 cm indicates the poor surface activity by the biosurfactant. In this study, all bacterial isolates can displace used motor oil layer more than 0.9 cm which indicated that good biosurfactants were produced by all bacterial isolates (Table 1). From Table 1, it was noticed that 8 produced biosurfactants displaced oil more than 2.5 cm of diameter with the greatest diameter was 4.0 cm shown by biosurfactant of isolate DSB7. Meanwhile, biosurfactants of isolates DWA5, DWC1, and DWC2 showed the diameter of oil displacement less than 2.0 cm represented the lower interfacial tension activity by these biosurfactants.

Table 1. Eleven bacterial isolates with their respective area of isolation and interfacial activity and emulsification assessment towards hydrocarbons with comparison to Sodium Lauryl Sulfate, SLS (1%).

Isolates	Samples and origin areas	Oil displacement test (cm) ± SD	Emulsification Index, EI_{24} (%) ± SD	
			Used motor oil	Kerosene
DSA 1	Soil, Area A	3.7 ± 0.29	73.81 ± 2.57	***NE***
DSA 6	Soil, Area A	3.3 ± 0.20	58.67 ± 4.16	39.34 ± 2.60
DSB 7	Soil, Area B	4.0 ± 0.06	30.00 ± 3.00	53.57 ± 0.00
DWA 5	Water, Area A	1.9 ± 0.36	75.61 ± 1.60	12.5 ± 0.00
DWA 9	Water, Area A	2.7 ± 0.29	***NE***	17.95 ± 2.22
DWB 5	Water, Area B	3.0 ± 0.50	56.67 ± 5.29	***NE***
DWC 1	Water, Area C	1.5 ± 0.50	85.67 ± 3.02	42.86 ± 0.00
DWC 2	Water, Area C	1.5 ± 0.50	62.28 ± 4.71	40.71 ± 4.35
DWC 7	Water, Area C	3.2 ± 0.29	25.4 ± 4.56	***NE***
DWC 8	Water, Area C	3.9 ± 0.06	15.00 ± 3.00	18.89 ± 3.85
DWC 9	Water, Area C	3.7 ± 0.46	***NE***	***NE***
SLS (1%)	Laboratory, UMK	6.0 ± 0.00	62.96 ± 2.03	62.47 ± 1.66

Note: NE: No emulsion. The measurements of each test were taken in triplicates

Emulsification Test

The emulsion stability of produced biosurfactants were evaluated by the emulsification index of two different oils; kerosene and used motor oil (Figure 1) in triplicates. From this test, it was noticed that most biosurfactants from bacterial isolates had better emulsification stability towards used motor oil rather than kerosene with the emulsification indices of all biosurfactants ranged from 15.0 % to 85.67 % for used motor oil and 12.5 % to 53.57 % for kerosene as shown in Table 1. Meanwhile, an anionic synthetic surfactant, SLS (1%) which was used as positive control has resulted in emulsification indices of 62.96 % and 62.47 % for used motor oil and kerosene, respectively.

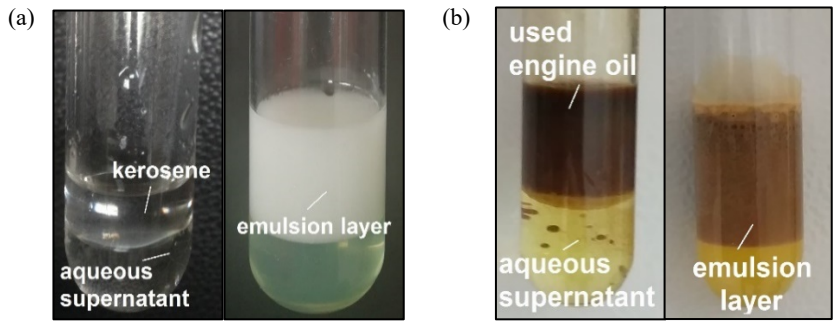


Figure 1: Emulsification of biosurfactants of two isolates towards two different hydrocarbons. Left (a): white layer represents the emulsion of kerosene by biosurfactant of *P.aeruginosa* DSB7. Right (b): light brown layer represents emulsion of used motor oil by biosurfactant of *A.taiwanensis* DWC1.

A significant positive emulsification activity was indicated by the emulsification index of 30 % or more [18]. The higher emulsification index indicates the better emulsification activity by the bacteria. Biosurfactant of isolate DSB7 showed the highest emulsification index of kerosene with index value of 53.57 % but less efficient in emulsifying used motor oil with only 30% of emulsification index. On the other hand, three isolates (DSA1, DWA5, and DWC1) showed emulsification index of used motor oil higher than SLS (1%) with index values of 73.81%, 75.61%, and 85.67%, respectively with biosurfactant of isolate DWC1 being the greatest bioemulsifier. Emulsification stability is influenced by several factors. The poor emulsification activity towards certain hydrocarbons might be due to the inability of biosurfactants to sustain the stable microscopic droplets in emulsion [19]. The emulsification process also is affected by the hydrophilic-lipophilic balance (HLB) of the biosurfactant compound [1]. The different molecular structures in a polar portion of biosurfactants lead to the specific action of emulsification towards different hydrocarbons. These could be the reasons for the variation of emulsification index by biosurfactants towards hydrocarbon oils. In fact, certain biosurfactants did not show any emulsification activity to the tested oils in this study. For instances, biosurfactants of DWA9 cannot emulsify used motor oil, biosurfactants of isolates DSA1, DWB5, and DWC7 showed no emulsion formation towards kerosene, and both oils cannot be emulsified by biosurfactant of DWC9. Since isolates DSB7 and DWC1 had the highest emulsification stability towards kerosene and used motor oil, respectively, these two noteworthy isolates were further identified based on the 16S rRNA gene sequencing and the produced biosurfactants by these isolates were characterized by FT-IR analysis.

16S rRNA Gene Identification and Phylogenetic Tree Analysis

In BLAST analysis, the 16S rRNA gene sequences of isolate DSB7 and DWC1 were compared to the ten highest matched sequences retrieved from the NCBI databases. We found that the isolate DSB7 was 99% homologous to *Pseudomonas aeruginosa* with the GenBank accession number of NR 113599.1, while the isolate DWC1 was 98% homologous to *Aeromonas taiwanensis* with GenBank accession number of NR 116585.1. The evolutionary relatedness histories of the bacterial species were further accomplished by the neighbor-joining based tree as shown in Figure 2.

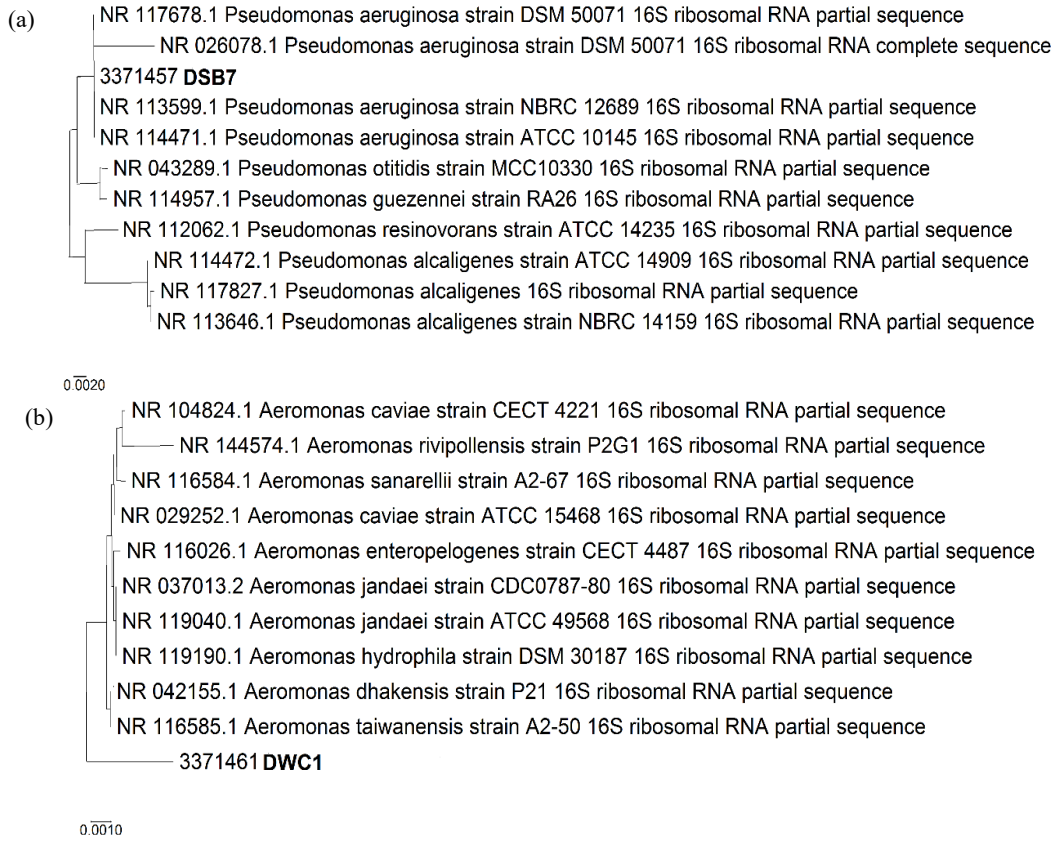


Figure 2: Phylogenetic tree by neighbor-joining method for isolates (a) *P.aeruginosa* DSB7 and (b) *A.taiwanensis* DWC1 constructed using MEGA X. The scale bar represent the nucleotide change per site.

FT-IR Analyses

For crude characterization, biosurfactants of *P.aeruginosa* DSB7 and *A.taiwanensis* DWC1 were subjected to FT-IR analysis. The FT-IR analysis of these two biosurfactants revealed two different patterns of IR spectra as shown in Figure 3 and Figure 4. In Figure 3, the broad medium spectrum that appeared at region 3385.53 cm^{-1} could be assigned to the -OH stretching vibration of the hydroxyl group in the chemical structures [20,21]. The sharp absorbance peaks observed at 2952-2852 cm^{-1} and 1458-1375 cm^{-1} indicated -CH stretches of aliphatic hydrocarbon chains [22,23]. The -CH bending vibrations could also be seen at 982 cm-1. The peak that appeared at 1735.14 cm^{-1} was indicative of the carbonyl group (C=O stretching of aldehyde) [24]. The C-O bending vibration which corresponded to sugar and ester bonding also could be seen at region 1155-1050 cm^{-1} [25]. The presence of -OH group, aliphatic hydrocarbon stretches, sugar, and ester bonding as well as the absence of peptide groups in the structure presumed that the biosurfactant of *P.aeruginosa* DSB7 was related to the glycolipid type [20,21].

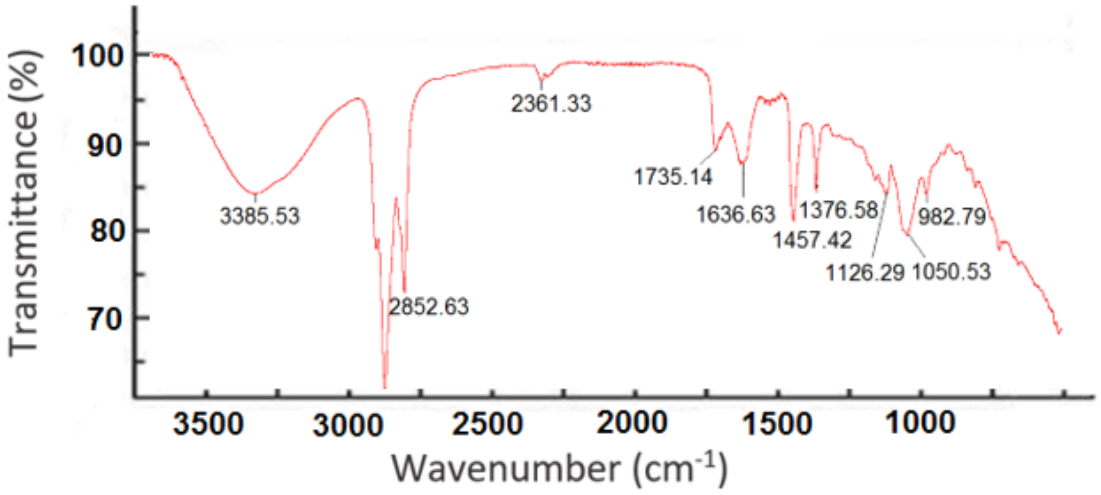
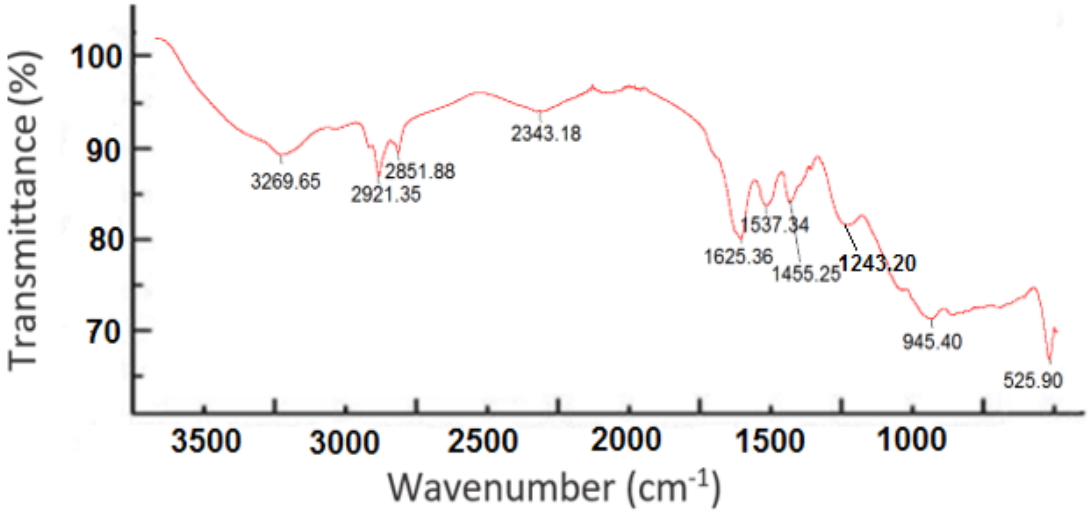


Figure 3: IR spectra of putative glycolipid produced by *P.aeruginosa* DSB7.

Meanwhile, in Figure 4, the spectrum showed the stretching signal around 3330-3250 cm^{-1} which indicated -NH stretch of the primary amine group [26,27]. Signals that appeared at the region between 3000-2850 cm^{-1} and around 1463-1377 cm^{-1} were due to $-CH_3$, $-CH_2$, and -CH stretches and CH deformation of methyl and methylene groups in the chemical structures [28] that reflect hydrocarbon chains which is the basic non-polar structure of biosurfactant. The absorbance signal that appeared at 1537 cm^{-1} represented the N-H stretches which showed the presence of protein. The indicative for -CH bending vibration from the proteins could be seen by absorbance signals in region 800-500 cm^{-1} [25]. Besides, absorbance signals in region 1300-1000 cm^{-1} correlated to the C-O stretch of sugar and esters [25] indicating the presence of carbohydrates in the chemical structures. This assumption was supported by [29] in their FTIR analysis of exopolymer biosurfactant which stated that the broad -OH stretch at 3346 cm^{-1} and the peak appeared at 1070 cm^{-1} which is close to the range of absorbance signals shown by biosurfactant of *A.taiwnensis* DWC1 were indicative for typical carbohydrates. Hence, this biosurfactant was putatively identified as polymeric compound that contain combination of protein and carbohydrates.

Biosurfactant of *P.aeruginosa* DSB7 that was putatively identified as glycolipid showed the highest emulsification index towards kerosene rather than used motor oil. This result was comparable with a study by Priya and Usharani (2009) [29], where biosurfactants of *B.subtilis* BS3 and *P.aeruginosa* PS3 showed higher emulsification index towards hydrocarbon fuels such as kerosene, petrol, and diesel with range values of 52-60% and 55-68%, respectively compared to vegetable oil. However, it was different with biosurfactant of *Pseudomonas* sp. P3 cultivated in media containing 4% molasses and 0.3% corn steep liquor where it can emulsify waste motor oil as high as 75.2 % [30]. Although the producers are from the same species group, the produced biosurfactants are substrate-dependent and their composition including congeners are determined by the carbon and nitrogen substrates [31]. For instance, *Bacillus amylofaciens* strain AR2 grown on dextrose,sucrose, and glycerol produced lipopeptides as a mixture of surfactin. iturin, and fengycin and had higher emulsification index towards kerosene with the indices range of 55-66%. However, the same strain when grown on maltose, lactose, and sorbitol, only iturin was produced with lower emulsification index of kerosene ranged from 32-52% [32]. Meanwhile, in used motor oil emulsification, the



highest index value was represented by polymeric type of biosurfactant from *A.taiwanensis* DWC1. Hydrocarbons with more complex molecular structures are more effectively emulsified by polymeric biosurfactants. This is due to the high number of reactive groups on the polymeric biosurfactant that bind tightly to the hydrocarbon molecules, and therefore stabilize the emulsion [33]. The high molecular weight compounds such as heteropolysaccharides, exopolysaccharides, carbohydrate-lipid-protein mixture, and other polysaccharide-protein complexes have commonly performed the emulsification [34,35]. However, they also demonstrated surface or interfacial tension activity which acknowledged them as biosurfactant as well. Several findings of previous studies have been highlighted regarding the ability of polymeric biosurfactants in emulsifying hydrocarbons. For instance, exopolymer produced by *Acinetobacter* M6 isolated from marine water sample has emulsified both kerosene and motor oil with emulsification indices of 67 % and 81 % respectively [36]. Besides, two other isolates named as DWA5 and DSA1 in this study also showed potential in stabilizing the emulsion of used motor oil where their emulsification index were 75.61% and 73.81%, higher than SLS 1%. This results suggested biosurfactants of these two isolates might be high molecular weight compound as well. However, further confirmation regarding their species identification and types of produced biosurfactants should be made in future study.

From these results, we came out with a conclusion that biosurfactant of *P.aeruginosa* DSB7 that was putatively identified as glycolipid was better in emulsifying kerosene (fuel oil), while putative polymeric compound from *A.taiwanensis* DWC1 had better emulsifying activity towards used motor oil (lubricant oil). Another two isolates, DWA5 and DSA1 can be highlighted for further studies as they showed the second and third highest emulsification index of used motor oil. These were opportunistic findings, where extent studies can be done to investigate more criteria of these bioemulsifiers to be applied as tools to clean-up the crude oil pollutants such as in environmental bioremediation or petroleum-related industries. In another point of view, the ability of bacteria to utilize oil wastes for growth and yield production has benefits in terms of production costs, where cheap substrates can be used as carbon sources for biosurfactant synthesis.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Higher Education, Malaysia for the funding of Research Acculturation Grant Scheme (RAGS), Faculty of Bioengineering and Technology, and Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus for the facilities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

REFERENCES

1. Yamashita, Y. and Sakamoto, K. (2016) Hydrophilic-Lipophilic Balance (HLB): Classical indexation and novel indexation of surfactant. in: H. Ohshima (Ed.), Encycl. *Biocolloid Biointerface Sci*., Wiley, pp. 570–574.

2. Ojinnaka, C.M., Ajienka, J.A., Abayeh, O.J., Osuji, L.C., and Duru, R.U. (2016) Formulation of best-fit hydrophile/lipophile balance-dielectric permittivity demulsifiers for treatment of crude oil emulsions. *Egyptian Journal of Petroleum*. 25 (4), 565–574.

3. Raya, S.A., Mohd Said, I., Abbas Ahmed, A., and Abubakar Umar, A. (2020) A critical review of development and demulsification mechanisms of crude oil emulsion in the petroleum industry. *Journal of Petroleum Exploration and Production Technology*. 10 (4), 1711–1728.

4. Ravera, F., Dziza, K., Santini, E., Cristofolini, L., and Liggieri, L. (2020) Emulsification and emulsion stability: The role of the interfacial properties. *Advances in Colloid and Interface Science*. 288 102344.

5. Campos, J.M., Stamford, T.L.M., Sarubbo, L.A., de Luna, J.M., Rufino, R.D., and Banat, I.M. (2013) Microbial biosurfactants as additives for food industries. *Biotechnology Progress*. 29 (5), 1097–1108.

6. Reis, R.S., Pacheco, G.J., Pereira, A.G., and Freire, D.M.G. (2013) Biosurfactants: Production and Applications. in: R. Chamy, F. Rosenkrantz (Eds.), Biodegrad. - Life Sci., InTechOpen, Londonpp. 31–61.

7. Usman, M.M., Dadrasnia, A., Lim, K.T., Mahmud, A.F., and Ismail, S. (2016) Application of biosurfactants in environmental biotechnology; remediation of oil and heavy metal. *AIMS Bioengineering*. 3 (3), 289–304.

8. Lamichhane, S., Krishna, K.C.B., and Sarukkalige, R. (2017) Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: A review. *Journal of Environmental Management*. 199 46–61.

9. Li, Z., Xu, D., Yuan, Y., Wu, H., Hou, J., Kang, W., et al. (2020) Advances of spontaneous emulsification and its important applications in enhanced oil recovery process. *Advances in Colloid and Interface Science*. 277 102119.

10. Ibrahim, H.M.M. (2016) Biodegradation of used engine oil by novel strains of *Ochrobactrum anthropi* HM-1 and Citrobacter freundii HM-2 isolated from oil-contaminated soil. *3 Biotech*. 6 (2), 1–13.

11. Kaczorek, E., Pacholak, A., Zdarta, A., and Smułek, W. (2018) The impact of biosurfactants on microbial cell properties leading to hydrocarbon bioavailability increase. *Colloids and Interfaces*. 2 (3), 35.

12. Beiras, R. (2018) Hydrocarbons and oil spills. in: Mar. Pollut. Sources, Fate Eff. Pollut. Coast. Ecosyst., Elsevier, pp. 1–408.

13. Bhat, R., Dayamani, K.J., Hathwar, S., Hedge, R., and Kush, A. (2015) Exploration on production of rhamnolipid biosurfactants using native Pseudomonas aeruginosa strains. *Journal of BioScience and Biotechnology*. 4 (2), 157–166.

14. Shah, M.U.H., Sivapragasam, M., Moniruzzaman, M., and Yusup, S. (2016) A comparison of recovery methods of rhamnolipids produced by *Pseudomonas aeruginosa*. *Procedia Engineering*. 148 494–500.

15. Saikia, R.R., Deka, H., Goswami, D., Lahkar, J., Borah, S.N., Patowary, K., et al. (2013) Achieving the best yield in glycolipid biosurfactant preparation by selecting the proper carbon/nitrogen ratio. *Journal of Surfactants and Detergents*. 17 (3), 563–571.

16. Siddiqui, S. and Bano, A. (2019) Hydrocarbon degradation. in: R.P. V.Kumar, M.Kumar (Ed.), Microb. Action Hydrocarb., Springer Nature Singapore Pte Ltd., pp. 615–641.

17. Syahriansyah, U.K.M. and Hamzah, A. (2016) Determination of optimum conditions and stability study of biosurfactant produced by Bacillus subtilis UKMP-4M5. Malaysian Journal of Analytical Science. 20 (5), 986–1000.

18. Shoeb, E., Ahmed, N., Akhter, J., Badar, U., Siddiqui, K., Ansari, F.A., et al. (2015) Screening and characterization of biosurfactant-producing bacteria isolated from the Arabian Sea coast of Karachi. *Turkish Journal of Biology*. 39 (2), 210–216.

19. Silva, R.C.F.S., Almeida, D.G., Rufino, R.D., Luna, J.M., Santos, V.A., and Sarubbo, L (2014) Applications of biosurfactants in the petroleum industry and the remediation of oil spills. *International Journal of Molecular Sciences*. 15 (7), 12523–12542.

20. Sabturani, N., Latif, J., Radiman, S., and Hamzah, A. (2016) Spectroscopic analysis of rhamnolipid produced by *Pseudomonas aeruginosa* UKMP14T. *Malaysian Journal of Analytical Sciences*. 20 (1), 31–43.

21. Ebrahimipour, G., Gilavand, F., Karkhane, M., Kavyanifard, A.A., Teymouri, M., and Marzban, A. (2014) Bioemulsification activity assessment of an indigenous strain of halotolerant Planococcus and partial characterization of produced biosurfactants. *International Journal of Environmental Science and Technology*. 11 (5), 1379–1386.

22. Varadavenkatesan, T. and Murty, V.R. (2013) Production of a lipopeptide biosurfactant by a novel Bacillus sp. and its applicability to enhanced oil recovery. *ISRN Microbiology*. 2013 1–8.

23. Karadi, R.V., Lokesh, K.N., Channarayappa, M.V., Venkataranganna, Sadanandan, B., and Siva Kiran, R.R. (2012) Standardization of biosurfactant enrichment process by factorial design and elucidating its physico-chemical and structural characteristics. *Journal of Biochemical Technology*. 3 (5), 161–166.

24. Chebbi, A., Hentati, D., Zaghden, H., Baccar, N., Rezgui, F., Chalbi, M., et al. (2017) Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated Pseudomonas sp. strain from used motor oil-contaminated soil. *International Biodeterioration and Biodegradation*. 122 128–140.

25. Hamza, F., Satpute, S., Banpurkar, A., Kumar, A.R., and Zinjarde, S. (2017) Biosurfactant from a marine bacterium disrupts biofilms of pathogenic bacteria in a tropical aquaculture system. *FEMS Microbiology Ecology*. 93 (11), 1–11.

26. Bezza, F.A. and Chirwa, E.M.N. (2015) Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by Bacillus subtilis CN2. *Biochemical Engineering Journal*. 101 (2015), 168–178.

27. Siriwong, M., Ayudhya, N., and Chuenchomrat, P. (2015) Screening and characterization of bacterial biosurfactant from Bangkok and Vicinities. *International Journal of Life Sciences Biotechnology and Pharma Research*. 4 (3), 168–171.

28. Bhardwaj, G., Cameotra, S.S., and Chopra, H.K. (2015) Isolation and purification of a new enamide biosurfactant from *Fusarium proliferatum* using rice-bran. *RSC Advances*. 5 (67), 54783–54792.

29. Priya, T. and Usharani, G. (2009) Comparative study for biosurfactant production by using *Bacillus subtilis* and *Pseudomonas aeruginosa*. 2 (4), 284–287.

30. Silva, M.A.M., Silva, A.F., Rufino, R.D., Luna, J.M., Santos, V.A., and Sarubbo, L.A. (2017) Production of biosurfactants by Pseudomonas species for application in the petroleum industry. *Water Environment Research*. 89 (2), 117–126.

31. Al-Araji, L., Rahman, R.N.Z.R.A., Basri, M., and Salleh, A.B. (2007) Minireview: Microbial surfactant. *Asia-Pacific Journal of Molecular Biology and Biotechnology*. 15 (3), 99–105.

32. Singh, A.K., Rautela, R., and Cameotra, S.S. (2014) Substrate dependent in vitro antifungal activity of Bacillus sp strain AR2. *Microbial Cell Factories*. 13 (1), 1–11.

33. Perfumo, A., Smyth, T.J.P., Marchant, R., and Banat, I.M. (2010) Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. in: K.N. Timmis (Ed.), Handb. Hydrocarb. Lipid Microbiol., Springer-Verlag Berlin Heidelberg, pp. 1502–1510.

34. Das, P., Mukherjee, S., Sivapathasekaran, C., and Sen, R. (2010) Microbial surfactants of marine origin: potentials and prospects. in: R. Sen (Ed.), Biosurfactants Adv. Exp. Med. Biol., Landes Bioscience, Texaspp. 88–101.

35. Vijayakumar, S. and Saravanan, V. (2015) Biosurfactants-types, sources and applications. *Research Journal of Microbiology*. 10 (5), 181–192.

36. Peele, K.A., Ravi Teja, V.C., and Kodali, V.P. (2016) Emulsifying activity of a biosurfactant produced by a marine bacterium. *3 Biotech*. 6 (2), 2–7.