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Biosurfactant and bioemulsifier production by hydrocarbonoclastic bacteria isolated from petroleum sludge as potential biodispersant for oil spill remediation

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ABSTRACT

The elevation of environmental pollution caused by the production and spillage of petroleum hydrocarbons is disastrous. Major compounds in petroleum hydrocarbon such as polycyclic aromatic hydrocarbons (PAHs) are of special concern since their molecules are persistent for a and highly toxic and carcinogenic to the ecosystems and humans. Although a lot of effort had been spent to treat these pollutants, the left-over products are still ubiquitous in the environment although they could be naturally degraded by indigenous microorganisms aided by biosurfactants or bioemulsifiers that they produce during hydrocarbon biodegradation process. Hydrocarbonoclastic bacteria were isolated from petroleum sludge obtained from a waste disposal company in Malaysia and investigated for their potential to produce biosurfactants (i.e., surface tension measurement) and bioemulsifiers (i.e., emulsification assay) together with their ability in total petroleum hydrocarbon (TPH) biodegradation (i.e., gas chromatography-mass spectrometry (GC-MS)). One isolate of potential bioemulsifier producing bacteria (BEP) exhibited emulsification index as high as 72.81% while the rest were between the range of 64%–68%. In addition, one isolate of potential biosurfactant producing bacteria (BSP) recorded a surface tension measurement as low as 18.92 mN/m while the rest were between the range of 45–28 mN/m. Further identification of selected potential isolates by 16s rRNA gene amplification and sequencing revealed their identity as from several genera such as *Achromobacter* sp., *Pseudomonas* sp. and *Serratia* sp. Isolate P15 was found as the best candidate for potential BSP identified as closely related to *Pseudomonas stutzeri* while isolate P18 which was the best candidate for potential BEP identified as closely related to *Serratia marcescens*. Biodegradation study conducted for both isolates showed the percentage of TPH loss of approximately 85% by BSP P15 and approximately 78% by BEP P18. Production of biosurfactant and bioemulsifier by both isolates supported by their ability to degrade total petroleum hydrocarbons validate the advantageous features of biosurfactants and bioemulsifiers in hydrocarbon biodegradation and this can be further exploited in designing bioremediation strategies for contaminated environment.

Keywords: Biosurfactant; Bioemulsifier; Petroleum hydrocarbon-degrading bacteria; Emulsification index; Surface tension

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1. Introduction

Biosurfactants are amphiphilic, surface-active compounds produced by microorganisms with almost similar properties as chemically synthesized surfactants. Compared to synthetic chemical surfactants, biosurfactants are low in toxicity, high in foaming activity, environmentally friendly and low cost in terms of their availability in nature, and therefore have shown a better and safer option to replace the use of chemical surfactants in food, edible oils, pharmaceuticals, and cosmetics [1]. Biosurfactants are also high in selectivity and specific activity under different temperature, pH and salinity conditions as well as can be synthesized by renewable sources [2]. In addition, biosurfactants are also known to have the properties of reducing surface tension, solubility or bioavailability and decreasing critical micelle concentrations (CMC), in which the maximum surfactant monomers concentration is affected by pH, temperature and ionic strength in water [1]. Biosurfactants are known to aid biodegradation of pollutant, as they can enhance the effective dispersion and bioavailability of hydrophobic pollutants for microbial access and degradation by the process of micelle solubilization [3]. Since biosurfactants have very low CMC than chemically made surfactants, the biosurfactants are effective at low concentrations and able to reduce the surface and interfacial tensions between different phases as well as forming stable emulsions [4].

Biosurfactants are divided into two categories with regards to their molecular weight. Low molecular weight biosurfactants such as glycolipids, lipopeptides and phospholipids are efficient in reducing interfacial tension while those with high molecular weight, such as lipoproteins, lipopolysaccharides, and complex biopolymers are more competent in stabilizing emulsion [5]. Among the well-known types of these biomolecules namely glycolipids, rhamnolipids and surfactin which are biosurfactants and emulsan, alasan and mannoproteins which are bioemulsifiers. High molecular weight biosurfactant often referred as bioemulsifiers which can emulsify two immiscible liquids such as hydrocarbons or other hydrophobic substrates similar to biosurfactants. Bioemulsifiers are more efficient in formation and stabilization of emulsions and are less effective at surface tension reduction compared to biosurfactants [3]. Some biomolecules however possess both surfactant and emulsifying properties which makes them highly potential candidates for industrial use [6].

Compared to other microorganisms, bacteria are known as major biosurfactants and bioemulsifiers producers that extensively produce them as secondary metabolites. Biosurfactants producer or called as biosurfactant-producing bacteria (BSP) and bioemulsifiers producer known as bioemulsifier-producing bacteria (BEP) are likely to be found in the environment that contains rich hydrocarbon and favourable condition such as oil reservoir [7], soil [8,9], marine environment [10], hot spring [11] or wastewater [12]. BSP and BEP in soils and sediments are generally dominated by a few main bacterial genera which are also known as hydrocarbon biodegraders such as *Pseudomonas*, *Bacillus*, *Sphingomonas* and *Actinobacteria* [13]. Other than that, microorganisms from palm oil industry such as palm oil mill effluent (POME) [14] and palm oil-contaminated soil [15] are also potential biosurfactant and bioemulsifier producers due to the presence of carbon source in the oil palm products.

Microbial biosurfactants and bioemulsifiers are being produced during pollutant biodegradation, increasing the bioavailability of organic pollutants such as hydrocarbons [16,17]. Microorganisms that have the ability to consume petroleum hydrocarbon as carbon source and simultaneously produce biosurfactants and bioemulsifiers can be manipulated for bioremediation, reduce heavy oil viscosity, clean oil storage tanks, increase petroleum transport in pipelines, and stabilize fuel water–oil emulsions and enhancing oil recovery in petroleum industry [13]. Biosurfactants can enhance the effective dispersion and bioavailability of hydrophobic pollutants for microbial access and degradation in oil polluted environments by the process of micelle solubilization. They have the ability to mobilize hydrophobic molecules bound on solid substrata increasing the flow rate. In addition, cell surface hydrophobicity of BSP increased and therefore aid for easy access and subsequent uptake of hydrophobic substrates by microbial cells [3]. Bioemulsifiers particularly involved in solubilization of poorly-soluble substrates, thus increasing their access and availability for biodegradation. In an oil polluted environment, these molecules play a specific role in stabilization of emulsion by binding tightly to dispersed hydrocarbons and oils preventing them from merging together and increasing their kinetic stability [3].

In this study, both biosurfactants and bioemulsifier producing activities were screened from bacterial isolates extracted from petroleum sludge sample and the best candidate for potential BSP and BEP were further identified using 16S rRNA gene amplification and sequencing together with their ability to bring about total petroleum hydrocarbon (TPH) biodegradation in minimal media supplemented with 1% (v/v) petroleum crude. These findings highlight the potential of BSP and BEP to be manipulated for biosurfactant and bioemulsifier production based on their characteristics of lowering surface tension, producing emulsion and degrading hydrocarbons. Besides, the commonly used commercial surfactants for the degradation of the pollutant may add up to another source of pollution to the environment. To help with the desorption of contaminants and the left-over products in the environment, biosurfactants and bioemulsifiers from hydrocarbonoclastic can be introduced in oil spill kit as a pollution control strategy based on biological approach (i.e., bioremediation) which offer more environmentally friendly solution.

2. Materials and methods

2.1. Sample collection

Petroleum sludge samples were obtained from Paka Petroleum Sdn Bhd, a waste disposal company in Kemaman, Terengganu, Malaysia. Samples were kept at 4°C for preservation prior to processing step. Previously, physical characteristics of the sludge sample such as texture, colour and moisture content were recorded. Subsequently, samples were sent to ALS Technichem (M) Sdn. Bhd. for further physicochemical analyses.

2.2. Enrichment culture of biosurfactant-producing bacteria

Biosurfactant and bioemulsifier-producing bacteria was isolated from the petroleum sludge sample by resuspending 10 g of petroleum sludge into maximum recovery diluent and incubated at 150 rpm for 30 min at room temperature followed by an enrichment culture in 50 mL Bushnell Hass broth supplemented with 1% (v/v) filter sterilized petroleum crude. The enrichment was done for 7 d at 30°C with the shaking speed of 150 rpm. Following enrichment, the suspension was plated onto Bushnell Haas agar supplemented with 1% (v/v) filter sterilized petroleum crude and incubated for 7 d at 30°C. Pure culture of isolates were obtained after several times of subculturing and further identification of the potential biosurfactant and bioemulsifier-producing bacteria was done by observing the morphological characteristics and gram staining. Isolates were then grown in Bushnell Haas broth supplemented with 1% (v/v) filter sterilized petroleum crude for preliminary screening of biosurfactant producing g activity. Measurement of emulsification index and surface tension of cell-free supernatant obtained from the culture of bacterial isolates were done as described in the following section.

2.3. Evaluation of biosurfactant production activity by isolated bacteria

Production of biosurfactant activity by bacterial isolates was evaluated based on their ability to emulsify petroleum crude and also lowering the surface tension of the media. Isolates were grown in Bushnell Haas broth supplemented with 2% (v/v) filter sterilized petroleum crude. The bacterial growth was measured by spectrophotometry analysis (OD_{600}) and when it reached the stationary phase, the bacterial suspensions were centrifuged at 9,000 x g and 4°C for 30 min to obtained the cell-free supernatant (CFS). Emulsification index and surface tension were measured for respective CFS obtained. Comparison of the measurements was done against sterile distilled water used as negative control and 1% (w/v) sodium dodecyl sulphate (SDS) as positive control.

2.4. Emulsification index measurement

Emulsification index (E24) was performed by adding 2 mL of CFS to 2 mL of sterilized petroleum crude in a 15 mL test tube, vortexed at high speed for 2 min to create optimum emulsion and were left to stand for overnight at ambient temperature [18,19]. The emulsification activity was calculated using the Eq. (1).

$$\text{Emulsification index, } E_{24} = \frac{\text{Height of emulsion layer (mm)}}{\text{Total height of mixture (mm)}} \times 100 \quad (1)$$

2.5. Surface tension measurement

The ability of the CFS in lowering surface tension of the media was monitored using a Surface Tension Analyser (Sigma 703D) with 1.9 cm platinum du Noüy ring method. The surface tension was measured at room temperature and in equilibrium condition by immersing the ring into the solution initially. For calibration, the surface tension of distilled water was first measured followed by the CFS [20].

2.6. Identification of selected biosurfactant and bioemulsifier-producing bacteria

Selected bacterial isolates which showed good biosurfactant and bioemulsifier production activity were further identified through the 16S rRNA gene amplification. Prior to that, genomic DNA of these bacterial isolates were extracted and PCR was carried out using the universal primers for bacterial 16S rRNA amplification. PCR products were sent for sequencing and results were compared to the available sequences in NCBI GenBank using BLAST.

2.7. Total petroleum hydrocarbon biodegradation

Selected bacterial isolates which showed good activity of biosurfactant and bioemulsifier production were further evaluated for their potential in biodegrading total petroleum hydrocarbon. Inoculum for selected isolates were prepared by inoculating 500 μL of bacterial cells from glycerol stock in 50 mL LB broth. They were incubated at 30° with shaking at 150 rpm until the OD_{600} measurement reaches 1.0. Bacterial inoculum was transferred into 1% (v/v) filter sterilized petroleum crude and residual crude oil were extracted using chloroform as solvent (1:1, v/v) at incubation day 3, 6, 9 and 12, respectively. Further separation was done by adding anhydrous sodium sulphate powder followed by the use of rotary evaporator. Subsequently, gas chromatography-mass spectrometry (GC-MS) analysis was done with the following parameters setup; the column oven temperature was set at 50°C, the injector and detector were maintained between 60°C and 300°C and the oven temperature was programmed to rise from 60°C to 300°C at 5.0°C/min increments and was held at 250°C for 5 min. 1 μL of sample was injected and analyzed for 45 min with nitrogen gas as carrier. The chromatograms were analyzed and the percentage of total petroleum hydrocarbon (TPH) was calculated using the Eq. (2):

$$\text{Total petroleum hydrocarbon, TPH}(\%) = \frac{\text{TPHcontrol-TPHsample (mm)}}{\text{TPHcontrol (mm)}} \times 100 \quad (2)$$

3. Results and discussion

3.1. Physicochemical properties of petroleum sludge

Petroleum sludge sample obtained was black semi-solid cake with texture of clay. It has a moisture content of 30.8% and total organic carbon of 70.4%. In terms of petroleum hydrocarbon composition (Table 1), 642 mg/kg are TPH compounds with a carbon chain C6-C9 which are the monoaromatic hydrocarbons benzene, toluene, ethylbenzene and xylene (BTEX). The highest concentrations amongst the BTEX group were found to be from meta- and para-xylene compounds which are up to 146 mg/kg. The total

Table 1
Concentrations (mg/kg) of hydrocarbon compounds in the oily sludge sample

	Hydrocarbon compounds	Concentrations (mg/kg)
Total petroleum hydrocarbon (TPH)	TPH C6-C9	642
	TPH C10-C14	174,000
BTEX	Benzene	<0/1
	Toluene	0.7
	Ethylbenzene	0.3
	Meta-, and para-xylene	146
	Ortho-xylene	62.6
Polycyclic aromatics hydrocarbons (PAHs)	Naphthalene	813
	Acenaphthylene	<0.5
	Acenaphthene	<0.5
	Fluorene	<0.5
	Phenanthrene	233
	Anthracene	<0.5
	Fluoranthene	<0.5
	Pyrene	<0.5
	Benz(a)anthracene	<0.5
	Chrysene	<0.5
	Benzo(b) & Benzo(k) fluoranthene	<1.0
	Benzo(a)pyrene	<0.5
	Indeno(1,2,3-cd) anthracene	<0.5
	Dibenz(a,h)anthracene	<0.5
	Benzo(g,h,i)perylene	<0.5

concentration of low molecular weight polycyclic aromatic hydrocarbons (PAHs) with a carbon chain C10-C14 was around 174,000 mg/kg in total. The two-ring naphthalene (813 mg/kg) and three-ring phenanthrene (233 mg/kg) were the two low molecular weight PAHs with the highest concentration. Other low molecular weight and high molecular weight PAHs were almost undetected.

3.2. Isolation and screening of potential biosurfactant and bioemulsifier-producing bacteria from petroleum sludge

Isolation of bacteria from petroleum sludge was done by enriching the hydrocarbonoclastic bacteria which are able to use hydrocarbon compounds in petroleum crude as their carbon source. These hydrocarbonoclastic group of bacteria are hypothesized to produce biosurfactant and bioemulsifier to aid biodegradation of hydrocarbons. Several isolates of biosurfactant-producing bacteria obtained from the petroleum sludge suspension were able to grow in the minimal Bushnell Haas media supplemented with 1% (v/v) filter sterilized petroleum crude. Enrichment of these bacterial culture was essential to select those who could use petroleum crude as carbon source by degrading the hydrocarbon compounds. Simultaneously, it can be hypothesized that they secrete biosurfactant or bioemulsifier to boost biodegradation process particularly involving high molecular weight hydrocarbons. Based on morphological and gram staining identification, these isolates were mostly rod-shaped gram-negative bacteria.

As a preliminary screening, the activity of biosurfactant and bioemulsifier production by these isolates was evaluated to select potential biosurfactant-producing bacteria and bioemulsifier-producing bacteria. Biosurfactant production activity was determined by conducting surface tension

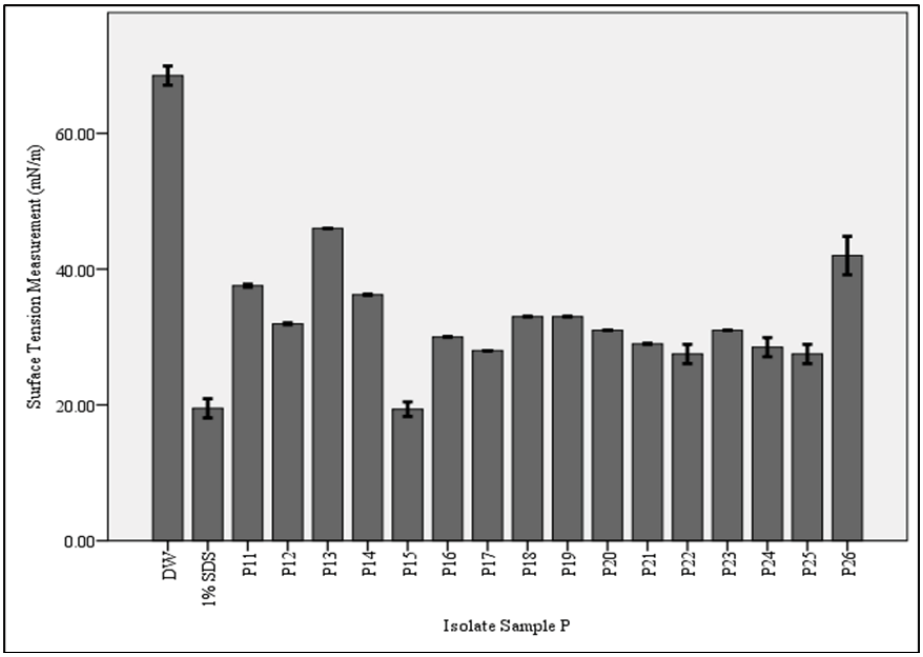

Fig. 1. Surface tension reduction of cell-free supernatant containing biosurfactants and bioemulsifiers produced by isolates. All values represent the mean ± standard deviation of three replicates. Positive control = 1% (w/w) SDS, negative control = distilled water.

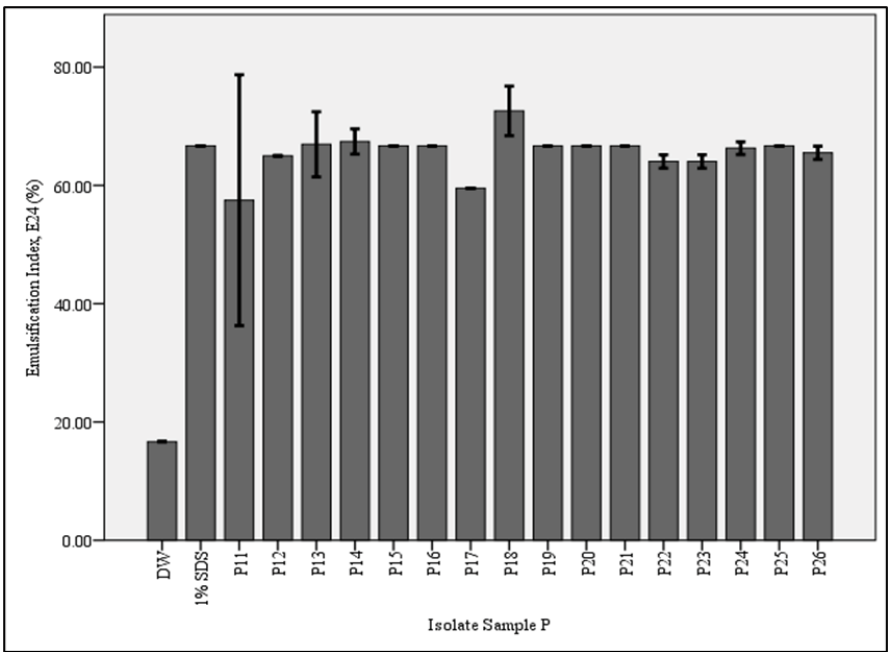

Fig. 2. Emulsification activity of cell-free supernatant (CFS) containing biosurfactants and bioemulsifiers produced by isolates. All values represent the mean ± standard deviation of three replicates. Positive control = 1% (w/v) SDS, negative control = distilled water.

measurement (Fig. 1) while bioemulsifier production activity was indicated by the calculation of emulsification index (E24) (Fig. 2). The presence of biosurfactant or bioemulsifier in the cell-free supernatant could be indicated by low surface tension measurement and high emulsification index, respectively. Their ability to lower surface tension and produce emulsion were used as strategies to screen both potential biosurfactant and bioemulsifier producing bacteria.

Surface tension measurement recorded the lowest reading of 18.92 mN/m exhibited by the isolate P15. This is also equivalent to the surface tension measurement of the synthetic surfactant (1% (v/v) SDS) while the rest were between the range of 45–28 mN/m and could be considered as moderate biosurfactant production activity when compared to the activity exhibited by the negative control distilled water. Almost all of these selected isolates showed equivalent emulsification index (E24) to the synthetic surfactant (1% (v/v) SDS) which is between 64% to 68%. They have distinctly higher emulsification index when compared to the negative control distilled water. One isolate P18 showed highest emulsification index (E24) which is 72.81%, higher than recorded by the SDS itself. From these two preliminary screenings, it can be concluded that the best candidate for potential BSP is P15 while the best candidate for potential BEP is P18. Molecular identification by 16S rRNA gene PCR amplification and sequencing revealed that the best candidate for potential BSP isolate P15 is closely related to *Pseudomonas stutzeri* while the best candidate for potential BEP isolate P18 is closely related to *Serratia marcescens* (Fig. 3).

Screening of potential BSP using the surface tension measurement method and BEP using the emulsification assay in this study were found to be more reliable to predict biosurfactant and bioemulsifier production activity compared to other types of screenings based on a strong correlation between them. Low surface tension measurement indicates a high surface activity and therefore a good biosurfactant production activity. Good biosurfactants' interfacial (oil/water) and surface tensions are respectively in the range of 1 and 30 mN/m [21]. A study by Phan et al. [22] revealed that the cell-free supernatant from *Pseudomonas aeruginosa* UKMP-14T, *Rhodococcus* sp. UKMP-5T, *Rhodococcus* sp. UKMP-7T, and *Acinetobacter baumannii* UKMP-12T showed higher surface activity than SDS by which is as low as approximately 25 mN/m.

In contrast, high emulsification index indicates high emulsion production and therefore a good bioemulsifier production activity. In the study by Deng et al. [23], they reported a maximum value of 70.3% emulsification index exhibited by *Achromobacter* sp. HZ01. Meanwhile, Ibrahim et al. [24] has reported emulsification index of 87% for an active crude oil degraders *Serratia marcescens* and Sun et al. [25] reported 60%–63% emulsion rate also by *Pseudomonas* sp. which has almost similar result to the average emulsion production in this current study. Preliminary screenings of biosurfactant and bioemulsifier activity from the isolates revealed some potential biosurfactant-producing and bioemulsifier-producing bacteria; which are found to be closely related to the genera of *Achromobacter*, *Pseudomonas* and *Serratia* as highlighted by other previous research.

3.3. Biodegradation potential of selected biosurfactant-producing bacterial isolates

In assessing biodegradation potential of BSP P15 and BEP P18, these isolates were grown in Bushnell Haas broth supplemented with 2% (v/v) filter sterilized petroleum crude. Growth of BSP P15 and BEP P18 were observed and residual petroleum crude were extracted using chloroform

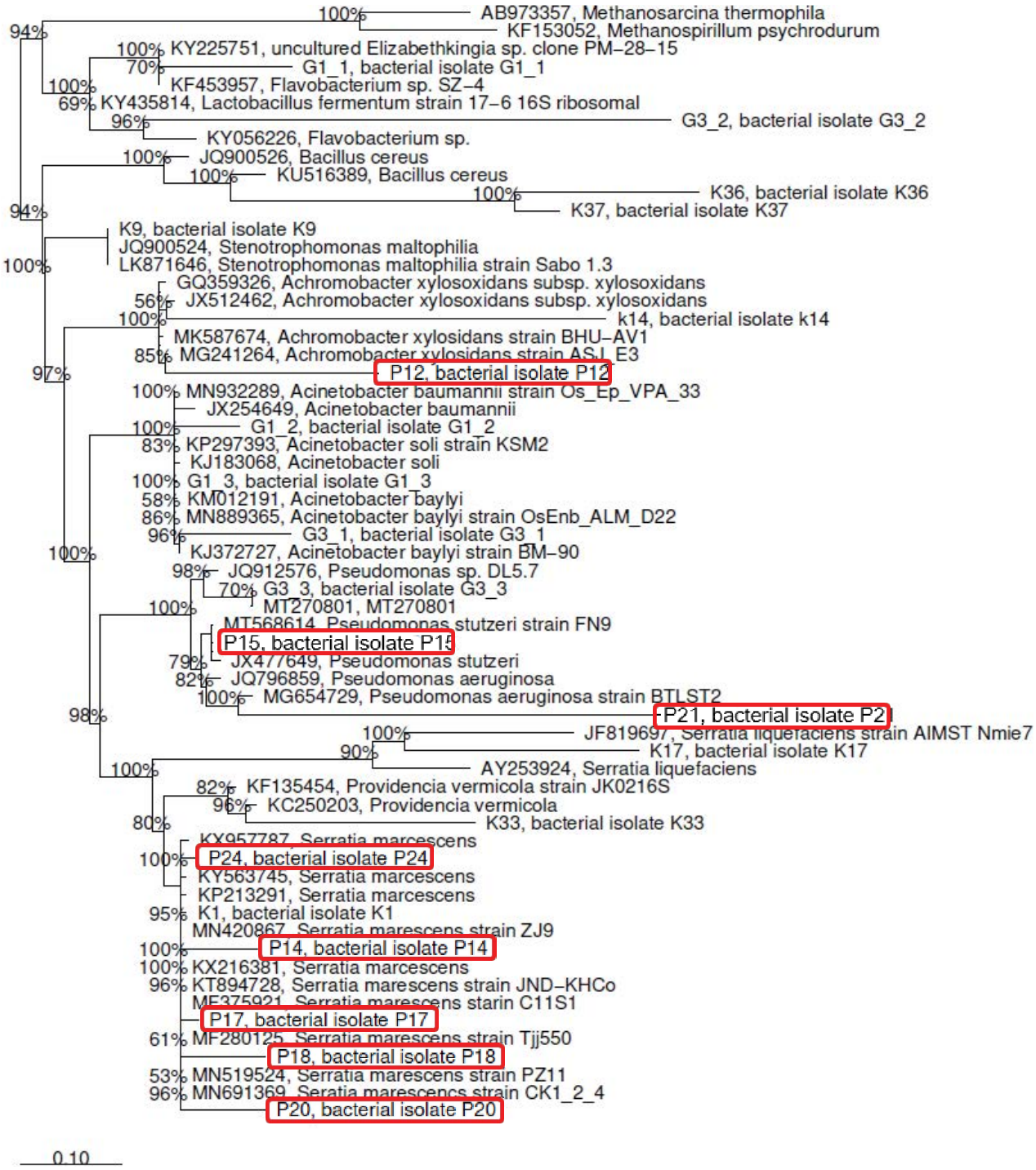

Fig. 3. Phylogenetic tree of selected bacterial isolates isolated from petroleum sludge of Paka, Terengganu, Malaysia. The red box represents the selected potential biosurfactant and bioemulsifier producing bacterial isolates in the study.

as solvent (1:1, v/v) at incubation day 3, 6, 9 and 12, respectively. Measurement of OD_{600} for BSP P15 and BSP P18 which plotted as a growth curve (Fig. 4) revealed that these isolates are slow-growing bacteria in a minimal media with petroleum crude as the sole carbon source environment. The growth was increasing throughout the incubation days until the end of the experiment.

As for the extracted residual petroleum crude at incubation day 3, 6, 9 and 12, the samples were analyzed using GC-MS which produced chromatograms. Analysis of the chromatograms revealed the reduction of peaks for total petroleum hydrocarbon as the degradation proceeds. Both BSP P15 and BEP P18 showed an increasing trend in degradation TPH throughout the incubation days (Fig. 5). As for

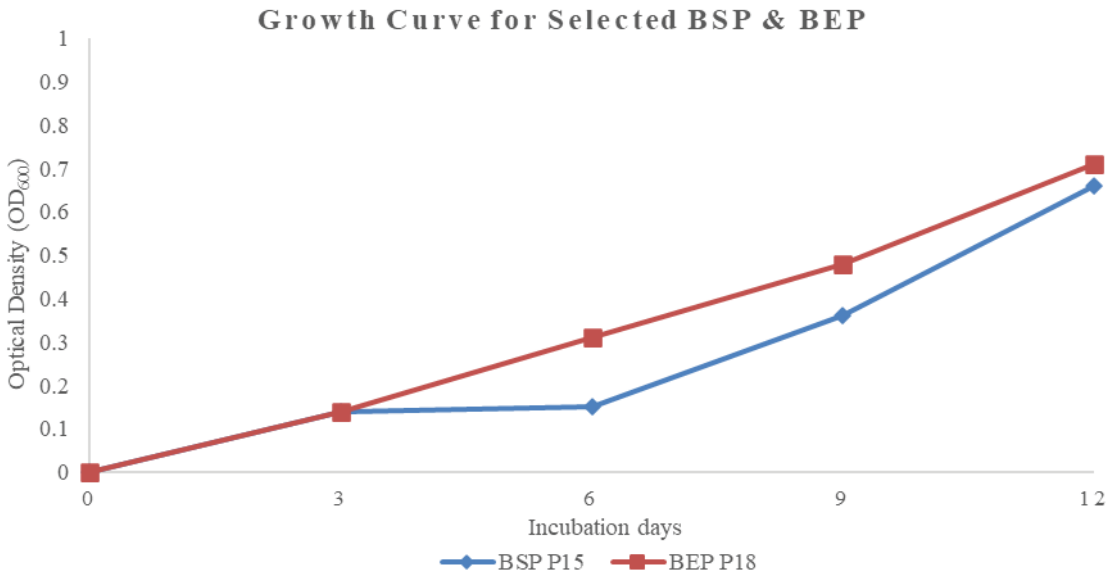

Fig. 4. Growth curve of BSP P15 and BEP P18. Optical density (OD_{600}) measurement was done for both culture during the course of TPH biodegradation using 1% (v/v) petroleum crude for 12 d.

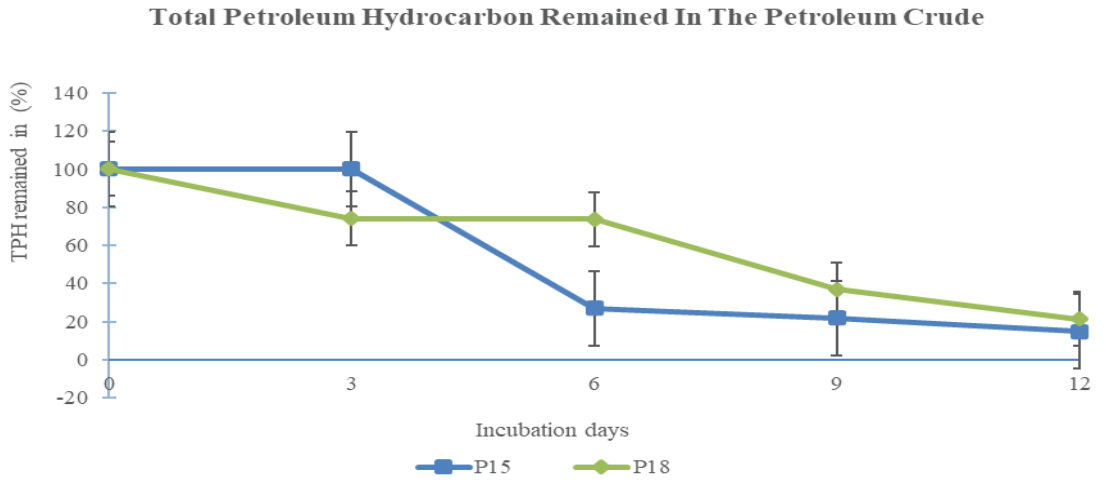

Fig. 5. Total petroleum hydrocarbon remaining in the course of TPH biodegradation using 1% (v/v) petroleum crude for 12 d. Extracted TPH at several incubation days were analyzed using GC-MS to get the TPH remained.

BSP P15, no evident disappearance of TPH had been detected in the initial period for up to more than 72 h of incubation, assuming growth-linked biodegradation period of acclimation. Rate of TPH loss became rapid afterwards and by day 6 of incubation, only 26.9% of TPH remained in the media suspension and was extracted, which shown up to approximately 73.1% of TPH biodegradation. By the 12th day of petroleum hydrocarbon biodegradation, the remaining TPH was 14.9% and the percentage of TPH loss was up to approximately 85%, showing the ability of BSP P15 to efficiently degrade TPH and potentially with the aid of biosurfactant produce by it during biodegradation. As for BEP P18, TPH remained was seen to be decreasing gradually from the very beginning but it was only after day 6 of incubation that the TPH remained went down further and reached 36.88% remaining at day 9 of incubation. Final measurement at day 12 of petroleum hydrocarbon biodegradation revealed the remaining TPH was 21.27% and that makes the percentage of TPH loss was up to approximately 78%. This also shows the ability of BEP P18 in TPH biodegradation and presumably the production and activity of bioemulsifier produced by BEP P18 during TPH biodegradation.

In terms of justifying the function of biosurfactant and bioemulsifier in assisting hydrocarbon degradation by microorganisms, previous researches have highlighted on a feasible production of biosurfactant and bioemulsifier from hydrocarbon-degrading microorganisms [26] such as bacterial isolates belonged to several genera such as *Pseudomonas*, *Rhodococcus*, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Acinetobacter* and *Serratia* [6]. As hydrocarbon uptake is highly related to the spontaneous release and function of biosurfactant or bioemulsifier, several bacterial strains which can degrade

petroleum and simultaneously produce biosurfactant have also been discovered such as *Chelatococcus daeguensis* HB-4, *Rhodococcus erythropolis* OSDS1, *Bacillus subtilis* RI4914 and *Bacillus licheniformis* [27]. These different genera of bacteria will produce different types of biosurfactant; for example, *Bacillus* sp. is the well-known species which produces lipopeptides group whereas *Rhodococcus* sp. is known for glycolipid production [28]. In the present study, approximately 85% of TPH loss was reported by BSP P15 and 78% loss for BEP P18. Assuming growth-linked biodegradation, the increase in growth of both BSP P15 and BEP P18 over time bring about an increase in petroleum hydrocarbon biodegradation too.

Previous study by Patowary et al. [5] also reported the biodegradation of various crude oil components and production of rhamnolipid biosurfactant by *P. aeruginosa* PG1 isolated from hydrocarbon contaminated garage soil. On the other hand, emulsan bioemulsifier was produced by a hydrocarbon-degrading *Acinetobacter venetianus* RAG-1 and was extracted in the late 1970s. The capacity of bioemulsifiers to stabilize emulsions by enhancing their kinetic stability has enhanced their application in the petroleum industries [29]. In addition, the TPH removal by several isolates *Acinetobacter baumannii* UKMP-12T, *P. aeruginosa* UKMP-14T, *Rhodococcus* sp. UKMP-5T, and *Rhodococcus* sp. UKMP-7T was also positively correlated to the ability of these bacterial isolates to reduce the surface tension of growth medium [22].

In the literature, the terms biosurfactant and bioemulsifier are considered interchangeable, but although all bioemulsifiers are considered biosurfactants, not all the biosurfactants produce stable emulsions [6]. Some biosurfactant producing bacteria, such as *Rhodococcus erythropolis* and *Rahnella aquatilis* strains isolated from heavily contaminated soil with petroleum hydrocarbons had been reported to have surface-active properties and to produce high emulsification rates and was among the bacterial strains that could degrade hydrocarbons [30]. Microorganism having both properties would be an excellent candidate for a complete and efficient petroleum hydrocarbon biodegradation. The association of the growth for best potential candidate of BSP and BEP with biodegradation of TPH over time has been revealed and previous study by Patowary et al. [5] also reported on the ability of strain PG1 which could degrade 81.8% of TPH; almost similar with the ability of BSP P15. Conclusively, both BSP P15 and BEP P18 are potent hydrocarbon biodegraders and that both biosurfactant and bioemulsifiers are unique microbial products showing advantageous features in hydrocarbon biodegradation.

4. Conclusion

Biosurfactant and bioemulsifier producing bacteria can be isolated from contaminated environmental samples like the petroleum sludge. Not only they produce unique biomolecules that can be harvested for many purposes, they also have the ability to remediate contaminated sites with their biodegradation potential. Therefore, both biosurfactants and bioemulsifiers production are vital in hydrocarbon biodegradation and screening of these two properties will lead to bioprospecting beneficial microorganisms for a lot of industrial purposes. Purified biosurfactants and bioemulsifiers can be used for various applications not limited to clean-up and bioremediation of contaminated environment but also in food and pharmaceutical industries. Once commercialized, biosurfactants and bioemulsifiers can be marketed to suits the needs of industries and also used in community without worrying about the effect they could bring to the environment as they are biodegradable and environmentally friendly in comparison with the synthetic surfactant. This has become a promising method that can be done to improve the effectiveness of hydrocarbon-contaminated environment bioremediation including the oil spill which is the most common pollution occurred.

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