

An Indigenous Biosurfactant Producing *Burkholderia cepacia* with High Emulsification Potential towards Crude Oil

Qadreyah Almatawah*

Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, PO Box 24885 Safat, 13109, Kuwait

Abstract

In this study, the isolation of a potent indigenous biosurfactant-producing bacterium with potential value in enhanced bioremediation of oil-contaminated soil and oil tertiary recovery from the soil was investigated. An indigenous biosurfactant producing Gram-negative-rod-shaped bacterial strain (KISRQC) was isolated from oil-contaminated soil in Kuwait. The strain was able to reduce the surface tension below 41.1 mN/m with all tested hydrocarbons and had excellent emulsification activity toward crude oil. The strain was identified as *Burkholderia cepacia*. Crude biosurfactant extract from this strain was found to contain lipid with 94.9% unsaturated fatty acids. The crude biosurfactant extract was able to reduce the surface tension to 24.6 mN/m at concentration 2 g/l. Characterization of the crude biosurfactant extract activities indicated that it was stable when exposed to high and low temperatures for a reasonable time period (>month) and in the presence of 5% NaCl. The crude biosurfactant extract was effective in recovering 99% of the residual crude oil from oil-saturated sand packs. This study shows the ability of *Burkholderia cepacia* KISRQC to produce a highly stable biosurfactant with the strong emulsification ability towards crude oil from non-toxic material. Suggesting its potential for commercial exploitation for enhanced bioremediation of oil-contaminated soil and oil recovery from the soil.

Keywords: Bioremediation; Biosurfactant isolation; *Burkholderia cepacia*; Crude oil

Introduction

Burkholderia cepacia, earlier known as *Pseudomonas cepacia*, is a motile aerobic oxidase positive Gram-negative bacillus commonly found in moist environments and liquid reservoirs. The strain was first described in 1950 by Burkholder [1] as the cause of soft rot of onions. *B. cepacia* is now gaining increasing interest in agriculture, biotechnology, and medicine. The reason for this particular interest includes the organism's abilities to promote plant growth by antagonizing soil borne plant pathogens [2-4]; to degrade hydrocarbons and thus contribute in the bioremediation of contaminated soil and water [5,6]; and to cause opportunistic human infections, mostly in patients with chronic granulomatous disease [7,8] and cystic fibrosis (CF) [9-12].

B. cepacia can degrade a wide variety of compounds as carbon and energy sources [13], including a variety of aromatic compounds [14,15] pesticides and herbicides [16]. It has the ability to degrade benzo(a) pyrene and other fused ring compounds [17], and biodegradation of heavy crude oil [18]. The organism had shown remarkable potential as an agent for environmental pollution bioremediation.

Biosurfactants are amphiphilic compounds produced on living surfaces frequently on microbial cell surfaces or excreted extracellularly with noticeable surface and emulsifying activities and have been described as anti-adhesive antimicrobial agents [19,20]. They contain hydrophilic and hydrophobic molecules that confer the ability to accumulate between fluid phases, hence reducing surface and interfacial tension at the surface and interface respectively [19,21]. Biosurfactants exhibit a broad variety of chemical structures such as lipopeptides and lipoproteins, glycolipids, lipopolysaccharides, phospholipids, fatty acids, and polymeric lipids [22,23]. In recent years, the importance of biosurfactants has increased remarkably as they became potential candidates for many marketing applications in the petroleum, pharmaceuticals, biomedical, and food processing industries [24]. They have many advantages over chemical surfactants including lower toxicity and higher biodegradability, healthier environmental compatibility, high selectivity, and effectiveness at extreme temperatures, salinities or pH values [24-27]. In the present study, the isolation of a

potent biosurfactant-producing bacterium, *Burkholderia cepacia* strain KISRQC with potential value in microbial enhanced oil recovery is reported. The effects of carbon sources on biosurfactant production and the biosurfactant chemical nature were also investigated. Furthermore, Microbial Enhanced Oil Recovery (MEOR) suitability tests and stability of the biosurfactant to environmental stresses are presented in this study.

Materials and Methods

Bacteria and growth conditions

The microorganism used in this study, code name KISRQC, is a Gram-negative-rod-shaped bacterium isolated from oil-contaminated soil in Kuwait by previously described method [28]. The strain was identified as *Burkholderia cepacia* by MIDI Labs laboratories (USA) using 16S rRNA gene sequence similarity, which was performed using a Perkin-Elmer Applied Biosystem's MicroSeq™ microbial analysis software and database. The sequence determined for the 16S rRNA gene resulted in 99.7% match with *Burkholderia cepacia* (Gene Bank accession no. KY047601). The strain was grown on minimal media containing (g/l distilled water): Na₂HPO₄, 2.7; KH₂PO₄, 1.4; NaNO₃, 3.0; K₂SO₄, 0.36; MgSO₄·7H₂O, 0.15; NaCl, 0.1; FeSO₄·7H₂O, 0.0007; 1 ml/l trace elements solution containing the following (g/l): ZnSO₄·7H₂O, 0.525; MnSO₄·4H₂O, 0.2; CuSO₄·5H₂O, 0.705; Na₂MoO₄·2H₂O, 0.015; CoCl₂·6H₂O, 0.200; H₃BO₃, 0.015; and NiSO₄·6H₂O, 0.027; and 3% yeast extract (pH 7.0). Olive oil was added to the media as a carbon source at a concentration of 3% (v/v). The strain was incubated at 37°C on the

*Corresponding authors: Qadreyah Almatawah, Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, PO Box 24885 Safat, 13109, Kuwait, Tel: 0096524989116; Fax: 0096524956659; E-mail: qmutawa@kisir.edu.kw

Received November 13, 2017; Accepted November 17, 2017; Published November 23, 2017

Citation: Almatawah Q (2017) An Indigenous Biosurfactant Producing *Burkholderia cepacia* with High Emulsification Potential towards Crude Oil. J Environ Anal Toxicol 7: 528. doi: 10.4172/2161-0525.1000528

Copyright: © 2017 Almatawah Q. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

rotary shaker at 250 rev/min for 24-48 h and checked for biosurfactant production by measuring the reduction in culture-broth surface tension with tensiometer.

Carbon source effect determination

To determine the effect of the carbon source on biosurfactant production, KISRQC strain was grown in minimal media containing 3% yeast extract and 3% olive oil and incubated at 37°C for 24 h. The 24 h culture was used to inoculate minimal media containing 3% yeast extract and different carbon source (2% crude oil; 1% diesel; 2% hexadecane; 1% kerosene; 3% olive oil; 3% paraffin; 1% xylene) and incubated for 96 h at 37°C. The growth was observed as an increase in absorbance at 660 nm and converted to g/l based on a predetermined calibration curve. After the incubation period, the broths surface tensions, emulsion indexes, and cell concentrations were measured.

Surface activity measurement

Surface tension and critical micelle concentration (CMC) were measured by the Du Nouy ring method [29] using Kruss K10T tensiometer (Kruss, Optische-Mechanische Werlostatten, Hamburg, W. Germany) with a 6 cm diameter platinum-iridium ring. The CMC is defined as the surfactant concentration necessary to initiate micelle formation. There will be no further decrease in the surface tension upon reaching the CMC when an additional surfactant is present. CMC was determined by diluting the whole culture broth and measuring the surface tension of each dilution [30,31]. The reciprocal of CMC is proportional to the total surface-active compound amount present in the solution and can be used as an approximate measure of biosurfactant concentration.

Determination of emulsification activity

The emulsification activity of the biosurfactant was determined by the addition of 6 ml hexadecane to 4 ml of the test broth or biosurfactant in a 15-ml graduated tube. The mixture was mixed vigorously for 2 min and allowed to stand for 24 h at room temperature. The emulsification index (E_{24}) was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying it by 100 [29,32,33].

Dry cell weight measurement

Biomass dry weights were determined by measuring the culture optical density at 660 nm, and then centrifugation of 100 ml sample of the culture broths at 10,000 xg for 10 min. The pellet was washed twice with distilled water, re-suspended in 10 ml of distilled water, filtered through a pre-washed and weighed 7-cm-diameter Whatman No.1 filter paper, placed on a pre-weighed aluminum dish, dried at 105°C overnight, and reweighed after 24 h. A standard curve was constructed from the dry cell weights and their corresponding OD_{660} values.

Biosurfactant extraction

Cells in culture broth were disrupted by sonication for 15 min using an MSE 150 W ultrasonic disintegrator at 14 amplitude and centrifuged at 15,000 xg for 15 min at 0°C. The hydrophobic layer located at the surface was extracted using the methyl tertiary-butyl ether (MTBE)-chloroform (1:1) solvent system. The solvent layer was separated from the aqueous phase and removed by rotary evaporation at 50°C under reduced pressure [34]. The resulting crude extract was stored at -20°C for later analysis.

GC analysis

The fatty acid composition of the crude biosurfactant extracts was analyzed by Hewlett Packard HP5890 gas chromatograph, equipped with a flame ionization detector and a capillary column fused with methyl silicone (50 m length, 0.25 mm internal diameter). The operating temperature of the detector was 300°C, and that of the injector was 280°C. The column temperature was set at 120°C for the first 5 min, then increased to 200°C at a rate of 5°C/min and then increased again to 225°C at the rate of 4°C/min until the final time (35 min).

Stability studies

The effects of several environmental parameters on the surface activity of the biosurfactant were determined. NaCl stability was determined by dissolving the crude biosurfactant in 5% NaCl (200 μ l/25 ml), and the surface tension was then measured. To determine the heat stability of the surface active compounds, the culture broth, the culture supernatant, the cell suspension, and the biosurfactant crude extract (200 μ l in 25 ml 0.9% NaCl) were heated at 100°C for 15 min and allowed to cool to room temperature, and the surface tension was then measured and compared to the corresponding values before heat treatment. Time stability was measured by incubating the biosurfactant crude extract at two different temperatures for five weeks, and the surface tension was then measured at one week intervals and compared to the corresponding values.

Sandpack test

A glass column (50 ml volume) was packed with acid washed sand (25-60 mesh 30 g). The column was then saturated with 6 ml crude oil and flooded with pore volumes of water until no further oil was released. The remaining residual oil was flooded with crude biosurfactant as a continuous flood of at least three pore volumes and released residual oil percentage was measured.

Results

Effect of carbon source on biosurfactant production

Table 1 showed the effect of carbon source on biosurfactant production. There were significant differences between the control and other tested carbon sources in the surface tension value. The surface tensions were below 41.1 mN/m for all tested carbon sources. Surface tension decreased from 41.1 to 27 mN/m, with the greatest reduction (34%) observed in culture enriched with 3% olive oil. Moreover, cell concentration was increased from 2.1 g/l to 20.4 g/l with olive oil enriched culture. Crude oil also showed an increase in cell concentration from 2.1 g/l to 17.1 g/l. The best emulsion index was 70% when olive oil used as carbon source. The ability of culture broth enriched with 3% olive oil to emulsify crude oil was tested. The broth was found to be able to emulsify crude oil (Figure 1).

Carbon Source	ST(mN/m)	E_{24} (%)	Cell Concentration (g/l)
Control*	41.1 \pm 0.3	30.0 \pm 0	2.1 \pm 0.08
Crude oil	31.3 \pm 0.7	25.0 \pm 15	17.1 \pm 1.6
Diesel	30.9 \pm 0.4	8.5 \pm 3.5	5.8 \pm 0.8
Hexadecane	30.0 \pm 0.3	7.5 \pm 2.5	4.4 \pm 0.7
Kerosen	33.1 \pm 0.45	5.5 \pm 0.5	1.8 \pm 0.4
Olive oil	27.1 \pm 0.05	70.0 \pm 0	20.4 \pm 0.2
Paraffin	30.6 \pm 0.35	10.0 \pm 5	7.1 \pm 0.1
Xylene	32.0 \pm 1.8	0	1.7 \pm 0.2

*Control containing minimal media, 3% yeast extract and bacteria

Table 1: Surface tensions (ST), emulsion index's (E_{24}) and cells concentrations for KISRQC strain grown on different carbon sources.

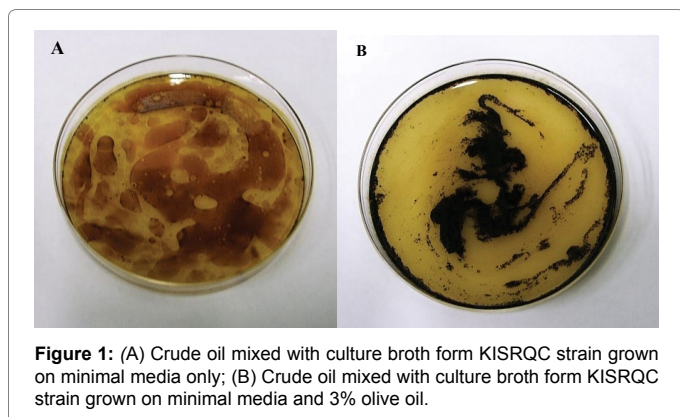


Figure 1: (A) Crude oil mixed with culture broth from KISRQC strain grown on minimal media only; (B) Crude oil mixed with culture broth from KISRQC strain grown on minimal media and 3% olive oil.

Effect of incubation period on biosurfactant production

The surface tension was monitored during the growth cycle of KISRQC strain to determine the optimum incubation period. The surface tension in the culture medium was found to be proportional to culture turbidity and biomass production (Figure 2). The reduction of culture surface tension occurred during the exponential growth in batch culture. The lowest value of surface tension (26.8 ± 0.1 mN/m) was recorded 96 h after culture inoculation.

Biosurfactant isolation

To estimate the effectiveness of the biosurfactant concentrates, samples of culture broth were taken at the logarithmic phase of growth (48 hr) and extracted according to the method described earlier. The concentrates obtained were re-dissolved in 0.9% NaCl, and the surface tensions for a series of dilutions of them were measured (Figure 3). The biosurfactant crude extract was found to reduce the surface tension to 24.6 ± 0.1 mN/m at a concentration of 2 g/l.

Biosurfactant characterization

The gas chromatography (GC/FID) analysis of the biosurfactant crude extract based on fatty acid showed that it contains saturated, and unsaturated fatty acids with the most predominate was the unsaturated fatty acid (Table 2). The fatty acid composition was C18:1 Trans (88%), C18:2 Trans (5.6%); C17:1 (5.0%), C18:3A (1.03%), C16:0 (0.32%), and C14:0 (0.12%). The biosurfactant crude extract was found to contain 14.4 g/l lipid.

Biosurfactant characterization

The crude biosurfactant produced was characterized according to five criteria: the capacity to reduce surface tension, heat stability, NaCl stability, time stability, and the ability to mobilize oil from sand pack. The isolated crude biosurfactant was able to reduce the surface tension to 24.6 ± 0.1 mN/m. Table 3 shows the results of experiments on the effect of heat treatment on the biosurfactant activity for KISRQC culture. No significant change in biosurfactant activity occurred when culture broth, supernatant, cells, and biosurfactant crude extract were exposed to heat treatment. The properties of the biosurfactant present in all tested samples, i.e., surface tension, remained stable after exposure to high temperatures of 100°C for 15 min.

Experimental results on the effect of 5% NaCl on the surface tension and the emulsification ability of the biosurfactant on crude oil showed no significant change of the surface tension value (27.1 ± 0.6 mN/m) and emulsification activity (Figure 4). The crude biosurfactant reduced 62.3% of the 5% NaCl solution original surface tension. The

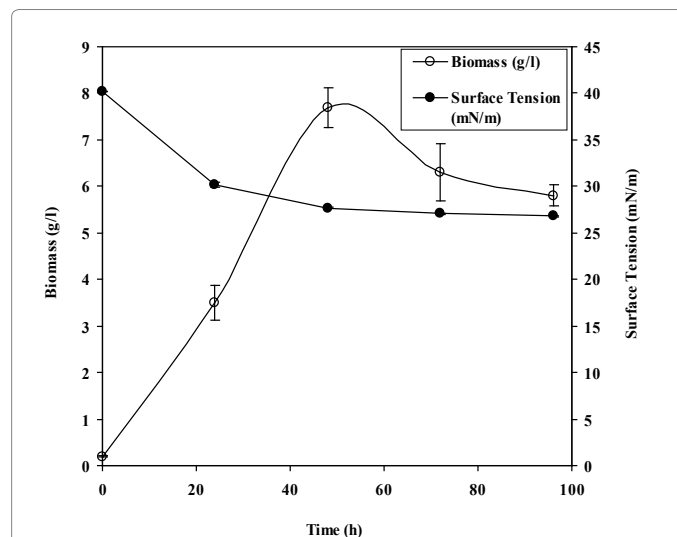


Figure 2: Growth curve for KISRQC strain showing the effect of incubation period at 37°C on biomass and culture broth surface tension.

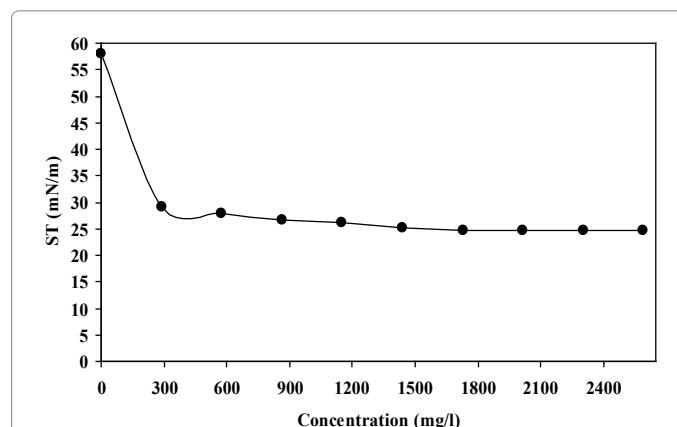


Figure 3: A plot for the surface tension versus the biosurfactant crude extract concentration.

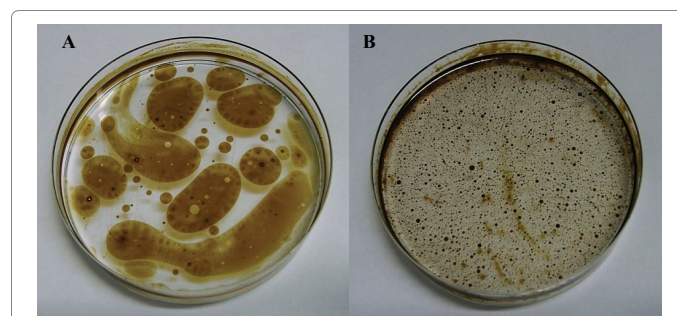


Figure 4: (A) Crude oil mixed with 5% NaCl solution only; (B) Crude oil mixed with 5% NaCl solution and 200 µl biosurfactant crude extract.

biosurfactant properties remained stable for a reasonable time period under low temperature (4°C) and high temperature (45°C). After five weeks, the crude biosurfactant was able to reduce the surface tensions to 28.6 ± 0.1 mN/m and 28.3 ± 0.2 mN/m at 4°C and 45°C respectively. In sand pack experiments, the crude biosurfactant was found to be able to elute nearly 99% of the crude oil from the sand packs (Figure 5).

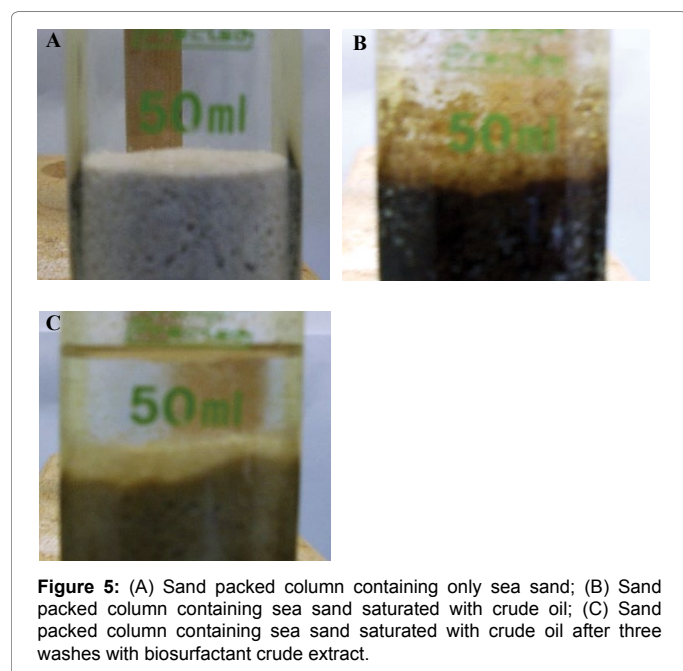


Figure 5: (A) Sand packed column containing only sea sand; (B) Sand packed column containing sea sand saturated with crude oil; (C) Sand packed column containing sea sand saturated with crude oil after three washes with biosurfactant crude extract.

Fatty Acid Profile*	Area %
C14	0.12
C16	0.32
C17:1	5.0
C18:1 Trans	88
C18:2 Trans	5.6
C18:3 A	1.03

*C14 (Myristic); C16 (Palmitic); C17:1 (cis-10-Heptadecenoic); C18:1 Trans (Elaidic); C18:2 Trans (Linolelaidic); C18:3 A (α -Linolenic)

Table 2: Fatty acid composition for the crude KISRQC strain biosurfactant (% of total fatty acids).

Biosurfactant	ST (mN/m) Before Heat Treatment	ST (mN/m) After Heat Treatment (100°C/15 min)
Culture Broth	28.4 ± 0.07	26.9 ± 0.04
Culture Supernatant	27.6 ± 0.07	26.6 ± 0.14
Cell Suspension	27.8 ± 0.07	26.0 ± 0
Biosurfactant Crude Extract	27.2 ± 0.32	27.8 ± 0.21

Table 3: Heat stability for KISRQC strain biosurfactant.

Discussion

In recent years, several studies have shown that a number of *Burkholderia* species such as *Burkholderia thailandensis* [34,35], *Burkholderia glumae* [36], *Burkholderia kururiensis* [37], *Burkholderia plantarii* [38], and *Burkholderia gladioli* [39] were able to produce biosurfactant. However, the ability of *Burkholderia cepacia* to produce a biosurfactant was not sufficiently documented in the literature. *Burkholderia cepacia* was reported as one of the most predominant culturable species in crude oil and for their ability to degrade both aliphatic and aromatic hydrocarbons [40]. Limited studies have focused on the production of biosurfactant by *Burkholderia cepacia*. One study, show the inability of *Burkholderia cepacia* to produce a biosurfactant [41] and other studies showed its ability to produce a biosurfactant [42,43]. A study on the bioavailability of non-aqueous phase liquids (NAPLs) for *Burkholderia cepacia*, provided no evidence for the release

of extracellular biosurfactants [41]. The study interrelated the release of lipopolysaccharides (LPS) in culture media for enhancement of cell surface hydrophobicity and adherence affinity to NAPLs. Another study reported the ability of *Burkholderia cepacia* MFW3 to produce biosurfactant when grown on whey waste water [42]. The biosurfactant was characterized as lipopeptide structure and reduced the surface tension by 12%, with maximum emulsification activity of 59% with mineral oil. Moreover, a study also reported the ability of *Burkholderia cepacia* to produce an active surface agent [43]. In this study, the ability of *Burkholderia cepacia* KISRQC to produce an active biosurfactant is documented. The biosurfactant reduced the surface tension by 34%, with maximum emulsification activity of 70% with hexadecane. Which is better than the previously reported results [42]. Furthermore, olive oil was found the best carbon source for the biosurfactant production. *Pseudomonas fluorescens* Migula 1895-DSMZ was reported to produce rhamnolipid biosurfactant with the highest yield when olive oil used as a carbon source [44]. Plant-derived oils have been documented as an excellent carbon substrates for biosurfactant production by *Pseudomonas aeruginosa* [45,46]. The *Burkholderia gladioli* culture produced most biosurfactant when enriched with 5% corn oil [39]. *Burkholderia thailandensis* produced a series of long chain rhamnolipids when grown in canola oil [35]. Although, diesel and hexadecane was also found to enhance biosurfactant production by *Burkholderia cepacia* KISRQC, one study reported the opposite [41]. This may be related to the application of different growth conditions.

The production of *Burkholderia cepacia* KISRQC biosurfactant is 2 g/l when grown at 37°C. This is nearly similar to the production of *Burkholderia thailandensis* E264 biosurfactant, 1.99 g/l biosurfactant when grown at 30°C [35]. *Burkholderia thailandensis* E264 culture grown at 30°C generated a dry cell biomass throughout the growth period with 7.71 g/l after 264 h, whereas *Burkholderia cepacia* KISRQC culture grown at 37°C generated a dry cell biomass throughout the growth period with 7.7 g/l after 48 h. *Burkholderia thailandensis* E264 was found to produce di-rhamnolipids with C14-C14 chain length fatty acid moiety in most abundance (41.88%) and others contained chains ranging from C10-C12 to C16-C16 chain length [34]. The potential of the long-chain rhamnolipids produced by *Burkholderia* species for lowering surface tension and decreasing the CMC was demonstrated [34]. Sophorolipids, a class of biosurfactants produced by *Candida bombicola*, was correlated its decreasing of the CMC to carbon chain length. The additional CH₂ groups were found to render the molecule more hydrophobic and facilitate micelle formation [47]. The ability of *Pseudomonas aeruginosa* to reduce the surface tension to 28.1 mK/m with maximum emulsification activity of 70% in culture medium enriched with 2% corn oil was reported [39]. Whereas, *Burkholderia gladioli* was found to reduce the surface tension to 45.4 mK/m with maximum emulsification activity of 69% in culture medium enriched with 5% corn oil. In this study, we reported the ability of *Burkholderia cepacia* KISRQC to reduce the surface tension to 27 mK/m with maximum emulsification activity of 70% in culture medium enriched with 3% olive oil. Most of the reported biosurfactant from *Burkholderia* species were rhamnolipids that have long alkyl chains than those produced by *Pseudomonas aeruginosa* [48]. Interestingly, *Burkholderia cepacia* KISRQC biosurfactant crude extract was found to contain 14.4 g/l lipid with C18 unsaturated fatty acids in the highest abundance (94.9%) followed by C17 (5%), C16 (0.32%) and C14 (0.12%). The highest abundance of C18 fatty acid in *Burkholderia cepacia* KISRQC biosurfactant means that the biosurfactant structure is different from the reported biosurfactant from *Burkholderia* species.

The isolated crude biosurfactant was able to reduce the surface tension, stable after exposure to high temperatures of 100°C, and when incubated at 4°C and 45°C for five weeks. The production of biosurfactant was in the exponential growth phase, which indicates that the production of biosurfactant is a primary product of metabolism and suggests that it would be possible to be produced effectively in a continuous process. Since Kuwaiti soil contains high concentrations of sodium chloride and other salts, it is essential that a biosurfactant should be effective under these conditions. The surface tension and the emulsification ability of the biosurfactant on crude oil was found stable at 5% NaCl. Moreover, the crude biosurfactant extract was effective in recovering 99% of the residual crude oil from oil-saturated sand packs. Several researchers have been using sand pack columns for evaluation of surfactant suitability for enhanced oil recovery [33,49,50].

So far, biosurfactants used has been limited due to the high cost of raw materials and processing. An inexpensive hydrophobic materials sources like vegetable oils or waste cooking oil and carbohydrates is a good approach for reducing industrial waste generation [51]. *B. cepacia* KISRQC seems a promising strain for large-scale biosurfactant production since it can grow on no toxic material such as olive oil; which is an advantage from an industrial point of view. Moreover, its biosurfactant showed a strong emulsification ability toward crude oil, suggesting its potential for commercial exploitation for enhanced bioremediation of oil-contaminated soil and oil recovery from the soil.

Acknowledgements

This research work was funded by the Kuwait Institute for Scientific Research (KISR).

References

- Burkholder WH (1950) Sour skin, a bacterial rot of onion bulbs. *Phytopathology* 40: 115-117.
- Hebbar KP, Davey AG, Dart PJ (1992) Rhizobacteria of maize antagonistic to *Fusarium moniliforme*, a soil-borne fungal pathogen: isolation and identification. *Soil Biology and Biochemistry* 24: 979-987.
- Hebbar KP, Davey AG, Merrin J, McLoughlin TJ, Dart PJ (1992) *Pseudomonas cepacia*, a potential suppressor of maize soil-borne diseases-seed inoculation and maize root colonization. *Soil Biology and Biochemistry* 24: 999-1007.
- McLoughlin TJ, Quinn JP, Bettermann A, Bookland R (1992) *Pseudomonas cepacia* suppression of sunflower wilt fungus and role of antifungal compounds in controlling disease. *Appl Environ Microbiol* 58: 1760-1763.
- Folsom BR, Chapman PJ, Pritchard PH (1990) Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. *Appl Environ Microbiol* 56: 1279-1285.
- Okoh A, Ajisebutu S, Babalola, G, Trejo-Hernandez, MR (2001) Potential of *Burkholderia cepacia* RQ1 in the biodegradation of heavy crude oil. *Int Microbiol* 4: 83-87.
- Lacy DE, Spencer DA, Goldstein A, Weller PH, Darbyshire P (1993) Chronic granulomatous disease presenting in childhood with *Pseudomonas cepacia* septicaemia. *J Infect* 27: 301-304.
- O'Neil KM, Herman JH, Modlin JF, Moxon ER, Winkelstein JA (1986) *Pseudomonas cepacia*: an emerging pathogen in chronic granulomatous disease. *J Pediatr* 108: 940-942.
- Anonymous edn (1992) *Pseudomonas cepacia* - more than a harmless commensal? *Lancet* 339: 1385-1386.
- Govan JR, Deretic V (1996) Microbial pathogenesis in cystic fibrosis: *Mucoid Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 60: 539-574.
- Isles A, MacLuskey I, Corey M, Gold R, Prober C, et al. (1984) *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 104: 206-210.
- Simmonds EJ, Conway SP, Ghoneim ATM, Ross H, Littlewood JM (1990) *Pseudomonas cepacia*: a new pathogen in patients with cystic fibrosis referred to a large centre in the United Kingdom. *Arch Dis Child* 65: 874-877.
- Lessie TG, Gaffney T (1986) Catabolic potential of *Pseudomonas cepacia*, pp: 439-448.
- Grifoll M, Selifonov SA, Gatlin CV, Chapman PJ (1995) Actions of a versatile fluorene-degrading bacterial isolate on polycyclic aromatic compounds. *Appl Environ Microbiol* 61: 3711-3723.
- Revathy T, Jayasri MA, Suthindhiran K (2015) Biodegradation of PAHs by *Burkholderia* sp. VITRSB1 isolated from marine sediments. *Scientifica*.
- Bhat MA, Tsuda M, Horiike K, Nozaki M, Vaidyanathan CS, et al. (1994) Identification and characterization of a new plasmid carrying genes for degradation of 2,4-dichlorophenoxyacetate from *Pseudomonas cepacia* CSV90. *Appl Environ Microbiol* 60: 307-312.
- Juhász AL, Britz ML, Stanley GA (1997) Degradation of benzo(a)pyrene, dibenz(a,h)anthracene and coronene by *Burkholderia cepacia*. *Water Sci Technol* 36: 45-51.
- Velraeds MC, Van der Mei HC, Reid G, Busscher HJ (1996) Physicochemical and biochemical characterization of biosurfactants released from *Lactobacillus* strains. *Colloids Surf. B Biointerfaces* 8: 51-61.
- Van Hamme JD, Singh A, Ward OP (2006) Physiological aspects: Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. *Biotechnol Adv* 24: 604-620.
- Rodrigues LR, Teixeira JA, Van der Mei HC, Oliveira R (2006) Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. *Colloids Surf. B Biointerfaces* 49: 79-86.
- Ahimou F, Jacques P, Deleu M (2000) Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. *Enzyme Microb Technol* 27: 749-754.
- Maier R, Soberon-Chavez G (2000) *Pseudomonas aeruginosa* rhamnolipids: Biosynthesis and potential applications. *Appl Microbiol Biotechnol* 54: 625-633.
- Desai J, Banat I (1997) Microbial production of surfactants and their commercial potential. *Microbiol Mol Biol Rev* 61: 47-64.
- Banat I, Franzetti A, Gandolfi I, Bestetti G, Martinotti M, et al. (2010) Microbial biosurfactants production, applications and future potential. *Appl Microbiol Biotechnol* 87: 427-444.
- Cameotra SS, Makkar RS (1998) Synthesis of biosurfactants in extreme conditions. *Appl Microbiol Biotechnol* 50: 520-529.
- Cameotra SS, Makkar RS, Kaur J, Mehta SK (2010) Synthesis of biosurfactants and their advantages to microorganisms and mankind. *Adv Exp Med Biol* 672: 261-280.
- Guerinik K, Al-Mutawah Q (2003) Isolation and characterization of oil-desulphurizing bacteria. *World J Microbiol Biotechnol* 19: 941-945.
- Abu-Ruwaida AS, Banat IM, Haditirto S, Salem A, Kadri M (1991) Isolation of biosurfactant-producing bacteria product characterization, and evaluation. *Acta Biotechnol* 11: 315-324.
- Carrillo PG, Mardaraz C, Pitta-Alvarez SI, Giulietti AM (1996) Isolation and selection of biosurfactant-producing bacteria. *World J Microbiol Biotechnol* 12: 82-84.
- Kim SH, Lim EJ, Lee SO, Lee JD, Lee TH (2000) Purification and characterization of biosurfactants from *Nocardia* sp. L-417. *Biotechnol Appl Biochem* 31: 249-253.
- Cooper DG, Goldenberg BG (1987) Surface-active agents from two *Bacillus* species. *Appl Environ Microbiol* 53: 224-229.
- Mohebbi G, Ball A, Kaytash A, Rasekh B (2007) Stabilization of water/gas oil emulsions by desulfurizing cells of *Gordonia alkanivorans* RIP190A. *Microbiology* 153: 1573-1581.
- Kuyukina MS, Ivshina IB, Philp JC, Christofi N, Dunbar SA, et al. (2001) Recovery of *Rhodococcus* biosurfactants using methyl tertiary-butyl ether extraction. *J Microbiol Methods* 46: 149-156.
- Dubeau D, Déziel E, Woods DE, Lépine F (2009) *Burkholderia thailandensis* harbors two identical *rhl* gene clusters responsible for the biosynthesis of rhamnolipids. *BMC Microbiol* 9: 263.
- Funston SJ, Tsaousi K, Rudden M, Smyth TJ, Paul SS, et al. (2016) Characterising rhamnolipid production in *Burkholderia thailandensis* E264, a non-pathogenic producer. *Appl Microbiol Biotechnol* 100: 7945-7956.

36. Costa SG, Déziel E, Lépine F (2011) Characterization of rhamnolipid production by *Burkholderia glumae*. Lett Appl Microbiol 53: 620-627.
37. Tavares LFD, Silva PM, Junqueira M, Mariano DCO, Nogueira FCS, et al. (2013) Characterization of rhamnolipids produced by wild-type and engineered *Burkholderia kururiensis*. Appl Microbiol Biotechnol 97: 1909-1921.
38. Hoermann B, Müller M, Syldatk C, Hausmann R (2010) Rhamnolipid Production by *Burkholderia plantarii* DSM 9509T. Eur J Lipid Sci Technol 112: 674-680.
39. Catter KM, de Oliveira DF, de Sousa OV, Barros Gonçalves LR, dos Fernandes Vieira RHS, et al. (2016) Biosurfactant production by *Pseudomonas aeruginosa* and *Burkholderia gladioli* isolated from mangrove sediments using alternative substrates. Orbital: Electron J Chem 8: 269-275.
40. Yoshida N, Yagi K, Sato D, Watanabe N, Kuroishi T, et al. (2005) Bacterial communities in petroleum oil in stockpiles. J Biosci Bioeng 99: 143-149.
41. Mohanty G, Mukherji S (2008) Enhancement of NAPL bioavailability by induction of cell-surface hydrophobicity in *Exiguobacterium aurantiacum* and *Burkholderia cepacia*. Indian J Biotechnol 7: 295-30.
42. Yilmaz F, Ergene A, Yalçın E, Tan S (2009) Production and characterization of biosurfactants produced by microorganisms isolated from milk factory wastewaters. Environ Technol 30: 1397-1404.
43. Ajao AT, Yakubu SE, Umoh VJ, Ameh JB (2014) Enzymatic studies and mineralization potential of *Burkholderia cepacia* and *Corynebacterium kutscheri* isolated from refinery sludge. J Microbiol Res 4: 29-42.
44. Abouseouda M, Maachib R, Amranec A, Boudergua S, Nabia A (2008) Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination 223: 143-151.
45. Rahman KS, Rahman TJ, McClean S, Marchant R, Banat IM (2002) Rhamnolipid biosurfactant production by strains of *Pseudomonas aeruginosa* using low-cost raw materials. Biotechnol Prog 18: 1277-1281.
46. Robert M, Mercadé ME, Bosch MP, Parra JL, Espuny MJ, et al. (1989) Effect of the carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44T1. Biotechnol Lett 11: 871-874.
47. Zhang L, Somasundaran P, Singh SK, Felse AP, Gross R (2004) Synthesis and interfacial properties of sophorolipid derivatives. Colloids Surf A Physicochem Eng Asp 240: 75-82.
48. Díaz De Rienzo MA, Kamalanathan ID, Martin PJ (2016) Comparative study of the production of rhamnolipid biosurfactants by *B. thailandensis* E264 and *P. aeruginosa* ATCC 9027 using foam fractionation. Process Biochem 51: 820-827.
49. Bordoloi NK, Konwar BK (2008) Microbial surfactant-enhanced mineral oil recovery under laboratory conditions. Colloids Surf B: Biointerfaces 63: 73-82.
50. Gudiña EJ, Pereira JFB, Costa Rita, Coutinho JAP, Teixeira JA, et al. (2013) Biosurfactant-producing and oil-degrading *Bacillus subtilis* strains enhance oil recovery in laboratory sand-pack columns. J Hazard Mater 261: 106-113.
51. Sarrubo LA, Farias CBB, Campos-Takaki GM (2007) Co-utilization of canola oil and glucose on the production of a surfactant by *Candida lipolytica*. Curr Microbiol 54: 68-73.