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PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANT PRODUCED BY OCHROBACTRUM ANTHROPI 2/3 USING DURIAN SEED POWDER AS A NOVEL SUBSTRATE

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ABSTRACT

Ochrobactrum anthropi 2/3 is a soil bacterial strain isolated from mangrove sediment collected from Satun Province in the south of Thailand. It produces promising yield of biosurfactant in mineral salts medium (MSM). Cellular growth and biosurfactant production in MSM were greatly affected by the medium components (carbon and nitrogen sources). Optimum carbon source was durian seed powder (45 g/L). The optimum nitrogen source was commercial monosodium glutamate (1.0 g/L). The MSM combining the optimum medium components was formulated and resulted in more than 14.1 folds increased in biosurfactant productivity (4.10 g/L or 0.08 g/L/h). The biosurfactant showed high surface tension reduction (43.5 mN/m) and emulsification activity toward xylene (55%). It also had a small critical micelle concentration value (10 mg/L), thermal and pH stability with activity of surface tension and emulsification and a high level of salt tolerance.

Keywords: Production; Characterization; Biosurfactant; Ochrobactrum anthropi; Durian seed

INTRODUCTION

Surface active compounds or surfactants are widely used in several fields including agriculture, cosmetic, food, pharmaceutical, environmental and petroleum industries [1]. Most of these compounds are chemically synthesized and are produced from petroleum-based industry. It is only in the past few decades that biosurfactants or surface active molecules from microor-ganisms have been described [2]. The demand of biosurfactants is steadily increas-ing since these compounds must be either environmental friendly or effective [1-2]. The advantages of biosurfac-tants over chemical surfactants include they possess high superficial activity and effec-tiveness at extreme temperatures, pH and salinity. Besides, it is being biodegradable, less in toxicity and the ability to be synthesized from renewable substrates [1-3]. Despite the advantages, the production of biosurfactant on a commercial scale has not been realized because of their relatively high production costs. In order to alleviate this problem, the choice of low-cost or cost-free raw materials is important to the overall economy of the process as they account for major portion of the final production cost [4]. Therefore, a possible strategy to reduce costs is the use of inexpen-sive substrates such as wastes or agroindustrial by products which generally contain the high levels of carbohydrates and lipids to support growth and biosynthesis of biosurfactants [2, 4]. Durain (Durio zibethinus) seed is an

example of an inexpen-sive substrate, since it is an agro-industrial residue which is usually discarded.

Durian, usually called as 'King of Fruit', is a valuable tropical fruit in some Asian countries. In 2012, production of durians in Thailand was more than one million tons [5]. Seeds make up around 5-15% of the total fruit mass. According to Srianta [6], fresh durian seeds contain high moisture (51.5%), carbohydrate (43.6%) and protein (2.6%). However, there is no research on the potentiality of durian seed as a substrate for biosurfactant production. The objective of the research was to study the durian seed powder as a new substrate for biosurfactant produc-tion from mangrove isolated Ochrobactrum anthropi 2/3.O. anthropi 2/3 used in this study was isolated and preliminarily characterized as a biosurfactant-producing strain as reported earlier [4].

MATERIALS AND METHODS

Microbial strain

Ochrobactrum anthropi 2/3 (accession number AB542934) was isolated from mangrove sediment collected from Thungwa sediment, Satun Province in the south of Thailand, during a screening study of biosurfactant-producing bacteria in mangrove sediment [4]. The entire 16S rRNA gene sequence from *O. anthropi* 2/3 showed 100 % homology to *O. anthropi* (accession number GQ368700). *O. anthropi* 2/3 was maintained on nutrient agar plates and transferred monthly.

Raw material

Durian seeds obtained from a local market in Phuket Province, Thailand were used as a substrate. Durian seeds were pre-treated to remove the sticky mucus by boiling in a $CaCO_3$ solution of 5% (w/v) for 10 min [6]. After pre-treatment, durian seeds were sliced into thin chips, dried at 60°C for 24 h and then ground into durian seeds powder (DSP). Proximate and starch content analysis of DSP were conducted by standard methods of AOAC [7].

Media and cultivation conditions

Nutrient broth was used for preparation of the inoculum. The composition of the nutrient broth used was as follows: beef extract 1.0 g; yeast extract 2.0 g; peptone 5.0 g; NaCl 5.0 g in a liter of distilled water. To make nutrient agar, 15.0 g of agar was added to the nutrient broth. The strain was cultivated in nutrient broth for 24 h at 30°C. This was used as inoculum at the 6% (v/v) level. For biosurfactant production, a mineral salt medium (MSM) with the following composition (g/L) was utilized: K_2HPO_4, 0.8; KH_2PO_4, 0.2; $CaCl_2$, 0.05; $MgCl_2$, 0.5; $FeCl_2$, 0.01; NaCl, 5.0 [4]. The pH of the medium was adjusted to 7.0. Carbon and nitrogen sources were added separately. Cultivation was performed in 250 mL flasks containing 50 mL medium at 30°C, and shaking in a rotary shaker at 150 rpm for 48 h.

Medium optimization

Four factors (DSP concentration (C), nitrogen source (N), C:N ratio and inoculum concentration) were used to obtain higher productivity of biosurfactant from *O. anthropi* 2/3. The concentration of DSP was varied (5-40%, w/v) by using $(NH_4)_2SO_4$ as a N source. For evaluation of the most appropriate N-source, $(NH_4)_2SO_4$, commer-cial monosodium glutamate (CMSG), NH_4Cl, $NaNO_3$ and peptone were used (conc., 1 g/L) with optimum concentration of DSP. The C:N ratio was varied (5-50) by keeping a constant N-source (conc., 1 g/L).

Recovery of biosurfactant

Four solvent systems; a mixture of chloroform: methanol (2:1), cold acetone, dichloromethane and ethyl acetate were examined for biosurfactant extraction [4]. The solvent showing the highest biosurfactant activity was chosen to recover biosurfactant from *O. anthropi* 2/3.

Stability of biosurfactant

The biosurfactant at critical micelle concentration (CMC) level in distilled water was prepared. To investigate the effects of pH, sodium chloride (NaCl) concentrations and temperature on biosurfactant activity, the biosurfactant solution was adjusted with 1.0 N HCl or NaOH to obtain the pHs of 2.0-12.0. NaCl was added to the sample to obtain the final concentrations of 1.0-11.0% (w/v). For thermal stability study, the biosurfactant solution was incubated at 4-80ºC for 24 h, 100°C for 1 h and at 121°C for 15 min and cooled to 25°C. The remaining activity was then determined.

Chemical analysis of biosurfactant

The chemical nature of the obtained biosurfactant was determined with thin layer chromatography (TLC). The obtained biosurfactant was spotted in triplicate on readymade silica gel TLC plates (Merck, Darmstadt, Germany) using $CH\text{-}Cl_3$:CH_3OH:H_2O (65:15:1,v/v) as solvent sys-tem. One of the plates was put into a jar saturated with rhodamine B reagent to detect lipids. Another plate was sprayed with anisaldehyde and ninhydrin reagent (0.2% ninhydrin solution in acetone) and dried. It was then heated at 120°C, 5 min for detection of sugars and peptides, respectively. Fourier transform infrared spectroscopy (FT-IR) of the obtained biosurfactant was done on a Nexus-870 FT-IR spectrometer (Thermo Electron Co., Yokohama, Japan) by the KBr pellet method. The dried biosurfactant samples (0.5 mg) were ground in about 80 mg of spectral grade KBr (Merck, Darmstadt, Germany) and pressed into pellets under about 6 tons/cm^2 pressure with the help of a hydraulic press (Specac, Orpington, Kent, UK).

Analytical methods

Bacterial cell growth was estimated using dry cell weight method. At different times of fermentation, samples were mixed in pre-weighted tubes with chilled distilled water and centrifuged at 8,500 rpm for 30 min. The biomass obtained was dried overnight at 105° and weighed [8].

Emulsification activity was performed accordingly to Satpute et al. [2]. Briefly, 4 mL of motor oil was added to 4 mL of aqueous solution of culture supernatant in a screw cap tube, and vortexed at high speed for 2 min. The emulsion stability was determined after 24 h, and the E24 was calculated by dividing the measured height of emulsion layer by the mixtures total height and multiplying by 100.

Surface tension was measured using a Model 20 Tensiometer (Fisher Science Instrument, PA, USA) at 25°C. CMC was determined by plotting the surface tension versus concentration of biosurfactant in the solution. All experiments were carried out in triplicate for the calculation of the mean value. Two well-defined synthetic surfactants, SDS and Tween80 at CMC (18 and 2 mg/mL, respectively) were used as positive controls, while distilled water and MSM medium were used as negative controls. All chemicals used were of analytical grade. Statistical analysis was performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of durian seeds powder concentration on growth and biosurfactant production

The result showed that durian seeds powder concentration affected the biosurfac-tant production of *O. anthropi* 2/3 (Table 1). The amount of bio-mass and biosurfactant production increased with increasing durian seeds powder concentration. Increasing the durian seeds powder concentration from 5 to 35% (w/v) resulted in an increase of more than 4.1 fold (0.90 to 3.68 g/L) and 11.7 folds (0.29 to 3.40 g/L) of biomass and biosur-factant yield, respectively. Thereafter, there was no marked change in biomass (3.68 to 3.70 g/L) and biosurfactant yield (3.40 to 3.41 g/L). This result suggested that 35% (w/v) was an optimal durian seeds powder concentration for growth and biosurfactant production of *O. anthropi* 2/3.

Table 1 Effect of carbon (C) concentrations, nitrogen (N) source and C:N ratio on growth and biosurfactant production by *Ochrobactrum anthropi* 2/3, cultivated in 250 mL flask containing 50 mL MSM at 30ºC in a shaking incubator at 200 rpm for 48 h

Factor	DCW (g/L)*	ST (mN/m)*	BS (g/L) *	EA (%) *
Durian seeds powder (g/L); [N-source, 1 g/L $(NH_4)_2SO_4$)]				
5	$0.90\pm0.02^{g**}$	68.2 ± 1.1^{a}	0.29 ± 0.01^{g}	15.15 ± 2.10^{g}
10	1.21 ± 0.54^{f}	65.8 ± 1.4^{b}	0.55 ± 0.02^{f}	20.32 ± 2.37^{f}
15	1.59 ± 0.01^{e}	57.5 ± 2.1^{c}	1.08 ± 0.03^{e}	24.24 ± 4.88^{e}
20	1.91 ± 0.11^{d}	52.0 ± 3.2^{d}	1.78 ± 0.02^{d}	30.47 ± 4.15^{d}
25	2.19 ± 0.15^{c}	48.6 ± 1.5^{e}	2.86 ± 0.05^{c}	33.18 ± 0.75^{c}
30	2.50 ± 0.12^{b}	43.4 ± 1.2^{f}	3.11 ± 0.04^{b}	35.09 ± 2.49^{b}
35	3.68 ± 0.24^{a}	42.5 ± 1.8^{g}	3.40 ± 0.06^{a}	42.21 ± 4.82^{a}
40	3.70±0.19a	42.3±2.7g	3.41±0.07a	42.14±6.05a
Nitrogen sources, 1 g/L (C-source, 35 g/L Durian seeds powder)				
$(NH_4)_2$ SO_4		42.5 ± 1.8^{a}	3.40 ± 0.06^{b}	42.21 ± 4.82^{b} 3.68 ± 0.24^{c}
CMSG		39.5 ± 1.5^{b}	3.80 ± 0.06^{a}	50.21 ± 4.82^{a} 4.14 ± 0.24^{b}
NH_4Cl		40.7 ± 2.4^{b}	2.59 ± 0.15^{c}	43.44 ± 3.18^{b} 2.52 ± 0.50^{d}
NH_4NO_3		42.3 ± 1.5^{a}	2.21 ± 0.09^{c}	44.90 ± 4.33^{b} 2.47 ± 0.74^{d}

Factor	DCW (g/L)*	ST (mN/m)*	BS (g/L) *	EA (%) *
Peptone 4.59±0.29[a]		41.5±1.3[a]	2.48±0.07[c]	43.62±6.50[b]
C: N ratio [N-source, 1 g/L CMSG]				
5 :1	1.85±0.12[g]	58.0±1.9[a]	1.81±0.01[f]	30.58±4.17[g]
10:1	2.32±0.10[f]	55.5±2.8[b]	2.19±0.15[e]	34.10±6.26[g]
15:1	2.50±0.52[e]	52.2±4.6[c]	2.63±0.18[d]	38.56±3.35[f]
20:1	2.95±0.24[d]	50.0±3.9[d]	2.89±0.16[d]	40.52±5.27[e]
25:1	3.18±0.19[c]	48.1±2.8[e]	3.10±0.15[c]	41.52±2.60[e]
30:1	3.45±0.32[c]	45.0±1.0[f]	3.29±0.10[c]	45.50±7.22[d]
35:1	4.14±0.24[b]	39.5±1.5[g]	3.80±0.06[b]	50.21±4.82[c]
40:1	4.34±0.12[b]	39.6±1.8[g]	3.91±0.21[b]	53.15±4.32[b]
45:1	4.84±0.20[a]	39.5±2.0[g]	4.00±0.15[a]	55.21±6.02[a]
50:1	4.90±0.73[a]	39.5±2.1[g]	3.93±0.43[b]	53.33±5.51[b]
Inoculum concentration (%, v/v)				
2	2.41±0.12[e]	48.7±1.0[a]	2.08±0.10[c]	42.15±4.10[c]
4	3.50±0.38[d]	43.6±2.4[b]	3.57±0.21[b]	50.57±3.24[b]
6	4.84±0.20[c]	39.5±2.0[c]	4.00±0.15[a]	55.21±6.02[a]
8	5.07±0.74[b]	40.1±1.8[c]	3.98±0.23[a]	56.18±5.57[a]
10	6.45±0.67[a]	39.8±3.2[c]	3.99±0.62[a]	55.57±4.87[a]

*DCW: dry cell weight; ST: surface tension; BS: biosurfactant
****Different letters in the same row indicate significant differences ($p<0.05$)

Effect of nitrogen source on growth and biosurfactant production

There is evidence that nitrogen plays an important role in the production of biosurfactant by microorganisms [9]. With durian seeds powder as a carbon source, the choice of how the nitrogen source affects the biosurfactant production is depicted in Table 1. After examining the most commonly used organic and inorganic nitrogen sources reported in the literature [10], it was found that commercial monosodium glutamate (CMSG) was the most efficient nitrogen source for O. anthropi 2/3 to produce biosurfactant. It gave a high biosurfactant yield of 3.80 g/L. This yield is about 1.7 fold of that obtained from using NH4NO3 as the nitrogen source. Moreover, using CMSG as the organic nitrogen source not only increased in biosurfactant yield but also improved the biomass and surface tension reduction. A similar result was reported in biosurfactant isolated from Deinococcus caeni PO5 [11] and Halobacteriaceae archaeon AS65 [12].

Effect of carbon: nitrogen (C:N) ratio on growth and biosurfactant production

In many fermentative processes, the C/N ratio is an extremely sensitive parameter, thus affecting metabolites accumulation. High C/N ratios, that are reduced levels of nitrogen, limit bacterial growth, favoring cellular metabolism towards the production of metabolites. On the other hand, an excess of the nitrogen source directs the substrate to the synthesis of cellular material, limiting relatively the accumulation of products [13]. The effect of C/N ratio on biosurfactant production was thus investigated by keeping a constant use of the nitrogen source (1.0 g/L of CMSG). The lowest C:N ratio used (C/N = 5) was limited in terms of carbon, since cell growth was about 62% lower compared with that using the highest C/N ratio (Table 1). The best biosurfactant yield (4.01 g/L) was obtained at a C/N ratio of 45, whereas the productivity tended to decrease as the C/N ratio increased from 45 to 50. Some reports mentioned that biosurfactant production is more efficient under nitrogen limiting conditions [14-15].

The results show that a possible inhibitory effect on the bacterial metabolism may occur due to a likely nutrient transport deficiency. That is, nitrate first undergoes dissimilatory nitrate reduction to ammonium and then is assimilated by glutamine-glutamate metabolism. It is likely that assimilation of nitrate as nitrogen source is so low, leading to a simulated nitrogen-limiting condition [9, 15].

Effect of inoculum size on biosurfactant production

To optimize the amount of inoculum for biosurfactant production, the concentration of the inoculum was varied

from 2 to 10% (v/v). As shown in Table 1, with an increase in inoculum size from 2.0 to 6.0%, growth and biosurfactant production by *O. anthropi* 2/3 strain was enhanced linearly. The result showed that the highest percentage of the biosurfactant yield (4.00 g/L) was obtained at 6% inoculum for this bacterial strain (Table 1). Although dry cell weight seemed to increase with an increase in the amount of inoculum, the excreted biosurfactants did not show superior surface activities and yield. These results was in accordance with Saimmai [15], who reported that 6% (v/v) of inoculum concentration was optimum for biosurfactant production by *Oleomonas sagaranensis* AT18.

Time course of growth and biosurfactant production

Times course studies were conducted on growth and biosurfactant production by *O. anthropi* 2/3 in MSM using 6% (v/v) of

inoculums, 45 g/L durian seeds powder and 1.0 g/L CMSG as the carbon and nitrogen source, respectively. It was cultivated in 250 mL flask containing 50 mL MSM at 30ºC in a shaking incubator at 200 rpm for 72 h (Figure 1).

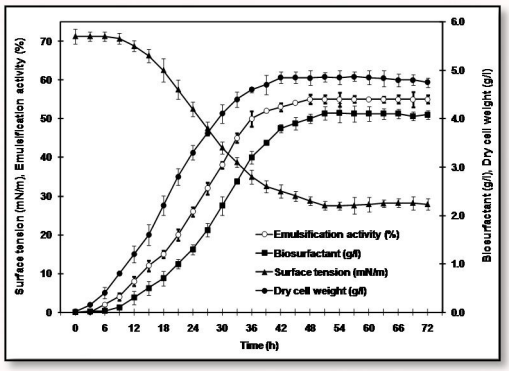

Fig.1 Time course of growth and biosurfactant production by Ochrobactrum anthropi 2/3 in optimal medium

It was observed that O. anthropi 2/3 started to excrete biosurfactant after the lag phase, which was after 6 h of cultivation, as indicated by a decrease in the surface tension of the culture medium. Under the study conditions, the log phase occurred and ranged from 9 to 36 h. In this period, the surface tension of the culture medium was markedly reduced and reached a minimum (28.5 mN/m). However, the highest biosurfactant yield (4.10 g/L) and emulsification activity (55%) was obtained as the cultivation time reached 51 h, which corresponded to the stationary phase of the microbial growth. Therefore, it can be concluded that the biosurfactant produced by O. anthropi 2/3 is a primary metabolite. Growth-associated production of biosurfactant has been reported for Pseudomonas sp. [16], Deinococcus caeni PO5 [11] and Sphingobacterium spiritivorum AS43 [17]. Chooklin et al. [12] also docu-mented that a biosurfactant synthesized by a strain of Halobacteriaceae archaeon AS65 was a primary metabolite produced during cellular biomass formation. From the result obtained, it can be seen that a cultivation time of 51 h gave the highest biosurfactant activity.

Recovery of biosurfactant

The ability of various solvent systems to recover surface-active components from the culture supernatant of *O. anthropi* 2/3 after 51 h of cultivation was examined. The use of ethyl acetate resulted in greater in activity of crude extract toward systems based on mixtures of chloroform and methanol, cold acetone or dichloromethane (data not shown). It was also reported previously that the extraction of bio-products with considerably high polarity by ethyl acetate is rather efficient [18]. Because the recovery and concentration of biosurfactants from fermentation broth largely determines the production cost, ethyl acetate is a better choice than the highly toxic chloro-organic compounds.

Surface tension and critical micelle concentration (CMC)

The relationship between surface tension and concentration of the obtained biosurfactant solution was determined by a du Nouy' a ring tensiometer (data not shown). The biosurfactant exhibited excellent surface tension reducing activity. The surface tension of water (72 mN/m) decreased to 28.5 mN/m by increasing the solution concentration up to 10 mg/L. Further increase in the concentra-tion of the biosurfactant solution did not reduce the surface tension of water, indicating that the CMC was reached at this concentra-tion. The biosurfactant from *O. anthropi* 2/3 showed a lower minimum surface tension and CMC value than that of the biosurfactant from *Lactobacillus paracasei* (41.8 mN/m, 2.5 mg/mL) [8] and from *Pseudomonas aeruginosa* Bs 20 (29.5 mN/m, 13.4 mg/L) [19].

Effect of temperature, pH and salinity on biosurfactant stability

The results obtained from thermal stability analysis of biosurfactant over a wide range of temperatures (4-121ºC) revealed that the biosurfactant from *O. anthropi* 2/3 was thermos-table (Table 2). Heating of the biosurfactant solution up to 100ºC (or its autoclaving at 121ºC) caused some effect on the biosurfactant performance and its emulsification capacity. About 15% reduction in E24, E48, and surface tension was observed after autoclaving at 121°C for 20 min. The surface tension (28-33 mN/m) and emulsification activity (E24=48-55% and E48=44-50%) of crude biosurfactant from *O. anthropi* 2/3 were relatively stable at the temper-atures used. The activity of biosurfactant from *O. anthropi* 2/3 was decreased obviously with decreasing pH (<4.0; Table 2). The surface tension of biosurfactant from *O. anthropi* 2/3 remained stable over a

pH of 5.0-10.0, although a marginal decrease at pH 11.0 was detected. At pH 7.0, the surface tension was lowest for biosur-factant (28.1 mN/m). Emulsification activity of the obtained biosurfactant increased when pH varied from 9.0 to 6.0 and reached its highest at pH 7.0. However, when the pH reached 2.0, the emulsification activity declined and came to its lowest point due to the precipitation of biosurfactant [12]. A negligible change occurred in biosurfactant activity with an increase in NaCl concentra-tion for the biosurfactant obtained (8%; Table 2). Similarly, an increase in NaCl concentra-tion up to 10% did not affect the emulsifica-tion activity of the biosurfactant obtained. However, at 12% of NaCl, E24 and E48 severely dropped to 30-32%, and the surface tension increased as well (47.8 mN/m).

Table 2 Influence of temperature, pH and salt concentration on surface tension and emulsification activity of biosurfactant produced by *Ochrobactrum anthropi* 2/3.

Parameters	Surface tension (mN/m)*	Emulsification activity (%)*	
		E24	E48
Temperature (ºC)			
4	28.5±2.0	55.0±4.8	50.5±3.7
20	28.5±1.7	55.5±5.1	50.7±3.3
40	28.4±1.8	55.4±4.0	50.3±4.1
80	29.2±2.0	55.7±2.4	50.4±2.2
100	30.9±1.1	54.3±3.0	49.2±3.1
110	32.5±3.1	50.1±5.6	47.1±4.5
121	33.1±2.2	48.2±4.8	44.0±3.4
pH			
2.0	50.2±1.2	37.8±2.8	25.6±5.0
3.0	45.1±2.4	40.5±5.5	35.4±4.9
4.0	37.5±1.0	48.5±3.5	40.2±2.3
5.0	28.3±2.1	51.5±4.8	47.8±5.3
6.0	28.6±2.5	55.7±2.9	50.3±6.0
7.0	28.1±2.3	55.5±5.5	50.8±2.2
8.0	28.5±1.4	54.3±6.0	50.2±3.5
9.0	28.3±1.8	53.5±4.5	49.1±5.3
10.0	28.5±2.0	50.8±3.0	45.0±4.0
11.0	35.3±2.3	44.5±4.7	40.2±3.2
12.0	47.8±1.5	36.0±3.9	33.0±4.1
NaCl (%, w/v)			
0	28.4±1.9	55.5±4.0	50.0±2.0
2	28.3±0.5	55.0±1.8	50.9±5.7
4	29.1±2.0	55.2±2.5	50.1±5.8
6	29.0±1.5	54.5±1.9	48.2±3.7
8	30.8±1.0	54.2±2.5	47.3±2.1
10	36.7±1.8	54.9±3.5	46.8±6.5
12	47.8±1.0	37.1±5.0	35.0±4.3
14	50.5±1.5	34.3±5.0	30.8±5.0
16	55.1±1.1	30.5±5.5	23.7±4.1

Emulsification properties of biosurfactant

To determine the hydrocarbon specificity for emulsification activity at 24 h (E24) and 48 h (E48), a wide range of pure and mixed substrates was investigated. Biosurfactant from *O. anthropi* 2/3 showed a good E24 and E48 against several hydrophobic substrates (Figure 3). Olive oil, soybean oil, palm oil, sun flower oil and toluene were good substrates for E24 and E48 produced by the biosurfactant from *O. anthropi* 2/3 and showed no significant differences. Kerosene, xylene and motor oil also formed stable emulsions. ULO, benzene, cyclohexane, hexadecane and hexane resulted in poor emulsification, probably due to the inability of the biosurfactant to stabilize the microscopic droplets of these compounds. The structure of ULO consisted of a mixture of paraffin, naphthalene, and aromatic hydrocarbon. Thus, it was difficult to emulsify by crude biosurfactant [20]. The ability of crude biosurfactant from *O. anthropi* 2/3 to emulsify various hydrophobic substrates indicates that it has a good potential for application in enhanced microbial oil removal and can also be used as an emulsifying agent in the food industry.

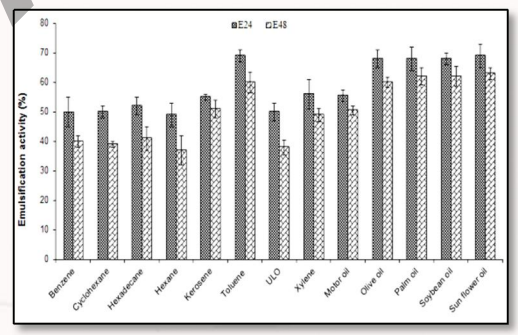

Fig.2 Emulsification activity of the biosurfactant produced by *Ochrobactrum anthropi* 2/3 against several hydrophobic compounds

Chemical characterization of the biosurfactant

Chemical nature of the biosurfactant from *O.* anthropi 2/3 was seen as a single spot on TLC (data not shown). This fraction showed positive reaction with ninhydrin reagent and rhodamine B reagent indicating the presence of peptide and lipid moieties in the molecule (data not shown). These results indicated the existence of lipopeptide biosurfactant. The FT-IR spectrum of biosurfactant from *O.* anthropi 2/3 showed strong absorption bands, indicating

the presence of peptides at 3304 cm-1 resulting from N-H stretching mode (Figure 2). The result shown that at 1727 cm-1, the stretching mode of a CO-N bond was observed, and at 1648 cm-1 the deformation mode of the NH bond combined with C-N stretching mode occurred. The presence of an aliphatic chain was indicated by the C-H stretching modes at 2958 to 2856 cm-1 and 1543-1208 cm-1. These results strongly indicate that the biosurfactant contains aliphatic and peptide-like moieties. The overall FT-IR spectrum of biosurfactant from *O.* anthropi 2/3 was very similar with cyclic lipopeptides produced by bacilli like surfactin (produced by Bacillus subtilis) and lichenysin (produced by Bacillus licheniformis) are the most effective biosurfactant discovered so far [21].

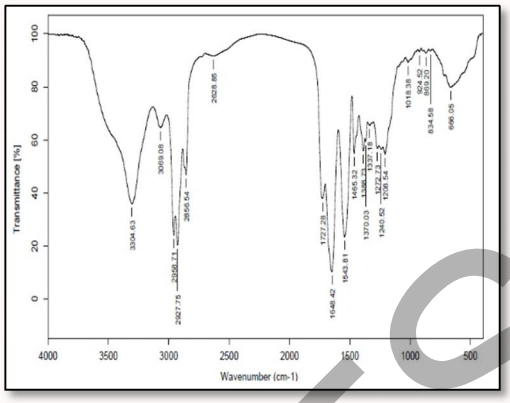

Figure 3 Fourier transform infrared spectrum of the biosurfactant produced by *Ochrobactrum anthropi* 2/3.

CONCLUSIONS

In the present study, the production of the biosurfactant from *O. anthropi* 2/3 by using free-cost substrate of durian seed is reported. The growth characteristics were obtained and studies on the properties of the biosurfactant indicate the possibility of its industrial application. The spectra obtained from FT-IR spectroscopy confirmed that the obtained biosurfactant was lipopeptide type biosurfactant. The potential of this biosurfactant for industrial uses was shown by studying its physical properties, i.e. the surface tension, CMC and emulsification activity, and its stability to environmental stresses such as salinity, pH and temperature. The surface tension of an aqueous solution of this biosurfactant at a CMC value of 10 mg/L, reached 28.5 mN/m. The properties of the obtained biosurfactant have potential applications especially for microbial enhanced oil recovery and/or reducing the intensity of environmental contamination.

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