Enhancing Oil Pollution Remediation Sites with Effective Emulsifying Property of Biosurfactant–Producing *Bacillus oceanisediminis* PM 08

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Abstract: *Bacillus oceanisediminis* PM 08, isolated from soil and seawater in southern Thailand, was evaluated as a potential biosurfactant with effective emulsifying properties. This strain produced a biosurfactant with a maximum emulsifying activity of 65% and a surface tension of 22.67 mN/m after 60 hours of cultivation under cultivation conditions. The yield of biosurfactant obtained by extraction with chloroform: methanol (2: 1, vol/vol) was 1.55 g/L. The stability of the biosurfactant was effective over a wide range of pH, temperature, and salinity. The high thermostability of the biosurfactant was determined using a thermal simultaneous. In addition, a preliminary chemical composition using FT-IR revealed that it is a glycolipid biosurfactant. The properties of this biosurfactant make it a good product for many industrial applications under extreme conditions.

Keywords: Biosurfactant, emulsifying, oil pollution remediation, *Bacillus oceanisediminis* PM 08

1. Introduction

Biosurfactants are amphipathic surface-active compounds consisting of structurally distinct biological macromolecules and functional groups produced by eukaryotic and prokaryotic microorganisms. [1] They are extracellular secondary metabolites that play an important role in the survival of the organisms that have them, either by interfering with host-microbe interactions or acting as antimicrobial agents. [2] These compounds are amphiphilic molecules with hydrophobic and hydrophilic moieties that act between fluids of different polarities, providing access to hydrophobic substrates, causing a reduction in surface tension, and an increase in contact area for insoluble compounds [3]

The lipophilic moiety may be a protein or peptide with a high proportion of hydrophobic side chains or a hydrocarbon chain of a fatty acid with 10 to 18 carbon atoms, although fatty acids with a higher molecular weight have also been reported. The hydrophilic moiety can be an ester, hydroxyl, phosphate, carboxylate

group, or sugar. [4] Biological surfactants are generally classified into low molecular mass molecules, effective in reducing surface and interfacial tensions, and high molecular mass polymers, more effective as emulsion stabilizers. The major classes of typical molecular mass surfactants are glycolipids, lipopeptides, and phospholipids, while high molecular mass surfactants include polymeric and particulate surfactants. [5] Biosurfactants offer a number of advantages over chemical surfactants, such as biodegradability due to their simple chemical structure, environmental compatibility, low toxicity allowing their use in the cosmetic, pharmaceutical, and food industries, high selectivity due to the presence of specific functional groups allowing specific detoxification of certain pollutants, and activity under extreme temperature, pH and salinity conditions. These properties contribute to the applicability of biosurfactants in various industries. Large quantities of crude oil entering the marine environment, groundwater, and soil can cause significant harm to the organisms living there. [6] Petroleum is a hydrophobic hydrocarbon with adverse effects on cell membranes' structural and functional properties in living organisms, posing a significant contamination risk to marine and terrestrial ecosystems. [7] When oil and its by-products come into contact with water, they spread and form a thin layer on the surface that prevents gas exchange between air and water, blocks the passage of sunlight, and impedes respiration and photosynthesis. In this way, hydrocarbon debris affects phytoplankton communities and causes a fundamental breakdown of the food chain. [8] The potential threat to human health from hydrocarbons is related to these compounds' physical and chemical properties, which are absorbed through the skin and spread rapidly through the organism when ingested or inhaled. The most common role of biosurfactants is to improve the dispersion of pollutants in the aqueous phase and increase the bioavailability of the hydrophobic substrate for microorganisms to remove these pollutants through biodegradation. [9] Based on the above facts, many studies have been conducted using different microbes to investigate the production of biosurfactants. Therefore, the present research focused on isolating biosurfactant-producing bacteria from palm oil-contaminated sites in southern Thailand. The isolate with the most powerful emulsifying property and petroleum-degrading strains from hydrocarbon-contaminated environments were identified and characterized.

2. Materials and method

2.1 Isolation and screening of biosurfactant-producing strains

Contaminated soil and seawater were collected from Pak Meng Port, Hat Yaw Port, and Libong Port in Trang Province in southern Thailand. Bacteria were isolated using mineral salt medium (MSM) containing (g/L): K_2HPO_4—0.8, KH_2PO_4—0.2, $CaCl_2$—0.05, $MgCl_2$—0.5, $FeCl_2$—0.01, $(NH_4)_2SO_4$—1.0 and NaCl—5.0 [10]. The pH of the medium was adjusted to 7.0 before autoclaving. One gram/mL of sample was added aseptically to 5 mL of 0.85% NaCl, and 100 µL of the suspension was spread on MSM agar supplemented with palm oil (1%, vol/vol) as a carbon source followed by incubation at 30 ± 3°C for 4–5 days. Subsequently, bacterial colonies with different morphologies were picked, purified on MSM agar, and kept in nutrient broth containing 20% glycerol at –20°C. The bacterial isolates were screened for their ability to produce biosurfactants. Inocula of cultures of isolated bacteria were prepared in nutrient broth (NB, HiMedia, India), and OD^{660} was adjusted to 0.5. Then, it was inoculated in 20 mL MSM medium supplemented with 1% (vol/vol) palm oil or 1% (wt/vol) glucose as a carbon source, followed by being kept on an incubator shaker (200 rpm) at 30 ± 3°C for 48 h.

2.2 16S rRNA gene sequence analysis

Species identification of selected bacterial isolates was made based on 16S rRNA gene sequencing. The genomic DNA was extracted, and the 16S rRNA sequence was amplified by using universal primer 27F and 1492R (5'-AGAGTTTGATCATGGCTCAG-3'; 5'-GGTACCTTGTTACGACTT-3') [11]. The resulting sequence was compared with lines in the GenBank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) using the nucleotide blast (BLASTn) network service.

2.3 Culture medium optimization and time course of biosurfactant production

MSM medium was used throughout this experiment. The medium optimization was conducted in a series of experiments changing one variable at a time, keeping the other factors fixed under specific conditions.

Two factors were chosen to obtain a biosurfactant's higher productivity: carbon source (C) and nitrogen source (N). The carbon sources used were 1% (wt/vol) of glucose, commercial sugar, molasses, soybean oil, palm oil, and used palm oil, with 0.1% (wt/vol) $(NH_4)_2SO_4$ as a nitrogen source. A medium with no carbon source was used as the control. For evaluation of the most appropriate nitrogen sources for the production of a biosurfactant, peptone, yeast extract, urea, $(NH_4)_2SO_4$, NH_4Cl, and $NaNO_3$ was employed at a concentration of 0.1% (wt/vol) with the optimum carbon source. A medium with no nitrogen source was used as a control assay. Finally, the optimum amount of carbon and nitrogen sources was determined. To find optimum conditions for biosurfactant production, the selected strain was grown in a 250 mL flask containing 50 mL of MSM medium using 5% (vol/vol) of inocula containing the optimum amount of carbon and nitrogen sources with pH 7.0, incubated in an orbital shaker (150 rpm) at 30 ± 3°C for 72 h. Samples were taken at different intervals to measure microbial growth by dry cell weight and its ability to emulsify palm oil by measuring emulsion activity (%EA and %EI).

2.4 Recovery of biosurfactant

For recovery of crude biosurfactant, cells were separated from the culture broth by centrifugation at 6000 × g for 10 min at 4°C. The supernatant was tested for biosurfactant extraction. Five precipitation methods (acid, acetone, $(NH_4)_2SO_4$, methanol, and ethanol) and solvent extraction (chloroform-methanol (2: 1, vol/vol)) for recovery of biosurfactant were performed according to Saimmai et al. [12] with modifications. The method showing the highest emulsion activity was used to recover the crude biosurfactant from *B. oceanisediminis* PM 08 Biosurfactant production in the culture broth was detected by the method described by Cooper and Goldberg [13]

2.5 Characterization of biosurfactant

Stability of biosurfactant. The crude biosurfactant (1 mg/L) was dissolved in distilled water. To investigate the effects of pH, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), and temperature on emulsion activity, the biosurfactant solution was adjusted with 1 N HCl or 1 N NaOH to obtain a pHs from 2.0 to 12.0. NaCl was added to the sample to get the final concentrations of 0–12% (wt/vol), and $MgCl_2$ and $CaCl_2$ were used in the range of 0–0.1% (wt/vol). For the thermal stability study, biosurfactant solution was incubated at 25–100°C for 1 h and at 110 and 121°C for 15 min and cooled to 30°C. The remaining activity was then determined.

2.6 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is very useful for identifying types of chemical bonds (functional groups) and can therefore be used to analyze the components of an unknown mixture. The obtained biosurfactant (10 mg) was ground with 100 mg of potassium bromide and pressed with 7,500 kg for 30 s to get translucent pellets. Infrared absorption spectra were recorded on a Thermo Nicolet AVATAR 330 FT-IR system with a spectral resolution and wave number accuracy of 4 and 0.01 cm^{-1}, respectively. Each set of measurements consisted of 500 scans and a potassium bromide pellet was used as a background reference [14]

2.7 Analytical methods

Tests for determination of the emulsification activity (%EA) and emulsification index (%EI) were performed to evaluate the emulsifying ability of culture supernatant following the method described by Cooper and Goldberg. [13] The activity was determined by mixing 1 mL palm oil with 1 mL culture supernatant in a test tube, vortexing at high speed for 2 min, and the mixture was allowed to stand for 1 h (%EA) and 24 h (%EI). Upon standing, a creamy emulsion was formed when an emulsifier was present. Then the total height of the suspension and the height of the emulsified layer were determined by a measuring scale. Emulsion activity is defined as the height of the emulsion layer divided by the total height and expressed as a percentage. The surface tension of the culture supernatant was examined [15]. All experiments were carried out in triplicate to calculate the mean value. All chemicals used were of analytical grade. Statistical analysis was performed using SPSS 10.0 for Windows (SPSS, USA).

3. Results and Discussion

3.1 Isolation and screening of biosurfactant-producing strains

Isolation and screening of biosurfactant-producing strains. MSM with 1% (wt/) palm oil as the sole carbon source was used to isolate biosurfactant-producing bacteria from soil and seawater collected from many harbor sites in Trang province, Southern Thailand. 31 bacterial isolates were obtained, characterized by different colony morphologies. All samples were analyzed for biosurfactant production using 1% (wt/vol) palm oil as the sole carbon source. Among them, 31 bacterial isolates were identified as biosurfactant-producing bacteria using the emulsifying activity test (% EA). Most isolated bacteria (75%) were Gram-negative (Table 1). It has been previously reported that most bacterial isolates from sites that have been contaminated with oil in the past are Gram-negative, and this could be a characteristic that contributes to the survival of these populations in such harsh environments [16]. The potential biosurfactant should have an emulsifying activity of more than 50%. Only 13 isolates showed EA of more than 50%. All bacterial isolates had varying emulsification activity, which depended primarily on the microbial strain, carbon and nitrogen source, pH and temperature, and adding water-immiscible substrates to the media. The isolate with the highest percentage of EA was isolated PM08 (59.23%)—identification of the selected strain. Isolate PM08 was identified by combining the alignment results of 16S rRNA sequence analysis with biochemical and physiological characteristics. Based on the 16S rRNA gene sequence and using the tool GenBank BLAST, this isolate PM08 was closely related to *B. oceanisediminis* with a percentage similarity of 100%. It is a Gram-negative, rod-shaped (coccobacillus, straight/curved), the non-spore-forming bacterium that shows motility through a single, non-enveloped polar flagellum and produces large amounts of a non-dialysable biosurfactant. [17]

3.2 Effect of carbon and nitrogen sources on growth and biosurfactant production of B. oceanisediminis PM 08

This study began by examining the effect of carbon sources on biosurfactant production. *B. oceanisediminis* PM 08 was grown on all the tested carbon and nitrogen sources. Table 2 represents the cell growth and emulsion activity produced by *B. oceanisediminis* PM08 using different carbon sources. The results showed that *B. oceanisediminis* PM08 grew better in water-soluble carbon sources. Molasses was the most appropriate carbon source, with the maximum growth and emulsion activity. This result can be compared with that of Abbasi et al. [18] demonstrated that water-insoluble substrates were more effective in biosurfactant production in *Pseudomonas aeruginosa*. Biosurfactant production is the most effective in the presence of vegetable oils. Maximum biosurfactant yield was obtained when corn and soybean oils were used as carbon sources. Moreover, there is evidence that nitrogen plays an important role in microorganisms' production of biosurfactant compounds. *B. oceanisediminis* PM08 could utilize a wide range of nitrogen sources for growth. Among the nitrogen sources used, organic nitrogen sources better support the growth than inorganic nitrogen compounds. The highest growth occurred in the presence of peptone. However, $NaNO_3$ exhibited the most increased emulsion activity (Table 3). Similar observations have been reported by Saimmai et al. [12], which showed that $NaNO_3$ was the most efficient nitrogen source for *Oleomonas sagaranensis* AT18 to produce biosurfactants.

3.3 Time course of growth and biosurfactant production by B. oceanisediminis PM 08

The growth characteristics and biosurfactant production of *B. oceanisediminis* PM 08 were studied by using MSM medium containing 5% (vol/vol) of inocula, 1% molasses as a carbon source, 0.1% $NaNO_3$ as a nitrogen source (pH 7.0) during incubation at 30 ± 3°C and 150 rpm for 72 h. Bacterial growth (DCW), biosurfactant concentration (g/L), %EA, %EI and pH of culture broth were monitored during incubation time (Fig. 1). The biosurfactant production by this strain started during the exponential phase beginning after 6 h of growth and continued up to the stationary phase. The maximum growth and biosurfactant production occurred after 60 h of cultivation. The highest biomass yield was 4.48 g/L of DCW, and the highest yield of biosurfactant production, as determined by acid precipitation, was found to be 3.55 g/L after 60 h of cultivation. Moreover, the culture was tested for %EA and surface tension. It can be seen that a cultivation time of 60 h gave the highest activity of 65% EA and 22.67 mN/m. It evidenced the growth-associated pattern of biosurfactant production. The production profile of biomass and biosurfactant as a function of time revealed that it was the primary metabolite. However, in other reports, a partial growth-associated biosurfactant production profile was observed, in which the biosurfactant production continued during the stationary growth phase.

Table 1. Gram's staining and emulsification activity (%EA) of isolated strains

Isolate	Gram's stain	EA, %
PM01	Negative	45.06 ± 5.17[b]
PM02	Negative	42.18 ± 4.11[c]
PM03	Positive	44.17 ± 7.46[b]
PM04	Negative	47.48 ± 5.48[b]
PM05	Negative	45.21 ± 7.25[b]
PM06	Negative	52.39 ± 8.29[a]
PM07	Negative	50.58 ± 6.58[a]
PM08	Positive	59.23 ± 5.26[a]
PM09	Negative	54.28 ± 5.25[a]
PM10	Negative	49.83 ± 7.49[b]
HY01	Negative	52.64 ± 6.12[a]
HY02	positive	55.26 ± 8.14[a]
HY03	Negative	50.56 ± 9.75[a]
HY04	Negative	49.46 ± 5.27[b]
HY05	Negative	51.09 ± 9.23[a]
HY06	Negative	48.37 ± 6.85[b]
HY07	Positive	44.12 ± 6.12[c]
HY08	Negative	55.36 ± 9.42[a]
HY09	Negative	50.56 ± 5.87[a]
HY10	Negative	49.25 ± 7.28[b]
HY11	Negative	42.38 ± 6.57[c]
HY12	Negative	40.59 ± 5.96[c]
LB01	Positive	48.27 ± 5.25[b]
LB02	Negative	43.49 ± 6.12[c]
LB03	Negative	57.23 ± 9.52[a]
LB04	Negative	48.27 ± 6.29[b]
LB05	Positive	52.26 ± 9.63[a]
LB06	Negative	44.28 ± 6.84[c]
LB07	Negative	47.29 ± 5.28[b]
LB08	Negative	44.58 ± 6.47[c]
LB09	Negative	55.08 ± 9.48[a]

* Different letters in the same column indicate significant different ($p < 0.05$)

Table 2. Effect of carbon source on biosurfactant production by *B. oceanisediminis* PM08 cultivated in MSM medium at 30°C and 150 rpm for 48 h

C-source (1%, wt/vol)	Dry cell weight, g/L	Final pH	EA, %	EI, %
Control**	0.07 ± 0.03[e]	6.72 ± 0.02[a]	2.86 ± 0.45[e]*	0
Glucose	2.12 ± 0.05[d]	5.28 ± 0.03[c]	32.58 ± 0.75[d]	17.56 ± 0.13[c]
Commercial sugar	2.84 ± 0.07[b]	5.47 ± 0.01[c]	45.29 ± 0.52[b]	30.14 ± 0.23[b]
Molasses	3.10 ± 0.08[a]	6.58 ± 0.05[a]	59.87 ± 0.58[a]	38.72 ± 0.15[a]
Soybean oil	2.50 ± 0.05[c]	6.75 ± 0.03[a]	39.72 ± 0.72[c]	15.56 ± 0.15[d]
Palm oil	2.86 ± 0.07[b]	6.49 ± 0.02[b]	45.27 ± 0.64[b]	19.84 ± 0.26[c]
Used palm oil	3.12 ± 0.06[a]	6.28 ± 0.04[b]	39.47 ± 0.55[c]	26.68 ± 0.27[b]

* Different letters in the same column indicate significant different ($p < 0.05$)

** Control: no carbon source. Results represented mean ± standard deviation from 3 determinations.

Table 3. Effect of nitrogen source on biosurfactant production by *B. oceanisediminis* PM08 cultivated in MSM medium at 30 °C and 150 rpm for 48 h (carbon source: 2.5% molasses)

N-source (1%, wt/vol)	Dry cell weight, g/L	Final pH	EA, %	EI, %
Control**	0.05 ± 0.01^{d}	5.23 ± 0.04^{c}	25.21 ± 0.48^{c} *	9.87 ± 5.02^{d}
Peptone	3.72 ± 0.03^{a}	8.35 ± 0.07^{a}	52.35 ± 0.17^{a}	45.14 ± 4.15^{a}
Yeast extract	3.24 ± 0.02^{a}	4.25 ± 0.06^{d}	48.14 ± 0.38^{b}	38.75 ± 5.24^{b}
Urea	3.08 ± 0.07^{b}	8.01 ± 0.03^{a}	45.28 ± 1.72^{b}	30.15 ± 3.86^{c}
$(NH_4)_2SO_4$	2.95 ± 0.02^{b}	4.53 ± 0.08^{d}	48.35 ± 0.25^{b}	30.35 ± 0.25^{c}
NH_4Cl	2.84 ± 0.08^{c}	4.32 ± 0.04^{d}	51.50 ± 2.56^{a}	40.83 ± 3.21^{b}
$NaNO_3$	2.91 ± 0.05^{b}	7.82 ± 0.05^{b}	46.05 ± 3.87^{b}	32.09 ± 5.10^{c}

* Different letters in the same column indicate significant different ($p < 0.05$)
** Control: no nitrogen source. Results represented mean ± standard deviation from 3 determinations.

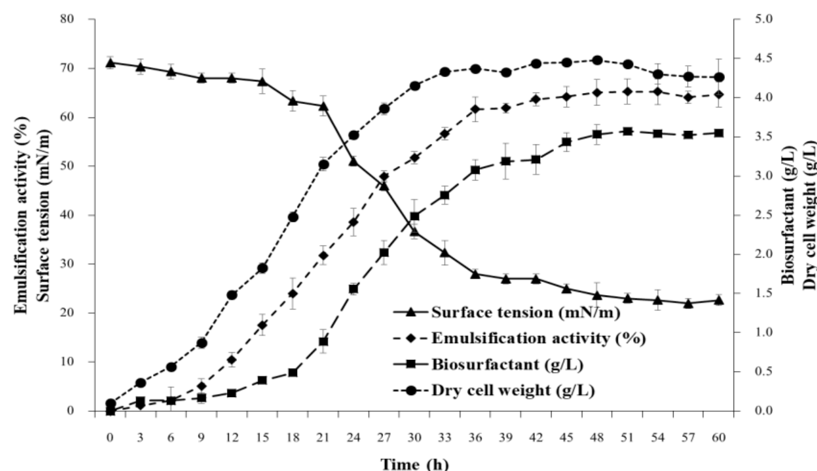

Figure 1. Time course of growth, biosurfactant production, and emulsion activity of *B. oceanisediminis* PM 08 under optimal medium conditions.

3.4 Recovery of biosurfactant

The biosurfactant was precipitated or extracted from the culture supernatant of B. oceanisediminis PM 08. Among six methods of precipitation and extraction, chloroform: methanol (2: 1) was the most efficient in biosurfactant recovery from the culture supernatant of this strain (Table 4). A recovery yield of 1.55 g/L was obtained from *B. oceanisediminis* PM 08. Panjiar et al. [19] reported the amount of emulsifier produced, as recovered by the acid precipitation method. It was found to be 3.07 ± 0.62 and 3.90 ± 0.3 g/L for *Lysinibacillus* sp. SP1025 and *Bacillus cereus* SP1035, respectively.

3.5 Characterization of biosurfactant. Stability of biosurfactant.

Crude biosurfactant was used to study the effect of pH, temperature, and salinity on emulsion stability. The impact of various pHs in the range of 2.0–12.0 on the emulsion activity (%EA and %EI) of biosurfactant are presented in Fig. 2a. Biosurfactant from *B. oceanisediminis* PM 08 showed a broad range of emulsion activity between pH from 4.0 to 10.0. The lowest emulsion activity was observed under acidic (pH < 4.0) and alkaline (pH > 11.0) conditions

Table 4. Effect of recovery method on yield, EA, and EI of the biosurfactant produced by *B. oceanisediminis* PM 08

Recovery method	Yield, g/L	EA, %	EI, %
Acid precipitation	5.87 ± 1.57[a]	40.65 ± 5.12[d]	35.17 ± 3.50[d]
Acetone precipitation	5.08 ± 1.42[b]	50.23 ± 4.11[c]	40.18 ± 3.49[c]
$(NH_4)_2SO_4$ precipitation	4.25 ± 1.34[b]	52.65 ± 5.52[c]	36.48 ± 2.58[d]
MeOH precipitation	2.88 ±1.35[c]	60.88 ± 5.35[b]	52.06 ± 4.28[a]
EtOH precipitation	1.98 ± 0.55[d]	65.32 ± 6.17[a]	49.82 ± 5.14[b]
CH_3Cl: MeOH extraction	1.55 ± 0.16[d]	69.81 ± 2.56[a]	55.26 ±5.86[a]

* Different letters in the same column indicate significant different ($p < 0.05$)

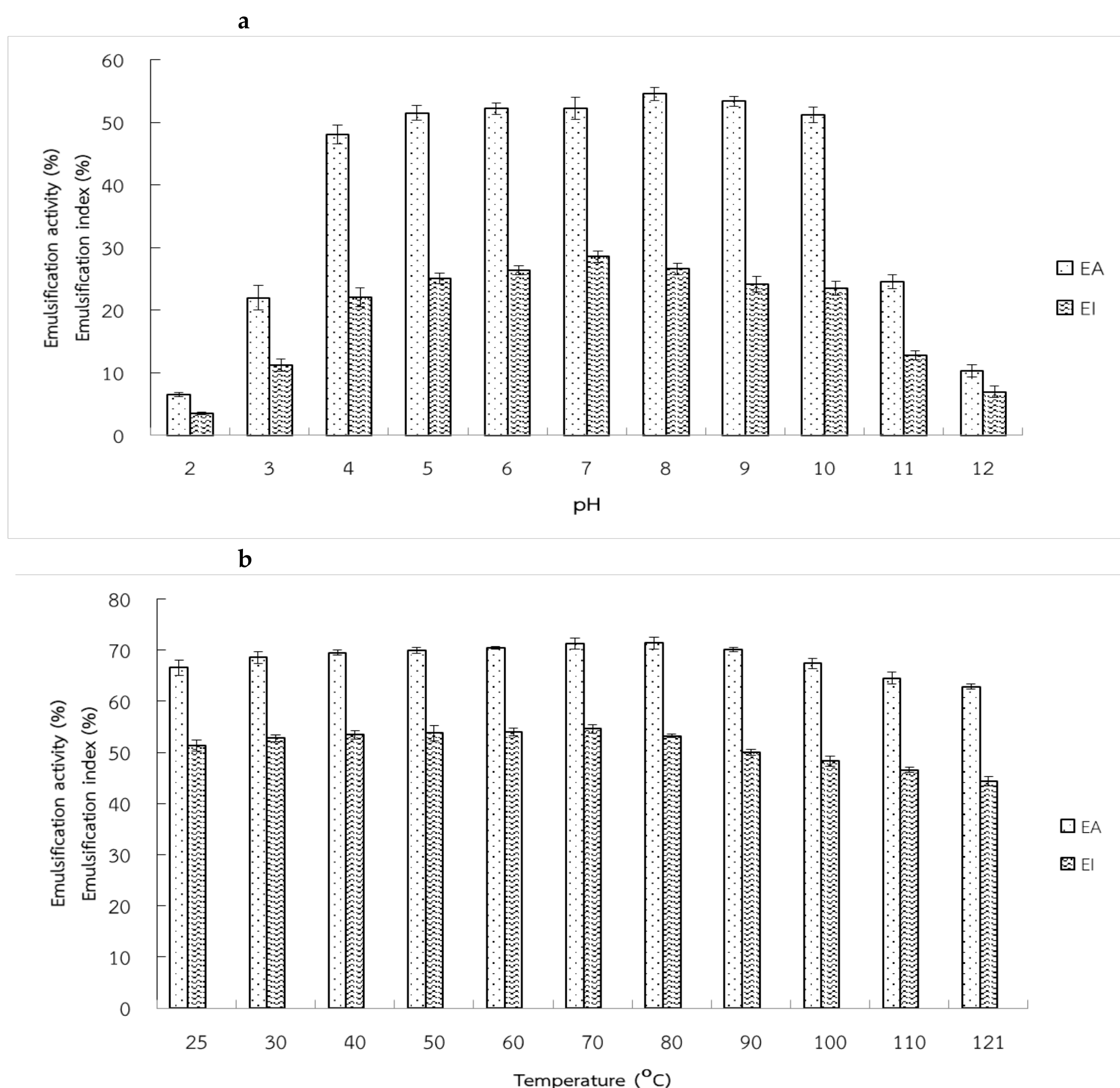

Figure 2. Effect of pH (a) and temperature (b) on emulsion activity of crude biosurfactant produced by *B. oceanisediminis* PM 08.

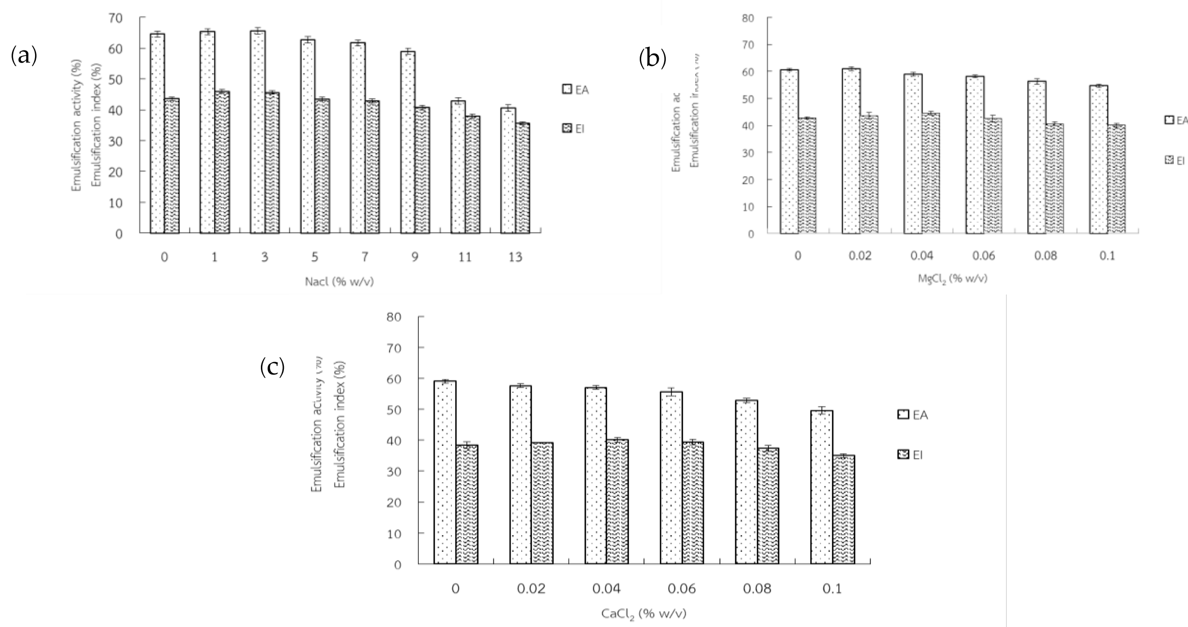

Figure 3. Effect of NaCl (a), $MgCl_2$ (b), and $CaCl_2$ (c) on emulsion activity of crude biosurfactant produced *B. oceanisediminis* PM 08.

Thermal stability analysis over a wide range of temperatures (25–121°C) showed that the biosurfactant had considerable stability under studied conditions (Fig. 2b). Furthermore, a study on the effect of salts and salt concentrations on emulsion activity was performed (Fig. 3). Emulsion stability retains up to 9% NaCl, 0.1% $MgCl_2$ and 0.06% $CaCl_2$. After that, a considerable reduction occurred at higher concentrations. Biosurfactants were stable at various temperatures, pH, and salinity. Gudina et al. [20] reported a novel bioemulsifier produced by a *Paenibacillus* strain isolated from crude oil. Its emulsifying ability was not affected by exposure to high salinities (up to 300 g/L), elevated temperatures (100–121°C), or a wide range of pH values (2.0–13.0). Similarly, the bioemulsifier from *Solibacillus silvestris* AM1 was to be thermostable and active in the pH from 5.0 to 9.0 and 0–5 M NaCl range [21]. Also, an *Ochrobactrum pseudintermedium* strain C1 that secrets an exopolysaccharide as a bioemulsifier showed its properties over a wide range of pH (2.0– 8.0), at moderate salinity (4–6% NaCl), and during exposure to elevated temperatures (100°C) [22] These exciting properties offer the opportunities for the biosurfactants to be investigated in the extreme environment for microbial enhanced oil recovery and in situ biodegradation of oil sludge. In addition, their use is possible in industrial processes for food and pharmaceutics, frequently involving exposure to extremes of temperature, pressure, pH, and ionic strength [23].

3.6 Biosurfactant Characteristics

It was submitted to FT-IR to identify the main functional groups present in the biosurfactant produced by B. oceanisediminis PM 08. Figure 4 shows the FT-IR spectrum of biosurfactants. The presence of a broad range band at 3082.20 cm−1 characteristic of the -OH group assigned to the carboxylic group of sugar moiety and a set of intense bands within the 1601–1451 cm−1 region (1601, 1583, 1541, 1493, and 1451 cm−1) were assigned to the vibration of the C–O and C–O–C glycosidic bands, demonstrating the occurrence of carbohydrates [24]. The 2923 and 2850 cm−1 spectra showed the presence of –CH aliphatic stretching and –CH2 methylene stretching [25]. In addition, the C–O stretching bands at 757 cm−1 and 700 cm−1 confirm the presence of the bands formed between a carbon atom and hydroxyl groups in the chemical structures of the glycoside part [26]. The FT-IR spectra showed the hydroxyl group of –OH stretching vibration, –CH aliphatic stretching, –CH2 methylene stretching, and an ester carbonyl group (C–O) revealed the lipid part in glycolipid biosurfactant such as rhamnolipid [27]. Overall, the FT-IR spectrum suggested that the biosurfactant produced

by B. oceanisediminis PM 08 has been classified as a glycolipid with a carbohydrate and lipid combination. The FT-IR spectra of this biosurfactant displayed a significant similarity in the adsorption of other glycolipids. [28]

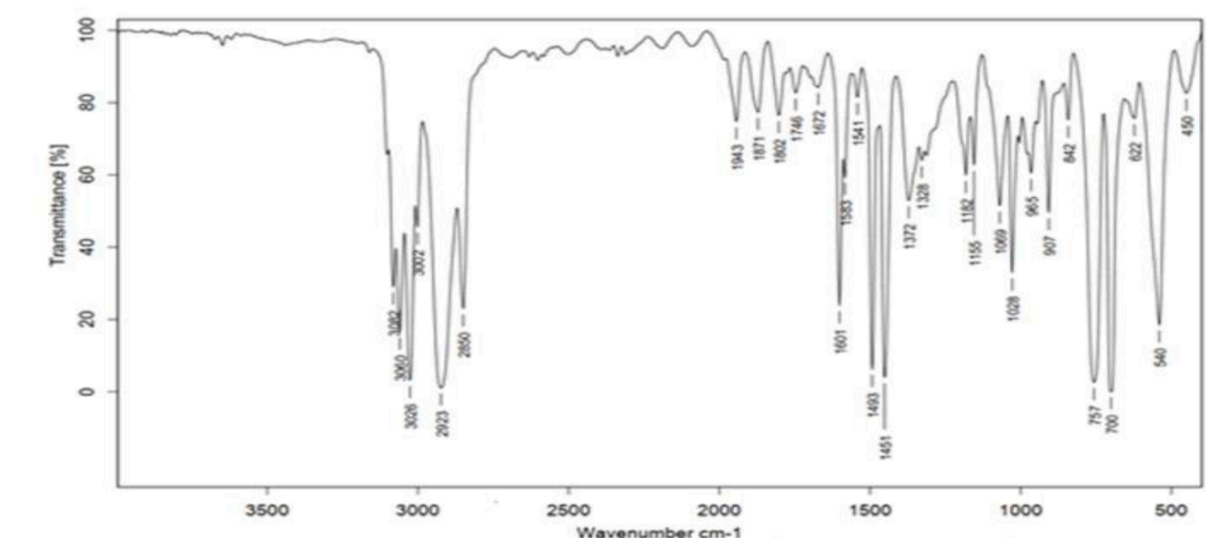

Figure 4. FT-IR spectrum of the biosurfactant produced by *B. oceanisediminis* PM 08

4. Conclusions

In this study, the biosurfactant produced by *B. oceanisediminis* PM 08 exhibited the highest emulsification activity after being grown under optimized growing conditions in MSM with molasses as a carbon source and $NaNO_3$ as a nitrogen source. The biosurfactant also proved stable under extreme pH, temperature, and salinity conditions. The biosurfactant from *B. oceanisediminis* PM 08 was extracted and characterized by FT-IR, indicating it is a glycolipid. The properties of this biosurfactant make it an interesting biotechnological product for many environmental and industrial applications.

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