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Characterization of Biosurfactants Produced by *Halobacillus Dabanensis* and *Pontibacillus Chungwhensi* Isolated from Oil-Contaminated Mangrove Ecosystem in Egypt[1]

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Abstract—The production and properties of biosurfactants synthesized by *Halobacillus dabanensis* and *Pontibacillus chungwhensi,* isolated from oil-contaminated mangrove sediments of *Avicennia marina*, Red Sea (Egypt) have been investigated. The two strains were classified as moderate halophile and halotolerant, respectively. The produced biosurfactants were considered as metabolites associated with the growth of *H. dabanensis* and *P. chungwhensi* and were able to reduce the interfacial tension to 20 and 19 mN/m, respectively. The partially purified biosurfactants showed high emulsiflcation value (E24) against n–hexadecane (68 and 72%, respectively). Biological degradation of n–alkane mixture and linear alkyl benzene after incubation of bacteria in Buslinell-Haas medium in presence and absence of the partially purified biosurfactants has been monitored. *H. dabanensis* and *P. chungwhensi* were capable of degrading 92.3 and 94.7% of n–alkane mixture and 76.5 and 76.7% of linear alkyl benzene, respectively. The biodegradation of n–alkane, achieved in the presence of biosurfactants was better than its degradation in control. The structural analysis of the biosurfactants proved them to be lipopeptides. Biosurfactant–producing halophilic/halotolerant microorganisms may play a significant role in accelerating remediation of oil–polluted saline environments.

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INTRODUCTION

Biosurfactants are chemical compounds produced by a variety of microorganisms [1] which have both clearly defined hydrophilic and hydrophobic groups (amphipathic compounds). Biosurfactants are a diverse group of surface–active biomolecules that can have some influence on the solid/water interfaces [2, 3]. These molecules are capable of lowering surface tension and/or interfacial tension in both aqueous solutions and hydrocarbon mixtures.

Originally, biosurfactants attracted attention as hydrocarbon solubilization agents in the late 1960s, and their applications have been greatly extended in the past five decades. Banat et al. [4] mentioned extension in potential biosurfactant applications as alternatives to chemical surfactants such as carboxylates, sulphonates and sulphate acid esters. In addition, biosurfactants play an important role in the bioremediation of organic pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and petroleum hydrocarbons [5]. The reason for their popularity as high value microbial products is primarily because of their specific action, low toxicity, high biodegradability, effectiveness at extreme temperatures, pH, salinity and widespread applicability [6].

Among the biosurfactant producing potential bacteria, different strains of *Bacillus* (*B. subtilis* SBS53 and *B. polymyxa* SR) isolated from mangrove forest, Abu-Menqar island and Safaga Red Sea–Egypt have been reported [7]. Various microorganisms are known to produce cyclic lipopeptides including surfactin, iturins, fengycins and lichenysins, which are the major classes of biosurfactants. Lipopeptides are of increasing interest in the pharmaceutical, food, cosmetic and petroleum industries due to their excellent surface properties and bioactivities [8]. Among these compounds, surfactin is one of the most powerful biosurfactants produced by several strains of *Bacillus* [9]. Besides that, the strain *Brevibacilhts brevis* HOB1, isolated from the formation water of an oil field, was found to produce lipopeptides, namely surfactin [9].

Halotolerant or halophilic microorganisms, able to live in saline environments, offer a multitude of actual or potential applications in various fields of biotechnology. Biopolymers, such as biosurfactants and exopolysaccharides, are of interest for microbially enhanced oil recovery. Other useful biosubstances are enzymes, such as new isomerases and hydrolases, that are active and stable at high salt concentration. As a result of adaptation to their environment, many extremophilic microorganisms have evolved unique properties of considerable biotechnological and, therefore, commercial significance [10].

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The aim of the study was the extraction, properties and structural analysis of biosurfactants produced by halophile *Halobacillus dabanensis* and halotolerant *Pontibacillus chungwhensi,* isolated from *Avicennia marina* community, in the mangrove forest, Red Sea (Egypt). The study finally demonstrated the biodegradation ability of bacterial isolates which coincided with biosurfactant production.

MATERIALS AND METHODS

Bacterial Strains and Their Identification. Two bacterial strains (HM1 and HM2) have been isolated from superficial mangrove sediment contaminated with petroleum hydrocarbons from *Avicennia marina* community, in Hamata at 17 km south to Safaga region on the Red Sea coast (Egypt).

The strains were identified using 16S rRNA sequencing according to the method of Rochelle et al. [11]. The gene sequencing was done in Macrogen (South Korea). DNA sequences were aligned using GeneMapper® v 4.1 and data collection v 3.1 Communication Patchl–August 2010 (http://www.appliedbiosystems.com/absite/us/en/home/support/software/dna-sequencing/genemapper.html). Bacterial 16S rRNA gene was amplified by using the following universal bacterial 16S rRNA primers: forward primer 27 F (5'–AG AGTTTGATCMTGGCTC AG–3') and reverse primer 1792 R (5'–TACGGYTACCTTGTTACGACTT–3'). Polymerase chain reaction was performed using kits with AmpliTaq® DNA polymerase (FS Enzyme) (Applied Biosystems, United States). Sequence analysis was performed in the National Centre for Biotechnology Information, using United States database BLASTN [12].

Media and Culture Conditions. Modified salt peptone (**SP**) medium [13] containing (g/L): peptone—10.0, yeast extract—1.0, $MgSO_4 \cdot 7H_2O$—1.0, KCl—0.5, $CaCl_2 \cdot 3HZ_2O$—0.7, $MnCl_2 \cdot 4H_2O$—0.05, K_2HPO_4—0.2, KH_2PO_4—0.2 supplemented with NaCl at the optimum concentrations (15% for HM1 and 5% for HM2) was used for the growth and inocula preparation of bacteria, Two loops of bacterial culture were inoculated in 100 mL of SP broth and incubated at 30°C for 24 h in the rotary shaker at 150 rpm until cell numbers reached 1×10^3 cfu/mL, this was used as a standard inoculum.

Halophile moderate (**HM**) medium [14] was utilized with modification for the biosurfactant production and had the following composition (g/L): yeast extract—10.0, proteose peptone—5.0, KC1—2.0, $MgSO_4 \cdot 7H_2O$—1.0, glucose—1.0, $CaCl_2 \cdot 3H_2O$—0.36, NaBr—0.23, K_2HPO_4—0.2, KH_2PO_4—0.2, $FeCl_3$—trace, supplemented with NaCl (15.0 % for HM1 and 5.0 % for HM2), pH of the medium was adjusted to 7.2.

Detection of Biosurfactant Activity. The bacterial strains were grown in 250 (mpflasks containing 50 mL HM broth at 35°C for 72 h with shaking at 150 rpm. The bacterial cells were removed from the culture broth by centrifugation at 42000 × g for 30 min and the cell free broth of each bacterium was used in the biosurfactant test.

Haemolytic Activity. Haemolytic activity of bacterial strains was screened on blood agar plates containing 5% (v/v) human blood and incubated at 30°C for 48–72 h. Haemolytic activity was detected as a defined clear zone around the bacterial colony [15].

Surface Activity. The surface activity of biosurfactants produced by *H. dabanensis* and *P. chungwhensi* was determined by measuring the interfacial tension of the culture supernatant after cell separation using a Kruss processor tensiometer (model K10ST, Germany) by ring detachment method at room temperature. Interfacial tension was performed against kerosene. To increase the accuracy an average of triplicates was used.

Oil Displacement Test. Twenty μl of weathered crude oil were placed on the surface of distilled water (50 μL) in a Petri dish (150 mm in diameter). Then, 10 μL of the culture supernatant were put gently on the centre of the oil film. Under visible light the diameter of clear halo visualized zones were measured and calculated after 30 s as described by Morikawa with coworkers [16].

Production and Extraction of Biosurfactants. Production of crude biosurfactants was performed according to Hsueh and collegues [15]. Briefly, 100 ml of HM broth was inoculated with 3 ml of an overnight culture of each bacterial strain and incubated at 35°C and 150 rpm for 3 to 10 days. We periodically measured OD_{620} (to follow bacterial growth), as well as surface tension and emulsification activity. The bacteria were removed by centrifugation at 16000 × g for 40 min at 4°C to get the cell free broth and the remaining supernatant liquid was sterilized through a 0.22 μm pore-size filter (Millipore, United States). The clear sterile supernatant served as the source of the crude biosurfactant. The surfactant was precipitated by adjusting the pH to 2.0 using 3.0 N HC1 and keeping for 30 min at 4°C. The precipitate was collected by centrifugation at 26000 × g for 20 min at 4°C [17].

For the further extraction of biosurfactant(s), 50 mL of chloroform/methanol (2 : 1 v/v) solvent system was added to 500 mg of the dry crude biosurfactant (air dried) and incubated at 30°C at 150 rpm, for 15 min. The mixture was filtered using a 0.45 u.m pore-size filter (Millipore, United States), then air dried overnight at room temperature. The pellet was dissolved in 200 μL methanol, evaporated and the extracted biosurfactant was determined gravimetrically [15].

Characterization of the Biosurfactant. *Emulsifwation* Activity Assay. The emulsification index (E24) provides a rapid and reliable measure of the quantity of biosurfactant. Emulsification activity was measured according to the method of Cooper and Goldenberg [18] with a slight modification. To 4 mL of the biosur-

factant crude extract (0.5%, w/v), 4 ml of n-hexadecane were added in a screw cap tube and vortexed at high speed for 2 min. The emulsion stability was determined after 24 h, and the emulsification index (E24) was calculated by dividing the measured height of emulsion layer by the mixture's total height and multiplying by 100.

Surface Tension and CMC Measurement. The surface tension of the crude biosurfactant was measured. The critical micelle concentration (**CMC**) was determined by measuring the surface tension at different concentrations of biosurfactant in distilled water at 25°C (up to a constant value of the surface tension). The value of CMC was obtained from plot of surface tension versus the log of the biosurfactant concentration [19].

Foaming Power. Foaming power was measured according to the method of Abouseoud et al. [20]. Briefly, 25 ml of 0.1% crude biosurfactant solution in bi-distilled water was shaken vigorously for 10 s in 100 mL glass stoppered graduated cylinder at 25°C. The solution was allowed to stand for 2 h and then the foam height was measured.

Identification of Biosurfactants. Infrared (Perkin–Elmer–1430, United States) spectroscopy was used for identifying types of chemical bonds (functional groups). One mg of freeze-dried partially purified biosurfactant was ground with 100 mg of KBr to form a very fine powder and pressed to obtain translucent pellets [21]. The spectrum was generated in the range of 500 to 4000 cm^{-1}.

Effect of Biosurfactants on Degradation of Hydrocarbons. The bacterial isolates were tested for their ability to grow on n–alkane mixture (alkanes from n–C_{11} to n–C_{39}) as aliphatic compounds and linear alkyl benzene as aromatic compound, using a liquid mineral medium, Bushnell–Haas (BH) medium, supplemented with 5 or 15% concentration of NaCl (the optimum concentration for moderate halophiles and halotolerant strain) in presence and absence of crude biosurfactant.

The isolate was inoculated into 10 mL SP medium and incubated at 35°C overnight at 150 rpm. The culture was centrifuged for 10 min at 25000 × g. The cell pellets were washed twice in potassium phosphate buffer (pH 7.6) and re-suspended in SP medium until they reached concentration of 5×10^5 cfu/mL as compared with 0.5 McFarland standard. One mL of bacterial inoculum was added to 250 mL Erlenmeyer flasks containing 100 ml of liquid BH medium supplemented with 0.5% carbon source. Uninoculated control flasks with 0.5% (v/v) hydrocarbon were incubated in parallel to monitor abiotic losses of the substrate. The culture and control flasks were incubated at 35°C at 150 rpm for 7 days.

The aliphatic and aromatic fractions were analyzed by gas chromatography with flame ionization detector using Agilent 6890 (Agilent Technologies, United States) with HP–1 (United States) for eluting of aliphatic compounds and HP–50 for eluting of aromatic compounds on 30-m long wide-bore column, 0.35 mm × 0.5 μm film thickness. The injector and detector were maintained at 250 and 300°C respectively, and oven temperature was programmed to rise from 80 to 250°C in 5°C/min increments and to hold at 250°C for 30 min. The flow rate was 2 ml/min, and injection volume was 1 μL. Individual compounds were determined in the aliphatic and aromatic fractions by matching the retention time with authentic standards.

RESULTS AND DISCUSSION

The production of biosurfactants by microorganisms has been a subject of increasing interest in recent years, especially due to their potential applications in enhanced oil recovery. Biosurfactants spontaneously released and functioned are often related to hydrocarbon uptake; therefore, they are predominantly synthesized by hydrocarbon-degrading microorganisms [22]. The two bacterial strains have been isolated from mangrove sediments in Red Sea coast (Egypt) contaminated with petroleum hydrocarbons, and identified using 16S DNA sequencing as *Halobacillus dabanensis* (MH1) and *Pontibacillus chungwhensis* (MH2). These strains were classified as moderate halophile and halotolerant, respectively.

The moderately halophilic bacteria and salt-tolerant microorganisms, in general, pose quite sufficiently interesting questions, especially those implied by their ability to grow over wide ranges of salt concentrations. To adapt to high saline conditions, halophilic microorganisms have developed various biochemical strategies, including compatible solute synthesis to maintain cell structure and function. These solutes plus other compounds (exopolysaccharides, hydrolases, biosurfactants) produced by halophilic microbes are clearly of industrial interest. Besides these metabolical and physiological features, halophilic microorganisms are known to play important roles in transforming and degrading waste and organic pollutants in saline wastewaters. Recently, there have been indications that halophilic and halotolerant bacteria may have greater potential in degradation of pollutants than was previously assumed. For instance, *Marinobacter hydrocarbonoclasticus* degraded a variety of aliphatic and aromatic hydrocarbons [23]. A halotolerant *Streptomyces* sp., isolated from an oil field in Russia, degraded crude petroleum [24].

Krepsky et al. [25] found that the biosurfactant-producing microbial consortia isolated from mangrove hydrocarbon-contaminated sediment were able to grow at a wide salinity range (8, 16 and 24 g/L). This salt concentration was 1.5 times lower than the minimal salinity range.of mangroves, which ranges from 25 to 45 g/L according to precipitation and river dynamics of the area. In our work, the *H. dabanensis* and *P. chungwhensi* isolates from mangrove sediment

Table 1. Biosurfactant activity of cultures of *H. dabanesis* and *P. chungwhensi* grown on HM medium

Strains	Haemolytic activity, cm	Oil displacement activity, cm	Interfacial tension, mN/m
H. dabanensis	4.65 ± 0.16	4.00 ± 0.25	20.00 ± 0.20
P. chungwhensi	3.40 ± 0.82	3.60 ± 0.10	19.00 ± 0.16

could grow at 50 and 150 g/L, respectively. These values are 2 to 10 times higher than the salinity range of mangroves.

Biosurfactant production from the microorganisms was screened by haemolytic activity, oil spreading, surface and interfacial measurements. The two strains of bacteria had haemolytic activity and showed the positive results with oil displacement and surface and interfacial tension. From the data presented in Table 1, *H. dabanensis* and *P. chungwhensi* exhibited a great haemolytic activity (4.65 and 3.40 cm, respectively) on blood agar plate and a high activity for oil displacement test toward weathered crude oil (4.0 and 3.6 cm) and caused a great decrease in the interfacial tension, it was 20 and 19 mN/m, respectively. Satpute et al. [26] suggested that single screening method is not suitable to identify all types of biosurfactants and recommended that more than one screening method should be included in the primary screening to identify potential biosurfactant producers. The sensitivity of oil displacement method enabled to assay of at least 10 μg (about 10 nmol) of biosurfactant [27]. Haddad et al. [9] found that the highest reduction of surface tension (32 mN/m) was achieved with a new bacterial strain (*Brevibacilis brevis* HOB1) from the formation water.

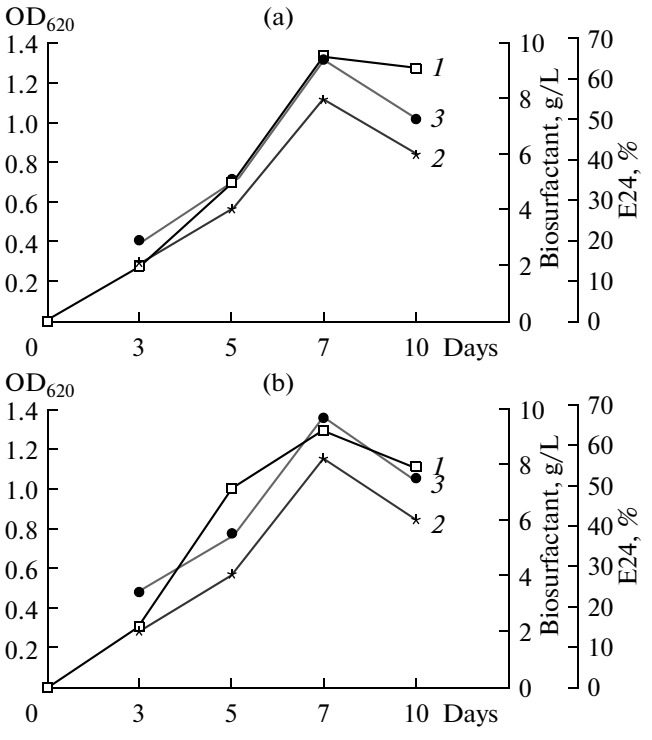

Fig. 1. Growth rate (*1*), biosurfactant production (*2*) and emulsification (*3*) for *H. dabanensis* (a) and *P. chungwhensi* (b) grown on HM medium.

Haemolytic activity has been used as a measure for the screening of microorganisms producing lipopeptide biosurfactants. Fiebig et al. [28] stated that biosurfactant producing capacity of bacteria grown in liquid media was associated with haemolytic activity. Therefore, measurement of haemolytic activity appears to be a good criterion for screening surfactant producing strains. Youssef et al. [29] reported that 13.5% of the haemolytic-active microorganisms lowered the surface tension to values below 40 mN/m. Different compounds are produced by microorganisms which can cause red blood cells lyses, however, they do not necessarily have to be surface-active molecules. For this reason, many authors suggested that measurement of haemolytic activity should be supported by other methods based on surface activity measurements [30]. Pyaza et al. [31] indicated that the oil spreading technique was reliable in detecting low levels of biosurfactant production, easy to carry out and less time-consuming.

A direct relationship between biosurfactant production and cell growth of *H. dabanensis* and *P. chungwhensi* on HM medium was observed in Fig. 1. Biosurfactant production started at the exponential phase and the production kinetics paralleled the biomass kinetics through the logarithmic phase and it was growth linked. In this concern, many authors proved growth dependent biosurfactant production in *Bacillus* [32].

Maximal surfactant concentration produced by *H. dabanensis* and *P. chungwhensi* was 8.0 and 8.2 g/L respectively after 7 days of cultivation (Fig. 1) and thereafter, production however was retarded after 10 days of bacterial growth. Das and Mukherjee [33] suggested that lipopeptide biosurfactant production by *Bacillus subtulis* DM–03 and DM–04 strains were stable over time.

Emulsification activity is an important property of biosurfactant-producing bacteria depending on measurement of the ability to emulsify oils or hydrocarbons forming emulsion. The results in Fig. 1 showed that the bacteria studied had emulsification activity of 66.5%, attained after 7 days of cultivation. Similar observations were reported by Bodour et al. [34] who stated that the stabilization of oil and water emulsion was commonly used as microbial surface activity indicator.

For the partial purified biosurfactant of *H. dabanensis* and *P. chungwhensi*, the emulsification activity revealed that it could be an emulsion-forming agent for hydrocarbons and oils, given highest stable emulsion value of about 68 and 72%, respectively (Table 2). Nitschke and Pastore [2] stated that the ability of bio-

surfactant to form emulsions with vegetable oils suggested its potential application as cleaning and emulsifying agent in food industry.

The time course of the microbial growth of *H. dabanensis* and *P. chungwhensi* on HM medium, the surface tension reduction of the cell free broth and CMC for cell free supernatants are shown in Fig. 2. A noticeable decrease in surface tension was observed after 3, 5 and 7 days of fermentation. The appearance of the surface tension reduction capacity of the bacteria corresponded to the initial phase of the exponential growth profile. The surface tension reduction behaviour was the greatest after a period of 7 days. The present results showed that the values for the minimum surface tension for *H. dabanensis* and *P. chungwhensi* were 30 and 29 mN/m, respectively. It is worth mentioning that the CMC was reached at the end of the exponential phase of the bacterial growth (Fig. 2). Although the production of the surfactant continued after exponential phase, surface tension remained constant. The CMC values of partially purified biosurfactants from *H. dabanensis* and *P. chungwhensi* were 0.25 and 0.05 mg/L, respectively (Table 2), which was low compared to other microbial surfactants.

Partially purified biosurfactants from *H. dabanensis* and *P. chungwhensi* were evaluated by FT–IR spectroscopy, which revealed that all spectra in both bacteria showed essentially similar absorption bands (Fig. 3). Strong absorption band at 3404 cm^{-1} indicated the presence of NH-groups in the molecule. Absorption

Table 2. Characteristics of the partial purified biosurfacants produced by *H. dabanesis* and *P. chungwhensi* grown on HM medium

Biosurfactant properties	Surfactant, 0.01 mg/mL	
	H. dabanensis	*P. chungwhensi*
Surface tension, mN/m	28	25
Interfacial tension, mN/m	10	6.0
CMC, mg/L	0.25	0.05
Emulsion stability, %	68	72
Foaming height, mL	2.5	3.0

valleys observed at 2960, 1450 and 1380 cm^{-1} demonstrated typical CH stretching vibration in the fatty acid chain. Absorption valleys appeared at 1652 cm^{-1} were stretching vibration of C = O in amide bond of peptide. From the FT–IR spectra obtained, it could be concluded that the biosurfactants consist of aliphatic hydrocarbons and peptide like moieties, which leads to the suggestion that they are lipopeptides [33].

The GC analysis showed that the degradation rate of n-alkane mixture (aliphatic compounds) and linear alkyl benzene (aromatic compounds) by *H. dabanensis* and *P. chungwhensi* after 7 days of incubation on BH medium (Table 3) increased in the presence of biosurfactant which reached 92.3 and 94.7% for the n–alkane mixture and 76.5 and 76.7% for aromatic com-

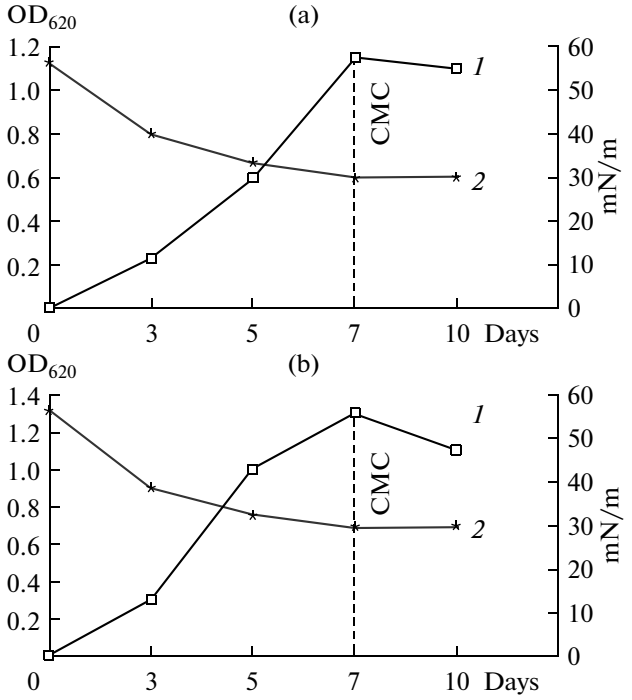

Fig. 2. Growth rate (*1*) and surface tension (*2*) for *H. dabanensis* (a) and *P. chungwhensi* (b) grown on HM medium.

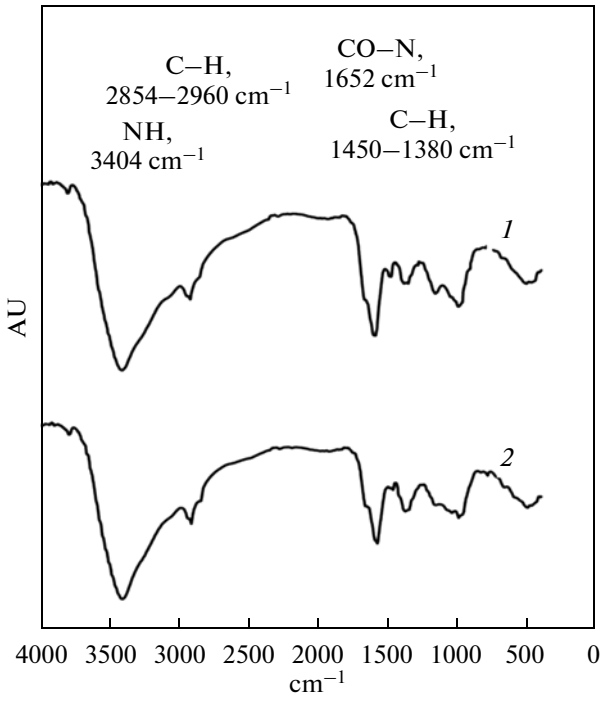

Fig. 3. Fourier transform infrared absorption spectra of partially purified biosurfactant produced by *H. dabanensis* (*1*) and *P. chungwhensi* (*2*).

Table 3. Degradation of aliphatic and aromatic groups by *H. dabanensis* and *P. chungwhensi* after 7 days of incubation on BH medium in presence and absence of the crude biosurfactant

Strain	% of degradation			
	paraffinic mixture, n-alkane		linear alkyl benzene	
	without	with	without	with
H. dabanensis	26.4	92.3	41.3	76.5
P. chungwhensi	66.9	94.7	60.6	76.7

pounds, respectively. The biodegradation of aliphatic compounds was higher than aromatic. Rocha et al. [35] suggested that biosurfactant-mediated dispersion of hydrocarbons played a very important role in the degradation of saturated compounds, regardless of the metabolic strategy used by the bacterial population.

In the present study new lipopeptide producers were isolated from oil-contaminated mangrove sediments of *Avicennia marina*, Red Sea, Egypt and identified as *H. dabanensis* and *P. chungwhensi* by analysis of 16S rRNA gene. Properties of the obtained biosurfactansts indicated the possibility of their industrial application. They showed high surface and interfacial tension reduction and low CMC values characterizing the product as a powerful surface active agent. Their emulsification activities resulted in high stable emulsion indices, therefore, they could be emulsion-forming agents for hydrocarbons and oils. The biosurfactants produced by *H. dabanensis* and *P. chungwhensi* were capable of increasing the degradability of n–alkane mixture and linear alkyl benzene. Biodegradation of aliphatic mixture by the two bacteria was higher in comparison to decay of aromatic compounds.

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REFERENCES

1. Healy, M.G., Devine, C.M., and Murphy, R., *Res. Conserv. Recycl.*, 1996, vol. 18, nos. 1–4, pp. 41–57.
2. Nitschke, M. and Pastore, G.M., *Bioresour. Technol.*, 2006, vol. 97, no. 2, pp. 336–341.
3. Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J., and Marchant, R., *Appl. Microbol. Biotechnol.*, 2010, vol. 87, no. 2, pp. 427–444.
4. Banat, I.M., Makkar, R.S., and Cameotra, S.S., *Appl. Microbiol. Biotechnol.*, 2000, vol. 53, no. 5, pp. 495–508.
5. Mulligan, C.N., *Environ. Pollut.*, 2005, vol. 133, no. 2, pp.183–198.
6. Hwang, Y.H., Kim, M.S., Song, I.B., Park, B.K., Lim, J.H., Park, S.C., and Yun, H.I., *J. Health Sci.*, 2009, vol. 55, no. 3, pp. 351–355.
7. Bayoumi, R.A. and El-Nagar, A.Y., *J. Appl. Sci. Res.*, 2009, vol. 5, no. 12, pp. 2435–2447.
8. Li, Y.M., Haddad, N.I.A., Yang, S.Z., and Mu, B.Z., *Int. J. Peptide Res. Therapeut.,* 2008, vol. 14, no. 3, pp. 229–235.
9. Haddad, N.I.A., Wang, J., and Mu, B.Z., *J. Ind. Microbiol. Biotechnol.*, 2008, vol. 35, no. 12, pp.1597–1604.
10. Margesin, R. and Schinner, F., *Extremophiles*, 2001, vol. 5, no. 2, pp. 73–83.
11. Rochelle, P.A., Will, J.A.K., Fry, J.C., Jenkins, G.J.S., Parkes, R.J., Turley, C.M., and Weightman, A.J., *Nucleic Acids in the Environment*, Trevors, J.T. and van Elsas, J.D., Eds., Berlin: Springer, 1995.
12. Altschul, S.F., Thomas, L.M., Alejandro, A.S., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J., *Nucleic Acids Res.,* 1997, vol. 25, no. 17, pp. 3389–3402.
13. Yoon, J.H., Kang, S.J., and Oh, T.K., *Int. J. Syst. Evol. Microbiol.,* 2007, vol. 57, no. 10, pp. 2406–2411.
14. Ventosa, A., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F., and Romos-Cormenzana, A., *J. Gen. Microbiol.*, 1982, vol. 128, pp.1959–1968.
15. Hsueh, Y.H., Somers, E.B., Lereclus, D., Ghelardi, E., and Wong, A.C.L., *Appl. Environ. Microbiol.,* 2007, vol. 73, no. 22, pp. 7225–7231.
16. Morikawa, M., Hirata, Y., and Imanaka, T., *Biochim. Biophys. Acta*, 2000, vol. 1488, no. 3, pp. 211–218.
17. Kim, P.I., Bai, H., Bai, D., Chae, H., Chung, S., Kim, Y., Park, R, and Chi, Y.T., *J. Appl. Microbiol.*, 2004, vol. 97, no. 5, pp.942–949.
18. Cooper, D.G. and Goldenberg, B.G., *Appl. Environ. Microb.,* 1987, vol. 53, no. 2, pp. 224–229.
19. Joshi, S., Bharucha, C., and Desai, A.J., *Bioresour. Technol.*, 2008, vol. 99, no. 11, pp. 4603–4608.
20. Abouseoud, M., Maachi, R., Amrane, A., Boudergua, S., and Nabi, A. *Desalination*, 2008, vol. 223, nos. 1–3, pp. 143–151.
21. Cooper, D.G., Macdonald, C.R., Duff, S.J., and Kosaric, N., *Appl. Environ. Microbiol.,* 1981, vol. 42, no. 3, pp. 408–412.
22. Banat, I.M., *Bioresour. Technol.,* 1995, vol. 51, no. 1, pp. 1–12.
23. Tiquia, S.M., Davis, D., Hadid, H., Kasparian, S., Ismail, M., Sahly, R., Shim, J., Singh, S., and Murray, K.S., *Environ. Technol.*, 2007, vol. 28, no. 3, pp. 297–307.
24. Kuznetnov, V.D., Zaitseva, T.A., Vakulenko, L.V., and Filippova, S.N., *Mikrobiologiya*, 1992, vol. 61, pp. 84–91.
25. Krepsky, N., Da Silva, F.S., Fontana, L.F., and Crapez, M.A.C., *Braz. J. Biol.,* 2007, vol. 67, no. 1, pp. 117–124.

26. Satpute, S.K., Bhawsarm, B.D., Dhakephalkar, P.K., and Chopade, B.A., *Indian J. Mar. Sci.*, 2008, vol. 37, no. 3, pp.243–250.
27. Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y., and Imanaka, T., *J. Bacteriol.,* 1993, vol. 175, no. 20, pp. 6459–6466.
28. Fiebig, R., Schulze, D., Chng, J.C., and Lee, S.T., *Biodegradation*, 1997, vol. 8, no. 2, pp. 67–75.
29. Youssef, N.H., Duncan, K.E., Nagle, D.P., Sava, K.N., Knapp, R.M., and McInerney, M.J., *J. Microbiol. Methods*, 2004, vol. 56, no. 3, pp. 339–347.
30. Makkar, R.S. and Cameotra, S.S., *J. Ind. Microbiol. Biotechnol.*, 1997, vol. 18, no. 1, pp. 37–42.
31. Pyaza, G.A., Zjawiony, I., and Banat, I.M., *J. Petrol. Sci. Eng.*, 2006, vol. 50, no. 1, pp. 71–77.
32. Sepahy, A.A., Assadi, M.M., Saggadian, V., and Noohi, A., *Int. J. Environ. Sci. Technol.,* 2005, vol. 1, no. 4, pp. 287–293.
33. Das, K. and Mukherjee, A.K., *Process Biochem.,* 2007, vol. 42, no. 8, pp. 1191–1199.
34. Bodour, A.A., Gerrero-Barajas, C., and Maier, M., *Appl. Environ. Microbiol.*, 2004, vol. 10, no. 6, pp. 1114–1120.
35. Rocha, C.A., Pedregosa, A.M., and Laborda, F., *AMB Express,* 2011, vol. 1, pp. 1–10.