Egyptian Journal of Aquatic Research xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Egyptian Journal of Aquatic Research

journal homepage: www.sciencedirect.com/locate/ejar

Full length article

Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt

Khouloud M. Barakat [a,*], Sahar W.M. Hassan [a], Osama M. Darwesh [b]

[a] *Microbiology Lab., National Institute of Oceanography & Fisheries, Alexandria, Egypt*
[b] *Department of Agriculture Microbiology, National Research Centre, Dokki, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 16 June 2017
Revised 4 September 2017
Accepted 6 September 2017
Available online xxxx

Keywords:
Biosurfactant
Haloalkaliphiles
Bacillus sp
SDS-PAGE
FTIR
GC–MS

ABSTRACT

This study aimed to produce biosurfactant by extremophilic marine bacteria. Twenty-one oil-spilled seawater samples were collected from Shalateen, Red Sea, Egypt. Two promising morphologically distinct biosurfactant-producing marine bacteria, SH20 and SH24, were selected. They were grown on minimal salt medium (MSM) and the biosurfactant production was evaluated and detected after 24 h using drop collapsing test, blood hemolysis, emulsification index and surface tension. SH20 and SH24 isolates showed the highest emulsification index 57 and 56%, respectively, and have positive results for hemolysis and drop collapse. The two isolates were identified using 16 S rRNA as *Bacillus amyloliquefaciens* SH20 and *Bacillus thuringiensis* SH24. Stability of biosurfactant production by both strains was observed at moderate temperature (30 °C), high alkalinity at pH (11) and high salt concentration (15%). An increase of emulsification index tended to be 60 and 69%, respectively, considering the two strains haloalkiphilic bacteria. To study the stability mechanism for extremophilic biosurfactant producers, the protein profile was determined using SDS-PAGE electrophoresis showing some new detected proteins depends on their culturing conditions. Partially purified biosurfactant from the most active strain *B. amyloliquefaciens* SH20 was chemically determined by FTIR and GC–MS analysis showed characteristic bands, revealed the presence of non-anionic didemnin surfactant.

Introduction

Surface-active compounds are chemically synthesized and commonly used in almost every sector of recent industry (Samadi et al., 2007). Environmental carefulness has expanded and led to alternative biological surfactants as the most promising existing product (Henkel et al., 2012). Biosurfactants are classes of high values using microbial activities which become important products that increase the demand of satisfy in wider applications (Sachdev and Cameotra, 2013). Biosurfactants are extracellular secondary metabolites, their structure depend on carbon and nitrogen ratio and influences on total production (Janek et al., 2013). The biosynthesis of these molecules with active surface occurs by new pathway or assembly from substrates (Syldatk et al., 1985).

Over synthetic surfactants, biosurfactants have several advantages: simplicity of syntheses, lower toxicity, action specificity and widespread applicability (Kumar et al. 2008). They are used as moistening, dispersing, emulsifiers and foaming agents. Also, the biosurfactant are the best environmental compatibility at extreme salinity, temperatures and pH (Datta et al., 2011). Therefore, hopefully future's extremophilic microbial surfactants appear to depend specifically on the use of plentiful and cheap substrates for optimization of the operational cultivation conditions, since they have particular adaptations to maintain stability in oppose environments (Makkar et al., 2011). The beneficial effects of this field were paid attention for isolation and characterization of extremophiles produced biosurfactants (Putri and Hertadi, 2015). Red Sea, the saltiest bodies of water in the world, has a wide salt preference diversity of slight (1.7–4.8%), moderate (4.8–20%) and extreme (20–30%) halophilic bacterial species having important compounds including biosurfactants (Ollivier et al., 1994; Kheiralla et al., 2013).

Thus, the present study intends to obtain haloalkali-bacterial species from Shalateen, Red Sea, Egypt, and explore them as a sustainable source for production of biosurfactants. Moreover, the two strains SH20 and SH24 were screened for their biosurfactant potentiality. Also, the mechanism of biosurfactant production stability was studied by SDS-PAGE electrophoresis techniques. Finally,

Peer review under responsibility of National Institute of Oceanography and Fisheries.

* Corresponding author.
E-mail address: kh2m2@yahoo.com (K.M. Barakat).

http://dx.doi.org/10.1016/j.ejar.2017.09.001
1687-4285/

Please cite this article in press as: Barakat, K.M., et al. Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt. Egyptian Journal of Aquatic Research (2017), http://dx.doi.org/10.1016/j.ejar.2017.09.001

characterization of the extracted biosurfactant product was chemically carried out using FTIR and GC–MS.

Materials and methods

Sampling and estimation of crude oil degrading marine bacteria

Three hundred ml water samples were collected from 21 oil contaminated sites (25 cm subsurface) at Shalateen, Red sea, Egypt (Fig. 1). Study area was characterized by 3.9–4.0% salt content and pH 8.0 – 8.2. Samples kept in sterile container were maintained at 4 °C and directly transferred to lab for further analysis.

Minimal salt medium (MSM) were prepared in filtered sea water according to Santos et al. (2014) containing (g/l): magnesium sulfate 0.2, dipotassium phosphate 1.0, ammonium nitrate 1.0, ferric chloride 0.05, calcium chloride 0.02, and agar 20. Five hundred μL of water samples were applied in petri dishes and swirled to ensure adequate mixing. One percent (v/v) of the sterile filtered crude oil was added as the sole carbon source. Plates were incubated for a period of 5–10 days at 25 °C. Isolates which produced clear zone of crude oil around colonies were counted, picked up and purified for further tests.

Screening for biosurfactant activities

Different bacterial colonies were inoculated in 100 ml of MSM broth supplemented by 2 drops of crude oil (Dutta and Harayama, 2001), and incubated under shaking condition (150 rpm) at 25 °C for 24–72 h. Bacterial cultures with emulsifying properties of crude oil as evidence for biosurfactant producing activity, represent as positive results. To confirm biosurfactant production by screened strains, further tests were carried out.

Drop collapsing test

The shape of the drop on the oil surface was examined after 1 min addition of 5 μL cultural supernatant to 2 μL of mineral oil surface. Positive (+) biosurfactant producing cultures gave flat drops, however, negative (-) were for those cultures with round drops i.e. the lack of biosurfactant production (Youssef et al., 2004)

Blood haemolysis

Blood agar plates containing 5% (v/v) human blood were used for screening haemolytic activity of the selected isolates. Occurrence of definite clear zones around a colony were detected after 24 h of incubation at room temperature (Carrillo et al., 1996).

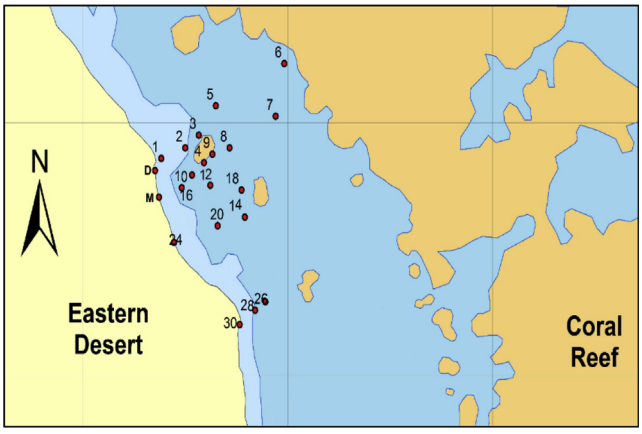

Fig. 1. Sampling sites at Shalateen, Red Sea, Egypt.

Emulsifying capacity

Emulsification index (E24) of cell-free supernatant was determined by adding equal volume of paraffin oil to the culture samples (v/v), then vortexing at high speed (2 min) and allowed to stand for 24 h. The percentage of E24 was calculated using the following equation (Techaoei et al., 2007).

$$E24 = \frac{\text{Height of emulsion formed (cm)} \times 100}{\text{Total height of solution (cm)}}$$

Surface tension

Surface tension measurements mN/m (mille Neuton/meter) were determined from cell-free culture obtained by centrifugation (Bodour and Maier, 2002). The mean of three measurements was recorded using surface tensiometer (TD 1 Lauda tensiometer, Germany).

Molecular identification of the potent biosurfactant producing marine bacteria

Genomic DNA was firstly extracted by lysozyme (20 mg/ml) and proteinase K (1 mg/ml). Total genomic DNA was purified using isopropanol buffer as described by Darwesh et al. (2014). Amplification reaction (PCR) of the 16 S rRNA genes was carried out using extracted DNA and the two primers F: (5′d AGAGTTTGATCCTGGCTCAG 3′) and R: (5′d TACGGTTACCTTGTTACGACTT 3′). The PCR amplification included initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s for complete extension (Kheiralla et al., 2016). PCR product was purified by QIAquick Gel Extraction Kit (QIAGEN, USA) and run on agarose gel for sequencing. Identification was achieved using the BLAST program (National Centre for Biotechnology Information). The sequences were aligned using Jukes Cantor Model. The phylogenetic reconstruction was done using the neighbour-joining (NJ) algorithm; with bootstrap values and submitted to Gene Bank.

Stability of biosurfactant activities

Biosurfactant stability of cultures growing on MSM at different temperatures (30, 40, 45, 50, 60 °C) and by changing pH (5, 6, 7, 9, and 11) were investigated. Salinity affecting on emulsification activity was determined by adding different concentrations of NaCl (0, 3, 5, 10, 15, 20, 25, and 30%) on the same growing medium. All the tested and control cultures were incubated under shaking condition at 150 rpm for three days. Emulsification activity was determined (El-Sersy, 2012).

Protein analyses for impacting of pH and salinity on SH20 and SH24 growth

SH20 and SH24 strains were grown into MSM and maintained at pH 11, salinity 15% NaCl. Culture media were inoculated and incubated at 30 °C for 3 days under shaking (150 rpm). For total proteins extraction, centrifuged cells were suspended in 200 μl of sample buffer then boiled for 10 min. After boiling, the mixture was centrifuged (5000 rpm for 5 min) (Darwesh et al., 2015). Total protein was separated by 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using mini-gel electrophoresis (BioRad, USA). The molecular weight of protein profile was estimated in comparison to standard markers (11–245 kDa; Sigma, USA). Using Coomassie Brilliant Blue R-250 stain, the protein bands were easily visualized (Laemmli, 1970).

Please cite this article in press as: Barakat, K.M., et al. Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt. Egyptian Journal of Aquatic Research (2017), http://dx.doi.org/10.1016/j.ejar.2017.09.001