Desalination and Water Treatment
www.deswater.com

24 (2010) 257–265
December

doi: 10.5004/dwt.2010.1569

Optimization and characterization of rhamnolipid biosurfactant from sponge associated marine fungi *Aspergillus* sp. MSF1

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Received 15 October 2009; accepted 13 June 2010

ABSTRACT

A sponge associated hydrocarbon emulsifying marine fungi *Aspergillus* sp. MSF1 was isolated from the marine sponge *Dendrilla nigra*. Production of biosurfactant was confirmed with different screening methods including hemolytic activity, oil displacement test, drop collapsing test, and emulsification index. The production was optimized under submerged fermentation conditions. An enhanced biosurfactant production was achieved with pH 7, 30°C, 2% NaCl, 1% $MgCl_2$, 1% asparagine and glucose and yeast extract as carbon and nitrogen sources, respectively. Among the different media, the Sabouraud's dextrose broth showed the highest emulsification activity. The surface active compound was separated using three step differential solvents fractionation and the active fraction was obtained in diethyl ether. Based on the estimation of macromolecules, TLC, FT-IR and HPLC analysis, the surface active compound was characterized as rhamnolipid. Emulsification activity of the cell free supernatant and different solvent extracts were compared with chemical surfactants including SDS and Tween80. The surface active compound showed potential activity against the pathogenic yeast *Candida albicans* and Gram negative bacteria.

Keywords: Biosurfactant; Marine-fungi; Optimization; Rhamnolipid; *Aspergillus*

1. Introduction

Biosurfactants have gained attention because of their biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and less-expensive substrates [1]. Biosurfactants are produced by many bacteria, actinomycetes, fungi and yeast. The molecular structure of these compounds comprise a hydrophilic portion, which consists of monosaccharides, oligosaccharides or polysaccharides, aminoacids or peptides or carboxylates or phosphate groups and a hydrophobic portion, which composed of saturated or unsaturated fatty acids (hydroxyl fatty acids and fatty alcohols). The main classes of biosurfactants are glycolipids, lipoaminoacids and lipopeptides, polymers phospholipids, monoglycerides, diglycerides and fatty acids [2].

The early interest in biosurfactants has arisen with the discovery of their antibiotic property [3]. Several biosurfactants have been shown antimicrobial action against bacteria, fungi, algae and viruses. Sophorolipids and rhamnolipids were found to be effective antifungal agents against plant and seed pathogenic fungi. Sophorolipids have the anticancer,

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immunomodulatory, anti-human deficiency virus and sperm immobilizing activities [4]. Environmental factors and growth conditions such as pH, temperature, agitation and oxygen availability are the critical control factors which influence the biosurfactant production. Salt concentration also affects the biosurfactant production depending on its effect of cellular activity [5]. The present study was aimed to explore pristine habitats for the isolation of potential biosurfactant producers. Marine organisms are good candidates for new pharmaceuticals and bioactive products [6]. Marine organisms are well-known to have the specific relationship with numerous microorganisms and sponges are no exception to this. From few decades the sponge-microbial association is a hot topic of research [7]. In addition to well-established sponge bacterial association, sponges got association with other microorganisms including marine fungi, which produce bioactive substances [8]. *Aspergillus* sp. MSF1 is a sponge-associated marine fungus has the ability to produce a wide range of enzymes and antimicrobial agents. In the present study, we report the optimization, purification and characterization of biosurfactant produced by marine fungi *Aspergillus* sp. MSF1, isolated from the marine sponge *Dendrilla nigra.*

2. Materials and methods

2.1. Sample collection and isolation of sponge associated fungi

Marine sponge *D. nigra* was collected from the Bay of Bengal region of the Indian peninsular coast by SCUBA diving at 10–15 m depth. To avoid cross contamination, only unbroken samples were used for microbiological analysis. The specimens were kept 2 h in sterilized aged seawater to remove loosely associated microorganisms from the inner and outer sponge surfaces. It has been hypothesized that this process may eliminate nonassociated bacteria from the host sponge by digestion. Environmental water representing the sponge habitat was taken prior to sponge sampling and filled up in 1 L sterilized glass bottles. Habitat water was used for isolation of fungi on Sabouraud's dextrose agar (SDA) and starch casein agar (supplemented with 2% NaCl) respectively [9].

2.2. Screening for potential producers

2.2.1. Hemolytic activity

Hemolytic activity was one of the preliminary assays for the biosurfactant production and it was performed in blood agar plates [10]. Blood agar plates containing 5% (v/v) human blood was used for the screening of isolated strains, 50 μl broth culture of MSF1 was inoculated on to blood agar plates and incubated at 37°C for 24 h. The plates were visually inspected for zone of clearance around the wells represented the positive results.

2.2.2. Drop collapsing test

Biosurfactant production was examined with drop collapsing test described by Youseff et al. [11]. In this method, 2 μl of mineral oil was added to 96 well microtitre plates. The plate was equilibrated for 1 h at 37°C and 5 μl of the culture supernatant was added to the surface of the oil. The shape of drop on the oil surface was observed after 1 min. The culture supernatant that make the drop collapsed was indicated as positive result and the drops remain beaded were scored as negative, which was examined with distilled water as control.

2.2.3. Oil displacement test

Biosurfactant production was screened by the zone of oil spreading which was observed macroscopically; the larger the zone of spreading oil, the higher is the surface-active property of the sample. To make an aliquot, 15 μl of weathered crude oil were placed on the surface of 40 μl distilled water in a Petri dish. Ten micorlitres of culture supernatant were gently put on the surface of oil film. After 30 s, the diameter of clear halo was visualized under visible light and the area was measured [12].

2.2.4. Emulsification index

The emulsification index was measured using the method as described by Cooper and Goldenberg [13]. In this method, 2 ml of the kerosene was added to equal volume of cell free supernatant (CFS) and homogenized in a vortex at high speed for 2 min. The emulsification stability was measured after 24 h and the emulsification index was calculated by dividing the measured height of the emulsion layer by the total height of the liquid layer multiplied by 100. The emulsification activity of the strain was compared with the standards such as SDS and Tween80.

2.2.5. Surface activity measurement

To extrapolate the surfactant activity of purified compound with emulsification index, the surface tension was measured with a tensiometer (Sigma) using the duNouy procedure with a platinum ring at 24°C. The surface tension–concentration plots were used to determine critical micelle concentrations [14]. In the present study, SDS was used as standard.

Table 1
Various culture conditions of MSF1 optimized for biosurfactant production. The factors were provided in combination with SDA medium

Factors	Ranges
pH	4, 5, 6, 7, 8, 9.
Temperature	10°C, 20°C, 30°C, 40°C, 50°C.
Salinity	1%, 1.5%, 2%, 2.5%, 3%, 3.5%.
Carbon sources (1%)	Glucose, paddy straw, olive oil, kerosene, vegetable oil.
Nitrogen source (1%)	Peptone, yeast extract, beef extract, $NaNo_3$, urea
Metals (0.1 mM)	$FeSO_4$, $CuSO_4$, $MnCl_2$, $CaCl_2$, $MgCl_2$
Aminoacids (0.1%)	Asparagine, valine, leucine, glycine, glutamic acid

2.3. Optimization of biosurfactant production

The fungal strain MSF1 was grown on different media such as SDA, PDA, ZMB and MSM medium in order to determine the growth conditions. The strain MSF1 was inoculated in a 250 ml Erlenmeyer flask containing 50 ml of appropriate medium and incubated for 120 h at 37°C. To predict the production, samples were collected at regular intervals for analysis. To optimize the culture conditions for biosurfactant production, the strain was optimized under the different culture conditions (Table 1).

2.4. Chemical characterization of surface active compound

2.4.1. Estimation of macromolecules

Solvent residue was subjected for the quantification of macromolecules such as protein, carbohydrate and lipid. Protein was estimated using the method of Lowry et al. [15] using bovine serum albumin as standard. Carbohydrate with phenol-sulfuric acid method by Chaplin and Kennedy [16], with glucose as standard and lipid was estimated for free fatty acid using the method of Sadasivam and Manickam, [17] with cholesterol as standard and the free fatty acid was calculated using the formula

$$\text{Estimation of free fatty acids} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample}}.$$

2.4.2. Glycolipid estimation

The orcinol assay was used for the direct assessment of the amount of glycolipids present in the sample [18]. Extracellular glycolipids concentration was evaluated in triplicates by measuring the concentration of rhamnosein 100 µl of sample by adding 900 µl of the solution containing 0.19% orcinol (in 53% H_2SO_4) was added. After heating for 30 min at 80°C the samples were cooled to room temperature and the OD was measured with rhamnose as standard.

2.4.3. TLC analysis

The concentrated extract was also analyzed by thin layer chromatography (TLC) on silica gel 60 plates (Merck). Chromatogram was developed with chloroform/methanol/acetic acid (15:5:1) and visualized by orcinol-sulfuric acid staining as described by Itoch et al. [19].

2.4.4. FT-IR analysis and HPLC analysis

Fourier transform infrared spectroscopy (FT-IR) is an analytical technique used to identify organic materials (in some cases inorganic material). This technique measures the absorption of various infrared light wavelengths by the material of interest. These infrared absorption bands identify specific molecular components and structures [20]. One milligram of freeze-dried partially purified biosurfactant was ground with 100 mg of KBr and pressed with 7,500 kg for 30 s to obtain translucent pellets. Infrared absorption spectra were recorded on a Thermo Niocolet, AVATAR 330 FTIR system with a spectral resolution and wave number accuracy of 4 and 0.01 cm^{-1}, respectively. All measurements consisted of 500 scans, and a KBr pellet was used as background reference.

For the HPLC analysis, the TLC spot was dissolved in 1 ml methanol and passed through a 0.22 µm pore filter. The TLC spot, which showed emulsification activity, was applied to liquid chromatography using semi-preparative HPLC (Shimadzu, Japan; HPLC RGM 011) on a reversed phase silica gel column. The column was eluted at a flow rate of 1.0 ml/min and observed at 254 nm. The concentration of biosurfactant was determined with peak calibration details of previous literatures. Eluted HPLC fractions were tested for emulsification activity and further analyzed by TLC for the characterization of the compound.

2.5. Antimicrobial activity of surface active compound

The extracted compound as well as the culture supernatant was tested for antimicrobial activity using well diffusion method and area of the zone was calculated [21]. Extracted active compounds were tested against human pathogens such as *Candida albicans*, *E. coli*, *Proteus mirabilis*, hemolytic *Streptococcus*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus*

subtilis and *Staphylococcus aureus*. Mueller Hinton agar plates were prepared and swabbed with pathogens. Using cork borer well was made and 50 µl of those extracted compound was added in wells, incubated at 30°C for 24 h. After incubation, the clear zone was measured and area of the clear zone was calculated.

2.6. Identification of the strain MSF1

Identification and characterization of the strain MSF1 was carried out with the methods described by Gilman [22]. The morphological and colony appearance was observed by naked eye examination of 3 days old culture grown on SDA medium. The micomorphology and sporulation was observed under light microscopy with LCB (lactophenol cotton blue) staining.

3. Results and discussion

Sponges are very fertile host animals for diverse symbiotic microorganisms and are simple multicellular invertebrate attached to solid surfaces in benthic habitats. They are filter feeders, numerous tiny pores on the surface allow water to enter and circulate through a series of canals where microorganisms and organic particles are filtered out and eaten. In some species of sponges, microorganisms can account for up to 60% of the sponge biomass (10^8–10^{10} bacteria per gram of sponge wet weight), exceeding seawater concentrations by two to three orders of magnitude [7].

3.1. Isolation and screening of biosurfactant producing fungi

Based on the colony morphology and stability in subculturing, eight sponge associated fungus were isolated from the marine sponge *D. nigra*. Among these, four strains showed positive for biosurfactant production particularly the strain MSF1 exhibited an emulsification activity (11.4%) in the CFS. Thus the strain MSF1 was selected for optimization and characterization of biosurfactant production.

Hemolytic activity of *Aspergillus* sp. MSF1 showed a clear zone diameter of 7 mm around the colony and no zone around the colony indicated the negative results. In the present study there is a relation between the hemolytic activity and the surfactant production. According to Carillo et al. [9] and Banat [23], biosurfactant production of the new isolates was preliminary screened by hemolytic activity however, in some cases hemolytic assay excluded many good biosurfactant producers [24] hence in the present investigation, oil displacement, drop collapsing and surface activity was included in the screening panel to confirm the biosurfactant production. The drop collapsing and oil displacement method was performed as a part of screening. In drop collapsing test, a flat drop was observed and in oil displacement method, a clear diameter of 5 mm was observed and the area was calculated as 78.50 mm^2, whereas beaded drop and no zone was noted in negative control. From the above two observations, it was confirmed that the strain *Aspergillus* sp. MSF1 was a biosurfactant producing marine fungi. Both the techniques have several advantages including small volumes of samples were required, rapid and easy to carry out and also do not require specialized equipment. Maneerat and Phetrong [25] revealed that about 15 isolates of marine bacteria showed positive results towards these screening methods.

The measurement of surface tension for the biosurfactant produced by MSF1 showed the reduction of surface tension of water (air–water interface) from 66 to 28 mN/m. This result was similar to other result produced by yeast from carbohydrate as substrate [14].

3.2. Optimization of biosurfactant production

3.2.1. Culture conditions

The strain MSF1 produced biosurfactant when grown in various nutrients. However, the amount of biosurfactant secreted varied in the presence of different nutrient sources of the four investigated culture medium, maximum biosurfactant production was observed in SDA (30%) followed by ZMB. There was no production of biosurfactant in MSM and PDA. Therefore SDA was selected as the biosurfactant production medium for the strain at 20°C for 96 h.

3.2.2. pH

The isolated strain *Aspergillus* sp. MSF1 was able to grow over a range of pH between 4 and 9. A better growth was obtained in pH 8 followed by pH 7 and pH 9. Maximum biosurfactant production was noted in pH 7 with E_{24} 20%. According to Carrillo et al. [9], the biosurfactant production was also correlated to the hemolytic activity as indicated in Fig. 1. This study also revealed that highest biomass concentration in pH 7–9 and lower in acidic condition. Abdel-Mawgoud et al. [26] indicated that the biosurfactant producing *Bacillus subtilis* isolate BS5 grew well at pH 6–9.

3.2.3. Temperature

The results displayed in Fig. 2 showed that this marine fungi *Aspergillus* sp. MSF1 could grows in all temperature. High cell growth was observed in 30°C

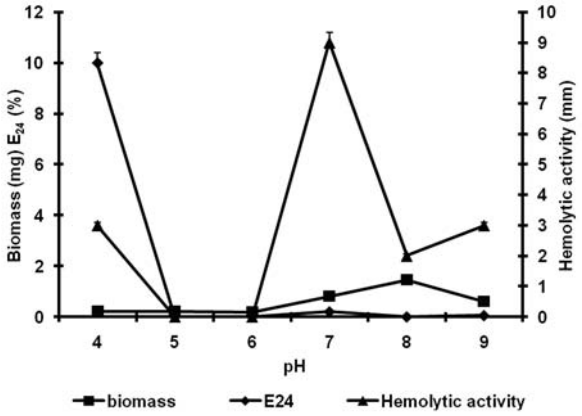

Fig. 1. Influence of pH on the production of biosurfactant, biomass and hemolytic activity of the strain MSF1. All the results are presented in triplicates.

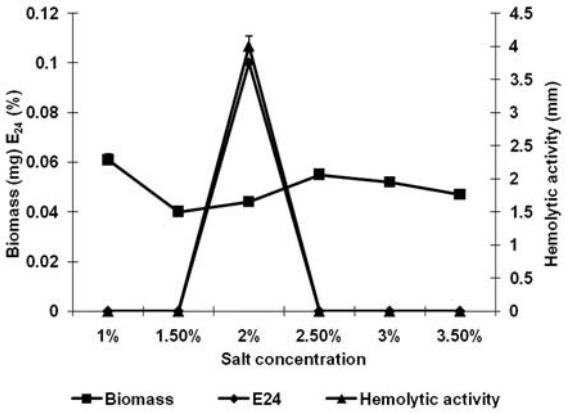

Fig. 3. Effect of salinity on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

followed by 50 and 10°C. Least cell growth was observed in 20 and 40°C. An emulsification activity of 20% was noted in 30°C. From the above results, it was confirmed that the biosurfactant production was higher at 30°C and lower at 40°C. Horowitz et al. [27] reported that biosurfactant production from *Bacillus lichenformis* was stable in the temperature ranges of 25–120°C.

3.2.4. Salinity

In this study, the salt concentration of 1% and 2.5% showed good growth, and better emulsification activity was observed in salinity of 2% (E_{24} 10%). A decrease in growth and emulsification activity was observed in other salt concentrations. According to Maneerat and Phetrong [25], the marine isolate showed a highest activity in the presence of 0–3% NaCl. In the present study, the highest activity was obtained in 2% NaCl (Fig. 3). Mulligan et al. [28] indicated that salt concentration of 4% gives the best growth and activity.

3.2.5. Carbon source

To determine the appropriate carbon source for the production of biosurfactant, four different carbon sources were used. Among carbon sources, better growth and emulsification activity was observed in paddy straw and glucose as substrate. Glucose showed a highest emulsification activity of 30% (Fig. 4). Many studies showed that glucose as the best carbon source. In the present study, paddy straw and vegetable oil

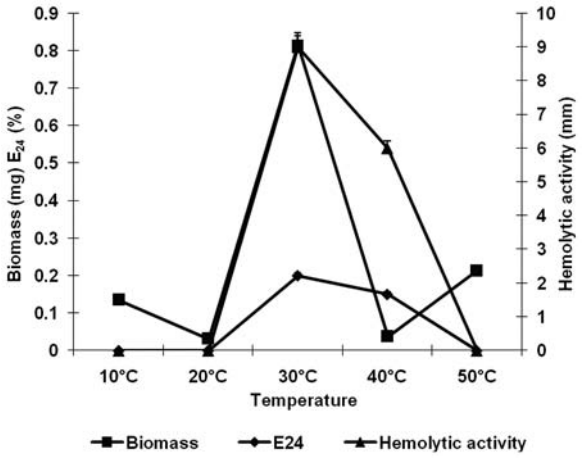

Fig. 2. Effect of temperature on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

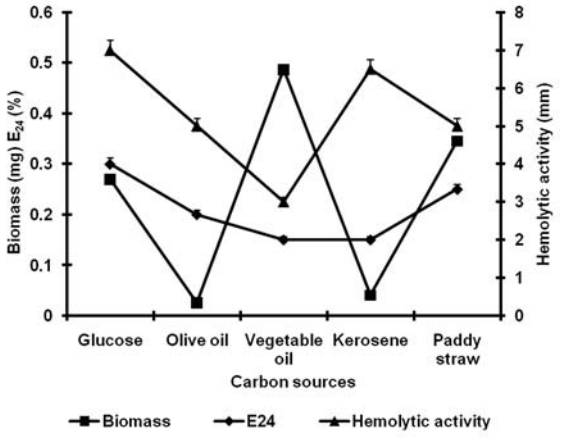

Fig. 4. Effect of carbon sources on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

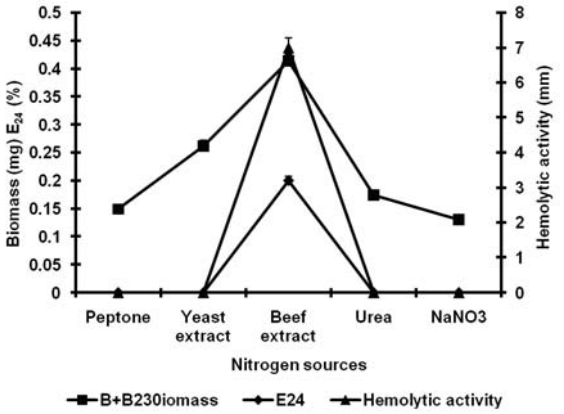

Fig. 5. Effect of nitrogen source on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

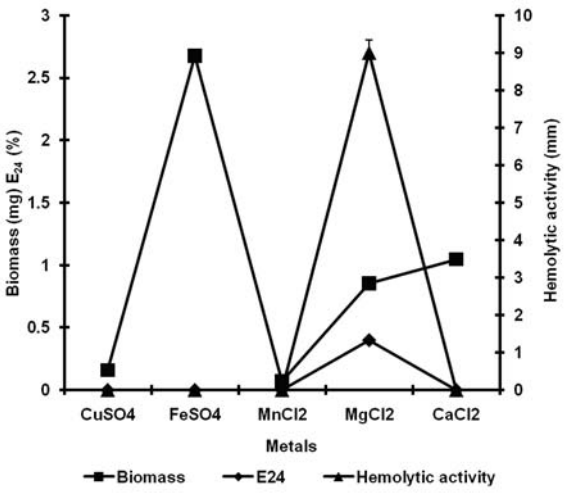

Fig. 6. Effect of metals on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

were appeared as best carbon sources with emulsification activity of 15% and 25%, respectively. A least growth was observed in olive oil followed by kerosene. The growth is less due to water immiscible substances. The results of Javaheri et al. [29] indicated that no growth was seen when using sunflower oil as a sole carbon source, but in our study the marine fungus MSF1 showed a maximum growth on vegetable oil, olive oil and kerosene as carbon source.

3.2.6. Nitrogen source

In this study, the nitrogen sources used are peptone, yeast extract, beef extract, urea and $NaNO_3$. Yeast extract gives a highest growth with a highest emulsification activity (Fig. 5). Ouled-Hadder et al. [30] reported that $NaNO_3$ was a good substrate for the growth but it gave a good productivity. It was reported that $NaNO_3$ and yeast extract used for the production of biosurfactant from different *Bacillus* sp. [13]. Many studies revealed that yeast extract promotes a good growth and emulsification activity. In our study yeast extract gives a better growth, followed by beef extract, urea, peptone and $NaNO_3$ and the emulsification activity was not observed in these substrates as nitrogen sources. These results are supported by Desai and Banat, [31] that nitrogen sources had the ability to limit the biosurfactant production.

3.2.7. Effect of heavy metals and amino acids on biosurfactant production

Fig. 6 showed the growth and activity of this strain using the metals. Here, the biomass was highest in $FeSO_4$ but no emulsification activity was observed, but in $MgCl_2$ a good growth was observed with higher activity ($E_{24} = 30\%$). Five different amino acids such as asparagine, glycine, glutamic acid, leucine and valine were optimized in this study. The aminoacids are provided in the Sabouraud's dextrose broth medium in the concentration of 1% (Fig. 7). As per Abdel-Mawgeued [26], surfactin optimization of metals with 1 mM gives higher growth and production, but manganese with 0.1 mM provides least growth, in our study 1 mM of manganese chloride gives a good growth with no emulsification activity. Cooper et al. [32] reported that no production was obtained by using the aminoacid as substrates, but in this study asparagine showed a

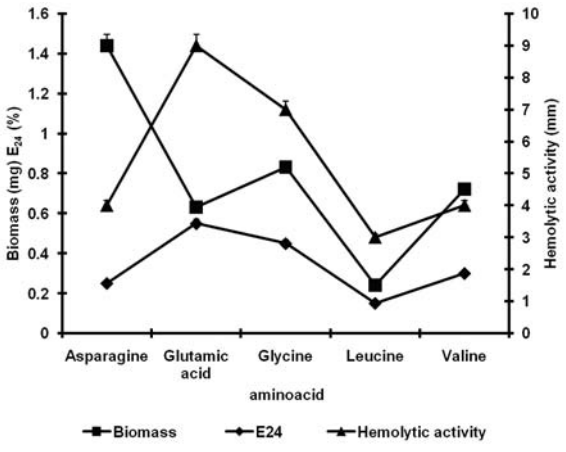

Fig. 7. Effect of aminoacids on biomass, hemolytic activity and production of biosurfactant by MSF1 in SDA medium. Emulsification activity was determined in terms of emulsification index E24 and the results are indicated in triplicates.

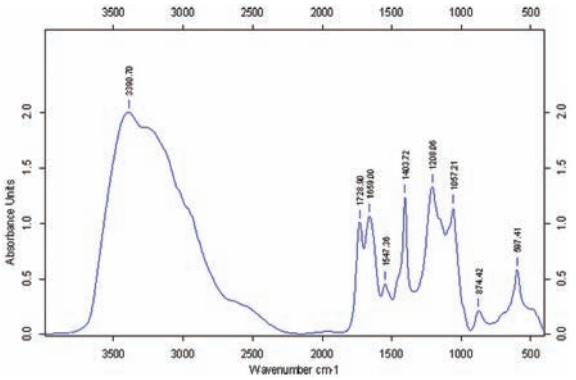

Fig. 8. FT-IR spectrum of biosurfactant produced by *Aspergillus* sp.

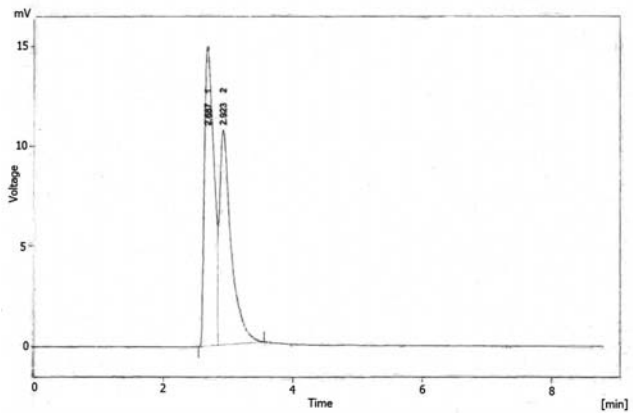

Fig. 9. HPLC chromatogram showed the surfactant produced by MSF1 isolated from the marine sponge.

good activity of 55% with a biomass concentration of 0.623 (Fig. 7).

3.2.8. Extract and characterization, chemical elucidation of biosurfactant

Acid precipitation of the CFS followed by solvent extraction was performed for the extraction of biosurfactant. Three solvents were used for this study including ethyl acetate, diethylether and dichloromethane. Of these solvent extracts, diethylether extract showed highest activity compared to other solvent extracts and culture supernatant. Emulsification activity of the pellet and supernatant of the extracts was also performed, the activity was observed only in the supernatant not in the pellet. Only two studies indicated the emulsification activity on the culture supernatant including a marine bacterium *Myroides* sp. SM1 [33] and *Renibacterium salmoninarum* 27BN [34]. In the present study, the emulsification activity was observed in the culture supernatant of the marine fungi *Aspergillus* sp. As observed in the present study, a previous report also indicated that the activity was higher in diethylether extract [34].

The concentrated diethylether extract was used for the estimation of protein, carbohydrate and lipid. Total protein was estimated as 3.82 mg/L, carbohydrate as 9.50 mg/L and lipid as 6.24 mg/L. Thus the overall percentage of macromolecules is as follows: 16% protein, 47.5% carbohydrate and 31% lipid. From the estimation it was revealed that the amount of carbohydrate and lipid was in higher percentage. The concentrated extract showed a total glycolipid concentration as 9.46 mg/L. The chromatogram of glycolipid showed an R_f value of 0.83 corresponding to the reference rhamnolipid from the strain *Pseudomonas aeruginosa* [19,34].

The molecular composition of the biosurfactant produced by the strain MSF1 was evaluated by FT-IR. Fig. 8 represents the spectra of the freeze dried biosurfactant sample from MSF1. The most important bands were located at 3390.70 cm^{-1} (OH bond, typical polysaccharides), 1728.90 and 1659.00 cm^{-1} (for C=O, C=O ester bond), 1,547 cm^{-1} (N-H), 1403.72 cm^{-1} (C–N amide groups), 1057.21 cm^{-1} (polysaccharides) and 874.42, 597.41 cm^{-1}(for the CH2 groups). Based on the important bands observed the compound responsible for the biosurfactant production was confirmed as the glycolipids moieties [32–35]. According to Gartshore et al. [19] FT-IR method was used for the quantitative analysis of biosurfactants. It is a quick and simple method used to quantify the concentration of most types of biosurfactants.

In the HPLC analysis, elution was done at 25°C with 100% methanol at a flow rate of 1 ml/min and observed with the absorbance at 254 nm (Fig. 9). Based on the important bands observed and by the retention time (2.5–3.5 min) [19], the compound responsible for the biosurfactant production was confirmed as the glycolipids moieties. Purified compound from HPLC was applied in TLC and it gives the R_f value of 0.83 which was compared with the glycolipids produced by *Pseudomonas*. The chemical analysis of the compounds such as the glycolipid estimation, TLC analysis, FT-IR and HPLC analysis confirmed the compound as rhamnolipid which is a class of glycolipid.

3.3. Comparison of synthetic surfactant with the biosurfactant

Emulsification activity of the biosurfactant was compared with three different solvent extracts as well as the CFS of MSF1 against the synthetic surfactants such as SDS and Tween80. In this marine fungi, the CFS showed 11.4% activity, diethyl ether extract showed a higher emulsification activity of 32% followed by ethyl acetate and dichloromethane. In SDS only 30% activity was observed, in which diethyl ether extract showed

Table 2
Antimicrobial activity of the marine fungus MSF1. All the tests were performed in triplicates and the standard error was calculated and tabulated

S. no.	Pathogenic culture	Area of zone (mm^2)
1	*Candida albicans*	200.96 ± 0.75
2	*E. coli*	153.86 ± 0.86
3	*P.mirabilis*	-
4	*H. Streptococcus*	200.96 ± 0.35
5	*P. aeruginosa*	803.84 ± 0.78
6	*M. luteus*	200.96 ± 0.56
7	*S.epidermidis*	78.5 ± 0.98
8	*E. faecalis*	200.96 ± 0.87
9	*K.pneumoniae*	15.86 ± 0.75
10	*Bacillus* sp.	-
11	*S. aureus*	153.86 ± 0.87

32% activity. 2% increase of emulsification activity was observed in this strain. Due to the low toxicity, easy degradability, and cheaper cost to produce compared to synthetic surfactant, this biosurfactant strain as well as this compound is a promising result to many of the research field.

3.4. Antimicrobial activity of surface active compound

Diethyl ether extract of *Aspergillus* sp. MSF1 showed a wide activity against the pathogens including *Candida albicans,* hemolytic *Streptococcus*, *Micrococcus luteus* and *Enterococcus faecalis* (Table 2).Therefore the future use of these biosurfactant as broad spectrum of antibiotics is highly promising. According to Tsuge et al. [36] lipopeptide surfactants are potent antibiotics mainly the surfactin, streptofactin, gramicidin produced by the microorganism had the wide antimicrobial activity compared to the glycolipid producing strain. A glycolipid surfactant from the *Candida antartica* has demonstrated antimicrobial activity against Gram positive bacteria. Therefore the future use of these biosurfactant as broad spectrum of antibiotics would be a highly promising venture.

3.5. Identification of the strain MSF1

Colonies of MSF1 was loose cottony, spreading surface, dark black sterile hyphae, hyaline, septate, ascending branched. In the culture plate the upper surface seems to be dark black and the substratum seems to be white in color. Conidiophores are simple and branched, width 3 μ, length 3.8 μ [22]. Based on the physiological and morphological observation, the strain MSF1 was identified as *Aspergillus* sp. MSF1.

In summary, sponge-associated marine microbes have been a goldmine for microbiologists and many research workers. The marine fungus was a boon to biodegradation of oil pollutants, production of antibiotics and various industrial applications. At present, due to high price of productivity and low yields, the biosurfactant are not competitive with chemical surfactants. Many researchers focus mainly on the development of novel recombinant hyperproducers for enhanced biosurfactants production. Therefore, the findings of the present study revealed that the biosurfactant from the marine fungi can be used for industrial and environmental applications.

Acknowledgements

This paper is an outcome of Department of Science and Technology's (DST-SERC) Young Scientist project of GSK. J.S. is thankful to P.A. Thomas for the identification of the sponge.

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