

Biosurfactant Production by Marine Actinomycetes Isolates *Streptomyces Althioticus* RG3 and *Streptomyces Californicus* RG8 as a Promising Source of Antimicrobial and Antifouling Effects

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Abstract

Background: Because of the ability of their bioactive metabolites production, many researchers were attracted to investigate and isolating marine actinomycetes from unique location with a unique environment. Information on antimicrobial activity and antifouling agents by *Streptomyces* sp. from the Ras Garib area, Gulf of Suez, Egypt is limited. One of the metabolites produced by the actinomycetes was biosurfactant. This paper describes the possibility of marine actinomycetes isolates for the production of biosurfactants, In addition to the possibility of using it as antimicrobial and antifouling agents.

Results: Marine actinobacterial isolates RG3 and RG8 had emulsification indexes of 76 and 68%, respectively. The two marine actinobacterial isolates were identified using 16srDNA as *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8, and submitted in the database of genetic information with accession number MW661230 and MW661234, respectively. Biosurfactants were stable at 10% NaCl, in case of *Streptomyces althioticus* RG3 and stable at 10%–15% NaCl in the case of *Streptomyces californicus* RG8. A temperature of 35°C was suitable for the stability of biosurfactants produced by both strains. Both strains produced the most biosurfactant when exposed to alkaline conditions. We characterized the biosurfactants produced by both strains including features such as the chemical composition of the biosurfactants and FTIR analysis. The antimicrobial activity of biosurfactants extract evaluated using a well diffusion method against *Vibrio alginolyticus* MK170250, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 4027, and *Staphylococcus aureus* ATCC 25923. *Streptomyces althioticus* RG3 biosurfactants have been shown to have better antimicrobial activity than *Streptomyces californicus* RG8, indicating that they may be used in pharmaceutical industries and in the manufacture of antifouling products.

Conclusions: *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8, isolated from Ras Garib, Gulf of Suez, Egypt, were able to develop very stable biosurfactants under stress conditions, which could be useful in a variety of industries such as pharmaceuticals and antifouling manufacturing.

Background

Surfactants help to form stable gels and foams by reducing surface tension at phase interfaces, emulsifying oil in water and water in oil mixtures, and emulsifying oil in water [1]. Synthetic precursors from the petrochemical and/or oleochemical industries can be used to make these amphiphilic compounds; however, surfactants can be produced by microbial organisms in a variety of forms. Biosurfactants and bioemulsifiers are surface- compounds that are active derived from sources biological such as yeasts, bacteria, and yeasts [2]. Microbial surface-active compounds' molecular structure is used to classify them. Lipoproteins, lipopolysaccharides, heteropolysaccharides, and proteins are examples of large molecular mass compounds known as bioemulsifiers. Compounds with a lower molecular weight known as biosurfactants include glycolipids such as rhamnolipids, sophorolipids, mannosylerythritol lipids, and trehalose lipids, as well as lipopeptides like surfactant and fengycin. [3]. High and low molecular weight surface active compounds may be utilised in a broad range of utilizations comprising

treatments for individuals and at home; chemicals used in agriculture; improved recuperation of microbials from oil; textiles and biomedicines. Bio-factants and bio-emulsifiers are an alternative to synthetically produced surfactants because of their low toxic, biodegradable content and an increased consumer awareness of sustainability and environmental protection [4]. Actinobacteria are members of the order Actinomycetales. Actinomycetes with characteristic filaments are gram-positive bacteria. Actinomycetes encompass a wide variety of habitats. They are present in different soil types, marine and ground water, and ecosystems of mangrove [5]. They can be alkali- and thermophilic [6], halophilic [7], or acidophilic [8]. Due to their ability to produce metabolite combinations that differ in structure and function, they played a significant position in several industries. The quest for metabolites of actinomycetes is made possible by exploration of certain areas, unique ecosystems and combinations of isolation methods [9]. Surfactants, active metabolites that reduce surface tension, are thought to be formed by the majority of microorganisms, including actinomycetes. The purpose of the research was to isolate actinomycetes from marine environments and explore their capability for producing commercially significant biosurfactants.

Methods

Sampling and isolation of marine actinomycetes

In sterile plastic bags, samples of marine sediment were obtained from Ras Garib area of the Gulf of Suez in Egypt (Fig.1). The coordinates and map work were obtained at the Marine Physics Laboratory, (NIOF), Red Sea, using the Surfer program version 15.2.305. This sediment sample was found to be high in oils and was therefore used to monitor for actinomycetes containing biosurfactants. A dry heating method was used to isolate actinomycetes. For eight days, sediment samples were dried in a 70°C oven, then mashed and filtered through a 1–5 mm sieve. The samples were shaken for two minutes and then left for one minute before being suspended in nine ml of sterilized seawater [10]. From these solutions, serial dilutions were made, up to 10^6 , then each dilution was added to the starch nitrate medium in 0.1ml increments. with the following components ($g\ l^{-1}$): 20 starch, 0.5 K_2HPO_4 , 1 KNO_3 , 0.5 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4$, 15 agar [11] at pH 7.0. It was fortified with griseofulvin and 0.05 ppm chloramphenicol. After that, the culture 7 days at $28^\circ C \pm 2^\circ C$ was incubated. Preliminary selection of actinomycete colonies was made based on a morphology of colony, and they were streaked on the previous medium. For further identification tests, in the refrigerator at $2^\circ C$ isolates were held.

Screening of biosurfactant producers

The selected isolates have been inoculated into a 500 mL flask of Erlenmeyer with 100 mL Kim biosurfactant medium. It consisting of ($g\ l^{-1}$): $NaNO_3$, 1.0; KH_2PO_4 , 0.1; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl_2$, 0.1; yeast extract, 0.2% and 3% olive oil as the carbon source at pH 6.0 [12]. For 5 days, the cultures of broth were incubated at $32^\circ C$ in a shaker incubator at 120 rpm min. Positive results were considered actinomycete cultures that emulsified oil as evidence of biosurfactant producing activity. Further tests were carried out to validate biosurfactant output by the screened strains. The existence of biosurfactant in cell-free

supernatant was investigated using drop collapsing tests, hemolytic tests, blue agar plates, emulsifying capacity, and surface tension measurements.

Drop collapsing test

After 1 minute of adding five mL of supernatant of culture to two mL of mineral oil surface, on the surface of oil, the drop shape was examined. Cultures that produced positive (+) biosurfactant produced flat drops, but cultures that produced round drops produced negative (-) results [13].

Hemolytic test

Actinomycetes isolates are grown in Blood Agar. 1.5 mL sterile human blood was pipetted into an Erlenmeyer flask containing 100 mL mineral agar medium. Until it became frozen and solid, the sterile blood medium was poured. The potential biosurfactant isolates were inoculated on blood medium, which was incubated at 32°C for 7 days. The hemolysis zone and changed of color produced by the behavior actinomycetes were tested [14].

Blood agar plate

To a glycerol yeast extract agar medium with methylene blue (0.2 mg/ml), carbon sources (2 % w/v) and cetyl trimethyl ammonium bromide (0.5 mg/ml) were added. A dark blue halo around the culture was deemed favorable for biosurfactant processing. [15].

Production of Lipase

Using tributyrin agar plates, the lipase formed by pure marine actinomycetes cultures was determined. After being inoculated, the plates were incubated at 30°C for 7 days. After the incubation period, A clear zone around the colonies was tested on the plates [16].

Emulsifying capacity

Emulsification index (E24) supernatant after adding volume of paraffin oil (v:v) was determined, then vortexed at highest speeds two min, to the sample cultures and allowed to stand for 24 hours. The E24 percentage with the following equation was determined [17].

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

Actinomycetes Isolate's identification

Morphological, physiological, biochemical, and molecular approaches were used to identify the most potent marine actinobacterial isolates [18]. DNA was isolated and purified from the selected actinomycetes. 16S sequence analysis was used to characterize the genotype., Finally, Bioedit was used to perform several alignments of the sequences of the members that are the most closely related [11].

Medium optimization for highest production of biosurfactant

By using series experiments, the medium was optimized by changing one variable at a time while maintaining the other variables constant at a set of conditions. To increase biosurfactant productivity, two factors were chosen: the source of carbon and the source of nitrogen. The sources of carbon which used is dextrose, sucrose, mannitol, xylose, maltose, glycerol, and glucose (20g/l). Separate filtration using bacteriological filters was used to sterilize the carbon sources, and then applied under aseptic conditions to the production medium. In order to determine the best nitrogen sources in biosurfactant production at concentrations of 10g/l with the optimal carbon source, yeast extract, urea, peptone, tryptone, ammonium sulfate, and beef extract were used [19].

Stability of biosurfactants activities

The stability of biosurfactants was studied in cultures grown on Kim biosurfactant medium at various temperatures (20°C, 30°C, 35°C, 45°C, 50°C, and 60°C) and pH (4, 5, 6,7,8, 9, 10, and 11). To the same growing medium, various NaCl concentrations were used to measure the salinity influencing emulsification activity (0%, 3%, 5%, 10%, 15%, 20%, 25%, and 30%). Both checked and medium were incubated for 7 days at 120 rpm under shaking conditions. The activity of emulsification has been determined [20].

Biosurfactant extraction

A solvent extraction method [21] was used to extract the biosurfactants provided by *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 from the filtrate. The biosurfactant was extracted and purified using 4L of culture broth centrifuged for the first time at 7000 rpm and 4°C for 25 minutes. The crude material was extracted by combining it with a similar equal quantity of chloroform: methanol at a 2:1 (v/v) ratio in a separator flask, shaking it vigorously, and letting it sit for 30 minutes. The bottom aqueous layer of the separation funnel was withdrawn, white bio-emulsifier layer immediately above it was collected in petri dishes and evaporated in a 40°C–45°C oven [10].

The biosurfactant's chemical composition

Lowry's method was used to assess concentration of protein from isolated biosurfactant [22], using bovine serum albumin as standard. By using the method of phenol sulfuric acid, carbohydrate content was determined [23]. D-glucose was the traditional tool for measured total lipid content [24].

Fourier Transform Infrared Spectroscopy (FTIR)

In (SRTA-City), the functional classes of isolated biosurfactants from *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 were identified using (FTIR) using a spectrum 400 instrument (PerkinElmer, Waltham Massachusetts MA, USA). Under ambient conditions, 400–4000 cm^{-1} were the spectral resolution and the precision of the wave number.

Antimicrobial Assay

The antimicrobial activity of the biosurfactants production against *Vibrio alginolyticus* MK170250, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 4027, and *Staphylococcus aureus* ATCC 25923 was determined using agar well diffusion method. In a nutshell, 100µl of aqueous biosurfactant solutions is poured into 6-mm-diameter wells in the pour plated nutrient agar. The plates were incubated at 37 °C for 24 h. The presence of a clear zone around the wells indicated antimicrobial activity [25]. These bacterial pathogens were kindly provided by the staff members of the National Institute of Oceanography and Fisheries (NIOF), Red Sea branch, Hurghada city, Egypt.

Antifouling activity

In a conical flask (50 ml) containing sterilized cover glass, seawater (1 ml) was mixed with nutrient broth medium (20 ml) (Oxoid Ltd, England) and incubated overnight at 28°C. About 500 ul of aqueous solutions of the biosurfactant was added into the flask (as an antifouling agent). The flask was filled with 500 µl of biosurfactant aqueous solutions (as an antifouling agent). The cover glass was washed in water, dried at room temperature, and examined under the microscope after ten minutes of dying with crystal violet solution (0.4 %) [11].

Data analysis

For data analysis, the R software (version 3.0.1) for Windows was used, and the results were interpreted as the mean ± standard deviation (SD) of the quintuple's samples.

Results And Discussion

Isolation and production of biosurfactants

Nine actinomycetes isolates were isolated from soil that has been tainted with fuel oil in the Ras Garib region of Egypt's Gulf of Suez and screened for biosurfactant production. Extracellular biosurfactant production of 3% olive oil from Kim's medium was initially screened for all these isolates as the only source of carbon. Out of nine isolates, only two isolates, RG3 and RG8, showed positive results. During the screening process, olive oil was used as the sole carbon source, as the biosurfactant production was increased using actinomycetes [26]. One of the parameters for selecting potential producer biosurfactants is the emulsification activity. Biosurfactant productivity is calculated by emulsifying activities [15]. The marine actinomycetes isolates RG3 and RG8 had the highest emulsification indices, as shown in Table 1, of 76% ± 2.5% and 68% ± 2.2%, respectively. Korayem [16] and his team found that, *Streptomyces* isolate 5S had an emulsification index of 31.74%. After initial screening by Zambry and his team [26], the emulsification index test (E24) has provided positive results for all isolates of actinomycete, the results varying between 84.11% to 95.80%. Selected isolates showing positive results against the collapse of the drop and hemolytic action. In this analysis, the biosurfactant producer was screened with lipase activity. Kokare says that [27], lipase in the water/oil surfaces was responsible for the operation, and therefore actinomycetes have shown that lipase is capable of producing bioemulsifiers. In this work, isolates RG3 and RG8 showed zones of lysis around the colonies (Fig.2). The

surface tension of the supernatant and purified biosurfactant was measured using a surface tensiometer. Both marine actinomycetes isolates (RG3 and RG8) reduced surface tension to values of 77.24 ± 2.4 mN/m and 69.27 ± 2.4 respectively, recording positive results for biosurfactant activity. Fig.3 shows the measurements of the surface tension effects and those caused by a decrease in the surface tension value. Arifiyanto *et al.*, [14] reported that three actinomycetes isolates belong to *Streptomyces* sp. named AF6, AB8, and AA1, which were 56.22, 57.27 and 57.33 mN/m surface tension values, respectively. If a bacterial isolate surface tension decreased to 40 mN/m or less, Cooper and Goldenberg [28] suggest that it could be a promising producer of biosurfactants. The marine organism *Virgibacillus salaries* displayed a decrease in tension on the surface (30 mN/m), an E24 = 80% of twenty, and three morphologically distinct colonies, collapse of drop, distribution of oil, and hemolysis of blood all showed promising effects [29].

Biochemical and molecular characterization

Table 2 displays the morphological, physiological, and biochemical characteristics of the selected isolates. The sequences of 16S rRNA for the selected RG3 and RG8 isolates were compared with the *Streptomyces* sp. sequence using multiple sequence alignment to validate the actinomycete isolate's identification. Using agarose gel electrophoresis, an experimental study of PCR amplification was performed. Table 3 shows the GenBank accession numbers of the closest neighbors of isolates RG3 and RG8. Isolate RG3 was assigned as *Streptomyces althioticus* RG3 with the accession number (MW661230) Fig.4(A). Isolate RG8 was given the name *Streptomyces californicus* RG8 with accession number (MW661234) as it is affiliated according to the genus *Streptomyces californicus* Fig.4(B).

Factors affecting the emulsification activity

There has been considerable research into the generation of biosurfactants by actinomycetes under extreme conditions for commercial use [30]. We investigated the effect of extreme conditions, including carbon sources, nitrogen sources, higher salt, temperature, and pH, on the production of biosurfactant from the marine isolates of *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8, to assess its stability.

Effect of various sources of carbon

The amount of biosurfactant generated was measured and found to be relying on the composition of medium. Changes in the carbon source in material was caused by biosurfactant secretion in shaken-flask experiments. Sucrose, dextrose, and glucose were found to be the most effective sources of carbon for production biosurfactant. Glucose (2% w/v) in the two strains *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 were the carbon sources producing the most biosurfactants (Fig.5). Nutrient substrates were screened, and we found that these isolates supported growth in all substrates, although xylose was limited in yield. Khopade and his team [19] said that, production of biosurfactant though marine *Streptomyces* sp. isolate B3 was decreased surface tension to 29 mN/m and the

emulsifying activity showed 80%. *Streptomyces* sp. generated the most biosurfactants when sucrose was used as the carbon source, according to previous research [16, 19].

Effectiveness of nitrogen sources

Production of biosurfactant is affected by the nitrogen source, as shown in Fig. 6. Macro-nutrients need specific conditions to produce high concentrations of biosurfactants [19]. In *Streptomyces althioticus* RG3 we discovered that the better use of nitrogen is required for growth and production of biosurfactant was yeast extract, which had an E24 of 69.25%, while ammonium sulfate was best for the isolation of *Streptomyces californicus* RG8, with an E24 of 59.21%. Other studies in which organic nitrogen sources were favored over inorganic sources produced similar findings [31, 32].

Stability of bio-surfactant activities

Effectiveness of different salt concentrations

Fig.7 indicates that the emulsification of *Streptomyces althioticus* RG3 was stable (70%) with 10% NaCl, and then gradually decreased to 15%–30% NaCl. *Streptomyces californicus* RG8 emulsification operation was steadily increased from 10% to 15% NaCl to 60%, and then decreased to 20%–30%. Khopade [19] reported that, in 4 % (w/v) NaCl, *Streptomyces* sp. B3 which isolated from marine resource generated the most biosurfactant (E24 = 78 %), and represented activity of emulsification (E24 = 60%) in the using of 9% (w/v) NaCl. Elkhawaga [10] showed, *Streptomyces griseoplanus* (MS1) have ability to produce biosurfactant stable at concentrations of the NaCl up to 10%. Elazzazya [29] stated that, in 4% (w/v) NaCl, biosurfactant synthesis was obtained.

Various temperatures effectiveness

One of the most critical parameters in the bioprocess was temperature. At 35°C, the highest emulsifying activity of *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 was observed, at emulsifying indices of 78% and 65%, respectively. Even so, there was still stable biosurfactant output from 45°C to 50°C, implying that both isolates were thermotolerant (Fig.8), and indicating that the biosurfactant is moderately thermostable. The composition of the biosurfactant in *Arthrobacter paraffineus* and *Pseudomonas* sp. changed as the temperature changed [13, 33]. A similar study indicated that the biosurfactant activity was highest when the marine *Streptomyces* species B3 was cultivated at 30°C (E24 = 80%) [19]. At 30°C–40°C, The surface tension of *Pseudomonas* sp. (MW2) culture broth was decreased, according to Dahil [34]. Deng and his team [35] showed that rising level of biosurfactant was generated by *Achromobacter* sp. HZ01 at 40°C, 60°C, and 80°C.

Effect of pH

Due to the large amount of use of biosurfactants in the production of detergents, it was necessary to choose an alkaline biosurfactant from isolated bacteria. [20]. As seen in Fig.9, the highest level of emulsification operation for *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 it was the

alkaline (pH 10) and reached 69% and 65%, respectively. This may be attributed to greater surfactant micelle stability with fatty acids in the presence of NaOH and secondary metabolites precipitation with rising values of pH. [19]. Operation for the emulsification was decreased due to a decrease in the value of pH moving from the basal to the acidic zone (9 to 5 pH), because of partial biosurfactant precipitation [36]. In alkaline pH (8–9), the bio-emulsifier activity was higher than in acidic pH. (5) [10]. Nadem et al, stated that *Streptomyces* sp. SS 20, isolated, soil contaminated with hydrocarbons has a high activity of bioemulsifying and stability over in a wide temperature spectrum (30°C–100°C) and pH level of 3–7 [37]. According to El-Sersy, the E24 of *B. subtilis* was stable in a pH range of 6 to 10 [38], with acidic pH decreased. The pH effect for biosurfactants of various microorganisms on surface activity has been recorded [39].

Biosurfactant chemical characterization

Chemical composition of the biosurfactants

S. althioticus RG3 produces a biosurfactants had a chemical structure of 30% protein, 20% carbohydrate, and 50% lipids. *S.californicus* RG8 provided a biosurfactant with a chemical composition of 18% proteins, 45% carbohydrates, and 37% lipids. Lipids, glycolipids, lipopeptides, and polysaccharide protein complexes are examples of biosurfactants isolated from microorganisms [40]. Biosurfactant biochemical composition is likely to be influenced by the substrates used in the growth medium. In the founded of refinery soybean oil, The biosurfactant production from *C. lipolytica* UCP0988 contained proteins in the rate of 50.0 %, carbohydrates in the rate of 8.0 %, and lipids in the rate of 20.0 %, according to Rufino and his colleagues [41]. Thavasi recorded other findings, such as the biosurfactant production from *L.delbrueckii* containing carbohydrates in the rate of 30.0 % and lipids in the rate of 70.0 % [42]. The biosurfactant of *C. Sphaerica* UCP0995 contained 15.0 % carbohydrate and 70.0 % lipids, according to Luna and his team [43].

Fourier Transform Infrared Spectroscopy (FTIR)

The biosurfactant developed by *S.althioticus* RG3 was analyzed using FTIR and showed the presence of 11 clear absorption peaks at 3343.1, 1653.7, 1401.8, 1084.9, 1007.2, 988.2, 832.6, 702.8, 663.4, 618.2, 543.8 cm^{-1} , as shown in Fig.10A. FTIR analysis of produced biosurfactant by *S. californicus* RG8 depicts the presence of eight clear absorption peaks at 3431.7, 2079.9, 1638, 1434.1, 1384.8, 1103.8, 983.5, 614.4 cm^{-1} , as shown in Fig.10B. Peaks at 3343.1 and 3431.7 cm^{-1} were amide groups, and a diketone group were apparent in 1653.7 and 1638.01 cm^{-1} . The peaks at 2079.9 and 1084.9 cm^{-1} were attributed to C-O bonds. The absorption peak at 1401.8 and 1434.1 cm^{-1} indicates for nitrosamine presence. In 1384.8 cm^{-1} peaks was isopropyl, whereas that at 1103.8 cm^{-1} refer to ester carbonyl group indicates. In 1007.2–983.5 cm^{-1} peaks were polysaccharides. The peak at 832.63 cm^{-1} was an aromatic group with absorption peaks at 702.8–543.8 cm^{-1} . These findings agree with the results of other studies [10, 44].

Antimicrobial activity

Because of their anti-adhesive agents and enzyme inhibitors, biosurfactants have operated as fungicidal, bactericidal, insecticidal, and antiviral ingredients [14, 19, 45]. In this study, we observed that an increase in the biosurfactants concentration led to improvement of its antimicrobial effects (Table 4). The highest antimicrobial effects of biosurfactants were observed against *Vibrioalginolyticus* MK170250 from *Streptomyces althioticus* RG3 (Fig.11) followed by the effects against *Escherichia coli* ATCC 8739. Gram negative bacteria are more antagonistic to *Streptomyces* VITSDK1 spp. surfactants, with an inhibition zone of 10.3 mm in *Klebsiellapneumoniae*. *Staphylococcus aureus* is inhibited by *Streptomyces* VITSDK1 spp. surfactants by 5.3 mm [46]. *Streptomyces* sp. strain AF1 has a distinct personality. It can grow at a high temperature of about 70°C and generate an antimicrobial biosurfactant [14].

Antifouling activity

Figure 12 illustrated the inhibitory action of *Streptomyces althioticus* (RG3) biosurfactants on bacterial biofilm formation. The biosurfactants production by *Streptomyces althioticus* RG3 reduced the density of bacterial cells and acted as an anti-biofouling agent, while *Streptomycescalifornicus* RG8 not achieved that. Napyradiomycin which production from marine *Streptomyces aculeolatus* isolate PTM-029 exhibited the higher antibacterial activity, the higher microfouling inhibitory activity, and the most potent antimicrofouling activity [47].

Conclusions

Two marine actinobacterial strains, *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8, isolated from Ras Garib, Gulf of Suez, Egypt was able to produce very stable biosurfactants under stress conditions, additionally that can be valuable in several industries like pharmaceutical industries and antifouling manufacturing.

Declarations

Authors' contributions

Mohammad R Alhuzani, Moaz M. Hamed, Mohamed H. Al-Agamy, and Asmaa M. Yousif performed the methodology. Moaz M. Hamed and Asmaa M. Yousif drafted the manuscript. Mohamed H. Al-Agamy, Moaz M. Hamed and Asmaa M. Yousif contributed in data analysis, writing, review, and editing of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All the data is presented in the manuscript and any additional data will be provided upon request. Please contact Mohamed Al-Agamy.

Consent for publication

Our manuscript does not contain any individual data in any form.

Ethics approval and consent to participate

No animals or human subjects were used in the above research.

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Tables

Table 1: Characterization of biosurfactant activities.

Isolate	Emulsification index (%)	Hemolysis	Drop collapse	Clear zone in blue agar plates	Lipase production (mm)	Surface tension mN/m
RG1	30 ± 1.7	-	-	-	+	-
RG2	-	-	-	-	-	-
RG3	76 ± 2.5	+	+	++	18	77.2 ± 2.4
RG4	-	-	-	-	-	-
RG5	25 ± 1.5	-	-	-	+	-
RG6	-	-	-	-	+	-
RG7	-	-	-	-	-	-
RG8	68 ± 2.2	+	+	+	16	69.2 ± 2.4
RG9	-	-	-	-	-	-

Results are expressed as mean ± SD ($n = 3$) where mean is significant at $p < 0.05$.

(+) positive result, (-) Negative result

Table 2: Biochemical identification of biosurfactant producing marine actinomycetes isolates

Test	Actinomycetes isolates	
	RG3	RG8
Substrate mycelium	Pale brown	Cream
Aerial mycelium	Cream	White
Diffusible pigments	Beige	-
Hydrolytic activity		
lipase	+	+
Starch	+	+
Lactose	+	+
Dextrose	+	+
Maltose	+	+
Cellulose	+	+
Mannitol	+	-
Sucrose	+	+
Glucose	+	-
Citrate utilization	+	-
H ₂ S production	-	-
Urase hydrolysis	+	+
Indole production	-	-
Voges Proskauer test	-	-
Gelatin hydrolysis	+	+

(+) positive result, (-) Negative result

Table 3 Accession numbers and percentages of similarity to the nearest known species

Isolate No.	Identification	Strain No & (Accession No.) of closely related species	Similarity (%)
RG3	<i>Streptomyces althioticus</i>	CSSP544 & NR_115392	95
RG8	<i>Streptomyces californicus</i>	CSSP711 & NR_115432	95

Table 4: Antimicrobial activities of the biosurfactants production by *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8

Microorganism	Antimicrobial assays	
	<i>Streptomyces althioticus</i> RG3	<i>Streptomyces californicus</i> RG8
<i>Vibrio alginolyticus</i> MK170250	+++	+
<i>Escherichia coli</i> ATCC 8739	++	+
<i>Pseudomonas aeruginosa</i> ATCC 4027	+	-
<i>Staphylococcus aureus</i> ATTC 25923	+	+

“+” sign demonstrates microbial growth inhibition, whereas “-” The failure to prevent the growth of the microorganisms under investigation. (+++) strongest effect, (++) moderate effect.

Figures

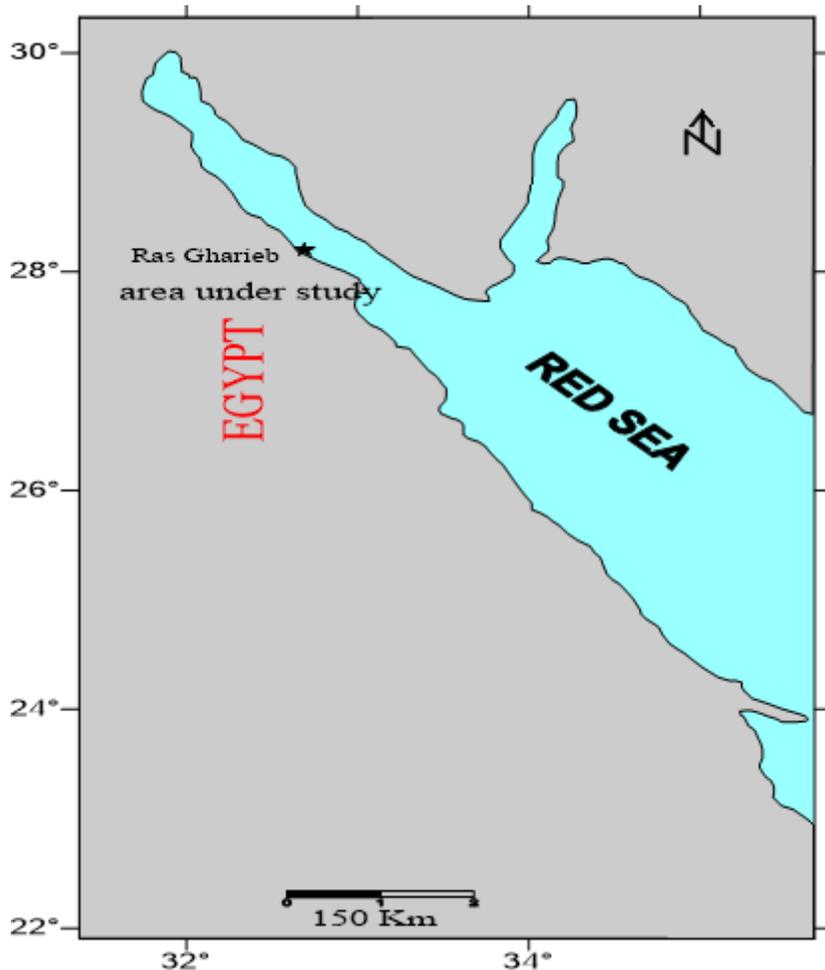


Figure 1

Map of the Ras Gharib area and the location of sampling site. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

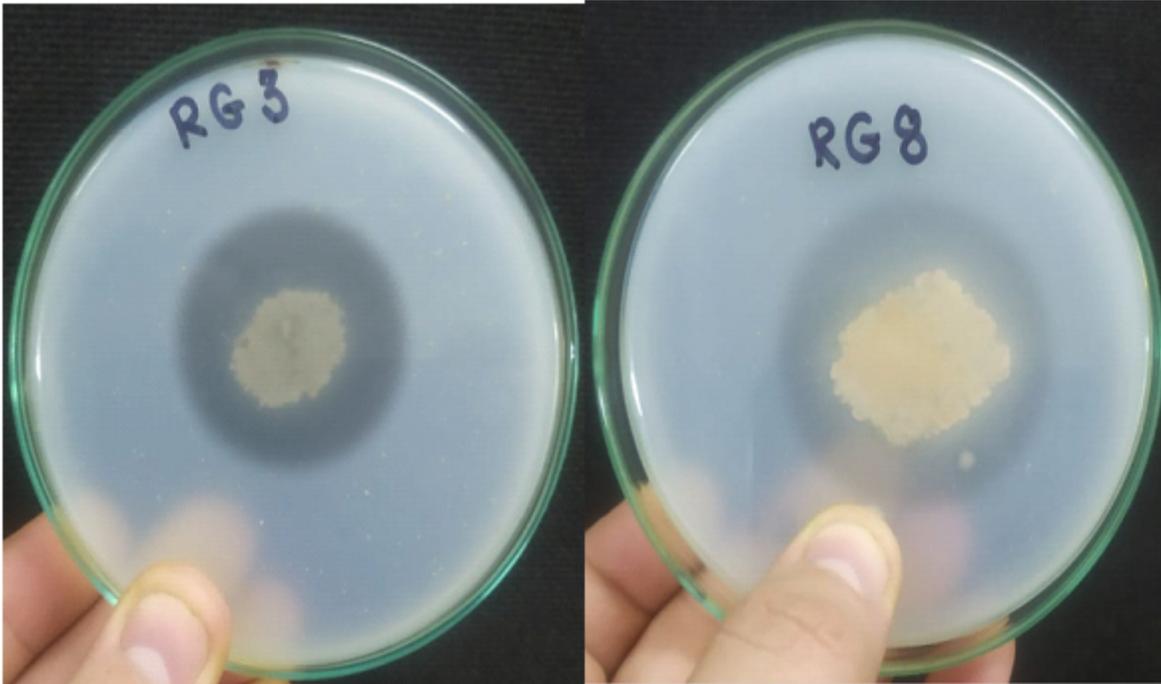


Figure 2

Screening of actinomycetes isolates (RG3 and RG8) for biosurfactant production: lipase activity test showing a clear zone.

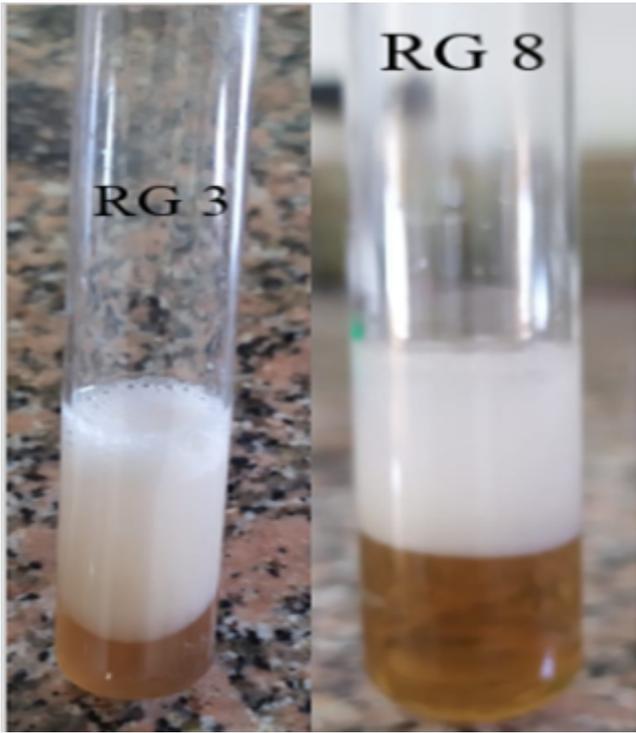


Figure 3

Emulsion formation using paraffin oil and inoculated marine actinomycetes supernatant for isolates (RG3 and RG8).

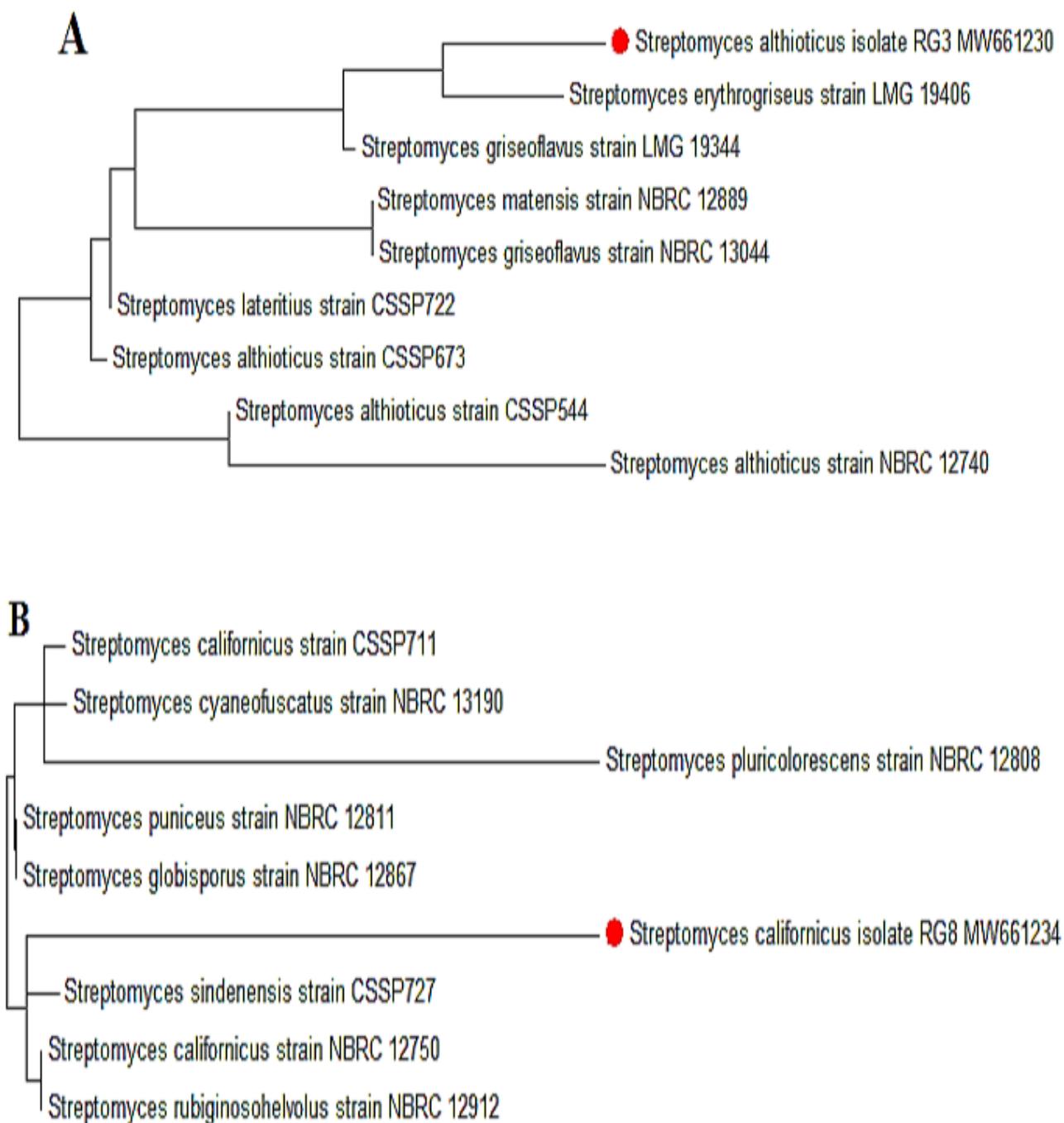


Figure 4

Phylogenetic tree of isolate RG3 (A) and RG8 (B) based 16S rRNA based dendrogram showing the phylogenetic position of isolates RG3 and RG8 among representatives of related bacterial species. The tree was constructed using Bioedit method Gene Sequence Analysis.

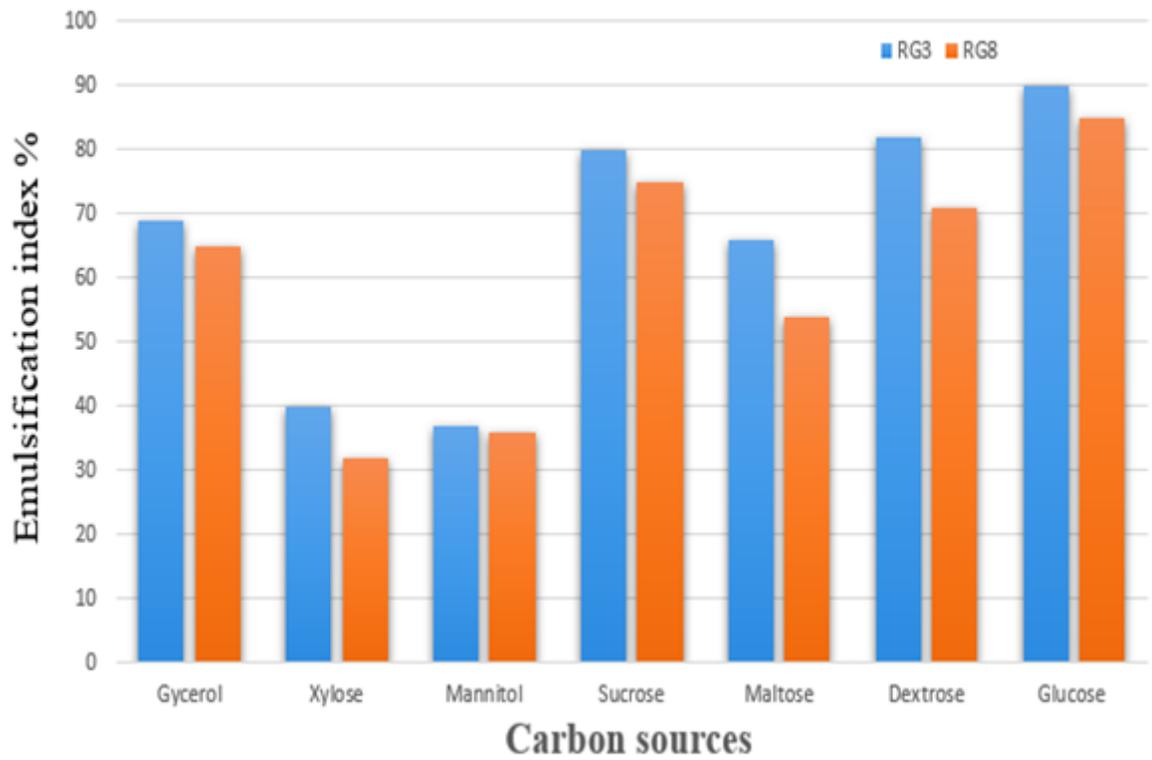


Figure 5

Effect of carbon sources on biosurfactant production by *Streptomyces althoticus* RG3 and *Streptomyces californicus* RG8.

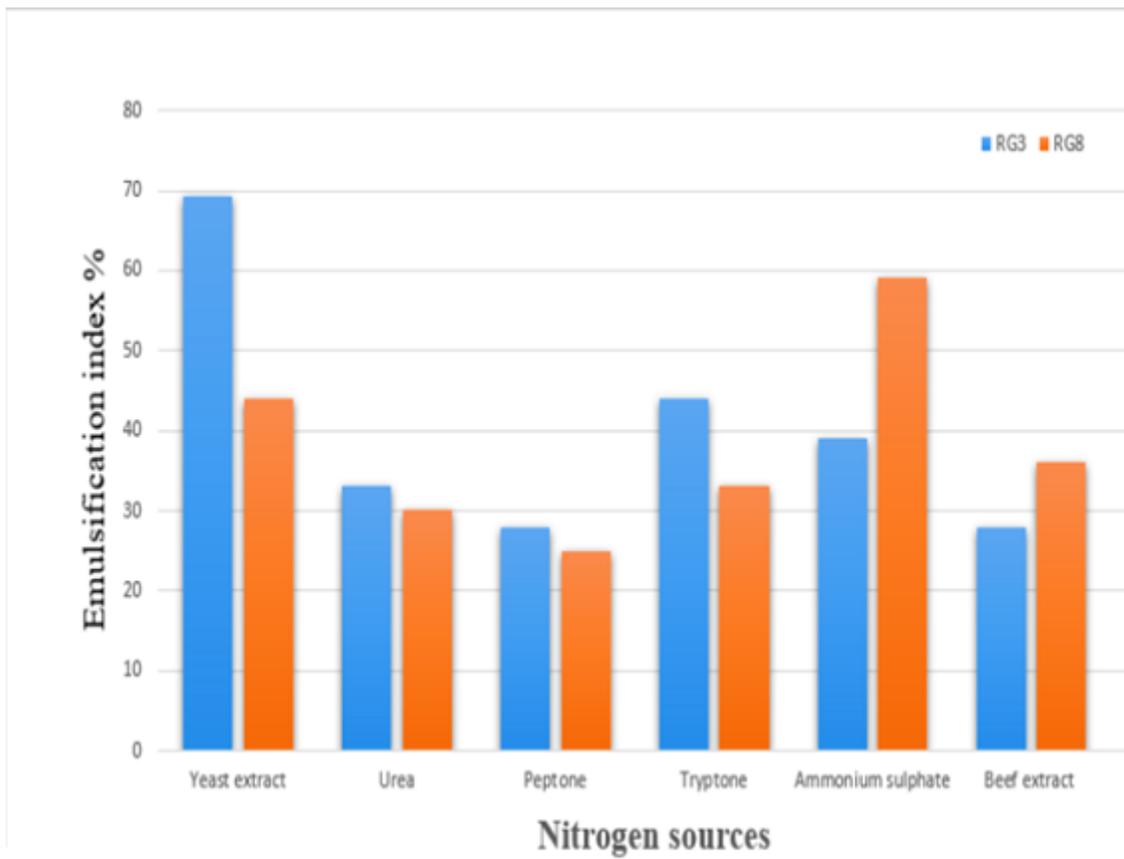


Figure 6

Effect of nitrogen sources on biosurfactant production by *Streptomyces althoticus* RG3 and *Streptomyces californicus* RG8.

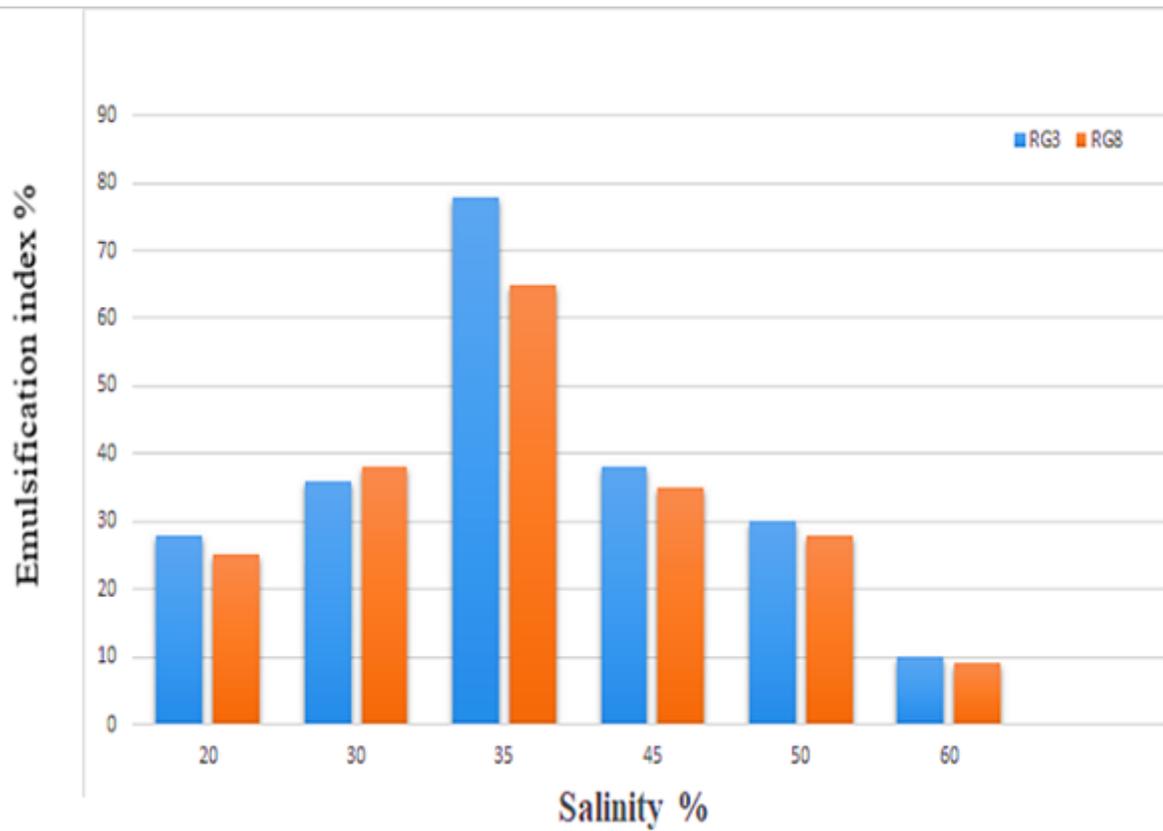


Figure 7

Effect of NaCl on biosurfactant production by *Streptomyces althoticus* RG3 and *Streptomyces californicus* RG8.

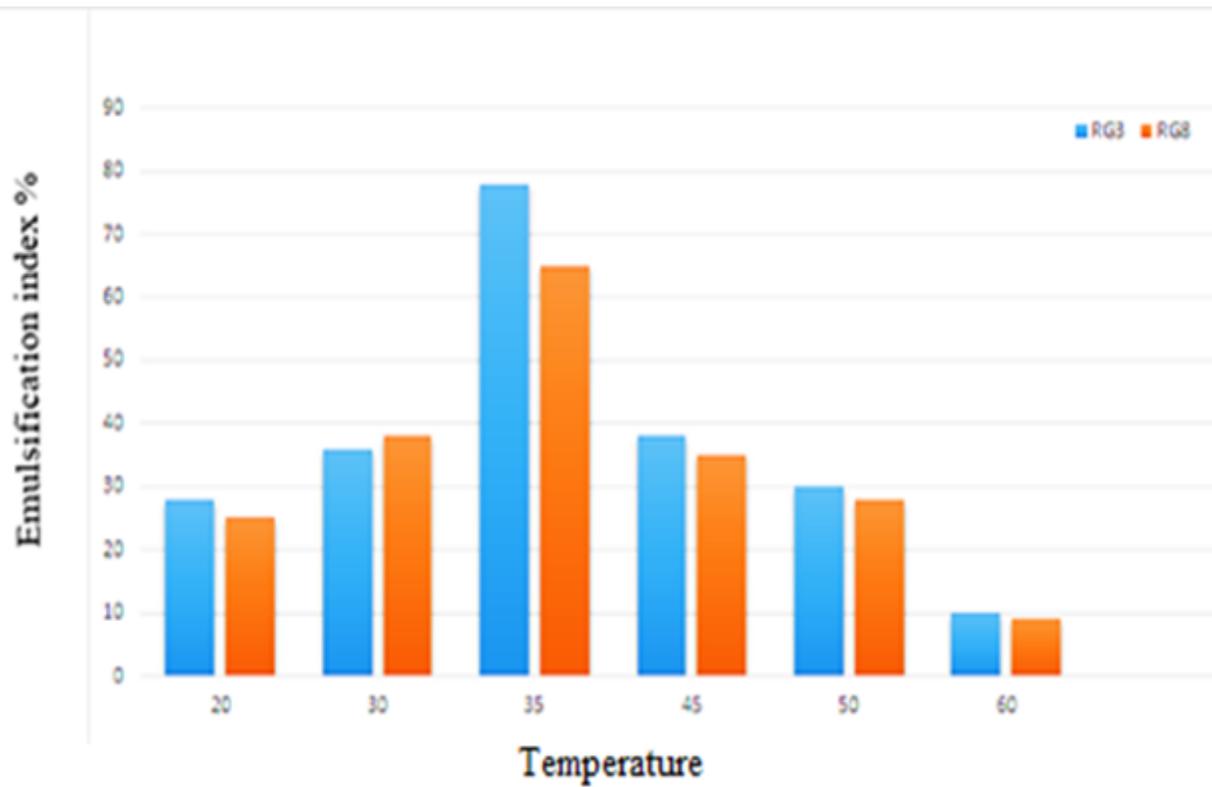


Figure 8

Effect of temperature on biosurfactant production by *Streptomyces althoticus* RG3 and *Streptomyces californicus* RG8.

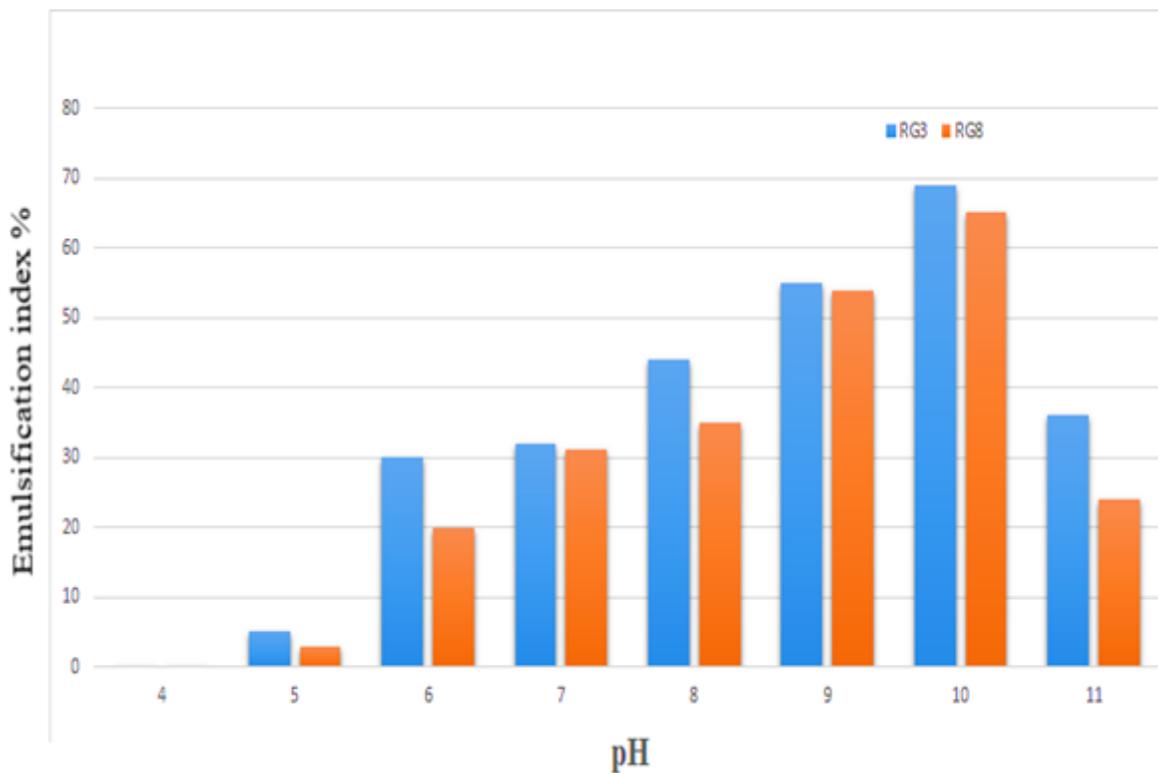


Figure 9

Effect of pH on biosurfactant production by *Streptomyces althoticus* RG3 and *Streptomyces californicus* RG8.

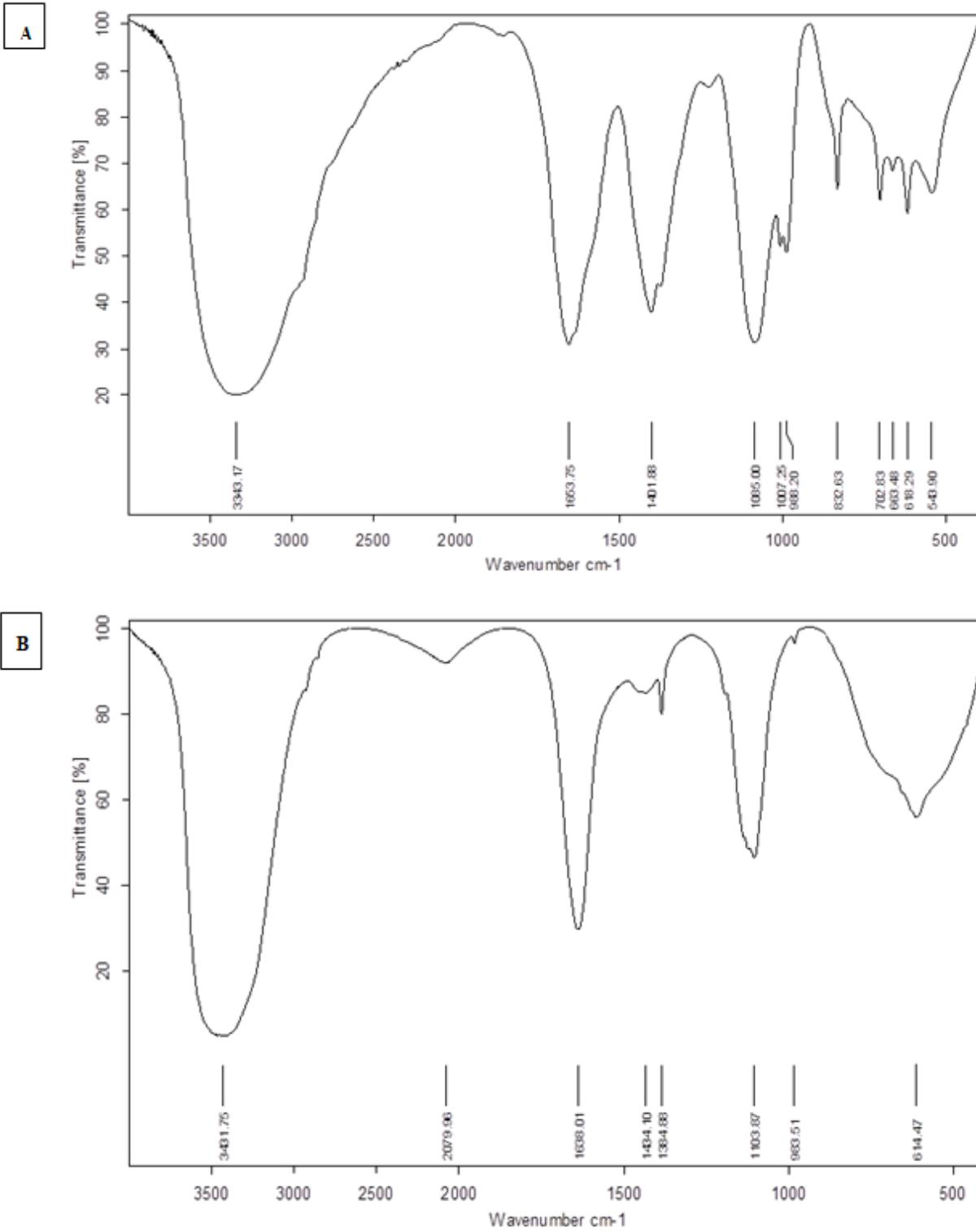


Figure 10

FTIR analysis of biosurfactant production by *Streptomyces althoticus* RG3(A) and *Streptomyces californicus* RG8 (B).

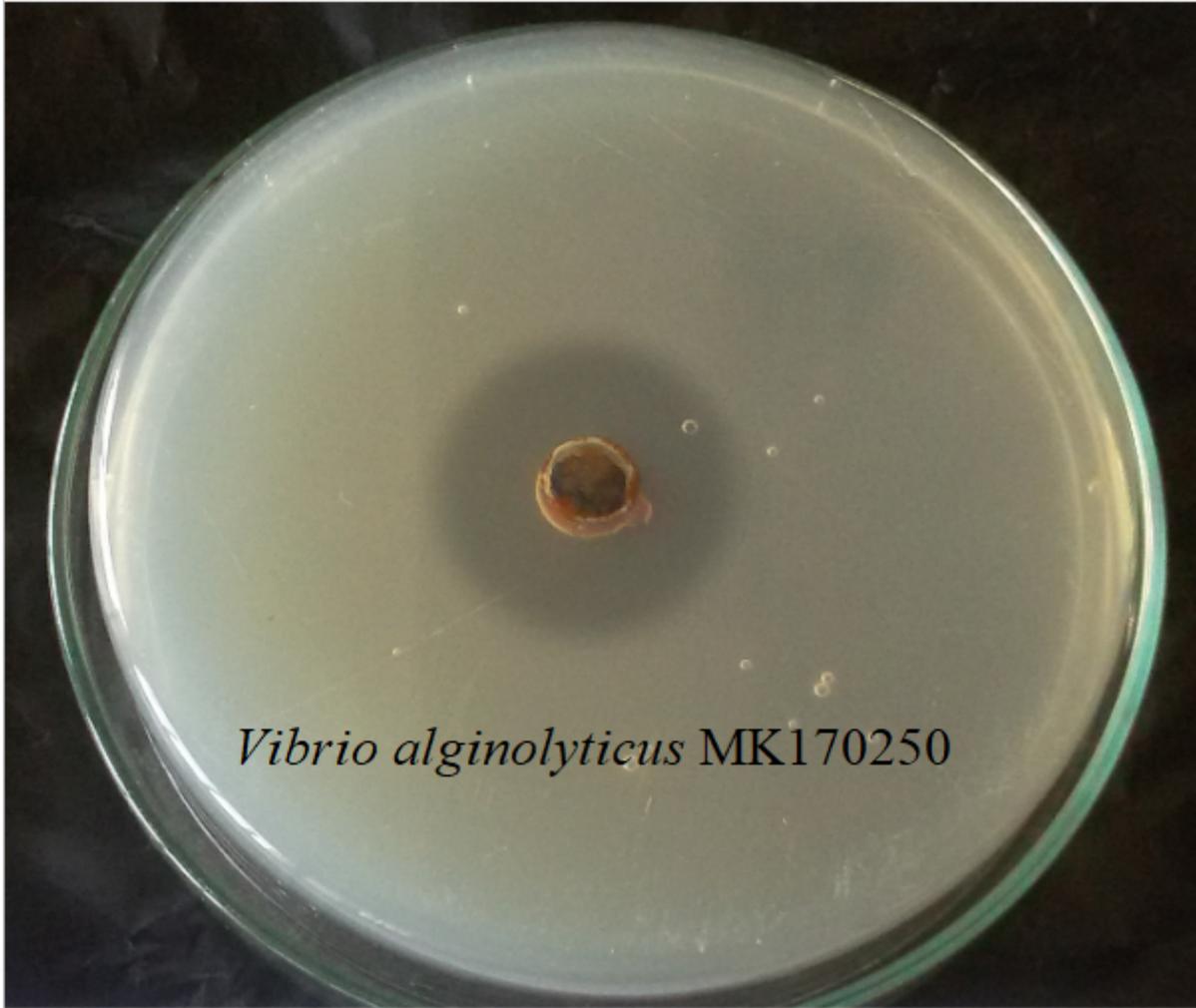
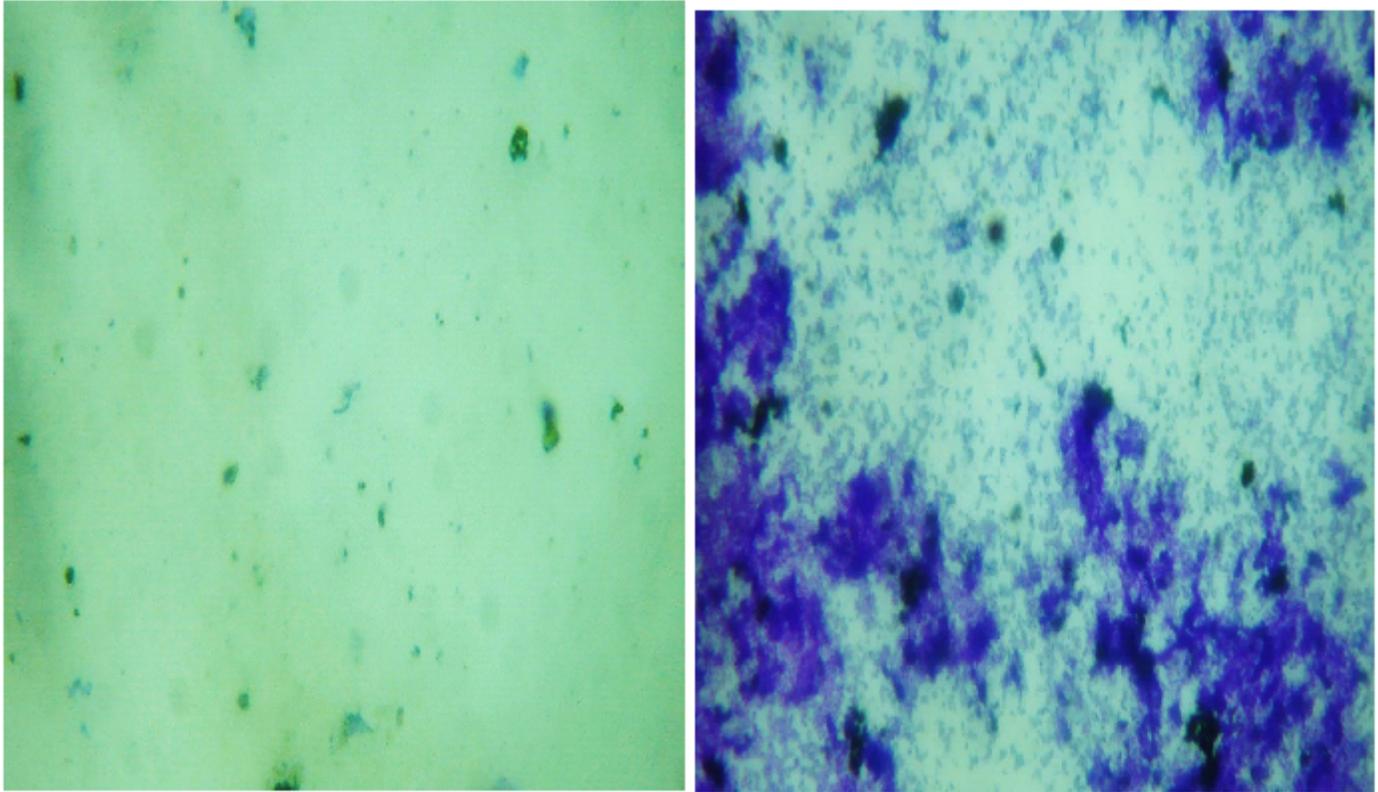


Figure 11

Antagonistic effects of biosurfactants production by *Streptomyces althoticus* RG3 against *Vibrio alginolyticus* MK170250



(A)

(B)

Figure 12

Photographs illustrating the antifouling effect of the biosurfactants from *Streptomyces althoticus* (RG3) on the biofilm formation, the emergence of a small number of bacterial colonies (A) and the effect without the biosurfactant compounds, the emergence of large numbers of bacterial colonies (B).