

Biosurfactant Production by Marine Actinomycetes Isolates *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 as Promising Sources of Antimicrobial and Antifouling Effects

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Two marine actinobacterial isolates, RG3 and RG8, were identified using 16Sr DNA as *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8 and submitted to the database of genetic information with accession numbers MW661230 and MW661234, respectively; they were found to have emulsification indexes of $60 \pm 2.5\%$ and $53 \pm 2.2\%$, respectively. The biosurfactants obtained were stable at a temperature of 35 °C for both strains; they were stable at 10% NaCl, in the case of *S. althioticus* RG3 and at 10–15% NaCl in the case of *Str. californicus* RG8; both strains produced the most biosurfactant when exposed to alkaline conditions. We characterized the biosurfactants, including features such as their chemical composition, using Fourier transform infrared spectroscopy analysis. The antimicrobial activity of the biosurfactant extracts was evaluated using the well diffusion method against *Vibrio alginolyticus* MK170250, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 4027, and *Staphylococcus aureus* ATCC 25923. *S. althioticus* RG3 biosurfactants were found to have better antimicrobial activity than those of *Str. californicus* RG8, indicating that they may be used in pharmaceutical industries and in the manufacture of antifouling products.

Keywords: Biosurfactants, actinomycetes, antimicrobial, red Sea, Egypt

Introduction

Surfactants help to form stable gels and foams by reducing surface tension at phase interfaces, emulsifying oil in water and water in oil mixtures [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. 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 sus-

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tainability 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. 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.

Materials and 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 (latitude 28° 21' 35"N and longitudes 033° 04' 39" E) (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 (MRC laboratory oven S36, UK), then mashed and filtered through a 1–5 mm sieve (AS 200 Control R-etsch Haan, Germany). The samples were shaken for two minutes and then left for one minute before being suspended in nine ml of sterilized seawater

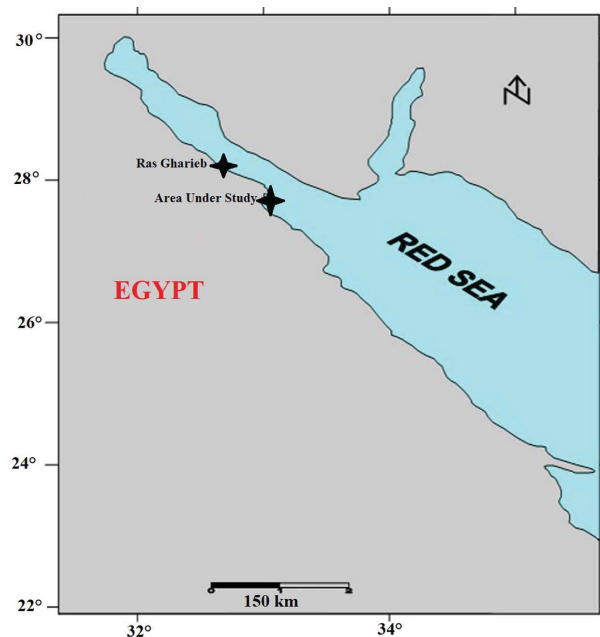


Fig. 1. Map of the Ras Gharib area and the location of sampling site.

[10]. From these solutions, serial dilutions were made, up to 10^6 , then each dilution was added to the starch nitrate medium in 0.1 ml increments. with the following components (g l^{-1}): 20 starch, 0.5 K_2HPO_4 , 1 KNO_3 , 0.5 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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 $28 \pm 2^\circ\text{C}$ was incubated in incubator (BDS-T 100/NS Biotec, Egypt). 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°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; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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°C in a shaker incubator (Benchmark Scientific Incu-Shaker 10L, USA) 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, emulsifying capacity, and surface tension measurements.

Drop collapsing test

After 1 min of adding five ml of supernatant of culture to two ml of mineral oil surface, 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 blood was pipetted into an Erlenmeyer flask containing 100 ml mineral agar medium (1.8 g K_2HPO_4 , 4.0 g NH_4Cl , 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $NaCl$, 0.01 g $FeSO_4 \cdot 7H_2O$ in 1 L and 15 g agar). 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].

Production of lipase

Using tributyrin agar plates (5.0 g Special peptone; 3.0 g Yeast extract and agar 12 g in 1 L), 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 [15].

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 h. The E24 percentage with the following equation was determined [16].

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

Surface tension measurement

Measurements of surface tension mN/m (mille Neuton/meter) have been determined using centrifugation of

cell-free actinomycetes culture by using surface tensiometer (TD 1 Lauda tensiometer, Germany) [17].

Actinomycetes isolate 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 (20 g/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 10 g/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 (20–60°C) and pH (4–11). To the same growing medium, various NaCl concentrations were used to measure the salinity influencing emulsification activity (0–30%). 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 *S. althioticus* RG3 and *Str. californicus* RG8 from the filtrate. The biosurfactant was extracted and purified using 4 L of culture broth

centrifuged for the first time at 7000 rpm and 4°C for 25 min. 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 min. 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 40–45°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].

Fourier Transform Infrared Spectroscopy (FTIR)

In City of Scientific Research and Technological, Alexandria, Egypt, the functional classes of isolated biosurfactants from *S. althoticus* RG3 and *Str. californicus* RG8 were identified using (FTIR) using a spectrum 400 instrument (PerkinElmer, 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* ATTC 25923 was determined using agar well diffusion method. In a nutshell, 100 μl 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 [24]. These bacterial pathogens were kindly provided by the staff members of the National Institute of Oceanography and Fisheries (NIOF), Egypt.

Antifouling activity

In a conical flask (50 ml) containing sterilized cover glass, seawater (1 ml) was mixed with sterile sweater (20 ml) and incubated overnight at 28°C. About 500 μl of aqueous solutions of the biosurfactant extract 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 dyeing with crystal violet solution (0.4%) [11].

Data analysis

All experiments were carried out in triplicate. The values are the mean \pm standard deviation (SD) of three separate experiments. The least significant difference ($\text{LSD}_{5\%}$) was used in a one-way analysis of variance.

Results and Discussion

Isolation and production of biosurfactants

Nine actinomycetes isolates were isolated from sediment 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 carbon source for biosurfactant production was increased using actinomycetes [25]. One of the parameters for selecting potential producer biosurfactants is the emulsification activity. Biosurfactant productivity is calculated by emulsifying activities [26]. The marine actinomycetes isolates RG3 and RG8 had the highest emulsification indices, as shown in (Table 1),

Table 1. Characterization of biosurfactant activities.

Isolate	Emulsification index (%)	Hemolysis	Drop collapse	Lipase production	Surface tension mN/m
RG1	30 \pm 1.7	-	-	+	-
RG2	-	-	-	-	-
RG3	60 \pm 2.5	+	+	+	77.2 \pm 2.4
RG4	-	-	-	-	-
RG5	25 \pm 1.5	-	-	+	-
RG6	-	-	-	+	-
RG7	-	-	-	-	-
RG8	53 \pm 2.2	+	+	+	69.2 \pm 2.4
RG9	-	-	-	-	-

Results are expressed as mean \pm SD ($n = 3$) where mean is significant at $p < 0.05$. (+) positive result, (-) Negative result.



Fig. 2. Screening of actinomycetes isolates (RG3 and RG8) for biosurfactant production: lipase activity test showing a clear zone.

of $60 \pm 2.5\%$ and $53 \pm 2.2\%$, respectively. Korayem [15] and his team found that, *Streptomyces* isolate 5S had an emulsification index of 31.74%. After initial screening by Zambry and his team [25], 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

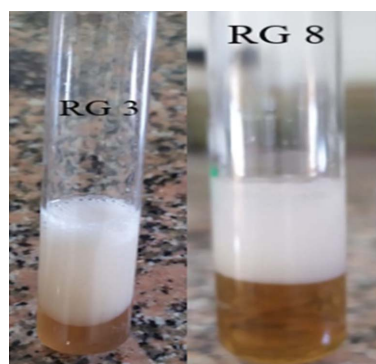


Fig. 3. Emulsion formation using paraffin oil and extraction of isolates (RG3 and RG8).

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%, 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

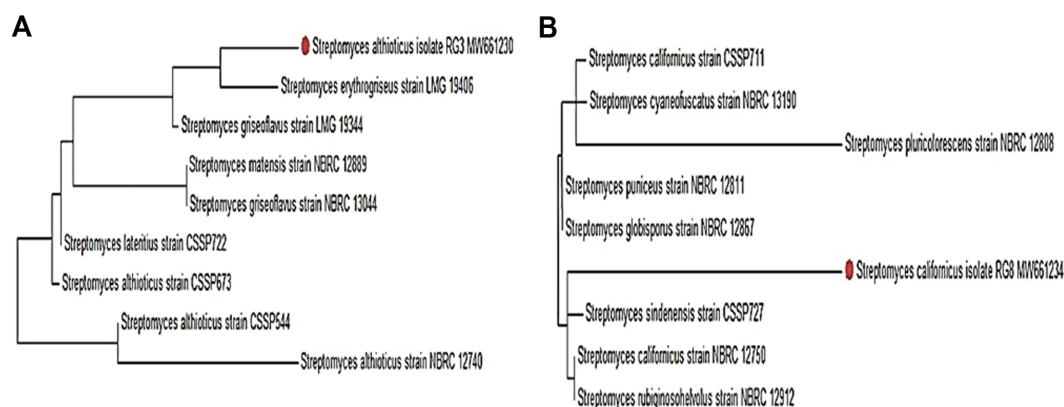
Table 2. Biochemical identification of biosurfactant producing marine actinomycetes isolates.

Characters	Actinomycetes isolates	
	<i>Streptomyces althoticus</i> RG3	<i>Streptomyces californicus</i> RG8
Morphological characters		
Substrate mycelium	Pale brown	Cream
Aerial mycelium	Cream	White
Diffusible pigments	Beige	-
Utilization of		
Lipase	+	+
Starch	+	+
Lactose	+	+
Dextrose	+	+
Maltose	+	+
Cellulose	+	+
Mannitol	+	-
Sucrose	+	+
Glucose	+	-
Biochemical test		
Citrate utilization	+	-
H ₂ S production	-	-
Urase hydrolysis	+	+
Indole production	-	-
Vogas Proskauer	-	-
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

**Fig. 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.

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 *S. althioticus* RG3 with the accession number (MW661230) Fig. 4A. Isolate RG8 was given the name *Str. californicus* RG8 with accession number (MW661234) as it is affiliated according to the genus *Str. californicus* Fig. 4B.

Factors affecting of fermentation condition

There has been considerable research into the generation of biosurfactants by actinomycetes under extreme conditions for commercial use [30]. The effect of conditions, including carbon sources, nitrogen sources, higher salt, temperature, and pH, on the production of biosurfactant from the marine isolates of *S. althioticus* RG3 and *Str. 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 culture media was caused by biosurfactant secretion in shaken-flask experiments. The statistical analysis revealed that the difference in biosurfactant activity at different carbon sources was statistically significant for selected isolates RG3 and RG8 (LSD 0.05 = 0.18 and 0.05 = 0.22). 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 *S. althioticus* RG3 and *Str. californicus* RG8 were the carbon sources producing the most biosurfactants (Fig. 5). Nutrient substrates were screened, and it was founded 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 [15, 19].

Effectiveness of nitrogen sources

Production of biosurfactant is affected by the nitrogen source, as shown in Fig. 5. Macro-nutrients need specific

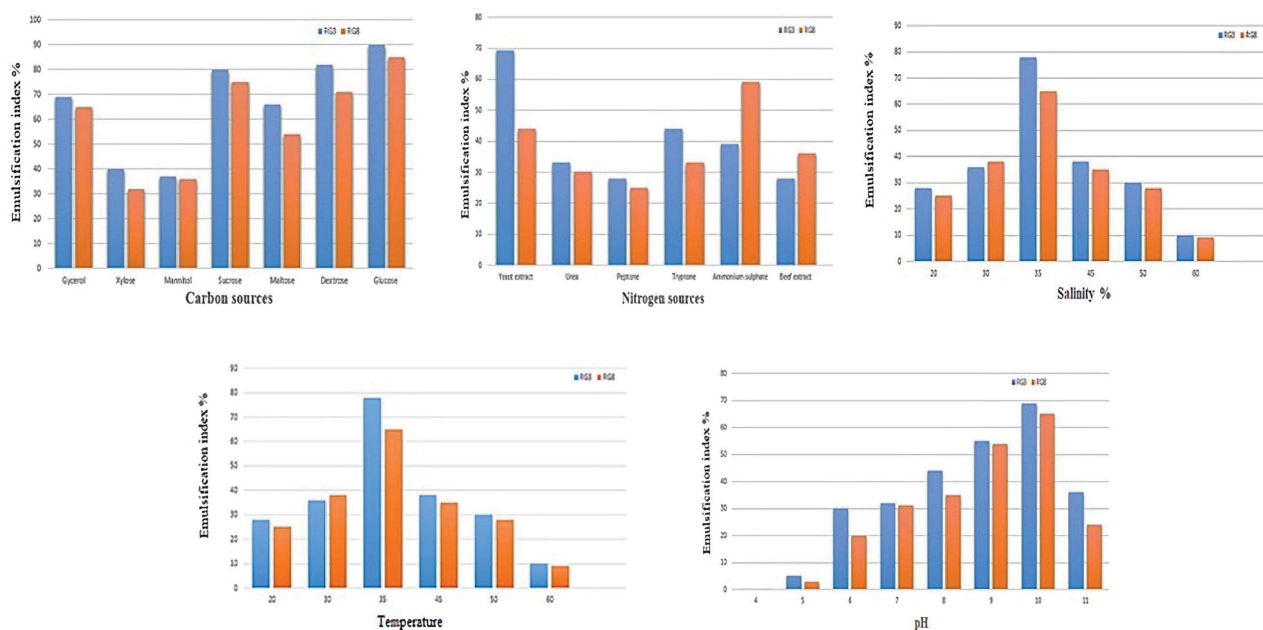


Fig. 5. Effect of culture conditions on emulsifying activity of broth media in biosurfactant relation production by *Streptomyces althioticus* RG3 and *Streptomyces californicus* RG8: carbon source, nitrogen source, NaCl concentration, temperature and pH. Three replicates of the tests were used; The data are shown in the form of a mean value with error bars representing the standard deviation (SD). Statically significant differences between the samples (LSD 5%) are estimated: (0.18 and 0.22), (0.18 and 0.14), (0.11 and 0.8), (1.3 and 1.2) and (0.24 and 0.21) respectively.

conditions to produce high concentrations of biosurfactants [19]. In significant variations between the different types of nitrogen sources (LSD 0.05 = 0.18) in isolate *S. althioticus* RG3 and it has been found that the 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 *Str. californicus* RG8, with an E24 of 59.21% and showed significant variation (LSD 0.05 = 0.14). 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. 5 indicates that the emulsification of *S. althioticus* RG3 was stable (70%) with 10% NaCl, and then gradually decreased to 15–30% NaCl with a significant variation LSD 0.05 = 0.11. *Str. californicus* RG8 emulsification operation was steadily increased from 10% to 15% NaCl to 60%, and then decreased to 20–30% with a significant variation LSD 0.05 = 0.8. 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) has 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. The statistical analysis indicated that the difference in activity of biosurfactant at different incubation temperatures was statistically significant (LSD 0.05 = 0.137) for isolate RG3 and (LSD 0.05 = 0.122) for isolate RG8. At 35°C, the highest emulsifying activity of *S. althioticus* RG3 and *Str. 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. 5), 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 the highest when the marine *Streptomyces* sp. B3 was cultivated at 30°C (E24 = 80%) [19]. At 30–40°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 40–80°C.

Effect of pH

Due to the large amount of using biosurfactants in the production of detergents, it was necessary to choose an alkaline biosurfactant from isolated bacteria [20]. As seen in (Fig. 5), the highest level of emulsification operation for *S. althioticus* RG3 and *Str. californicus* RG8 was the alkaline (pH 10) and reached 69% and 65%, respectively. This result indicated a significant variation between the results (LSD 0.05 = 0.24 and 0.21) However, no significant difference was observed in the pH range 4–9. This may be attributed to greater surfactant micelle stability with fatty acids in the present 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 a wide temperature spectrum (30–100°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]. The pH effect for biosurfactants of various microorganisms on surface activity has been recorded [39].

Chemical composition of the biosurfactants. *S. althioticus* RG3 produces a biosurfactants had a chemical structure of 30% protein, 20% carbohydrate, and 50% lipids. *Str. 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

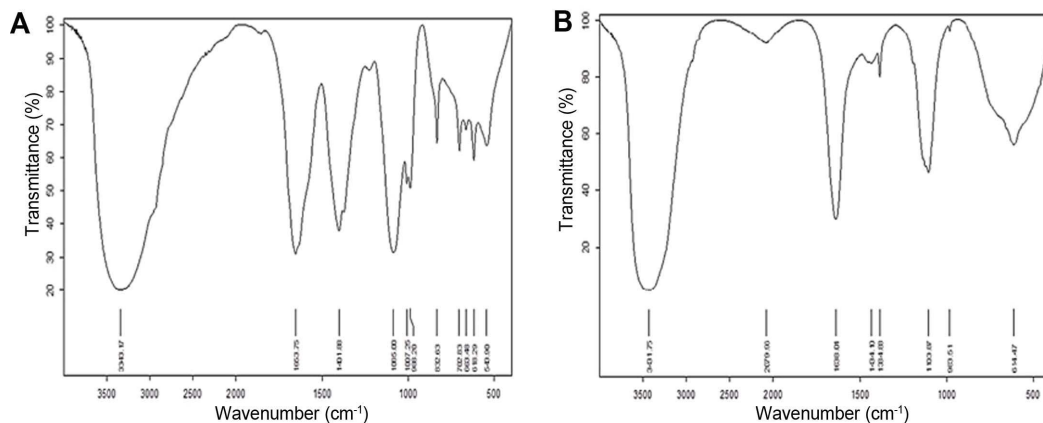


Fig. 6. FTIR analysis of biosurfactant production by *Streptomyces althioticus* RG3 (A) and *Streptomyces californicus* RG8 (B).

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> ATCC 25923	+	+

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

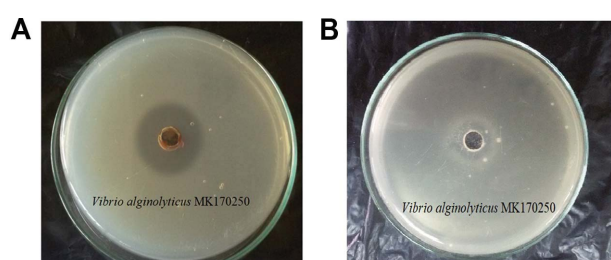


Fig. 7. Antagonistic effects of biosurfactants production by *Streptomyces althioticus* RG3 (A) and *Streptomyces californicus* RG8 (B) against *Vibrio alginolyticus* MK170250.

(Fig. 6A). 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. 6B). 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 *Vibrio alginolyticus* MK170250 from *S. althioticus* RG3 (Fig. 7) followed by the effects against *E. coli* ATCC 8739. Gram negative bacteria are more antagonistic to *Streptomyces* VITSDK1 spp. surfactants, with an inhibition zone of 10.3 mm in *Klebsiella pneumoniae*. *Staphylococcus aureus* is inhibited by *Streptomyces* VITSDK1 spp. surfactants by 5.3 mm [46]. *Streptomyces* sp. strain AF1 has a distinct personality. It

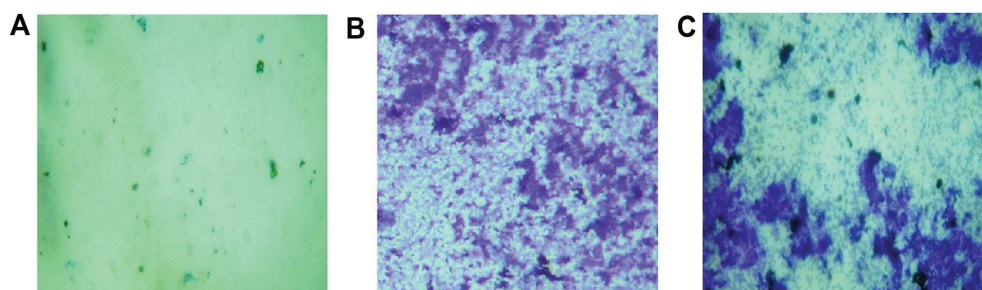


Fig. 8. Photographs illustrating the antifouling effect of the biosurfactants from *Streptomyces althioticus* (RG3) on the biofilm formation, the emergence of a small number of bacterial colonies (A), *Streptomyces californicus* RG8 a large number of bacterial colonies and the effect without the biosurfactant compounds (Control), the emergence of large numbers of bacterial colonies (C).

can grow at a high temperature of about 70°C and generate an antimicrobial biosurfactant [14].

Antifouling activity

Fig. 8 illustrated the inhibitory action of *S. althioticus* (RG3) biosurfactants on bacterial biofilm formation. The biosurfactants production by *S. althioticus* RG3 reduced the density of bacterial cells and acted as an anti-biofouling agent, while *Str. californicus* RG8 not achieved that. Napyradiomycin which production from marine *Streptomyces aculeolatus* isolate PTM-029 exhibited the higher antibacterial activity, the higher micro-fouling inhibitory activity, and the most potent antimicrofouling activity [47]. Two marine actinobacterial strains, *S. althioticus* RG3 and *Str. 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.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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