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Isolation and Characterization of Marine Actinomycetes from Specific Red Sea Algae: Evaluation of Their Antimicrobial and Antioxidant Activities

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Abstract Bacteria associated with algae play a crucial role in the ecological balance of marine ecosystems. Actinomycetes have many advantageous characteristics that increase the bioavailability of vital nutrients in soil and regulate the growth of microbial pathogens. This study aimed to identify the culturable actinomycetes, associated with some algal samples from the Red Sea and investigate their antimicrobial and antioxidant activities. The actinomycetes were isolated from four algal species collected from Yanbu cityon starch nitrate agarmedium supplemented with 10% NaCl and tested for their antimicrobial activity against a panel of pathogenic multidrug-resistant bacteria and for their antioxidant potential using appropriate assays. The collected Algae were identified as *Ulva lactuca* and *Codium tomentosum* (green algae), *Padina gymnospora* (brown algae) and *Hypnea valentia* (red algae). The results demonstrated that out of 4 different algal samples collected from the Red Sea, 15 filamentous bacterial isolates were obtained, purified and screened for antibacterial activities. Five isolates showed detectable antibacterial activities using the agar plug diffusion assay. Excellent antibacterial activities were recorded by the isolate RAD3 against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis* in addition to *Escherichia coli.* At the same time, it exhibited lower antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and *Serratia marcescens*. The Minimal Inhibitory Concentrations (MICs) of the RAD3 extract were ranged from 50-100 µg/ml for the tested bacterial pathogens. No antibacterial activity was recorded against *Klebsiella pneumonia* and *Acinetobacter baumannii*. The isolate RAD3was the most active in bacterial inhibition and prevented *E. faecalis* biofilm formation by 63% and exopolymeric substances (EPS) by 47%. The ethyl acetate extracts of the five selected bacteria showed promising antioxidant potential by scavenging DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Out of the 5 chosen bacteria, RAD3 showed 66.37% free radical scavenging with EC_{50} = 29.0 µg/ml and the results were compared to the positive control and the synthetic antioxidant Butylated Hydroxytoluene (BHT) which showed 96% free radical scavenging with EC_{50} = 19.7 µg/ml. The 5 chosen bacterial isolates were morphologically characterized and genetically identified by the analysis of 16S rDNA as species belonging to the genus *Streptomyces*. In conclusion, these findings highlight the potential of bacteria associated with the Red Sea algae as a source of novel antimicrobial and antioxidant compounds for food and medicine industries.

Key Words Red Sea Streptomyces, antimicrobial agents, Biofilm inhibition, DPPH

INTRODUCTION

The marine environment encompasses 70% of the Earth's surface and comprises highly productive zones that contain various subsystems supporting nearly 80% of the world's biota, including microorganisms, animals, algae, sponges and protozoa [1-3]. Its vast phylogenetic diversity exceeds the terrestrial environment due to location, temperature and salinity variations [4]. There are widespread symbiotic relationships between algae and bacteria, considered an ancient survival strategy that provides microorganisms with advantages for proliferation, such as access to nutrient-rich habitats and increased environmental stability. Additionally, microbial aggregates on the algal surface offer greater protection against external stresses [5]. The isolates of the genus *Endozoicomonas* exhibit highly diverse and adaptable symbiotic relationships with a wide array of marine hosts, such as sponges, fish and corals and play a role in protein and carbohydrate transport and cycling. Furthermore, they control high bacterial growth inside their hosts through competition and exclusion methods [6].

Marine bacteria are a diverse group of microorganisms that can live in close association with various types of algae. They play essential roles in the ecology and physiology of their hosts through processes such as nutrient cycling, symbiosis and disease resistance. Secondary metabolites released by different types of macroalgae can selectively attract and enhance the growth of these specific bacteria [7]. In a study from Finland, 17 bacterial species were isolated from four algae cultures: *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Isochrysis* sp. and *Nitzschia microcephala*. The bacterial community of *Isochrysis* sp. differs significantly from the other tested algae [8,9]. Bacteria associated with brown algae have emerged as a promising source of bioactive metabolites. In a study on Panjang Island, Jepara, twenty-three marine bacterial strains were isolated from *Sargassum* using marine agar and they showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. epidermidis*. The most active bacterial strain was identified as *Bacillus subtilis* [10]. From Red Sea sediments, 27 strains of actinomycetes were isolated and the most active isolate was identified as *Streptomyces* sp., which showed antimicrobial and anticancer activities with no cytotoxic effect, likely due to the presence of many anthraquinone compounds [11]. Also, bacteria from seaweed surfaces and invertebrates produce many antibacterial compounds that inhibit the most dangerous resistant bacteria, MRSA and vancomycin-resistant *Enterococcus* [12,13]. Moreover, marine *Streptomyces* from India's Bengal coast inhibit the growth of multidrug-resistant (MDR) bacteria which became a global threat and a matter of concern [14]. Similarly, isolated *Streptomyces* sp. MK388207 is from the Gulf of Suez and it has a strong antimicrobial agent against some pathogens, such as *Candida albican* [15]. Moreover, out of 21 crude extracts of marine bacterial strains associated with sponges, *Rhodococcus erythropolis* showed excellent antibacterial activities against MRSA [16] while marine *Streptomyces* sp. Al-Dhabi-100, isolated from Jazan region of Saudi Arabia, showed growth inhibition of *Enterococcus faecalis*, *Bacillus subtilis*, *S. aureus*, *S. epidermidis* and *Klebsiella pneumoniae*, with MIC ranging from 32.5-250 µg/ml. Additionally, the strain showed anti-tubercular and antioxidant potentials [17]. These findings suggest the therapeutic potential of marine-derived bacterial extracts against bacterial infections.

Different contaminants from certain hotspot sources like wastewater, sludge and natural fertilizer scan potentially spread, which enhances the emergence of new antimicrobial-resistant bacterial strains that contribute annually to 700,000 deaths worldwide and are likely to increase to 10 million in the year 2050 [18,19]. These bacteria have developed biochemical and physiological mechanisms, including the bioactive compounds with distinctive structural features accumulation, serving various functions such as reproduction, communication and defense against predation, infection and competition [20]. This is why, it is essential to search for new biologically active compounds from distinct sources to obtain novel antimicrobial agents. Marine water needs more precious studies to discover new species with excellent marine metabolites with structurally unique structures and action [21,22]. The Red Sea, with its extreme salinity and temperature, hosts unique microbial communities that have evolved to produce diverse bioactive compounds. Actinomycetes, especially those associated with algae, are prolific sources of antimicrobial and antioxidant metabolites with therapeutic potential. Thus the study was aimed to isolate and characterize the marine actinomycetes from Red Sea algae, highlighting their significance as an untapped resource for novel drug discovery and strategies against antimicrobial resistance and oxidative stress-related diseases [23-27].

METHODS

Algal Sample Collection and Actinobacterial Isolation

Algae were chosen as a source for actinomycete isolation because their nutrient-rich surfaces provide micro-niches that support diverse microbial communities capable of producing bioactive metabolites. Red algae, in particular, were prioritized as they exhibited distinct colony morphologies-colored, powdery and filamentous growth-characteristic of actinomycetes, making them strong candidates for antimicrobial and antioxidant screening. Building on this rationale, isolating marine actinomycetes from algae was a multi-step process that required careful sterile techniques and specialized media to obtain pure cultures for further study and potential biotechnological applications.

In this research, algal samples were collected from their natural habitat in sterile bags and washed several times with sterile water to avoid contamination, identified and classified. Algae were collected during winter 2022 by a local diver from a 3-4 m depth of Yanbu shores, Saudi Arabia and transferred to the laboratory in an ice box. To remove sand and necrotic parts, algal samples were washed with seawater followed by distilled water [28] and then identified at the Biology Department, Faculty of Science, King Abdulaziz University, according to color and morphological features.

All algal samples were processed to isolate actinomycetes using two selective media: Zobell Marine Agar (ZMA2216; Himedia, India) and modified starch nitrate agar supplemented with 10% NaCl [29]. Zobell Marine Agar is widely used for the isolation of marine bacteria, while starch nitrate agar provides nutrients that favor actinomycete growth and inhibit most other true bacteria. Sterilized surfaces of the collected algae were cut into 1 cm² squares and inoculated directly onto the agar surface of both media. The plates were incubated at 25°C for four days, after which individual colonies were selected for further purification by streaking, based on colony morphology. Colonies displaying characteristic actinomycete features-colored, powdery appearance and filamentous growth-were purified, examined microscopically for mycelial structures and preserved on

slants of the same medium or in glycerol stocks at -80°C for future use [30]. Following successful isolation, the purified actinomycete cultures were subjected to phytochemical screening to evaluate their capacity for secondary metabolite production. Standard qualitative tests for alkaloids, flavonoids, tannins and saponins were performed using reagents such as Mayer's reagent, ferric chloride and foam tests, enabling the detection of key bioactive compounds with potential antimicrobial and antioxidant activity.

Actinobacterial Characterization

Various methods, such as microscopy, biochemical tests and molecular techniques (like DNA sequencing), are used to identify the isolated actinomycetes to the genus or species level and characterize their properties. Out of 15 actinobacteria, five were examined and identified using morphological examination on Oatmeal agar at 25°C and biochemical tests. Gram stain detected after examination with a light microscope (CX21FS1 Olympus, China), the aerial and substrate mycelia colors and some biochemical tests like oxidase and catalase were carried out by conventional methods used in the microbiology laboratories [31].

Molecular Identification

According to the instructions of the manufacturer (QIAamp DNA Mini Kit, Qiagen, Germany), the DNA of the selected colonies was extracted, purified and preserved at -20°C for further analysis. Its purity was confirmed using the NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) [32]. For each DNA bacterial extract,16S rRNA gene sequences were amplified using two internal Universal Primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 511 R (5'-GCG GCT GCTGGC ACR KAG T -3') and PCR products was obtained, purified (QIA quick PCR purification kit) and sequenced (Big Dye Terminator). Nucleotides blast of NCBI and phylogenetic analysis were performed.

The Tested Bacterial Pathogens

The pathogenic Gram-positive and negative bacteria, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella oxitoca*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Shigella sonnei*, *Enterococcus faecalis*, *Staphylococcus aureus* (MRSA) and *Streptococcus pyogenes* were collected from KAU hospitals on plate agar. All isolates were examined using Gram stain and preserved on agar slants of nutrient agar at 4°C until used.

Bacterial Extract Preparations

The bacterial isolates were cultured in 500 ml flasks containing 100 ml of broth medium and incubated under shaking at 120 rpm for 7 days, at 25°C. After the fermentation period, the cells were separated by centrifuging at 5000 rpm for 10 min. The supernatants were mixed with an equal amount of ethyl acetate and agitated for 12 hours at room temperature. The ethyl acetate extract was evaporated under reduced pressure at 50°C by a Rotary evaporator (HS-2005S-N, Hahnvapor, Hahnshin Scientific, Korea). The collected dried extracts were saved in dark glass bottles and stored at -20°C for antimicrobial, antioxidant and phytochemical analysis [33].

The Antimicrobial Activity of the Isolated Actinobacteria

The isolates were screened for their antimicrobial activity using an agar plug diffusion assay. All actinomycete isolates (15) were grown on SNA medium at 25°C for 5 days. After incubation, the medium was cut aseptically with a sterile corkborer (6 mm) and the obtained discs were deposited on the opposed side on the Mueller Hinton agarplate, inoculated with 0.1 mL of suspensions of the tested pathogenic bacteria and all plates were incubated for 24 hours at 37°C. The substances diffuse from the plug to the agar. Then, the antimicrobial activity of the isolates was detected by the appearance of an inhibition zone (mm) around the agar plug. Moreover, a disc (6 mm diameter) fromRAD3, grown on SNA medium at 25°C for 5 days was transferred to 250 ml conical flasks containing 48 ml SN broth medium. The flasks were incubated in an incubator shaker (100 rpm) at 25°C for 5 days and the cultures were centrifuged at 4,000 rpm for 15 min at 4°C. The supernatant was collected, extracted with the same volume of ethyl acetate (v/v), dried and re-suspended in 2 ml DMSO and the antibacterial activity of the extract was detected using Agar well diffusion method and paper disc diffusion methods against MRSA and *E. faecalis* [34-36]. The mean diameter of the produced inhibition zone was determined. Broth microdilution assays were used to detect the minimal inhibitory concentration of the tested actinobacteria against a panel of pathogenic bacteria [36].

Effect of the Extract of Actinobacteria on Biofilm and Exopolysaccharide Formation by *E. faecalis*

All collected actinobacterial isolates were screened for inhibition of biofilm and exopolysaccharide formation using Crystal Violet Staining method [37,38], respectively as described before [39].

Antioxidant (DPPH Free Radical Scavenging) Activity

The five selected Actinobacteria were grown in the SNA medium at 25°C for 4 days (pre-culture) and 2 ml of the pre-culture (6×10^6 CFU/ml) was transferred to 250 ml conical flask containing 48 ml of fresh sterile SNB medium and the flasks incubated at 100 rpm and 25°C for 7 days. Cells were removed and the supernatant was used to determine the Antioxidant (free radical scavenging) activity [28]. Different concentrations of the actinobacteria extract were prepared from a stock solution of 10 mg/ml in methanol. In a 96-well plate containing 100 µl of 0.1 mM DPPH (Sigma-Aldrich, USA) in methanol and 100 µl of the tested bacterial extract (500, 400, 200, 100, 50 and 25 µg/ml). The plate was incubated for 30 min. at 20°C in a dark place [40]. Ascorbic acid and butylated hydroxytoluene (BHT) (Sigma-Aldrich, USA) were used as positive controls while DPPH solution

(200 µl) was considered as negative control. The absorbance at 515 nm was measured using a Micro-titer plate reader (HT Synergy, USA) and the percentage scavenge activity was calculated [41]:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

Where, A_c: Absorbance of control (DPPH), A_s: Absorbance of the sample.

The effective concentration of the extract produced a 50% reduction of DPPH activity (EC_{50}) which was detected by drawing the relationship between inhibition percentage and extract concentrations.

Preliminary Phytochemical Analysis

The dry extracts (0.1 g) were dissolved in 5% dimethyl sulfoxide (DMSO) and analyzed for the presence of saponins, flavonoids, anthocyanins, betacyanins, steroids and tannins [42,43].

Statistical Analysis

All experiments were carried out in triplicate and data are expressed as Mean±standard deviation (SD). Antimicrobial activity data (zone of inhibition) were analyzed using one-way ANOVA followed by Tukey's post-hoc test to compare differences between isolates. Antioxidant activity assays across different concentrations were evaluated using two-way ANOVA to assess both isolate- and concentration-dependent effects. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA)/GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA).

RESULTS

Isolation of Different Actinobacteria from Algal Samples

The following information is about the collection and identification of marine macroalgae from the coastal area of the Red Sea of Yanbu, Saudi Arabia (Figure 1). Four types of marine macroalgae were identified: two species of Chlorophyte (Green Algae), one species of Phaeophyte (Brown Algae) and one species of Rhodophyte (Red Algae). The specific algae identified were *Ulva lactuca* and *Codium tomentosum* from green algae, *Padina gymnospora* from brown algae and *Hypnea valentia* from red algae as shown in Figure 2. Two different media were used to isolate associated actinobacteria with the algae: Zobell Marine agar and SNA with 10% NaCl. The Zobell Marine agar was unsuitable for actinobacteria isolation and give weak growth and undefined colonies of actinobacteria, while the SNA with 10% NaCl was excellent for actinobacterial isolation as the colonies were good separated and colored. Fifteen different actinobacteria were isolated, purified and studied from SNA with 10% NaCl while no isolate was recovered from Zobell Marine agar. Seven isolates were from *Ulva*, three from *Codium*, three from *Padina* and two from *Hypnea* (Figure 3). The isolates were named RAD1 to RAD15.

Screening of the Isolated Actinobacteria for Antagonistic Activities

Antimicrobial activities of the 15 bacterial isolates were detected using agar disc diffusion assay and 9 bacterial pathogens. Moderate activity was recorded against *E. coli* as test organism by isolate RAD1, RAD3 and RAD14 while no activity was recorded by the other isolates (Figure 4a). Of the 15 isolates, 5 (33%) showed potent antimicrobial activity against more than one tested pathogens. Approximately 60% of the isolates showed inhibitory activity against *Streptococcus pyogenes* and 50% showed inhibitory activity against *Staphylococcus aureus*. Only 10% inhibited *Acinetobacter baumannii* and 19-20% inhibited *Klebsiella oxitoca* and *Escherichia coli* which was used as control bacteria (Figure 4b).

Some isolates showed antimicrobial activities against all tested pathogens, with one isolate (RAD3) showing the most potent antimicrobial activity (Table 1). The bacterial index was calculated for each actinobacterial isolate and the highest index was recorded for isolate RAD3, followed by RAD7. The lowest minimum inhibitory concentration (MIC)

Figure 1: The study area of the algal collection, Yanbu city, Saudi Arabia

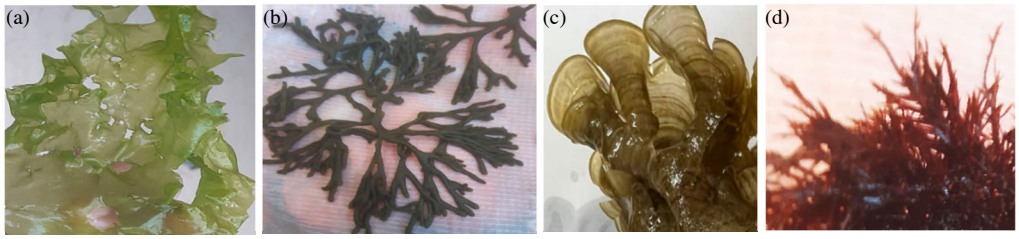

Figure 2(a-d): The green algae, (a) *Ulva lactuca*, (b) *Codium tomentosum*, the brown algae, (c) *Padina gymnospora* and the red algae and (d) *Hypnea valentia*

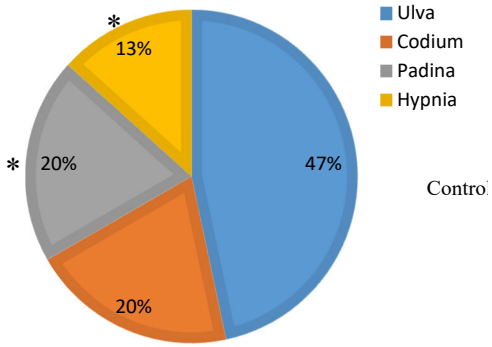

Figure 3: Percentage of isolated actinobacteria from *Ulva lactuca*, *Codium tomentosum*, *Padina gymnospora* and *Hypnea Valentia*
*Significant bacterial counts compared to control (*Ulva*)

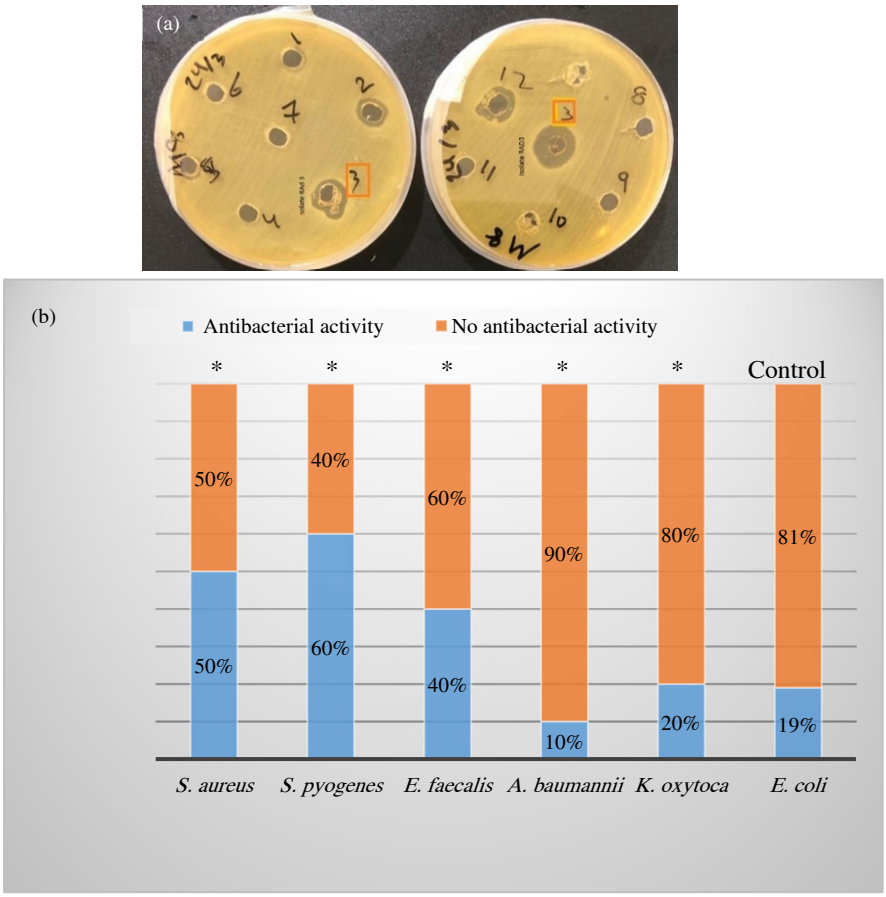

Figure 4: Screening of the 15 isolated actinobacteria for (a) Antimicrobial activities against *E. coli* using agar well diffusion assay and (b) The antimicrobial activities of the actinobacteria (15 isolates) against different bacterial pathogens
*Significant antibacterial activity compared to control (*E. coli*)

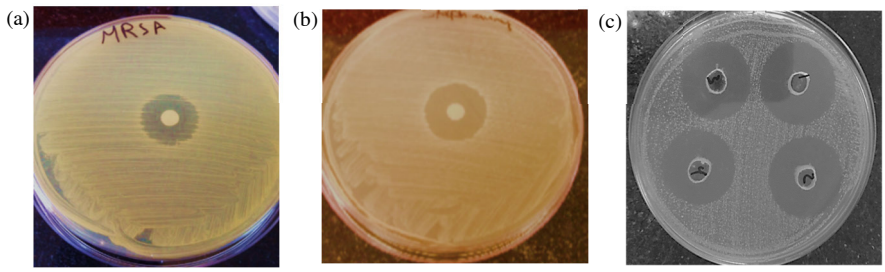

Figure 5(a-c): The antimicrobial activity of the crude extract of the isolate RAD3 using paper disc diffusion assay and against (a) MRSA and (b) *E. faecalis* and using agar well diffusion assay against (c) *E. faecalis*

Table 1: The antimicrobial activities of five selected actinobacteria against different bacterial pathogens using paper disc diffusion assay

Tested bacteria	Antimicrobial activity of five selected actinomycetes					Ampicillin (Control)	MIC (µl/ml)	
	RAD1	RAD3	RAD7	RAD9	RAD14		RAD3 extract	Ampicillin (Control)
Acinetobacter baumannii	ND	ND	11±2.9	ND	ND	ND	ND	ND
Escherichia coli	12±2.0	13±1.4	ND	ND	14±1.0	20±1.1	75.0±25.0*	7.5±1.5
Klebsiella oxitoca	ND	ND	10±0.8	11±0.5	13±0.6	21±10	ND	7.5±1.0
Pseudomonas aeruginosa	10±2.4	13±2.0	11±0.9	11±0.5	13±0.4	29±2.0	75.0±25.0*	7.5±1.0
Salmonella typhi	11±0.6	13±0.8	10±1.0	11±0.4	11±0.6	22±1.6	100±25.0*	7.5±1.0
Serratia marcescens	10±2.0	16±0.8	14±0.33	16±1.1	13±0.8	30±1.4	75.0±25.0*	5.0±0.5
Shigella sonnei	11±2.0	13±0.3	10±2.2	14±0.7	11±0.8	26±0.4	100.0±25.0*	7.5±0.5
Enterococcus faecalis	17±1.0	21±0.29	14±0.57	10±0.3	18±1.0	30±0.4	50.0±2.5	5.0±1.5
Staphylococcus aureus (MRSA)	19±2.1	22±1.45	11±0.49	14±1.4	14±2.4	40±0.6	50.0±2.5*	5.0±1.5
Streptococcus pyogenes	17±1.9	23±2.7	11±0.54	13±1.2	15±2.5	37±0.6	50.0±2.5*	5.0±1.5
Bacterial index**	13.95*	17.20*	12.75*	14.8*	16.8*	30.7	ND	
LSD	1123	229	1158	720	543	619	11290	411

Table 2: The inhibition of biofilm and exopolysaccharide formation using *E. faecalis* as test strain and the antioxidant activity detected by DPPH and compared to the control (ascorbic acid and BHT) of the selected actinobacteria extracts

Actinobacteria extract	Biofilm inhibition (%)	EPS inhibition (%)	DPPH reducing %
RAD1	19^{e}	23^{e}	55.08±0.51^{e}
RAD3	63^{a}	47^{a}	66.38±0.72^{a}
RAD7	39^{c}	27^{c}	60.16±0.52^{c}
RAD9	30^{d}	24^{e}	50.10±0.32^{e}
RAD14	28^{d}	25^{e}	58.02±0.12^{c}
Negative control (no extract)	0	0	0
BHT	ND	ND	96.0±1.19^{d}
Ascorbic acid	ND	ND	100

*Significant results compared to negative control, The results are the mean of three replicates, Values within a column followed by different alphabets are significantly different at p≤0.05, ND: Not detected

was recorded for *S. aureus*, *S. pyogenes* and *Enterococcus faecalis* and it was ranged from 50 µg/ml while for *Pseudomonas aeruginosa*, *Shigella sonnei*, *Serratia marcescens*, *E. coli* and *Salmonella typhi*, it was ranged from 75-100 µg/ml. The active material of isolate RAD3 was extracted with ethyl acetate, showing excellent antimicrobial activity against MRSA and *E. faecalis* using two different methods, paper disc diffusion assay and agar well diffusion assay (Figure 5).

Actinobacterial Extracts Inhibited of Biofilm Formation and EPS Secretion of the Bacterial Pathogens

Additionally, the actinobacteria extracts inhibited biofilm formation to different degrees. The isolate RAD3 extract was the most active in bacterial growth inhibition and prevented biofilm formation by 63% and exopolysaccharide (EPS) by 47% while the lowest activities were recorded by isolate RAD1 extract (Table 2).

The Antioxidant Activities of the Actinobacterial Extracts

Also, the antioxidant activities of the selected actinobacteria were detected by DPPH free radical scavenging activity and the results were significantly different compared to the control, ascorbic acid ($p<0.05$). The highest scavenging percentage was recorded for RAD3 extract, which demonstrated notable antioxidant activity, the lowest was by extract RAD 9 and extracts of isolate RAD1, RAD7 and RAD14 recorded moderate activities. The percentage of reduced DPPH radical by bacterial ethyl acetate extracts at a concentration of 1mg/ml is shown in Table 2. The most potent scavenger among bacterial species was RAD3 extract which recorded 66% activity of the standard antioxidant. EC_{50} of the actinobacteria was detected and compared to controls (Figure 6). RAD 3 extract has EC_{50} = (29.14 µg/ml) relatively higher than the synthetic antioxidant BHT EC_{50} = (17.22 µg/ml).

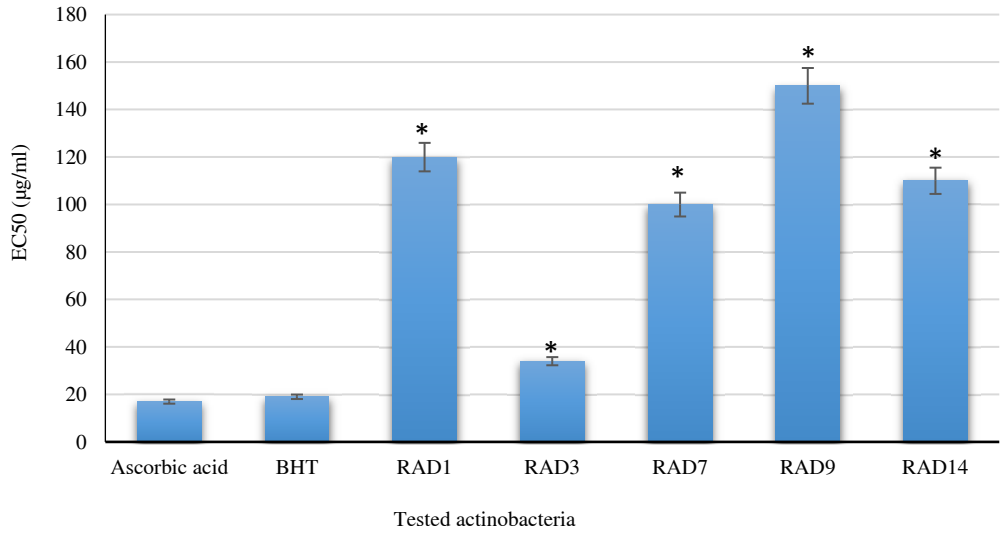

Figure 6: Effective concentration (µg/ml) of the actinobacteria extracts, reduced DPPH radicals by 50% (EC_{50} values)

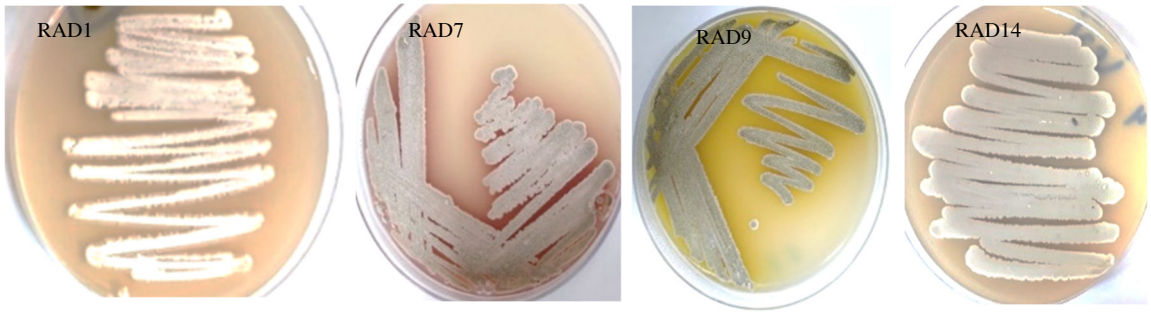

Figure 7: The growth of the most active antibacterial producer actinobacteria on starch nitrate agar after 10 days of growth at 25°C

Table 3: Preliminary phytochemical analysis of ethyl acetate extract of the isolate RAD3

	Phytochemicals					
Bacterial extract	Saponins	Flavonoids	Anthocyanin	Betacyanin	Tannins	Steroids
Isolate RAD3	-	+	+	+	-	+

+: The presence of the material, -: The absent of the material

The Primary Analysis of the Actinobacterial Extracts

The primary analysis revealed the presence of flavonoids, anthocyanins, betacyanins and steroids but no saponins or tannins, among the extract of the isolate RAD 3, as shown in Table 3.

Characterization and Identification of the Selected Actinomycetes

The growth of the selected actinobacteria (RAD1, RAD7, RAD9 and RAD14) on oatmeal agar is illustrated in Figure 7 while the growth, Gram stain and shape of aerial and substrate mycelia, examined under a scanning electron microscope of the isolate RAD3, are summarized in Figure 8. It's evident that all isolates displayed good growth on oatmeal agar and belonged to the gray series, except isolate RAD1, which belonged to the white series. Some morphological, biochemical and physiological characteristics of the selected actinobacteria are presented in Table 4. The spores are in chains that are straight, spiral or closed spiral shapes with smooth or rough surfaces and the aerial and substrate mycelia were well-developed with different colors. Soluble pigments of different colors were detected for all strains and no production of catalase and urease enzymes was observed. The actinobacteria showed no visible change on blood agar, such as staining or clearing around the bacterial colony (gamma hemolysis), as they produced no hemolytic enzymes. Additionally, the selected actinobacteria were identified through molecular analysis as species belonging to the genus *Streptomyces* (Table 5). Isolate RAD1 was isolated from *Ulva* while RAD3 and RAD7 were from *Codium*. Similarly, RAD 9 and RAD14 were from *Padina* and *Hypnea,* respectively. All isolates, RAD1, RAD3, RAD7, RAD9 and RAD14 were identified as a filamentous bacteria of the genus *Streptomyces*. The phylogenetic tree analysis of the obtained bacteria is shown in Figure 9 and the isolates were identified as *S. filamentosus* RAD1, *S. glaucescens* RAD3, *S. chilikensis* RAD7, *S. pseudogriseolus* RAD9 and *Streptomyces* sp.

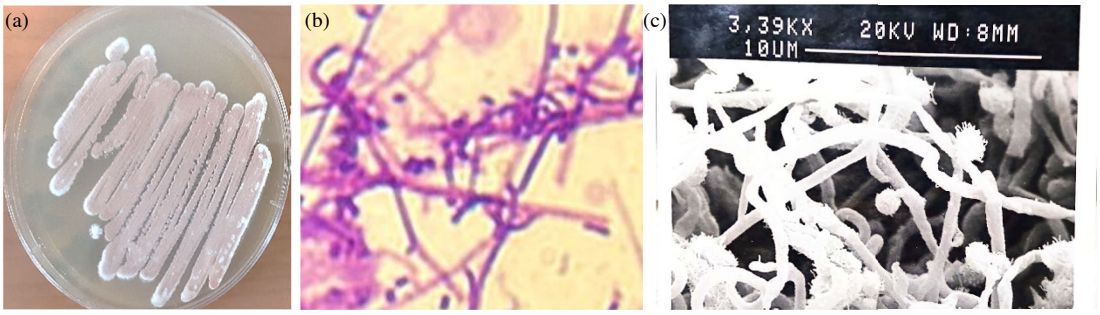

Figure 8(a-c): The isolate RAD 3 on (a) Oatmeal agar, (b) Examined under a light microscope after Gram staining x1000 and (c) Examined under a scanning electron microscope

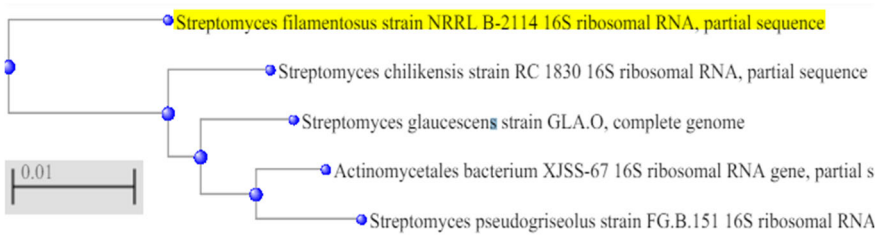

Figure 9: The phylogenetic tree of the obtained actinobacteria on 16S rRNA sequences

Table 4: Some morphological, biochemical and physiological characteristics of the selected actinobacteria grown on Oat meal agar at 25°C

Isolate	Growth	Spore chain	Spore surface	Color	Aerial mycelia	Substrate mycelia	Soluble Pigment	Catalase	Urease	Hemolysis
RAD1	+	Straight	Smooth	White to gray	White	Dark brown	Pale brown	-ve	-ve	γ
RAD3	++	Straight	Smooth	Gray	Reddish gray	Reddish brown	Pale brown	-ve	-ve	γ
RAD7	++	Closed spiral	Rough	Gray	Pale gray	Dark gray	Reddish gray	-ve	-ve	γ
RAD9	++	Spiral	Rough	Gray	Gray	Dark gray	Yellowish gray	-ve	-ve	γ
RAD14	++	Spiral	Smooth	Gray	Dark gray	Black	Dark gray	-ve	-ve	γ

γ: Gamma hemolysis, -ve: Negative result, +: Moderate growth, ++: High growth

Table 5: Molecular identification of actinomycetes associated with algal samples based on 16S rRNA gene sequence analysis

Isolate No.	Source	Close relative in GenBank	Closest species	Identity (%)	G+C (%)
RAD1	*Ulva lactuca*	NR_115665.1	*Streptomyces filamentosus*	95	60
RAD3	*Codium tomentosum*	NR_118246.1	*Streptomyces glaucescens*	95	67
RAD7	*Codium tomentosum*	CP009438.1	*Streptomyces chilikensis*	97	70
RAD9	*Padina gymnospora*	EU598259.1	*Streptomyces pseudogriseolus*	95	67
RAD14	*Hypnea valentia*	KF991642.1	*Streptomyces* sp. XJSS-67	97	66

RAD14. The similarity levels ranged from 95-97%, while the percentages of G+C were high, ranging from 60-70%.

DISCUSSION

The Red Sea is one of the most important and unique marine environments, hosting diverse habitats such as sea-grass, macroalgae, sponges and mangroves with rich microbial communities[24]. Four algae were collected during winter to obtain special bacterial isolates with excellent antimicrobial activity. The absence of these activity was usually recorded during summer and autumn due to the unfavorable and dry conditions that prevailed during these successive seasons in seawater and sediments [44,45]. The Red Sea is known for its rich biodiversity, its high salinity and unique physical and chemical conditions making it a valuable source of epiphytic bacteria, which have been defined as non-parasitically populations and can survive, live and multiply on the surface of various organs of plants, algae and animals. These epiphytic bacteria occupy ecological niches that could be occupied by pathogens and can produce bioactive compounds for pharmaceutical industries [46]. Epiphytic actinobacteria are known for their filamentous growth, broad antagonistic effects against pathogens and ability to produce bioactive compounds with various biological activities, such as antimicrobial, anticancer and immunosuppressive properties [47]. However, the isolation of actinomycetes from algae in the Red Sea is not well studied. Out of fifteen actinobacterial isolates associated with the four collected algae, five were selected for their antimicrobial activities and identified based on their morphology and sequences of the 16 S rRNA. DNA was extracted and amplified for molecular identification of the strains to the species level. The 16S rRNA sequence was compared to the GenBank database. Similarly, various strains of actinobacteria, such as

S. chilikensis, *S. glaucescens* and *S. pseudogriseolus*, have been isolated from different marine sediment in India and *S. pseudogriseolus* was found to produce several bioactive compounds with antimicrobial activity [48]. Understanding and isolating these bacteria is crucial, especially in light of the growing concern about antibiotic-resistant bacteria, which cause a significant number of deaths annually and are projected to cause even more in the future [18]. Therefore, the search for new biologically active compounds from various sources is essential to develop novel antimicrobial agents. Different marine bacteria play a pivotal role as primary colonizers on the surfaces of organisms within the marine environment and have been isolated from a diverse range of biological and ecological niches, including seaweeds, marine invertebrates, sediments and water and produce antimicrobial agents effective against multidrug-resistant bacteria [49]. Similarly, five marine bacteria with inhibitory effects against *E. coli*, *S. aureus* and *Listeria monocytogenes* were isolated by Chbel *et al.* [50]. The relationship between bacteria and their algae is considered an ancient survival strategy that provides microorganisms with advantages for proliferation, such as access to nutrient-rich habitats and increased environmental stability. Microbial aggregates on the algal surface offer higher protection against external stresses, play a role in host-associated protein and carbohydrate transport and cycling and control high pathogen growth inside their host through competition and exclusion methods [5,6].

Secondary metabolites released by different types of macroalgae can selectively attract and enhance the growth of specific bacteria [10]. Four bacterial isolates from red algae and among them, *Lysinibacillus odyssey* exhibited broad-spectrum antibacterial activity against *E. coli*, *Klebsiella pneumoniae*, *Shigella* strains [50]. Similarly, 15 actinomycetes were isolated from 4 different sponge samples collected from the Red Sea and *Streptomyces* sp. Sp9 extract showed potent antitumor activity, due to the presence of heliomycin and tetracenomycin D [51].

The antioxidants are natural phytochemicals present in plants and macroalgae. The discovery of new species with significant antimicrobial and antioxidant activities in marine water is recommended because these microorganisms may be new species and have developed mechanisms to synthesize bioactive compounds with distinctive structures and features that help them defend against predators and competition [52-55]. In this study, the five selected isolates exhibited great potential as antioxidants, with RAD3 showing the most promising results. As far as we know, this could be the first study on the antioxidant activity of associated actinobacteria with algae in Saudi Arabia. It was known that Actinobacteria produce some pigments with significant antioxidant activity against DPPH radicals [56]. Similar to our results, the bacteria associated with the brown algae revealed ≥60% DPPH scavenging activity [30,56] and can act as a potential probiotic. The presence of Steroids, flavonoids and tannins in most bacteria extracts may explain the antioxidant activities, in addition to others such as polysaccharides, fatty acids and proteins [57-59] and many studies indicated a positive correlation between the antioxidant activity and the total phenolic compounds [60,61].

This study had few limitations. Sampling was restricted to a few algal species and locations in the Red Sea, which may not reflect the full actinobacterial diversity. Only culturable isolates were examined, potentially overlooking non-culturable strains with bioactive potential. Antimicrobial and antioxidant activities were tested in vitro without *in vivo* validation and the chemical structures of the active compounds were not characterized. Additionally, advanced analyses such as metabolomics or biosynthetic gene cluster identification were not performed, limiting mechanistic insights.

Further investigations are recommended to uncover the potential hidden in the Red Sea shores. Finally, the identified isolates belonged to the genus *Streptomyces*, family Streptomycetaceae and order Actinomycetales of the class Schizomycetes. The bacteria of this genus are mainly found in soil and occasionally isolated from marine sources and showed antimicrobial and antioxidant activities [62-64].

CONCLUSIONS

This study highlighted the untapped bioactive potential of Red Sea algal-associated actinobacteria, with *Streptomyces glaucescens* emerging as a particularly promising isolate. Identified through morphological and molecular methods, its extract exhibited strong antibacterial activity, effectively inhibited biofilm formation and EPS production and showed notable antioxidant effects. These properties position it as a valuable candidate for the development of novel therapeutic agents targeting infectious diseases and oxidative stress-related disorders. To fully realize its potential, future research should focus on characterizing the bioactive compounds responsible, elucidating their mechanisms of action and assessing their applications in medicine, pharmaceuticals and related industries.

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Conflicts of Interest

The author reports no conflict of interest.

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