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Bioactivity assessment of the Saudi Arabian Marine *Streptomyces* sp. Al-Dhabi-90, metabolic profiling and its *in vitro* inhibitory property against multidrug resistant and extended-spectrum beta-lactamase clinical bacterial pathogens

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ABSTRACT

Background: Metabolites obtained from the marine microorganisms were known for their important role in microbial inhibition. Interestingly, bioprospecting of secondary metabolites from marine derived actinomycetes has huge demand especially in the treatment of multi drug resistant clinical pathogens. The present study subjected towards the identification of promising antimicrobial actinomycetes from the Arabian Gulf regions and metabolic profiling of the crude organic solvent extract by chromatographic techniques.

Methods: The strains were characterized by 16S rRNA sequencing. Extracellular metabolites were profiled by performing GC–MS analysis. MIC values of the compounds were detected using broth dilution technique.

Results: A Gram positive, spore forming filamentous *Streptomyces* sp. Al-Dhabi-90 possessed good antibacterial activities against the drug resistant pathogens were confirmed by 16S rRNA gene sequencing. Further, the gas chromatography coupled with mass spectrum analysis data revealed that the organic solvent extract of the fermented *Streptomyces* sp. Al-Dhabi-90 contained major components such as 3-methylpyridazine, *n*-hexadecanoic acid, indazol-4-one, octadecanoic acid and 3a-methyl-6-((4-methylphenyl) sul respectively. The Minimum Inhibitory Concentration (MIC) of the extract against *Staphylococcus aureus* and *Klebsiella pneumoniae* were 12.5 and 50 μg/ml respectively. Against drug resistant ESBL pathogens such as *Escherichia coli, Pseudomonsa aeroginosa* and *Proteus mirabilis* were 12.5, and 25 μg/ml respectively. Interestingly, the extract showed promising activity against the vancomycin resistant *Enterococcus faecium* at 50 μg/ml. The increased level of cellular constituents after the extract treatment evidenced that the metabolites altered the membrane integrity of the pathogens.

Conclusion: Conclusively, the marine *Streptomyces* sp. Al-Dhabi-90 is an ideal source for the treatment of multi drug resistant clinical pathogens.

Introduction

Diversity of the natural products obtained from plants and microbes were interesting because of their unique chemical structure with wide level of applications in medical fields. Considering the wide level importance of the natural products with less side effects, numerous studies have been performed towards the identification of promising active molecules from different environmental samples [1,2]. However, the presences of natural compound producing microbes were achieved by exploiting to the unexplored or non-exploiting environments [3,4]. Among the environments, huge number of different promising microbes with bioactive compounds were identified from marine samples because of their ability to adapt to harsh conditions, and the extreme conditions reflects to the microbes for novel metabolic pathways and novel structures and the novel compounds represented a great importance in field of development of new drugs [5]. Since 1985, more than 15,000 new compounds were derived from marine

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organisms; many of the identified metabolites possess interesting biological activities [6]. Dramatically, during the last two decades the studies on marine microbes have been increased in different countries with respect to the bioactive metabolites [7]. Several novel bioactive compounds have been derived from actinobacteria in different marine environments with a broad spectrum of biological activities including antitumor, antimicrobial, anti-malarial, antiviral, anti-inflammatory and anti-parasitic respectively [8]. Many reports claimed that marine actinomycetes produced several novel active compounds such as pacificanones, salinipyrones [9], rifamycins, arenimycin [10], cyanosporaside A [11] and saliniketals A and B [12]. Espinosa et al. extracted marinomycin A–D from *Marinispora* with antibacterial and anticancer activities [13].

Many interesting works were done in other continents related to the identification of novel molecules from actinomycetes, whereas, the in-depth work on the identification of active actinomycetes with promising activities from Saudi Arabia is limited. Abdelmohsen et al. isolated 48 sponge-associated Actinobacteria strains from the Red Sea in Saudi Arabia [14]. Among all isolated strains, extracts of 9 strains exhibited antimicrobial and antiviral activities. Two strains are SA6 and SA22 belonged to *Salinispora* sp. almost inhibited the growth of all tested pathogenic microbes. The objective of the present study was to isolate novel actinomycetes from the Arabian Gulf in Saudi Arabia and investigating its potential applications in particular against multi-drug resistant bacteria.

Materials and methods

Chemicals and reagents

The nutrient medium used for the isolation of the actinomycetes was procured from Himedia, India. Glucose, starch, peptone and yeast extracts were obtained from Scharlan, S.L, Spain. Solvents and other acids were procured from the Somatco, Riyadh, Saudi Arabia. Standard antimicrobial agents such as, streptomycin, nalidixcic acid, actidione and ampicillin were provided from Himedia, Mumbai. Water used in this study were double distilled and sterilized.

Collection of marine samples and isolation of actonomycetes

Different samples such as, soil, sediment, water from the seashore, back water samples, different sponges and died crabs were collected from the Arabia Gulf regions in Saudi Arabia in November 2017. All the collected samples were carefully transferred into the sterile collecting bottles and stored in the cold room. After that, 10 g or 10 ml of the samples were mixed with 90 ml of sterile distilled water and kept in the shaking incubator for 60 min for the even mixing of the samples. Further, the samples were serially diluted and 100 μl of the samples were spread of the sterile plates containing starch casein agar medium consisted of starch, casein, potassium hydrogen phosphate, di-pottassium hydrogen orthophosphate, magnesium sulfate, ferric chloride, sodium chloride, agar together with filter sterilized streptomycin, nalidixcic acid, actidione and amplicillin [15]. The antibiotics were used to inhibit unwanted growth of bacteria and fungi. Further, the plates were incubated at 30 °C for 21 days. The visible colonies were picked and purified in ISP-2 medium, later the pure single actinomycetes strains were used for the antimicrobial compound production screening using MNGA medium by perpendicular cross streak method. Eleven, active antimicrobial compound producing actinomycetes strains against microbial pathogens were identified. Among that, strain Al-Dhabi-90 displayed promising activity against all the tested pathogens was selected for further mass production study. Initially, various biochemical, physiological, morphological, scanning electron microscopic (SEM) studies and 16S rRNA gene amplification studies were performed for the identification of the selected actinomycetes.

Cultivation of actinomycetes and extraction of antimicrobial metabolites

Modified nutrient glucose broth added with 10% of glucose was used as the base for the bulk level production of the antimicrobial metabolites. Approximately, 20 l of the broth was prepared and distributed 500 ml into 2 l Erlenmeyer flasks and sterilized. After sterilization, 10 ml of the spore suspension was aseptically transferred into the fermentation medium and kept in the orbital incubator shaker at 150 RPM, 30 °C for 40 days. After the incubation periods, the cell suspensions were centrifuged at 10,000 RPM for 20 min at room temperature to separate the supernatant and the biomass. The supernatant was checked for its antimicrobial activity against microbial pathogens by well diffusion method. After the confirmation of the antimicrobial activities, the pH of the supernatant was adjusted to 4 using 1 N H_2SO_4 and the crude antimicrobial metabolites were extracted using ethyl acetate with the help of separating funnel. The active components containing the solvent phase was concentrated using IKA@RV10 evaporator at 35 °C and then the collected crude extracts were tested for antimicrobial activities.

Identification of major metabolites using gas chromatography-mass spectrometry (GC–MS) analysis

The chemical compositions of the active extract were determined by gas chromatography–mass spectrometry (GC–MS) analysis using Agilent GC–MS [4]. The instrument was built with capillary column (30 × 0.25 mm inner diameter and 50 m length) and 5 HP pressure was maintained during the operating stages. Helium gas (99.9%) was used as the carrier gas with the flow rate of 0.5 ml/30 s. The mass spectrometer (RI 70 eV and ion source 200 °C) programmed at 40–280 °C with the variation range of 4 °C/min. The injection volume was 3 μl and the evaporation temperature of 280 °C with flowing velocity of 36 cm/s. The chemical composition of the extract was determined by measuring the peak area and the retention time by comparing the NIST 11 library.

In vitro *antimicrobial activity*

Microbial pathogens

Microbial pathogens such as *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis, Staphylococcus aureus* (ATCC 29213), multidrug resistant *S. aureus* (WC 25 V 880854), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC25922), *E. coli* (ESBL 4345), *E. coli* (ATCC 35218), *Klebsiella pneumoniae* (ATCC70063), *Pseudomonas aeruginosa* (ATCC27853), *Proteus mirabilis* (DR 4753), *Acinetobacter baumannii* (MDR 4414) and *Enterococcus faecium* (VRETC 773) studied in this research was obtained from the Medical Microbiology Department of National Guard Military Hospital, and King Khalid University Hospital Riyadh, Saudi Arabia. The pathogenic strains were maintained in the nutrient agar slant for routine laboratory use and stored in 20% sterile glycerol at −4 °C for the long term usages.

Minimum Inhibitory Concentration (MIC) of the metabolite

Minimum Inhibitory Concentration (MIC) of the crude ethyl acetate extract was performed by double fold dilution technique using the liquid broth [15]. Briefly, 40 mg of the extract was mixed with 150 μl of sterile distilled water and 50 μl of sterile DMSO and then sonicated for even distribution of the compounds. For the experiment, each well of the sterile 96 well plates contained 185 μl of sterile MH broth, 10 μl of the compounds and 5 μl of

the freshly prepared mid log phase microbial pathogens. The well without compounds was treated as the control and the wells with standard antibiotics streptomycin and only DMSO were considered as the positive control and negative control. After proper mixing, the plates were coved with sterile aluminium foil and kept under shaking incubator at 37 °C for 17 h. After the time periods, from each well 5 μl of the cells were spotted on the MH agar medium containing 2.2% agar and placed it in the incubator for the observation of the visible growth. Similar methodology was followed for the positive, negative and antibiotic control. The spot without bacterial growth was noted as the concentration of the compounds for complete killing of the bacteria. The compounds MIC were confirmed after the experiment for three times.

Cell suspension inhibition assay

Microbial biomass inhibition assay of the extracts was determined by slightly modifying the methodology of Pangestuti et al. and mixing the extracts with the growing cells [5]. Briefly, 100 μg/ml of the extract were mixed with 5 ml nutrient broth containing microbial pathogens. After that, the cell suspensions were incubated at 150 rpm for 37 °C for 17 h. Alternatively, 5 ml nutrient broth without the extracts was also considered as the positive control. After 17 h the cells were centrifuged at 10,000 RPM for 10 min and the cell pellets were washed and re-suspended for measuring the cell growth at 660 nm. The difference in the cell growth between the control and treated compounds were used for the calculation of the percentage of the cell suspension inhibition.

Measurement of intra cellular cell constituents' release

The rigid integrity of the outer cell membrane of the Gram positive and Gram negative microbial pathogens were evaluated by measuring the total intracellular constituents such as proteins, sugars, nucleic acids, and metabolites by slightly modified methodology of Rhayour et al. [16]. Briefly, 50 ml total volume of the freshly prepared cell suspension with the concentration of 10^8 CFU/ml was washed thoroughly will distilled water and then treated with different concentration of the extract and incubated at 37 °C for 5 h. After that the cell constituents were measured at absorbance at 260 nm. The cells without the extracts were considered as the positive control. The experiments were repeated for final confirmation.

Results

Identification of antagonistic marine actinomycetes

Actinomycetes from marine sources have been studied widely throughout the world and many reports were available related to the identification, characterization of novel molecules with biomedical applications. However, very limited studies were performed from the Arabian Gulf regions in Saudi Arabia with respect to antimicrobial metabolites from actinomycetes. Therefore, studies have been initiated related to the isolation and identification of novel antimicrobial actinomycetes from the marine region of Arabian Gulf, Dammam and extraction of novel antimicrobial molecules with promising inhibiting properties towards drug resistant pathogens. A total of 55 suspected actinomycetes were selected based on their ability to grow on the starch casein agar medium and purified on ISP-2 medium. Primary antimicrobial screening against microbial strains revealed promising activity against the tested strains. Promising level of antimicrobial activities was detected against *S. epidermidis, S. aureus, K. pneumoniae* and *E. coli*. Screening analysis showed that 56 percentages of the actinomycetes strains documented activity against *S. aureus* and 56% against *S. epidermidis.* Among the tested Gram positive pathogens, *S. aureus* activity (56%) was dominant and *E. faecalis* showed least activities (32.7%). However, a total of 23 actinomycetes strains revealed activity against the Gram negative bacteria *K. pneumoniae* and 13 strains showed activity against *P. aeruginosa* respectively. Approximately, 5–13% of the actinomycetes isolate documented antibacterial activity against the tested drug resistant strains. Interestingly, more number of actinomycetes strains (12.7%) was able to inhibit the growth of the multidrug resistant *S. aureus*. Among the actinomycetes active strains, a strain named as Al-Dhabi-90 revealed comparatively better activity, Gram negative and multi drug resistant strains were selected and identified by biochemical, morphological and genetic level studies, and was cultivated for the extracellular active molecules extraction.

Biochemical characterization studies confirmed that the isolate was Gram positive and filamentous, spore forming. The individual colonies appeared as dry and powdery on SCA agar medium. The macro morphological characteristic of the isolate was noted by growing the strain on different cultivation medium for 21 days. It was observed that the isolate was able to grew well on the cultivation medium containing glucose as the main carbon source and displayed white and light grey color shade on the agar plates; initially the spores were white color; whereas after 14–21 days the spores were turned into dark grey with white color on the surfaces, the individual colonies were rough elevated and powdery (Fig. 1a). Microscopic observations noted that the isolate contained both aerial and elevated mycelia with spores on the surfaces. SEM image displayed that the size was 1 μ diameter and 6 μ length and spores were arranged as connecting chains (Fig. 1b). Biochemical characteristics revealed that the isolate was non motile, not able to produce hydrogen sulfide and able survive up to 8% of Sodium chloride. Additionally, the isolate was able to grow on different carbohydrates such as glucose, galactose, rhamnose, lactose, arabinose, sucrose, fructose, maltose and mannitol and able to ferment sugars such as glucose and sucrose respectively. The growth pattern and carbohydrate fermentation a characteristic of the isolate was coincides with the standard *Streptomyces* species. Also, the isolate was able to grow in the presence of various antibiotics such as nalidixcid acid, ampicillin, streptomycin, and actidione indicated its antibiotic production and withstanding capacity. The 16S rRNA gene amplification and sequencing guided 1464 base pairs and the BLSTn analysis of the sequence resulted that the sequence showed 100% identity and sequence similarity with the *Streptomyces smyrnaeus* strain SM350. The maximum sequence bit score and E values were 2680 and 0 respectively. Further the sequence was deposited in National Center for Biotechnology Information, USA under the accession number MH201370 for public availability. Conclusively the 16S rRNA gene sequence showed more similarity to the genus *Streptomyces* from the marine origin. Therefore, the isolate was designated as *Streptomyces* sp. strain Al-Dhabi-90.

Identification of major metabolites using gas chromatography–mass spectrometry (GC–MS) analysis

The chemical compositions of the active extract of the *Streptomyces* sp. strain Al-Dhabi-90 were determined by gas chromatography–mass spectrometry (GC–MS). The detailed individual components were summarized in Table 1 and Fig. 2. The major chemical constituent was 3-methylpyridazine (94.2%) (Fig. 3), *n*-hexadecanoic acid (3.2%), indazol-4-one (10.5%) (Fig. 4), and 3a-methyl-6-((4-methylphenyl) sul (7.42%) respectively. The other minor detected compounds were showed in Table 1 with respect to the retention time and the percentage of the areas.

Antimicrobial activity of the extracellular metabolite

Initially, the antimicrobial properties of the crude extracellular metabolite were determined by the disc diffusion method

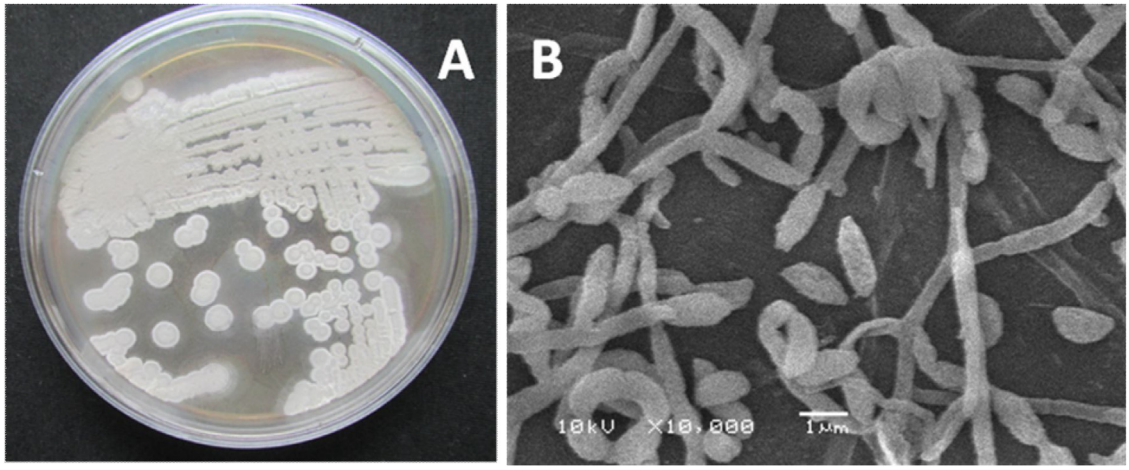

Fig. 1. Micro morphology of the marine *Streptomyces* sp. Al-Dhabi-90.
(A) growth of the marine *Streptomyces* sp. Al-Dhabi-90 on MNGA medium.
(B) scanning electron microscopic image of the marine *Streptomyces* sp. Al-Dhabi-90.

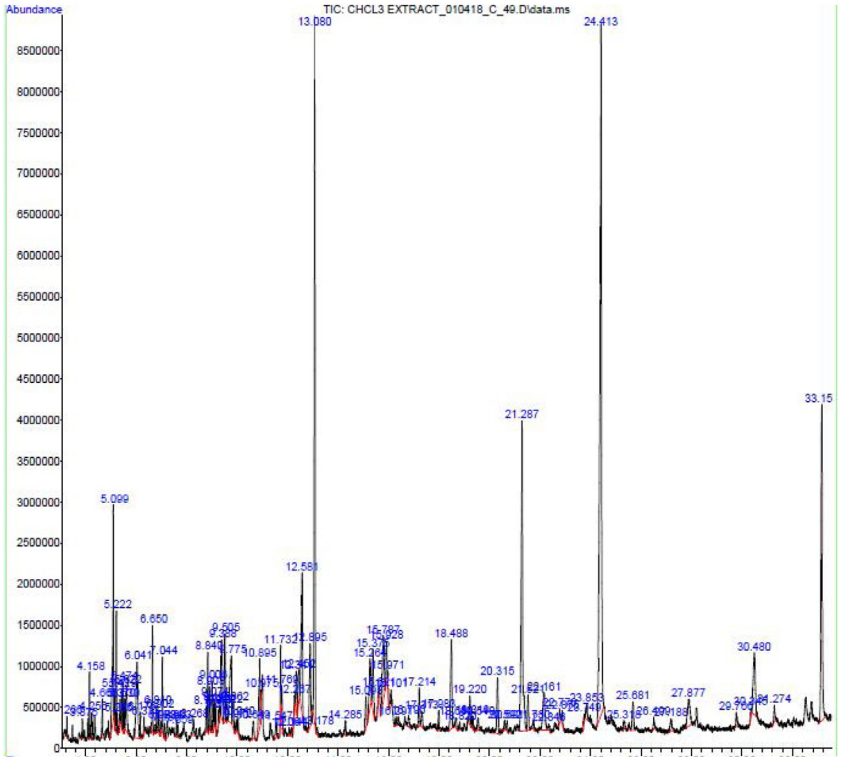

Fig. 2. Gas chromatography mass spectrometry (GC–MS) spectrum of the extracellular metabolite produced from marine *Streptomyces* sp. Al-Dhabi-90.

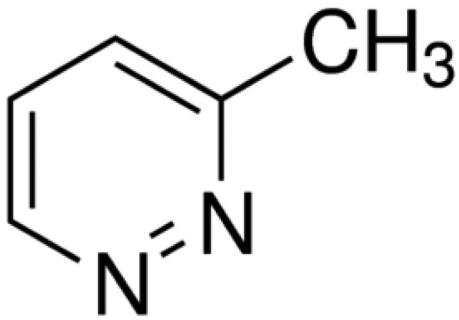

Fig. 3. Chemical structure of 3-methylpyridazine obtained from the fermentation broth of marine *Streptomyces* sp. Al-Dhabi-90.

against the pathogenic microbes. After that the MIC values of the extract was determined by standard methodology. As displayed in Figs. 5 and 6, the studied extract revealed variable level of antimicrobial activity. Among the Gram positive bacteria *S. aureus* showed MIC of 12.5 μg/ml and *S. epidermidis* displayed MIC of 25 μg/ml. *B. subtilis* and *E. faecalis* documented 50 μg/ml each. Whereas the Gram negative bacteria such as *P. aeruginosa*, *K. pneumoniae* and *E. coli* documented the MIC of 100 and 50 μg/ml respectively. Among the drug resistant pathogens, the strains of *A. baumannii* showed MIC of 6.25 μg/ml and multidrug resistant *S. aureus* and ESBL producing *E. coli* displayed the MIC of 12.5 μg/ml respectively. Drug resistant strains of *E. faecium, S. aureus* and *E. coli* had the highest MIC value (100 μg/ml).

Table 1
Individual metabolite composition of marine *Streptomyces* sp. Al-Dhabi-90 using gas chromatography mass spectrometry (GC–MS) analysis.

S. no	Name of the compounds	RT	Peak area	Molecular weight
1	Hydrazine, propyl-	3.266	0.15	43.20
2	Guanidine	3.875	0.16	57.10
3	Undecane	4.158	0.34	43.10
4	1-Octene	4.256	0.28	41.20
5	Benzoic acid	4.662	0.47	122.10
6	2-Coumaranone	5.099	3.06	78.10
7	Heptane, 2-methyl-	5.142	0.19	43.10
8	Pentadecane	5.222	0.77	43.20
9	Tricosane	5.265	0.12	43.20
10	Heptadecane, 2,6,10,15-tetramethyl-	5.370	0.27	43.10
11	Indole	5.419	0.26	41.20
12	Tetradecane, 2-methyl-	5.474	0.44	43.20
13	Methyl valerate	5.530	0.22	74.10
14	Butanoic acid	5.622	1.05	31.10
15	Ethyl ether	6.041	2.35	73.10
16	1-Pentanol, 3,4-dimethyl-	6.176	0.19	43.10
17	2,3-Hexanedione	6.317	0.16	43.10
18	Tetracosane	6.650	0.89	43.20
19	Phenol, 3,5-bis(1,1-dimethylethyl)-	6.810	0.29	191.20
20	Acetamide, *N*-(2-phenylethyl)-	6.902	0.30	43.10
21	3-Methyl-5-hydroxy-isoxazole	6.982	0.12	43.20
22	Octadecane, 2-methyl-	7.043	0.72	43.10
23	Heptacosane	7.136	0.16	43.30
24	2-Butenoic acid, 3-methyl-	7.234	0.26	43.20
25	Cyclohexane, 1,2-dimethyl-, cis-	7.603	0.14	43.20
26	Oxirane, phenyl-	7.659	0.14	89.10
27	2,5-Piperazinedione, 3-methyl-6-	8.268	0.33	128.00
28	Acetamide, *N*-methyl-*N*-(4-methylp...	8.779	0.29	121.20
29	Octadecane	8.840	0.77	43.20
30	2-Bromo-3′-methoxyacetophenone	8.908	0.44	135.20
31	Phenol, 2-(1,1-dimethylethyl)-	9.006	0.68	135.10
32	1,3-Cyclohexadiene, 1,2,3,4,5,6-...	9.074	0.56	149.20
33	Phenol, 4-(1-methylpropyl)-	9.148	0.21	149.20
34	Cyclohexene, 2-ethenyl-1,3,3-tri...	9.265	0.18	135.20
35	1,3-Dimethyl-3,4,5,6-tetrahydro-...	9.320	0.19	43.10
36	3,6-Undecandione	9.388	1.42	43.20
37	Phenol, *m-tert*-butyl-	9.505	1.42	135.20
38	4-Hydroxyphenylpyruvic acid, met...	9.622	0.12	149.20
39	Pyrrolo[1,2-a]pyrazine-1,4-dione...	9.775	2.06	83.10
40	Methylphosphonothioic acid, S-[2...	9.862	0.44	114.10
41	Thiophene, 2-formyl-2,3-dihydro-	9.972	0.20	114.20
42	3-Ethylidene- heptan-2,6-dione	10.040	0.18	41.20
43	*N*-Acetyltyramine	10.649	0.38	120.20
44	L-Proline, *N*-valeryl-, pentadecy	10.895	1.47	154.20
45	Propanoic acid, phenyl ester	10.975	0.13	94.10
46	3-Methylpyridazine	11.547	94.20	94.20
47	7,9-di-*tert*-Butyl-1-oxaspiro(4,5...	11.732	0.87	217.20
48	Tetracosane	11.769	0.21	43.20
49	[5-[(Furan-2-carbonyl)amino]-3-m...	12.064	0.12	277.30
50	8H-[1,2,5]Thiadiazolo[3,4-e][1,4...	12.144	0.13	170.10
51	L-Proline, *N*-butoxycarbonyl-, is...	12.267	0.28	70.20
52	Benzenemethanol, 3-hydroxy-5-met...	12.341	0.57	154.20
53	Atis-16-ene, (5.beta.,8.alpha.,9...	12.452	0.80	257.20
54	*n*-Hexadecanoic acid	12.581	3.28	41.10
55	3,5-di-*tert*-Butyl-4-hydroxypheny...	12.895	1.81	263.30
56	Indazol-4-one, 3,6,6-trimethyl-1...	13.079	10.50	291.30
56	Carbazole, *N*-trifluoroacetyl-	13.178	0.13	43.10
58	Propanamide, *N*-heptyl-*N*-octyl-2,...	14.285	0.20	57.20
59	Heptacosane, 1-chloro-	15.098	0.50	43.20
60	9,12-Octadecadienoic acid (Z,Z)-	15.264	1.37	41.20
61	Cyclopropaneoctanal, 2-octyl-	15.375	0.97	41.20
62	2-Pentynoic acid, 4-[(t-butoxyca...	15.621	0.26	91.20
63	Octadecanoic acid, 2-(2-hydroxye...	15.787	1.96	43.20
64	2,5-Piperazinedione, 3,6-bis(2-m...	15.928	0.74	170.10
65	L-Norleucyl-L-norleucine, *N*-ally...	15.971	0.21	170.20
66	2,5-Piperazinedione, 3-(phenylme...	16.101	0.28	91.20
67	Cholestan-6-en-3-ol, *O*-acetyl-24...	16.291	0.14	43.10
68	2,5-Piperazinedione, 3-benzyl-6-...	16.790	0.25	43.20
69	Thiocyanic acid, phenylmethyl ester	17.214	0.66	91.10
70	2,5-Piperazinedione, 3-benzyl-6-...	17.313	0.32	91.10
71	Cyclohexadecadiene-1,6-dione, 3,...	17.983	0.34	43.20
72	Pyrrolo[1,2-a]pyrazine-1,4-dione...	18.488	2.03	125.10
73	1H-1,2,3,4-Tetrazole-1,5-diamine...	18.660	0.23	91.00
74	4(1H)-Pyrimidinone, 2-amino-6-hy...	18.820	0.13	43.10
75	Benzonitrile, 3-(2,4-dihydroxybe...	19.220	0.44	238.10
76	Pyrrolidine, 1-(1-cyclohexen-1-yl)-	19.319	0.24	91.20
77	15-Hydroxydehydroabietic acid, m...	19.540	0.24	43.30
78	Crinan-1-ol, (1.alpha.)-	20.315	1.21	273.30
79	Tricyclo[4.2.1.1(2,5)]decan-9-ol...	20.592	0.16	55.30
80	Isothiazole, 4-nitro-	20.691	0.19	43.20
81	Glycerol 1-palmitate	21.287	7.71	43.20
82	Acetic acid, 8-(2-cyanoethyl)-1,...	21.521	0.71	43.20
83	Heneicosane	21.730	0.17	71.20
84	Urea, *N*-(2-chloro-3-methylphenyl...	22.161	1.31	91.10
85	Eicosane, 9-octyl-	22.346	0.12	43.20
86	Tricosanoic acid	22.776	0.28	43.10
87	Formamide, *N*-(2,4-diamino-1,6-di...	22.875	0.17	91.20
88	Formic acid, (4-fluoro-3-nitroph...	23.749	0.13	107.10
89	2-Ethyl-1,3,4-trimethyl-3-pyrazo...	23.853	0.33	107.10
90	Octadecanoic acid, 2,3-dihydroxy...	24.413	19.25	43.20
91	4-Thiazoline-5-carboxylic acid, ...	25.318	0.17	91.00
92	Acetamide, *N*-[2-[[2-[2-(2-nitrop...	25.681	0.65	43.20
93	Cyclononasiloxane, octadecamethyl-	26.499	0.20	73.20
94	1,2-Dipalmitoylphosphatidylcholine	27.188	0.31	55.10
95	Benzoic acid, 2,5-bis(trimethyls...	27.877	1.14	147.20
96	Silane, [[4-[1,2-bis[(trimethyls...	27.877	1.14	147.20
97	1H-Pyrrol-2-amine, 4,5-dimethyl-...	30.345	0.17	334.10
98	Methylenebis(2,4,6-triisopropylp.	30.480	2.23	441.40
99	Cyclodecasiloxane, eicosamethyl-	31.274	0.31	281.10
100	3a-Methyl-6-((4-methylphenyl)sul...	33.156	7.42	441.40

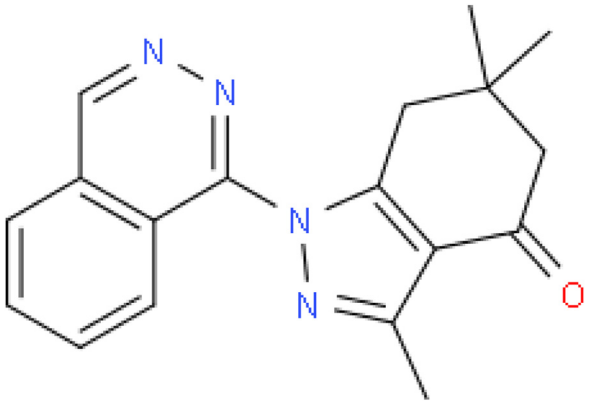

Fig. 4. Chemical structure of indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-obtained from the fermentation broth of marine *Streptomyces* sp. Al-Dhabi-90.

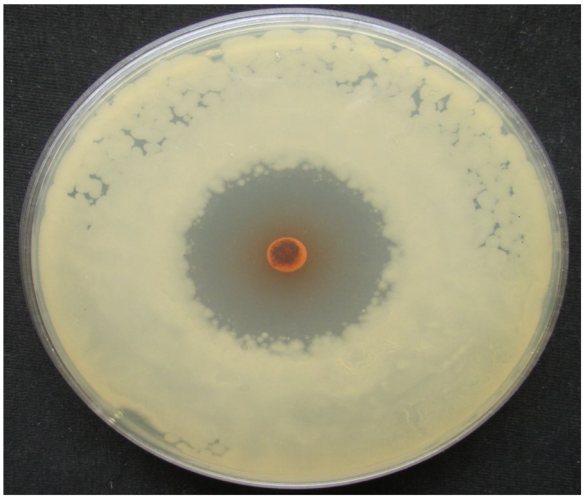

Fig. 5. Antimicrobial activity of extracellular metabolite produced from marine *Streptomyces* sp. Al-Dhabi-90 against *Staphylococcus epidermidis*.

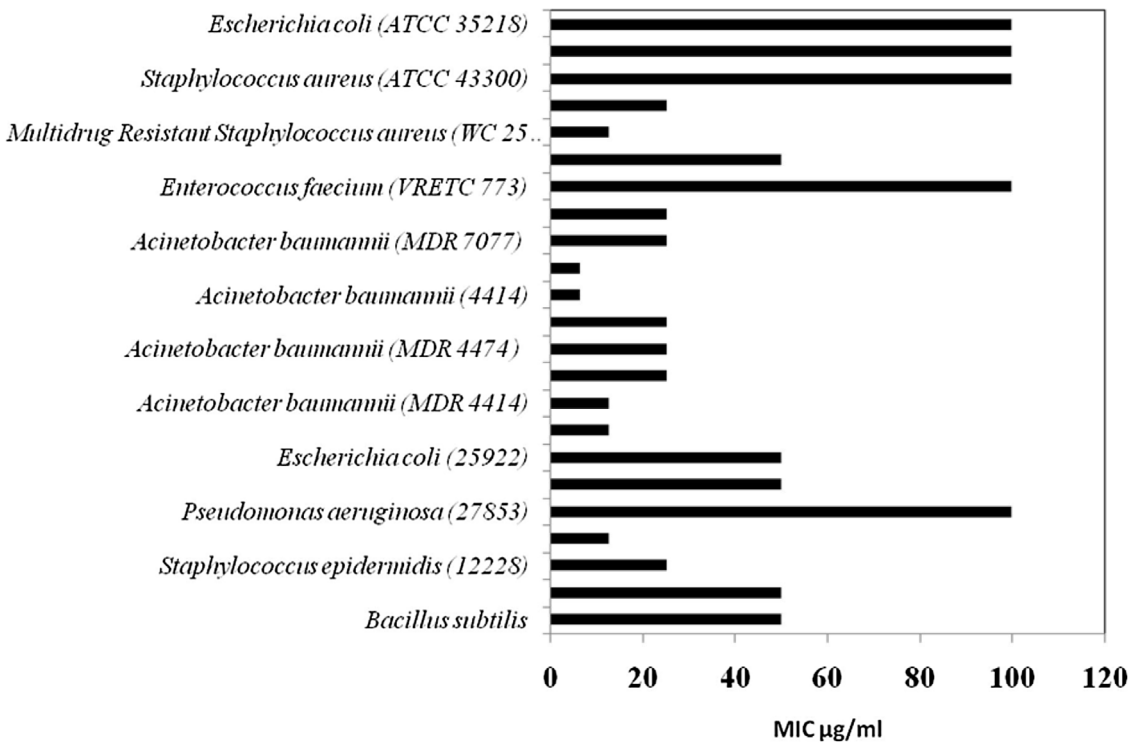

Fig. 6. Minimum Inhibitory Concentration of the crude ethyl acetate extract obtained from the fermentation broth obtained from the marine derived *Streptomyces* sp. Al-Dhabi-90 against the bacterial pathogens.

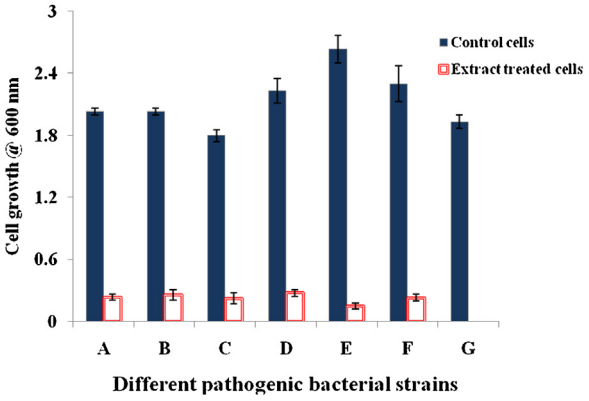

Fig. 7. Cell suspension inhibition properties of the extracellular metabolite produced from marine *Streptomyces* sp. Al-Dhabi-90 against various bacterial pathogens.

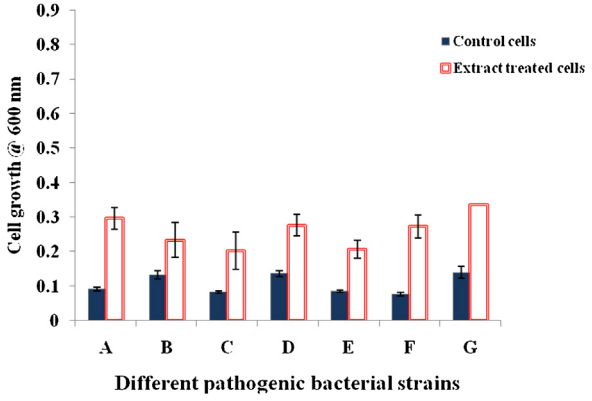

Fig. 8. Intra cellular cell constituents release of various bacterial pathogens after treating the extracellular metabolite produced from marine *Streptomyces* sp. Al-Dhabi-90.

Cell suspension inhibition assay

The effects of extract on the cell suspension of the studied pathogens are presented in Fig. 7. Results clearly indicated that 100 µg/ml of the crude extracts on the suspension of the cells were completely killed the microbial strains, such as *E. coli, P. aeruginosa,* and *K. pneumoniae*. The results clearly indicated that the extract was very active towards the Gram negative bacteria, where as the Gram positive bacteria namely *S. aureus* (killing percentage 88.3), *S. epidermidis* (killing percentage 87.2), *E. faecalis* (killing percentage 87.6), and *B. subtilis* (killing percentage 87.61), documented comparatively better activity. Conclusively, the results were in coincides with the primary screening of the actinomycetes against microbial pathogens and the MIC values.

Measurement of intra cellular cell constituents' release

The total intracellular constituents such as proteins, sugars, nucleic acids, and metabolites were measured for the determination of the crude extract on the compact integrity of the outer cell membrane of the microbial pathogens. The activity was measured by measuring the cellular constituents at 260 nm absorbance. The results indicated that the intra cellular constituents of the control and the treated pathogens were significantly varied (Fig. 8). The level of constituents was observed in the following, *E. coli* (72%), *S. aureus* (69%), *K. pneumoniae* (58.8%), *P. aeruginosa* (58.41%), *B. subtilis* (50.6%) and *S. epidermidis* (42.85%) respectively. Conclusively, the assay clear evidence of the activity of the extract was related to the release of the intra cellular constituents.

Discussion

Seventy-one percentages of the Earth's area were known for the existence of unbelievable entities with various chemical diversities [17]. Recent reports claimed that over 15,000 novel secondary metabolites act as antibiotics, pigments, enzyme inhibitors, and toxins were recovered from the marine microorganisms [18,19]. Because of the typical environmental conditions such as nutrient availability, pressure, temperature and dissolved oxygen levels, enhanced the metabolic level of the marine microorganism to produce novel chemically different molecules namely 12-deoxo-12-hydroxy-8-*O*-methyltetrangomycin, salinosporamide, marizomib and warkmycin with various activities [20,21]. Especially, the secondary metabolites recovered from the marine actinomycetes were highly used in preventing the spreading of drug resistant clinical pathogens. Recently, Rab et al. reported germicidin and zoumbericin from marine *S. ambofaciens* BI0048 were highly active against the multidrug-resistant strains of *E. coli* and *S. aureus* [22]. The characterization of the marine actinomycetes documented well, however, the number of actinomycetes strains with regards to the marine environment is very less. In our recent report Al-Dhabi et al., marine actinomycetes were isolated using starch casein agar medium supplemented with different proportion of the sea water [15]. Therefore, the starch casein Agar medium supplemented with sea water was used as a base for the isolation of novel actinomycetes isolates from the marine sediment samples from the Arabian Gulf region in Saudi Arabia. The active isolate Al-Dhabi 90, revealed potential activity against, Gram positive, Gram negative and multidrug resistant strains and it was selected and identified based on their biochemical, morphological and physiological characteristics. Molecular level characterization of the isolate was performed by 16S rRNA gene sequencing as described previously [23,24]. Present study indicated that more than 50% of the marine actinomycetes strains revealed promising antibacterial activity against the tested Gram positive bacteria. This clearly suggested that the Arabian Gulf region represented potential area for the exploration of active actinomycetes with variable activities which was coincides with the report of [25] stating that the marine sediment was potential organic rich habitats for the actinomycetes bacteria [25]. Also, recently, Cumsille et al. reported promising actinomycetes from the Chilean marine ecosystems [26]. Nevertheless, antimicrobial metabolite producing actinobacteria have been isolated from the back water area of the coastal regions [27,28].

The antimicrobial properties of the extracts confirmed that the extract was completely inhibiting the growth of both Gram positive and Gram negative bacteria. Interestingly, the extract showed more promising activities against the tested various drug resistant clinical strains. In addition, the antimicrobial properties of the organic extracts were confirmed by the cell suspension inhibition assay and the measurement of the intracellular constituents. The action of the extract on the cell wall of the microbial pathogens altered the cellular membrane and released the intracellular components clearly confirmed that the extract has the potential to kill the microbial pathogens. It is predicted that the extract acts on the outer lipopolysaccharide membrane of the pathogens and transport the extracts inside the cells and might change the inner cellular components which resulted in the increase cellular components in the treated samples [29]. GC–MS analysis authenticated the presence of 3-methylpyridazine, *n*-hexadecanoic acid, octadecanoic acid and 3a-methyl-6-((4-methylphenyl) sul in the crude extract. Recently, Lacret et al., identified 4-dehydro-4a-dechloronapyradiomycin and 3-chloro- 6,8-dihydroxy-8-lapachone marine-derived actinomycete strain CA-271078 revealed antimicrobial activity towards *S. aureus* and *E. coli*, similarly the metabolites present in the *Streptomyces* sp. Al-Dhabi-90 revealed activity against MRSA at 25 μg/ml, drug resistant *A. baumannii* at 6.25 μg/ml, ESBL producing *E. coli* at 12.5 μg/ml respectively [30–35].

Conclusion

The present study concluded that the Saudi Arabian marine derived *Streptomyces* sp. Al-Dhabi 90 possesses strong antimicrobial activity against the tested clinical pathogens. The isolate was confirmed based on the 16S rRNA gene sequence. The extracellular compound secreted by the isolate was extracted using organic solvents displayed antimicrobial activity at lower concentrations. GC–MS analysis of the extracts guided to detect 3-methylpyridazine and *n*-hexadecanoic acid as the major components. The MIC values of the extracts against the Gram positive and Gram negative pathogens were comparable with the other reported studies. Interestingly, the extract showed significant activity towards the drug resistant clinical isolates. Cell suspension inhibition and cell constituent's measurement studies evidenced that the real activity of the extract on the pathogen. Overall, the report herein microorganism recovered from unexplored marine regions of Saudi Arabia were the promising sources for the recovery of secondary metabolites with wide level of activities which guided for the production of new antibiotics.

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Competing interests

None declared.

Ethical approval

Not required.

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