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Original article

Chemical profiling of *Streptomyces* sp. Al-Dhabi-2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications

Naif Abdullah Al-Dhabi *, Galal Ali Esmail, Veeramuthu Duraipandiyan, Mariadhas Valan Arasu

Department of Botany and Microbiology, College of Sciences, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

Filamentous bacterial belonged to *Streptomyces* species were novel drug source for medical and industrial applications. However, the detailed identification of *Streptomyces* species from Saudi Arabian extreme environment for the identification novel drug source for medical and industrial applications were rarely studied. The *Streptomyces* strain Al-Dhabi-2 obtained from the thermophilic region kingdom of Saudi Arabia, exhibited antimicrobial potentials against the pathogenic microorganism were characterized. Biochemical and phylogenetic analysis confirmed that the strain was closely associated to the *Streptomyces* species. The chromatogram of GC-MS analysis of this ethyl acetate extract (EA) had diverse of chemical compounds namely benzene acetic acid (7.81%), acetic acid, methoxy-, 2-phenylethyl ester (6.01%) were the major compounds. EA of Al-Dhabi-2 showed inhibition zone ranged from 14 to 25 mm at 5 mg/well concentration against the tested microbial pathogens. Results revealed that the significant MIC values were observed against *B. cereus,* and *E. faecalis* by (less than 39 μg/ml) and against *S. agalactiae* with (78 μg/ml). Minimum inhibitory concentrations (MIC) for fungi: were also reported against *Cryptococcus neoformans* and *Trichophyton mentagrophytes* by (156 μg/ml), whilst *Candida albicans* and *Aspergillus niger* by (312 μg/ml). Results of this study showed that thermophilic actinobacteria could be promise source in the context of searching for unique antimicrobial agents with novel properties.

1. Introduction

Saudi Arabia has a good source of clean and sustainable energy (Aljuhani, 2012), nearly ten geothermal hot springs available in Saudi Arabia (Khiyami et al., 2012). Thermophilic bacteria and archaea are populated and diverse in geothermal environments (Kaur et al., 2008). Microbes that thrive in harsh ecosystems are called extremophiles. During the last four decades, these microorganisms have been isolated form extreme sites (Khalil, 2011). Thermal systems scattered on different locations on the planet harbour several of thermophilic archaeal genera (Arab et al., 2000). Recently, thermophilic actinobacteria have gained notable interest by the researchers owing to the promising results they showed to produce variety of substances with biological activities and the economic potential for that. Theremophilic actinobacteria can be used in biodegradation and other biological processes (Kleeberg et al., 1998), produce bioactive molecules (Takeuchi et al., 1991) or enzymes (Uzel and Hames-Kocabas, 2007; Hames-Kocabas and Uzel, 2007). The genus belonged *Streptomyces* were known for the production of bioactive compounds such as streptothricin, streptomycin, actinomycin, chloramphenicol, tetracycline, erythromycin, leucomycin, vancomycin, gentamicin, teicoplanin, fortimicin, rosamicin, nocardicin and salinosporamide A respectively (Waksman et al., 2010; Arasu et al., 2013b; Al-Dhabi et al., 2014; Arasu et al., 2015; Al-Dhabi et al., 2019).

Actinobacteria, Gram positive bacteria, are the pioneers among all microbes in term of producing antimicrobial agents in drug discovery programs (Ellaiah et al., 2004; Stackebrandt et al., 1997; Arasu et al., 2013a; Balachandran et al., 2015; Al-Dhabi et al., 2018a, 2018b). In nature, the distribution of actinobacteria are very diverse and represent a main source for commercially active molecules (Andrew and Cook Paul, 2003; Valli et al., 2012). Actinobacteria represent prolific source of several active compounds

* Corresponding author.
E-mail address: naldhabi@ksu.edu.sa (N.A. Al-Dhabi).
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(Demain, 1995), with great impact in clinical treatment and other applications of human medicine (Watve et al., 2001). Thermophilic actinobacteria are potential as producer of such bioactive compounds due to they can grow rapidly, thus rapid mycelium autolysis in compared to mesophilic actinobacteria (Moreira et al., 1981). The genus *Streptomyces* produces the vast majority of the bioactive compounds more than any other genera within the actianobacteria phylum, therefore, *Streptomyces* has been extensively investigated and reported as a prolific mine for unique and new antibiotics (Watve et al., 2001). The current project aim to isolate thermophilic actianobacteria from the sediment sample collected from thermal spring located in kingdom of Saudi Arabia. *Streptomyces* Al-Dhabi-2 identified based on cultural, physiological, molecular level, biological parameters and sensitivity to different antibiotics. The secondary metabolites of the strain have been extracted to investigate their antimicrobial activities against pathogenic bacteria and fungi.

2. Materials and methods

2.1. Chemicals and medium

Cultivation media such as starch casein agar, nutrient agar, muller hinton agar and muller hinton broth were purchased from Himedia, India. Solvents such as hexane, chloroform, ethyl acetate, methanol, acetone and DMSO were obtained from Somato, Riyadh, Saudi Arabia. Commercial antibiotics such as, nalidixcic acid, actidione, streptomycin was procured from Himedia, India. Experiments were performed in mill Q water for the routine experiments.

2.2. Source of actinomycetes

Sediments from hot spring of Tharban (19°2′20.37″N, 41°40′45.13″E) located in the southern west of Al-Mjardah province, in Aseer region, Saudi Arabia was collected aseptically (Fig. 1). The sediments samples were collected with sterilized scoop into the place. Each sample was taken in depth of 6–15 cm from ground's surface. Sediments samples were put into sterile polythene bags and tightly covered. Immediately, the samples transferred to the our research laboratory, the samples were kept until to be dried and finally the dried sediments stored in a refrigerator until further use.

2.3. Isolation, cultivation and maintenance thermophilic actinomycetes

A portion of sediment sample was dried at 100 °C by using hot air oven for one-hour support for the isolation of thermophilic actinobacteria (Uzel et al., 2011) dried sediment added to sterile DD water (10 g/100 ml) and thoroughly shaking for half hour (Kuster and Williams, 1981). Thermophilic actinobacteria were isolated via spreading dilutions of samples by adding 500 µl onto isolation media, nalidixic acid (25 µg/ml) and cycloheximide (50 µg/ml) antibiotics were supplemented to the both media to inhabit the grow of unwanted microes. The incubation of the plates were at 55 °C for 3–5 days with presence of humidity. The strains were repeatedly sub-cultured on SCA until obtain the pure culture. Pure isolates were stored in 50% (v/v) glycerol at freezer for long time storage.

2.4. Morphological characteristics of Streptomyces sp. Al-Dhabi-2

A loopful from the broth culture of strain Al-Dhabi-2 was spread on a clean glass slide to prepare the smear and then drying by heat. Crystal violet and iodine were added and washed with water and treated with alcohol at appropriate times. Finally, safranin stain was added onto the smear for 30 sec, the excessed of the stain was discard and the slide was drying. Slide was examined using phase-contrast microscope at 100×. *Streptomyces* sp. Al-Dhabi-2

Fig. 1. Hot spring of Saudi Arabia, taken from Google earth.

was inoculated on 4 different ISP media (ISP2-ISP5) and MNGA medium, the incubation temperature was at 50 °C for 3–5 days. The morphological properties were observed under a magnifying lens, which include aerial, substrate mycelium, colour and the branching (Shirling and Gottlieb, 1966).

2.5. Characterizations of the strain by physiological and biochemical analysis

Standard method was followed for this analysis (Valan Arasu et al., 2008). Gelatin hydrolysis, starch hydrolysis, production of DNase, hydrogen sulphide production and ability to grow in different temperatures (29–55 °C), range of pH (5–9) and concentrations of NaCl (4% to 13%) on medium were tested using standard methods.

2.6. Molecular identification of Al-Dhabi-2

Thermophilic actinobacteria Al-Dhabi-2 inoculated in yeast peptone glucose (YPG) medium at 50 °C for 3–5 days. The extraction the DNA of strain Al-Dhabi-2 was performed by using the commercial kit and amplifications were done using the following primers [F 27 (5′-AGAGTTTGATCCTGGCTCAG-3) and R 1492 (5′-TACGGCTACCTTGTTACGACTT-3′)]. The PCR products were sequenced and analysed.

Clustal W software was used for aligning of the strain Al-Dhabi-2 16S rRNA sequences to those retrieved genes sequences form the NCBI. The resulted data was processed via neighbour-joining method used for analysed and analysing (Saitou and Nei, 1987). The evaluation of the topology of the resultant tree using bootstrapping assay by 1000 replications of the neighbour-joining tree.

2.7. Antimicrobial activity

2.7.1. Test microbes

The following pathogenic microorganisms were taken for preliminary screening and Minimum inhibitory concentration (MIC) studies. *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Psudomonas aeruginosa*. Fungi: *Candida albicans and Cryptococcus neoformans* were used. The tested microbes are ATCC except *Cryptococcus neoformans* (clinical isolate).

2.7.2. Inoculum preparation

Inoculums of the selected pathogenic bacteria inoculated into growth medium and incubated for 24 h at 35 °C. the inoculums were diluted via using sterile MHB medium to desired cell counts. Inoculums of fungi were prepared by grown in SDA slants for 10 days at 28 °C. Sterile D.D. water (5 ml) added to the fungal growth on slants and homogenized, Yeasts were inculcated into contain SDB media.

2.7.3. Preliminary antimicrobial activity

The inhibitory potential of strain Al-Dhabi-2 were screened and determined by cross-streak method (Duraipandiyan and Ignacimuthu, 2009). Secondary screening of fermented broth was tested against microbe by agar well diffusion method.

2.7.4. Optimization of media and antimicrobial metabolites production

The thermophilic strain Al-Dhabi-2 was inoculated into several broth media to evaluate the appropriate medium for bioactive secondary metabolite production (150 rpm at temperature 45 °C) in an orbital shaker. These media are; Antibiotic production medium, M6 medium, M3 medium-*Micromonospora* medium, yeast extract malt medium and MNG broth media. We have followed standard protocol which was published our previous paper (Al-Dhabi et al., 2016).

2.7.5. Extraction of antibacterial metabolites

Streptomyces Al-Dhabi-2 was grown in a shaker using modified nutrient glucose (MNG) broth medium, based on media optimization, for extraction of antimicrobial compounds for 13 days at 45 °C. Growth culture was filtered by using Whatman filter paper. About 5000 ml of culture filtrate was collected. The filtrate pH was reduced to 2 pH using HCl (0.1 N) and the organic solvent ethyl acetate was used as for extraction of antimicrobial metabololites by adding two different equal volume (v/v) of culture filtrate and organic solvent. Two layers were formed; the upper layer contains the organic solvent and the extracted secondary metabolites. Organic phase was separated by using separating funnel and the crude metabolites were collected after removing the organic solvent at 60 °C by using vacuum evaporator (IKA Rotary Evaporator model). The crude extract was stored in dark and cold place for further tests.

2.7.6. Preparation of stock crude extracts

Stock solution was prepared by dissolving 100 mg of EA into 0.5 ml of DMSO.

2.7.7. Agar-well diffusion method

Antibacterial and antifungal activities of the strain Al-Dhabi-2 were screened against selected human pathogenic microbes via using agar-well diffusion method**.** Wells with 5 mm diameter were made on the agar media using sterile cork borer followed by filling 25 μl of the prepared ethyl crude extract with concentration 5 mg/well. The standard positive control for bacteria and fungi are Streptomycin (25 μg) and caspofungin (200 μg) respectively, whilst the negative control is dimethyl sulfoxide (DMSO).

2.7.8. Determine MIC

Test concentrations were prepared via mixing Crude extract of strain Al-Dhabi-2 with DMSO:water (1:9). The evaluation of minimum inhibitory concentration (MIC) has conducted by previously published procedures (Duraipandiyan et al., 2010). The required concentrations (mg/ml) of the test extract (0.0781, 0.156, 0.312, 0.625, 1.25, 2.5 and 5 mg/ml) were placed into the 96 well plate which contain MHB medium. From each inoculum culture suspension 5 μl added into the wells and the 10^5 CFU/ml was the final inoculum size. The positive controls were caspofungin and streptomycin whilst DMSO was included as a negative control. The lowest concentration of the extract inhibited growth of microbe was used to determine the MIC values.

2.8. Chemical profile of crude extract

The profile of substances contained bioactive compounds in the crude extract of the strain were investigated via using gas chromatography (GC-MS) as described by Al-Dhabi et al. (2016), Valsalam et al. (2019).

3. Results

Actinobacteria have been extensively studied, earlier researchers have been published several reports regarding to the morphology, physiology, biochemical and biological properties of the actinobacteria (Al-Dhabi et al., 2016). However, there is not much report on thermophilic actinobacteria with antibacterial and antifungal properties from the extreme environmental regions of Saudi Arabia (Fig. 1).

3.1. Cultural properties

Strain Al-Dhabi-2 was Gram positive and filamentous. Cultural properties results revealed that the strain can grow well on various ISP and MNGA media. Arial mycelium of Al-Dhabi-2 was grey and brown color. Diffusible pigment was not produced in the agar plate (Table 1).

3.2. Biochemical and physiological characteristics

Physiological and biochemical traits of Al-Dhabi-2 were studied. The results showed that the strain could be cultured at a salt concentrations ranging from 4% to 7%. The isolated strain Al-Dhabi-2 grew normally at the pH ranging between 5 and 9 and the optimal pH was 7.0. The strain grew in a range of the different temperatures (29 °C to 55 °C) on all tested media. The optimal growth temperature was 50 °C. Based on these results the isolated actinomycete Al-Dhabi-2 was classified as thermophilic actinobacteria. The tolerance of temperature, pH, variation of NaCl concentrations, utilization of different carbon source, production of amylase, DNase, gelatinase and sensitivity to the different antibiotics indicated that the strain belongs to actinobacteria (Table 2).

3.3. DNA extraction, sequencing, and phylogenetic analysis

Based on phenotypic characteristics and according to the Bergey's Manual, Al-Dhabi-2 was identified as *Streptomyces* sp. 1238 bp was resulted from the sequencing of 16S rRNA gene was and resulted, the sequences were deposited to NCBI and got the accession number (KF815081). 16S rRNA gene sequences showed high similarity to those sequences of 16S rRNA gene deposited in NCBI, sequences of *Streptomyces pseudogriseolus* was the closest to Al-Dhabi-2 sequences with 100% (E Value: 0.0) identity. Phylogenetic analysis confirmed these results as shown in phylogenetic tree of strain Al-Dhabi-2 (Fig. 2). Eventually the molecular results are consistent and supported the phenotyping identification of the strain Al-Dhabi-2 as *Streptomyces* sp.

3.4. Antibacterial and antifungal activities

Preliminary antimicrobial screening showed that *Streptomyces* Al-Dhabi-2 inhibited the growth of tested microbes in streak method. Significant antimicrobial activities observed against *Escherichia coli*, *Bacillus cereus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Staphylococcus epidermidis, Proteus vulgaris*, *Streptococcus agalactiae, Salmonella typhimurium*, *Psudomonas aeruginosa*, *Staphylococcus aureus*, *C. albicans* and *Cryptococcus neoformans* (Table 3). Based on the results of preliminary screening, further studies have been conducted in the secondary screening. The levels of bioactive compounds production in different growth media were determined. Results showed that the medium MNG was the best one for antibiotic production. Thus, strain Al-Dhabi-2 was grown on MNG broth medium. Ethyl acetate was used to extract bioactive compounds. Crude extract yielded 800 mg. Agar well-diffusion assay was used for screening the crude extract of Al-Dhabi-2 against bacterial and fungal pathogens. Al-Dhabi-2 exhibited good antimicrobial activity against the determined ATCC microbes (Table 4). The highest antimicrobial activities were reported against *E. Faecalis* (16 mm) followed by, *S. epidermidis, B. cereus* and *S. agalactiae* by 15 mm zone of inhibition. However, the activity against filaments fungi was interested by 19 mm for *A. niger* followed by 16 mm zone of inhibition for *T. mentagrophytes* (Fig. 3).

Based on the well diffusion assay results the extract was also studied for minimum inhibitory concentrations for tested bacteria and fungi. The MIC evaluation the extract was conducted using standard method. The values of MIC of ethyl acetate extract have been ranged between less than 0.039 to 0.625 mg/ml as shown in Table 5. The highest values were observed against *B. cereus*, *E. faecalis* with <0.039 mg/ml and *S. agalactiae* by 0.078, while in fungi the highest value is 0.156 mg/ml against *C. neoformans* and *T. mentagrophytes*.

3.5. GC-MS profile of crude extract

GC-MS chromatograph has been used for analysis the diversity of the compounds contained within the extract of *Sterptomyces* sp. Al-Dhabi-2. Results showed that crude extract consists of many

Table 2
Biochemical and physiological traits of the strain Al-Dhabi-2.

Characteristics	Results
Gram stain	Positive
Growth	Filamentous aerial growth
diffusible pigment production	–
Melanoid production	–
Growth temperature range	29 °C to 55 °C
Growth optimal temperature	50 °C
Growth pH range	5–9
Optimal pH for growth	7.0
Production of H_2S	–
Amylase	+
Protease	–
Gelatinase	+
deoxyribonuclease (DNase) production	+
NaCl tolerant	4% to 7%
Carbon sources utilization	
Negative control; none carbon source	–
Positive control; glucose	++
Sucrose	–
D-fructose	+
D-rhamnose	++
D-Xylose	++
D-arabinose	++
D-manitol	++
Sensitivity to antibiotics	
Ciprofloxacin (5 μg)	S
Gentamicin (10 μg)	S
Penicillin (10 μg)	R
Ampicillin (25 μg)	R
Chloramphenicol (30 μg)	S
Vancomycin (30 μg)	S
Tetracycline (30 μg)	S
Nystatin (100 μg)	R

+: presence; –: absence; S: Sensitive; R: Resistance.

Table 1
Morphological traits of the strain Al-Dhabi-2.

Media	Growth	Colour of aerial mycelium	Reverse side colour	Pigment
MNGA	+++	Grey	Yellow-brown	Absent
ISP-2	++	Grey	Yellow-brown	Absent
ISP-3	++	Grey	Brown	Absent
ISP-4	++	Grey	White	Absent
ISP-5	+++	Whitesh-grey	White	Absent

+++: prolific growth and ++: good growth.

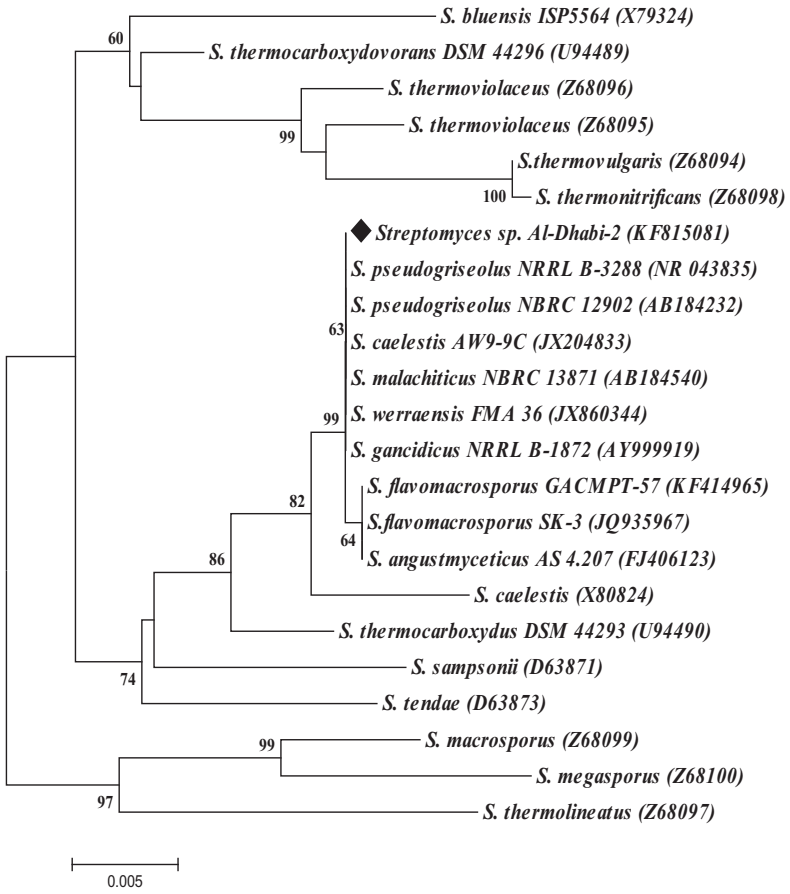

Fig. 2. Neighbour-joining tree based on partial 16S rRNA gene sequences showing the relationship between strain Al-Dhabi-2 and 22 species of the genus *Streptomyces*. Values at nodes point to bootstrap support (%) levels based on analysis of 1000 resampled datasets the values >50% are given only. Numbers between parentheses are NCBI accession numbers for each sequence.

Table 3
Antimicrobial activity of Al-Dhabi-2 in preliminary screening.

Tested microbes	ATCC* strain No.	Inhibition activity
B. cereus	11778	++
S. epidermidis	12228	++
S. aureus	6538P	+
E. faecalis	49532	+++
S. agalactiae	27956	++
E. coli	10536	++
P. vulgaris	33420	+
P. aeruginosa	27853	++
S. typhimurium	13311	++
K. pneumonia	13882	++
C. albicans	2091	++
C. neoformans	Clinical strain	++

ATCC: American type culture collection, +: moderate; ++: good; +++: Significant.

Table 4
The activity of Al-Dhabi-2 ethyl acetate extract against list of human pathogens.

Microorganisms	ATCC No.	Zone of inhibition in mm	
Bacteria		Al-Dhabi-2 (5 mg/well)	Streptomycin 10 μg
B. cereus	11778	15	22
S. epidermidis	12228	15	–
S. aureus	6538P	11	12
E. faecalis	49532	16	24
S. agalactiae	27956	15	20
E. coli	10536	14	15
P. vulgaris	33420	10	15
P. aeruginosa	27853	14	16
S. typhimurium	13311	10	17
K. pneumoniae	13882	11	17
Fungi Caspofungin 200 μg			
A. niger	16888	19	27
T. mentagophytes	9533	16	–
C. albicans	2091	10	22
C. neoformans	Clinical strain	10	–

–: no activity.

compounds (Table 6). The acetic acid, 2-phenylethyl ester (10.45%), benzene acetic acid (7.81%), acetic acid, 3,6-bis(2-methylpropyl)- (6.62%), acetic acid methoxy-2-phenylethyl ester (6.01%) and diisopropyl ether (5.25%) were the major compounds (Fig. 4).

4. Discussion

Since the beginning of the revolutionary era for antibiotic production in the last century, actinobacteria come to the forefront as a prolific source for bioactive compounds production. Thousands of bioactive compounds have been extracted from microorganisms, actinobacteria are produced around 50% of these compounds as secondary metabolites (Berdy, 2005). In present study, the thermophilic *Streptomyces* strain was isolated from thermal spring in Saudi Arabia and identified based on to standard methods via using phenotypic and molecular identification techniques. The phenotypic and molecular characteristics of Al-Dhabi-2 are consistent with those of the genus *Streptomyces*. Al-Dhabi-2 exhibited moderate antibacterial and antifungal activities in the streak method. Sequences analysis of the gene 16S rRNA was used for

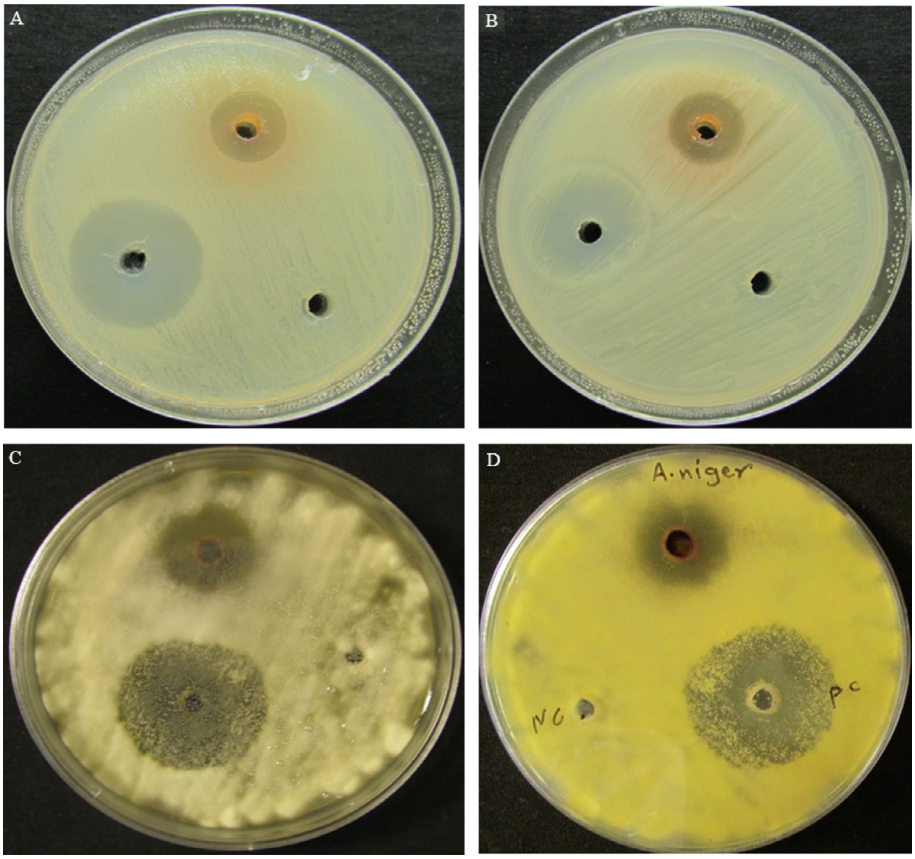

Fig. 3. Antimicrobial activity of Al-Dhabi-1 ethyl acetate extract against human pathogenic microbes. (A) *E. faecalis*; (B) *S. agalactiae*; (C) *A. niger* and (D) *A. niger* (reverse side of the plate). PC; positive control and NC; negative control.

Table 5
MIC values of Al-Dhabi-2 ethyl acetate extract using micro-broth dilution method.

Microorganisms	ATCC No.	MIC	
Bacteria		Al-Dhabi-2 (mg/ml)	Streptomycin μg/ml
B. cereus	11778	<0.039	0.156
S. epidermidis	12228	0.156	>10
S. aureus	6538P	0.625	2.5
E. faecalis	49532	<0.039	1.25
S. agalactiae	27956	0.078	1.25
E. coli	10536	0.312	0.625
P. vulgaris	33420	0.625	5
P. aeruginosa	27853	0.625	1.25
S. typhimurium	13311	0.312	5
K. pneumoniae	13882	0.156	0.625
Fungi Caspofungin μg/ml			
A. niger	16888	0.312	62.5
T. mentagophytes	9533	0.156	>500
C. albicans	2091	0.312	<4
C. neoformans	Clinical strain	0.156	250

molecular characterization of *Streptomyces* Al-Dhabi-2. The sequencing of 16S rRNA illustrated that *Streptomyces* sp. Al-Dhabi-2 (accession No. KF815081) had close family relationship with sequences of different species belong to *Streptomyces* when compared to Gen Bank data also.

Thermophilic actinobacteria can be isolated form the hot environments. They were isolated from various hot springs in several countries over the world and and identified by different techniques from the numerical taxonomy (Şahin et al., 2002), analysis of the composition of fatty acids (McNabb et al.,1997), through the use analysis of ribosomal protein (Ochi, 1992) and to phylogentic analysis the sequences of 16S rRNA (Song et al., 2001; Yoon and Park, 2000). It can be said that, identification of bacteria, includes actinobacteria, via sequencing of 16S rRNA represent a robust and accurate technique among the previous methods for identification (Songara and Swarnjeet, 2013; Woese, 1987). Morphological characteristics still represent one of the leading traits for identifying strains of the genus *Streptomyces* at species level, such as the colour of the aerial mycelium (Pridham and Tresner, 1974).

Singh and Kapoor (2013) used the biochemical characterization to identify thermophilic *Streptomyces* sp. MSC702 strain was isolated from mushroom compost samples. The strain Al-Dhabi-2 was grown at different temperatures from 29 °C to 55 °C. The optimum temperature was 50 °C. Production of antimicrobial metabolites was observed at 45 °C for Al-Dhabi-2. James and Edwards (1988, 1989) reported that *S. thermoviolaceus* utilized a broad spectrum of substances as sole source for carbon. Several of these compounds enhanced the production of antibiotics at temperature range from 30 to 55 °C, However the optimal production was at 50 °C. The strain Al-Dhabi-2 produced good enzyme activity. Amylase, gelatinase and deoxyribonuclease (DNase) enzymes were observed in the agar plate using appropriate media. Stress molecules can be produced by the microorganisms that thrive in extreme environments by which these microbes are surviving in such systems, therefore these unique molecules can be extracted as novel compounds and can be investigated in many aspects.

Hence, thermophilic actinobacteria isolates were screened against pathogenic microbes for their antimicrobial activities (Uzel et al., 2011). Thermophilic actinobacteria Al-Dhabi-2 showed

Table 6
Chemical profile of ethyl acetate extract.

Sl. no	RT	Compound	Quality	Molecular weight	Peak area %
1	4.059	Diisopropyl ether	45	102.104	5.25
2	5.700	Hexylene glycol	59	118.099	3.06
3	8.082	Phenylethyl Alcohol	94	122.073	2.59
4	9.084	Benzoic acid	53	122.037	3.00
5	10.463	Acetic acid, 2-phenylethyl ester	90	164.084	10.45
6	10.594	Benzene acetic acid	94	136.052	7.81
7	12.526	1-Tetradecene	99	196.219	0.85
8	13.136	Trans-Cinnamic acid	96	148.052	1.95
9	13.557	Acetic acid, methoxy-, 2-phenylethyl ester	83	194.094	6.01
10	14.878	N-Acetyltyramine	50	179.095	0.95
11	15.241	Cetene	99	224.25	2.41
12	16.853	7-Acetyl-1,7-diazabicyclo[2.2.0]he ptane	22	140.095	1.26
13	17.652	1-Octadecene	99	252.282	2.26
14	17.783	Coumarin	58	146.037	0.88
15	18.044	Phenol, 3,5-dimethoxy-	49	154.063	2.94
16	18.262	N-Acetyltyramine	91	179.095	1.85
17	19.105	l-Proline, N-allyloxycarbonyl-, heptyl ester	43	297.194	3.32
18	19.293	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	95	210.137	5.45
19	19.831	E-15-Heptadecenal	99	252.245	1.46
20	21.573	2,5-Piperazinedione, 3-methyl-6-(p henylmethyl)-	95	218.106	1.01
21	21.733	2,5-Piperazinedione, 3,6-bis(2-met hylpropyl)-	43	266.168	6.62
22	21.820	1-Docosene	97	308.344	1.11
23	22.227	2-(3-Amino-1,2,4-triazol-2-yl)(4,5H)imidazoline	35	152.081	0.64
24	23.331	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	91	244.121	2.64
25	23.389	9-Octadecenamide, (Z)-	99	281.272	2.44
26	25.422	Fumaric acid, propyl 2,3,6-trichlo rophenyl ester	35	335.972	1.63
27	25.800	Formamide, N-(2,4-diamino-1,6-dihy dro-6-oxo-5-pyrimidinyl)-	37	169.06	3.13
28	26.264	Bicyclo[3.1.1]heptan-2-one, 6,6-di methyl-, (1R)-	55	138.104	1.49
29	26.511	Tartaric acid, dimenthyl ester	64	426.298	2.60
30	27.150	Squalene	99	410.39	0.63

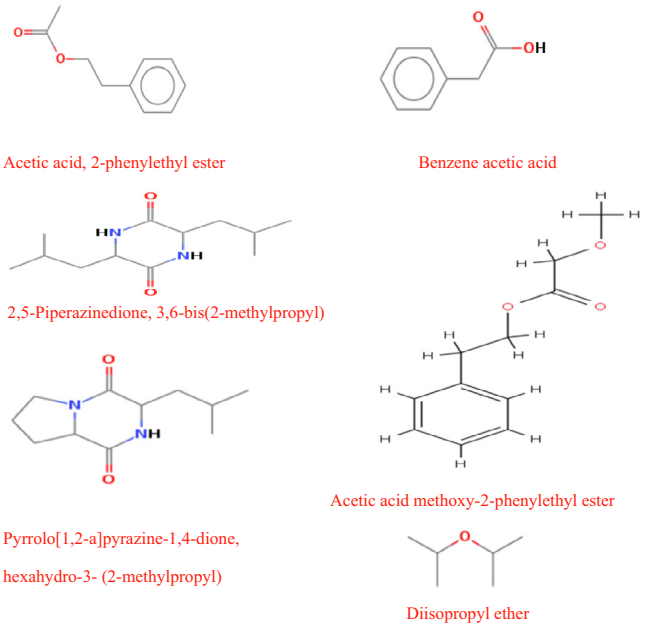

Fig. 4. Chemical structure of major compounds of Al-Dhabi-2 crude extract.

inhibitory activity against tested microbes. Extract of Al-Dhabi-2 inhibited the growth of tested microbes at 5 mg/ml concentration. The appropriate carbon source was glucose for antimicrobial agent production by Al-Dhabi-2. Temperature and pH are important factors for the growth of actinobacteria and production of antimicrobial metabolites. Al-Dhabi-2 grew optimally at 45–50 °C; optimum pH was 7.0. El–Abyad et al. (1996) reported that media composition is very specific for the antimicrobial potentials of the *Streptomyces* species.

Thermomycin and granaticin are antibiotics produced by thermophilic strains belonging to *Streptomyces thermophiles* and *Streptomyces thermoviolaceus* respectively (Edwards, 1993; Arokiyaraj et al., 2015). Songara and Swarnjeet (2013) reported that thermophilic actinobacteria identified as *Streptomyces* sp. from Rajasthan exhibited antibacterial activity to *Klebsiella* sp and *S. aureus*. The extracts of thermophilic *Thermoactinomyces* sp. isolated suppressed the growth of methicillin resistant *S. aureus* (MRSA) (Uzel et al., 2011; Korkmaz et al., 2007). The crude extract of the strain Al-Dhabi-2 exhibited antimicrobial activities which inhibited the growth of almost all tested microbes including *K. pneumonia, B. cereus, S. typhi S. aureus, E. coli* at 5 mg level. The chemical profile of the extract of Al-Dhabi-2 was analysed by GC-MS. The results showed that 30 compounds were present in the extract. Benzene acetic acid was one of those compounds found. A previous study has reported that benzene acetic acid exhibited antifungal and antibacterial properties (Tayade and Jadhao, 2012; Arasu et al., 2017, 2019). Therefore, further chemical analyses will be conducted on crude extract of Al-Dhabi-2 to determine the compound/compounds responsible for the antimicrobial activity.

5. Conclusion

This study was conducted in attempts searching for new antimicrobial compounds that produced via microorganisms such as *Streptomyces* from unexplored environments in particular the extreme habitats. The antimicrobial activities results of crude extract antagonistic to bacteria and fungi were promising. GC-MS techniques guided to detect acetic acid, 2-phenylethyl ester (10.45%), benzene acetic acid (7.81%), acetic acid, 3,6-bis(2-methylpropyl)-(6.62%), methoxy- 2-phenylethyl ester (6.01%) and diisopropyl ether (5.25%) from the crude extract. The future work is aiming to isolate the bioactive compound, followed by testing its antimicrobial activity against antibiotic resistant bacteria.

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