

Isolation, identification and screening of antimicrobial thermophilic *Streptomyces* sp. Al-Dhabi-1 isolated from Tharban hot spring, Saudi Arabia

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Received: 13 May 2015 / Accepted: 15 October 2015 / Published online: 30 October 2015
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Abstract The strain *Streptomyces* sp. Al-Dhabi-1 was isolated from soil sediments collected from Tharban hot spring in the southern west of Saudi Arabia using actinomycetes isolation agar and starch casein agar at 55 °C. Identification of the isolate was done according to morphological, physiological and biochemical characteristics and 16S rRNA sequence similarity as well. 16S rRNA sequence and blast analyses confirmed that the isolate belonging to the genus *Streptomyces*. The sequence was submitted to GenBank with accession number (KF815080). Ethyl acetate extract of *Streptomyces* sp. Al-Dhabi-1 showed good antimicrobial activities against tested pathogenic microbes. Minimum inhibitory concentration results showed that the best values were observed against *S. agalactiae* (<0.039 mg/ml) and *Klebsiella pneumonia* (0.125 mg/ml). Minimum inhibitory concentration of Al-Dhabi-1 against fungi; *Cryptococcus neoformans* (0.078 mg/ml), *C. albicans* (0.156 mg/ml), *A. niger* (0.625 mg/ml), and *T. mentagrophytes* (0.156 mg/ml). GC–MS analysis was used for the chemical profile of ethyl acetate extract. Benzeneacetic acid (16.02 %) and

acetic acid 2-phenylethyl ester (10.35 %) were the major compounds among 31 substances found the ethyl acetate extract. According to the results of antimicrobial activity against pathogenic microbes, it is clear that the actinomycetes from hot springs with extreme environments are promising source for antimicrobial compounds.

Keywords Thermophilic · *Streptomyces* sp. · Hot springs · Antimicrobial · GC–MS

Introduction

Discovering of novel metabolites from natural sources which have a potent effect against resistant pathogenic bacteria is considered today as an important field of research. The diversity of natural products makes it one of the most important sources for novel structures with useful biological activities. Several studies are oriented towards isolation of new microorganisms from different habitats in the context of the search for novel antibiotics from new sources (Sujatha et al. 2005).

In Saudi Arabia, there are ten geothermal springs available (Khiyami et al. 2012). Geothermal systems are populated by diverse thermophilic bacteria and archaea (Kaur et al. 2008).

Actinomycetes produced a variety of bioactive compounds (secondary metabolites) such as antibiotics, herbicides, pesticides, anti-parasitic, and enzymes (Oskay et al. 2004; Hozzein et al. 2011). *Streptomyces* is the greatest producer genus for discovering antibiotics in the microbial world so far; approximately 80 % of the total antibiotics were produced by *Streptomyces* sp. (Ravel et al. 2000; Procópio et al. 2012). As a result of the emergence of resistant pathogens and the side effects of antimicrobial agents

Communicated by M. da Costa.

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used at the present time, it becomes necessary to search for new antibiotics continuously (Arai et al. 1976). Therefore, in recent years thermophilic actinomycetes have attracted the attention of interested scientists. Owing to their diversity in biological activities and production of novel chemical compounds such as antibiotics like other microorganisms that inhabit extreme environments actinomycetes are promising (Kumar and Kannabiran 2010; Uzel et al. 2011).

The present study was aimed at isolating *Streptomyces* sp. Al-Dhabi-1 from hot spring and testing it for its antimicrobial activity. Identification of the isolate was done based on morphological properties, physiological and biochemical tests, in addition to analysis of 16S rRNA sequencing.

Materials and methods

Isolation, cultivation and maintenance of thermophilic actinomycetes

The strain *Streptomyces* sp. Al-Dhabi-1 was isolated from Tharban hot spring (52 °C) in Asir region southern west of Saudi Arabia (19°2'20.37"N, 41°40'45.13"E). Ten grams of dry sediments were added into 100 ml sterile distilled water and shaken for 30 min. *Streptomyces* sp. Al-Dhabi-1 was isolated using micro dilution technique. The sediment sample (0.5 ml from the dilution) was spread over onto following media actinomycetes isolation agar (AIA) (Himedia) and starch casein agar (SCA) (per liter: starch 10 g, KNO₃ 2 g, casein 0.3 g, NaCl 2 g, K₂HPO₄ 2 g, MgSO₄ 7 H₂O 0.05 g, CaCO₃ 0.02 g, FeSO₄ 7 H₂O 0.01 g, agar 18 g, and pH 7.2) supplemented with 0.05 % cycloheximide to prevent the growth of fungi. The plates were incubated at 55 °C for 3–5 days. *Streptomyces* sp. Al-Dhabi-1 was subcultured on AIA and SCA until pure culture was obtained. The pure culture with 50 % (v/v) glycerol was prepared and stored in the freezer (Uzel et al. 2011).

Morphological, physiological and biochemical Characteristics

The pure isolate of *Streptomyces* sp. Al-Dhabi-1 grown in starch casein broth at 50 °C for 3 days. After 3 days Al-Dhabi-1 smear of culture was taken in a sterile glass slide and heated gently over a flame. The smear was covered with crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was applied over the smear for 1 min and washed with running tap water. Alcohol solution was used to decolorize the smear. The slide was washed with water and counter stain safranin was flooded over the smear for 2 min, then the slide was washed, drained, air dried, and viewed under microscope.

The culture retaining the violet color indicated that it was Gram-positive organism.

Morphological, physiological and biochemical properties were determined based on standard methods. The International *Streptomyces* Project (ISP) media were used as following ISP medium 2, ISP medium 3, ISP medium 4 and ISP medium 5 to determine the Aerial mass color and soluble pigments; ISP medium 6 and ISP medium 7 were used for melanoid pigments, whereas ISP medium 9 was used to determine utilization of carbon source as recommended by Shirling and Gottlieb (1966). Utilization of nitrogen source, degradation of starch, gelatin and DNA were done according to Williams et al. (1983) and sensitivity to antibiotics was carried out as described by Petrova and Vlahov (2007). Ability to grow at different NaCl concentrations, temperatures and pH were done using ISP medium 2.

Molecular identification of active isolate using 16S rRNA

Streptomyces sp. Al-Dhabi-1 was grown in yeast peptone glucose broth (5 g peptone, 3 g yeast extract, 3 g NaCl, and 10 g glucose in 1 liter distilled water) until sufficient mycelium was formed for DNA extraction. High molecular weight genomic DNA was extracted from the isolate using NucleoSpin® Tissue kit (Macherey–Nagel, Duren, Germany) according to the manufacturer's instructions. The 16S rRNA was amplified and sequenced by (Macrogen Inc., Korea) using primers F 27 (5'-AGAGTTTGATCCTGGCTCAG-3) and R 1492 (5'-TACGGCTACCTTGTTACGACTT-3') (Dalisay et al. 2013).

Sequence of 16S rRNA of strain Al-Dhabi-1 was aligned with others retrieved from GenBank using Clustal W (Thompson et al. 1994). Phylogenetic analysis was performed using the software package MEGA 5 (Tamura et al. 2011). The resulting data were processed using the maximum-parsimony method (Fitch 1971) and the neighbor-joining method (Saitou and Nei 1987) for analysis of resultant data as well. The topology of the resultant unrooted tree was evaluated by bootstrap assay of the neighbor-joining tree, performed in 1000 replications.

Antimicrobial activity

Preliminary antimicrobial activity

Preliminary Antimicrobial screening assay was carried out for isolate Al-Dhabi-1 on Modified Nutrient Glucose Agar (MNGA) medium. The spore suspension of thermophilic *Streptomyces* sp. Al-Dhabi-1 was inoculated in a straight line on prepared agar plates and incubated at 50 °C for 7 days. After 7 days the following pathogenic microbes

(ATTC) were used for preliminary screening; *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus agalactiae*, and yeast: *Cryptococcus neoformans*, *Candida albicans*. The above microbes were grown in nutrient broth at 37 °C for 24 h. The grown microbes were streaked perpendicular ('T'-streak) to the strain *Streptomyces* sp. Al-Dhabi-1. The plates were incubated at 37 °C for 24 h. After the incubation period the antimicrobial activity was determined based on the distance of inhibition between the margin of thermophilic actinomycetes and pathogenic microbes. (Duraipandiyam and Ignacimuthu 2009).

Media optimization and antimicrobial metabolites production

For the production of bioactive compounds from the isolate *Streptomyces* sp. Al-Dhabi-1, the following media were used; Antibiotic Production Medium (APM), Micromonospora Medium (M3), Modified nutrient glucose medium (MNG), M6 media and yeast extract malt medium (YM). The pH of the medium was adjusted to pH-7.0 using 1 M HCl and 1 M NaOH. The culture was grown with continuous shaking on a rotary shaker (150 rpm) at 45 °C for 13 days. After growth the fermented broth was centrifuged and the supernatant was used as crude antibiotic extract. To study the influence of incubation periods the culture was maintained in the optimized production media for up to 16 days (Valan Arasu et al. 2012).

Extraction of antimicrobial metabolites

Modified nutrient glucose medium (MNG) was used for the extraction of antimicrobial compounds based on media optimization result. The strain *Streptomyces* sp. Al-Dhabi-1 was cultivated in MNG broth medium in a rotary shaker (150 rpm) at 45 °C for 10 days. To obtain the supernatant, the filtration of broth culture was done using Whatman No. 1 filter paper. The filtrate pH was adjusted to two using 0.1 N HCl to extract the majority of compounds in the filtrate. The ethyl acetate as an organic solvent was used for extracting the bioactive compounds by adding an equal volume (v/v) of the solvent to culture filtrate. The mixture of filtrate with organic solvent was appearing in two layers, the organic layer which contained the secondary metabolites and the aqueous layer. The crude extract was obtained after concentrating the solvent using rotary evaporator (IKA Rotary Evaporator model) at 60 °C and stored at 4 °C for antimicrobial assay.

Cup plate method

Antimicrobial activities for crude extracts were determined using the agar well diffusion method (Chaudhary et al. 2013). Crude extract of the isolate Al-Dhabi-1 was prepared for antibacterial activity as follows: 100 mg of crude extract was dissolved in 500 µl of dimethyl sulfoxide (DMSO). Sterile Mueller–Hinton Agar (MHA) (Hi-media, Mumbai) (25 ml) was poured into petri plates. 100 µl of suspension containing 10⁸ CFU/ml of test bacteria was swabbed onto solidified media. Wells were made using sterile cork borer (5 mm in diameter) and filled with 25 µl of prepared extract (the concentration is 5 mg), 25 µl of streptomycin (the concentration is 10 µg) as positive control and 25 µl of DMSO as negative control and left at room temperature for 30 min for allowing the compounds to diffuse through the agar. The plates were incubated at 37 °C for 24 h. The zone of inhibition was recorded in millimeters.

Minimum inhibitory concentration

The crude extract (10 mg) was dissolved in 1 ml of dimethyl sulfoxide (DMSO): water (1:9) and used for minimum inhibitory concentration (MIC), using standard broth microdilution method (Duraipandiyam and Ignacimuthu 2009) and the MIC was calculated. Mueller–Hinton broth (Himedia, Mumbai) was prepared and sterilized by autoclaving at 121 °C, 15 lbs for 15 min. The required concentration of the extract (5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.0781 mg/ml) was added to the 96 well micro titer plate containing 0.2 ml broth. The 5 µl of inoculum culture suspension was introduced into the respective wells and the final inoculum size was 1 × 10⁵ cfu/ml. The plates were incubated at 37 °C for 24 h. Positive control and solvent control (DMSO) were also included. MIC was determined as the lowest concentration of the extract which inhibited complete growth.

Identification of active principle using gas chromatography-mass spectrometry (GC–MS) analysis

The active ethyl acetate extract was quantified using gas chromatograph (GCMS-Shimadzu) equipped with a CPB-capillary column (mm inner diameter × 50 m length) mass spectrometer (ion source 200 °C, RI 70 eV) programmed at 40–280 °C with a rate of 4 °C/min. Injector temperature was 280 °C; carrier gas was He (20 psi). Sample volumes of 1 µl were injected with a split ratio of 25:1 using a hot needle. GC–MS analysis was carried out at Sargam Laboratory Service, Private Ltd, Chennai-600089, India.

Fig. 1 The growth of *Streptomyces* sp. Al-Dhabi-1 on different media **a** on ISP-2, **b** on ISP-3, **c** on ISP-4, **d** on ISP-5 and **e** on MNGA

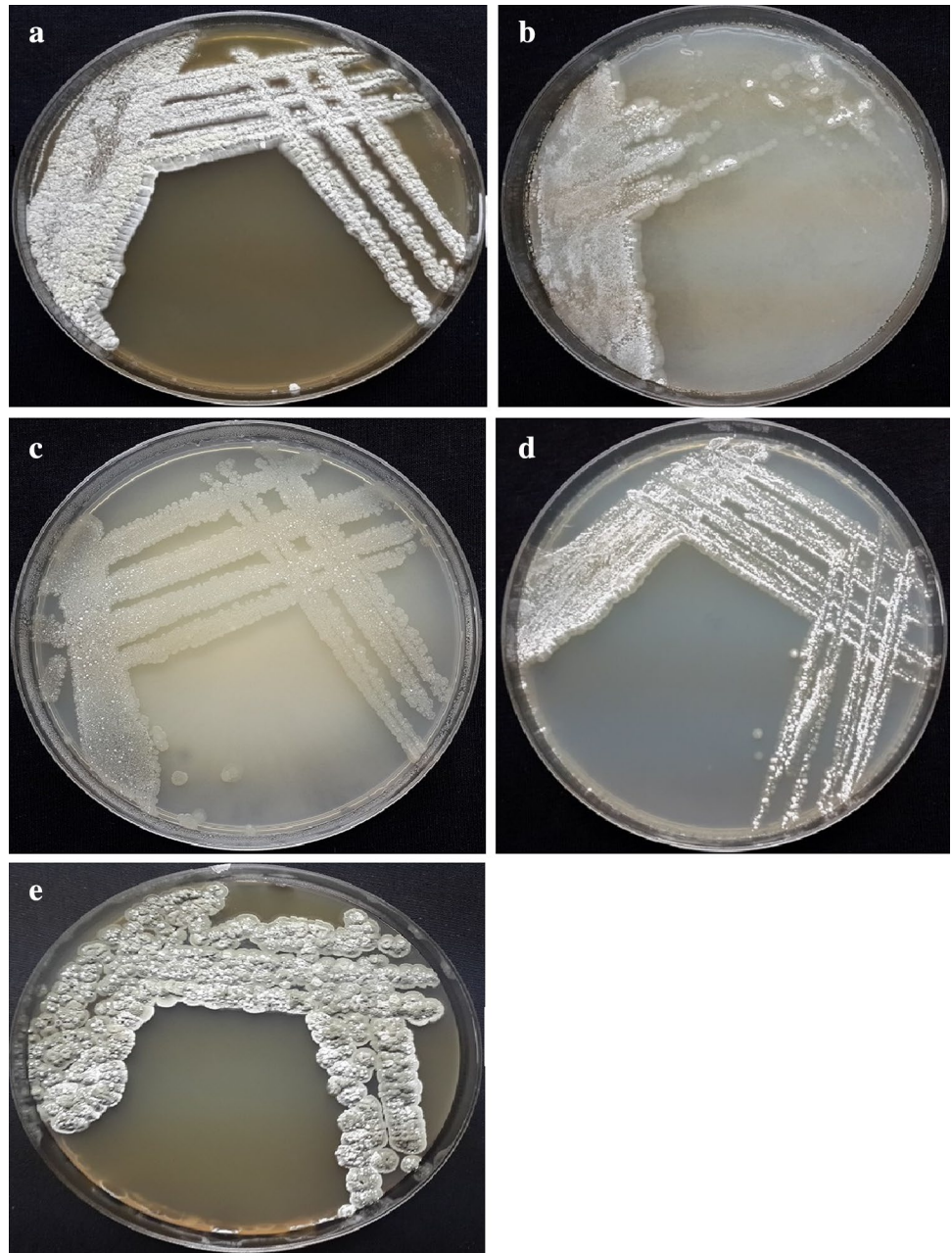


Table 1 Culture characteristics of *Streptomyces* sp. Al-Dhabi 1 on different media

Medium	Growth	Substrate mycelium	Aerial mycelium	Reverse	Pigment
ISP 2	Good	Present	Greenish-white	Yellowish-brown	–
ISP 3	Moderate	Present	Greyish-white	White	–
ISP 4	Moderate	Present	Greyish-white	White	–
ISP 5	Good	Present	White	Yellow	–
MNGA	Good	Present	White	Yellowish-brown	–

ISP2-5, International *Streptomyces* Project; MNGA, modified nutrient glucose agar, +, present; –, absent

Results

The genus *Streptomyces* has been studied and many reports have been published by earlier researchers relating to their morphology, physiology, biochemical, and biological properties in the medical field. Very limited reports have been published on thermophilic actinomycetes dealing with antimicrobial properties. Especially, in Saudi Arabia, there is not much more report on actinomycetes, particularly on thermophilic *Streptomyces* sp. Thermophilic *Streptomyces* sp. Al-Dhabi-1 was isolated from sediment soil collected from Tharban hot spring, Asir region, Saudi Arabia, using actinomycetes isolation agar (AIA) (Himedia, Mumbai) and starch casein agar.

Morphological, physiological and biochemical characteristics

The strain *Streptomyces* sp. Al-Dhabi-1 was filamentous gram-positive actinobacteria. Cultural characteristic of Al-Dhabi-1 showed that the isolate grew on different media (ISP2, ISP3, ISP4, ISP5, and MNGA) (Fig. 1); it formed green and whitish color mycelium and on the reverse side it showed yellowish-brown and white colors. The strain Al-Dhabi-1 never showed diffusible and melanoid pigments on any medium (Table 1).

The results of physiological and biochemical properties revealed that the strain *Streptomyces* sp. Al-Dhabi-1 grew at temperature between 37 and 55 °C; the optimal temperature was 50 °C, at pH between 6 and 11 with the normal pH of 7.0 and growth at 4 % NaCl concentration. The obtained results (Table 2) were compared to the relevant characteristics in Bergey's Manual of Systematic Bacteriology (Whitman et al. 2012) and numerical classification of thermophilic streptomycetes (Goodfellow et al. 1987) for identifying the strain Al-Dhabi-1. This showed that the thermophilic strain Al-Dhabi-1 belonged to the genus *Streptomyces*.

Sequencing and phylogenetic analysis

The sequencing result of 16S rRNA gene of strain Al-Dhabi-1 revealed that a total 1314 bp were obtained (Fig. 2). The sequence was submitted to GenBank with accession number (KF815080). The phylogenetic tree of strain Al-Dhabi-1 (Fig. 3) based on the neighbor-joining method formed a single clade with two strains of *Streptomyces megasporae* (16S rRNA gene sequence similarity of 98 %). The molecular characterization result of Al-Dhabi-1 revealed that the strain belonged to *Streptomyces*.

Table 2 Physiological and biochemical characteristics of *Streptomyces* sp. Al-Dhabi-1

Characteristics	Results
Gram staining	Positive
Shape and growth	Filamentous aerial growth
Production of diffusible pigment	–
Range of temperature for growth	37–55 °C
Optimum temperature	50 °C
Range of pH for growth	6–11
Normal pH	7.0
Growth in the presence of NaCl	4 %
H ₂ S production	–
Amylase	+
Protease	–
Gelatinase	+
Production of deoxyribonuclease (DNase)	+
Growth in the presence of NaCl	4 %
<i>Utilization of carbon sources</i>	
None carbon source (–ve control)	–
Glucose (+ve control)	++
D-Galactose	++
Sucrose	–
D-Fructose	++
D-Arabinose	+
D-Xylose	++
D-mannitol	+
D-Rhamnose	+
<i>Utilization of nitrogen sources</i>	
L-Serine	++
L-Phenylalanine	++
L-Threonine	++
L-Valine	++
<i>Standard antibiotics</i>	
Ciprofloxacin 5 µg	S
Gentamicin 10 µg	S
Ampicillin 25 µg	R
Tetracycline 30 µg	S
Vancomycin 30 µg	S
Penicillin 10 µg	R
Chloramphenicol 30 µg	S
Nystatin 100 µg	R

++, prolific growth; +, moderate growth; –, absence; S, sensitive, R, resistance

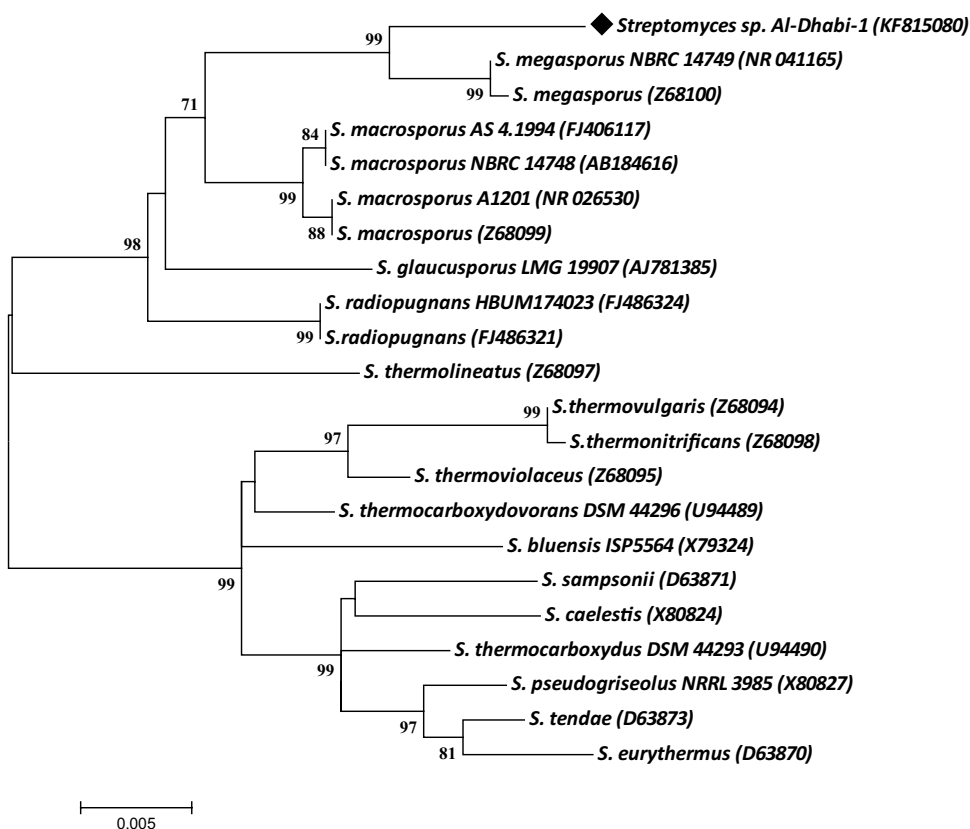
In vitro antimicrobial assay

In primary screening, the antimicrobial activity of *Streptomyces* sp. Al-Dhabi-1 was determined by the cross-streak method on Modified Nutrient Glucose Agar (MNGA)

Fig. 2 16S ribosomal RNA gene sequence of the *Streptomyces* sp. Al-Dhabi-1

AGTGGCGAACGGGTGAGTAACACGTTGGGCAATCTGCCCTGCACTTCGGGATAACTCCGGGAAACCGGAGC
 TAATACCGGATACGACCACCGGGGGCATTCTCGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGC
 GCCTATCAGCTGGTTGGTGGGGTGACGGCCACCAAGGCGACGACGGGTAGCCGGCTGAGAGGGCGACC
 GGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG
 CGCAAGCCTGATGCAGCGACCGCGGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGA
 AGAAGCGGAAGTGACGGTACCTGCAGAAGAAGCACCCGGCTAACCTACGTGCCAGCAGCCGCGGTAATACGT
 AGGGTGGAGCGTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGGCGCTGTCCGCTCGGATGTGAA
 AGCCCGGGGCTTAACCCCGGGTGGCATTTCGATACGGGCAGGCTGGAGTTTCGGCAGGGGAGATCGGAATT
 CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGAT
 ACTGACCGTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCCACGCCGTAAACG
 TTGGGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCCGTAGCTAACGCATTAAGTGCCCGCCCTGG
 GGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCGCACAAGCGGGGAGCATGTGGCT
 TAATTTCGACGCAACCGGAAGAACCCTACCAAGGCTTGACATACCGCCGACGGCTCCGGAAGACGGGTTT
 CCCTTTGGGCGGTGTACAGGTGGTGCATGGCTGTCTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCC
 CGCAACGAGCGCAACCCTGTTCCGTGTTGCCAGCAGCTCTCTCTTTGGGGGTGGTGGGGACTCACGG
 GAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCTTATGTCTTGGGC
 TGCACACGTGCTACAATGGCCGGTACAATGAGCTGCCAGGCCGTGAGGCCGAGCGAATCTCAAAAAGCCG
 GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCCGAGTCCGCTAGTAATCGCAGATCAGCA
 GTGCTGCGGTGAATACGTTCTCCGGCCTGTACACACCGCCCGTCACGTCACG

Fig. 3 Relationship between *Streptomyces* sp. Al-Dhabi-1 and the closest phylogenetic relative (21) species in the single best tree inferred from neighbor-joining analysis based on partial 16S rRNA sequences (1314 nt). Numbers on the nodes indicate bootstrap values. The number between parentheses is the GenBank accession number



medium. The strain exhibited the significant activity against ATTC cultures (Table 3). Results of media optimization revealed that MNG medium was found to be the most suitable medium for antimicrobial compounds production. About 1 g was obtained as crude extract using ethyl acetate.

The ethyl acetate extract of *Streptomyces* sp. Al-Dhabi-1 was tested against pathogenic microbes using the well diffusion method. Results revealed that good antimicrobial

activity was observed in the ethyl acetate extract of strain Al-Dhabi-1 (Fig. 4). The extract inhibited the growth of ATTC bacteria and fungi at a concentration of 5 mg/well for *B. cereus* (15 mm), *E. coli* (11 mm), *E. Faecalis* (16 mm), *K. Pneumoniae* (9 mm), *P. vulgaris* (9 mm), *S. epidermidis* (15 mm), *S. typhimurium* (14 mm), *S. aureus* (14 mm), *P. aeruginosa* (14 mm), *S. agalactiae* (18 mm), *C. neoformans* (15 mm), *C. albicans* (14 mm), *A. niger* (24 mm), and *T. mentagrophytes* (17 mm) (Table 4).

Table 3 Preliminary screening of *Streptomyces* sp. Al-Dhabi-1 (streak method)

Sl. no.	Tested microbes	Inhibition	
		Strain number	Strain Al-Dhabi-1
1	<i>B. cereus</i>	ATCC 11778	++
2	<i>E. coli</i>	ATCC 10536	++
3	<i>E. faecalis</i>	ATCC 49532	+++
4	<i>K. pneumoniae</i>	ATCC 13882	++
5	<i>P. vulgaris</i>	ATCC 33420	++
6	<i>S. epidermidis</i>	ATCC 12228	++
7	<i>S. typhimurium</i>	ATCC 13311	+
8	<i>S. aureus</i>	ATCC 6538P	+
9	<i>P. aeruginosa</i>	ATCC 27853	++
10	<i>S. agalactiae</i>	ATCC 27956	++
11	<i>C. neoformans</i>	Clinical	++
12	<i>C. albicans</i>	ATCC 2091	++

+, moderate activity; ++, good activity; +++, significant activity

Minimum inhibitory concentration (MIC) was carried out to determine the active concentration of *Streptomyces* sp. Al-Dhabi-1 ethyl acetate extract that inhibited the growth of tested microorganism. MIC of the strain *Streptomyces* sp. Al-Dhabi-1 extract against pathogenic microbes was as follows; *B. cereus* (0.312 mg/ml), *E. coli* (0.625 mg/ml), *E. faecalis* (0.625 mg/ml), *K. pneumoniae* (1.56 mg/ml), *P. vulgaris* (0.312 mg/ml), *S. epidermidis* (0.312 mg/ml), *S. typhimurium* (0.312 mg/ml), *S. aureus* (0.312 mg/ml), *P. aeruginosa* (0.312 mg/ml) and *S. agalactiae* (0.078), *C. neoformans* (0.078 mg/ml), *C. albicans* (0.156 mg/ml), *A. niger* (0.625 mg/ml), and *T. mentagrophytes* (0.156 mg/ml) (Table 5). Significant MIC values of the strain *Streptomyces* sp. Al-Dhabi-1 extract were observed against *S. agalactiae* (<0.039 mg/ml), *K. pneumoniae* (1.25 mg/ml) and *C. neoformans* (0.078 mg/ml).

GC–MS analysis of ethyl acetate extract

The active ethyl acetate extract of *Streptomyces* sp. Al-Dhabi-1 was studied using GC–MS chromatograph (Fig. 5). The GC–MS spectrum revealed the presence of many compounds (Table 6). The major compounds were: butanoic acid, 3-methyl ester (1.72 %), butanoic acid, 2-methyl ester (1.71 %), isopropyl alcohol (4.56 %), phenylethyl alcohol (1.11 %), acetic acid, 2-phenylethyl ester (10.35 %), benzeneacetic acid (16.02 %), benzoic acid 2-amino-methyl ester (5.19 %), 6-amino-1,3,5-triazine-2,4(1H,3H)-dione (1.08 %), pyrrolo[1,2-a]pyrazine-1,4-dione (2.34), L-leucine, N-cyclopropylcarbonyl (3.92 %), pyrrolo[1,2-a]pyrazine-1,4-dione (5.74 %), sebacic acid 2,6-dimethoxyphenyl (2.40 %), 2,5-piperazinedione, 3-methyl-6-[phenylmethyl (1.00 %), 2,5-piperazinedione,

3,6-bis(2-methylpropyl) (3.73 %), pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-[phenylmethyl (2.07 %), pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl) (1.98 %), and cyclohexanecarboxylic acid, 4-heptyl-, 4-fluorophenyl ester (1.48 %).

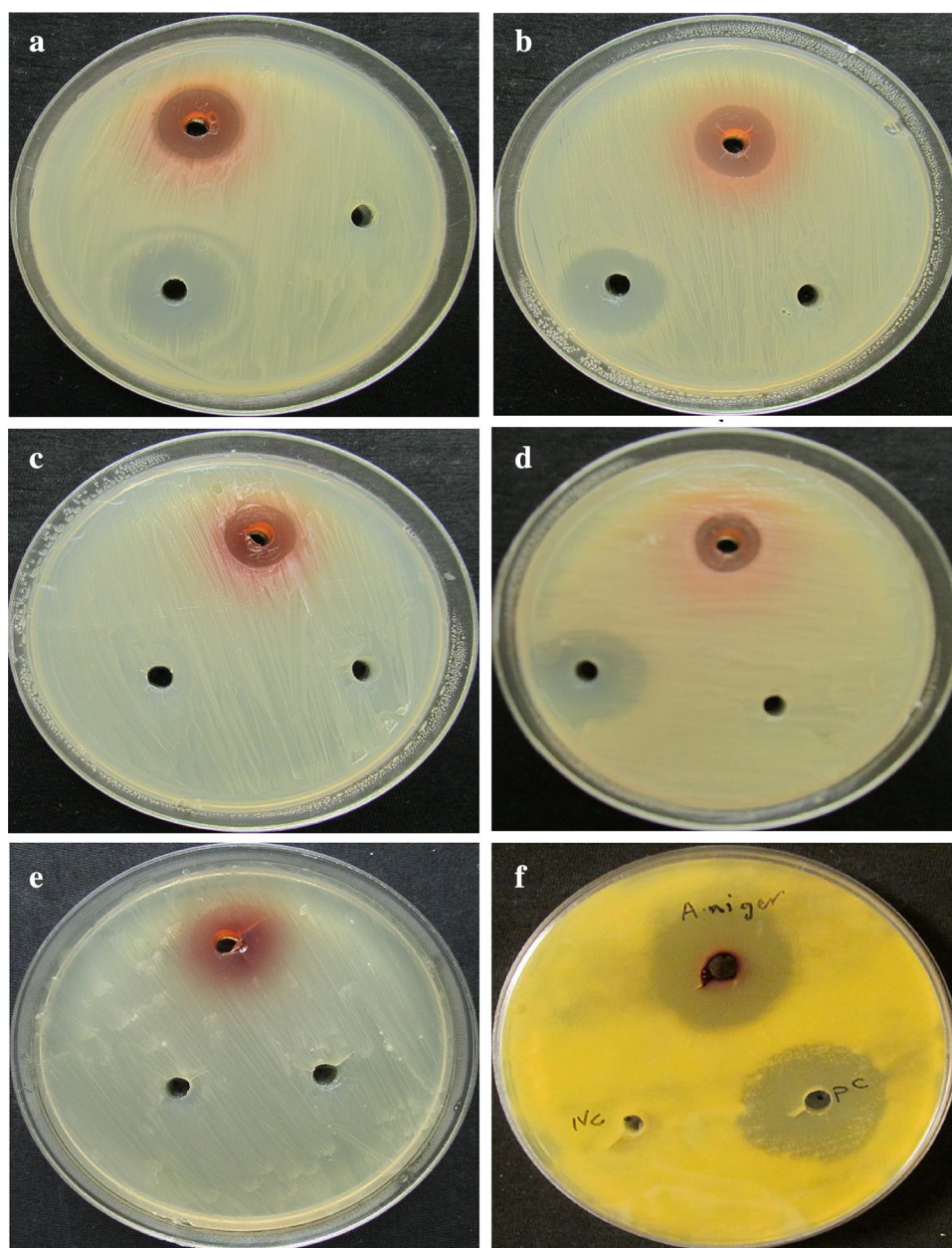
Discussion

Actinomycetes are widespread in natural environments and mesophilic species have been and continue to be extensively screened for their potential for producing useful natural products. The isolated thermophilic *Streptomyces* sp. Al-Dhabi-1 showed good antimicrobial activity against tested microbes. Previously many researchers have reported that *Streptomyces* produced antimicrobial metabolites (Boudemagh et al. 2005; Fguira et al. 2005; Saravana Kumar et al. 2014; Govindarajan et al. 2014).

The isolate was confirmed as *Streptomyces* sp. by morphology, physiology and molecular characteristics. The *Streptomyces* sp. Al-Dhabi-1 was identified by the use of 16S rRNA gene sequence analysis for the molecular identification. The results showed high similarity of 98 % (*E* value 0.0) with 16S rRNA gene of *S. megasporae*. Contrarily to the traditional methods of actinomycetes identification, which are slow, difficult and very expensive, the molecular approach for identification is often used for their speed and efficiency (Kim et al. 1999; Labeda and Kropfenstedt 2000). The ethyl acetate extract of *Streptomyces* sp. Al-Dhabi-1 inhibited the growth of bacteria and fungi. Maximum zone of inhibition was observed against *S. epidermidis* (15 mm), *S. typhimurium* (14 mm), *S. aureus* (14 mm), *P. aeruginosa* (14 mm), *S. agalactiae* (18 mm), *C. neoformans* (15 mm), *C. albicans* (14 mm), *A. niger* (24 mm), and *T. mentagrophytes* (17 mm). Abussaud et al. (2013) investigated the antimicrobial activity in 8 thermophilic *Streptomyces* strains isolated from hot springs; the strains inhibited the growth of *E. coli*, *S. aureus* and *C. albicans*. Sahin (2003) reported that a total of 74 different *Streptomyces* isolates were recovered from 46 soil samples. From these isolates, 45.9 % isolates exhibited antibacterial activity and 29.4 % of the *Streptomyces* isolates inhibited the growth of *S. epidermidis*.

The production of antimicrobial metabolites was optimized using different media. The isolate Al-Dhabi-1 grew well in MNGA medium and produced metabolites. The media optimization study has been reported in our earlier work (Al-Dhabi et al. 2014). Most *Streptomyces* and other Actinomycetes are used in the production of a diverse array of antibiotics including aminoglycosides, macrolides, β -lactams, peptides, polyenes, polyether, tetracyclines, etc. (Subramani and Aalbersberg 2012). Al-Bari et al. (2005) reported that thermophilic *Streptomyces bangladeshensis*

Fig. 4 Inhibition zone of the strain Al-Dhabi-1 extract against pathogenic bacteria and fungi. **a** *B. cereus*, **b** *E. faecalis*, **c** *S. epidermidis*, **d** *S. agalactiae*, **e** *C. neoformans*, and **f** *A. niger*



produced bis-(2-ethylhexyl) phthalate which exhibited antibacterial and antifungal activities against pathogenic microbes. *Streptomyces* sp. Al-Dhabi-1 produced an array of secondary metabolites; the major secondary metabolites were Acetic acid, 2-phenylethyl ester (10.35 %), benzoic acid (16.02 %), and benzoic acid 2-amino-methyl ester (5.19 %). Previous researcher has shown that benzoic acid showed antimicrobial properties (Tayade and Jadhao 2012).

The present study revealed that *Streptomyces* sp Al-Dhabi-1 isolated from hot spring of Saudi Arabia showed good antimicrobial activity against tested microbes in preliminary screening. Fandi et al. (2014) reported that

thermophilic isolates isolated from hot spring of Jordan. The isolates were showed antimicrobial activity against pathogenic bacteria. Maataoui et al. (2014) have studied that antimicrobial *Streptomyces* strain isolated from deteriorated wood. The isolate was identified by 16S rRNA technique. The ethyl acetate extract of *Streptomyces* sp-H2 inhibited the growth of bacteria *S. aureus*. Anusuya and Geetha (2012) reported that isolation of thermophilic actinomycetes from banana waste. The isolates isolated at 50–55 °C temperature. The isolate *Streptomyces* sp. Al-Dhabi-1 also isolated same condition of temperature of 52 °C.

Table 4 Antimicrobial activity of *Streptomyces* sp. Al-Dhabi-1 ethyl acetate extract using well diffusion method

Sl. no.	Tested microbes	Zone inhibition in mm		
		Bacteria	Strain number	Al-Dhabi-1 5 mg/well
1	<i>B. cereus</i>	ATCC 11778	15	22
2	<i>E. coli</i>	ATCC 10536	11	15
3	<i>E. faecalis</i>	ATCC 49532	16	20
4	<i>K. pneumoniae</i>	ATCC 13882	9	17
5	<i>P. vulgaris</i>	ATCC 33420	9	15
6	<i>S. epidermidis</i>	ATCC 12228	15	0
7	<i>S. typhimurium</i>	ATCC 13311	14	17
8	<i>S. aureus</i>	ATCC 6538P	14	12
9	<i>P. aeruginosa</i>	ATCC 27853	14	16
10	<i>S. agalactiae</i>	ATCC 27956	18	25

Sl. no.	Tested microbes	Zone inhibition in mm		
		Fungi	Strain number	Al-Dhabi-1 5 mg/well
11	<i>C. neoformans</i>	Clinical	15	–
12	<i>C. albicans</i>	ATCC 2091	14	22
13	<i>A. niger</i>	ATCC 16888	24	27
14	<i>T. mentagrophytes</i>	ATCC 9533	17	–

–, no activity; *Streptomyces* sp. Al-Dhabi-1, isolate; streptomycin, antibacterial agent; caspofungin, anti-fungal agent

Table 5 Minimum inhibitory concentrations of *Streptomyces* sp. Al-Dhabi-1 ethyl acetate extract against pathogenic microbes

Sl. no.	Tested microbes	MIC		
		Bacteria	Strain number	Mg/ml
1	<i>B. cereus</i>	ATCC 11778	0.312	0.156
2	<i>E. coli</i>	ATCC 10536	0.625	0.625
3	<i>E. faecalis</i>	ATCC 49532	0.625	1.25
4	<i>K. pneumoniae</i>	ATCC 13882	0.156	0.625
5	<i>P. vulgaris</i>	ATCC 33420	0.312	5
6	<i>S. epidermidis</i>	ATCC 12228	0.312	>10
7	<i>S. typhimurium</i>	ATCC 13311	0.312	5
8	<i>S. aureus</i>	ATCC 6538P	0.312	2.5
9	<i>P. aeruginosa</i>	ATCC 27853	0.312	1.25
10	<i>S. agalactiae</i>	ATCC 27956	<0.039	1.25

Sl. no.	Tested microbes	MIC		
		Fungi	Strain number	Mg/ml
11	<i>C. neoformans</i>	Clinical	0.078	250
12	<i>C. albicans</i>	ATCC 2091	0.156	<4
13	<i>A. niger</i>	ATCC 16888	0.625	62.5
14	<i>T. mentagrophytes</i>	ATCC 9533	0.312	>500

The ethyl acetate extract was taken from fermented broth of Al-Dhabi-1; it showed significant antimicrobial activity against bacteria and fungi at lowest concentration. The

chemical profile was analysed using ethyl acetate extract; it revealed the diversity of secondary metabolites. In future we may isolate the active molecules from *Streptomyces* sp.

Fig. 5 GC-MS analysis of *Streptomyces* sp. Al-Dhabi-1 ethyl acetate extract

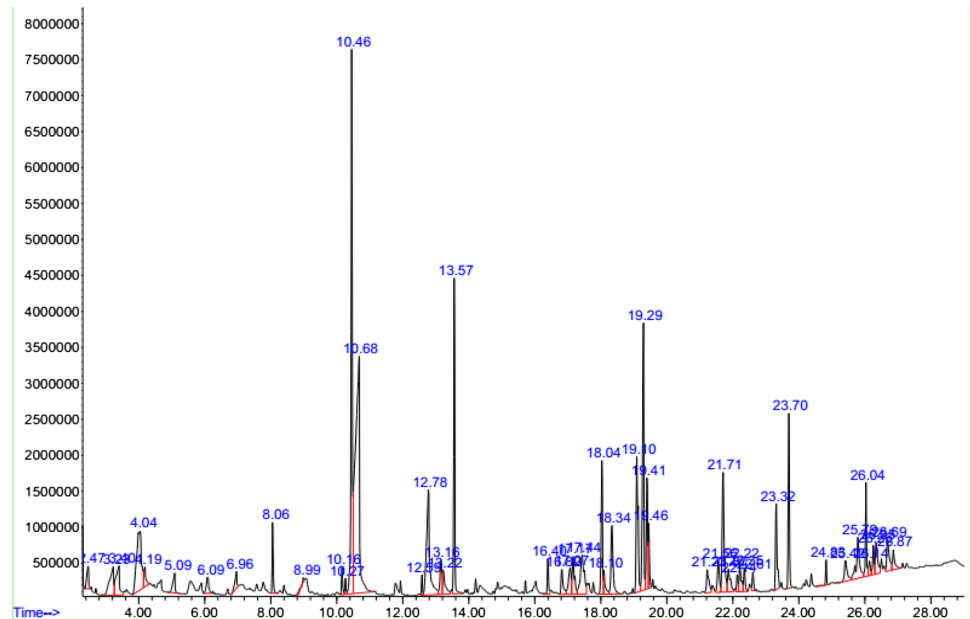


Table 6 GC-MS analysis of *Streptomyces* sp. strain Al-Dhabi-1 ethyl acetate extract

Sl. no.	Time	Name of the compound	Quality	Peak area %
1	2.476	Butanoic acid	90	0.66
2	3.231	Butanoic acid, 3-methyl ester	78	1.72
3	3.405	Butanoic acid, 2-methyl ester	78	1.71
4	4.044	Isopropyl alcohol	50	4.56
5	4.189	Formic acid, 1-methylpropyl ester	45	0.65
6	5.090	Methoxyacetic acid, heptyl ester	43	0.66
7	6.092	2-Methylthioacetic acid	53	0.62
8	6.963	3-Methylbutan-2-yl propyl carbonate	43	0.47
9	8.067	Phenylethyl alcohol	94	1.11
10	10.158	2-Coumaranone	96	0.48
11	10.274	Cyclohexanone	47	0.26
12	10.463	Acetic acid, 2-phenylethyl ester	83	10.35
13	10.681	Benzeneacetic acid	94	16.02
14	12.787	benzoic acid 2-amino- methyl ester	95	5.19
15	13.165	Benzenebutanoic acid	87	0.98
16	13.223	Benzenebutanal	30	1.05
17	13.571	Oxalic acid, di(2-phenylethyl) ester	80	5.25
18	16.403	2(1H)-Naphthalenone, octahydro-4	49	0.72
19	16.824	Piperidin-4-one, 1-tert-butyl-2	27	0.81
20	17.173	Tridemorph	50	1.37
21	17.434	Pyrrolo[1,2-a]pyrazine-1,4-dione	97	2.34
22	18.044	Phenol, 3,5-dimethoxy-	38	2.78
23	18.335	5,5-Dimethylbarbituric acid	41	2.34
24	19.090	L-Leucine, N-cyclopropylcarbonyl	50	3.92
25	19.293	Pyrrolo[1,2-a]pyrazine-1,4-dione	94	5.74
26	19.409	sebacic acid 2 6-dimethoxyphenyl	43	2.40
27	21.704	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)	49	3.73
28	23.316	Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3	99	2.07
29	25.800	Pyrimidine-2(1H)-thione, 4,4,6-trimethyl	45	1.98
30	26.032	Cyclohexanecarboxylic acid, 4-heptyl-, 4	52	1.48
31	26.700	L-Proline, N-allyloxycarbonyl-	38	1.23

Al-Dhabi-1 ethyl acetate extract to be used as drugs for the control of microbes causing infectious diseases.

Acknowledgments This work was supported by the National Plan for Science, Technology and Innovation, (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number (11-BIO1873-02).

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