



Full Length Research Paper

Antimicrobial activity of soil actinomycetes Isolated from Alkharj, KSA

^{1,2*}Muharram, M M; ^{1,3}Abdelkader, M S; ⁴Alqasoumi, S I

¹Department of Pharmacognosy, College of Pharmacy, Salman Bin Abdulaziz University, 11942 Alkharj, KSA

²Department of Microbiology, College of Science, Al-Azhar University, Nasr city, 11884, Cairo, Egypt

³Department of Pharmacognosy, College of Pharmacy, Alexandria University, Alexandria 21215, Egypt

⁴Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, KSA

Abstract

Thirty three soil-isolates of *actinomycetes* were screened for their antimicrobial activities. Three potent isolates, (SA110, SC110 and SF202), showed antibacterial and antifungal activity against one or more strain of *Escherichia coli*, ATCC 8739; *Candida albicans* ATCC 66027; *Candida albicans* ATCC 2091, *Aspergillus niger*, ATCC 16404 *Staphylococcus aureus*, ATCC 29213; *Bacillus subtilis*, ATCC 11774 and *Streptococcus epidermidis* ATCC 12228. Screening of the antimicrobial activity was conducted by Cross-streak, Cork-borer and agar well methods. The isolates of SA110, SC110 and SF202 were identified as *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces roche*, respectively based on a variety of morphological, physiological and molecular analysis of 16S rDNA gene sequence isolates. Factors affecting the biosynthesis of antimicrobial agent like different inoculum size, pH values, temperatures, incubation period, and different carbon and nitrogen sources were analyzed.

Keywords: *Actinomycetes*, *Streptomyces*, Antimicrobial, KSA.

INTRODUCTION

Actinomycetes occur in a multiplicity of natural and man-made environments (Goodfellow and Williams, 1983). They also exhibit diverse physiological and metabolic properties. Approaches to the search for and discovery of new antibiotics are generally based on screening of naturally occurring actinomycetes (Okami and Hotta, 1988). Vast numbers of these antimicrobial agents are discovered from actinomycetes by screening natural habitat such as soils and water bodies (Duraipandiyar et al., 2010; Gallagher et al., 2010; Zotchev, 2011). A wide taxonomic range of actinomycetes have the ability to produce secondary metabolites with biological activities such as antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant and other industrially useful compounds (Baltz, 2007; Olano et al., 2009; Demain and Sanchez, 2009; Kekuda et al., 2010; Naine et al., 2011; Newman and Cragg, 2007)

Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered. Around 7,600 compounds are produced by *Streptomyces* species. Many of these secondary metabolites are potent antibiotics, vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors which has made streptomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Bonjar, 2004; Berdy, 2005).

Among the different types of drugs prevailing in the market, antifungal antibiotics are a very small but significant group of drugs and have an important role in the control of mycotic diseases. Fungal infections have been gaining prime importance because of the morbidity of hospitalized patients (Beck-Sague and Jarvis, 1993). In particular, candidiasis and aspergillosis have remained the opportunist fungal infections that occur most frequently. Presently, they represent a major area of concern in the medical field; however, the occurrences of

*Corresponding Author E-mail: magdimoahmed72@yahoo.com

invasive fungal diseases, particularly in AIDS and other immune compromised patients, are life-threatening and they increase economic burden (Beck-Sague and Jarvis, 1993; Talbot *et al.*, 2006). The need for new, safe and more effective antifungal compounds are a major challenge to the pharmaceutical industry.

Presently, there is little documented information on the biodiversity of actinomycetes and their bioactivity for potential production of antimicrobial compounds in KSA. Actinomycetes in KSA are as yet poorly studied. Therefore the aim of the present work was to screen and identify actinomycetes in Alkharj governorate (KSA), as a virgin research area, and to investigate their antifungal activity.

MATERIALS AND METHODS

Actinomycete Isolates used in this study

Actinomycete isolates of this study were isolated from different soil sample at six different locations in kingdom of Saudi Arabia. The soil sample was collected from a depth of 5cm in sterile pouch, dried, serially diluted and plated on Starch casein agar. The inoculated plates were incubated aerobically at 30°C for a week. The isolate was subcultured on fresh plates of starch casein agar.

I-Screening for Antimicrobial Activity of the Isolates

Preliminary screening for inhibitory metabolite producing ability of the isolate was tested by Cross streak, Cork-borer and Agar well methods. Study of the antimicrobial activity in the three used protocols was conducted against all of *Staphylococcus aureus*, ATCC 29213; *Bacillus subtilis*, ATCC 11774; *Streptococcus epidermidis* ATCC 12228; *Escherichia coli*, ATCC 8739; *Candida albicans* ATCC 66027; *Candida albicans* ATCC 2091 and *Aspergillus niger*, ATCC 16404

Cross-streak method

The isolates of were inoculated as a single streak in the centre of the petridish containing media and incubated at 30°C for 3-4 days to permit growth and antibiotic production. Later the test bacteria and fungi were inoculated by streaking perpendicular to the growth of isolate. The plates were incubated for 24-48 hours at 37°C in case of bacteria, 72 hours at room temperature in case of fungi. After incubation, inhibition of test bacteria and fungi around the growth of isolate was taken as positive for inhibitory activity.

Cork-borer method

Cultures of bacteria or yeasts were inoculated in the form

of a loopful of each test organism into about 20 ml of nutrient agar medium for bacteria and sabaroud agar medium for yeast at 45°C, tilted and poured into sterile plates and left to solidify. In case of fungi, Dox agar medium was used (spore suspension technique). Plugs were cut out of actinomycete cultures with a Cork-borer and placed onto the surface of the agarized medium seeded with test organisms. The plates were kept in a refrigerator for two hours to permit homogenous diffusion of the antimicrobial agent before growth of the test organism, and then plates were incubated at 37 °C for 24 hours in case of bacteria, 30 °C for 24 hours in case of yeast and 25 °C for 72 hours in case of fungi.

Agar well method

At the end of incubation period the antimicrobial activities in the filtrates were assessed using the agar well method. One-tenth ml of the clear filtrates was transferred into each hole in the test plate. The petri dishes were kept in refrigerator for diffusion just before incubation at 37°C for 24 hours for bacteria and at 30 °C for 24-48 hours for yeast and 72 hours at 25 °C for fungi.

II-Identification of the Most Potent Actinomycete Isolates

A-Morphological Studies

For this purpose different media were used. These media were Starch-nitrate agar medium; Glycerol-asparagine agar medium; Inorganic salt-starch agar medium; Yeast extract-malt extract agar medium and Oatmeal agar medium. The medium was dispensed into sterilized plates and after solidification the plates were inoculated with the isolates under study. Incubation was carried out at 28°C for 7 days and the morphological examination was carried out under the bright field of a phase contrast. For studying the morphological characteristics of the actinomycetes, the cover slip technique was used. Cover slip cultures were fixed using few drops of absolute methanol for 15 minutes, and then washed with tap water. The cover slips were stained using 0.15 crystal violet for one minute, then washed by tap water, dried and examined by oil emersion lens.

B-Analysis of Cell Hydrolysate

The organisms under investigation were grown in a starch-nitrate liquid medium at 30°C for a period of 5-10 days; cells were collected by filtration, washed with water and then dried in the open air at room temperature.

i- Detection of Diaminopimelic Acid (DAP)

This procedure was carried out according to Becker *et*

al., (1964) and Lechevalier and Lechevalier, (1968). For the detection of diaminopimelic acid (DAP), 10 mg of the dried cells of an actinomycete isolate under study were hydrolysed for 18 hours with 1 ml of 6 N HCl in a sealed pyrex tube held at 100°C in sand bath. After cooling, the tubes were opened and its contents filtered through Whatman No. 1 filter paper, the sediment was washed with 3 drops of distilled water and the filtrate was dried 3 consecutive times on a water bath to remove the HCl, the residue was taken into 0.3 ml distilled water and 20 microlitres of the liquid were loaded on Whatman No. 1 filter paper. A spot of 10 Microlitres of 0.01 M of a mixture of meso- and L-diaminopimelic acid was loaded on the paper to run alongside the sample to serve as a reference standard. Descending chromatography was carried out over-night using the solvent system, (Methanol - water - 10NHCl - pyridine) in the ratio of 80:175:2.5:10 (v/v). Amino acids were detected by spraying the air dried chromatograms with ethanolic ninhydrin (0.2%,w/v) followed by heating for 2 minutes at 100°C. Diaminopimelic acid spots give olive green fading to yellow colour, other amino acid in the hydrolysate gave purple spots with this reagent and moved DAP. L-DAP moved faster than *meso*-DAP.

ii- Sugar pattern analysis

It was carried out according to Becker *et al* (1964) and Lechevalier and Lechevalier (1968). Fifty mg of air dried cells were weighed into a pre constricted pyrex tube and 1 ml of 1 N H₂SO₄ was added. The tube was placed in a steam cone for 2 hours. Contents were rinsed into a polyethylene centrifuge tube and adjusted at 5-5.5 with saturated aqueous barium hydroxide. The white precipitate formed was then centrifuged at 3000 rpm for five minutes, and the supernatant was filtered into a clean 100 ml beaker. The beaker was placed in a vacuum oven to dry for 2-3 days. The residue was taken up in 0.4 ml of distilled water and 20 microlitres were loaded on a sheet of Whatman No. 1 paper. Then Microlitres of a sugar standard containing 5 mg/ml of the following sugars: galactose, arabinose, xylose mannose, rhamnose, and ribose. In 10% aqueous isopropanol (v/v in distilled water) was also spotted against the residue spot. Descending chromatography was carried out overnight using the solvent system (n-butanol-pyridine-water-toluene) in the ratio of 5:3:3:4 (v/v). Sugars were detected by spraying with aniline-phthalate (1 volume), Diphenylamine 1% in acetone (100 volume) and phosphoric acid 85% (10 volume) followed by heating for 2 minutes at 100°C.

C-Physiological and Biochemical Characteristics

Lecithinase was detected using egg-yolk medium

according to the method of (Nitsh and Kutzner, 1969); Lipase (Elwan, *et al.*, 1977); Protease (Chapman, 1952); Pectinase (Hankin *et al.*, 1971); α -amylase (Ammar, *et al.*, 1998) and Catalase Test (Jones, 1949). Melanin pigment (Pridham, *et al.*, 1957). Esculin broth and xanthine have been conducted according to (Gordon *et al.*, 1974). Nitrate reduction was performed according to the method of (Gordon, 1966). Hydrogen sulphide production was carried out according to (Cowan, 1974). The utilization of different carbon and nitrogen sources was carried out according to (Pridham and Gottlieb, 1948).

D-Color characteristics

The ISCC-NBS color –Name Charts illustrated with centroid detection of the aerial, substrate mycelia and soluble pigments (Kenneth and Deane, 1955) was used.

E-Molecular analyses of the most potent of the Actinomycetes isolates

i- DNA Extraction (Sambrook *et al.*, 1989).

Actinomycete strains were grown in 10 ml International Streptomyces Project Medium 1 (ISP 1)[18] with agitation at 30°C for 18–24 h and examined by Gram stain. Cells (4 ml) were harvested by centrifugation (7500 g for 2 min), washed once with 500 ml of 10 mM Tris-HCl/1 mM EDTA (TE) buffer (pH 7.7) and resuspended in 500 ml TE buffer (pH 7.7). The samples were heated in boiling water for 10 min, allowed to cool for 5 min and centrifuged (7500 g for 3 min). The supernatant (300 ml) was transferred to a clean tube and stored at 4°C. If melanin or other pigments were produced during growth in ISP-1, cultures were grown in Middlebrook 7H9 broth, as these pigments interfered with the PCR.

ii- PCR Amplification

PCR was carried out in 50 μ l volumes containing 2 mM MgCl₂, 2U *Taq* polymerase, 150 mM of each dNTP, 0.5 μ M of each primer and 2 μ l template DNA. Primers used in this study were F1 (5'-AGAGTTTGATCITGGCTCAG-3'; I=inosine), R1 (5'-ACGGITACCTTGTTACGACTT-3') for the amplification of the 16S rDNA gene, and AZF1 (5'-AGCAACCAACGATGGTGTGTCCAT-3) and AZF2 (5'-CAACTTGTGCAACCGCATACCCT-3) for the amplification of the heat shock protein gene (*HSP*-65). The PCR programme used was an initial denaturation (96°C for 2 min), 30 cycles of denaturation (96°C for 45 s), annealing (56°C for 30 s) and extension (72°C for 2 min), and a final extension (72°C for 5 min). The PCR products were electrophoresed on 1% agarose gels,

containing ethidium bromide (10 µg ml), to ensure that a fragment of the correct size had been amplified.

RESULTS

Screening for the antimicrobial activities

Screening for antimicrobial activity of the actinomycete isolates SF202, SA110, and SC1012 isolate exhibited antibacterial activities against gram positive and gram negative species and antifungal activity against unicellular and filamentous fungi. Screening for these activities of, by Cork-borer method and agar well methods, was recorded by appearance of inhibition zone around the growth of actinomycetes isolates and was taken as '+'. Result of for these activities is depicted in Table 1.

Identification of the actinomycete isolates

Identification steps were started by examining the morphological characteristics of the three cultures like their growth on different ISP media, Spore chain, spore surfaces, color of their mycelia and diffusible pigments.

Analysis of their cell wall hydrolysate exhibited meso-diaminopimelic acid galactose as sugar pattern in two isolates. The three isolates were able to hydrolyze protein; Starch, Egg-yolk (lecithin) and cellulose but they exhibited negative result for catalase enzyme. The isolate (SA110) could not hydrolyze both of Pectin and Lipid. The three strains were moderately able to grow in the presence of Cephaoridine and Rifampicin and poorly in the presence of streptomycin but all of them were sensitive to Vancomycin (50) and Gentamycin (100). Melanin pigment was detected only in case of the isolate SC1012 with all three tested media as given in table 2.

All of three strains were able to degrade xanthin. However, only the isolate SA110 failed to degrade aesculin, to reduce nitrate, to produce H₂S gas. Milk could be coagulated by the three strains. The three isolates were examined for utilization of different carbon and nitrogen sources, growth on different degrees and temperature, different values of pH as given in table 3.

Taxonomy of actinomycete isolates

It was performed according to the recommended international Keys (Buchanan and Gibsons, 1974; Williams, 1989; and Hensyl, 1994). Based on collected data of the three isolates, it could be stated that the isolates (SA110, SC110 and SF202) belonging to *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces rochei*. This result was supported by

sequence of the 16S rDNA gene for the three isolates. Sequence analysis was compared to the sequences of *Streptomyces* spp. In order to determine the relatedness of the three isolates to these *Streptomyces* strains a phylogenetic tree was depicted (data not shown). This tree revealed that the three isolates of SA110, SC110 and SF202 are closely related to *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces rochei* by similarity percentage of 98, 94 and 95, respectively.

Parameters controlling the biosynthesis of the antimicrobial agent

For the actinomycete isolates SA110 and SC1012 addition of different equimolecular carbon sources for production of antimicrobial agent revealed that starch is the best carbon source for biosynthesis antimicrobial agent with concentration 2.0 g/100 while glucose was preferred by the isolate SF202 at the same concentration. However, the addition of different nitrogen sources exhibited an increase production level of the antimicrobial agent where sodium nitrate was found to be the best nitrogen source for the three isolates at a concentration of 0.25 g/100 ml.

In case of actinomycetes isolate SF202, Maximum Inhibition zones of 32mm was recorded with four discs in case of *Candida albicans* ATCC 66027; *Candida albicans* ATCC 2091 and *Aspergillus niger*, ATCC 16404 as given in figure 1. Inhibition zones of the same inoculum varied from 27mm to 25mm in case of isolates SC1012 and SA110 when tested against *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 66027, respectively. Data illustrated in figure 2 showed the relation between antibiotic productivity and time of incubation. Maximum inhibition zone values of 32.0 and 26.0 were recorded by the end of the fifth day of incubation for isolates SF202 and SA110, respectively. However, an inhibition zone of 28 was recorded for the actinomycete isolate SC1012 by the end of the sixth day of incubation.

The results represented in figure 3 illustrated that the optimum initial pH value capable of promoting antimicrobial biosynthesis by the actinomycete isolate SF202 was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antimicrobial agent productivity reached up to 32.0. Optimum antimicrobial biosynthesis by the actinomycete isolate SA110 and SC1012 was achieved at pH value of 8.0 where the diameter of inhibition zone was at 25.0 and 27.0 for both isolates, respectively. The optimum temperature capable of promoting antimicrobial agent biosynthesis by the three actinomycetes isolates was at 30°C which resulted in an inhibition zone of 25.0, 27.0 and 32.0 for SA110, SC1012 and SF202, respectively.

Table 1. Screening for the antimicrobial activity of the actinomycetes isolates

Test organism	Actinomycete isolates		
	SA110	SC1012	SF202
<i>Staphylococcus aureus</i> , ATCC 29213	+	++	++
<i>Bacillus subtilis</i> , ATCC 11774	++	+	+
<i>Streptococcus epidermidis</i> ATCC 12228	++	++	+
<i>Escherichia coli</i> , ATCC 8739	+++	++	+
<i>Candida albicans</i> ATCC 66027	+++	+++	+++
<i>Candida albicans</i> ATCC 2091	++	++	+++
<i>Aspergillus niger</i> , ATCC 16404	+++	+++	+++

Table 2. Morphological and biochemical characteristics of actinomycete isolates.

Characteristic	SA110	SC1012	SF202
	Morphological characteristic		
Aerial hyphae	Straight/Rectiflexibiles	Rectiflexibiles	Spiral /Retinaculiaperti
Spore mass	White	grey	grey
Spore surface	Smooth	smooth	Smooth
Color of substrate ycelium	Red/Orange	Red/Orange	grey
Diffusible pigment	-	Red/Orange	-
Motility	-ve		-ve
Cell wall hydrolysate:			
Diaminopimelic acid (DAP)	<i>meso</i> -DAP	<i>meso</i> -DAP	<i>meso</i> -DAP
Sugar Pattern	Galactose	--	Galactose
Protein; Starch , Egg-yolk (lecithin) and cellulose	+	+	+
Pectin and Lipid	-	+	+
Catalase test	-	±	-
Resistance of different antibiotics			
Gentamycin (100)	-	-	-
Neomycin (50)	-	-	+
Streptomycin (100)	±	±	±
Tobramycin (50)	-	-	±
Rifampicin (50)	+	+	+
Cephaoridine (100)e	+	+	+
Vancomycine (50)	-	-	-
Production of melanin pigment on:			
Peptone yeast- extract iron agar (ISP-6)	-	+	-
Tyrosine agar medium(ISP-7)	-	+	-
Tryptone – yeast extract broth (ISP-1)	-	±	-

+=Positive , - = Negative and ± = doubtful result.

DISCUSSION

Actinomycetes play an important role in the production antimicrobial agents. In this study 33 actinomycete soil isolates were evaluated for their antimicrobial activity. Out of 33 actinomycete isolates, only three isolates, SA110,

SC1012 and SF202, exhibited a wide spectrum antimicrobial agent against Gram positive and Gram negative bacteria and unicellular and filamentous Fungi. Those three isolates were isolated from soil samples collected from Al-kharj governorate, KSA and allowed to grow on starch nitrate agar medium. All of *Staphylococcus*

Table 3. Physiological characteristics of actinomycete isolates.

	SA110	SC1012	SF202
Degradation of: Xanthin	+	+	+
Degradation of: Aesculin	-	+	+
H ₂ S Production	-	+	+
Nitrate reduction	±	+	+
Citrate utilization	-	+	+
Urea test	-	+	+
Coagulation of milk	+	+	±
Utilization of: different carbon sources			
D-Xylose	+	+	+
D- Mannose	+	+	+
D- Glucose	+	+	+
D- Galactose	+	+	+
Sucrose	±	+	+
Rhamnose	±	+	+
Raffinose	+	-	+
Mannitol	+	+	+
L- Arabinose	+	+	+
<i>meso</i> -Inositol	-	-	-
Lactose	+	+	+
Maltose	-	±	+
Trehalose	+	+	+
L-Melzitose	+	-	+
D-fructose	-	+	+
Sodium citrate	+	+	+
Utilization of different amino acids			
L-Cycteine			
L-Valine	-	+	-
L-Histidine	-	+	+
L-Phenylalanine	-	+	+
L-Arginine	+	+	+
L-Lysine & L-Hydroxproline	-	-	-
L-Glutamic acid	±	+	+
Growth inhibitors:			
Thallos acetate (0.001)	-	-	-
Sodium azide (0.01)	-	+	+
Phenol (0.1)	-	+	+
Growth at different temperatures (°C):			
10	±	+	-
20	+	+	+
30- 45	+	+	+
50	-	-	±
Growth at different pH values:			
4	-	±	±
5-9	+	+	±
10	-		
Growth at different concentrations of NaCl (%)			
4	+	+	+
7	+	+	+
12	-	-	-

+=Positive , - = Negative and ± = doubtful result.

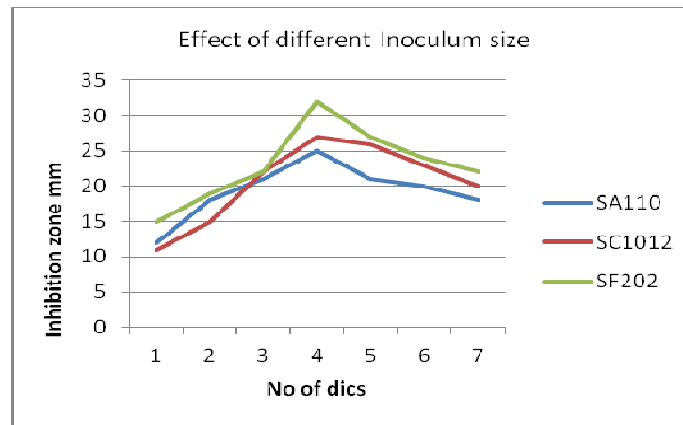


Figure 1. Effect of different inoculum size on the antimicrobial agent(s) biosynthesis produced by *Streptomyces spp*, SA110, SC1012 and SF202

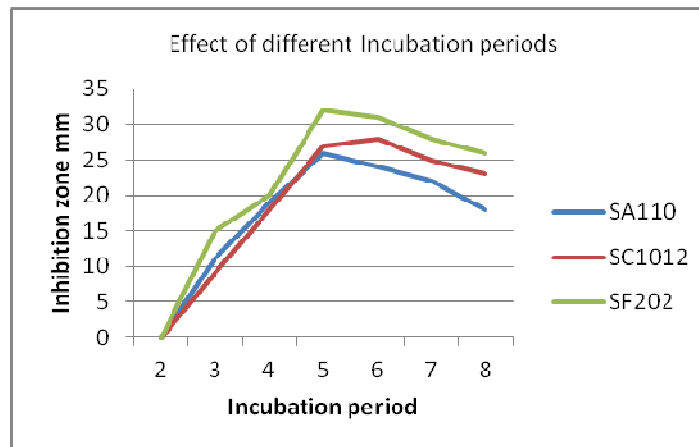


Figure 2. Effect of different inoculum size on the antimicrobial agent(s) biosynthesis produced by *Streptomyces spp*, SA110, SC1012 and SF202

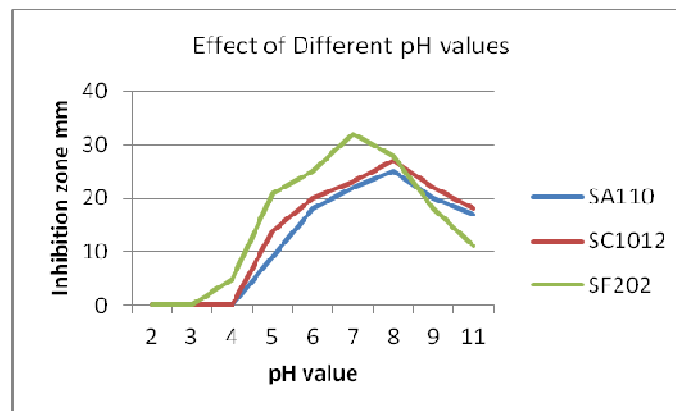


Figure 3. Effect of different pH values on the antimicrobial agent(s) biosynthesis produced by *Streptomyces spp*, SA110, SC1012 and SF202

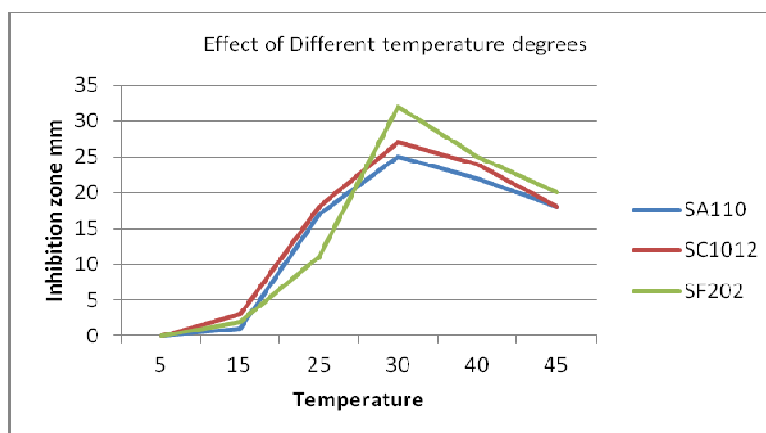


Figure 4. Effect of different incubation temperature on the antimicrobial agent(s) biosynthesis produced by *Streptomyces* spp, SA110, SC1012 and SF202

aureus, ATCC 29213; *Bacillus subtilis*, ATCC 11774; *Streptococcus epidermidis* ATCC 12228; *Escherichia coli*, ATCC 8739; *Candida albicans* ATCC 66027; *Candida albicans* ATCC 2091 and *Aspergillus niger*, ATCC 16404 were recorded to be the most sensitive strains affected by the three actinomycetes isolates. Identification process had been carried out according to the Key's given in Bergey's Manual of Determinative Bacteriology 8th edition (Buchanan and Gibbons, 1974), Bergey's Manual of Systematic Bacteriology, vol. 4 (Williams, 1989) and Bergey's Manual of Determinative Bacteriology, 9th edition (Hensyl, 1994).

For identification of actinomycete isolates, morphological characteristics and microscopic examination emphasized that the spore chain were straight, spiral and retinaculiaperti for SA110, SC1012 and SF202, respectively. The three isolates exhibited smooth surface for spores. Substrate mycelium was red-orange in case of SA110 and SC1012 and grey in case of the isolate SF202. Red to orange pigment was produced only in case of SC1012 ISP-media No. 1 to 5. The results of physiological, biochemical characteristics and cell wall hydrolysate of actinomycetes isolate, exhibited that the cell wall containing LL-diaminopimelic acid (DAP). These results emphasized that the actinomycetes isolate related to a group of *Streptomyces* (Williams, 1989 and Hensyl, 1994).

In view of all the previously recorded data of the three isolates, it could be stated that the isolates (SA110, SC1012 and SF202) belonging to *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces rochei*. This result was supported by sequence analysis of the 16S rDNA gene for the three isolates. Sequence analysis was compared to the sequences of *Streptomyces* spp. The resulted sequence was aligned with available almost complete sequence of type strains of family streptomycetaceae. These data

revealed that the three isolates of SA110, SC1012 and SF202 are closely related to *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces rochei* by similarity percentage of 98, 94 and 95, respectively (Williams et al., 1983; Augustine et al., 2005; Kavitha and Vijayalakshmi, 2007; Jain and Jain, 2007; Muharram et al., 2010; Thenmozhi and Kannabiran, 2010).

Different cultural conditions such as inoculum size, pH, temperature, and incubation period, effect of different carbon and nitrogen sources were studied for optimizing the biosynthesis of the antimicrobial agent from the three strains of *Streptomyces samposonii*, *Streptomyces albidoflavus* and *Streptomyces rochei*. *Streptomyces rochei* (SF202) *Streptomyces samposonii* (SA110) and *Streptomyces albidoflavus* (SC1012) preferred starch and sodium nitrate as best carbon and nitrogen sources for the produced antimicrobial agent. These similar results have been recorded by various workers: (Howells et al., 2002; El-Naggar et al., 2003; Criswell et al., 2006; Sekiguchi, et al., 2007). The maximum biosynthesis was achieved at the end of an incubation period of 5 days at pH value of 7.0 for the *Streptomyces rochei* (SF202) and 8.0 for *Streptomyces samposonii* (SA110) and *Streptomyces albidoflavus* (SC1012). Similar results had been recorded by various workers; (Jain and Jain, 2007; Joseph et al., 2009 and Prema et al., 2009). Maximum yield of the antimicrobial agent occurred at the end of an incubation temperature of 30°C was in complete accordance with those reported by (Selvin et al., 2004; El-Naggar et al., 2007 and Atta, 2010).

ACKNOWLEDGMENTS

This work was kindly supported by University of Salman bin Abdulaziz, KSA (Grant No. 2/S/1432).

REFERENCES

- Ammar MS, EL- Esawey M, Yassin M, Sherif YM (1998). Hydrolytic enzymes of fungi isolated from certain Egyptian Antiquities objects while utilizing the industrial wastes of Sugar and Integrated Industries Company (SIIC). *Egypt. J. Biotechnol.*, 3. (1998):60-90.
- Atta HM (2010). Production, Purification, Physico-Chemical Characteristics and Biological Activities of Antifungal Antibiotic Produced by *Streptomyces antibioticus*, AZ-Z710. *American-Eurasian Journal of Scientific Research*. 5 (1):39-49.
- Augustine SK, Bhavsar SP, Kapadnis BP (2005). A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23. *J Biosci*. 2005 Mar; 30 (2):201-11.
- Baltz RH (2007). Antimicrobials from Actinomycetes. Back to the future. *Microbe* 2: 125-131.
- Becker B, Lechevalier, MP, Gordon RE, Lechevalier HA (1964). Rapid Differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. *APPL. Microbiol.*, 12: 421 – 423.
- Beck-Sague CM, Jarvis WR (1993). Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980-1990. *J. Infect. Dis*. 167: 1247-1251.
- Bérdy J (2005). Bioactive microbial metabolites. *J Antibiot (Tokyo) Jan*; 58(1):1-26.
- Bonjar S, Fooladi MH, Mahdavi MJ, Shahghasi A (2004). Broadspectrim, a novel antibacterial from *Streptomyces* sp. *Biotechnol*. 3, 126–130.
- Chapman GS (1952). A simple method for making multiple tests on a microorganism. *J. Bacteriol*. 63:147.
- Cowan ST (1974). Cowan and Steel, s Manual for the Identification of Medical Bacteria 2 nd. Edition Cambridge, Univ. Press.
- Criswell D, Tobiason VL, Lodmell JS, Samuels DS (2006). Mutations Conferring Aminoglycoside and Spectinomycin Resistance in *Borrelia burgdorferi*. *Antimicrob. Agents Chemother*. 50:445-452.
- Demain AL, Sanchez S (2009). Microbial drug discovery: 80 years of progress. *J. Antibiot.*, 62:5-16.
- Duraipandiyar V, Sasi AH, Islam VIH, Valanarasu M, Ignacimuthu S (2010). Antimicrobial properties of actinomycetes from the soil of Himalaya. *J. Med. Mycol.*, 20: 15-20.
- El-Naggar, MY, Hassan MA Said WY, El-Aassar SA (2003). Effect of support materials on antibiotic MSW2000 production by immobilized *Streptomyces violatus*. *J. Gen. Appl. Microbiol*. 49:235-243.
- Elwan SH, El-Nagar MR, Ammar MS (1977). Characteristics of Lipase(s) in the growth filtrate dialystate of *Bacillus stearothermophilus* grown at 55 °C using a tributyrin- cup plate assay. *Bull. Of the Fac. of Sci ., Riyadh Univ .*, 8:105–119.
- Gallagher KA, Fenical W, Jensen PR (2010). Hybrid isoprenoid secondary metabolite production in terrestrial and marine actinomycetes. *Curr. Opin. Biotechnol.*, 21: 794-800.
- Goodfellow M, Williams ST (1983). Ecology of actinomycetes. *Annu Rev Microbiol*. 1983;37:189-216.
- Gordon RE, Barnett DA, Handehan JE, Pang CH (1974). *Nocardia coeliaca*, *Nocardia autotrophica* and *Nocardia* Strain. *International Journal of Systematic Bacteriology*. 24:54-63.
- Gordon RE (1966). Some Criteria for The Recognition of *Nocardia madura* (Vincent) Blanchord. *J. General Microbiology*, 45:355-364.
- Hankin L, Zucker M, Sands DC (1971). Improved solid medium for the detection and enumeration of proteolytic bacteria. *Appl. Microbiol.*, 22:205-509.
- Hensyl WR (1994). *Bergey's Manual of Systematic Bacteriology* 9 th Edition. John. G. Holt and Stanley, T. Williams (Eds.) Williams and Wilkins, Baltimore, Philadelphia, Hong kong, London, Munich, Sydney, Tokyo.
- Howells JD, Anderson LE, Coffey GL, Senos GD, Underhill MA, Vogler DL, Ehrlich JB (2002). A new Aminoglycosidic Antibiotic Complex: Bacterial Origin and Some Microbiological Studies. *Antimicrob Agents Chemother*. Aug; 2 (2):79–83.
- Jain PK, Jain PC (2007). Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS1322. *Indian journal of experimental biology*. 45:203-206.
- Jones K (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristics. *J. Bacteriol.*, 57: 141-145.
- Joseph S, Shanmughapriya S, Gandhimathi R, Seghal Kiran G, Rajeetha Ravji T, Natarajaseenivasan K, Hema TA (2009). Optimization and production of novel antimicrobial agents from sponge associated
- Kavitha A, Vijayalakshmi M (2007). Studies on Cultural, Physiological and Antimicrobial Activities of *Streptomyces rochei*. *J. Appl. Sci. Res.*, 12: 2026-2029.
- Kekuda TRP, Shobha KS, Onkarappa R (2010). Fascinating diversity and potent biological activities of actinomycetes metabolites. *J. Pharm. Res.*, 3:250-256.
- Kenneth LK, Deane BJ (1955). *Color universal language and dictionary of names*. United States Department of Commerce. National Bureau of standards. Washington, D.C., 20234.
- Lechevalier MP, Lechevalier HA (1968). Chemical composition as a criterion in the classification of aerobic actinomycetes. *J. Systematic Bacteriology*. 20: 435-443.
- Muharram M Atta H, Bayoumi R, 3Mounir MS (2010). Taxonomic Utility of PCR-Restriction Pattern Analysis for Rapid Identification of Clinical Isolates of aerobic Actinomycetes to the Genus Level. *Aust. J. Basic and Appl. Sci.*, 4(2): 151-159.
- Naine J, Srinivasan MV, Devi SC (2011). Novel anticancer compounds from marine actinomycetes: a review. *J. Pharm. Res*. 4:1285-1287.
- Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.*, 70: 461-477.
- Nitsh B, Kutzner HJ (1969). Egg-Yolk agar as diagnostic medium for *Streptomyces* sp., 25:113.
- Okami B, Hotta AT (1988). Search and discovery of new antibiotics. In *Actinomycetes in biotechnology*, Goodfellow M, ST, Williams and M Mordarski (Eds.) Pergamon Press, Oxford: 33-67.
- Olano C, Gómez C, Pérez M, Palomino M, Pineda-Lucena A, Carbajo RJ, Braña AF, Méndez C, Salas JA. (2009). Deciphering biosynthesis of the RNA polymerase inhibitor streptolydigin and generation of glycosylated derivatives. *Chem Biol*. 2009 Oct 30;16(10):1031-44.
- Prema P, Audline SP, Suja Ranjani SS, Immanuel G (2009). UV/VIS, FTIR spectrum and Anticandidal activity of *Streptomyces* strains. *The Internet J. Microbiol*. 7 (2).
- Pridham TG, Gottlieb D (1948). The utilization of carbon compounds by some actinomycetes as an aid for species determination. *J. Bacteriol.*, 56(1):107-114.
- Pridham TG, Anderson P, Foley C, Lindenfesler LA, Hesselting CW, Benedict RG (1957). A section of media for maintenance and taxonomic study of *Streptomyces*. *Antibiotics Ann*. pp. 947-953.
- Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular cloning. A laboratory Manual* 2ed Cold Spring, Harbor Laboratory press, Cold Spring Harbor, New York, USA.
- Seviguchi JI, Miyoshi-Akiyama T, Augustynowicz-Kopec E, Zwolska Z, Kirikae F, Toyota E, Kobayashi I, Morita K, Kudo K, Kato S, Kuratsuji T, Mori T, Kirikae T (2007). Detection of Multidrug Resistance in *Mycobacterium tuberculosis*. *J. Clin. Microbiol*. 45: 179-192
- Selvin JS, Joseph KR, Asha WA, Manjusha VS, Sangeetha DM, Jayaseema MC, Antony AJ, Denslin V (2004). Antibacterial potential of antagonistic *Streptomyces* sp. Isolated from marine sponge *Dendrilla nigra*. *FEMS Microbiology Ecology* 50, 117–122.
- Talbot GH, Bradley J, Edwards JEJ, Gilbert D, Scheld M, Bartlett JG (2006). Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis*. 1; 42(5):657-68.
- Thenmozhi M, Kannabiran K (2010). Studies on Isolation, Classification and Phylogenetic Characterization of Novel Antifungal *Streptomyces* sp. VITSTK7 in India. *Current Research J. Biol. Sci*. 2(5): 306-312, 2010.
- Williams ST (1989). *Bergey's Manual of Systematic bacteriology* Vol. 4, Stanley T., Williams.
- Williams and Wilkins (Eds.), Baltimore, Hong kong, London, Sydney.
- Williams ST, Goodfellow M, Alderson G, Wellington EM, Sneath PH, Sookin MJ (1983). Numerical classification of *Streptomyces* and related genera. *Gen. J. Microbiol.*, 129: 1743–813.
- Zotchev SB (2011). Marine actinomycetes as an emerging resource for the drug development pipelines. *J. Biotechnol.*, doi:10.1016/j.jbiotec.2011.1006.1002.