Taxonomic Studies and Phylogenetic Characterization of *Streptomyces rimosus* - KH-1223-55 Isolated from Al-Khurmah Governorate, KSA

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Abstract: This work was carried out in the course of a screening program for specifying the bioactive substances that demonstrated inhibitory affects against microbial pathogenic. Twenty-eight actinomycete strains were isolated from soil samples collected from Al-Khurmah governorate, KSA. One of the actinomycete culture, symbol KH-1223-55 from six cultures was found to produce a wide spectrum antimicrobial agent against (bacterial Gram positive and Bacteria Gram negative and unicellular and filamentous Fungi). The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 98% similarity with *Streptomyces rimosus*. From the taxonomic features, the actinomycetes isolate KH-1223-55 matched with *Streptomyces rimosus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces rimosus*. The parameters controlling the biosynthetic process of antimicrobial agent formation including: different inoculum size, pH values, temperatures, incubation period, and different carbon and nitrogen sources were fully investigates.
[**Houssam M**. **Atta**; Bayoumi R.; El-Sehrawi M. and Gehan F. Galal]. Taxonomic Studies and Phylogenetic Characterization of *Streptomyces rimosus* - KH-1223-55 Isolated from Al-Khurmah Governorate, KSA. [Researcher, 2011;3(9):27-40]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher.

Key words: *Streptomyces rimosus*; Taxonomy and Phylogenetic Characterization; Parameters of antimicrobial agent biosynthesis.

1. Introduction

An extensive literature review concerning the taxonomic status of the species of the genus *Streptomyces* has been made on the base of classical microbiological and chemo taxonomical methods (Christova *et al*., 1995) proved that the result of the analysis of 200 strains for 100 characters, grouped them to 25 variant groups. The analysis showed that many of the characteristics used for the classification of *Streptomyces* species are strongly variable and hard for interpretation (Ismail, 2006). A considerable step ahead is the numerical classification of (Williams *et al*., 1983), which used 475 strains among them 394 *Streptomyces* type cultures from ISP and other 14 *actinomycete* genera. (Ochi, 1992) proved the efficiency of protein analysis as a novel approach for taxonomy of 11 *streptomyces* strains by using numerical methods. (Bouchek-Mechiche *et al*., 1998) reported that phenotypic characteristics and numerical analysis clearly differentiated all the 31 *streptomyces* strains isolated from common and netted scabs in France. (Doumbou *et al*., 2001) applied numerical taxonomy to compare 16 non-pathogenic actinomycetes isolated from common scab lesion on potato tuber with *Streptomyces scabiei*, they reported that the use of phenotypic traits to differentiate pathogenic streptomycetes from non-pathogenic ones is difficult; in contrast none of the non-pathogenic isolates could be confused with *Streptomyces scabiei* in regard to 16S r-DNA sequence. (Trujillo and Goodfellow, 2003) used numerical taxonomic data to generate a frequency matrix designed to facilitate the identification of clinically significant *Actinomadura*, *Nocardiopsis* and *Streptomyces* stains to the species level.

The importance of soil sources for the discovery of novel natural products with a pharmaceutical potential has been proved during the last decade and was highlighted in various excellent review articles (Blunt *et al*., 2003). Bacteria within the order Actinomycetales (actinomycetes) are common soil inhabitants with an unprecedented ability to produce clinically useful antibiotics (William and Paul, 2006). Most of the microbial antibiotics discovered so far are originated from actinomycete bacteria, only a few of them from soilderived genera (*Streptomyces* and *Micromonospora*). Actinomycetes produce a wide range of secondary metabolites and more than 70% of the naturally derived antibiotics that are currently in clinical use are derived from soil actinomycetes (Pimentel-Elardo *et al*., 2009). Among the 140 described actinomycete genera, only a few are responsible for the majority of over 20,000 microbial natural products identified so far. In particular, the genus *Streptomyces* accounts for about 80% of the actinomycete natural

products reported to date (Bull and Stach, 2007).

In the present study were describe the isolation of an actinomycete strain Twenty-eight from Al-Khurmah governorate, KSA, which generates a production the bioactive substances that demonstrated inhibitory affects against microbial pathogenic. The identification of this strain based on the cultural, morphology, physiology and biochemical characteristics, as well as 16s rRNA methodology. The primary bioactive substances were tested against Gram positive and Gram negative bacteria and unicellular and filamentous fungi. The parameters controlling the biosynthetic process of antimicrobial agent biosynthesis were fully investigates.

2. Material and Methods

2.1. Actinomycete isolate

The actinomycete isolate KH-1223-55 was isolated from soil sample collected from Al-Khurmah governorate, KSA. It was purified using the soil dilution plate technique described by (Williams and Davis, 1965).

2.2. Test organisms

2.2.1. Gram Positive: *Staphylococcus aureus*, NCTC 7447; *Bacillus subtilis*, NCTC 1040; *Bacillus pumilus*, NCTC 8214; *Micrococcus luteus*, ATCC 9341.

2.2.2. Gram Negative: *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB 9111; *Pseudomonas aeruginosa*, ATCC 10145.

2.2.3. Unicellular fungi: *Saccharomyces cerevisiae*, ATCC 9763, *Candida albicans* IMRU 3669.

2.2.4. Filamentous fungi: *Aspergillus niger*, IMI 31276; *Fusarium oxysporum*, *Botrytis fabae*, *Rhizoctonia solani* and *P. chrysogenum*.

2.3. Screening for antimicrobial activity

The anti- microbial activity was determined according to (Kavanagh, 1972).

2.3. Characterization studies of actinomycete isolate

2.3.1. Morphological characteristics

Morphological characteristics of aerial hyphae, spore mass, spore surface, color of aerial and substrate mycelia and soluble pigments production were conducted by growing the organism on ISP- media.

2.3.2. 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).

Determination of Diaminopimelic acid (DAP) and sugar pattern was carried out according to (Becker *et al*., 1964 and Lechevalier and Lechevaier, 1968).

2.3.3. 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.

2.3.4. DNA isolation and manipulation

The locally isolated actinomycete strain was grown for 6 days on a starch agar slant at 30°C. Two ml of a spore suspension were inoculated into the starch- nitrate broth and incubated for 4 days on a shaker incubator at 200 rpm and 30°C to form a pellet of vegetative cells (pre-sporulation). The preparation of total genomic DNA was conducted as described by (Sambrook *et al*., 1989**).**

2.3.5. Amplification and sequencing of the 16S rRNA gene

PCR amplification of the 16S rRNA gene of the local actinomycete strain was conducted using two primers, StrepF; 5.-ACGTGTGCAGCCCAAGACA-3. and Strep R; 5.ACAAGCCCTGGAAACGGGGT-3., (Edwards *et al*., 1989**).** The PCR mixture consisted of 30 pmol of each primer, 100 ng of chromosomal DNA, 200 µM dNTPs, and 2.5 units of Taq polymerase, in 50 µl of polymerase buffer. Amplification was conducted for 30 cycles of 1 min at 94°C, 1 min of annealing at 53°C, and 2 min of extension at 72°C. The PCR reaction mixture was then analyzed via agarose gel electrophoresis, and the remaining mixture was purified using QIA quick PCR purification reagents (Qiagen, USA). The 16S rRNA gene was sequenced on both strands via the dideoxy chain termination method (Sanger *et al*., 1977).

2.3.6. Sequence similarities and phylogenetic analysis

The BLAST program (www.ncbi.nlm.nih.gov/blst) was employed in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software (Hall,

1999). The phylogenetic tree was displayed using the TREE VIEW program.

2.4. Parameters controlling of antimicrobial agent biosynthesis

These included inoculum size, incubation period, pH values, incubation temperatures; different carbon and nitrogen sources have been determine by the standard methods.

3. RESULTS

3.1. Screening for the antimicrobial activities

The metabolites of twenty-eight actinomycete isolates exhibited one of the actinomycete culture, symbol KH-1223-55 from six cultures was found to produce a wide spectrum antimicrobial agent against (bacterial Gram positive and Bacteria Gram negative and unicellular and filamentous Fungi) (Table 1).

3.2. Characterizations of the actinomycete isolate

3.2.1. Morphological characteristics

Spore chain was spiral, non motile, spore mass was yellow or white, spore surface was smooth; substrate mycelium was light yellowish brown and no diffusible pigment was occur on ISP-media 1 to 5 (plate 1).

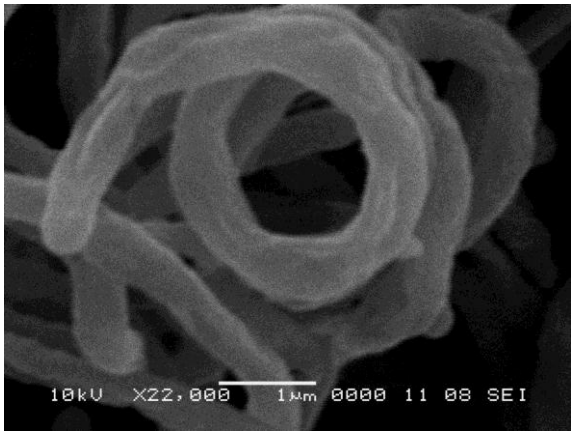

Plate 1. Scanning electron micrograph of the actinomycete isolate KH-1223-55 growing on starch nitrate agar medium spore chain is spiral shape and spore surfaces is smooth (X22.000).

3.2.2. Cell wall hydrolysate

The cell wall hydrolysate contains LL-diaminopimelic acid (LL-DAP) and sugar pattern not detected.

3.2.3. Color and culture characteristics

The isolate KH-1223-55 exhibited good growth on yeast extract agar medium (ISP-2) was good, aerial mycelium was yellowish gray and substrate mycelium was light yellowish brown. The growth on inorganic salt starch agar medium (ISP-4) was very good, aerial mycelium was yellowish gray and substrate mycelium was light yellowish Brown. The growth on tyrosine agar medium (ISP-7) was very good, aerial mycelium is white, substrate mycelium was deep yellow and diffusible pigment was light yellowish Brown. The growth on starch nitrate agar medium was very good, aerial mycelium was light gray, substrate mycelium was moderate brown and diffusible pigment was deep yellowish brown. The growth on Peptone-yeast extract iron agar medium (ISP-6) was moderate, aerial mycelium is white, substrate mycelium was Light yellowish brown and diffusible pigment was deep yellowish brown. No growth of actinomycete isolate was detected on tryptone yeast extract broth medium (ISP-1), Glycerol asparagine agar medium (ISP-5) and Oat meal agar medium (Table 2).

3.2.4. Physiological and biochemical characteristics

The actinomycete isolate KH-1223-55 could hydrolyzes protein, starch, lipid, lecithin and casein, whereas pectin hydrolysis and catalase test are negative, melanin pigment is negative, degradation of esculin & xanthin was positive, nitrate reduction, citrate utilization, urea and KCN utilization were positive, whereas, production of H_2S is negative.

The isolate KH-1223-55 utilizes mannose, mannitol, glucose, fructose, *meso*-inositol, galactose, maltose, lactose, starch, sodium malonate, phenylalanine, valine, arginine, histidine, glutamic acid and, but do not utilize xylose, Rhamnose, sucrose and cyctein. Growth was detected in presence of up to (7%) NaCl. The growth was inhibited in the presence of sodium azid (0.01%), phenol (0.01%); but not inhibit in thallous acetate (0.001). Good growth could be detected within a temperature range of 25 ^{o}C to 50 ^{o}C. Good growth could be detected within a pH value range of 5 to 9. The actinomycete isolate KH-1223-55 not sensitive to Ampicillin (25ug/ml) Nalidixic acid (30 ug/ml) Cefoperazone (75ug/ml) and Fusidic acid (10 ug/ml, Gentamicin (10 ug/ml) and Kanamycin (30 ug/ml) (Table 3)

3.3. Taxonmy of actinomycete isolate- KH-1223-55

This was performed basically according to the recommended international Key's viz. (Buchanan and Gibsons, 1974; Williams, 1989; and Hensyl, 1994). On the basis of the previously collected data and in view of the comparative study of the recorded properties of KH-1223-55, it could be stated that KH-1223-55 is suggestive of being belonging to *Streptomyces rimosus*,

and thus given the name *Streptomyces rimosus*, KH-1223-55 (Table 4).

3.4. Molecular phylogeny of the selected isolate

The 16S rDNA sequence of the local isolate was compared to the sequences of *Streptomyces* spp. In order to determine the relatedness of the local isolate to these *Streptomyces* strains. The phylogenetic tree (as displayed by the Tree View program) revealed that the locally isolated strain is closely related to *Streptomyces* sp., the most potent strain evidenced an 98% similarity with *Streptomyces rimosus* (Fig. 1).

3.5. Parameters controlling the biosynthesis of the antimicrobial agent

3.5.1. Inoculum size

Data illustrated in (Fig. 3) showed the relation between antibiotic productivity and different inoculum sizes. Maximum antimicrobial activity production could be recorded that a different inoculum sizes for four discs, after this maximum values 27.0, 26.0, 26.0, 25.0 and 23.0 in case of *Staph. aureus*, NCTC 7447, *Bacillus subtilis* NCTC 1040, *Fusarium oxysporum*; *Saccharomyces cerevisiae* ATCC 9763 and *Klepseilla pneumonia* NCIMB, 9111, respectively.

3.5.2. Incubation period

Data illustrated in (Fig. 4) showed the relation between antibiotic productivity and time of incubation. The level of antimicrobial agent yield increased gradually with increasing the incubation period up to the end of 5 days, after this maximum values 27.0, 26.0, 26.0, 25.0 and 23.0 in case of *Staph. aureus*, NCTC 7447, *Bacillus subtilis* NCTC 1040, *Fusarium oxysporum*; *Saccharomyces cerevisiae* ATCC 9763 and *Klepseilla pneumonia* NCIMB, 9111, respectively.

3.5.3. pH value

The results represented in (Fig. 5) that, the optimum initial pH value capable of promoting antimicrobial agents biosynthesis by *Streptomyces rimosus*, KH-1223-55 was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antimicrobial agent productivity reached up to 28.0, 27.0, 27.0, 26.0 and 24.0 in case of *Staph. aureus*, NCTC 7447, *Bacillus subtilis* NCTC 1040, *Fusarium oxysporum*; *Saccharomyces cerevisiae* ATCC 9763 and *Klepseilla pneumonia* NCIMB, 9111, respectively.

3.5.4. Incubation temperature

Data represented in (Fig. 6) showed that, the optimum temperature capable of promoting antimicrobial agent biosynthesis by *Streptomyces rimosus*, KH-1223-55 was at 30ºC, where, the diameter of inhibition zone resulted from antimicrobial agent productivity reached up to 28.0, 27.0, 27.0, 26.0 and 24.0 in case of *Staph. aureus*, NCTC 7447, *Bacillus subtilis* NCTC 1040, *Fusarium oxysporum*; *Saccharomyces cerevisiae* ATCC 9763 and *Klepseilla pneumonia* NCIMB, 9111, respectively.

3.5.5. Carbon source

Data given in (Fig. 7) indicated that the addition of different equimolecular carbon sources for production of antimicrobial agent revealed that starch is the best carbon source for biosynthesis antimicrobial substances with concentration 2.0 g/100. The effect of the used carbon sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces rimosus*, KH-1223-55, starch> glucose> D-mannose> Galactose> mannitol> *meso*-Insitol> fructose>.

3.5.6. Nitrogen source

Data given in (Fig. 8) indicated that the addition of different nitrogen sources exhibited an increase in the level of antimicrobial agent production by *Streptomyces rimosus*, KH-1223-55 where sodium nitrate was found to be the best nitrogen source for the antimicrobial agent production with concentration 0.25 g/100 ml. The effect of the used nitrogen sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces rimosus*, KH-1223-55, $NaNo_3$> KNO_3> peptone> $(NH_4)_2SO_4$> NH_4Cl> urea.

Table 1. Screening tests for antimicrobial activities producing of actinomycete isolates

*Organism number	* Mean values of inhibition zones (in mm) against													
	Bacteria							Fungi						
	S. aureus, NCTC 7447	*Bacillus subtilis*,	*Bacillus pumilus*, NCTC 8214	*M. luteus*, ATCC 9341	*E. coli* NCTC 10416	*Klebsiella pneumonia*, NCIMB 9111	*P. aeruginosa*, ATCC 10145	*Candida albicans*, IMRU 3669	*S. cervicea* ATCC 9763	*Rhizoctonia solani*	*Botrytis fabae*	*Asp. niger*, IMI 31276	*Fusarium oxysporum*	*P. chrysogenum*
KH-1223-55	27.0	26.0	25.5	25.0	23.0	23.0	21.0	023.	25.0	22.0	23.0	22.0	26.0	20.0
KH-1223-7	25.0	24.0	23.0	25.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KH-1223-32	20.0	18.0	18.0	18.0	17.0	15.0	12.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KH-1223-64	13.0	12.0	12.0	0 .0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KH-1223-67	21.0	20.0	20.0	22.0	20.0	0.0	0.0	00.	15.0	0.0	0.0	0.0	0.0	0.0
KH-1223-82	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0	18.0	17.0	18.0	12.0

Mean values of determination was calculated

Table 2. Cultural characteristics of the actinomycete isolate KH-1223-55.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
1-Starch nitrate agar medium	Good	264-1. gray light gray	58-m-Br moderate brown	deep-yBr deep yellowish brown
2-Tryptone yeast extract broth (ISP-1)	No growth	-	-	-
3-Yeast extract malt extract agar medium (ISP-2)	Good	93-y-gray yellowish gray	76-1-y-br Light yellowish Brown	-
4-Oatmeal agar medium (ISP-3)	No growth	-	-	-
5-Inorganic salts starch agar medium (ISP-4)	moderate	93-y-gray yellowish gray	76-1-y-br Light yellowish Brown	-
6-Glycerol – asparagine agar medium (ISP-5)	No growth	-	-	-
7-Peptone yeast extract iron agar medium (ISP-6)	moderate	263 white white	76-1-y-br Light yellowish Brown	deep-yBr deep yellowish brown
8-Tyrosine agar medium (ISP-7)	Good	263 white white	76-1-y-br Light yellowish Brown	deep-yBr deep yellowish brown

The color of the organism under investigation was consulted using the ISCC-NBS color - Name charts II illustrated with centroid color.

Table 3. The morphological, physiological and biochemical characteristics of the actinomycete isolate, KH-1223-55.

Characteristic	Result	Characteristic	Result
Morphological characteristics:		Mannitol	++
Spore chains	Spiral	L- Arabinose	+
Spore mass	White and yellowish gray	*meso*-Insitol	++
Spore surface	smooth	Lactose	+
Color of substrate mycelium	Light yellowish Brown	Maltose	+
Diffusible pigment	Deep yellowish brown	D-fructose	+
Motility	Non-motile	Sodium malonate	+
Cell wall hydrolysate		**Utilization of amino acids:**	
Diaminopimelic acid (DAP)	LL-DAP	L-Cycteine	-
Sugar Pattern	Not-detected	L-Valine	+
Physiological and biochemical properties:		L-Histidine	+
Hydrolysis of:-		L-Phenylalanine	+
Starch	+	L-Arginine	+
Protein	+	L-Glutamic acid	+
Lipid	-	**Growth inhibitors**	
Pectin	-	Sodium azide (0.01)	+
Casein & Lecithin	+	Phenol (0.1)	+
Catalase test	-	Thallous acetate (0.001)	-
Production of melanin pigment on:		**Growth at different temperatures (˚C):**	
Peptone yeast- extract iron agar	-	10	-
Tyrosine agar medium	-	20	±
Tryptone – yeast extract broth	-	25-50	+
Degradation of:		55	-
Xanthin	+	**Growth at different pH values:**	
Esculin	+	3 - 4.5	-
H_2S Production	-	5-9	+
Nitrate reduction	+	9.5-12	-
Citrate utilization	+	**Growth at different concentration of NaCl (%)**	
Urea test	+	1-7	+
KCN test	+	10	-
Utilization of carbon sources		**Resistance to:**	
D-Xylose	-	Ampicillin (25ug/ml) and	+
D- Mannose	+	Nalidixic acid (30 ug/ml)	+
D- Glucose	+	Cefoperazone (75ug/ml)	+
D- Galactose	+	Gentamicin (10 ug/ml)	+
Sucrose	-	Kanamycin (30ug/ml)	+
L-Rhamnose	-	Fusidic acid (10 ug/ml)	+
Raffinose	+		
Starch	++		

*+=**Positive , - = Negative, ++ = moderate growth and +++= good growth results.***

Table 4. A comparative study of the characteristic properties of KH-1223-55 in relation to reference strain *Streptomyces rimosus* (C.F. Hensyl,1994, Page693 and Table 27.5).

Characteristics	KH-1223-55	Hensyl (1994) *Streptomyces rimosus*
Morphological characteristics:		
Spore mass	White and yellowish gray	White and Yellow
Spore surface	Spiral	Spiral
Color of substrate mycelium	Light yellowish brown	Yellowish brown
Spore surface	Smooth	Smooth
Motility	Non-Motile	Not-Motile
Cell wall hydrolysate:		
- Diaminopimelic acid (DAP)	LL-DAP	LL-DAP
- Sugar pattern	Not-detected.	Not– Detected
Melanin pigment	-	-
Hydrolysis of:		
Casein	+	+
protein	+	+
Pectin	-	-
Starch	+	+
Degradation of:		
Esculine	+	+
Xanthine	+	+
H_2S production	-	-
Nitrate reduction	+	+
Utilization of:		
Sucrose	-	-
Mannitol	+	+
meso-Inositol	+	+
Rhamnose	-	-
L-Cysteine	-	-
L-Valine	+	+
L-Phenylalanine	+	+
L-Histidine	+	+
Growth temperature	25-50^{0}C	**25**-50^{0}C
Growth at pH:	5-9	6-9
Growth at NaCl (7.0 %)	+	+
Growth inhibitors:		
Sodium azide (0.01)	+	+
Phenol (0.1)	+	+
Thallous acetate (0.001)	-	-

+=Positive, - =Negative.

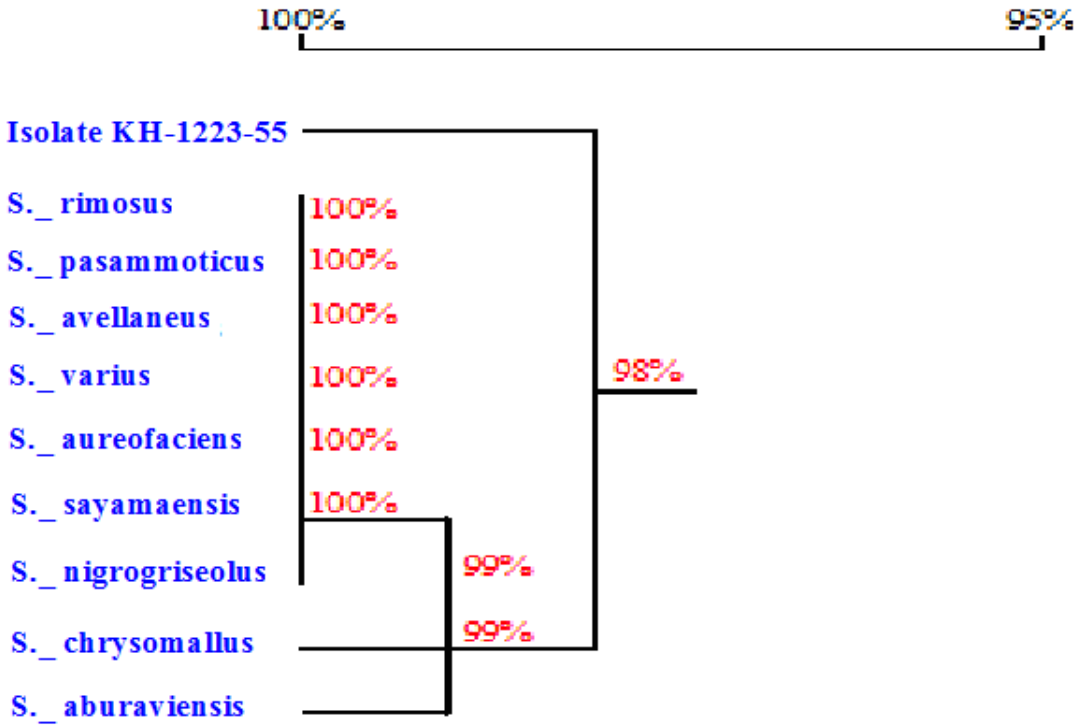

Fig. 1. The phylogenetic position of the local *Streptomyces* sp. strain among neighboring species. The phylogenetic tree was based on the multiple alignments options of 16_S rDNA sequences.

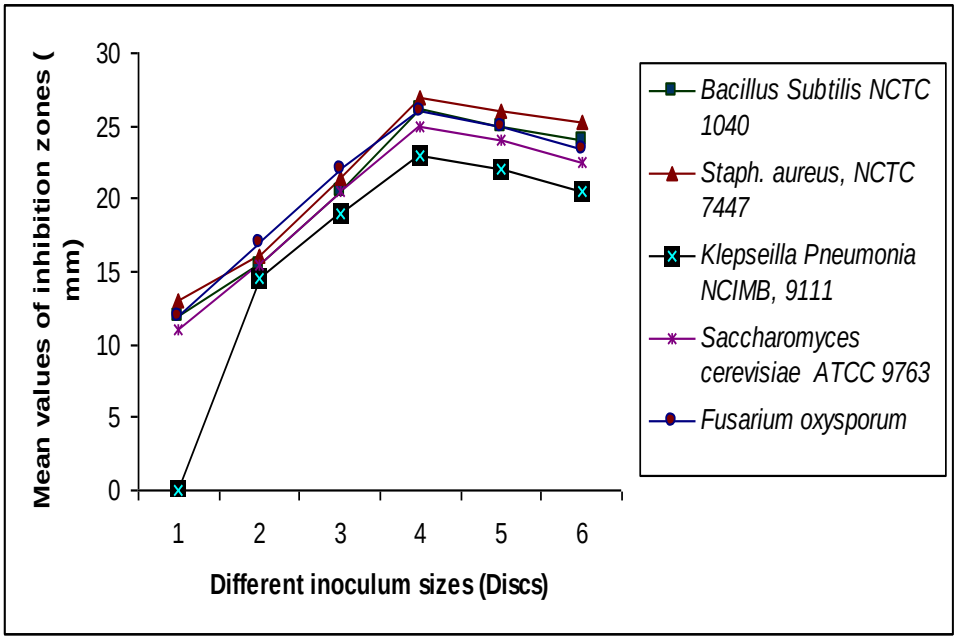

Fig. 2. Effect of different inoculum size on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, KH-1223-55.

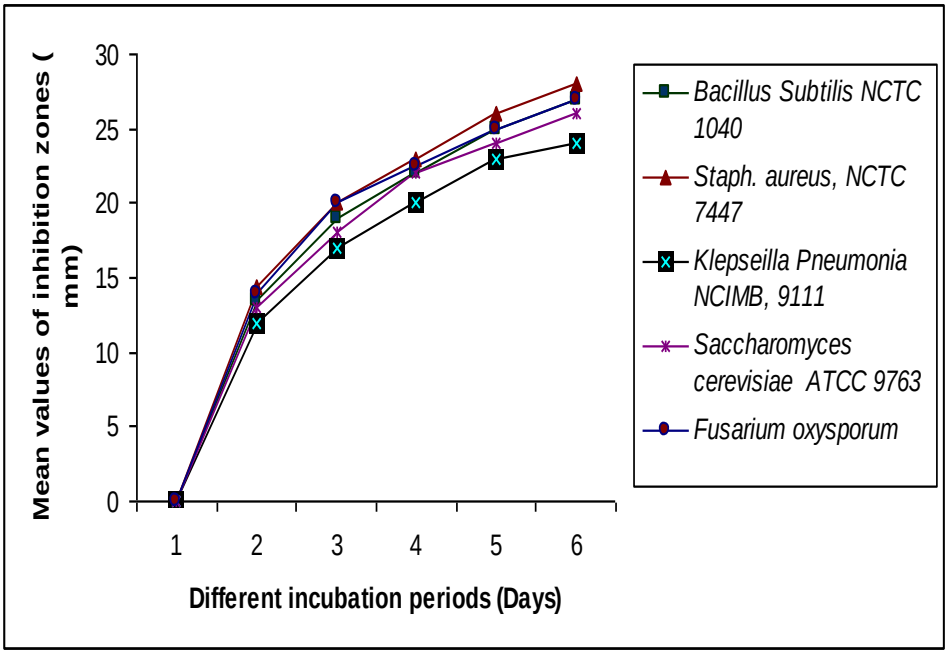

Fig. 3. Effect of different incubation periods on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus,* KH-1223-55.

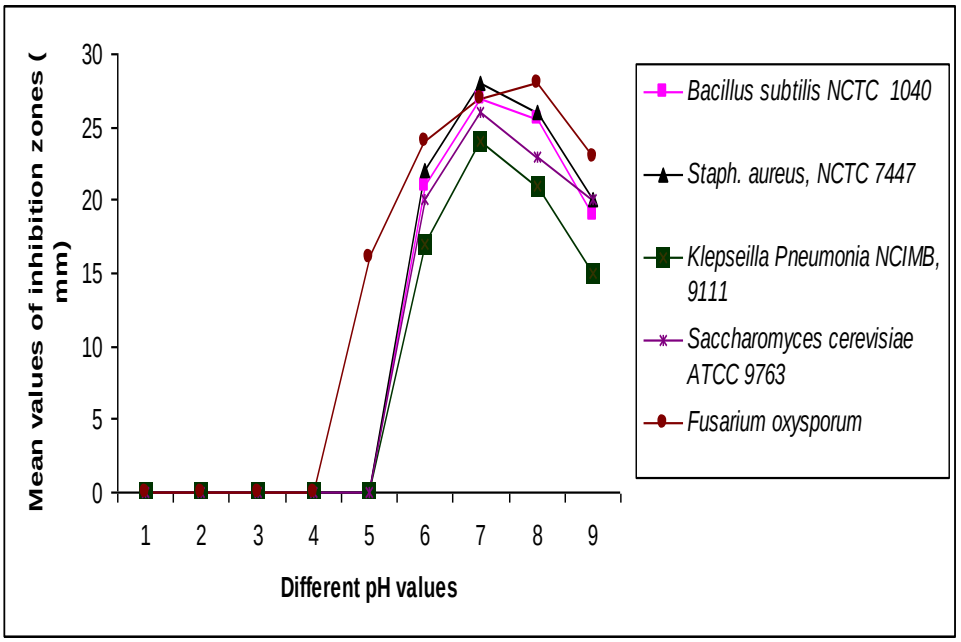

Fig. 4. Effect of different pH values on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus,* KH-1223-55.

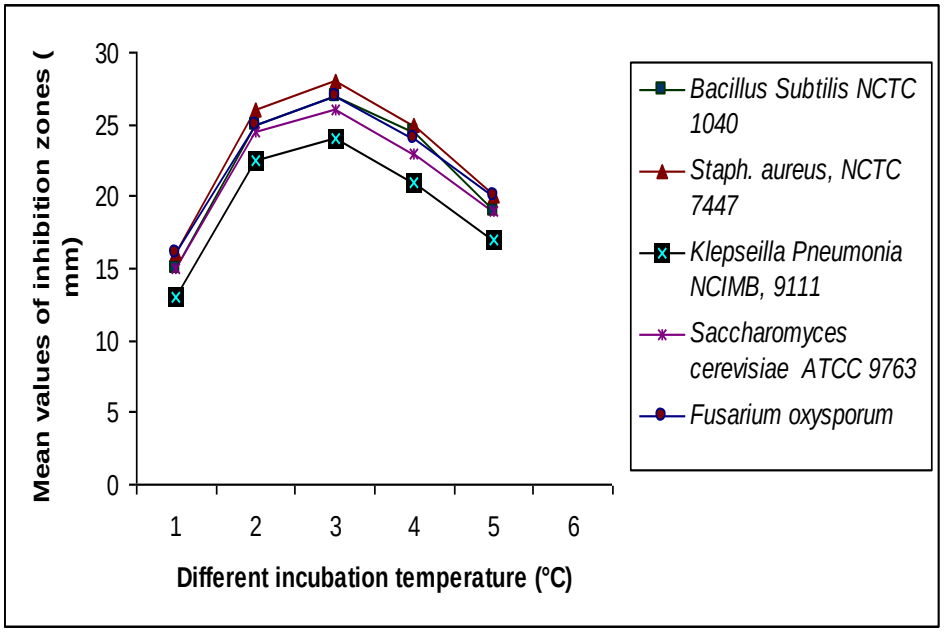

Fig. 5. Effect of different incubation temperature on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus,* KH-1223-55.

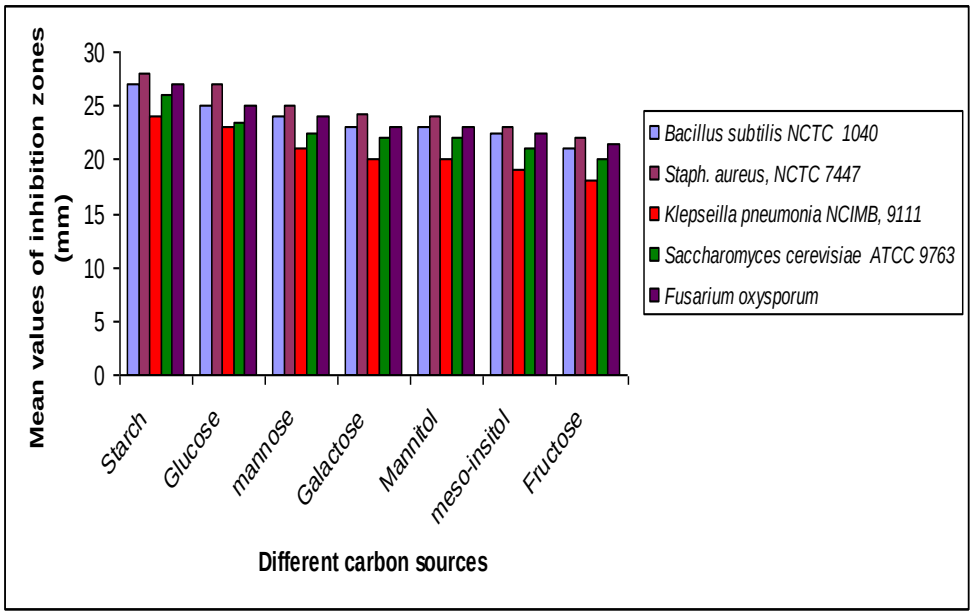

Fig. 6. Effect of different carbon sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus,* KH-1223-55.

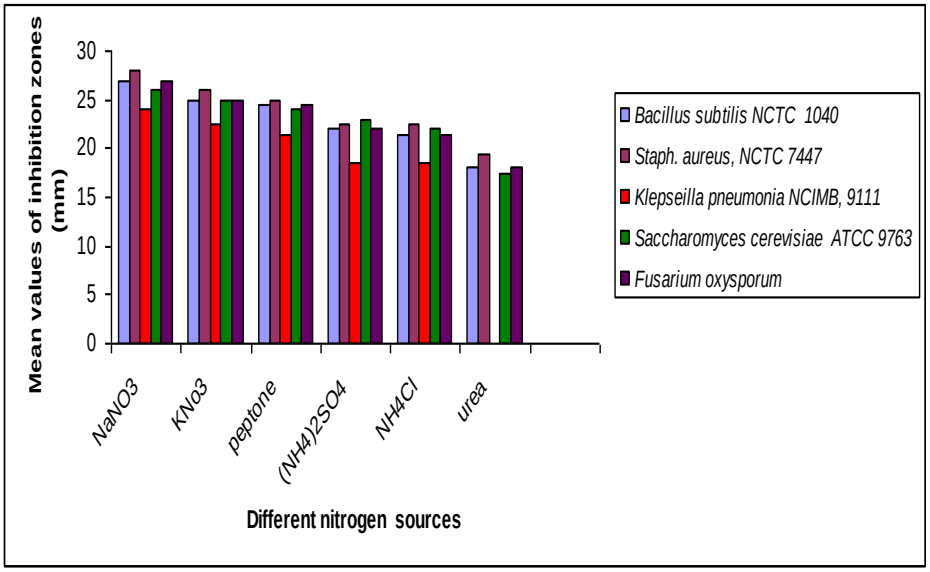

Fig. 7. Effect of different nitrogen sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus,* KH-1223-55.

4. DISCUSSION

The actinomycete isolate, KH-1223-55 was isolated from a soil sample collected from Al-Khurmah governorate, KSA. The isolate was growing on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. The actinomycete isolate, KH-1223-55 exhibited a wide spectrum antimicrobial agent against (bacterial Gram positive and Bacteria Gram negative and unicellular and filamentous Fungi). (Asha devi *et al.,* 2006). Identification process had been carried out according to the Key's given in Bergey's Manual of Determinative Bacteriology 8th edition (Buchanan and Gibbsons, 1974), Bergey's Manual Of Systematic Bacteriology, vol. 4 (Williams, 1989) and Bergey's Manual Of Determinative Bacteriology, 9th edition (Hensyl, 1994). For the purpose of identification of actinomycete isolate, the morphological characteristics and microscopic examination emphasized that the spore chain is spiral. Spore mass is yellow or white; while spore surface is smooth, substrate mycelium is light yellowish brown and no diffusible pigment was produced on 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) and sugar pattern of cell wall hydrolysate could not detected. 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, the identification of the actinomycete isolate KH-1223-55 was suggestive of being belonging to *Streptomyces rimosus,* KH-1223-55 which can produce a broad-spectrum antimicrobial agent (Kavitha and Vijayalakshmi, 2007**;** Atta *et al.,* 2011). The resulted sequence was aligned with available almost compete sequence of type strains of family streptomycetaeae. The phylogenetic tree (diagram) revealed that the local isolate is closely related *Streptomyces rimosus,* KH-1223-55 (similarity matrix is 98%) (Augustine *et al.,* 2005 and Thenmozhi and Kannabiran, 2010). For optimizing the biosynthesis of the antimicrobial agent from *Streptomyces rimosus,* KH-1223-55, different cultural conditions such as inoculum size, pH, temperature, and incubation period, effect of different carbon and nitrogen sources was studied. The maximum biosynthesis was achieved at the end of an incubation period of 6 days at pH 7.0 for the antimicrobial agent production using four discs of actinomycete culture. Similar results had been recorded by various workers; (Joseph *et al.,* 2009 and Prema *et al.,* 2009). The fact that maximum yield of the antimicrobial agent occurred at the end of an incubation temperature of 30ºC was in complete accordance with those reported by (Selvin *et al.,* 2004; El-Naggar *et al.,* 2007 and Atta, 2010). Data of the effect of different carbon and nitrogen sources on the production of the antimicrobial agent indicated that *Streptomyces rimosus,* KH-1223-55 require starch and

sodium nitrate at 2.0.0 g/100 ml; 0.25 g/100 respectively. 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 and Atta *et al.* 2009 and 2011).

5. Conclusion

The present study mainly involved in the isolation of Actinomyces based on its morphology, physiology, biochemical and cultural characteristics. Further work should be focused in most potent *Streptomyces* isolate for production the antimicrobial activities against bacterial Gram positive and Bacteria Gram negative and unicellular and filamentous Fungi and studies the parameters controlling the biosynthetic process of antimicrobial agent formation.

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9/12/2011