Screening, Identification, Phylogenetic Characterization and Optimization of Antimicrobial Agents Biosynthesis Produced By *Streptomyces rimosus*

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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 from actinomycetes strains. Eighty eight actinomycete strains were isolated from twelve soil samples collected from different localities in Egypt. Only one actinomycete culture AZ-146 from eight cultures was found exhibited to produce wide spectrum antimicrobial activities. It is active in *vitro* against some microbial pathogenic viz: *Staph. aureus*, NCTC 7447; *Micrococcus lutea*, ATCC 9341; *Bacillus subtilis*, NCTC 10400; *Bacillus pumilus*, NCTC; *Klebsiella pneumonia*, NCIMB 9111; *Escherichia coli*, NCTC 10416; *Pseudomonas aeruginosa*, ATCC 10145; *S. cerevisiae* ATCC 9763; *Candida albicans*, IMRU 3669; *Aspergillus niger* IMI 31276; *Fusarium oxysporum*;. The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 99% similarity with *Streptomyces rimosus*. From the taxonomic features, the actinomycetes isolate AZ-146 matches with *Streptomyces rimosus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces rimosus*, AZ-146. 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.
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Key words: *Streptomyces rimosus*, Phylogenetic Characterization, Optimization of antimicrobial activity.

1. Introduction

New antibiotics that are active against resistant bacteria are required. Bacteria have lived on the Earth for several billion years. During this time, they encountered in nature a wide range of naturally occurring antibiotics. To survive, bacteria developed antibiotic resistance mechanisms (Rogers, 2008). Therefore, it is not surprising that they have become resistant to most of the natural antimicrobial agents that have been developed over the past 50 years (Hancock, 2007). The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal (Cowen, 2008). Antibiotics like penicillin and erythromycin, which used to be one-time miracle cures are now less effective because bacteria have become more resistant (Pearson and Carol, 2008).

Actinomycetes are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites (Berdy, 2005), notably antibiotics (Strohl, 2004). Actinomycetes are prokaryotes with extremely various metabolic possibilities. They produce numerous substances essential for health such as antibiotics enzymes (Bachmann and McCarthy, 1991) immunomodulators. If we include secondary metabolites with biological activities other than antimicrobial, Actinomycetes are still out in front, with over 60% *Streptomyces* sp. accounting for 80% of these (Hopwood *et al.*, 2000). Psychrophiles or Cryophiles (adj. cryophilic) are extremophilic organisms that are capable of growth and reproduction in cold temperatures (Law *et al.*, 2007). Antibiotics are the best known products of actinomycete. The morphology of an actinomycete growing on agar can provide useful and rapid clues to its identity, but viewing isolated colonies can give little worthwhile information. Morphological characters are still widely used for characterizing genera, for example, the presence or absence of spores on the substrate mycelium or the formation of zoospores in specialized spore vesicles or sporangia. The ability to produce motile spores is more widespread in the actinomycetes (Brun and Skimkets, 2000).

The present study described the isolation of an actinomycete strain from Mansoura districted, 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. On the other hand, studies the optimization parameters controlling on the biosynthetic process of antimicrobial agent formation.

2. Material and Methods

2.1. Actinomycete strain: Strain AZ-146 was isolated from a suspension of a soil sample **(**Williams and Davis, 1965**)** inoculated onto a Starch-nitrate agar it was composed of (g/l) starch, 10; $NaNO_3$, 2; K_2HPO_4 1; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; microelement, 1 ml; and agar 20. The pH was adjusted to 7.2 before sterilization using 1 N NaOH or 1 N HCl. Stock solution was composed of (g/500 ml) $FeSO_4 \cdot 7H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.5; and $ZnSO_4 \cdot 7H_2O$, 0.5. Plates and incubated at 30°C for five days. The soil samples were collected from the Zagazig district. The isolates were individually maintained on Starch-nitrate agar at 4°C and stored as a mixture of hyphae and spores in 20% glycerol at 80°C. Each isolated strain was cultured in a Starch-nitrate broth: This medium contained the same ingredients as mentioned above for starch-nitrate agar with the omission of agar. After clarification of the culture broths, the supernatant tested for antimicrobial activity.

2.2. Test organisms:

2.2.1. Bacteria:

2.2.1.1. Gram-positive Bacteria: *Staphylococcus aureus*, NCTC 7447; *Bacillus subtilis*, NCTC 1040; *Bacillus pumilus*, NCTC 8214 and *Micrococcus luteus*, ATCC 9341.

2.2.1.2. Gram-negative Bacteria: *Escherichia coli*, NCTC 10416; *Klebsiella pneumonia*, NCIMB 9111 and *Pseudomonas aeruginosa*, ATCC 10145

2.2.2. Fungi:

2.2.2.1. Unicellular Fungi: *Candida albicans*, IMRU 3669 and *Saccharomyces cervisiae* ATCC 9763

2.2.2.1. Filamentous Fungi: *Asp. niger*, IMI 31276; *Aspergillus flavus*, IMI 111023, *Aspergillus fumigatous*, ATCC 16424; *Fusarium oxysporum and Penicillium chrysogenium***.**

2.3. Screening for antimicrobial activity: The anti-microbial activity was determined by cup method assay according to (Kavanagh, 1972).

2.4. Taxonomic studies of actinomycete isolate:

2.4.1. Morphological characteristics of the most potent produce strain AZ-146 grown on starch nitrate agar medium at 30 ºC for 4 days was examined under scanning electron microscopy (JEOL Technics Ltd.,).

2.4.2. Physiological and biochemical characteristics: The ability of the strain to produce different enzymes was examined by using standard methods. Lecithinase was conducted on egg–yolk medium according to the method of (Nitsh and Kutzner, 1969); Lipase (Elwan *et al.*, 1977); Protease (Chapman, 1952); Pectinase according to the method of (Hankin *et al.*, 1971); α-amylase according to the method of (Cowan, 1974) and Catalase test according to the method of (Jones, 1949). Melanin pigment according to the method of (Pridham *et al.*, 1957). Degradation of Esculin and xanthine according to the method of (Gordon *et al.*, 1974). Nitrate reduction according to the method of (Gordon, 1966). Hydrogen sulphide production and oxidase test according to the method of (Cowan, 1974). The utilization of different carbon and nitrogen sources according to the methods of (Pridham and Gottlieb, 1948). Cell wall was performed by the method of (Becker *et al.*, 1964 and Lechevalier and Lechevalier, 1970). Cultural characteristics such as color of aerial mycelium, color of substrate mycelium and pigmentation of the selected actinomycete were recorded on ISP agar medium (Shirling and Gottlieb, 1966). Colors characteristics were assessed on the scale developed by (Kenneth and Deane, 1955).

2.4.3. DNA isolation and manipulation:

The locally isolated actinomycete strain was grown for 5 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 3 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 in accordance with the methods described by **(**Sambrook *et al*., 1989**).**

2.4.4. 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., in accordance with the method described by **(**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 electro-phoresis, 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, as described by **(**Sanger *et al.*, 1977**).** The 16S rRNA gene (1.5 kb) sequence of the PCR product was acquired using a Terminator Cycle Sequencing kit (ABI Prism 310

Genetic Analyzer, Applied Biosystems, USA).

2.4.5. 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 evaluating using BioEdit software **(**Hall, 1999**).** The phylogenetic tree was displayed using the TREE VIEW program.

2.5. Factors effecting on the biosynthesis of the antimicrobial agent: 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: One of the actinomycete cultures AZ-146 from eight cultures were found exhibited various degrees of activities against Gram-positive and Gram-negative bacteria and unicellular and filamentous fungi (Table 1).

3.2. Identification of the actinomycete isolate:

3.2.1. Morphological characteristics: The vegetative mycelia grew abundantly on both synthetic and complex media. The aerial mycelia grew abundantly on Starch-nitrate agar medium and Inorganic salts starch agar medium (ISP-4). The Spore chains were spiral, and had a smooth surface (Plate 1). Neither both sclerotic granules and sporangia nor flagellated spores were observed.

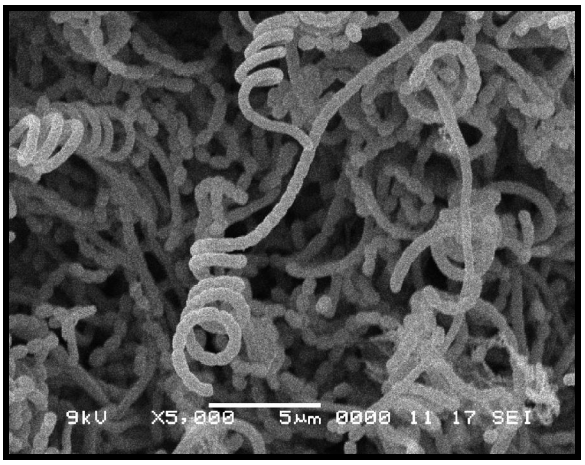

Plate (1). Scanning electron micrograph of the actinomycete isolate AZ-146 growing on starch nitrate agar medium showing spore chain Spiral shape and spore surfaces smooth (X15,000).

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: As shown in Table (2), the growth of actinomycete isolate AZ-146 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.

3.2.4. Physiological and biochemical characteristics: The actinomycete isolate AZ-146 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 AZ-146 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 6 to 9. (Table 3).

3.2.5. Taxonomy of actinomycete isolate, AZ-146: This was performed basically according to the recommended international Key's viz. (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 AZ-146 in relation to the most closest reference strain, viz. *Streptomyces rimosus*, it could be stated that actinomycetes isolate, AZ-146 is suggestive of being likely belonging to *Streptomyces*

rimosus, AZ-146 (Table 4).

3.2.6. Amplification of the 16S rDNA gene: The 16S rDNA gene was amplified by polymerase chain reaction (PCR) using the universal primers. The primers that was used to 16S rDNA sequencing were 16F357 of the sequence strepF; 5'-ACGTGTGCAGCCCAAGACA-3' and strpR; 5'-ACAAGCCCTGGAAACGGGGT-3', the product of the PCR was analyzed on 1.5% ethidium bromide gel.

3.2.7. 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., rather than to *Streptomyces rimosus* (Fig. 1). Multiple sequence alignment was conducted the sequences of the 16_S rDNA gene of *Streptomyces rimosus*. Computer assisted DNA searches against bacterial database similarly revealed that the 16_S rDNA sequence was 99% identical *Streptomyces rimosus* (Fig. 1).

3.3. Factors effecting on the biosynthesis of the antimicrobial agent produced by *Streptomyces rimosus*, AZ-146

3.3.1. Effect of different inoculum size: Data illustrated graphically in (Fig. 2) showed the relation between antibiotic productivity, inoculum size. The maximum inhibition zones of produced antimicrobial agents against tested microorganisms reached up to 27.0, 24.0, 20.0 & 18.0 in case of *Staph. aureus*, NCTC 7447, *Klepseilla pneumonia* NCIMB 9111, *Candida albicans* IMRU 3669 and *Aspergillus niger*, IMI 31276 respectively at an inoculum size of 4 (discs per 100 media) in all cases.

3.3.2. Effect of different incubation periods: Data illustrated graphically in (Fig. 3) showed the relation between antibiotic productivity and time of incubation. The level of antimicrobial agents yield increased gradually with increasing the incubation period up to the end of four days, after this maximum values 27.4, 26.5, 20.5 & 19.5 in case of *Staph. aureus*, NCTC 7447, *Klepseilla pneumonia* NCIMB 9111, *Candida albicans* IMRU 3669 and *Aspergillus niger*, IMI 31276 respectively.

3.3.3. Effect of different incubation temperature (°C): Data represented graphically in (Fig. 4) showed that, the optimum temperature capable of promoting antimicrobial agents biosynthesis by *Streptomyces rimosus*, AZ-146 was at 30 ^oC, whereas, the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 27.5, 26.5, 20.5 & 19.5 in case of *Staph. aureus*, NCTC 7447, *Klepseilla pneumonia* NCIMB 9111, *Candida albicans* IMRU 3669 and *Aspergillus niger*, IMI 31276 respectively.

3.3.4. Effect of different pH values: The results represented graphically in (Fig. 5) that, the optimum initial pH value capable of promoting antimicrobial agents biosynthesis by *Streptomyces rimosus*, AZ-146 was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 28.5, 26.4, 21.8 & 20.0 in case of *Staph. aureus*, NCTC 7447, *Klepseilla pneumonia* NCIMB 9111, *Candida albicans* IMRU 3669 and *Aspergillus niger*, IMI 31276 respectively.

3.3.5. Effect of different carbon sources: Data given in (Fig. 6) indicated that the addition of different equimolecular carbon sources for production of antimicrobial agents revealed that starch is the best carbon source for biosynthesis antimicrobial substances. The effect of the used carbon sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces rimosus*, AZ-146, starch> glucose> mannitol > *meso*-insitol > mannose> galactose >fructose

3.3.6. Effect of different nitrogen sources: The nitrogen sources exhibited an increase in the level of antimicrobial agent production by *Streptomyces rimosus*, AZ-146. The effect of the used nitrogen sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces rimosus*, AZ-146, KNO_3> $NaNo_3$> NH_4Cl> $(NH_4)_2SO_4$> peptone> urea. (Fig. 7).

Table 1. Antimicrobial potentialities of the antibiotic-producing microorganisms isolated from various localities

[+]Organism number	* Mean values of inhibition zones (in mm) against													
	Bacteria							Fungi						
	Staph. aureus, NCTC 7447	*Bacillus subtilis*, NCTC 1040	*Bacillus pumilus* NCTC 8214	*M. luteus*, ATCC 9341	*E. coli*, NCTC 10416	*K. pneumonia* NCIMB 9111	*P. aeruginosa* ATCC 10145	*Candida albicans*, IMRU 3669	*S. cerevisiae* ATCC 9763	Asp. niger, IMI 31276	*Asp. fumigatus*	*Asp. flavus*, IMI 111023	*F. oxysporum*	*P. chrysogenum*
AZ-146	**27.0**	**26.0**	**28.0**	**28 .0**	**26.0**	**24.0**	**20.0**	**20.0**	**20.0**	**18.0**	**0.0**	**0.0**	**17.0**	**0.0**
AZ-65	25.0	25.0	274.5	25.0	22.0	18.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-102	24.0	23.0	23.0	24.0	22.0	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-111	21.0	20.0	20.0	22.0	20.0	16.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-124	18.0	17.0	16.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-128	21.0	20.0	20.0	21.0	17.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-132	27.0	26.0	27.0	26.0	25.0	24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AZ-139	18.0	18.0	17.0	17.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Culture characteristics of the actinomycete isolate AZ-146.

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 with the ISCC-NBS color –name charts illustrated with centroid color

Table 3. The morphological, physiological and biochemical characteristics of the actinomycete isolate AZ-146

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			
D-Xylose	-		
D- Mannose	+		
D- Glucose	+		
D- Galactose	+		
Sucrose	-		
L-Rhamnose	-		
Raffinose	+		
Starch	+++		

+ =Positive , - = Negative , ± = doubtful results, , ++ = moderate growth & +++ = good growth.

Table 4. A comparative study of the characteristics of actinomycete isolate, AZ-146 in relation to reference *Streptomyces rimosus* (C.F. Hensyl,1994, Page693 and Table 27.5).

Characteristics	AZ-146	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:	6-9	6-9
Growth at NaCl (7.0 %)	+	+
Growth inhibitors:		
Sodium azide (0.01)	+	+
Phenol (0.1)	+	+
Thallous acetate (0.001)	-	-

+=Positive, - =Negative.

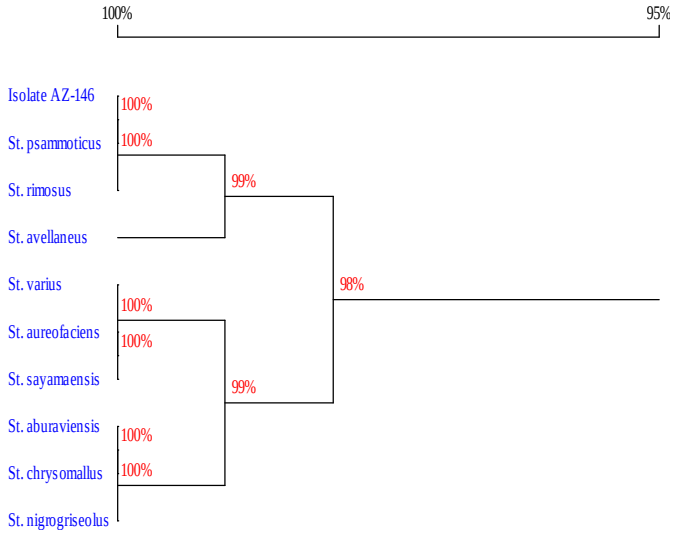

Fig. 1. The phylogenetic position of the local *Streptomyces* sp. strain among neighboring species. The phylogenetic tree was based on the multiple sequence alignment comparisons of 16_S rDNA sequences.

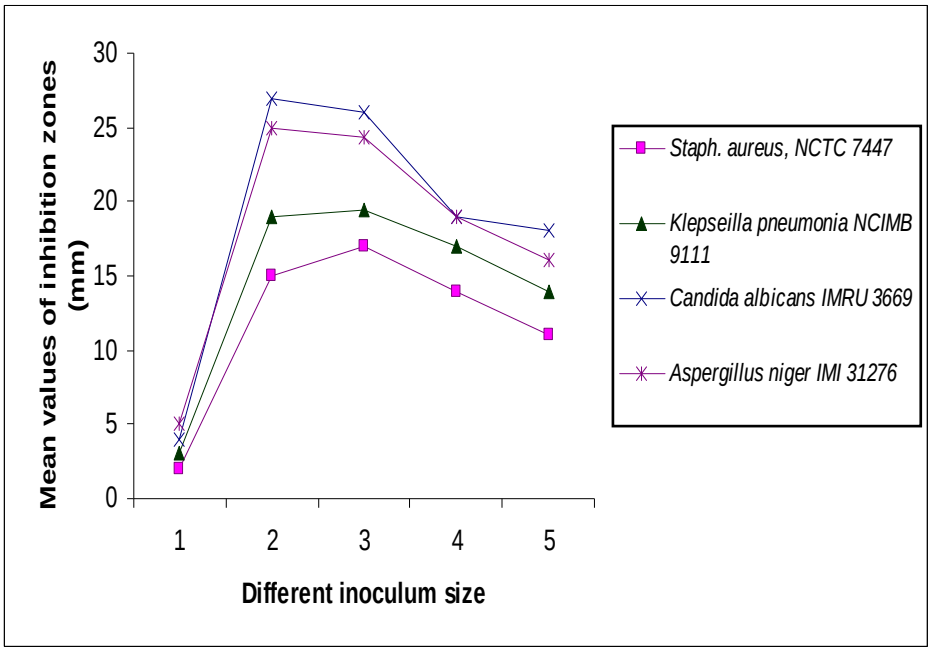

Fig. 2. Effect of different inoculum size on the antibiotic yield produced *Streptomyces rimosus*, AZ-146.

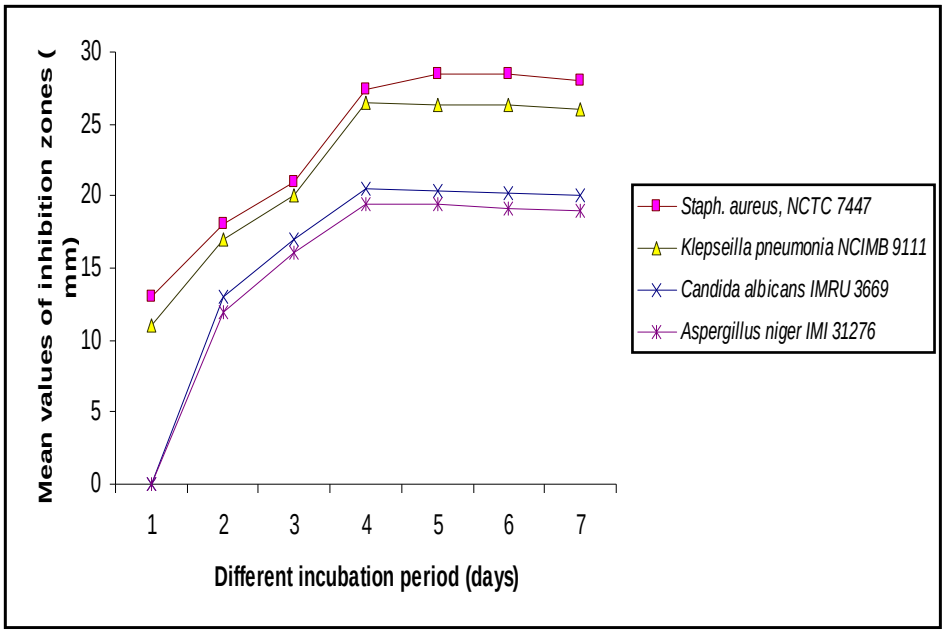

Fig. 3. Effect of different incubation periods on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, AZ-146.

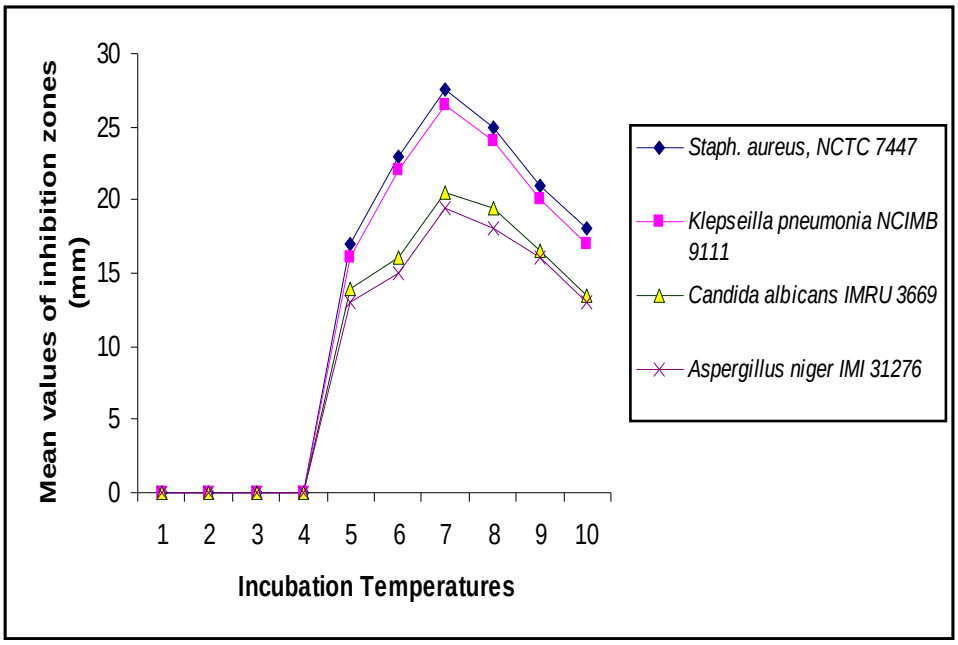

Fig. 4. Effect of different incubation temperature on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, AZ-146 [1=5; 2=10; 3=15; 4=20; 5=25; 6=30; 7=35; 8=40; 9=45 and 10=50]

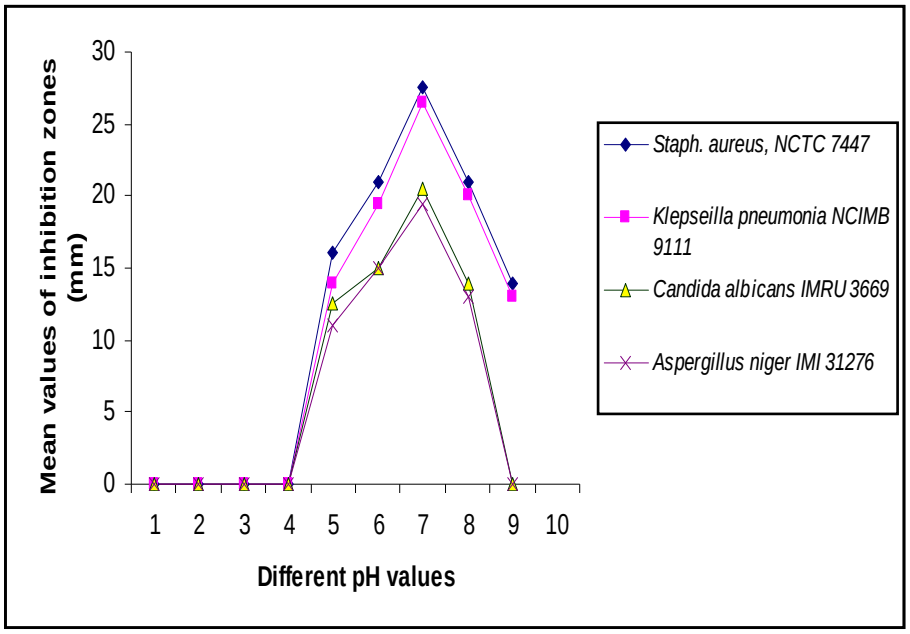

Fig. 5. Effect of different pH values on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, AZ-146.

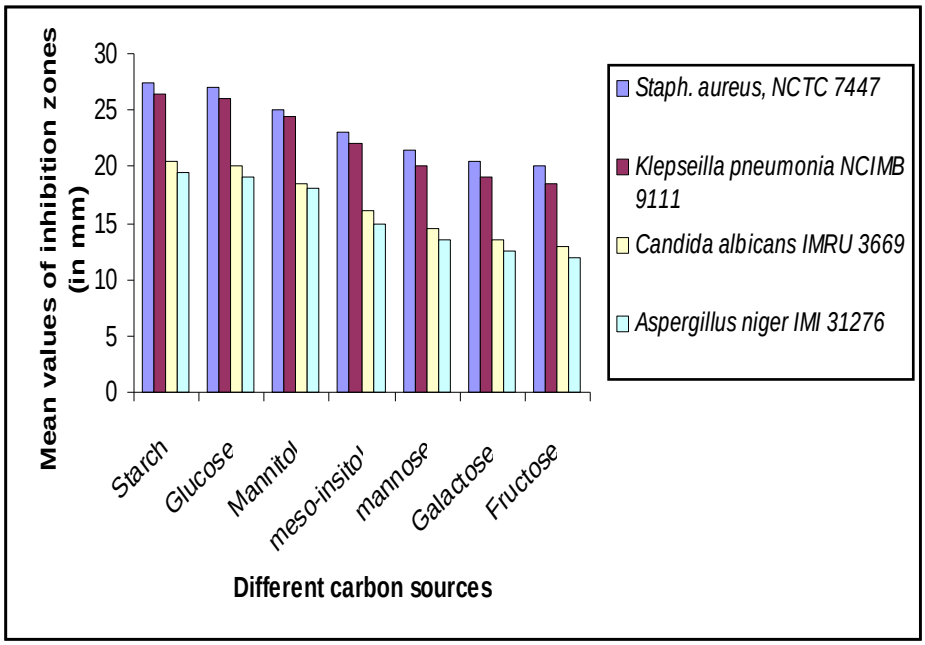

Fig. 6. Effect of different carbon sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, AZ-146.

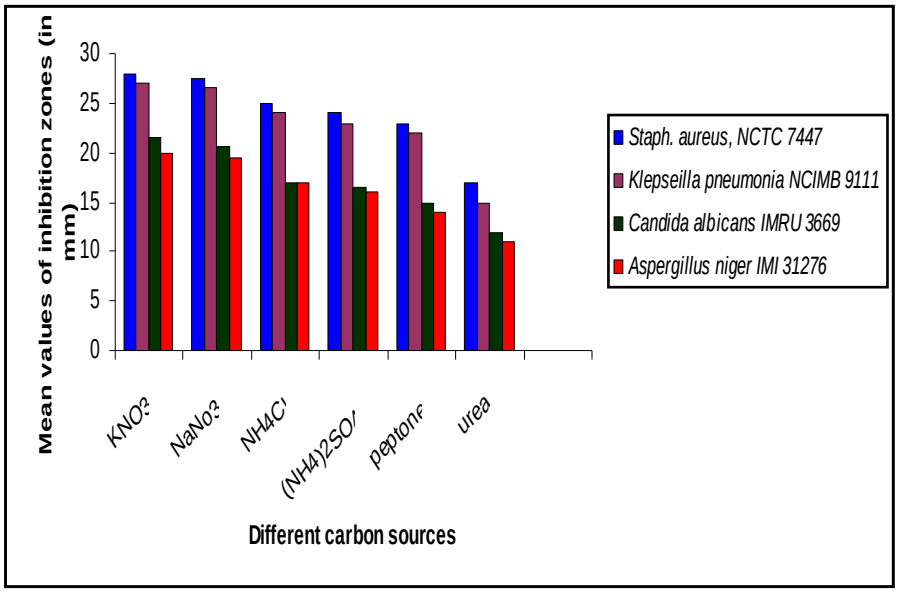

Fig. 7. Effect of different nitrogen sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces rimosus*, AZ-146.

4. DISCUSSION

It has long been known that some of the actinomycete strains of the same species could generate different antibiotics, whereas some other strains belonging to different species generated the same antibiotics (Lechevalier, 1975). The production of antibiotics by actinomycetes, therefore, may not be species-specific, but rather strain-specific. Antibiotics of actinomycete origin evidence a wide variety of chemical structures, including aminoglycosides, anthracyclines, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyketides, actinomycins, and tetracyclines (Baltz, 1998). Eighty-eight actinomycete strains were isolated from twelve soil samples collected from Mansoura districted, Egypt. Only one actinomycete culture AZ-146 from eight cultures was found exhibited to produce wide spectrum antimicrobial activities. Identification process has been carried out according to (Williams, 1989 and 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 white and yellow; while spore surface is smooth, substrate mycelium is Light yellowish brown and diffusible pigment was deep yellowish brown produced on ISP-6 and 7 media. 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*. In view of all the previously recorded data, the identification of actinomycete isolate AZ-146 was suggestive of being belonging to *Streptomyces rimosus*, AZ-146. The resulted sequence was aligned with available almost compete sequence of type strains of family streptomycetaeae. It formed phyloletic line that was closely related to *Streptomyces rimosus*, AZ-146, sharing 16s rRNA gene similarity matrix is 99%.

Nutritional requirements of Streptomyces play an important role during metabolite synthesis process (Yu *et al.*, 2008). Amongst various nutritional requirements, carbon source and nitrogen source are generally regarded as important factors of metabolism, and several examples of the production of metabolites in media with optimized contents of these components are also described in the literature (Purama and Goyal, 2008; Yuan *et al.*, 2008). Maximum antimicrobial activity biosynthesis could be recorded that a different inoculum sizes for four discs; incubation period for four days (Mu *et al.*, 2009); pH 7.0 (Atta, 2009 and 2010); temperature 30ºC (Khalifa, 2008); starch best carbon source (Lazim *et al.*, 2009); KNO_3 best nitrogen source (Atta *et al.*, 2011).

5. Conclusion

Actinomycetes are producers of potent metabolic compounds used commercially as antibiotics and other novel drugs The present study shows the present data focusing on obtaining microbial local isolates which have the ability to produce antimicrobial agent. An

interesting scope for further research would be to improve antimicrobial agent production by *Streptomyces rimosus*, AZ-146 against pathogenic microorganisms (Gram positive and Gram negative bacteria and unicellular and filamentous fungi) and studies the parameters controlling the biosynthetic process of antimicrobial agent formation.

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