Taxonomic Study, Phylogenetic Characterization and Factors Affecting on the Biosynthesis Antimicrobial Agents Produced By *Streptomyces lydicus*

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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-55 from eight cultures was found exhibited to produce wide spectrum antimicrobial activities. The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 99% similarity with *Streptomyces lydicus*. From the taxonomic features, the actinomycetes isolate AZ-55 matches with *Streptomyces lydicus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces lydicus*, AZ-55. 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 lydicus*, Taxonomic study, Phylogenetic Characterization, factors affecting antimicrobial activity.

1. Introduction

Actinomycetes is one of the most attractive families of industrial bacteria on account of their superior potential for producing valuable secondary metabolites including antibiotics, anti-cancer drugs, immunosuppressors and enzyme inhibitors (Zitouni *et al*., 2004a and Sanasam and Ningthoujam, 2005). The species belonging to the genus Streptomyces constitute 50% of the total population of soil actinomycetes and 75-80% of the commercially and medicinally useful antibiotics have been derived from this genus (Lazzarini *et al*., 2000). The list of novel microorganisms and products derived from poorly explored areas of the world like China, Australia, Antarctica and Jordan suggests that a careful exploration of new habitats might continue to be useful (Zitouni *et al*., 2004a). The search for new antibiotics continues to be almost importance in research programs around the world because the increase of the resistant pathogens and toxicity of some used antibiotics. Among microorganisms actinomycetes are one of the most investigated groups particularly members of the genus *Streptomyces* from which, a large number of antibiotics was obtained and studied (Gupte *et al*., 2000). The vast majority of actinomycetes have originated from soil (Shearer, 1997) and their isolation method deal almost exclusively with those suitable for *Streptomyces* species which grow rapidly on soil dilution plates. However, in recent years, the rate of discovery of new antibiotics in the genus *Streptomyces* was declining and isolation of other actinomycete genera, appeared to be necessary to assess the health hazard and to and novel strains producing commercially valuable antibiotics. With the discovery of new antibiotics from strains of *Actinomadura*, *Micromonospora*, *Saccharothrix* and *Streptosporangium*, increased emphasis was placed on developing methods for the isolation and identification of non-streptomycete actinomycetes (Shearer, 1997). During a screening program for *Streptosporangium*, strains can produce valuable substances of biotechnological interest search of potent antimicrobial products, was focused on antibiotic producing rare actiomycetes. Selective methods were used to isolate new strains producing new antibiotics (Zitouni *et al*., 2004b). It has been also found that *Streptosporangium* strains can produce valuable substances of biotechnological interest (Ammor *et al*., 2008).

The present study described the isolation of an actinomycete strain from Zagazig 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. Whereas, studies the parameters controlling on the biosynthetic process of antimicrobial agent formation.

2. Material and Methods

2.1. Actinomycete strain: Strain AZ-55 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-55 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-55 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; Oatmeal agar medium (ISP-3) 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.

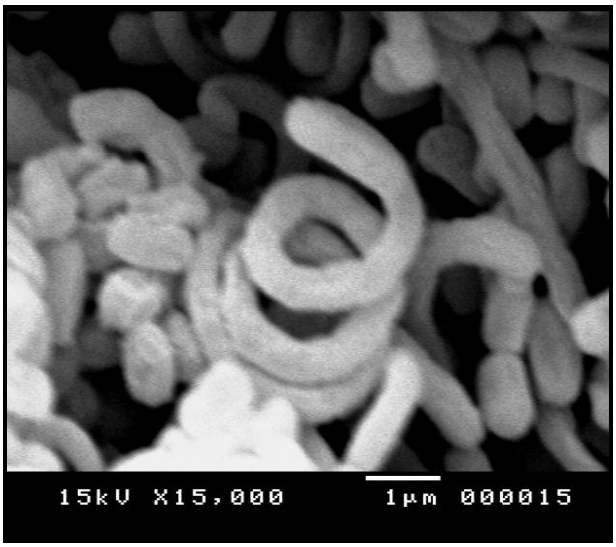

Plate (1). Scanning electron micrograph of the actinomycete isolate AZ-55 growing on starch nitrate agar medium showing spore chain Spiral shape and spore surfaces smooth (X15,000).

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 AZ-55 grew on the ISP-media. The isolate exhibited good growth on starch-nitrate agar medium, the aerial mycelium showed dark gray color, substrate mycelium is moderate yellowish brown and the diffusible pigment is dark grayish yellowish brown. No growth on tryptone- yeast extracts broth (ISP-1) and yeast extract –malt extract agar medium (ISP-2). Good growth was detected on Oat- meal agar medium (ISP-3). Aerial mycelium is light gray, and substrate mycelium is moderate yellowish brown and no diffusible pigments are seen. Moderate growth was detected on inorganic salts- starch agar medium (ISP-4). Aerial mycelium is light gray and substrate mycelium is light brown and no diffusible pigments are visible. Moderate growth was detected on glycerol–asparagine agar medium (ISP-5). Aerial mycelium is light gray; substrate mycelium is brown and no diffusible pigment. Poor growth was detected on peptone yeast extract-iron agar medium (ISP-6), aerial mycelium is light gray; substrate mycelium is light yellowish brown, and diffusible pigment moderate brown. Poor growth was detected on tyrosine agar medium (ISP-7), aerial mycelium is light gray, substrate mycelium is light yellowish brown, and diffusible pigment is moderate yellowish brown.

3.2.4. Physiological and biochemical characteristics: The actinomycete isolate AZ-55 could hydrolyze protein, starch, lecithin and casein hydrolysis are positive whereas lipid, pectin and Catalase test are negative. Melanin pigment, production of H_2S, KCN test, and nitrate reduction are negative. Degradation of esculin, xanthine, utilization of citrate, and decomposition of urea are positive. The isolate utilizes, mannose, glucose, galactose, sucrose, mannitol, raffinose, *meso*-insoitol, arabinose, lactose, maltose, fructose, sodium malonate, L-phenylalanine, L-arginine, L-glutamic acid, xylose and L-cysteine whereas it failed to utilize L-valine, histidine and rhamanose. Good growth could be detected within a temperature range of 20 to 45 ˚C. Growth in the presence of NaCl up to 7% was recorded and growth at different pH values from 5 to 8 was also recorded and finally no growth in the presence of growth inhibitors; sodium azide (0.01 w/v), phenol (0.1 w/v) and thallous acetate (0.001 w/v) (Table 3).

3.2.5. Taxonomy of actinomycete isolate, AZ-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 AZ-55 in relation to the most closest reference strain, viz. *Streptomyces lydicus*, it could be stated that actinomycetes isolate, AZ-55 is suggestive of being

likely belonging to *Streptomyces lydicus*, AZ-55 (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 related to *Streptomyces* sp., rather than to *Streptomyces lydicus* (Fig. 1). Multiple sequence alignment was conducted the sequences of the 16_S rDNA gene of *Streptomyces lydicus*. Computer assisted DNA searches against bacterial database similarly revealed that the 16_S rDNA sequence was 99% identical *Streptomyces lydicus* (Fig. 1).

3.3. Factors effecting on the biosynthesis of the antimicrobial agent produced by *Streptomyces lydicus*, AZ-55

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 28.0, 26.0, 21.0 & 19.0 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 5 days, after this maximum values 28.5, 27.4, 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.

3.3.3. 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 lydicus*, AZ-55 was at 30 ^oC, whereas, the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 28.8, 27.5, 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.

3.3.4. 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 lydicus*, AZ-55 was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 28.8, 27.5, 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 sucrose 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 lydicus*, AZ-55, sucrose> starch> mannitol> *meso*-insitol> Glucose> mannose> fructose.

3.3.6. Effect of different nitrogen sources: The nitrogen sources exhibited an increase in the level of antimicrobial agent production by *Streptomyces lydicus*, AZ-55. The effect of the used nitrogen sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces lydicus* AZ-55, $NaNo_3$>KNO_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. cervicea* ATCC 9763	Asp. niger, IMI 31276	*Asp. fumigatus*	*Asp. flavus*, IMI 111023	*F. oxysporum*	*P. chrysogenum*
AZ-55	28.0	28.0	28.0	29.5	27.0	26.0	22.0	**٠21.**	**21.0**	**19.0**	**0.0**	**17.0**	**20.0**	**0.0**
AZ-65	25.0	25.0	274.5	25.0	22.0	18.0	0.0	٠.٠	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	٠.٠	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	٠.٠	0.0	0.0	0.0	0.0	0.0	0.0
AZ-124	18.0	17.0	16.0	16.0	٠.٠	0.0	٠.٠	٠.٠	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	٠.٠	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	٠.٠	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	٠.٠	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Culture characteristics of the actinomycete isolate AZ-55.

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
1-Starch nitrate agar medium	Good	266-d.gray dark gray	77-m.ybr moderate yellowish brown	81-d.gy-ybr dark grayish yellowish brown
2-Tryptone yeast extract broth (ISP-1)	No growth	-	-	-
3-Yeast extract malt extract agar medium (ISP-2)	No growth	-	-	-
4-Oatmeal agar medium (ISP-3)	Good	264-1. gray light gray	77-m.ybr moderate yellowish brown	-
5-Inorganic salts starch agar medium (ISP-4)	moderate	264-1. gray light gray	57-1.br light brown	-
6-Glycerol – asparagine agar medium (ISP-5)	Good	264-1. gray light gray	57-1.br light brown	-
7-Peptone yeast extract iron agar medium (ISP-6)	Poor	264-1. gray light gray	77-m.ybr moderate yellowish brown	٥٨ m-br moderate brown
8-Tyrosine agar medium (ISP-7)	Poor	264-1. gray light gray	76-1-y-br Light yellowish Brown	77-m.ybr moderate 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-55.

Characteristic	Result	Characteristic	Result
Morphological characteristics:		Mannitol	++
Spore chains	Spiral	L- Arabinose	+
Spore mass	gray	*meso*-Insitol	++
Spore surface	smooth	Lactose	+
Color of substrate mycelium	Yellowish brown	Maltose	+
Diffusible pigment	Moderate 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	-	15	±
Tryptone – yeast extract broth	-	20-45	+
Degradation of:		50	-
Xanthin	+	**Growth at different pH values:**	
Esculin	+	3 - 4.5	-
H_2S Production	-	5-8	+
Nitrate reduction	-	8.5-12	-
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-55 in relation to reference strain ، *Streptomyces lydicus* (C.F. Hensyl,1994, Page693 and Table 27.5).

Characteristics	AZ-55	Hensyl (1994) *Streptomyces lydicus*
Morphological characteristics:		
Spore mass	Gray	Gray
Spore surface	Spiral	Spiral
Color of substrate mycelium	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	+	+
Egg-Youk	+	+
Degradation of:		
Esculine	+	+
Xanthine	+	+
H_2S production	-	-
Nitrate reduction	-	-
Utilization of:		
Sucrose	+	+
Mannitol	+	+
meso-Inositol	+	+
Rhamnose	-	-
L-Cysteine	+	+
L-valine	-	-
L-Phenylalanine	+	+
L-Histidine	-	-
Optimum growth temperature	30^0C	٢٥^{0}C
Optimum pH	٧	٧
Growth at NaCl (7.0 %)	+	+
Growth inhibitors		
Sodium azide (0.01)	-	-
Phenol (0.1)	-	-
Thallous acetate (0.001)	-	-

+=Positive, - =Negative.

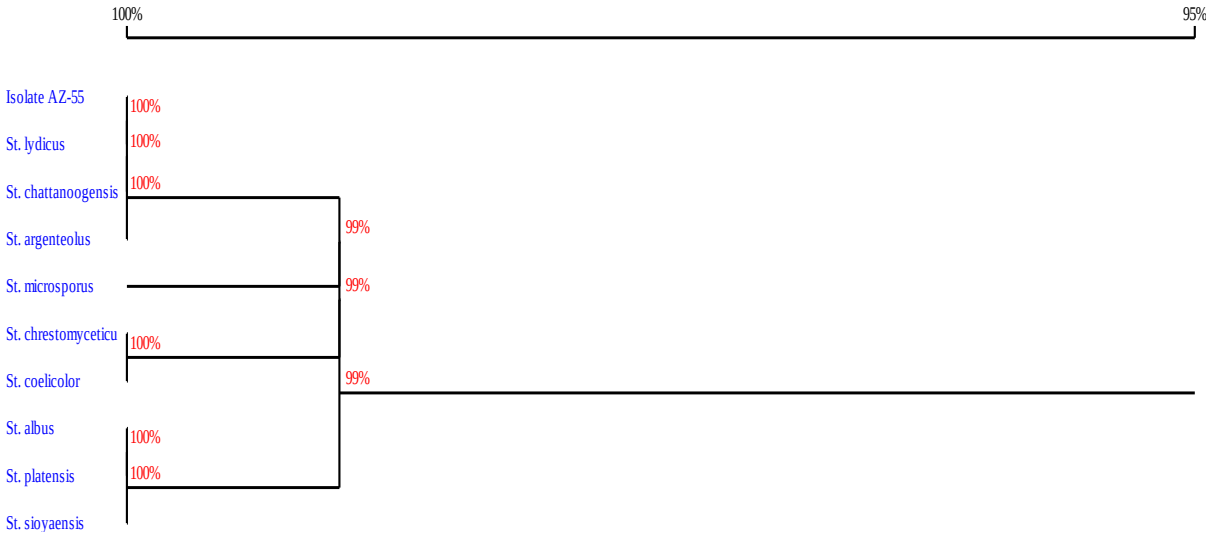

Figure 1. The phylogenetic position of the local Streptomyces sp. strain among neighboring species. The phylogenetic tree was based on the multiple sequence alignment comparisons of 16S rDNA sequences.

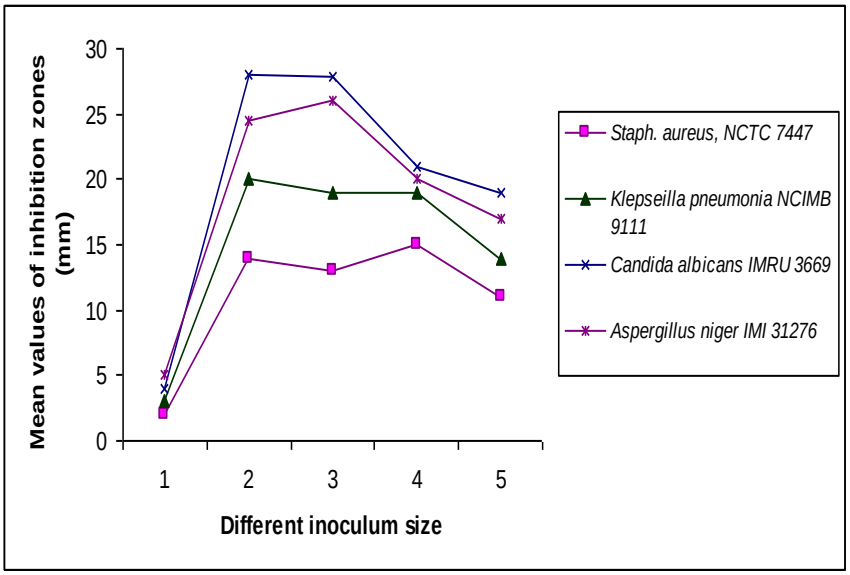

Figure 2. Effect of different inoculum size on the antibiotic yield produced by *Streptomyces lydicus*, AZ-55.

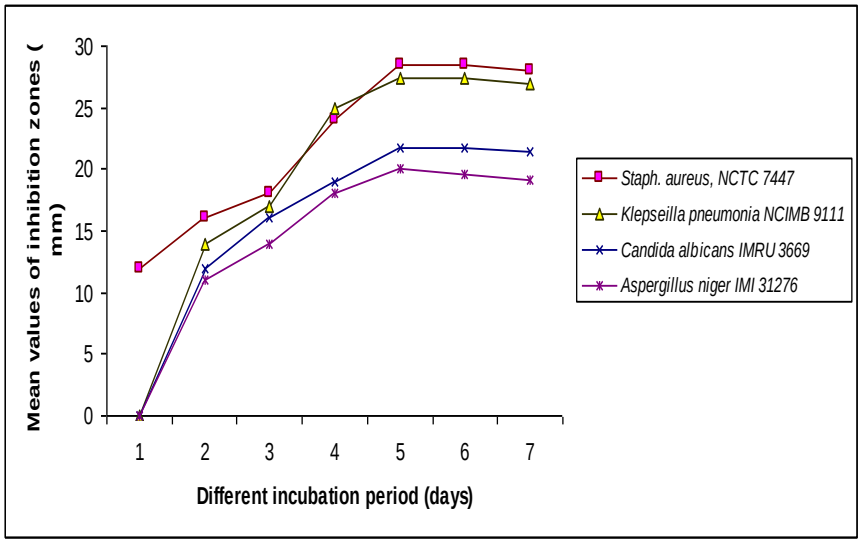

Figure 3. Effect of different incubation periods on the antimicrobial agent(s) biosynthesis produced by *Streptomyces lydicus*, AZ-55.

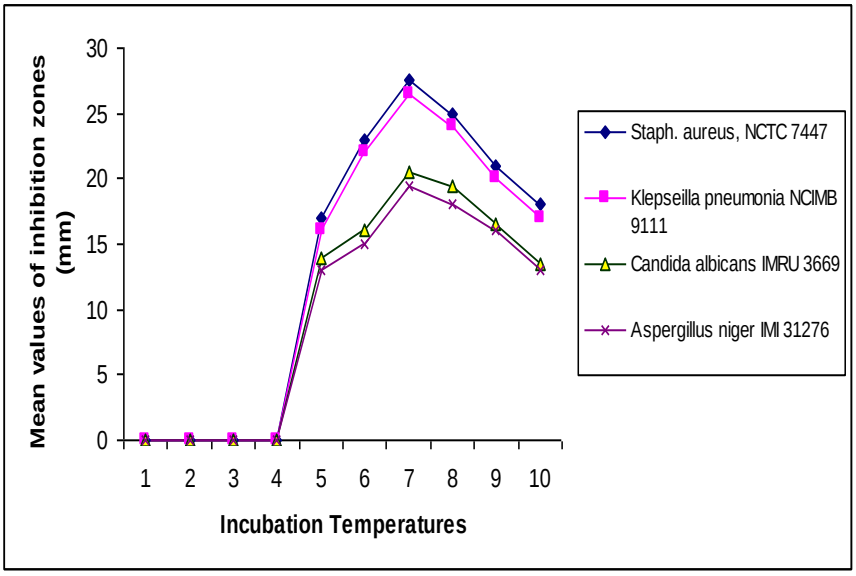

Figure 4. Effect of different incubation temperature on the antimicrobial agent(s) biosynthesis produced by *Streptomyces lydicus*, AZ-55 [1=5; 2=10; 3=15; 4=20; 5=25; 6=30; 7=35; 8=40; 9=45 and 10=50]

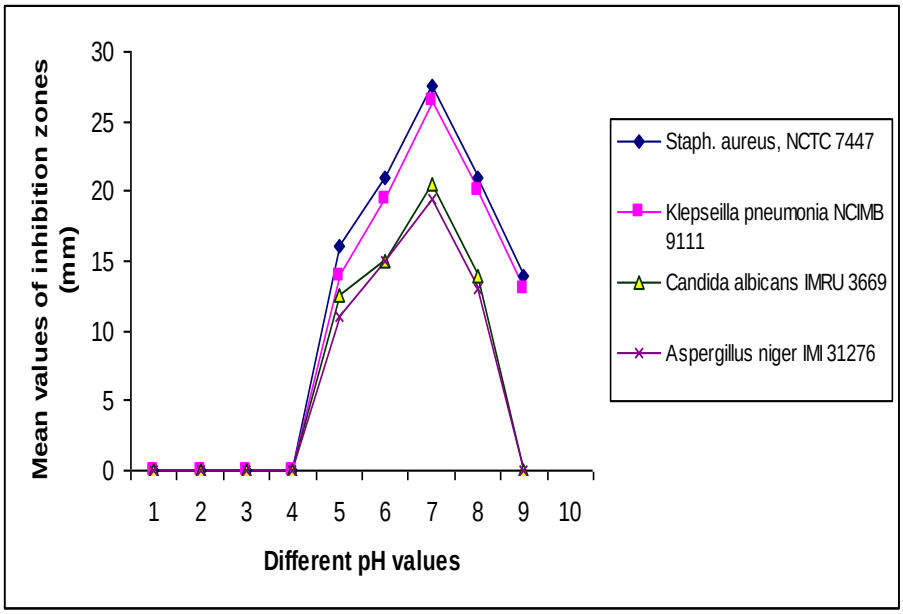

Figure 5. Effect of different pH values on the antimicrobial agent(s) biosynthesis produced by *Streptomyces lydicus*, AZ-55.

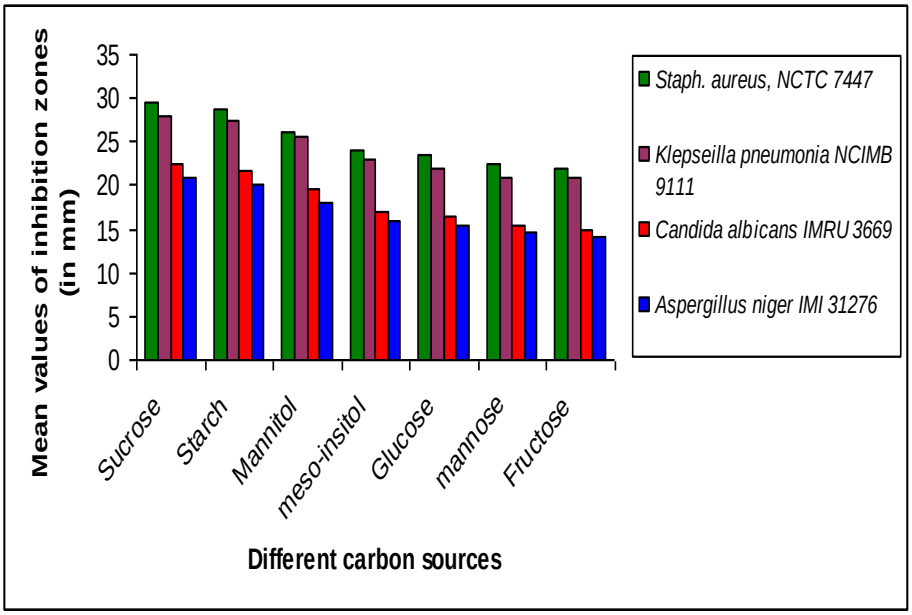

Figure 6. Effect of different carbon sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces lydicus*, AZ-55.

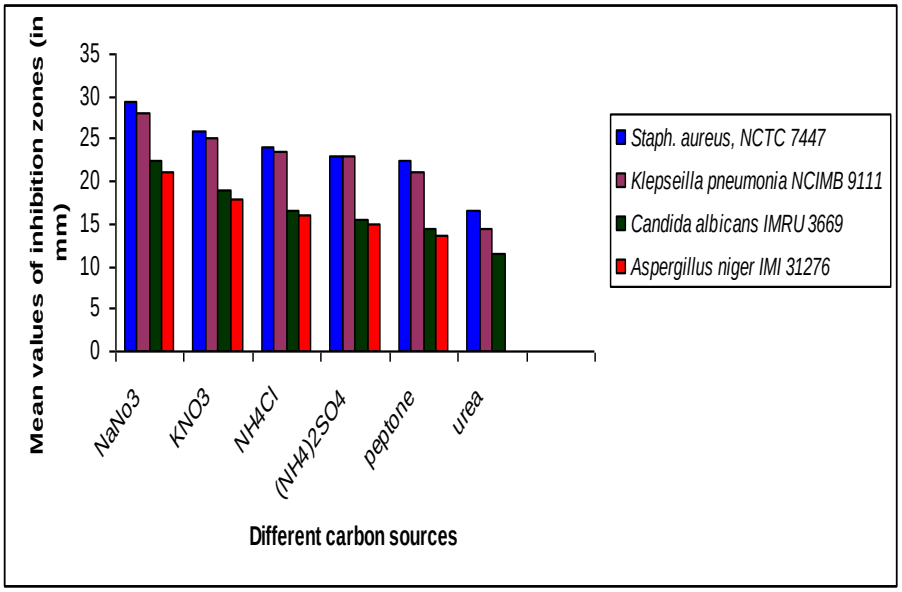

Figure 7. Effect of different nitrogen sources on the antimicrobial agent(s) biosynthesis produced by *Streptomyces lydicus*, AZ-55.

4. DISCUSSION

The increase in the frequency of multi-resistant pathogenic bacteria is created an urgent demand in the pharmaceutical industry for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity, which resist the inactivation processes exploited by microbial enzymes (Motta *et al.*, 2004). Eighty-eight actinomycete strains were isolated from twelve soil samples collected from Zagazig districted, Egypt. Only one actinomycete culture AZ-55 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 light gray; while spore surface is smooth, substrate mycelium is yellowish brown and no diffusible pigment was produced on ISP-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-55 was suggestive of being belonging to *Streptomyces lydicus*, AZ-55. 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 lydicus*, AZ-55, sharing 16s rRNA gene similarity matrix is 99%.

Maximum antimicrobial activity biosynthesis could be recorded that a different inoculum sizes for four discs; incubation period for five days (Adinarayana *et al.*, 2002); pH 7.0 (Atta, 2009 and 2010); temperature 30ºC (Khalifa, 2008); sucrose best carbon source (Hoshino *et al.*, 2004); $NaNO_3$ 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 lydicus*, AZ-55 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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