Studies on Isolation, Classification and Phylogenetic Characterization of antifungal substance produced by *Streptomyces albidoflavus*-143

Houssam M. Atta [1], Bahobail A.S.[2] and El-Sehrawi, M.H.[2]

[1.] Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. The present address: Biotechnology Department. Faculty of Science and Education- Al-Khurmah, Taif University; KSA.
[2.] Biology Dept. Faculty of Science - Taif University; KSA.
houssamatta@yahoo.com

Abstract: This work was carried out in the course of a screening program for specific actinomycetes bioactive substances that demonstrated inhibitory effects against some pathogenic strains. Twenty-eight actinomycete strains were isolated from soil sample collected from Farm Jabbar districted, Al-Khurmah governorate, KSA. One of the actinomycete culture, symbol 143 from two cultures was found to produce a wide spectrum antifungal agent (unicellular and filamentous fungi). The nucleotide sequence of the 16s RNA gene (1.5 Kb) of the most potent strain evidenced an 77% similarity with *Streptomyces albidoflavus*. From the taxonomic features, the actinomycetes isolate 143 matched with *Streptomyces albidoflavus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name *Streptomyces albidoflavus*, 143. The parameters controlling the biosynthetic process of antifungal agent formation including: inoculum size, different pH values, different temperatures, different incubation period, and different carbon and nitrogen sources were fully investigates.
[Houssam M. Atta, Bahobail A.S. and El-Sehrawi M.H. Studies on Isolation, Classification and Phylogenetic Characterization of antifungal substance produced by *Streptomyces albidoflavus*-143. New York Science Journal 2011;4(3):40-53]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork.

Key words: *Streptomyces albidoflavus*, Isolation, Classification and Phylogenetic Characterization, Parameter controlling antifungal activities.

1. Introduction

Streptomyces spp. are capable of producing microbial antibiotics with a wide variety of chemical structures. In particular, approximately 60% of antibiotics developed for agricultural use were isolated from *Streptomyces* spp. (Tanaka and Mura, 1993). It is interesting that *Streptomyces* strains continue to provide a larger number and wider variety of new antibiotics than any other actinomycete genus, suggesting that substantial numbers of *Streptomyces* species or strains with novel antibiotic productivity exist in nature (Okami and Hotta, 1988). In searches for bioactive antibiotics, *Streptomyces* strains have been isolated from various types of soils, including rice paddy, lake mud and water, deciduous forest, tropical forest, wasteland, and cave soils (Bhattacharya *et al.*, 2007).

Actinomycetes, particularly *Streptomyces* spp. have been a widely exploited group of microorganisms in the production of secondary metabolites and enzymes of commercial importance in medical and agricultural applications (Kumar and Gupta, 2006). Several chitinolytic enzymes have been identified in several *Streptomyces* spp. including *S. antibioticus, S. griseus, S. plicatus, S. lividans,S. aureofaciens* and *S. halstedii* (Taechowisan e*t al.,* 2003, Joo, 2005). Chitinase has received attention due to its use as a biocontrol agent (Mathivanan e*t al.*, 1998, Zhu e*t al.,* 2008) and also for developing transgenic plants (Dahiya e*t al.,* 2006). They have been used in a biological research for the generation of fungal protoplasts to degrade the fungal cell wall and also for being employed in human health care such as making ophthalmic preparations with chitinases (Narayana e*t al.,* 2007).

In the present study were describe the isolation of an actinomycete strain 143 from Farm Jabbar districted, Al-Khurmah governorate, KSA, which generates a production the bioactive substances that demonstrated inhibitory affects against fungal 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 antifungal agent formation were fully investigates.

2. Material and Methods

2.1. Actinomycete isolate

The actinomycete isolate 143 was isolated from soil sample collected from Farm Jabbar districted, 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.

2.2.2. Gram Negative

Klebsiella pneumonia, NCIMB, 911; *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, *Aspergillus flavus*, IMI 111023, *Fusarium oxysporum* and *Penicillium 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 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 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 antifungal 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 antifungal activities

The metabolites of the actinomycete isolate 143 exhibited various degrees of activities against unicellular fungi and filamentous fungi (Table 1).

3.2. Characterizations of the actinomycete isolate

3.2.1. Morphological characteristics

Spore chains are retiflexibiles, spore mass is white, yellowish gray; spore surfaces are smooth plate (1);

substrate mycelium is light yellowish brown and diffusible pigment absent or moderate yellowish brown plate (1).

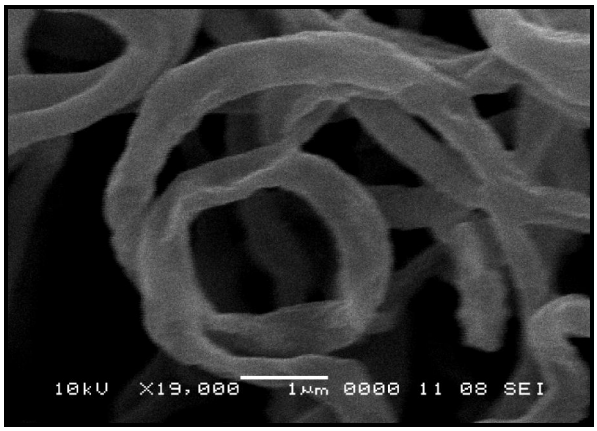

Plate 1. Scanning electron micrograph of the actinomycete isolate 143 growing on starch nitrate agar medium spore chain is retiflexibiles shape and spore surfaces is smooth (19.000).

3.2.2. Cell wall hydrolysate

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

3.2.3. Physiological and biochemical characteristics

The actinomycete isolate 143 could hydrolyze; starch, lipid and protein, but pectin, lecithin and catalase were negative. Melanin pigment not produced, degradation of xanthine, esculine, production of H_2S, decomposition of urea, and utilization of citrate were positive but nitrate reduction were negative. The isolate 143 could utilize mannose, glucose, galactose, mannitol, arabinose, trehalose, fructose, starch, cycteine, histidine, phenylalanine, lysine, arginine and tyrosine, but not utilize xylose, rhamenose, raffinose, *meso*-inositole, lactose, maltose, sucrose, valine and serine. The actinomycete isolate 143 grows well on 0.5% to 7% NaCl concentrations, but no growth in the presence of 10% NaCl. Good growth could be detected within a temperature range of 25 to 40ºC. The growth of actinomycete isolate 143 is not inhibited in the presence of sodium azide, phenol and thallus acetate. The actinomycete isolate 143 are active against *Saccharomyces cerevisiae,* ATCC 9763, *Candida albicans* IMRU 3669 and *Asp. niger,* IMI 31276 (Table 2).

3.2.4. Color and culture characteristics

The actinomycete isolate 143 good growth on starch nitrate agar medium, aerial mycelium was white, substrate mycelium was light yellowish brown, and diffusible pigment was moderate yellowish brown. Moderate growth on Yeast extract - Malt extract agar medium (ISP-2), the aerial mycelium was yellowish gray, substrate mycelium was grayish yellow, and the diffusible pigment was moderate yellowish brown. Moderate growth on oat meal agar medium (ISP-3), aerial mycelium was white, substrate mycelium was light yellowish brown, and diffusible pigment not produced. Good growth on inorganic salts-starch agar medium (ISP-4), the aerial mycelium was white, substrate mycelium was light yellowish brown, and the diffusible pigment not produced. Moderate growth on glycerol-asparagine agar medium (ISP-5), the aerial mycelium was yellowish gray, substrate mycelium is Light yellowish brown and the diffusible pigment not produced. Moderate growth on Peptone yeast extract-malt extract iron agar medium (ISP-6), the aerial mycelium is white, the substrate mycelium is light yellowish brown and the diffusible pigment is moderate yellowish brown. Moderate growth on tyrosine agar medium (ISP-7), the aerial mycelium is white, substrate mycelium is light yellowish brown, and the diffusible pigment is moderate yellowish brown, Moderate growth on Tryptone-yeast extract broth (ISP-1), the aerial mycelium is white, substrate mycelium is light yellowish brown and the diffusible pigment not produced (Table 3).

3.3. Identification of actinomycete isolate- 143

This was performed basically according to the recommended international Key's viz. (Buchanan and Gibsons, 1974; Williams, 1989; and Hensyl, 1994) and Numerical taxonomy of *Streptomyces* species program (PIB WIN). On the basis of the previously collected data and in view of the comparative study of the recorded properties of 143 in relation to the most closest reference strain, viz. *Streptomyces albidoflavus* it could be stated that the actinomycetes isolate143 is suggestive of being likely belonging to *Streptomyces albidoflavus* -143 (ID Score 0.98094) (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 77% similarity with *Streptomyces albidoflavus* (Fig. 1).

3.5. Parameters controlling the biosynthesis of the antifungal agent

3.5.1. Inoculum size

Data illustrated in (Fig. 2) showed the relation between antibiotic productivity and different inoculum sizes. Maximum antifungal activity production could be recorded that a different inoculum sizes for three discs, after this maximum values 26.0, 24.0, 23.0 and 22.5 in case of *Candida albicans* IMRU 3669; *Aspergillus niger*, IMI 31276*; Aspergillus flavus*, IMI 111023 ; *Fusarium oxysporum*, respectivily.

3.5.2. Incubation period

Data represented in (Fig. 3) showed that, the optimum temperature capable of promoting antifungal agent biosynthesis by *Streptomyces albidoflavus* -143 was at 30ºC, where, the diameter of inhibition zone resulted from antifungal agent productivity reached up to 26.2, 24.2, 23.0 and 22.8 in case of *Candida albicans* IMRU 3669; *Aspergillus niger*, IMI 31276*; Aspergillus flavus*, IMI 111023 ; *Fusarium oxysporum*, respectivily.

3.5.3. pH value

The results represented in (Fig. 4) that, the optimum initial pH value capable of promoting antifungal agents biosynthesis by *Streptomyces albidoflavus* -143 was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antifungal agent productivity reached up to 26.2, 24.2, 23.0 and 22.8 in case of *Candida albicans* IMRU 3669; *Aspergillus niger*, IMI 31276*; Aspergillus flavus*, IMI 111023 ; *Fusarium oxysporum*, respectivily.

3.5.4. Incubation temperature

Data illustrated in (Fig. 5) showed the relation between antibiotic productivity and time of incubation. The level of antifungal agent yield increased gradually with increasing the incubation period up to the end of 5 days, after this maximum values 26.2, 24.2, 23.0 and 22.8 in case of *Candida albicans* IMRU 3669; *Aspergillus niger*, IMI 31276*; Aspergillus flavus*, IMI 111023; *Fusarium oxysporum*, respectivily.

3.5.5. Carbon source

Data given in (Fig. 6) indicated that the addition of different equimolecular carbon sources for production of antifungal agent revealed that starch is the best carbon source for biosynthesis antifungal substances with concentration 2.0g/100. The effect of the used carbon sources in production of antifungal agent could be arranged in the following descending manner; for *Streptomyces albidoflavus*-143, starch> mannitol> glucose> fructose> arabinose> galactose.

3.5.6. Nitrogen source

Data given in (Fig. 7) indicated that the addition of different nitrogen sources exhibited an increase in the level of antifungal agent production by *Streptomyces albidoflavus* -143 where sodium nitrate was found to be the best nitrogen source for the antifungal 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 albidoflavus* -143, $NaNo_3$>KNO_3> NH_4Cl> peptone> urea> $(NH_4)_2SO_4$.

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

Test organisms / Actinomycete isolates	*Mean values of inhibition zones (in mm)									
	Gram positive bacteria		Gram negative bacteria		filamentous fungi				Unicellular fungi	
	Bacillus Subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Klebsiella pneumonia,* NCIMB, 9111	*P. aeruginosa,* ATCC 10145	*Asp. flavus IMI 111023*	*Asp. niger IMI 31276*	*P. chrysogenum*	*Fusarium oxysporum*	*S. cerevisiae,* ATCC 9763	*Candida albicans* IMRU 3669
143	**0.0**	**0.0**	**0.0**	**0.0**	**23.0**	**24.0**	**21.0**	**22.4**	**25.0**	**26.0**
166	0.0	0.0	0.0	0.0	21.0	22.0	17.0	20.0	23.0	23.0

Mean values of determination was calculated

Table 2. The morphological, physiological and biochemical characteristics of the actinomycete isolate, 143

Characteristic	Result
Spore mass	White, Yellowish gray
Spore surface	Smooth
Spore chain	Retiflexibiles
Color of substrate mycelium	Light yellowish brown
Diffusible pigment	Absence or moderate yellowish brown
Diaminopimelic acid (DAP)	LL-DAP
Sugar Pattern	Not detected
Hydrolysis of**:** Protein, Starch and Lipid Pectin and Egg-yolk (lecithin)	+ -
Catalase test	-
Production of melanin pigment on: Peptone yeast- extract iron agar and Tyrosine agar medium	 -
Degradation of**:** Esculin and Xanthin	+
H_2S Production	+
Nitrate reduction	-
Citrate utilization	+
Urea test	+
Utilization of	
D-Xylose	-
D- Mannose	+
D- Glucose	++
D- Galactose	+
Rhamnose	-
Raffinose	-
Mannitol	+++
L- Arabinose	+
meso-Inositol	-
Lactose	-
Maltose	-
Trehalose	+
D-fructose	++
Sucrose	-
Starch	+++
L-Cycteine	+
L-Valine	-
L-Histidine	++
L-Phenylalanine	+
L-Lysine	+
L-Arginine	+
L-Serine	-
L-Tyrosine	+
Growth with: Thallous acetate (0.001), Sodium azide (0.01) and Phenol (0.1)	 +
Growth temperature	30 ºC (25-40 ºC)
Growth at 7% NaCl concentration	+
Antifunfal activity against	
Saccharomyces cerevisiae, ATCC 9763 and *Candida albicans* IMRU 3669	+
Asp. niger, IMI 31276	+

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

Table 3. Cultural characteristics of the actinomycete isolate 143

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
1- Starch-nitrate agar medium	Good	*263 - white white	*76.1.y Br light yellowish brown	77 m-y Br moderate yellowish brown
2- Yeast extract - Malt extract agar medium (ISP-2)	Moderate	*93-y-Gray yellowish gray	*90-gy-y grayish yellow	77 m-y Br moderate yellowish brown
3- Oat-meal agar medium (ISP-3)	Moderate	263 - white white	76.1.y Br light yellowish brown	-
4- Inorganic salts-starch agar medium (ISP-4)	Good	263 - white white	76.1.y Br light yellowish brown	-
5- Glycerol-Asparagine agar medium (ISP-5)	Moderate	93-y-Gray yellowish gray	76.1.y Br light yellowish brown	-
6- Melanin test:				
a- Peptone yeast extract-iron agar medium (ISP-6)	Moderate	263 - white white	76.1.y Br light yellowish brown	77 m-y Br moderate yellowish brown
b- Tyrosine agar (ISP-7)	Moderate	263 - white white	*77-m-y Br moderate yellowish brown	77 m-y Br moderate yellowish brown
c- Tryptone-yeast extract broth (ISP-1)	Moderate	263 - white white	76.1.y Br light yellowish brown	-

The color of the organism under investigation was consulted using the ISCC-NBS color - Name charts II illustrated with centroid color. **ISP** = international *Streptomyces* project; **76.1.y Br** =light yellowish brown; **77-m-y Br** = moderate yellowish brown ; **90-gy-y**= grayish yellow; **93-y-Gray**= yellowish gray**; 263 – white**= white

Table 4. Numerical taxonomy of *Streptomyces* species program (PIB WIN) (*Streptomyces* species) J. Gen Microbiol. 1989 13512-133 lang

Characteristic	143	*Streptomyces albidoflavus*
Dianinopimelic acid (DAP)	LL-diaminopimelic acid	LL-diaminopimelic acid
Sugar pattern	Not detected	Not detected
Spore chain rectiflexibles	+	+
Spore mass Spiral	-	-
Spore mass red	-	-
Spore mass gray	-	-
Diffusible pigment red/orange	-	-
Diffusible pigment yellow/brown	+	+
Melanin pigment 1-Peptone yeast extract-iron agar medium (ISP-6) 2-Tyrosine agar medium (ISP-7)	 - -	 - -
Lecithinase activity	-	-
Lipolysis activity	+	+
Pectin hydrolysis	-	-
Nitrate reduction	-	-
H_2S production	+	+
Degradation of Xanthin	+	+
Growth at 45°C	-	-
Growth at NaCl 7% (w/v)	-	-
Growth with Phenol (0.1 % w/v)	+	+
Utilization of:		
L- Cysteine	+	+
L- Valine	-	±
L- phenylalanine	+	+
L- Histadine	-	±
Sucrose	-	±
meso-Inositol	-	±
Mannitol	+	+
Rhamnose and Raffinose	-	-

No.	Key	Source	Identification	ID Score
1	**143**	**Farm Jabbar districted**	***Streptomyces albidoflavus***	**0.98094**

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

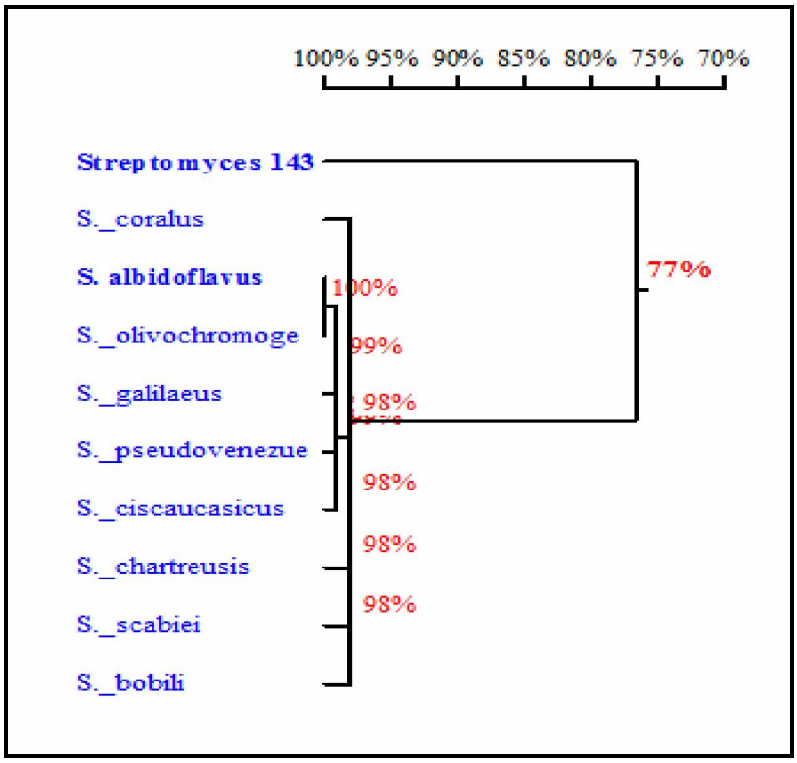

Figure 1. The phylogenetic position of the local *Streptomyces* sp. strain among neighboring species. The phylogenetic tree was based on the multiple alignments options of 16_S rDNA sequences

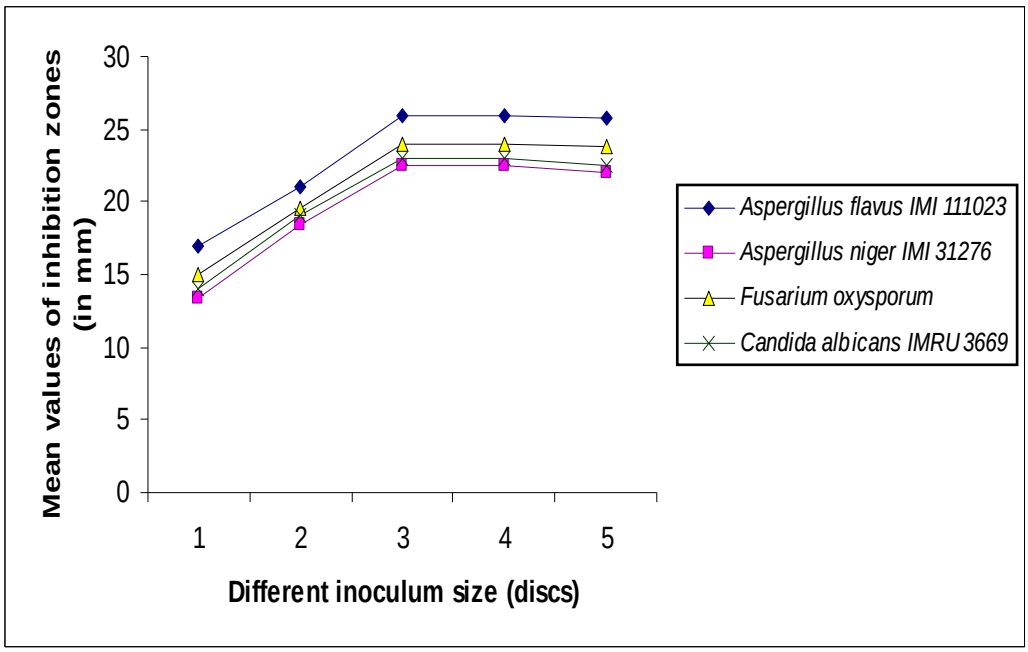

Figure. 2. Effect of different inoculum size on the antifungal production by *Streptomyces albidoflavus*, 143

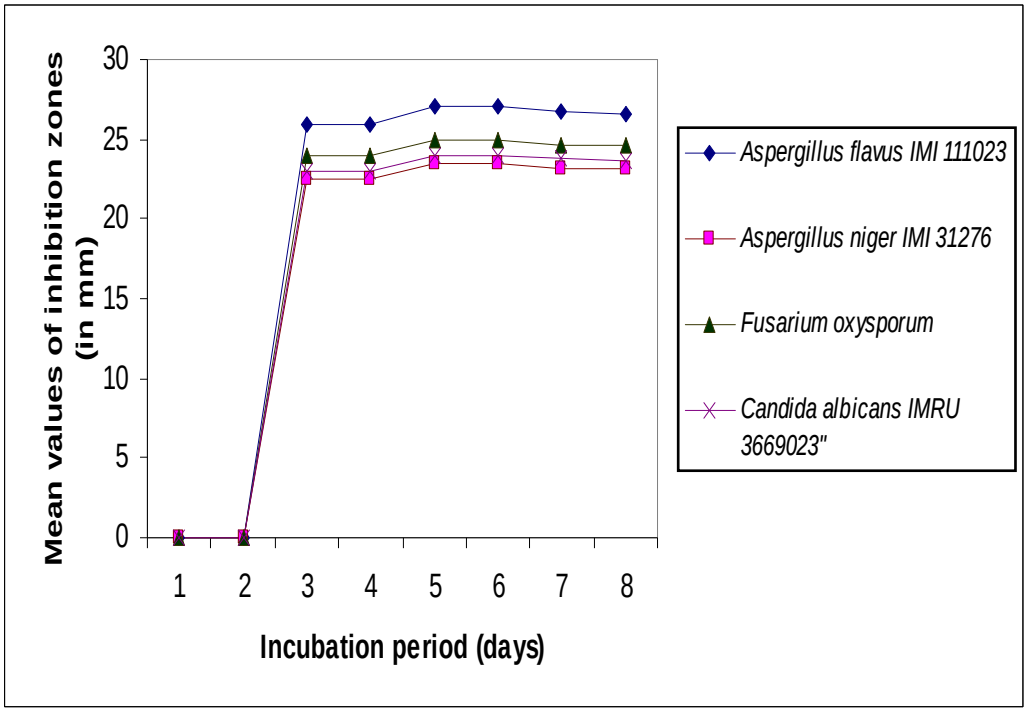

Figure 3. Effect of incubation period on the antifungal production by *Streptomyces albidoflavus*, 143

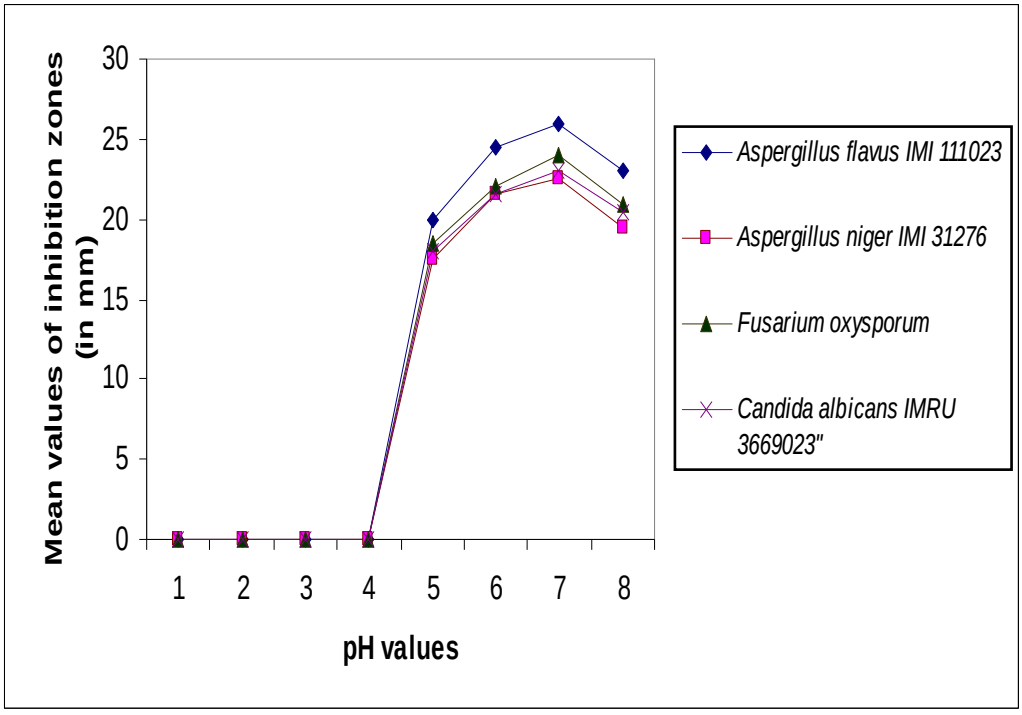

Figure 4. Effect of pH values on the antifungal production by *Streptomyces albidoflavus*, 143

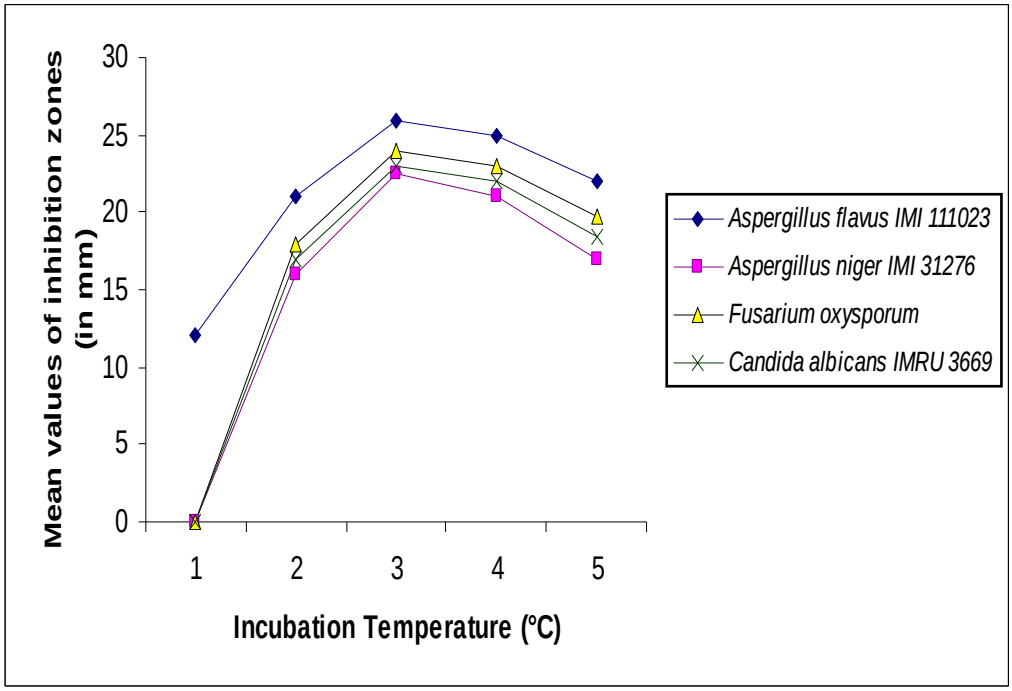

Figure 5. Effect of different incubation Temperature (°C) on the antifungal production by *Streptomyces albidoflavus,* 143

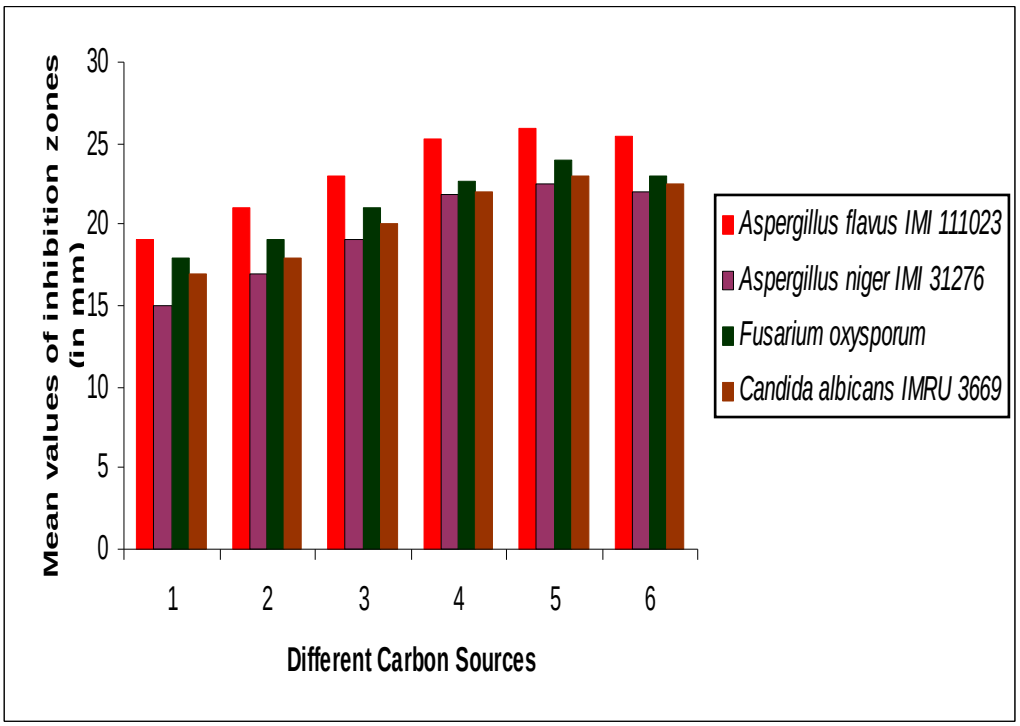

Figure 6. Effect of different carbon sources on the antifungal production by *Streptomyces albidoflavus,* 143 (1= Galactose; 2= Arabinose; 3= Fructose; 4= Glucose; 5= Starch and 6= Mannitol)

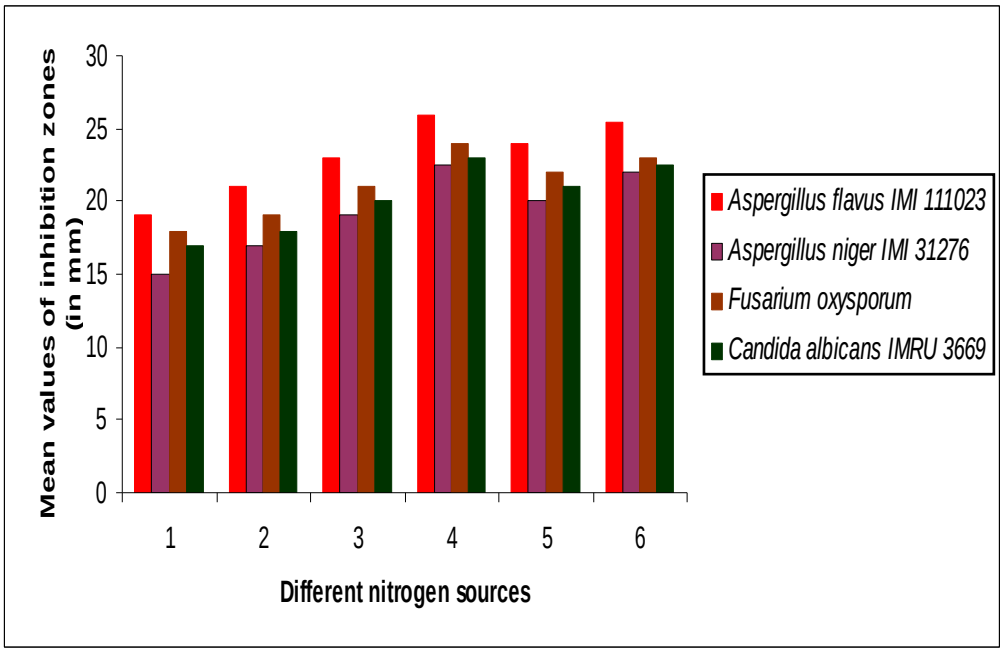

Figure 7. Effect of different carbon sources on the antifungal production by *Streptomyces albidoflavus,* 143 (1= Urea; 2= $(NH_4)_2SO_4$; 3= Peptone; 4= $NaNo_3$; 5= NH_4Cl and 6 = KNO_3)

4. DISCUSSION

The actinomycete isolate, 143 was isolated from a soil sample collected from Farm Jabbar districted, Al-Khurmah governorate, KSA. The isolate was growing on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. The actinomycete isolate, 143 exhibited a wide spectrum antifungal agent. 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) and Numerical taxonomy of *Streptomyces* species program (PIB WIN). For the purpose of identification of actinomycete isolate, the morphological characteristics and microscopic examination emphasized that the spore chain is Retiflexibiles. Spore mass is white and yellowish gray; while spore surface is smooth, substrate mycelium is light yellowish brown and no diffusible pigment was produced on ISP-media No. 3, 4 & 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 143 was suggestive of being belonging to *Streptomyces albidoflavus,* 143 (ID Score 0.98094) which can produce a broad-spectrum antifungal agent (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 albidoflavus* (similarity matrix is 77%). Similar results had been recorded by various workers (Augustine *et al.,* 2005 and Thenmozhi and Kannabiran, 2010). For optimizing the biosynthesis of the antifungal agent from *Streptomyces albidoflavus,* 143, different cultural conditions such as inoculum size, pH, temperature, and incubation period, effect of different carbon and nitrogen sources, sodium nitrate, was studied. The maximum biosynthesis was achieved at the end of an incubation period of 5 days at pH 7.0 for the antifungal agent production using three discs of actinomycete culture. Similar results had been recorded by various workers (Adinarayana *et al.,* 2002; Kharel *et*

al., 2004; Augustine *et al.,* 2005 and Prema *et al.,* 2009). The fact that maximum yield of the antifungal 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 antifungal agent indicated that *Streptomyces albidoflavus,* 143 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 and identification based on the physiology, biochemical and cultural characteristics. Further work should be focused in most potent *Streptomyces* isolate for production the antifungal activities against pathogenic fungi (unicellular and filamentous fungi) and studies the parameters controlling the biosynthetic process of antifungal agent formation.

Corresponding Author:
Prof. Dr. Houssam M. Atta
Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. The present address: Biotechnology Department. Faculty of Science and Education. Al-Khurmah, Taif University; KSA.
E-mail: houssamatta@yahoo.com

References

1. Adinarayana K.; Ellaiah P.; Srinivasulu B.; Bhavani, R. and Adinarayana G. (2002). Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces marinensis* under solid-state fermentation. Andhra University, Process Biochemistry 38, 1565-1572.
2. Ammar, M.S.; EL- Esawey, M.; Yassin, M. and Sherif, Y.M. (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., Vol. 3. Jan. 1998: PP. 60 - 90.
3. Asha devi, N.K., Jeyarani, M. and Balakrishnan, K. (2006). Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. Pak. J. Biol. Sci., 9(3): 470-472.
4. Atta, H. M. (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, 2010.
5. Atta, H. M.; Abul-hamd A. T. and Radwan, H. G. (2009). Production of Destomycin-A antibiotic by *Streptomyces* sp. using rice straw as fermented substrate. Comm. Appl. Biol. Sci, Ghent University, 74 (3): 879-897, 2009.
6. Atta, H.M.; El-Sehrawi, M. H.; Awny, N.M. and El-Mesady, N.I. (2011). Microbiological Studies on Cultural, Physiological Characteristics and Antimicrobial Activities of *Streptomyces Cyaneus*-AZ-13Zc. Researcher, 2011;3 (2): 80-90.
7. Augustine, S.K.; Bhavsar, S.P. and Kapadnis, B.P. (2005). A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23**.** J Biosci. 2005 Mar;30 (2):201-11.
8. Becker, B.; Lechevalier , M. P . Gordon, R. E. and Lechevalier, H. A. (1964). Rapid Differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. APPl. Microbiol., 12: 421 – 423.
9. Bhattacharya, D.; Nagpure, A.; Gupta, R.K. (2007). Bacterial chitinases: properties and potential. Critical Rev. Biotechnol., 27, 21-28.
10. Buchanan, R. E. and Gibbson, N. E (Ed.) (1974). Bergey's Manual of Determinative bacteriology 8th edition. The Williams & Wilkins company/ Baltimore.
11. Chapman, G.S. (1952). A simple method for making multiple tests on a microorganism. J. Bacteriol. 63:147.
12. Cowan, S .T. (1974). Cowan and Steel' s Manual For The Identification Of Medical Bacteria 2 nd. Edition Cambridge, Univ. Press.
13. Criswell, D.; Tobiason, V. L.; Lodmell, J. S. and Samuels, D. S. (2006). Mutations Conferring Aminoglycoside and Spectinomycin Resistance in Borrelia burgdorferi. Antimicrob. Agents Chemother. 50: 445-452.
14. Dahiya, N.; Tewari, R.; Hoondal, G.S. (2006). Biotechnological aspects of chitinolytic enzymes: a review. *Appl. Microbiol. Biotechnol.*, 71, 773-782.
15. Edwards, U., Rogall, T.; Bocker, H.; Emade, M. and Bottger, E. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16s ribosomal DNA. Nucleic Acid Res. 17: 7843-7853.

16. El-Naggar M. Y. (2007): Kosinostatin, a Major Secondary Metabolite Isolated from the Culture Filtrate of *Streptomyces violaceusniger* Strain HAL64, The Journal of Microbiology, p. 262-267.
17. El-Naggar, M.Y., Hassan, M.A. Said W.Y., and El-Aassar. S.A. (2003). Effect of support materials on antibiotic MSW2000 production by immobilized *Streptomyces violatus*. J. Gen. Appl. Microbiol. 49, 235-243.
18. Elwan, S .H.; El-Nagar, M. R. and Ammar, M. S. (1977). Characteristics of Lipase(s) in the growth filtrate dialystate of *Bacillus stearothermophilus* grown at 55 ºC using a tributryin- cup plate assay. Bull. Of the Fac. of Sci ., Riyadh Univ ., vol .8 : 105 – 119.
19. Gordon ,R.E., Barnett, D.A. , Handehan, J.E. and Pang, C.H. (1974). *Nocardia coeliaca, Nocardia autotrophica* and *Nocardia* Strain. International Journal of Systematic Bacteriology. 24:54-63.
20. Gordon, R.E. (1966). Some Criteria for The Recognition of *Nocardia madura* (Vincent) Blanchord. J. General Microbiology, 45:355-364.
21. Hankin, L.; Zucker, M. and Sands, D.C. (1971). Improved solid medium for the detection and enumeralion of proteolytic bacteria. Appl. Microbiol., 22:205-509.
22. Hensyl, W. R. (1994). Bergey's Manual of Systematic Bacteriology 9 th Edition. John. G. Holt and Stanley, T. Williams (Eds.) Williams and Wilkins, Baltimore, Philadeiphia, Hong kong, London, Munich, Sydney, Tokyo.
23. Howells, J. D.; Anderson, L. E.; Coffey, G. L.; Senos, G. D.; Underhill, M. A.; Vogler, D. L.; Ehrlich, J. B. (2002). A new Aminoglycosidic Antibiotic Complex: Bacterial Origin and Some Microbiological Studies. Antimicrob Agents Chemother. Aug; 2 (2):79–83.
24. Jones, K. (1949). Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristics. J. Bacteriol., 57: 141-145.
25. Joo, J.G. (2005). Purification and characterization of an extracellular chitinase from the antifungal biocontrol agent *Streptomyces halstedii*. Biotechnol. Lett., 27, 1483-1486.
26. Kavanagh, F. (1972). Analytical Microbiology. Vol. 2, Acad. Press, New York.
27. Kenneth, L.K. and Deane, B.J. (1955). Color universal language and dictionary of names. United States Department of Commerce. National Bureau of standards. Washington, D.C., 20234.
28. Kharel, M. K.; Basnet, D. B.; Lee, H. C.; Liou, K.; Woo, J. S.; Kim, B. G. and Sohng, J. K. (2004). Isolation and characterization of the tobramycin biosynthetic gene cluster from *Streptomyces tenebrarius*. FEMS Microbiol. Lett. 230:185–190.
29. Kumar, D.; Gupta, R.K. (2006). Biocontrol of wood rotting fungi. Indian J. Biotechnol., 5, 20-25.
30. Lechevalier, M.P and Lechevalier, H.A. (1968). Chemical composition as a criterion in the classification of aerobic actinomycetes. J. Systematic Bacteriology. 20: 435-443.
31. Mathivanan, N.; Kamilan, V.; Murugesan, K. (1998). Purification, characterization and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis*. Canadian J. Microbiol., 44, 646-651.
32. Nitsh, B. and Kutzner, H.J. (1969). Egg-Yolk agar as diagnostic medium for *Streptomyces*. sp., 25:113.
33. Narayana, K.J.P.; Prabhakar, P.; Vijayalakshmi, M.; Vekateswarlu, Y.; Krishna, P.S.J. (2007). Biological activity of phenylpropionic acid from a terrestrial *Streptomycetes*. *Polish J. Microbiol.*, 56, 191- 197.
34. Okami, Y., and Hotta, K. (1988). Search and discovery of new antibiotics, p. 33-67. In M. Goodfellow, S. T. Williams, and M. Mordaski (ed.), Actinomycetes in biotechnology. Academic Press, London, United Kingdom.
35. PIB WIN (1989). Numerical taxonomy of *Streptomyces* species program (PIB WIN) (*Streptomyces* species J. Gen Microbiol. 1989 13512-133.
36. Prema, P.; Audline, S. P.; Suja; Ranjani, S. S. and Immanuel, G. (2009). UV/VIS, FTIR spectrum and Anticandidial activity of *Streptomyces* strains. The Internet Journal of Microbiology. 2009 Volume 7 Number 2.
37. Pridham, T.G., and Gottlieb, D. (1948). The utilization of carbon compounds by some actinomycetes as an aid for species determination. J. Bacteriol., 56(1):107-114.
38. Pridham, T.G.; Anderson, P.; Foley, C.; Lindenfelser, L.A.; Hesselting, C.W. and Benedict, R.G. (1957). A section of media for maintenance and taxonomic study of *Streptomycetes*. Antibiotics Ann. pp. 947-953.
39. Sekiguchi, J.I.; Miyoshi-Akiyama, T.; Augustynowicz-Kopec, E.; Zwolska, Z.,; Kirikae, F.; Toyota, E.; Kobayashi, I.; Morita, K.; Kudo, K.; Kato, S.; Kuratsuji, T.; Mori, T. and Kirikae, T. (2007). Detection of Multidrug Resistance in Mycobacterium tuberculosis. *J. Clin. Microbiol.* 45: 179-192
40. Sambrook, J.; E. F. Fritsch and T. Maniaties,

(1989). Molecular cloning. A laboratory Manual 2ed Cold Spring, Harbor Laboratory press, Cold Spring Harbor, New York, USA.
41. Sanger, F., S. Nicklen and A.R. Coulson (1977). DNA sequencing with chain terminator inhibitors. *Proc. Natl.Acad. Sci*. 74: 5463-5467.
42. Selvin J. S.; Joseph, K.R.; Asha, W.A.; Manjusha, V.S.; Sangeetha, D.M.; Jayaseema, M.C.; Antony, A.J. and Denslin V. (2004). Antibacterial potential of antagonistic *Streptomyces* sp. Isolated from marine sponge Dendrilla nigra. FEMS Microbiology Ecology 50, 117–122.
43. Taechowisan, T.; J.F. Peberdy,; S. Lumyong, (2003). Chitinase production by endophytic *Streptomyces aureofaciens* CMU Ac 130 and its antagonism against phytopathogenic fungi. Annal. Microbiol., 53, 447-461.
44. Tanaka, Y. T., and S. Mura, (1993). Agroactive compounds of microbial origin. Annu. Rev. Microbiol. 47:57-87.
45. Thenmozhi, M. and K. Kannabiran, (2010). Studies on Isolation, Classification and Phylogenetic Characterization of Novel Antifungal *Streptomyces* sp. VITSTK7 in India. Current Research Journal of Biological Sciences 2(5): 306-312, 2010.
46. Williams, S.T. (1989). Bergey's Manual of Systematic bacteriology Vol. 4, Stanley T., Williams. Williams and Wilkins (Eds.), Baltimore, Hong kong, London, Sydney.
47. Williams, S.T. and F. L. Davies (1965). Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. Gen. Microbiol., 38:251-262.
48. Zhu, Y.; P. Jieru; S. Junzhi.; G. Xiong (2008). Isolation and characterization of a chitinase gene from entomopathogenic fungus *Verticillium lecanii*. Braz. J. Microbiol., 39, 314-320.

1/21/2011