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Actinomycetes Antagonizing Plant and Human Pathogenic Fungi. II. Factors Affecting Antifungal Production and Chemical Characterization of the Active Components

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ABSTRACT

A study was carried out to investigate the factors affecting the antifungal production of four actinomycetes species i.e. *Streptomyces lydicus*, *S. ederensis*, *S. erumpens* and *S. antimycoticus.* Glycerol was the best carbon source for antifungal production by the selected species followed by starch. Glutamic acid was found to be the best nitrogen source for antifungal production by *S. lydicus* and *S. erumpens*, ammonium sulphate for *S. ederensis*, while soybean for *S. antimycoticus*. The optimum temperature for antifungal production by *S. lydicus* and *S. ederensis* was 24°C, while for *S. erumpens* and *S. antimycoticus* it was 28°C. The optimum pH value for antifungal production by the four selected species was 7.0. It was noticed that *S. lydicus*, *S. erumpens* and *S. antimycoticus* each produces four active components, while *S. ederensis* produces only three active components. The chemical analysis of the culture filtrates of the species revealed the presence of 13 chemical compounds in the culture filtrate of *S. lydicus*, 12 chemical compounds in the culture filtrate of each of *S. ederensis* and *S. erumpens* and 11 chemical compounds in the culture filtrate of *S. antimycoticus*.

Key Words: Actinomycetes; Human pathogens; Plant pathogens; Antifungal; Production

INTRODUCTION

Readily metabolized carbon sources, such as glucose, can suppress antibiotic production by preventing the synthesis of a key enzyme in the biosynthetic pathway. Because of its resemblance to the glucose effect on catabolic pathways, the phenomenon has been referred to as "catabolite repression" (Gallo & Katz, 1972).

Biosynthesis of antibiotics is often regulated by carbon catabolites, nitrogen catabolites, phosphates and other metabolites. High production of antibiotics has generally been achieved by cultivating organisms in media containing slowly utilized carbon and/ or nitrogen sources or under conditions which allow a slow supply of these nutrients (Drew & Demain, 1977).

Chatterjee *et al*. (1983) studied the nutritional requirements for chloramphenicol biosynthesis in *Streptomyces venezuelae* and they indicated that in a media with ammonium sulphate as a non-restricting source of nitrogen, chloramphenicol production varied with the carbon source chosen. Production occurred during the growth was highest on galactose, lactose, cellobiose, and starch. Variation of the nitrogen source with glucose as a non-restricting source of carbon showed that the highest antibiotic titres were obtained with poorly utilized compounds such as isoleucine or phenylalanine.

One thermophilic species, *S. thermoviolaceus*, produces a colored antibiotic and has been used to study the effects of temperature on growth and secondary metabolism. This organism grows between 25 and 57°C despite evidence indicating that some of the reactions of energy production are thermolabile at temperatures upwards of 50°C (Edwards & Ball, 1987). *S. thermoviolaceus* utilizes a wide range of carbon sources for growth and many of these also support antibiotic synthesis at temperatures between 30 and 55°C, but optimally at 45°C (James & Edwards, 1988, 1989).

In 1997, Chattopadhyay and Sen studied the induction of higher amount of antifungal antibiotic by *Streptomyces rochei* G 164 through variation of cultural parameters. The maximum effectivity was found in sucrose as carbon source, peptone as nitrogen source and at pH 7.0. The effect of other selected factors such as incubation period, temperature and aeration was also studied and it was indicated that the isolate G 164 produced the antifungal antibiotic optimally at the 9th day of fermentation and in less aerated condition, the optimum temperature for production was reported to be 30°C.

Jonsbu *et al*. (2000) investigated cell growth and production of nystatin by *Streptomyces noursei* (ATCC 11455) on the three different nitrogen sources ammonium sulphate, ammonium nitrate and sodium nitrate. *S. noursei* was able to utilize all of the three tested nitrogen sources for the growth and production of nystatin. High ammonium concentration had a negative effect on production of nystatin when phosphate and glucose were in excess. There was an increased production of nystatin when the cultures became ammonium limited.

The present study was carried out to investigate the

factors that play a role in the process of antifungal production, and to detect the active compound in the culture filtrate of tested streptomyces species and to detect their chemical structure.

MATERIALS AND METHODS

Antagonizing actinomycetes. Four actinomycete species from rhizosphere and non-rhizosphere soil, identified and tested for their antagonizing activity against both human and plant pathogenic yeast and mold fungi from our previous study (Abd-Allah *et al*., 2004). These species are *Streptomyces lydicus*, *S. ederensis*, *S. erumpens* and *S. antimycoticus*.

Tested fungal species. Four human pathogenic yeast and mold fungi species (*Aspergillus niger, Candida albicans, C. parapsilosis* and *Saccharomyces cerevisiae*) and four plant pathogenic fungal species (*Alternaria solani, Cephalosporium maydis*, *Fusarium oxysporum f.sp. lycopersicis* and *Penicillium digitatum*) were chosen. All the identified fungal species were received from Dr. David P. Labida at ARS culture collection (NRRL) National Center for Agriculture Utilization Research, United States department of agriculture, Peoria, Illinios, USA.

Factors affecting the production of antifungal compounds. The antifungal compound was assayed against the tested fungi as follows: A spore suspension of the tested fungus was prepared in the agar medium (malt extract agar for yeast fungi and potato dextrose agar for mold fungi) and 15 ml were poured into each plate (Anwar, 1949; Patrick, 1954). The antagonistic actinomycetes were cultivated for 10 days, the antagonistic mycelial plug was then placed at the center of the seeded medium and the plates were incubated at 28±2^{o}C for 2-5 days. After incubation, the diameter of inhibition zone around the antagonistic plug was measured.

Carbon source. The carbon source (glucose) of Hussein's fishmeal extract agar (HFMEA) was substituted by 5 different carbon sources (with the same concentration, 20 g L^{-1}) to identify the most suitable one for antifungal production, these were fructose, lactose, sucrose, glycerol and starch (Chattopadhyay & Sen, 1997).

Nitrogen source. To find out the most suitable nitrogen source, five different nitrogenous compounds substituted the nitrogen source (peptone) of HFMEA (with the samr concentration, 5 g L^{-1}). These were potassium nitrate, ammonium sulphate, arginine, glutamic acid and soybean flour (Chattopadhyay & Sen, 1997).

Temperature. Temperature effect on antifungal production was determined by allowing different sets to grow between 20 to 36^{o}C interval, the antifungal was then assayed against the tested fungal species as described above (Chattopadhyay & Sen, 1997).

Effect of pH. To study the effect of pH, HFMEA was adjusted to pH values from 5 to 9 with one set up (Chattopadhyay & Sen, 1997).

Preliminary Chemical Characterization of the Antifungal Compounds

Extraction of the antifungal compounds. The selected species were cultivated on HFMEA broth, culture broth harvested from 2 L flasks was then filtered and extracted with a mixture of chloroform-ethyl acetate (1:1 v/v) (Pridham *et al.*, 1956).

Chemical characterization of the antifungal compounds. The active components in the culture filtrates of the actinomycete species were studied by means of descending paper chromatography; the solvent system was a 6: 4: 3 mixture of 1-butanol, pyridine and water. After development, the paper strips (Whatman No 1) were air dried and placed on agar plates seeded with the tested organisms. The plates were incubated at 28^{o}C for 2 days in case of yeast fungi and for 5 days in case of mold fungi, the the antifungal compounds were detected according to their R_f value (Pridham *et al.*, 1956).

Chemical analysis of the culture filtrates. This experiment was carried out according to Abd-Allah and El-Mehalawy (2002), using mass spectroscopy. Varian gas chromatography coupled with a mass selective detector. Finningan mat SSQ 7000 and equipped with Chem. Station software and NIST spectral data was used with DB-5 fused silica capillary column (30 x 0.25 um i.d., o.25 um film thickness). The chromatographic conditions were as follows: column temperature 60^{o}C (30 min), raised from 60 to 260^{o}C (5^{o}C / min) and isothermal at 260^{o}C for 10 min, interface, 260^{o}C; injector temperature 250^{o}C, ionization energy 70 eV; mass range 50-750; volume injected 1uL. This experiment was performed at the Mass Spectroscopy Unit, Central Scientific Laboratory at the National Research Center.

RESULTS

Carbon compounds constitute the major requirement for growth and enter in the different metabolic processes resulting in the production of primary metabolites and secondary metabolites including antifungal compounds. Fig. 1-4 show that media supplemented with different carbon sources showed high antifungal yields compared to that not supplemented with a carbon source (negative control. It is also shown that glycerol was the best carbon source for antifungal production and consequently the antagonistic activities of the four selected *Streptomyces* species, followed by starch, while lactose gave the poorest antifungal yield.

Nitrogen constitutes the second major requirement for growth and the different metabolic activities as it enters in the synthesis of cell structural and functional proteins. Fig. 5-8 show that supplementation of culture media with different nitrogen sources led to an increase in the antifungal yields compared to that not supplemented with nitrogen source (negative control). It is also shown that ammonium sulphate was the best nitrogen source for antifungal production by both *Streptomyces lydicus* and *S. ederenses*.

Table I. Chemical Characterization of the active components in the culture filtrates of the selected *Streptomyces* species

The active components of Streptomyces	Tested fungal species							
species	1	2	3	4	5	6	7	8
S. lydicus R_f: 0.15	+	-	-	-	+	+	+	+
R_f: 0.34	-	+	+	-	-	+	+	-
R_f: 0.65	-	-	+	-	+	+	+	+
R_f: 0.9	+	+	-	+	+	+	+	+
S. ederensis R_f: 0.15	+	-	-	-	+	+	+	+
R_f: 0.45	-	-	+	-	+	+	+	-
R_f: 0.8	+	+	+	+	-	+	+	+
S. erumpens R_f: 0.2	+	+	+	-	-	+	-	-
R_f: 0.65	-	-	+	-	+	+	+	+
R_f: 0.8	+	+	+	+	-	+	+	-
R_f: 0.9	+	+	-	+	+	+	+	+
S. antimycoticus R_f: 0.15	+	-	-	-	+	+	+	+
R_f: 0.45	-	-	+	-	+	+	+	-
R_f: 0.8	+	+	+	+	-	+	+	-
R_f: 0.9	+	+	-	+	+	+	+	+

1: *Aspergillus niger;* 2: *Candida albicans*; 3: *C. parapsilosis;* 4: *Saccharomyces cerevisiae;* 5: *Alternaria solani;* 6: *Cephalosporium maydis;* 7: *Fusarium oxysorum;* 8: *Penicillium digitatum*.

Table II. Chemical analysis of the culture filtrate of *S. lydicus*

Chemical name	Chemical formula	Molecular weight
Tritetracontane	$C_{43}.H_{88}$	604
Dioxane,4-(hexadecyclo)2-pentasecyl-	$C_{35}.H_{70}.O_3$	538
Octacosane	$C_{28}.H_{58}$	394
2(3H)-Furanone,dihydro-5-tetradecyl-	$C_{18}.H_{34}.O_2$	282
Phenol,2-methyl-4-(1-propenyl)-acetate	$C_{12}.H_{14}.O_3$	206
Phenol,2,4,4-dimethoxy,acetate	$C_{10}.H_{12}.O_4$	196
Cyclopentane,1-pentyl-2-propyl	$C_{13}.H_{26}$	182
2-Hexenyl diethyl	$C_{10}.H_{20}.O_2$	172
2-Pentanone,5-phenyl-	$C_{11}.H_{10}.O$	162
2-Methyl-5-propanoylthiophrne	$C_8.H_{10}.O.S$	154
2-(Methylamino) benzimidazole	$C_8.H_{11}.N_3$	149
Hexanoic acid,2-methyl-	$C_7.H_{14}.O_2$	130
Pentanoic acid,4-methyl-	$C_6.H_{12}.O_2$	116

Glutamic acid was the best nitrogen source for *S. erumpens*, while soybean was the best nitrogen source for *S. antimycoticus*. For all the tested *Streptomyces* species, potassium nitrate was the poorest nitrogen source for supporting antifungal production.

Temperature is a physical factor that plays an important role in growth and metabolism. It is shown from Fig. 9-12 that increasing the temperature led to an increase in the antifungal production and antagonistic activity which was the optimum temperature, above which any increase in temperature was accompanied by a decrease in the antifungal production and a decrease in the antagonistic activity. Fig. 9 to 12 show that 24°C was the optimum temperature for antifungal production and antagonistic activities of *S. lydicus* and *S. ederensis*, while 28°C was the optimum temperature for *S. erumpens* and *S. antimycoticus* to produce antifungal compounds and to antagonize the tested fungal species.

pH is another factor that plays an important role in growth and metabolism as it affects the enzymatic reactions involved in the different metabolic processes. Fig. 13-16 show that increasing the pH value led to an increase in the

Table III. Chemical analysis of the culture filtrate of *S. ederensis*

Chemical name	Chemical formula	Molecular weight
Tritetracontane	$C_{43}.H_{88}$	604
Dotriacontane	$C_{32}.H_{66}$	450
12-Tricosanone	$C_{17}.H_{36}$	338
Di-n-butyl azelate	$C_{17}.H_{32}.O_4$	300
1,3-Dioxolane,4-hexyl-2-2-dimethyl-5-octyl	$C_{19}.H_{38}.O_2$	298
Dutadrupine	$C_{17}.H_{15}.N.O_3$	281
5-Indacene-1,7-dione,2,3,5,6-tetra-hydro-3,3,4,5,5,8-hexamethyl	$C_{18}.H_{22}.O_2$	270
Lumiflavine	$C_{13}.H_{12}.N_4.O_2$	256
2-Methyl-6-tert-octylphenol	$C_{11}.H_{24}.O$	220
Silane,(4-ethylphenoxy)trimethyl-	$C_{11}.H_{18}.O.Si$	194
Dodecane	$C_{12}.H_{26}$	170
Benzene1,-ethyl-3-methyl	$C_9.H_{12}$	120

Table IV. Chemical analysis of the culture filtrate of *S. erumpens*

Chemical name	Chemical formula	Molecular weight
Ergotaman-3´,6´,18-trione,9,10´-dihydro-12´- hydroxy-2´-methyl-5´-(1-henyl) Octacosane	$C_{33}.H_{37}.N_5.O_5$	583
12-Tricosanone	$C_{28}.H_{58}$	394
2(3H)-Furanone,dihydro-5-tetradecyl-	$C_{17}.H_{37}$	338
Lumiflavine	$C_{18}.H_{34}.O_2$	282
1-hydroxy-4-methylanthraquinone	$C_{13}.H_{12}.N_4.O_2$	256
2,5-Cyclohexadiene-1,4-dione,2,6-bis(1,1-dimethyl ethyl)-	$C_{15}.H_{10}.O_3$	238
Metharcital	C14.$H_{20}.O_2$	220
3,4-Dichlorophenylacetonitrile	$C_9.H_{14}.N_2.O_3$	198
Methyl paraben	$C_8.H_5.N.Cl_2$	185
Furan,2,5-dihydro-,2,5-dimethoxy	$C_8.H_{10}.O_3$	152
Benzene,1-fluro-2-methyl-	$C_6.H_{10}.O_3$	130
	$C_7.H_7.F$	110

Table V. Chemical analysis of the culture filtrate of *S. antimycoticus*

Chemical name	Chemical formula	Molecular weight
Tritetracontane	$C_{43}.H_{88}$	604
Dotriacontane	$C_{32}.H_{66}$	450
12-Tricosanone	$C_{17}.H_{36}$	338
2-(3H)-Furanone,dihydro-5-tetradecyl	$C_{18}.H_{34}.O_2$	282
Lumiflavine	$C_{13}.H_{12}.N_4.O_2$	256
Benzene,[(2-phenylethenyl)sulfonyl]-	$C_{14}.H_{12}.O_2.S$	244
2-Cyclohexene-1-one,2,4,4-trimethyl-(3-oxo-1-butenyl)-	$C_{13}.H_{18}.O_2$	206
3,4-Dichlorophenylacetonitrile	$C_8.H_5.N.Cl_2$	185
2-Methyl-5-propanoylthiophene	$C_8.H_{10}.O.S$	154
N-(3-methybutyl)acetamide	$C_7.H_{15}.N.O$	129
Acetamide, N-(2-methylpropyl)-	$C_6.H_{13}.N.O$	115

Fig. 1. Effect of different carbon sources on the antagonistic activity of *S. lydicus* (a) human-pathogenic yeast and mold fungi and (b) plant pathogenic fungi

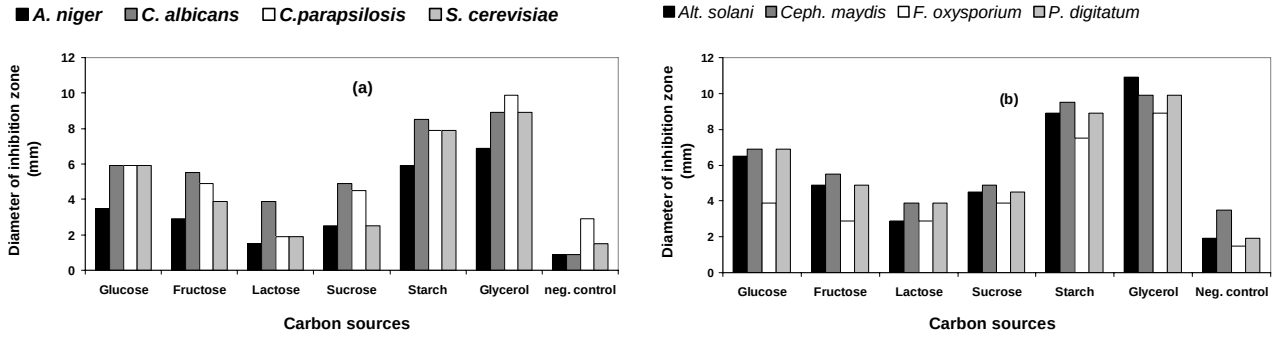


Fig. 2. Effect of different carbon sources on the antagonistic activity of *S. ederensis*

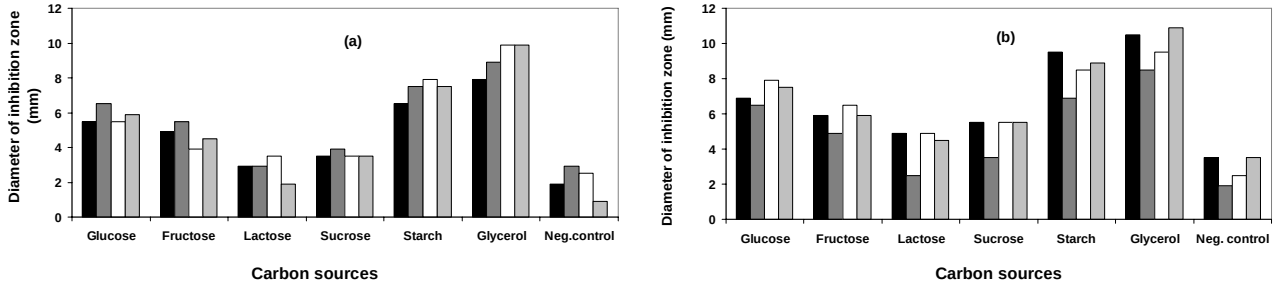


Fig. 3. Effect of different carbon sources on the antagonistic activity of *S. erumpens*

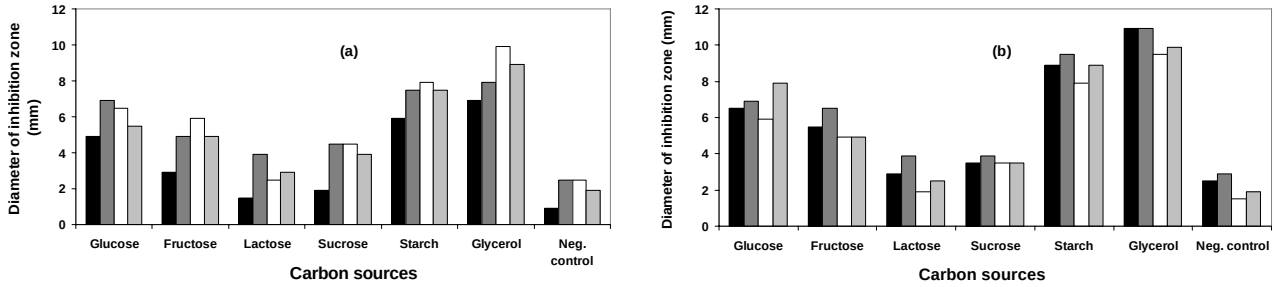


Fig. 4. Effect of different carbon sources on the antagonistic activity of *S. antimycoticus*

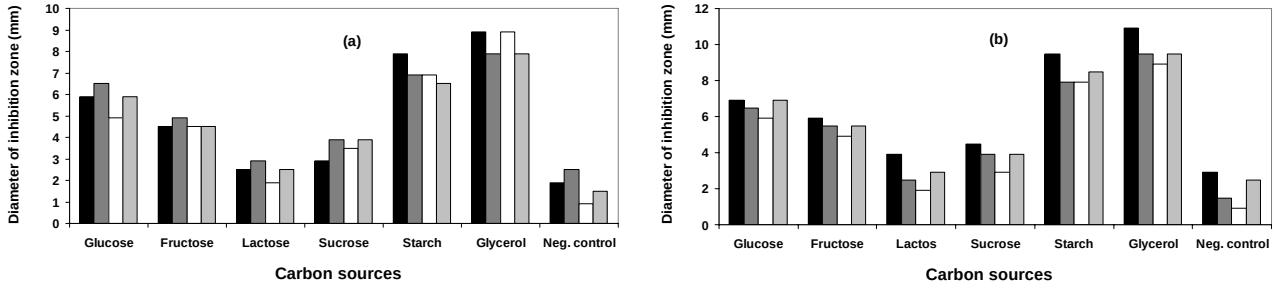


Fig. 5. Effect of different nitrogen sources on the antagonistic activity of *S. lydicus*

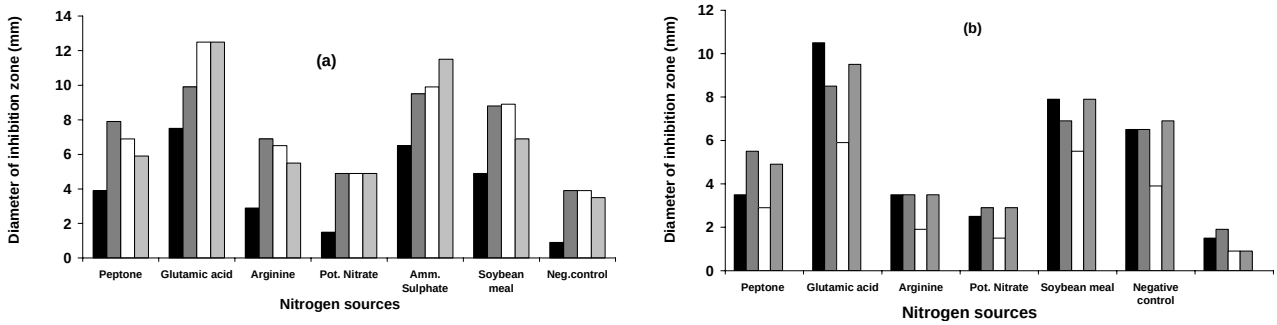


Fig. 6. Effect of different nitrogen sources on the antagonistic activity of *S. ederensis* .
(a) human-pathogenic yeast and mold fungi and (b) plant pathogenic fungi

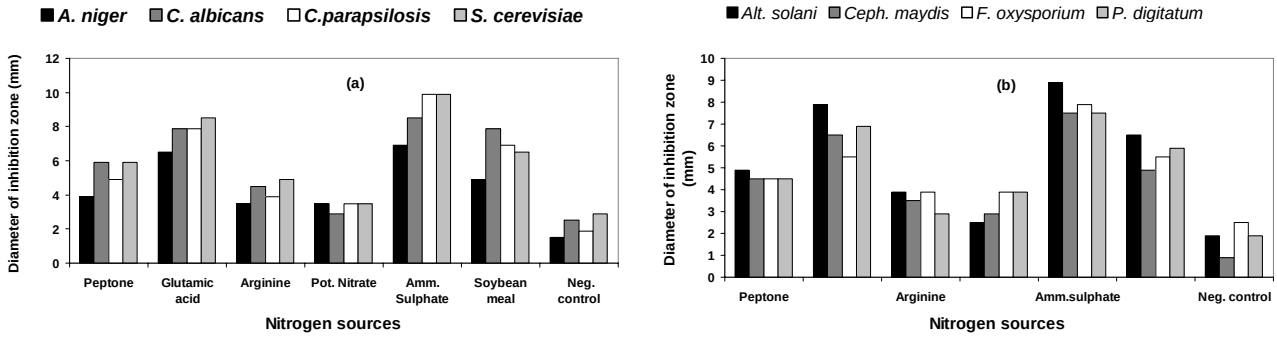


Fig. 7. Effect of different nitrogen sources on the antagonistic activity of *S. erumpens*

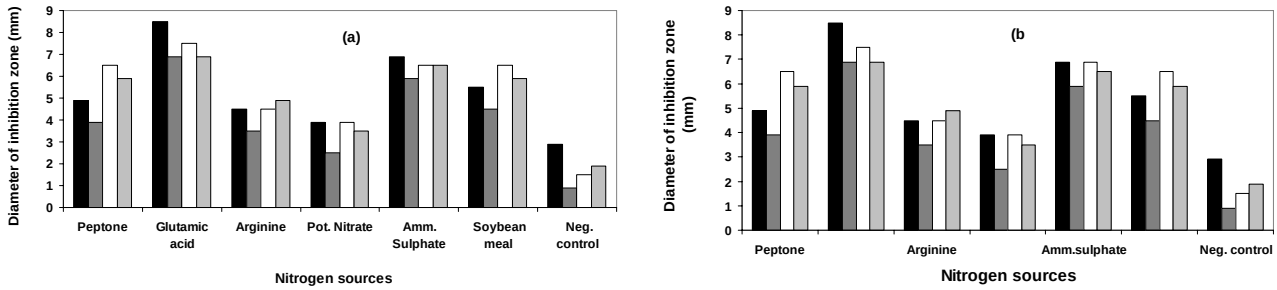


Fig. 8. Effect of different nitrogen sources on the antagonistic activity of *S. antimycoticus*

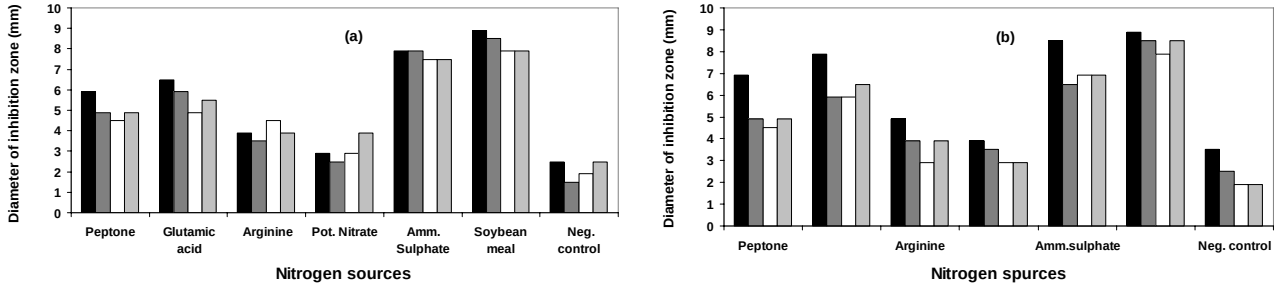


Fig. 9. Effect of different temperatures on the antagonistic activity of *S. lydicus*
(a) human pathogenic yeast and mold fungi and (b) plant pathogenic fungi

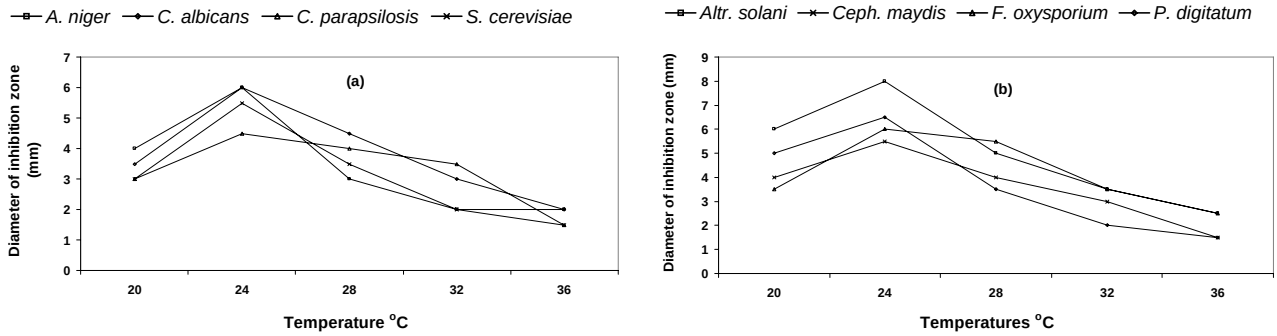


Fig. 10. Effect of different temperatures on the antagonistic activity of *S. ederensis* (a) human pathogenic yeast and mold fungi and (b) plant pathogenic fungi

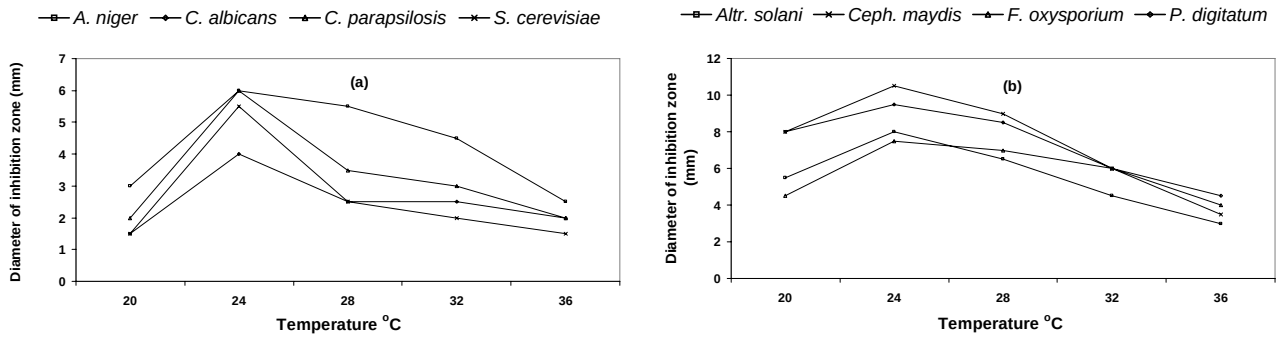


Fig. 11. Effect of different temperatures on the antagonistic activity of *S. erumpens*

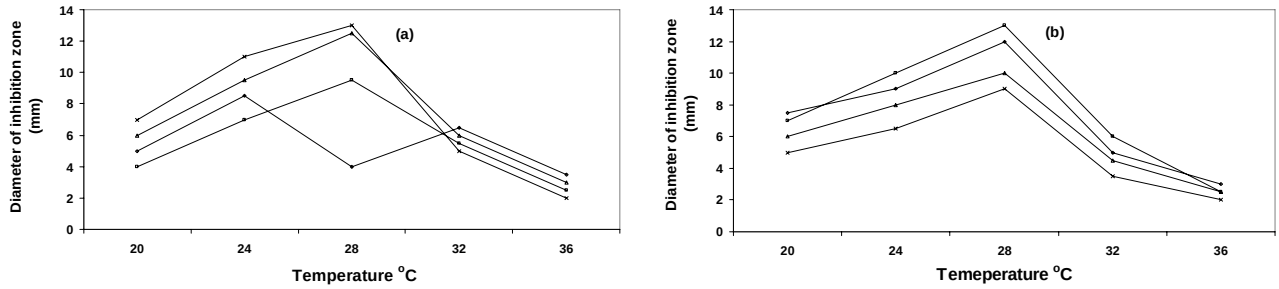


Fig. 12. Effect of different temperatures on the antagonistic activity of *S. antimycoticus*

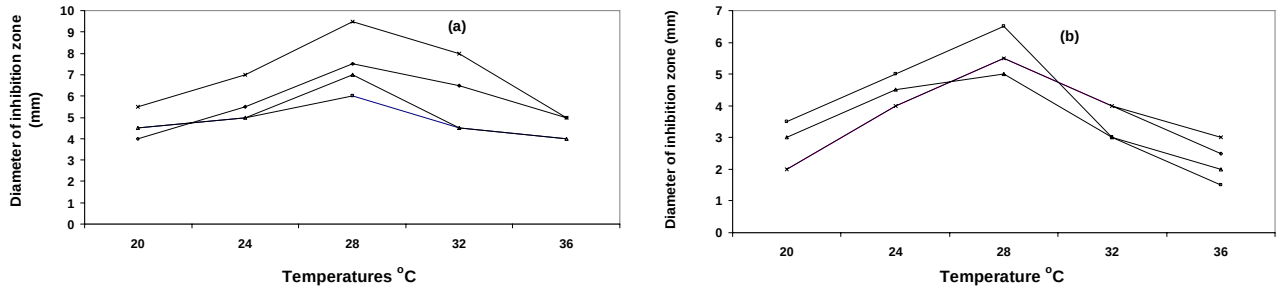


Fig. 13. Effect of different pH values on the antagonistic activity of *S. lydicus*

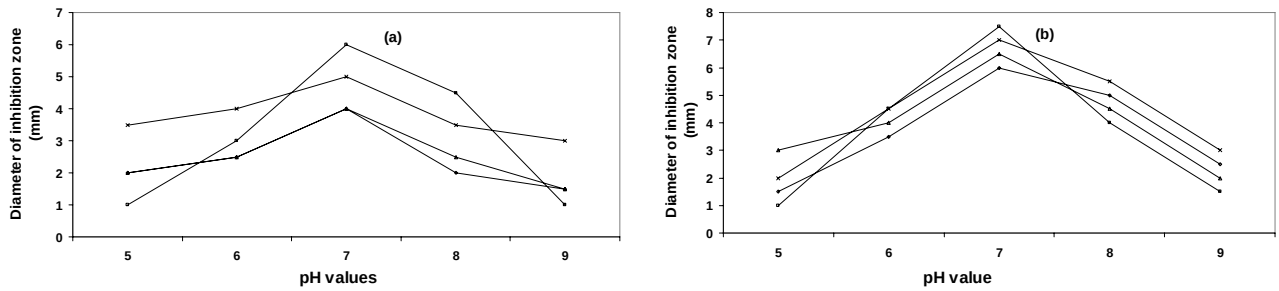


Fig. 14. Effect of different pH values on the antagonistic activity of *S. ederensis*
(a) human-pathogenic yeast and mold fungi (b) plant pathogenic fungi

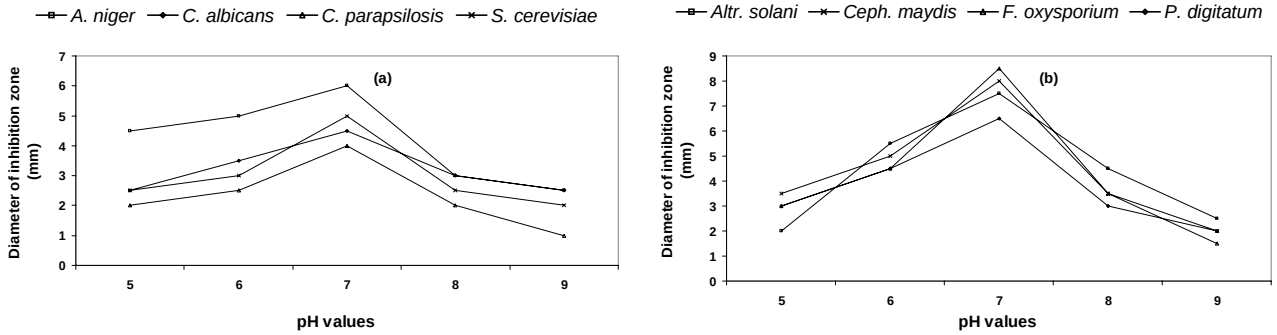


Fig. 15. Effect of different pH values on the antagonistic activity of *S. erumpens*

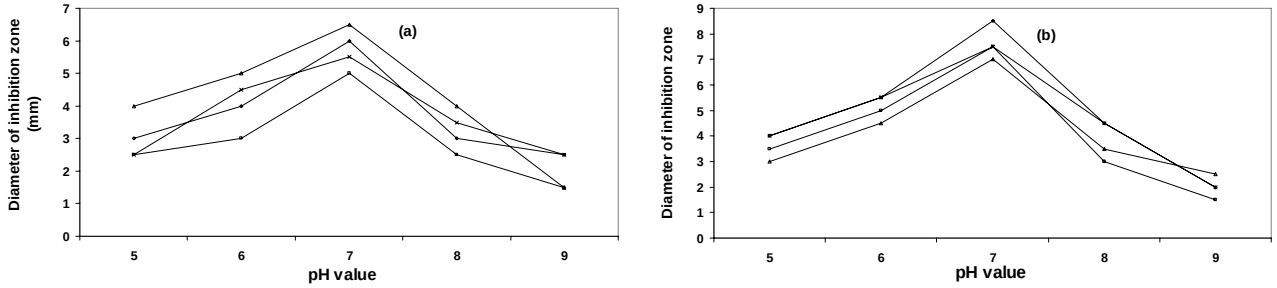
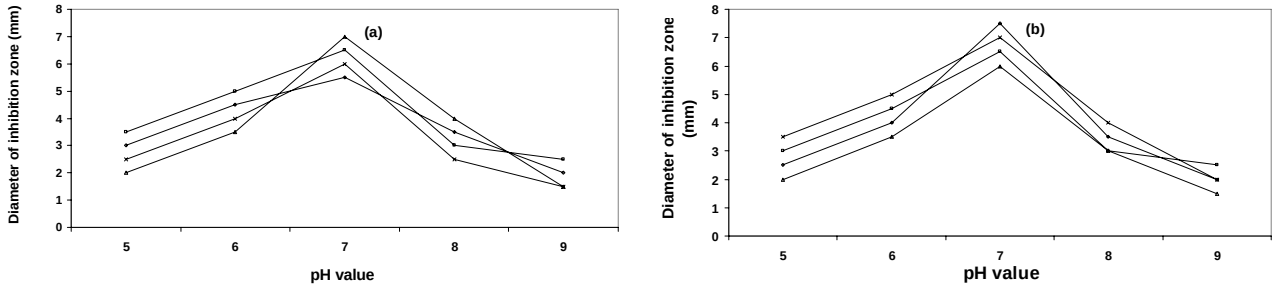


Fig. 16. Effect of different pH values on the antagonistic activity of *S. antimycoticus*



antifungal production up to certain limit above which any increase in the pH value was accompanied by a decrease in the antifungal production and consequently the antagonistic activity. pH 7.0 was found to be the optimum pH value for antifungal production by the four selected *Streptomyces* species.

The four selected *Streptomyces* species were grown under the optimum cultural conditions on broth media for the production of antifungal compounds. The active components were detected using paper chromatography by the inhibition zones around their spots and were determined by their R_f values as show in Table I. It is shown from this table that four active components were detected in the culture filtrates of *S. lydicus*, *S. erumpens* and *S. antimycoticus*, while three active components were detected in the culture filtrate of *S. ederendis*. It is also shown from the table that the active component of R_f 0.9 was effective against seven of the eight tested fungal species. The active component of R_f 0.8 was effective against six of the eight tested fungal species, while the active component of R_f 0.15 was effective against five of the eight tested fungal species. The active components of R_f values 0.02, 0.34, o.45 and 0.65 each was effective against four of the eight tested fungal species. It is worth noting that all the active components detected in the culture filtrates of the four selected *Streptomyces* species were effective against *Cephalosporium maydis*.

Tables II-V show the chemical analysis of the culture

filtrates of the four selected *Streptomyces* species. The chemical analysis of the culture filtrate of *S. lydicus* (Table II) revealed the presence of 13 chemical compounds arranged descendingly according to their molecular weights, the chemical analysis of the culture filtrates of *S. ederendsis* and *S. erumpens* revealed the presence of 12 chemical compounds, while the chemical analysis of the culture filtrate of *S. antimycoticus* revealed the presence of 11 chemical compounds.

It is evident from Tables II-V that Tritetracontane of high molecular weight was detected in the culture filtrates of both *S. lydicus, S. ederensis* and *S. antimycoticus*. 2(3H)-Furanone, dihydro-5-tetradecyl- of molecular weight 282 was detected in the culture filtrates of *S. lydicus, S. erumpens* and S. antimycoticus. The culture filtrates of both *S. ederensis, S. erumpens* and *S. antimycoticus* revealed the presence of 12-Tricosane of molecular weight 338.

It is well known that a component o high molecular weight has a low R_f value and vice versa. Therefore, the active component of R_f 0.15 may be Tritetracontane, the active component of R_f 0.2 may be Ergotaman 3´,6´, 18-trion, 9,10´dihydro-12´hydroxy-2´-methyl-5´(1-henyl), the active component of R_f 0.34 may be 1,3-dioane, 4-(hexadecyl)2-pentadecyl-. The active component of R_f 0.45 may be Dotriacontane, the active component of R_f 0.65 may be Octacosane, the active component of R_f 0.8 may be 12-tricosanone and finally the active component of R_f 0.9 may be 2(3U) furanone-dihydro-5- tetradecyl-.

DISCUSSION

Concerning the effect of different carbon sources on the antifungal production by the selected *Streptomyces* species, glycerol was found to be the best carbon source followed by starch. These findings are in agreement with the fact that secondary metabolite production in actinomycetes is often stimulated by slowly assimilated complex carbohydrates or oils in the production media and is decreased when more rapidly utilized monosaccharides such as glucose are present (Bertasso *et al*., 2001). A possible explanation of this phenomenon is that glucose or other carbon sources cause catabolite repression in which the production of an enzyme of secondary metabolite biosynthesis is inhibited. This explanation is in accordance with those reported by Drew and Demain (1977) and Iwai and Omura (1982).

The selected *Streptomyces* species varied with respect to the best nitrogen source that supported maximal antifungal production. Glutamic acid was found to be the best nitrogen source for antifungal production by *S. lydicus* and *S. erumpens*. This may be due to the fact that amino acids can supply both carbon and nitrogen from which antifungal precursors could be derived. This finding runs parallel by those reported by Liao *et al*. (1995) and Junker *et al*. (1998).

Soybean meal was found to be the best nitrogen source for antifungal production of *S. antimycoticus*. Similar results were obtained by Tereshin (1976) for the production of candicidin by *S. halstedii*. Glutamic acid and soybean meal that proved to be the best nitrogen sources for antifungal production by the three *Streptomyces* species are in line with the recommendation by many workers that organic nitrogen sources increased the production of antimicrobial compounds by many *Streptomyces* species (Agwa, 1985; El-Abyad *et al*., 1996). Ammonium sulphate was found to be the best nitrogen source for antifungal production by *S. ederensis*. Similar results were obtained by Cimburkova *et al*. (1988). The effect of ammonium ions, whether repression or stimulative, is highly dependent upon specific culture system as well as the levels of other key factors and of course on the genetic information of the producing organism (Brana & Demain, 1988) and Shapiro (1989).

Concerning the effect of different temperatures on the antifungal production by the selected *Streptomyces* species, it was found that 24°C was the optimum temperature for antifungal production by *S. lydicus* and *S. ederensis*, while 28°C was the optimum for antifungal production by *S. erumpens* and *S. antimycoticus*. This is because the selected *Streptomyces* species are mesophilic and thus the above mentioned temperatures are suitable for growth and consequently for the activity of enzymes responsible for secondary metabolite biosynthesis. This finding runs parallel with those reported by Bhattacharyya *et al*. (1998) and Nadkarni *et al*. (1998).

pH value of the culture medium is another factor that influences the antifungal production. It was found that the optimum pH-value for antifungal production by the four selected *Streptomyces* species was 7.0. It was found by El-Abyad *et al*. (1996) that highly acidic or basic media, whether adjusted initially or buffered after autoclaving were not suitable for the antifungal production by many *Streptomyces* species and that neutral media (pH 7.0) were the most favorable for antifungal production.

Three *Streptomyces* species produce four active components, while one species produces three active components only. Actinomycete cell metabolism under conditions of nutritional excess is directed towards the generation of cell mass rather than the production of secondary metabolites and when depletion of key nutrients occurs, it shifts the cell cycle to the stationary phase and signals the transition from primary to secondary metabolism in which these active components are produced. This finding agrees with those reported by Abanat *et al*. (1999) and Abd-Allah and El-Mehalawy (2002).

The culture filtrates of two *Streptomyces* species revealed the presence of 12 chemical compounds, the culture of one species revealed the presence of 13 chemical compounds and the culture filtrate of the last one revealed the presence of 11 chemical compounds. It is suggested that the organism may divert its metabolic effort making antibiotics only when it is in competition for a substrate

which it requires for growth. This finding is in accordance with that reported by Bushell (1989).

CONCLUSIONS

It was concluded from this investigation that actinomycetes play an important role in antagonizing both human and plant pathogenic fungi, and may be used in an agricultural and medicinal scales after being further studied.

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