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Antibiotic Biotamycin Produced by *Streptomyces antibioticus* sub.sp. $11M_w$

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THE ANTIMICROBIAL activity of 101 actinomycete isolates obtained from different localities in El-Sharkia and El-Minia Governorates were tested against 12 microorganisms including 6 Gram-positive & Gram-negative bacteria and 6 fungal organisms.

The most potent actinomycete isolate was isolated from the rhizosphere of *Triticum aestivum* (wheat) cultivated in El-Minia governorate, Egypt and identified as *Streptomyces antibioticus* sub.sp. $11M_w$. The optimum culture conditions for the production of the antimicrobial metabolites from this organism were studied. The optimum incubation temperature was 30 °C after incubation period 6 days at pH 8.0. Moreover, the production of the antimicrobial metabolites produced from *S. antibioticus* sub.sp. $11M_w$. decreased by exposure increasing to irradiation dose of Gamma radiation. The most favourable carbon and nitrogen sources were starch (20g/l) and $NaNO_3$ (2g/l), respectively. The antimicrobial substance was extracted by using different solvents followed by chromatographic purification. The active fractions were concentrated and identified on the basis of the recommended key's for identification of antibiotic. The physicochemical analysis revealed the imperical formula is $C_{18}H_{28}O_5N$. The collected data emphasized that the purified antibiotic have characters likely similar to biotomycin and thus it was given the name biotomycin $11M_w$ belonging to glutarimide group (protomycin). The maximum antibiotic biosynthesis was attained by adding barium chloride (0.05 mg/100 ml) to starch nitrate medium and was completely inhibited by copper sulfate (0.05 mg/100 ml).

Keywords : *Streptomyces antibioticus*, Antimicrobial activity, Test organisms.

Microbial production of antibiotics is a rapidly expanding branch of industrial microbiology. Nearly all bacterial infectious diseases that were prior to the antibiotic era, major causes of human death have been brought under control by the use of these drugs. The potentialities of the utilization of antibiotics in the life of modern man are still far from exhausted (Ellaiah, 1998).

Actinomycets have gained great economic and public health importance as producers of antibiotics, vitamins and enzymes. Among all the genera of actionmycets *Streptomyces* is represented in nature by the largest number of species and varieties. The majority of antibiotic producing actinomycetes are found in this species and genus led to growing economic importance of these organisms which resulted in the isolation and description of number of new species.

However, for maximum productions specific media and specific conditions must be developed not only for each antibiotic, but also for each strain of the producing organism (Krassilnikov, 1960). The concept of extracellular nutrients for the regulation of secondary metabolism has provided a framework for commercial process optimization. Carbon catabolites, nitrogen metabolites and phosphate regulation have all been observed in antibiotic biosynthesis (Martin & Demain, 1980; Vilches *et al.*, 1990 and Mansour *et al.*, 1997). Therefore, the medium constitution together with the metabolic capacity of the producing organism greatly affect the antibiotic biosynthesis. Several investigators studied the effect of incubation period, incubation temperature and PH of growth medium on production of antimicrobial metabolites (Nadkarni *et al.*, 1998; Mansour *et al.*, 1996; Ramadan, 2000 and Corvini *et al.*, 2000). On the other hand, the effect of Gamma irradiation on the growth and metabolic activity of microorganisms have been studied by many workers (El-Fouly *et al.*, 1987; Abdel-Aal, 1980; El-Zawahry *et al.*, 1982 and Abdel-Rahim, 1988).

The aim of the present work, is to study the most potent antimicrobial activity among 101 actinomycetes isolated from soil samples collected from different localities in Minia and Sharkia governorates, Egypt. Characterization and identification of the selected actinomycete isolates were performed. Furthermore, the optimum conditions for the antimicrobial activity production from the selected actinomycete isolate were studied. The antimicrobial substance produced by the selected isolate was extracted, precipitated and purified. The chemical properties of the pure antimicrobial substance has been also investigated.

Material and Methods

Isolation and identification of the most potent actinomycete isolate

Media used

Growth media : Starch nitrate media (Tadashi, 1975) g/l; soluble starch, 20.0; $NaNO_3$, 2.0; K_2HPO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; agar,20.0, the pH was adjusted at 7.0 before sterilization.

Isolation of actinomycetes from soil samples

About 101 actinomycete isolates were isolated from five soil samples which collected from different localities of two governorates namely El-Minia (60 isolates) and El-Sharkia (41 isolates) in Egypt, by using the method recommended by Johnson *et al.* (1960).

Antagonistic activity of the actinomycete isolates

Actinomycete isolates (101 cultures) were tested for their antimicrobial activity using filter paper disc method, well method and mycelial disc method against the following test organisms:

Bacteria

1) *Gram-negative*: *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.*
2) *Gram-positive*: *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus.*

Fungi

1) *Unicellular fungi* : *Saccharomyces cerevisiae.*
2) *Filamentous fungi* : *Aspergillus niger, Aspergillus flavus, Fusarium solani, Fusurium oxysporum* and *Penicillium* sp.

Five isolates showed high antimicrobial activity, three isolates from El-Minia Governorate. (4 M_T, 11 M_w and $12M_w$) and two from El-Sharkia Governorate ($29SH_s$ and $30SH_s$). The most potent isolate ($11M_w$) was isolated from El-Minia Governorate from rhizosphere of *Triticum aestivum* (wheat).

Identification of the most potent isolate

Morphological, physiological and biochemical characters for the identification of the most potent five isolates were carried according to Bergy's Manual of Determinative Bacteriology (Shirling & Gottlieb., 1968a, b, 1969 & 1972; Williams *et al.*, 1984 and Lechevalier, 1989). Micrographs of spore surface chain of the five isolates were obtained by transmission electron microscope TEM 100 CX (Jeol electron microscope) using the spor-print technique (Trsener *et al.*, 1961).

Different media were used to study culture properties of the five actinomycete isolates. These media were starch nitrate agar, inorganic salts starch agar, glycerol asparagines agar, malt yeast extract agar, oat meal agar, czapek's dox agar and casein starch agar. These culture properties were expressed as colour of aerial mycelium, colour of substrate mycelium and melanoid pigments formation. Physiological properties also was carried out, production of melanin pigments, utilization of different carbon sources, H_2S production, nitrate reducation, gelatin liquefaction, formation of enzymatic activities including amylase; caseinase & lipase activities, antimicrobial activity and sensitivity to growth at 10°C and 50°C.

Out of these five isolates, the most potent one $11M_w$ was selected for further investigation.

Effect of certain environmental and nutritional factors on the antimicrobial activity of the selected isolates

Effect of incubation period, incubation temperature and pH values

Inoculated starch nitrate agar slants were used to determine the optimum incubation period after 2, 4, 6, 8 and 10 days at incubation temperature 10, 20,

25, 30, 40 and 45 °C. The study of pH values ranging from 5.5 up to 8.5 using citrate-phosphate and phosphate buffers was carried out using mycelial disc method.

Effect of gamma-irradiation

Source of gamma irradiation :Gamma irradiation process was conducted at the National Centre for Radiation Research and Technology (NCRAT) at Nasr City, Cairo, Egypt, using Co^{60} isotope in Co^{60} gamma champer 4000 A (Indian). The dose rate of the source was 25 KGy /hour during experimental time.

Six days old slants of isolate $11M_w$ grown on starch nitrate agar were irradiated at the doses of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 KGY. Spore suspension in sterile distilled water from the irradiated slants were used after incubation for 4 days to inoculate starch nitrate agar medium, then used to test antimicrobial activity.

Effect of carbon, nitrogen sources and microelements

Carbon sources: Effect of different carbon sources was tested at concentration g/100 ml. Starch (2.0), D-glucose (2.1), sucrose (2.0), D-mannitol (2.0), D-galactose (2.1), lactose (2.0), cellulose (2.1) and D-fructose (2.1). Each carbon source was fortified singly as a substitute to starch in nitrate agar medium lacking starch, inoculated with the selected isolate $11M_w$ and tested for antimicrobial activity formation after 6 days using mycelial disc method.

Nitrogen sources: Effect of different nitrogen sources as g/100 ml. $NaNO_3$ (0.2), KNO_3 (0.24), NH_4 Cl (0.11), urea (0.07), beef extract (0.2), yeast extract (0.2), yeast extract (0.2) and peptone (0.2) was incorporated singly in starch nitrate agar medium as substitutes for $NaNO_3$, inoculated with the isolate, and tested for antimicrobial acitivity.

Microelements: Eight microelements namely $MnCl_2$, $BaCl_2$, $CaSO_4$, $MgSO_4$, $CuSO_4$, $ZnSO_4$, $MnSO_4$, KCl and $FeSO_4$ were incorporated at concentration of 0.05 mg/100 ml medium. The medium inoculated with the selected isolate and tested for antimicrobial activity after growth period of 6 days at 30 °C incubation temperature.

Extraction and purification of the antimicrobial metabolite from the selected isolate

Extraction

The fermentation medium used is starch nitrate liquid medium, inoculated with the actinomycete spore suspension, incubated for 6 days at 30 °C and pH 8.0. Then extracted with seven solvents (butanol, chloroform, benzene, ethylacetate, petroleum-ether, diethyl ether and xylene). The solvents were added to fermentation broth at the level of 1 : 1 (v/v). The extract was obtained by separating funnel three times. The organic phase was collected, concentrated to

dryness under vacuum by using a rotary evaporator. The extraction with different solvents were tested for their extractability of the antibiotic from broth (Hussein *et al.,* 1998).

Thin layer chromatography (TLC)

The previous extracts were analyzed by (TLC) on silica gel plates using chloroform : methanol (24 : 1) (v/v) as the developing solvent (Eshita *et al.,* 1995 and Winkelman *et al.,* 1983).

Purification

The purifications of the antibiotic was carried out using column chromatography of silica gel. Chloroform and Methanol 9 : 1 (v/v) was used as an eluting solvent. The fractions were collected and antimicrobial activities were determined for each separate fraction (Kenichi *et al.,* 2001 and Ueno *et al.,* 2002).

Physico-chemical properties of the pure antibiotic

Elemental analysis: The elemental analysis carbon, hydrogen, oxygen, nitrogen and sulphur was carried out by the microanalytical centre of Cairo University, Egypt.

Reaction of the antibiotic with certain chemical test: For this purpose, the following reactions were carried out according to Ramadan (2000): Molish's, fehling, Sakaguchi, Ninhydrin, Ehrlish, Nitroprusside, Ferric chloride, Meyer reaction, Million's reaction, Tollen's reaction and Lead sulphide reaction.

Spectroscopic analysis

The IR, UV, Mass spectrum and HPLC were determined at the Micro Analytical Centre of Cairo University, Egypt.

Biological activity of the pure antibiotic

The minimum inhibitory concentration (MIC) was carried out using the pure antimicrobial substance using nutrient broth for bacteria, where the antibiotic serially diluted from 0.0 µl /ml to 200 µl /ml. One drop of bacterial suspension is inoculated in each tube, incubated at 35°C for 18/24 hr, then examined for turbidity. The antimicrobial activity was investigated by the diffusion method (Havangh, 1963). The minimal Bactericidal concentratoin were carried out according to Victor Lorian (1986).

Results

Screening of antimicrobial activities of actinomycete isolates

Out of 101 actinomycete isolates, only 80 were selected to study their antimicrobial activity according to Waksman (1948). Of these, only 32 showed antimicrobial activity against the tested organisms. Out of 12 isolates which

exhibited antimicrobial good activity while others showed poor to moderate activities. Five isolates were antimicrobial chosen for further study. The antimicrobial activity of these 5 isolates is presented in Table 1(a, b). among these isolates, the isolate number $11M_w$ showed the maximum activity against both Gram-positive and Gram-negative bacteria as well as, yeast and fungi. Therefore isolate $11M_w$ was selected for further study.

Identification of the most potent isolates

The morphological, physiological and biochemical tests for the characterization of the selected five isolates has been made by the procedure of Shirling & Gottlieb, (1968 a, b, 1969 & 1972), William *et al.* (1984) and Lechevalier (1989). According to the international *Streptomyces* project (I.S.P) for identification, the most potent isolates were identified as *Streptomyces antibioticus* (isolate $11M_w$), *Streptomyces pyridomyceticus* isolate $30SH_s$ (Fig. 1a, b, c, d, e).

Factors affecting antimicrobial activity of Streptomyces antibioticus sub.sp. $11M_w$

The maximum biosynthesis of the antimicrobial substance could be recorded using starch nitrate medium within an incubation period of 6 day at incubation temperature 30°C, and pH8.0. Moreover, the antagonistic activity were decreased as the gamma irradiation dose increased (Fig. 2a, b, c, d).

The results for all the tested carbon sources showed that starch is the best carbon source (Fig. 3a, b), and sodium nitrate is the best nitrogen source (Fig. 4a, b), for the antimicrobial substance production. The results for studying the effect of addition of microelements to the growth medium on the antagonistic activity of isolate *S. antibioticus*, recorded also, that barium chloride is the best microelement added to the medium and showed the highest antagonistic effect against all tested organisms except *Fusarium solani* where zinc sulphate was the best one (Fig. 5a, b).

Extraction, purification and identification of the antimicrobial substance

Separation of the antibiotic produced by the most potent isolate into individual components has been carried out by thin layer chromatography on silica gel plates using chloroform : methanol (24 : 1v/v) as the solvent developing system. Spots were detected by UV according to (Eshita *et al.,* 1995). The obtained results showed that, the R_f of the antimicrobial substance was determined at 0.625. Purification of the antibiotic was carried out by using silica gel column chromatography.

The crude antimicrobial substance in the least amount of chloroform and petroleum ether was injected into the column and the active fractions was eluted with system of MeoH : chloroform (1 : 9). The active fractions 5ml each were collected and tested for antimicrobial activity. The active fractions obtained ranged between fraction number 5 to 18. The active fractions were concentrated, then used for more studies.

TABLE 1a . Antimicrobial activity of the most potent actinomycete isolates against Gram- negative and Gram-positive bacteria.

Tested orgenisms / Actinomycete Isolates	Inhibition Zones (mm)																	
	Gram-negative bacteria									Grampositive bacteria								
	E-coil			*Salmonella typhi*			*Psudomonas aeruginosa*			*Bacillus cereus*			*Bacillus subtilis*			*Staphylococcus aureus*		
	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W
$11M_W$	9.0	7.0	12.0	7.0	8.0	12.0	0.0	5.0	7.0	1.0	10.0	15.0	9.0	25.0	21.0	6.0	4.0	11.0
$4M_T$	9.0	12.0	8.0	0.0	8.0	0.0	1.0	9.0	11.0	6.0	11.0	6.0	5.0	6.0	7.0	11.0	12.0	12.0
$29SH_S$	6.0	6.0	11.0	0.0	0.0	0.0	6.0	6.0	8.0	3.0	6.0	11.0	0.0	0.0	0.0	2.0	4.0	6.0
$30SH_S$	2.0	3.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	7.0	11.0	0.0	0.0	0.0	1.0	2.0	2.0
$12M_W$	0.0	3.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	5.0	3.0	0.0	0.0	0.0	8.0	7.0	12.0

TABLE 1b . Antimicrobial activity of the most potent actinomycete isolates against fungi.

Tested orgenisms / Actinomycete Isolates	Inhibition Zones (mm)																	
	Molds															Yeast .		
	Fusarium solani			*Fusarium oxysporum*			*Penicillium spp.*			*Aspergillus niger*			*Aspergillus flavus*			*Saccharomyces cereviciae*		
	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W	Fp	M.D	W
$11M_W$	10.0	19.0	17.0	9.0	19.0	29.0	30.0	23.0	30.0	6.0	8.0	8.0	7.0	12.0	8.0	0.0	9.0	2.0
$4M_T$	0.0	8.0	0.0	7.0	9.0	8.0	8.0	8.0	9.0	0.0	5.0	0.0	0.0	0.0	0.0	5.0	7.0	6.0
$29SH_S$	2.0	5.0	2.0	8.0	6.0	10.0	10.0	21.0	12.0	9.0	12.0	15.0	12.0	9.0	20.0	0.0	0.0	0.0
$30SH_S$	0.0	5.0	0.0	20.0	8.0	24.0	24.0	0.0	0.0	0.0	6.0	0.0	4.0	3.0	2.0	0.0	0.0	0.0
$12M_W$	0.0	0.0	0.0	15.0	30.0	18.0	18.0	1.0	2.0	20.0	23.0	17.0	4.0	10.0	23.0	0.0	0.0	0.0

Fp = Filter paper
M.D = Mat Disc
W = Well

A) Isolate 11M$_w$ of grey colour. *S.antibioticus.*

B) Isolate 4M$_t$ of grey colour. *S.recifensis.*

C) Isolate 12M$_w$ of white colour. *S.grisobrunneu.*

D) Isolate 29SH$_s$ of white colour. *S.auromonophdiales.*

E) Isolate 30SH$_s$ of white colour series. *S.pyridomyceticus.*

(1) Photographs of micromorpholgy of spore chain

(2) Electron micrograph of spore surface

Fig. 1. Photographs of morphology of spore chain and electron micrographs of spore surface of the most potent actinomycete isolates (using transmission microscope).

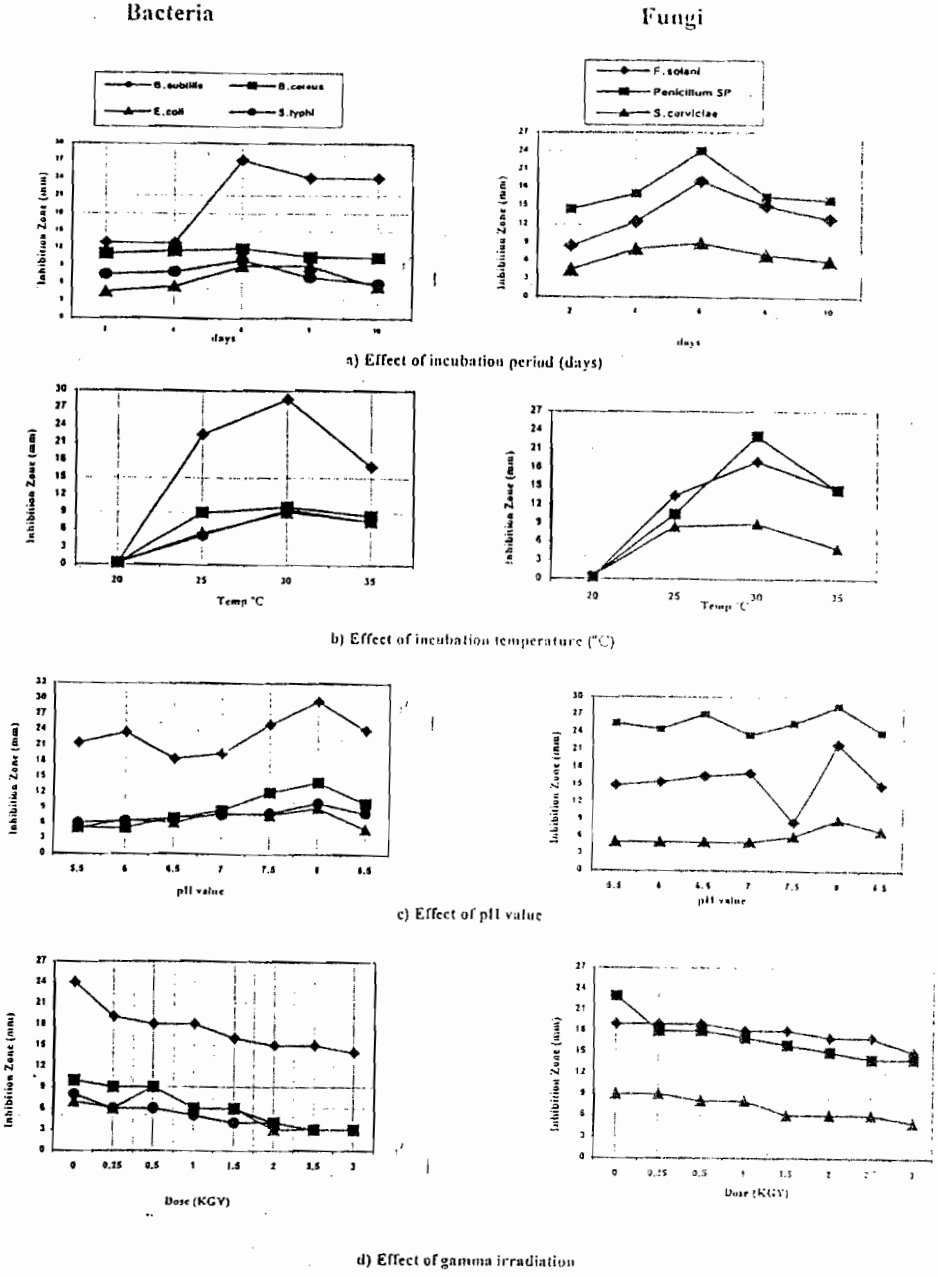

Fig.2. Effect of a) incubation period, b) incubation temperature, c) pH values and d) Gamma irradiation on the antimicrobial activity of S.antiboiticus. sub. Sp. 11 M. against the tested Gram-positive & negative bacteria.

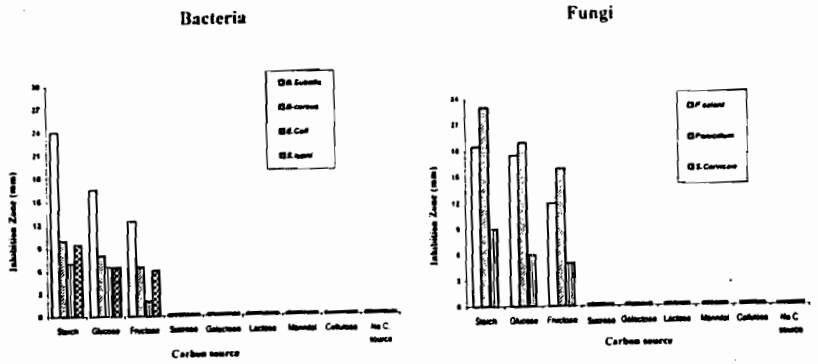

Fig. 3. Effect of supplying different carbon sources on the biosynthesis of antimicrobial substance produced by *Streptomyces antibioticus* sub. sp.11M$_w$.

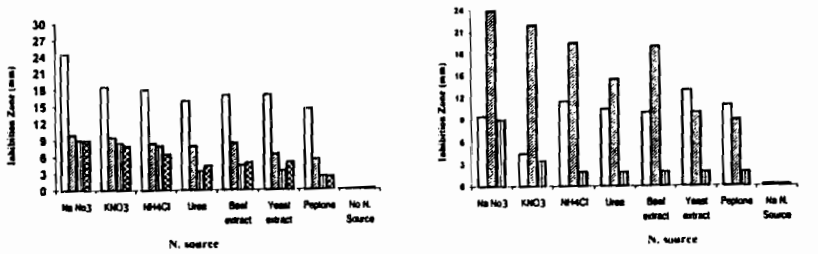

Fig. 4. Effect of supplying different nitrogen sources on the biosynthesis of the antimicrobial substance produced by *Streptomyces antibioticus* sub. sp.11M$_w$.

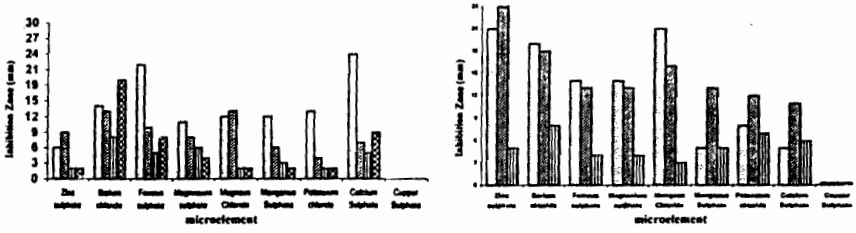

Fig. 5. Effect of different microelement on the biosynthesis of the antimicrobial substance produced by *Streptomyces antibioticus* sub. sp.11M$_w$

The characteristics of the purified antibiotic showed that, it is a yellow viscous syrup with no characteristic odour and soluble in chloroform, petroleium ether & methanol, insoluble in n-butanol, xylene, Diethyl ether, n-hexan, toluene, acetone & isopropanol and it is immiscible with water.

The elemental analytical data of the antibiotic indicated the presence of carbon, hydrogen, nitrogen and oxygen in the following percentages :

C = 63.41%, H = 8.46%, N = 4.73%, O = 23.4% and S = 0.0.
The analysis gives the imperical formula of $C_{18}H_{28}O_5N$.

Biochemical reactions of the antibiotic

The reactions revealed the detection of certain groups, in the investigated molecules. The antibiotic showed positive results with ferric chloride reaction, tollen's reaction and ninhydrin test and negative results with Molish's, Sakaguchi, Ehrlish's, Fehling, Meyer and lead sulphide reactions (Table 2).

TABLE 2. Blochemical reactions of the antimicrobial agent.

Chemical test	Results	Comment
1-Molish's reaction	-	Absence of sugar moiety
2-Sakaguchi reaction	-	Argianine is absent
3-Ehrlish's reaction	-	Absence of indolic group
4-Nitroprusside reaction	-	Amines are absent
5-Ferric chloride reaction	+	Di-ketons or enolic group are present
6-Fehling reaction	-	Absence of free aldehyde or keto sugars
7-Meyer's reaction	-	Absence of sulphur
8-Lead sulphide reaction	-	Absence of sulphur
9-Tollen's reaction	+	Presence of oromatic aldehydes diketons or aromatic amines
10-Ninhydrin test	+	Presence of protein, aminoacids and/or free amino group

+ = positive results - = negative result

Spectroscopic characteristics

The Infrared (IR) Spectrum of the antibiotic showed a characteristic band corresponding to 22 peaks (Fig. 6). The ultraviolet (UV) absorption spectrum of the antibiotic recorded a maximum absorption peak at 170 nm. (Fig.7). The HPLC spectrum of the antibiotic showed that the maximum peak at 1.309 (Fig.8). The mass spectrum of antibiotic showed that the molecular weight is 355 (Fig. 9, 10) and NMR spectrum of antibiotic (Fig. 11).

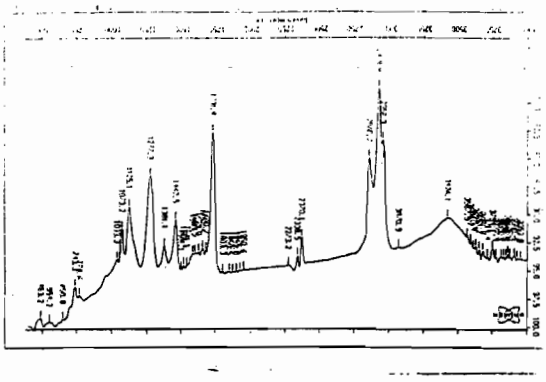

Fig. 6. The IR Spectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub. sp. 11 M_w.

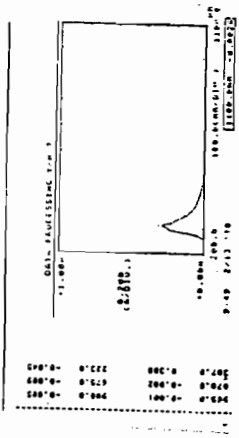

Fig. 7. The UV Spectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub.sp. 11 M_w.

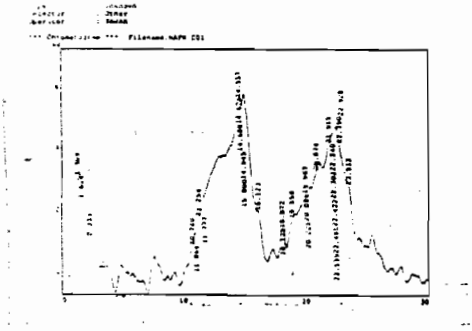

Fig. 8. The HPLC spectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub. sp. 11 M_w.

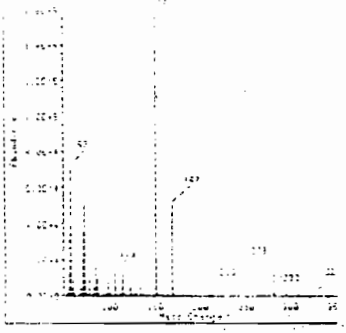

Fig. 9. The mass spectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub.sp. 11 M_w.

Biological activities of the purified antibiotic

Studying of the antimicrobial activity of antibiotic indicated that, the antibiotic is active against the tested Gram-positive, Gram-negative bacteria and fungal species (Table 3).

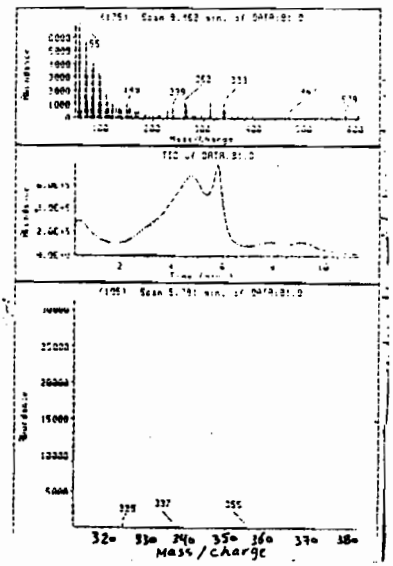

Fig. 10. The massspectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub. sp. 11 M_w.

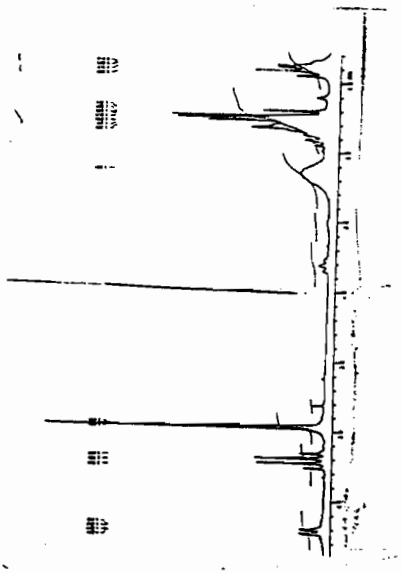

Fig. 11. The NMR Spectrum of the purified antimicrobial substance produced by *Streptomyces antibioticus* sub. sp. 11 M_w.

TABLE 3. Antimicrobial spectrum of the purified antibiotic produced from *Streptomyces antibioticus* sub. Sp. 11M_w.

Test Organism	MIC ul / ml	MBC ul / ml
A) Bacteria		
Escherichia coli.	200	> 200
Salmonella typhi	180	> 200
Bacillus subtilis	200	> 200
Bacillus cereus	180	180
B) Fungi		
Fusarium solani	140	180
Penicillium sp.	200	> 200

MIC = Minimal Inhibitory concentration.
MBC = Minimal Bactericidal concentration.

Identification of the purified antibiotics

On the basis of the recommended keys, for the identifications of antibiotics and in view of the comparative study of the recorded properties of the antibiotics, it could be stated that the antibiotic is suggestive of being belonging to

Glutarimide group (Protomycin) antibiotic (Table 4), (Umezwa, 1977 and Berdy, 1974, 1980a, b & c). It was suggested to give the name Biotomycin $11M_w$.

TABLE 4. A comparative study of the characteristic properties of antibiotic $11M_w$ antibiotic in relation to reference antibiotic (Protomycin).

Characteristic	Protomycin	Antibiotic (under test)
1-Molecular weight	351	355
2-Chemical anaylsis		
C	64.93	63.41
H	8.32	8.46
N	3.99	4.73
O	22.76	23.4
S	ND	0.0
3-Ultraviolet	232	170
4-Formula	$C_{19}H_{29}O_5N$	$C_{18}H_{28}O_5N$

N.D = Not Deteted Protomycin related to group Glutarimide

Discussion

More efforts have been done for producing new substances instead of chemicals through using some microorganisms which can produce some antimicrobial agents (Daquen *et al.,* 1996). Most of the known antibiotics were isolated from some species belonging to actinomycets. More than 50% of the antibiotics described are in fact produced by members of only one bacterial order actinomycetales. Among the various genera of actinomycetes, the genus streptomyces has yielded the greatest number of antibiotics (Betina, 1983).

Among the various genera of actinomycetes, the geneus *Streptomyces* had yielded the largest number of antibiotics (Betina, 1983).

Out of 101 actinomycete isolates, obtained in the present work that were isolated from El-Minia Governorate and El-Sharkia Governorate, only fifth isolates $4M_T$, $11M_w$, $12M_w$, $29SH_s$ and $30SH_s$ showed high antimicrobial activity against the tested bacterial and fungi when grown on starch nitrate medium. Fortunately, the actinomyate isolate No. $11M_w$ exhibited the most antagonistic activity against the tested organisms. Identification of the selected five actinomycete isolates revealed that all five species belongs to the genus *Striptomyces* and identified as *Streptomyces antibioticus, Streptomyces recifensis, Streptomyces griseobrunneus, Streptomyces aureomonopodiales* and *Streptomyces pyridomyceticus*. The identifications carried out according to Shirling & Gottlieb (1968a, 1968b, 1969 and 1972), Williams *et al.* (1984) and Lechevalier (1989).

For the purpose of optimizing the biosynthesis of the antibiotic under study different environmental conditions and physical factors such as incubation periods, pH values, incubations temperature and gamma irradiation effect were studied. Here in the maximum biosynthesis occurred after incubation period of 6 days for the antibiotic production. These results are in agreement with El-Shirbiny (1990) and Nadkarni *et al.* (1998) for the production of caeseorhodomycin and methomycin produced by *Streptomyces* spp. and *Actinomyces* sp. It was also noticed that, a slight decrease in antagonistic activity of *Streptomyces antibioticus* sub.sp. $11M_w$ recorded after 7 and 10 days of incubation. This observation is in agreement with the results of Samoilov *et al.* (1967) for the production of corminomicin by *Actinomyces lavendula* culture.

The recorded optimum incubation temperature for the selected isolate *St. antibioticus* sp. 11 M_w was 30°C, meanwhile lower and higher temperature seemed to be inconducive. These results are in agreement with those reported by El-Shirbiny (1990), Mansour *et al.* (1996) and Ramadan (2000), for the production of caeseorhodomycin, demethyltetracyline and AZ.AH 2B Produced by *S. caesius, S. aureofacier* and *Streptoalloteichus hindustans,* respectively.

The maximum biosynthesis of antibiotic was found to be in a production medium adjusted at pH 8.0 for the experimental isolate *Streptomyces antibioticus* sub.sp. $11M_w$. These results agree with those of Nguyen *et al.* (1995) and Corvini *et al.* (2000) for the production of tylosin and pristinomycin produced by *Striptomyces pristinaespiralis* at pH ranging from 6.3 and 8.7 and those reported by Ramadan (2000) for the production of antibiotic produced by *Striptoalloteichw hindustanus* sub.sp. AZ. AH 2H.

The results showed that as the dose level of gamma rays increased a significant decrease in both viability and antagonistic activity were obtained. In this connection several worders proved the inhibitory effect of high doses of gamma rays on both growth and metabolic activity of actinomycetes (Abou-Elkhair, 1986 and Tohamy, 1991).

For obtaining maximum yield of the antibiotic produced by *Streptomyces antibioticus* sub.sp. $11M_w$, the effect of certain nutritional requirements (*e.g.* carbon, nitrogen and certain micro elements) were tested. Starch at 20 g/l level was the best carbon source at this concentration for maximum production of antibiotic by *Streptomyces antibioticus* sub.sp. $11M_w$ where as sodium nitrate was the optimum nitrogen source, when used at 2 g/l level. These results were in agreement with those of Mansour *et al.* (1996), Ghazal (1984), Lee & Dewey (1980) and El-Shirbiny (1990) for production of dimethyltetracycline, AZ-SA501 antibiotic, antibacterial compounds and caesorhodomycin by *S.aureofaciens* sub sp. *Viridalaus, Streptoverlicillium, Lovenduligriseus, Streptomyces* sp., *Micromonospora Purpurea* and *Streptomyces casesus var., egyptica,* respectively.

The present results showed that the selected isolate *Streptomyces antibioticus* sub. sp. 11 Mw was affected by addition of different microelement ions to the fermented medium such as Ca^{+2}, K^{+2} , Ba^{+2}, Fe^{+3}, Zn^{+2}, Mg^{+2}, Mn^{+2} and Cu^{+2}. Maximum antibiotic biosynthesis was obtained by adding barium chloride to starch nitrate medium except with *F. solani* required zinc sulphate, the addition of cupper sulphate completely inhibited the growth of *Strep. antibioticus* sub sp. 11 Mw and hence no antibiotic produced. These results agreed with Egorov (1985) for the production of tetracycline and chloram-phenicol and El-Shirbiny (1990) for the production of caesorhodomycin and many other enzymes, respectively. Shaukat and Sideliqui (2003) reported that, zinc addition remarkably improved antifungal activity of fluorescent *Pseudomonads* against *Macrophomina phaseolina*.

The band with an R_f value of 0.625 indicated the presence of one compound. Similarly many workers use this method (Taro *et al.*, 1998) for the purpose of purification process, the antibiotic was allowed to pass through a column chromatography. Fifty fractions were collected and tested for their activity. The activity was recorded at fractions between No 5 to 18. Similarly, many workers used a column chromatography (Toshio *et al.*, 2000; Honda *et al.*, 2001; Kenichi *et al.*, 2001; Ueno *et al.*, 2002 and Yoko *et al.*, 2001).

Studying the elemental analysis of the antibiotic resulted in an empirical formula of $C_{18}H_{28}O_5N$. The spectroscopic characteristics of antibiotic revealed the presence of the maximum absorption peak in UV at wave length 170 nm, infra red absorption spectrum represented by 22 peaks. HPLC-Spectrum high peak at 1.309 and Mass spectrum showed that the molecular weight is 355. The biochemical tests of antibiotic gave positive reaction with ferric chloride reaction, tollen and Ninhydrin reaction. Similar studies were conducted by Atta (1999), Akihkiko *et al.* (2000), Naki *et al.* (2000),Toshio *et al.* (2000), Ammar *et al.* (2001), Honda *et al.* (2001), Yutaka *et al.* (2001) and Ueno *et al.* (2002).

Identification of antibiotic was carried out according to recommended international keys for the identification of antibiotics and in view of the comparative study of the recorded properties of the antibiotic, it could be stated that the antibiotic may belong to Glutarimide group (Protomyein antibiotic) and give the name biotomycin $11MM_w$.

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مضاد حيوى جديد ينتجه *ستربتوميسيس أنتيبيوتيكاس* تحت نوع جديد ١١ إم دبليو

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يهدف البحث إلى دراسة النشاط الضد ميكروبى لمائة وواحد سلالات أكتينوميسينية والتى تم عزلها من مناطق مختلفة من محافظات الشرقية والمنيا على اثنى عشر كائن دقيق (٦ سلالات بكتريا موجبة وسالبة الجرام و ٦ سلالات فطريات). وتم عزل أكثر العزلات الأكتينوميسينية فاعلية من محيط جذر القمح المزروع فى محافظة المنيا – مصر وتم تعريفه *Streptomyces antibioticus sub. sp. 11M*$_w$ ، وتوصل الباحثون إلى أفضل الظروف لإنتاج الأيض الضد ميكروبى للكائن *Streptomyces antibioticus sup. sp. 11* M_w يقل بزيادة جرعات أشعة جاما، وكان أنسب مصدر كربونى ونيتروجينى هما النشا ونترات الصوديوم ، على التوالى. وباستخدام مذيبات مختلفة بواسطة الفصل اللونى تم فصل المادة الضد ميكروبية وتركيز الأجزاء النشطة وتعريفها باستخدام مفتاح التعريف لمضادات الأحياء، وتشير التحاليل الكيموفيزيائية إلى الصيغة C_{18} H_{28} O_5 N وتؤكد الدلائل أن مركب المضاد الحيوى المنقى والذى اقترح تسميته بيوتوميسين ينتمى إلى مجموعة جلوتاريميد (بروتوميسين).

وأوضحت النتائج أن أعلى تخليق لمضادات حيوية كان بإضافة كلوريد الباريوم بنسبة (٠,٠٥ مللى جرام/ ١٠٠ مللى) إلى الوسط الغذائى نترات النشا ومثبط بإضافة كبريتات النحاس (٠,٠٥ مللى جرام / ١٠٠ مللى).