Microbiological Studies on Cultural, Physiological Characteristics and Antimicrobial Activities of *Streptomyces Cyaneus*-AZ-13Zc

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Abstract: Hundred and five actinomycete isolates could be isolated from different soil samples collected from different localities in Egypt. One of the actinomycete cultures (AZ-13Zc) from three cultures was found to produce a wide spectrum antimicrobial agent (anti-Gram-positive and Gram-negative bacteria and unicellular fungi). The actinomycete AZ-13Zc could be isolated from a soil sample collected from Zefta district, Egypt. From the taxonomic features, the actinomycete isolate AZ-13Zc matches with *Streptomyces cyaneus* in the morphological, physiological and biochemical characters. Thus, it was given the suggested name Streptomyces cyaneus-AZ-13Zc. The parameters controlling the biosynthetic process of antimicrobial agent formation including: inoculum size, different pH values, different temperatures, different incubation period, and different carbon and nitrogen sources, potassium nitrate, K_2HPO_4, $MgSO_4.7H_2O$ and KCl concentrations were fully investigates.
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1. Introduction

Of all the known microbes, actinomycetes represent a rich source of biologically active metabolites such as antibiotics, immunosuppressant's, antiphrastic and anticancer agents (Berdy, 2005). Actinomycetes have provided many important bioactive compounds of high comercial value and continue to be routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes (Okami and Hotta, 1988). Emergence of drug resistant pathogens especially in immunodeficient patients revealed the need for new and novel antibiotics (Jain. and Jain, 2005 and Kadar, 2005). Majority of the antibiotics so far reported are obtained from *Streptomyces*, which are common inhabitants of soil (Ravel *et al.*, 2000). Numerically, they cover about 80% of total antibiotic products as compared to other genera (Kieser *et al.*, 2000). *Streptomyces*, genus of the order *Actinomycetales* constitute a distributed group of bacteria. They have many properties that favor their predominance among other saprophytic microorganisms. They are best known for their economic importance as producers of antibiotics, vitamins and enzymes, and are certain to have a significant role in future biotechnology (Goodfellow *et al*; 1984). Moreover, the majority of antibiotics in use today were discovered in the 1950's, 1000 antibiotics are known today and most of them (58%) are produced by Actinomycetales especially the genus *Streptomyces*, (Edward, 1980). Some species of *Streptomyces* are causative agents of important human and animal diseases, plant pathogens and the rest are involved in the turnover of organic matter (Goodfellow, *et al*; 1984). Revising the literature (Waksman and Henrici, 1957) revealed that *Streptomyces sp.* had been investigated and their antagonistic properties were known and some species produced famous antibiotics. This research was aimed to isolate bacterial strains from soil able to produce antibiotic and then to study the different conditions affecting its productivity.

In the present study were describe the isolation of an actinomycete strain AZ-13Zc from Zefta district, which generates an production the bioactive substances that demonstrated inhibitory affects against microbial pathogenic. The identification of this strain based on the cultural, morphology, physiology and biochemical characteristics. The primary bioactive substances were tested against Gram positive and Gram negative bacteria and unicellular fungi.

2. Material and Methods

2.1. Actinomycete isolate

The actinomycete isolate AZ-13Zc was isolated from soil sample collected from Zefta district. It was purified using the soil dilution plate technique described by (Williams and Davis, 1965).

2.2. Screening for antimicrobial activity

The anti- microbial activity was determined according to (Kavanagh, 1972).

2.3. Characterization studies of actinomycete isolate (AZ-13Zc)

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.

2.4. Parameters controlling antimicrobial agent biosynthesis

These included inoculum size, incubation period, pH values, incubation temperatures; different carbon and nitrogen sources, starch, potassium nitrate, K_2HPO_4, $MgSO_4.7H_2O$ and KCl have been determine by the standard methods.

3. RESULTS

3.1. Screening for the antimicrobial activities

The metabolites of the actinomycete isolate AZ-13Zc exhibited various degrees of activities against Gram positive and Gram negative bacteria and unicellular fungi.

3.2. Characterizations of the actinomycete isolate

3.2.1. Morphological characteristics

Spore chains are spiral, spore mass are gray and red; spore surfaces are smooth; substrate mycelium is yellowish brown and diffusible pigment yellowish brown to brown plate (1).

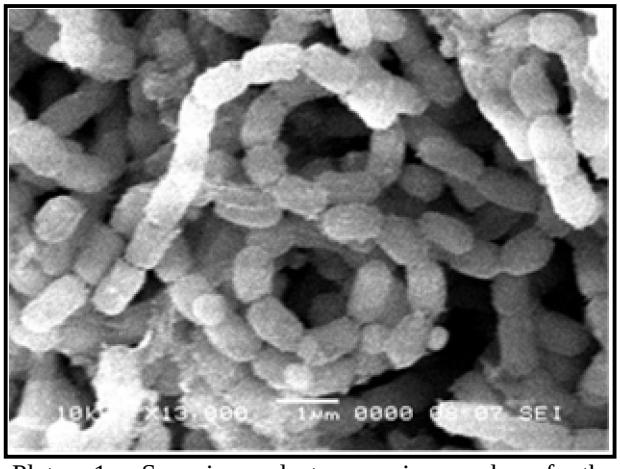

Plate 1. Scanning electron micrograph of the actinomycete isolate AZ- 13Zc growing on starch nitrate agar medium spore chain is spiral shape and spore surfaces is smooth (13.000).

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 AZ- 13Zc could hydrolyze pectin, starch, lipid and cellulose, but lecithin, protein and catalase were negative. Melanin pigment was positive, degradation of xanthine, esculine, production of H_2S, decomposition of urea, and utilization of citrate were positive but KCN and nitrate reduction were negative.The isolate AZ- 13Zc could utilize maltose, fructose, rhamenose, lactose, raffinose, mannitole, and xylose. Moderate growth on arabinose,

sodium acetate, sucrose, glucose, L-asparagine, L-leucine, L-cystiene and glycine.. Only trace growth could be detected using L-argenine, L-phenylalanine, L-alanine, L-tryptophan, L-valine and L-lysine, but week growth on L-serine. The actinomycete isolate AZ-13Zc can ferment fructose and xylose with the formation of acid, whereas fermentation of glucose, cellobiose, maltose, sodium acetate, sodium citrate, rhaminose, mannitol and arabinose are negative. The actinomycete isolate AZ- 13Zc grows well on 0.5% to 5% NaCl concentrations, but no growth in the presence of 6% NaCl. Good growth could be detected within a temperature range of 20 to 40ºC and pH range could be detected within a pH range of 5 to 9. The growth of actinomycete isolate AZ- 13Zc is not inhibited in the presence of crystal violet and phenol.

3.2.4. Color and culture characteristics

The actinomycete isolate AZ-13Zc failed to grow on tryptone-yeast extract broth medium (ISP-1) and yeast extract-malt extract agar medium (ISP-2). Good growth on oat meal agar medium (ISP-3), aerial mycelium was medium red, substrate mycelium was light yellowish brown, and diffusible pigment was moderate yellowish brown. Good growth on inorganic salts-starch agar medium (ISP-4), the aerial mycelium was medium gray, substrate mycelium was light brown gray, and the diffusible pigment was moderate yellowish. Moderate growth on glycerol-asparagine agar medium (ISP-5), the aerial mycelium is medium red, substrate mycelium is Light yellowish brown and the diffusible pigment is deep red orange. Moderate growth on Peptone yeast extract-malt extract iron agar medium (ISP-6), the aerial mycelium is light gray, the substrate mycelium is moderate yellowish brown and the diffusible pigment is deep brown. Good growth on tyrosine agar medium (ISP-7), the aerial mycelium is light gray, substrate mycelium is moderate yellowish brown, and the diffusible pigment is deep brown, Good growth on starch nitrate agar medium, the aerial mycelium is light gray, substrate mycelium is light yellowish brown and the diffusible pigment is moderate yellowish brown.

3.3. Identification of actinomycete isolate- AZ-13Zc

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 AZ- 13Zc in relation to the most closest reference strain, viz. *Streptomyces cyaneus* it could be stated that the actinomycetes isolate, AZ- 13Zc is suggestive of being likely belonging to *Streptomyces cyaneus*-AZ- 13Zc (ID Score 0.94083) (Table 2).

3.4. Parameters controlling the biosynthesis of the antimicrobial agent

3.4.1. Inoculum size

Data illustrated in (Table. 3) showed the relation between antibiotic productivity and different inoculum sizes. Maximum antimicrobial activity production could be recorded that a different inoculum sizes for three discs, after this maximum values 30.0, 29.5, 28.3, 30.5 and 29.0 in case of *Bacillus subtilis* NCTC 1040 ; *Staph. aureus,* NCTC 7447 ; *Escherichia coli* NCTC 10416; *Salmonella typhi* NCIMB 9331; *Candida albicans* IMRU 3669, respectivily.

3.4.2. Incubation period

Data illustrated in (Table. 4) showed the relation between antibiotic productivity and time of incubation. The level of antibiotic yield increased gradually with increasing the incubation period up to the end of 3 days, after this maximum values 31.0, 30.0, 28.5, 30.5 and 29.3 in case of *Bacillus subtilis* NCTC 1040 ; *Staph. aureus,* NCTC 7447 ; *Escherichia coli* NCTC 10416; *Salmonella typhi* NCIMB 9331; *Candida albicans* IMRU 3669, respectivily at an, a steadness of antimicrobial agents productivity was observed.

3.4.3. pH value

The results represented in (Table. 5) that, the optimum initial pH value capable of promoting antimicrobial agents biosynthesis by *Streptomyces cyaneus*-AZ- 13Zc was found to be at the value of 7.0 since the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 31.0, 29.5, 28.6, 30.6 and 29.0 in case of *Bacillus subtilis* NCTC 1040 ; *Staph. aureus,* NCTC 7447 ; *Escherichia coli* NCTC 10416; *Salmonella typhi* NCIMB 9331; *Candida albicans* IMRU 3669, respectivily.

3.4.4. Incubation temperature

Data represented in (Table. 6) showed that, the optimum temperature capable of promoting

antimicrobial agents biosynthesis by *Streptomyces cyaneus*-AZ- 13Zc was at 30ºC, where, the diameter of inhibition zone resulted from antimicrobial agents productivity reached up to 31.0, 29.5, 28.6, 30.6. and 29.0 in case of *Bacillus subtilis* NCTC 1040 ; *Staph. aureus*, NCTC 7447 ; *Escherichia coli* NCTC 10416; *Salmonella typhi* NCIMB 9331; *Candida albicans* IMRU 3669, respectivily.

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

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	*Escherichia coli* NCTC 10416	*Salmonilla typhi NCIMB 9331*	*Aspergillus flavus IMI 111023*	*Aspergillus niger IMI 31276*	*Candida albicans* IMRU 3669
2Sc	24.5	20.5	21.0	20.5	0.0	0.0	0.0
6Sc	19.0	14.5	17.0	15.0	0.0	0.0	0.0
7Sc	23.5	21.0	0.0	0.0	16.0	14.0	13.5
4Su	20.3	19.5	30.0	25.6	0.0	0.0	0.0
6Su	13.5	14.6	12.0	16.0	22.5	21.0	20.0
8Su	22.0	19.5	20.0	18.2	0.0	0.0	0.0
11Su	24.5	23.0	0.0	0.0	0.0	0.0	0.0
12Su	20.5	22.0	0.0	0.0	0.0	0.0	0.0
3Sb	22.0	23.5	11.5	11.0	12.5	20.5	13.5
2Zc	28.5	26.5	14.0	16.0	13.0	13.5	0.0
3Zc	24.5	20.5	20.5	19.0	14.5	0.0	0.0
5Zc	19.0	20.5	17.5	19.0	0.0	0.0	0.0
7Zc	21.0	26.0	15.0	22.6	0.0	0.0	0.0
9Zc	16.0	16.5	13.5	0.0	21.0	17.0	0.0
11Zc	13.0	15.0	19.5	19.0	0.0	0.0	0.0
13Zc	**31.0**	**30.0**	**28.3**	**30.6**	**0.0**	**0.0**	**29.0**
17Zc	21.0	19.5	23.0	22.3	0.0	0.0	13.0
19Zc	23.5	24.8	23.0	28.0	24.7	28.5	17.0
1Zb	0.0	0.0	0.0	0.0	25.0	24.3	22.0
9Zb	20.6	15.5	24.2	20.0	0.0	0.0	0.0
3Ns	25.0	22.5	0.0	0.0	0.0	0.0	0.0
4Ns	0.0	0.0	0.0	0.0	22.5	19.8	0.0
3Ks	17.5	20.3	15.7	19.5	0.0	0.0	0.0
6Ks	18.0	18.5	18.5	0.0	17.5	19.5	21.0
8Ks	25.6	21.0	24.2	25.5	0.0	0.0	20.0
1As	21.0	22.0	22.5	24.5	28.5	23.0	17.0
2As	18.5	20.0	11.5	15.5	0.0	0.0	0.0

.

*Mean values of determination was calculated

Table 2. Numerical taxonomy of *Streptomyces* species program (PIB WIN) (*Streptomyces* species) J. Gen Microbiol. 1989 13512-133 lang.

Characteristic	AZ-13Zc	*Streptomyces cyaneus*
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 inhibitors Phenol (0.1 % w/v)	 +	 +
Utilization of		
L- Cysteine	+	+
L- Valine	+	+
L- phenylalanine	+	+
L- Histadine	+	+
Sucrose	+	+
meso-Inositol	+	+
Mannitol	+	+
Rhamnose	+	+
Raffinose	+	+
Melezitose	+	+

No.	Key	Source	Identification	ID Score
1	**AZ-13Zc**	**Zefta district**	***Streptomyces cyaneus***	**0.94083**

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

3.4.5. Carbon source

Data given in (Table 7) indicated that the addition of different equimolecular carbon sources for production of antimicrobial agents revealed that starch is the best carbon source for biosynthesis antimicrobial substances with concentration 2.5g/100. The effect of the used carbon sources in production of antimicrobial agent could be arranged in the following descending manner; for *Streptomyces cyaneus*-AZ- 13Zc, starch> mannitol> sucrose> glucose> fructose> maltose> sucrose> D-galactose> lactose> cellulose.

3.4.6. Nitrogen source

Data given in (Table 8) indicated that the addition of different nitrogen sources exhibited an increase in the level of antimicrobial agent production by *Streptomyces cyaneus*, AZ- 13Zc where potassium nitrate was found to be the best nitrogen source for the antimicrobial 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 cyaneus*-AZ- 13Zc, KNO_3> NH_4Cl> $NaNo_3$> peptone> urea> $(NH_4)_2SO_4$> yeast extract> beef extract.

3.4.7. Concentration of K_2HPO_4, $MgSO_4.7H_2O$ and KCl

The best concentration of K_2HPO_4, $MgSO_4.7H_2O$ and KCl for the biosynthesis of the antimicrobial agent is 1.0 g/l; 0.7 g/l and 0.5 g/l respectively table (9, 10 and 11).

Table 3. Effect of different inoculum size on the antimicrobial production by *Streptomyces cyaneus*, AZ-13Zc, against the tested strains.

Inoculum size (discs)	*Mean values of inhibition zones (in mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus*, NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
1	26.0	25.5	23.5	25.4	0.0	0.0	20.5	0.33
2	27.8	27.6	25.5	28.0	0.0	0.0	25.6	0.37
3	**31.0**	**29.5**	**28.3**	**30.5**	**0.0**	**0.0**	**29.0**	**0.40**
4	30.7	29.2	28.0	30.0	0.0	0.0	28.0	0.39
5	29.0	28.5	27.5	29.4	0.0	0.0	27.5	0.39

Table 4. Effect of different incubation periods on the antimicrobial production by *Streptomyces cyaneus*, AZ-13Zc, against the tested strains.

Incubation Period (days)	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus*, NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
1	18.0	17.5	18.5	20.0	0.0	0.0	15.5	0.22
2	26.0	25.0	23.5	26.5	0.0	0.0	23.0	0.33
3	**31.0**	**30.0**	**28.5**	**30.5**	0.0	0.0	**29.3**	**0.39**
4	31.0	30.0	28.5	30.5	0.0	0.0	29.5	0.39
5	31.0	29.8	28.5	30.5	0.0	0.0	29.3	0.39
6	31.0	30.0	28.5	30.5	0.0	0.0	29.0	0.39
7	31.0	30.0	28.5	30.5	0.0	0.0	29.3	0.39
8	30.8	30.0	28.5	30.4	0.0	0.0	29.3	0.39
9	30.8	29.8	28.3	30.5	0.0	0.0	29.0	0.39
10	30.6	29.5	28.3	30.1	0.0	0.0	29.0	0.39

Table 5. Effect of different incubation temperatures (°C) on the antimicrobial production by *Streptomyces cyaneus*, AZ-13Zc, against the tested strains.

Incubation Temperature (°C)	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
20	18.7	17.3	16.7	19.8	0.0	0.0	21.0	0.22
25	27.1	25.8	26.5	27.0	0.0	0.0	25.3	0.35
***30**	**31.0**	**29.5**	**28.6**	**30.5**	**0.0**	**0.0**	**29.0**	**0.39**
35	29.5	26.0	27.0	28.8	0.0	0.0	24.5	0.37
40	27.0	24.8	24.5	24.5	0.0	0.0	18.5	0.34
45	19.6	18.0	17.8	18.9	0.0	0.0	15.0	0.29
50	15.2	13.1	14.0	13.1	0.0	0.0	12.5	0.20
55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.20

*Mean values of 3 determination was calculated * 0.0 = No growth

4. DISCUSSION

The actinomycete isolate, AZ-13Zc was isolated from a soil sample collected from Zefta distract, Egypt. The isolate was grown on starch nitrate agar medium for investigating its potency to produce antimicrobial agents. The growth of the actinomycete isolate exhibited antimicrobial activities against (Gram-positive and Gram-negative bacteria and unicellular fungi). 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). In view of all the previously recorded data, the identification of the actinomycete isolate AZ-13Zc was suggestive of being belonging to *Streptomyces cyaneus*-AZ-13Zc (ID Score 0.94083) which can produce a broad-spectrum antimicrobial agent. For optimizing the biosynthesis of the antimicrobial agent from *Streptomyces cyaneus*-AZ-13Zc, different cultural conditions such as inoculum size, pH, temperature, and incubation period, effect of different carbon and nitrogen sources, potassium nitrate, K_2HPO_4, $MgSO_4.7H_2O$ and KCl was studied. The maximum biosynthesis was achieved at the end of an incubation period of 3 days for the antimicrobial agent production using three discs of actinomycete culture. Similar results had been recorded by various workers; (Adinarayana *et al.,* 2002 and Kharel *et al.,* 2004) . The fact that maximum yield of the antimicrobial 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 antimicrobial agent indicated that *Streptomyces cyaneus*-AZ-13Zc require starch, potassium nitrate, K_2HPO_4, $MgSO_4.7H_2O$ and KCl at concentrations 2.5.0 g/100 ml; 0.25 g/100; 1.1 g/l; 0.7 g/l and 0.5 g/l respectively. Similar results have been recorded by various workers: (Howells *et al.,* 2002; El-Naggar *et al.,* 2003 and Criswell *et al.,* 2006).The active metabolites were extracted by ethyl acetate at pH 7. Similar results were obtained by (Sekiguchi, *et al.,* 2007 and Atta *et al.* 2009).

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 antimicrobial activities against pathogenic microorganisms (Gram positive and Gram negative bacteria and unicellular fungi) and studies the parameters controlling the biosynthetic process of antimicrobial agent formation.

Table 7. Effect of different equimolecular carbon sources on the production of antimicrobial agents produced by *Streptomyces cyaneus*, AZ-13Zc, against tested strains.

Different carbon sources	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
Cellulose	19.3	18.5	22.0	20.0	0.0	0.0	17.0	0.23
Lactose	21.5	20.5	22.5	22.0	0.0	0.0	18.0	0.26
Galactose	23.0	21.5	24.0	22.5	0.0	0.0	18.9	0.27
Sucrose	23..0	22.0	24.0	23.0	0.0	0.0	23.5	0.27
Maltose	24.5	25.5	26.0	26.0	0.0	0.0	24.0	0.29
Fructose	27.5	27.0	28.0	26.8	0.0	0.0	24.0	0.35
Glucose	29.0	27.3	28.5	29.0	0.0	0.0	26.3	0.37
Starch	**31.1**	**30.0**	**29.5**	**30.6**	**0.0**	**0.0**	**29.0**	**0.40**
Mannitol	30.4	29.0	28.0	28.5	0.0	0.0	27.0	0.39

Table 8. Effect of different nitrogen sources on the production of antimicrobial agent produced by *Streptomyces cyaneus*, AZ-13Zc, against tested strains.

Different nitrogen sources	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
Beef ext.	25.5	24.0	26.0	24.5	0.0	0.0	21.2	0.37
Yeast ext.	27.0	25.0	26.3	25.0	0.0	0.0	23.6	0.39
$(NH_4)_2SO_4$	27.5	26.7	27.0	26.5	0.0	0.0	25.0	0.39
Urea	29.0	28.0	27.5	27.0	0.0	0.0	25.3	0.40
Peptone	30.8	29.8	30.0	29.5	0.0	0.0	28.0	0.41
$NaNo_3$	31.5	30.3	29.9	31.0	0.0	0.0	29.6	0.43
NH_4Cl	31.6	30.5	30.9	31.4	0.0	0.0	29.8	0.43
KNO_3	**32.1**	**31.0**	**31.2**	**32.0**	**0.0**	**0.0**	**30.5**	**0.44**

Table 9. Effect of different concentration of K_2HPO_4 on the production of antimicrobial agents produced by *Streptomyces cyaneus*, AZ-13Zc, against tested strains.

Different conc. (g/l) of K_2HPO_4	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
0.2	24.5	25.0	25.5	23.8	0.0	0.0	21.5	0.36
0.5	27.8	26.0	28.5	27.0	0.0	0.0	27.3	0.38
0.8	30.0	29.5	31.0	30.3	0.0	0.0	29.0	0.39
1.0	**32.2**	**31.5**	**32.0**	**32.5**	**0.0**	**0.0**	**31.2**	**0.44**
1.4	31.0	30.5	30.8	29.5	0.0	0.0	31.8	0.41

*Mean values of 3 determination was calculated * 0.0 = No growth

Table 10. Effect of different concentration of $MgSO_4.7H_2O$ on the production of antimicrobial agents produced by *Streptomyces cyaneus*, AZ-13Zc, against tested strains.

Different conc. (g/l) of $Mgso_4.7H_2o$	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
0.1	26.3	27.0	27.3	28.0	0.0	0.0	26.0	0.37
0.3	29.5	30.0	30.5	30.0	0.0	0.0	27.0	0.41
0.5	32.0	31.5	32.0	32.6	0.0	0.0	31.0	0.44
0.7	**32.5**	**31.5**	**32.8**	**33.5**	**0.0**	**0.0**	**31.6**	**0.46**
1.0	31.0	31.0	30.5	33.0	0.0	0.0	31.5	0.45

Table 11. Effect of different concentration of KCl on the production of antimicrobial agent produced by *Streptomyces cyaneus*, AZ-13Zc, against tested strains.

Different conc. (g/l) of KCl	Mean values of inhibition zones (mm)							Dry weight (gm/50ml)
	Gram positive bacteria		Gram negative bacteria		Filamentous fungi		Unicellular fungi	
	Bacillus subtilis NCTC 1040	*Staph. aureus,* NCTC 7447	*Escherichia coli* NCTC 10416	*Salmonella typhi* NCIMB 9331	*Aspergillus flavus* IMI 111023	*Aspergillus niger* IMI 31276	*Candida albicans* IMRU 3669	
0.1	27.0	26.6	28.0	27.5	0.0	0.0	21.0	0.38
0.3	30.7	30.0	31.0	31.0	0.0	0.0	28.0	0.42
0.5	**32.5**	**31.6**	**32.7**	**33.5**	**0.0**	**0.0**	**31.5**	**0.46**
0.7	32.6	31.0	31.8	32.5	0.0	0.0	31.0	0.47
1.0	31.8	29.5	30.0	31.0	0.0	0.0	30.3	0.45

*Mean values of 3 determination was calculated * 0.0 = No growth

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