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Effects of some Environmental Conditions on Biomass and Antimicrobial Metabolite Production by *Streptomyces* Sp., KGG32

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ABSTRACT

The purpose of the present study was concerned with the taxonomy and influences of some cultural conditions on growth and antimicrobial metabolite production of a strain of *Streptomyces*, designated KGG32, which is a highly stable antimicrobial metabolite producer against the Gram-positive and Gram-negative bacteria under *in-vitro* growth conditions. In order to optimize the culture conditions for the production of antimicrobial metabolite, such as the effect of different temperatures, nature of carbon and nitrogen sources, pH value and time incubation were determined. Effects of nutritional compounds on production of antimicrobial compounds showed that the highest antimicrobial activities were obtained when sucrose at 1.0% (w/v) level was used as sole carbon source, under aerobic conditions at temperature 30°C, pH at 7.5. Bacteriological peptone was identified as nitrogen source that significantly affected antibiotic production followed by meat extract. Phenotypic characters of the strain strongly suggested that this strain belongs to the genus *Streptomyces*. The strain was grey colored with rectiflexibiles spore chains, exhibited resistance to neomycin, vancomycin and penicillin. It had the ability to produce enzymes such as caseinase amylase and xylanase. The results showed that strain KGG32 was a potential soil microorganism with antimicrobial activity and may be used for biotechnological purposes. © 2011 Friends Science Publishers

Key Words: Environmental conditions; Carbon sources; Antimicrobial metabolites; Fermentation; Biomass; *Streptomyces*

INTRODUCTION

The search for novel natural products with useful pharmacological activities often includes the isolation of actinomycetes, such as *Streptomyces* species, from soil samples (Ritacco *et al.,* 2003; Sembiring & Goodfellow, 2008). Actinomycetes have been especially useful to the pharmaceutical industry for their seemingly unlimited capacity to produce secondary metabolites with diverse chemical structures and biological activities. Searching for novel actinomycetes constitutes an essential component in natural product-based drug discovery (Valan Arasu *et al.,* 2008). Actinomycetes are also the focus of attention due to their production of secondary metabolites that may have a range of pharmaceutical and biotechnological applications.

Microbial natural products still appear to be the most promising source of the future antibiotics that society expects to be developed and they are the origin of most of the antibiotics on the market today (Kaltenpoth, 2009; Thumar *et al*., 2010). The search for new antibiotics or new microorganism strains producing antibiotics continues to be of utmost importance in research programs around the world, because of the increase of resistant pathogens and toxicity of some used chemical antibiotics. Therefore, there is an alarming scarcity of new antibiotics currently under development in the pharmaceutical industry. Still, microbial natural products remain the most promising source of novel antibiotics, although new approaches are required to improve the efficiency of the discovery process (Thumar *et al.,* 2010). In the past two decades however, there has been a decline in the discovery of new lead compounds from common soil-derived actinomycetes (Valan Arasu *et al.,* 2008).

It is well known that designing an appropriate fermentation medium is of critical importance in the production of secondary metabolites (Gao *et al.,* 2009). Prior knowledge and experience in developing a suitable basal medium may play an important role in further medium optimization (Jia *et al.,* 2008). Production of secondary metabolites through fermentation is influenced by various environmental factors including nutrients (nitrogen, phosphorous & carbon source), growth rate, feedback control, enzyme inactivation and variable conditions (oxygen supply, temperature, light & pH) (Lin *et al.,* 2010; Ruiz *et al.,* 2010; Sánchez *et al.,* 2010). In addition, production of valuable metabolites by actinomycetes differs qualitatively and quantitatively depending on the strains used in fermentation. As one of the most significant

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components in the medium, carbon source plays a critical role as sources of precursors and energies for synthesis of biomass building blocks and secondary metabolite production (Wang *et al.,* 2008 & 2010; Jia *et al.,* 2009). Therefore, influences of medium components and environmental conditions are an initial and important step to improve metabolite production of the genus *Streptomyces*.

This research work is concerned with identify of the strain KGG32 based on morphological, physiological, biochemical characteristics and determine the effects of various environmental factors on antimicrobial metabolite production and cell growth, under fermentation conditions.

MATERIALS AND METHODS

Microorganisms, media, and growth conditions: The *Streptomyces* sp., KGG32 was used in this investigation. The strain was isolated from the soil sample collected from Geçitköy Pond, North Cyprus as described previously (Oskay *et al.,* 2010). Spore and mycelia suspensions of *Streptomyces* sp., KGG32 was maintained in sterile 15% glycerol deposited at –20°C in culture collection of Biology Department, Celal Bayar University, Manisa Turkey.

Target Microorganisms: Different test microorganisms (15) were used throughout this study (Table I). Cultures of test bacteria were grown in Mueller-Hinton Broth (Oxoid) at 37°C for 24 h and stored in nutrient agar slants at 4°C. Yeasts were cultured on yeast extract malt extract broth at 30°C for 48 h and they maintained on the potato dextrose agar (PDA, Oxoid). The bacteria were obtained from the Department of Biology, Ege University (İzmir/Turkey).

Characterization of the *Streptomyces* sp., KGG32

Cultural and phenotypic characteristics: A set of morphological and physiological characteristics of the strain were examined after incubation for 7-14 days at 28°C on various media described by the International *Streptomyces* Project (ISP) (Shirling & Gottlieb, 1966) and the Bergey's Manual of Systematic Bacteriology (Williams *et al.,* 1983a; Cross, 1989). Mature aerial mycelium and substrate mycelium pigmentation were recorded on ISP5, aerial mass color on ISP3 and ISP4, melanin production on ISP6 and ISP7 following incubation at 27°C for 21 days using a reference color key (Prauser, 1964). For morphology of spore bearing hyphae with entire spore chain the strain was grown on different ISP media (ISP 3–6) and observed with a light microscope using cover-slip method. Temperature range, pH range, and NaCI tolerance for growth were recorded on Bennet's agar plates that were incubated at 27°C for up to 21 days. Resistance to antibiotics was estimated by the conventional paper disc agar diffusion bioassay. Utilization of carbohydrates was investigated on ISP9 medium using glucose as positive control. The ability to utilize nitrogen sources was determined in a basal medium containing glucose 10 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4.7H_2O$ 0.01 g, K_2HPO_4 1.0 g, NaCI 0.5 g, agar 3.0 g and distilled water 200 mL; after 15 days. Other physiological and biochemical characteristics were determined using the method described by Williams *et al.* (1983a & b). All tests were performed at 27°C.

Chemotaxonomic analysis: Analysis of the whole-cell diaminopimelic acid isomers (*LL*-DAP or *meso*-DAP) and the sugars was done by the method of Lechevalier and Lechevalier (1970) using the thin layer chromatography (silica gel plates (20X20, 60 F_{254}, Merck, Darmstadt, Germany).

Selection of suitable culture conditions for the optimum production of antimicrobial metabolite: Bioactive metabolite production of the strain was optimized by using different cultural parameters such as pH, temperature, carbon and nitrogen sources and time incubation in hours.

Effect of carbon and nitrogen sources: The influence of different carbon (glucose, glycerol, starch, maltose & sucrose) and nitrogen sources (meat extract, yeast extract, peptone, tryptone & L-asparagine) were studied to standardize the antibiotic production. The carbon and nitrogen sources were added to the pre-optimized medium before sterilization at a concentration of 1% (w/v) and 0.5% (w/v), respectively. The carbon and nitrogen source supporting the maximum production of metabolite were selected for the further studies. The temperature and pH were set to 30°C and at 7.5, respectively.

Effect of initial pH of the culture medium: To determine, influence of initial pH value of culture medium on growth and bioactive metabolite production; the strain was cultivated in the medium with different initial pH values (6–9). The pH was adjusted using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. The initial pH of culture medium achieved maximum antibiotic production was used for subsequent study.

Effect of incubation temperature: The optimum temperature for cell growth and bioactive metabolite yield was assayed by incubating the production medium at different temperatures varying from 20-37°C, maintaining all other conditions at optimum levels at original concentration.

Preparation of Fermentation medium: For maximum production of antimicrobial metabolite from the *Streptomyces* sp. KGG32; was inoculated in ISP2 medium that was used for development of inoculums. The seed culture was conducted in 250 mL Erlenmeyer flasks containing 40 mL of medium (same with fermentation medium) by inoculating 2.0 mL of spore suspension containing 1.0×10^6 spores/mL and cultivated under agitation (180 rpm) at 30°C for 2 days. After optimization of the fermentation parameters, a sample of 2 mL (5%, v/v) of spore suspension containing 1.0×10^6 spores/mL was inoculated to 200 mL of into the desired medium containing carbon source 10.0 g, meat extract 3.0 g, yeast extract 1.0 g, bacteriologic peptone 5.0 g, $CaCO_3$ 1.0 g, K_2HPO4 1.0 g, KH_2PO_4 1.0 g, trace element solution (Shirling & Gottlieb, 1966) 1 mL and distilled water 1000 mL. The initial pH was adjusted to 7.5 with 0.1 N NaOH or 0.1 N HCI as required

prior to sterilization. The inoculated flasks were incubated with agitation (180 rpm) at 30°C for 120 h.

During fermentation, at 12 h intervals, samples were taken for pH, biomass, and antibiotic analysis. The pH was measured with a digital pH meter (Hanna pH 211). Growth was monitored by determining the dry cell weight (DCW). Fermentation broth was taken aseptically and the cells were separated from the culture filtrate by centrifugation at 8000 rpm for 10 min and dried at 100°C for 24 h. DCW was calculated by the difference in the weights. The cell free supernatant of fermentation broth was harvested by centrifugation and assayed for antimicrobial activity.

Antimicrobial spectrum: Primary antimicrobial studies of *Streptomyces* sp. KGG32 on solid media were determined by conventional cross-streak method. Briefly, strain KGG32 was streaked near the periphery of a plate in straight line on Mueller-Hinton agar (MHA, Oxoid) (for bacteria) or PDA (for yeasts) and incubated at 30°C for five days. After incubation time, the test organisms were inoculated perpendicular to the antagonist on the agar medium, but not in contact with colony of the strain. The plates were incubated at 37°C for 24 h and at 30°C for 48 h, respectively for bacteria and yeasts. The antimicrobial activity was observed by determining the distance of inhibition between target microorganisms and KGG32 colony margin.

The effect of fermentation culture media containing different carbon sources on the antimicrobial activity was carried out. Antibiotic activity of liquid samples of fermentation were determined by a well diffusion method (Perez *et al.,* 1990) against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) at 12 h intervals. For this purpose, the cell free supernatant of fermentation broth was harvested by centrifugation at 8000 rpm for 10 min and subjected to antimicrobial activity. Shortly, bacterial strains grown on nutrient agar at 37°C for 24 h were suspended in a saline solution (0.85% NaCI) and adjusted to a turbidity of 0.5 MacFarland standards. 50 µL (1.0×10^6-cfu/mL) inoculum was used to inoculate 90 millimeters (mm) diameter petri plates containing 25 mL MHA, with a sterile non-toxic cotton swab on a wooden applicator. Small wells (6 mm diameter, 2 cm apart from each other) were punched in the medium and filled with 50-µL clear supernatant. The dishes were preincubated at 4°C for 2 h to allow uniform diffusion of possibly produced compound(s) into the agar. After preincubation, agar plates incubated at 37°C for 24 h. After the incubation period, the antibacterial activity was measured by the zone diameter (mm) of inhibition of the test organism observed. Negative controls included only liquid culture media. The effect of temperature and pH on the antimicrobial activity was also monitored. Each experiment was repeated three times and the mean of inhibitory zones recorded.

Statistical analysis: All tests and analyses were run in triplicate and results were means of triplicate determinations. Statistical analysis was carried out using Minitab-pc software package (version 13.20, Minitab Inc., 2000).

RESULTS AND DISCUSSION

A bacterium designated as *Streptomyces* sp., isolate KGG32 was isolated from a soil sample collected in Geçitköy Pond, North Cyprus showed antimicrobial activity against some Gram-positive bacteria, Gram-negative bacteria and some yeasts. *Streptomyces* (97.6%) was a dominant genus of actinomycetes isolated from 23 soil samples as reported by Atalan *et al.* (2000). A great variety of cultural and phenotypic features of the strain KGG32 was studied. According to the cultural characteristics, KGG32 is a gram-positive filamentous bacterium with extensively branched aerial mycelia, mesophilic and grew well on readily on most of the nutrient media described (Waksman, 1961; Shirling & Gottlieb, 1966; Locci, 1989; Manfio *et al.,* 1995; Atalan *et al.,* 2000; Sembiring & Goodfellow, 2008) (Table II) and the colonies were spreading. Aerial mycelium of strain appears grayish on ISP media (ISP2-4 & ISP6-7) **(**Fig. 1a) and the reverse side of the colony appears olive green, grey or cream (Fig. 1b). The strain was not produced melanoid pigment on both ISP6 and ISP7. During observation with microscopy on the ISP 3–6, spore-bearing hypha of the strain was rectiflexibiles chain (Fig. 2). The number of the spores was higher than 20, which indicated they refer to the long chains of spores.

On the other hand, the vegetative hyphae was branched but not fragmented. The results of the phenotypic and chemotaxonomic characteristics of the isolate are presented in Table III. Utilization of various carbon sources by strain indicated a wide pattern of carbon source assimilated. D-fructose, mannitol, L-rhamnose, raffinose, adonitol, lactose, xylose, glycerol, starch and glucose were readily utilized. Some differences occurred in utilization of nitrogen sources; DL-α-n-butyric acid, L-phenylalanine, L-histidine, L-proline and L-asparagine were used. This isolate also produced extracellular enzymes such as lecithinase, xylanase and amylase. Temperature for growth ranged from 15 to 37°C and an optimal temperature was 30°C, but not grew at 4 and 45°C. It showed good growth on medium supplemented with sodium chloride up to 7%; but no growth was observed at 10 and 13%. KGG32 not reduced nitrates; H_2S was not produced whereas elastin, xanthine and oxalate degraded. DAP existed as isomers with *LL* types. Cells contained no diagnostic sugar components. These characteristic properties strongly suggested that KGG32 belonged to the genus *Streptomyces* (Williams *et al*., 1983a & b).

The ability of *Streptomyces* isolate KGG32 to produce antibiotics is not consistent, but could be increased or decreased remarkably under different cultural conditions. The isolate had different antimicrobial activities against the test bacteria grown on solid media (Table I) and in liquid media. The results of the primary antimicrobial screening assays indicated that, KGG32 was the most antagonistic streptomycete strain with an inhibition zones ranged from 12-34 mm against eleven human pathogenic bacteria

Table I: Comparative antimicrobial activity of strain KGG32 in solid media with some standard antibiotics

	Test microorganisms[a]														
	SA	**BC**	**KR**	**EC**	**PF**	**BS**	**STYP**	**SM**	**SE**	**EF**	**MRSA**	**CA**	**SC**	**YL**	**GP**
KGG32[b]	34	24	30	26	-	30	15	-	-	34	12	12	-	12	14
NA	20[c]	28	10^R	26	30	32	6^R	30	6^R	30	24	ND	ND	ND	ND
NV	32	25	28	6^R	20	13^R	40	6^R	26	28	34	ND	ND	ND	ND
AMP	15^R	6^R	26	6^R	6^R	10^R	6^R	6^R	16	16	16^R	ND	ND	ND	ND
P	24	10^R	20^R	6^R	6^R	$8^{\pm R}$	6^R	6^R	11^R	24	6^R	ND	ND	ND	ND
VA	12	15	15	6^R	6^R	6^R	28	6^R	15	16	14	ND	ND	ND	ND
CLH	20	28	30	26	12^R	30	40	30	25	30	25	ND	ND	ND	ND
NYS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22	18	26	20

[a] *SA* - *Staphylococcus aureus* ATCC 6538P, *BC* -*Bacillus cereus* CCM 99, *KR* - *Kocuria rhizophila* ATCC 9341, *EC* - *Escherichia coli* ATCC 39628, *PF* - *Pseudomonas fluorescens*, *BS* - *Bacillus subtilis* ATCC 6633, *STYP* - *Salmonella typhimurium* CCM 5445, *SM* - *Serratia marcescens* CCM 583, *SE* - *Staphylococcus epidermidis* ATCC 12228, *EF* - *Enterococcus faecalis* ATCC 29212, *MRSA* - methicillin-resistant *Staphylococcus aureus* ATCC 95047, *CA* - *Candida albicans*, *SC* - *Saccharomyces cerevisiae*, *YL* - *Yarrowia lipolytica*, *GP* - *Geotrichum penicillatum*

[b] *KGG32* - *Streptomyces* sp. KGG32; *NA* - Nalidixic acid (30 µg/disc); *NV* - Novobiocin (30 µg/disc); *AMP* - Ampicillin (10 µg/disc); *P* - Penicillin G (10 i.u./disc); *VA* -Vancomycin (30 µg/disc); *CLH* - Chloramphenicol (30 µg/disc); *NYS* – Nystatin (10 µg/disc)

[c] Diameter of inhibition zone in mm including disc diameter (6 mm) -, negative, [R]resistant, 6 - no activity

Table II: Cultural characteristics of strain KGG32 on different culture media

Medium	**Growth***	**HMC**	**SMC**	**SP**	**SCM**
Yeast malt extract agar (ISP2)	++++	Grey	Olive green	+/-	Rf
Oat meal agar (ISP3)	++++	Grey	Cream	-	Rf
Inorganic salt-starch agar (ISP4)	++++	Grey	Olive green	?	Rf
Glycerol-asparagine agar (ISP5)	+++	Cream	Cream	-	Rf
Peptone yeast extract iron agar (ISP6)	++++	Grey	Olive green	-	Rf
Tyrosine agar (ISP7)	+++	Grey	Grey	-	ND
Starch-asparagine agar	++++	Grey	Olive green	-	Rf
Glucose-nitrate agar	+++	Grey	Grey	?	ND
Glucose-asparagine agar	+++	Grey	Grey	-	Rf

*****HMC; aerial mycelium color, SMC; substrate mycelium color, SP; soluble pigments other than melanin, +; sparse growth, ++; poor growth, +++; moderate growth, ++++; abundant. +/-; variable, -; negative; ?, doubtful diffusible pigment. SCM; Spore chain morphology, Rf; Rectiflexibiles, ND; not determined

(except *Pseudomonas fluorescens*, *S. epidermidis* & *S. marcescens*) that are resistant to some antibiotics. It shows promising antibacterial activity that is comparable to nalidixic acid, chloramphenicol as well as novobiocin. It is also found to be more potent than penicillin G, ampicillin and vancomycin against human pathogens. In addition, KGG32 inhibited in different degree the test yeasts (except *S. cerevisiae*) with an inhibition zones ranging from 12 to 14 mm. Previous studies showed that many species of *Streptomyces* had antimicrobial activity (Manivasagan *et al.,* 2009; Ting *et al.,* 2009; Kariminik & Baniasadi, 2010; Mohd-Fuat *et al.,* 2010; Sibanda *et al.,* 2010; Reddy *et al.,* 2011).

In order to develop effective medium composition, the role of different carbon and nitrogen sources were evaluated for their influence on growth (Fig. 3) and antibiotic production (Table IV) by this actinomycete strain. The results indicated that among the various carbon sources studied, the strain grew well in the medium containing glucose, starch and glycerol, which are usually used for enumeration of streptomycetes. The strain grew within the temperature range 15–37°C. When the strain was cultured at 30°C, mycelial growth was the most favorable in the medium supplemented starch (Fig. 3), while the production of antimicrobial compound(s) was maximal in medium containing sucrose as a sole carbon source. Therefore, we used the filtrate of the culture grown in sucrose-supplemented medium at 30°C. El-Enshasy *et al.* (2008) reported glucose and sucrose in pure or in polymer forms were the best C-sources for erythromycin production. The results indicated that among the various carbon sources studied, the strain produced the maximum antibiotic activity in sucrose, followed by maltose and glycerol (Table IV).

More than 30 examples of secondary metabolites are reported to be suppressed by the presence of the carbon source. Glucose and other carbohydrates, such as glycerol, maltose, mannose, sucrose and xylose, have been reported to interfere with the synthesis of secondary metabolites (Ruiz *et al.,* 2010; Sánchez *et al.,* 2010). A study of the induction of higher amount of antifungal antibiotic by *Streptomyces rochei* G 164 through variation of cultural parameters was showed that the maximum effectively was found in sucrose as carbon source, peptone as nitrogen source and at pH 7.0 (Chattopadhyay & Sen, 1997). The present study confirms this situation. However, sucrose $(NH_4)_2SO_4$ and yeast extract repressed antimicrobial agent production according to Laidi *et al*. (2008) findings. A few reports have demonstrated that maximum growth and antibiotic production were observed in glycerol and glucose at 1% (w/v) was used as sole carbon source (Wu *et al.,* 2008; Oskay, 2009; Oskay *et al.,* 2010). The best C-sources for the production of actinomycin-D by *S. halstedii* and *S. anulatus*, were fructose and galactose, respectively, Addition of glucose and mannose above 1% decreased

Table III: Phenotypic characteristics of strain KGG32

Characteristics[a]	KGG32	Characteristics	KGG32
Whole cell sugar composition	No diagnostic	Whole cell hydrolysate	L-DAP
Aerial mycelium	+	**Resistance to:**	
Spore chain morphology[b]:		Neomycin (50 µg/mL)	R
Rectinaculiaperti	-	Rifampicin (5 µg/mL)	R
Rectiflexibiles	+	Penicillin G (10 IU)	R
Spirals	-	**Growth at** 45°C	-
Verticillat	-	37°C	+
Spore mass color:		4°C	-
Red	-	**Growth with (% w/v) :**	
Grey	+	NaCI (4%)	+
Mycelium pigment red-orange	-	NaCI (7%)	+
Diffusible pigment produced	+	Sodium azide (0.01)	-
Diffusible pigment yellow-brown	-	Phenol (0.1)	-
Melanin production on:		Potassium telluride (0.001)	+
Peptone yeast iron agar	-	Crystal violet (0.0001)	-
Tyrosine agar	-	**Utilization of nitrogen sources:**	
Mycelium fragmentation	-	DL-α-n-butyric acid	+
Substrate mycelium sporulation	-	L-Cysteine	-
Antibiosis against to:		L-Valine	-
B. subtilis	+	L-Phenylalanine	+
M. luteus	+	L-Histidine	+
C. albicans	+	L-Hydroxyproline	-
S. cerevisiae	-	KNO_3	-
A. niger	-	L-Proline (positive control)	+
Enzyme activity:		L-Lysine	-
Lecithinase	+	L-Tyrosine	-
Lipolysis[c]	+	L-Asparagine (positive control)	+
Nitrate reduction	-	L-Arginine	-
H_2S production	-	**Utilization of carbon sources:**	
Starch reduction	+	Sucrose	+
Gelatin reduction	-	*Meso*-Inositol	-
DNase	-	Mannitol	+
Urease	-	L-Rhamnose	+
Xylanase	+	Raffinose	+
CMC	-	D-Melesitose	-
Proteolitic activity	+	Adonitol	+
Degradation:		D-Mellibiose	-
Hippurate	-	Dextran	-
Elastin	+	Xylitol	-
Xanthine	+	Lactose	+
Arbutin	-	Xylose	+
Oxalate	+	D-Fructose	+
Chitosanase	-	Glucose (positive control)	+

[a] CMC; carboxy methyl cellulase, IU; international units, [b] result on ISP 4 medium after 14 days incubation, [c] egg-yolk medium results, +; positive, -; negative, +/-; variable, S; sensitive, R; resistant

actinomycin production in both strains. In same study yeast extract had the strongest effect on antibiotic activity (Praveen *et al.,* 2008).

In the present study, the antimicrobial activity of the culture filtrate under optimized conditions started after 44 h of fermentation. Higher spectrum of broadness reached after 120 h of incubation, so the cultivation was terminated after 120 h and the filtrate was used to determine antimicrobial activity (Fig. 4**)**. When the initial pH was 6.0, the antimicrobial activity was low (16 & 10 mm against *S. aureus & E. coli*, respectively). As the pH increased, antimicrobial activity increased and reached a maximum at pH 7.5 (32 & 26 mm against *S. aureus & E. coli*, respectively). A further increase in pH decreased the antimicrobial activity (Table V). The highest biomass and antimicrobial production was observed when initial temperature of culture medium set at 30°C. Consequently, the optimal temperature for maximum antimicrobial was found to be 30°C and an incubation time of 120 h. Similar results were obtained in the study of Kavitha and Vijayalakshmi (2009) in the determination of the effect of various cultural parameters on the antibiotic activity of *Nocardia levis* MK-VL_113. They found medium containing 2% sucrose supported high levels of biomass and bioactive metabolite production by the strain. However, El-Mehalawy *et al.* (2005) have reported that the factors affecting the antifungal production of *S. lydicus*, *S. ederensis*, *S. erumpens* and *S. antimycoticus.* Glycerol was found positive effects for antifungal production followed by starch. In addition, it was noticed that 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 these species was 7.0.

Table IV: Influence of some carbon and nitrogen sources on antimicrobial activity of *Streptomyces* sp. KGG32

Carbon sources	Antimicrobial activity (mm)		Nitrogen sources	Antimicrobial activity (mm)	
	Sa	Ec		Sa	Ec
Glucose	10	0	Meat extract	24	22
Glycerol	12	10	Yeast extract	22	18
Starch	10	10	Bacteriological peptone	26	22
Sucrose	32	26	Tryptone	22	16
Maltose	14	16	L-asparagine	18	12

(Culture conditions; pH: 7.5, temperature: 30°C)

Table V: Effect of initial temperature and pH on antimicrobial activity of *Streptomyces* sp. KGG32

Temperature (°C)	Antimicrobial activity (mm)		pH	Antimicrobial activity (mm)	
	Sa	Ec		Sa	Ec
20	14	10	6	16	10
25	18	12	7	22	16
30	28	20	7.5	30	22
37	12	10	8	12	10
			9	0	0

(Carbon source: sucrose)

Fig. 1: Colony colors of strain KGG32 on yeast-malt extract agar (ISP2) a) Aerial mass color, b) Reverse side color

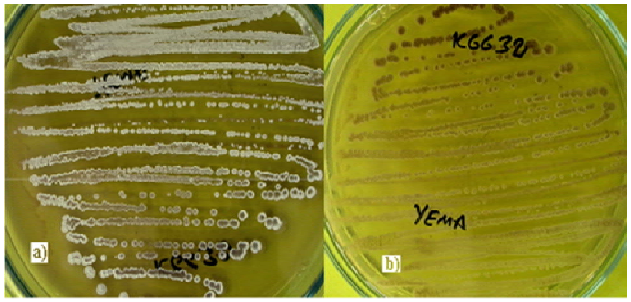
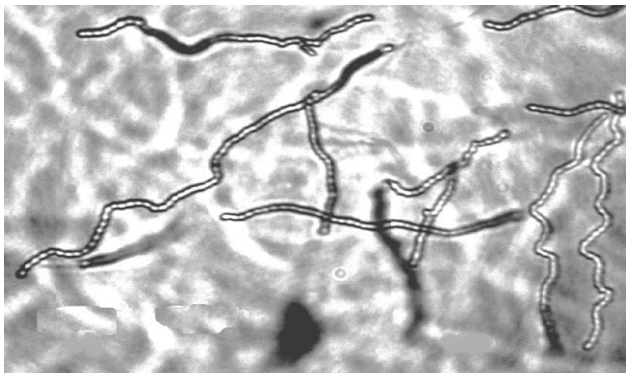


Fig. 2: Spore chain morphology of strain KGG32 grown in ISP3 at 28°C for 14 days (1000X)



Five nitrogen sources were employed in the submerged cultures of *Streptomyces* sp. KGG32. As shown in Table IV, among nitrogen sources, bacteriological peptone, yeast extract, meat extract and tryptone favored

Fig. 3: Effect of different carbon sources and incubation time for biomass of KGG32 (pH: 7.5, temperature 30°C)

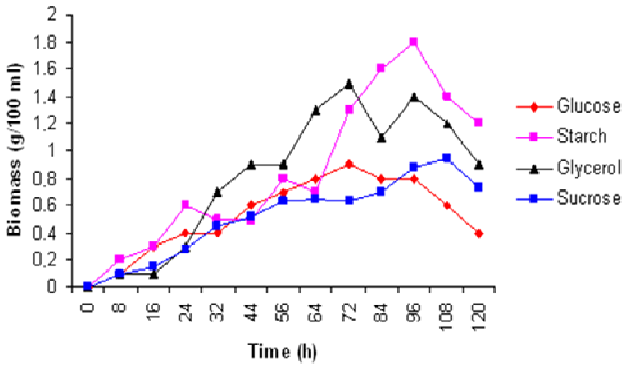
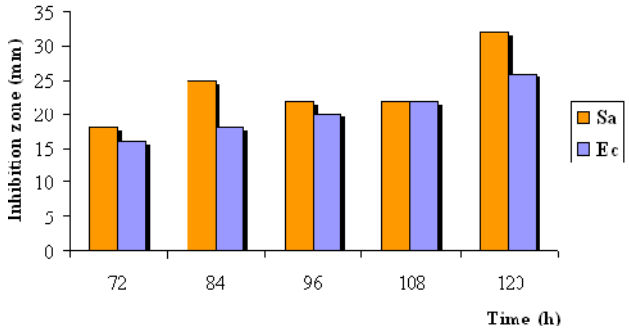


Fig. 4: Effect of incubation time in hours for antimicrobial activity of strain KGG32, Sa; *S. aureus*, Ec; *E. coli* (pH: 7.5, temperature: 30°C, carbon source: sucrose)



antibiotic production and the antimicrobial activity had no significant differences. Lower antibiotic activities were observed with L-asparagine. Hence, sucrose and bacteriological peptone were chosen as the source of carbon and nitrogen for further experiments, respectively. The influence of medium composition, incubation temperatures, and initial pH on microbial growth and antibiotic production was also reported in various *Streptomyces* strains (Banga *et al.*, 2008; Kavitha & Vijayalakshmi, 2009; Sallam *et al.*, 2010). Gao *et al.* (2009) reported a maximum production of avermectin B1a with corn starch (149.57 g/L) and yeast extract (8.92 g/L) by *Streptomyces avermitilis* 14-12A. Narayana and Vijayalakshmi (2008) studying with *Streptomyces albidoflavus*, found that basal medium amended with maltose and soybean meal as carbon and nitrogen sources, respectively was proved to be the best for the production of bioactive metabolites at pH 7.0 and temperature at 35°C. In another study, the best pH and temperature for antifungal antibiotic production with *Streptomyces rimosus* MY02 were 6.0 and 28°C, respectively (Yu *et al.*, 2008). A study of the influence of the temperature, pH, agitation, soybean flour and glycerol concentrations, on the clavulanic acid production by *Streptomyces* DAUFPE 3060 has been investigated by

Viana *et al.* (2010). They reported that the highest antibiotic concentration (494 mg/L) was obtained after 48 h at 150 rpm, 32°C, pH 6.0, 5.0 g/L glycerol, and 20 g/L soybean flour concentrations. Wu *et al*. (2008) detected the optimal cultural condition for fungichromin (FC, a polyene macrolide antibiotic) production from *Streptomyces padanus* PMS–702 in a shake flask as follows: glucose 11.2 g/L, soybean meal 11.2 g/L, $CaCO_3$ 0.46 g/L, temperature 31.7°C and an initial pH 5.5. In the present study, some of the dissimilar results attributed to different strain and different culture conditions used.

CONCLUSION

Streptomyces sp. KGG32 that is potent effective against pathogenic test bacteria *in vitro*. Further studies will have to be made to determine of additional characteristics concerning purification, characterization, and identification of active compound before it becomes a medical treatment. These findings may provide beneficial information on secondary metabolites for utilizing industrial microorganisms.

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