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Study effects of some parameters on antifungal activity for Streptomyces spp.

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Abstract:

Twenty –two soil samples were collected from different places in Hilla city. Four isolates of *Streptomyces* spp. were isolated. These isolates were tested for antifungal activity against *Aspergillus niger* . The results showed that *Streptomyces*2 with high antifungal activity against *A. niger* with 22 inhibition zone. *Streptomyces* .2 was selected for study to the parameters which effect on antifungal activity such as(pH, temperature, nitrogen source and carbon source). The results showed that high inhibition zone against test pathogen by using glucose as carbon source and praline as nitrogen source .The optimal pH for antifungal activity was 7.5. Antifungal activity was observed at 30 ^{o}C .

الخلاصة:

جمعت اثنتان وعشرون نموذج تربة من مناطق مختلفة في مدينة الحلة.حيث تم عزل اربعة عزلات من الستربتومايسس.فحصت هذه العزلات الاربع للفعالية ضد الفطر *A.niger* واظهرت النتائج ان العزلة *Streptomyces.2*تمتلك فعالية ضد الفطر *A.niger* مع قطر تثبيط mm22.اختيرت هده العزلة لدراسة البراميترات التي توثر على الفعالية الفطرية ك(الرقم الهيدروجيني, درجة الحرارة, مصدر النتيروجين ,والمصدر الكربوني).
اظهرت النتائج فعالية عالية ضد الممرضات بواسطة استعمال الكلوكوز كمصدر كاربوني, والبرولين كمصدر نتروجيني, وايضا اظهر الرقم الهيدروجيني المثالي هو 7.5 ولوحظت فعالية فطرية عالية مع درجة حرارة 30 درجة سيليزية.

Introduction:

Actinomycetes a group of filamentous, gram positive and branching unicellular microorganisms. These microorganisms were produce branching mycelium which may be of two kinds: substrate mycelium and aerial mycelium. *Streptomycetes* are the dominant, members of the actinomycetes, which live in natural environment [4]. Actinomycetes have capacity to produce secondary metabolites with diverse chemical structures and biological activities. Among the genera of Actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities. *Streptomyces* is widely distributed in nature and is a source of antibiotics and pharmaceutically useful compounds [13].Two new compounds, isostreptazolin and sannaphenol, were isolated from the culture broth of *Streptomyces sannanensis* [36].*Streptomycetes,* the gram positive, areobic filamentous bacteria, are widely living in natural and manmade environments, constituting a significant component of the microbial population in most soils [1].It produce branching substrate and aerial mycelium. *Streptomyces* are distinguished ability to produce an array of secondary metabolites [5]. *Streptomyces hygroscopicus* BS-112 showed broad-spectrum antifungal and antibacterial activities [35].The genus *Streptomyces* is represented in nature by the largest number of species and varieties among the family Actinomycetaceae. They differ greatly in their morphology, physiology and biochemical activities, producing the majority of known enzymes [11,27].

Secondary metabolites production was 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) [4,16, 21].The nutritional requirements of *Streptomyces* play an important role during metabolite synthesis process. Amongst various nutritional requirements, antifungal substance production has been known to be influenced by media components and cultural conditions, such as aeration, agitation, pH,

temperature, carbon, nitrogen source and incubation time, which vary from organism to organism [3,7, 33].
This study aimed to isolation of *Streptomyces* spp. from soil samples with antifungal activity and study the effects of some parameters on antifungal activity.

Materials and methods:

Isolation of actinomycetes

Soil samples were collected from different places in Hilla city during the period from December,2012 to Janurary,2013. Samples were placed in a Petri dish and tightly sealed. The samples were then pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37°C for 4 days and subjected to serial dilution (up to 10^{-6}dilution. About 1.0 ml of diluted samples were plated on starch casein agar and incubated at 28°C for 7 to10 days [6 , 23].

Screening for the antifungal activity

All isolates were screened for their *in vitro* antifungal activity against *A. niger*. A 7 mm diameter disk from 5-day-old culture of the actinomycete isolate being tested was placed in the centre of starch nitrate agar plate seeded with the tested fungus.. The starch-nitrate plates were then incubated at 30 ± 1°C. The inhibition zone, if any,was measured in mm diameter after 24, 48 and 72 h [31]. The soil suspension was then diluted and 1 ml of diluted soil suspension was spread onto starch-nitrate-agar plates that contained (g/L): Starch; 10, NaNO3; 2.5, K2HPO4; 1, KH2PO4; 1, MgSO4·7H2O; 0.5, KCl; 0.5, trace salt solution 1 ml CuSO4·5H2O (0.64 g/L), FeSO4·7H2O (0.11 g/L), MnCl2·4H2O (0.79 g/L), and ZnSO4·7H2O (0.15 g/L)], agar; 20 and distilled water, 1 L.The medium was adjusted to the initial pH 7 prior to sterilization [31].

Optimization of nutritional factors

Effect of different carbon sources, glucose, fructose, sucrose, lactose, manose and galactose were studied by used as substitutive carbon sources. Soluble starch of starch-nitrate medium was substituted by one of the previous sugars. Erlenmeyer flasks containing medium were inoculated by *Streptomyces* .2. The initial pH of the various media was adjusted at 7.5, before sterilization and the flasks were incubated for 5 days at 30°C on shaker incubator. For each, 5 ml of the culture filtrate were then taken aseptically and the antifungal activity was measured by the inhibition zone method. The effect of different nitrogen sources was carried out by the same method. Ammonium nitrate, ammonium sulphate, proline, alanine, and histidine were tested as substitutive nitrogenous sources. Potassium nitrate was substituted by one of the previous nitrogen sources [29].

Optimization of environmental factors

The effect of incubation period was studied by using Erlenmeyer flasks (250 ml) containing 100 ml sterile starch-nitrate broth, each of which was inoculated *Streptomyces* .2 isolate and incubated on shaker incubator at 30 ± 2°C for 7 days. At each incubation period, 5 ml of the culture filtrate were then taken aseptically and the antifungal activity was measured using the inhibition zone method described earlier. The effect of incubation temperature was studied by using the same previous steps and incubated at different temperatures (20, 25, 30, 35,40°C). For each, 5 ml of the culture filtrate was then taken aseptically and the antifungal activity was measured using the inhibition zone method. Also the effect of pH value was +studied by the same method and incubated at 30°C on the 5^{th} day. The various levels of pH (6, 6.5, 7, 7.5 and 8) were adjusted using phosphate buffer. For each, 5 ml of the culture filtrate were then taken aseptically and the antifungal activity was measured using the inhibition zone method [14].

Statistical analysis:

ANOVA (LSD) least significance difference was used for statistical analysis.

Results and Discussion:

Screening of *Strepomyces* isolates for antifungal activity:

Four different Streptomyces spp. were isolated from twenty –two soil samples in Hilla city. These isolates were tested for antifungal activity against Aspergillus niger . The results showed that Streptomyces2 with high antifungal activity against A. niger with 22 inhibition zone. Based on this results , the Streptomyces .2 was selected for study to the parameters which effect on antifungal activity such as(pH, temperature, nitrogen source and carbon source) (Table 1).

Table(1): Antifungal activity of Streptomyces isolates against A. niger

Strepomyces isolates	Inhibition zone
Streptomyces. 1	18
Streptomyces. 2	20
Streptomyces. 3	16
Streptomyces .4	13

Effect of different pH on antifungal activity of *Streptomyces* .2

The antifungal activity of *Streptomyces*.2 against *A. niger* was tested . The results showed that a high inhibition zone at pH equals to 7.5(21mm). The lowest inhibition zone at pH equals to 6 (14mm) (Table 2).

Table (2): Antifungal activity of *Streptomyces* .2 at different pH.

pH	Inhibition zone (mm) $\bar{M} + SD$
6	14.2 (0.25)
6.5	16.2 (0.25)
7	18.3 (0.30)
7.5	21.3 (0.32
8	12.2 (0.20)

$P<0.05$

Statistical analysis showed that significance differences between different pH.

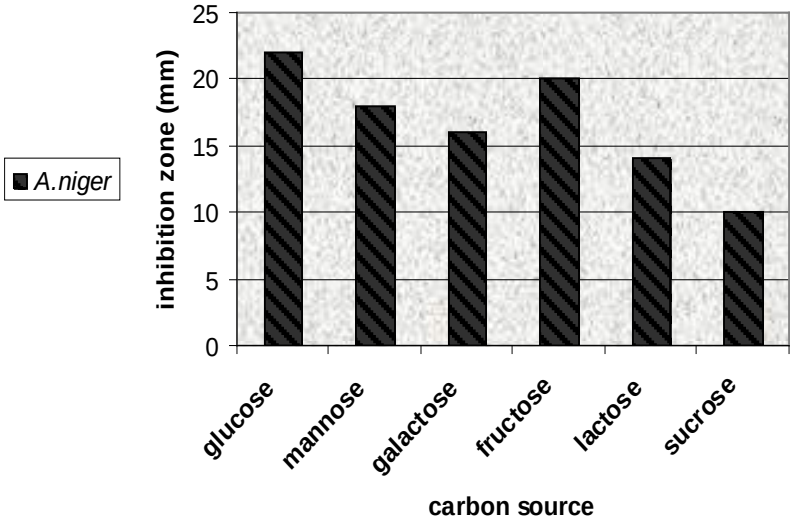
Our results agreed with results which showed that the optimum pH value that gave the maximum antifungal activity of *S. spororaveus* RDS28 against all of the tested fungi was 7.5 [2].

The obtained results were in agreement with that of [10] who recorded that the highest antibiotic production by *Streptomyces violatus* was obtained at initial pH value of 7.5. [9] found 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) were the most favorable for antifungal production.

The optimal conditions for antifungal production by *S. noursei* were performed on starch nitrate medium at pH 7 after 7 days of growth at 30°C [1].

Effect of different carbon sources on antifungal activity of *Streptomyces* .2

The higher antifungal activity showed that by using glucose as carbon source with inhibition zone (23mm).The using of sucrose as carbon source due to lowest inhibition zone. This results agreed with results obtained by [28] who found that, antibiotic production by *Streptomyces* sp. M4018 was higher in glucose medium when compared also by The highest antibacterial activity of *Streptomyces sannanensis* strain RJT-1 was obtained when glucose at 1% (w/v) was used as a carbon source followed by xylose and arabinose [30]. Similar results were obtained by [18] and [20], in which glucose proved to be the best carbon source for antibiotic production (Figure1). According to figure (1), there were a significance difference between different type of carbon source.

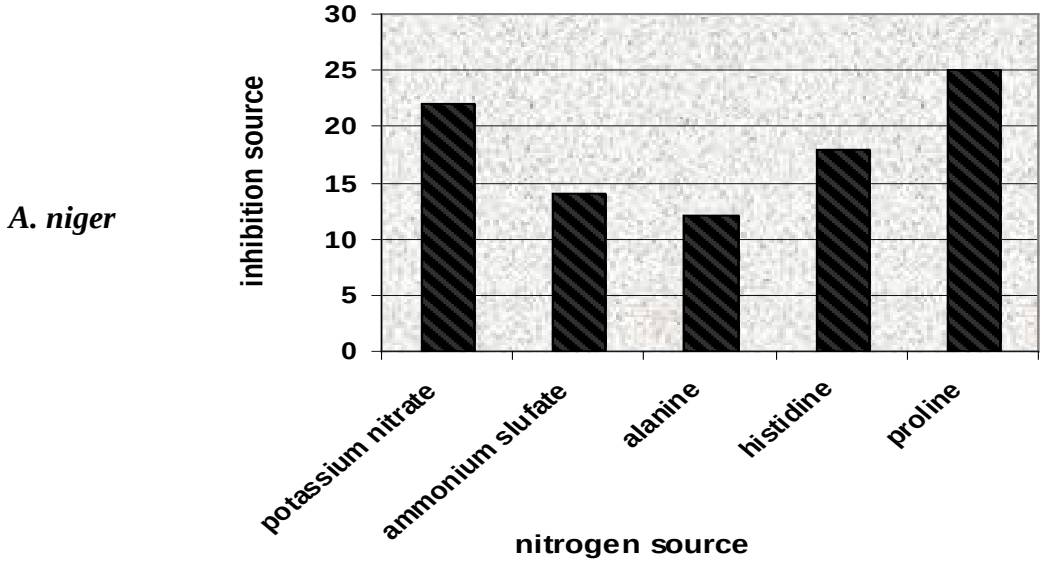


Figure(1):Influence of various carbon sources on antifungal activity of Streptomyces .2 against A.niger

Effect of nitrogen source on antifungal activity of *Streptomyces* .2

The results showed that the best nitrogen source for antifungal production were proline followed by potassium nitrate, while alanine was the poorest nitrogen source for supporting antifungal production(Figure 2). The obtained results were comparable with that reported by [24] who described the effect of different nutrients on the production of the macrolide polyene antibiotics (PA-5 and PA-7) produced by *Streptoverticillium* sp. 43 to 16. Optimal production yields have been achieved with L-proline and glycine as nitrogen sources, respectively. Proline and humic acid (0.1 %) had been listed as selective carbon and nitrogen sources for the isolation of actinomycetes [12]. Moreover, proline had also been recommended for the selection of antibiotic producing actinomycetes. The best nitrogen sources for production of melanin pigment (secondary metabolite) in *Streptomyces* species were proline [8].

According to figure (2), there were a significance difference between different type of nitrogen source.



Figure(2): Influence of various nitrogen sources on antifungal activity of *Streptomyces* .2 against *A.niger*

Effect of temperature on antifungal activity of *Streptomyces* .2

The results showed that , 30 ^{o}C were the optimal temperature which give high activity against *A.niger* with inhibition zone equals to 25 mm.(table 3).

Table(3) Antifungal activity of *Streptomyces* .2 at different temperature

Temperature ^{o}C	Inhibition zone (mm) $\bar{M}$ + SD
20	16.5 (0.50)
25	18.2 (0.20)
30	25.2 (0.20)
35	22.3 (0.30)
40	14.2 (0.25)

$P<0.05$

Statistical analysis showed that significance differences by using different temperature.

Our results agreed with results which showed that ,the optimal temperature for maximum antimicrobial was found to be 30.0°C and an incubation time of 96 h. [17].

The obtained results were in agreement with that achieved by [25] who found that the best growth and antibiotic production by Streptomyces sammoticus were recorded when incubation was carried out at temperature of 30°C. The incubation of S.spororaveus RDS28 for 72 h at 31°C and initial pH 7.5 on a medium containing glucose as a carbon source and proline as a nitrogen source gave the best antifungal antibiotic production [2]. The culture actinomycetes, and more specifically, Streptomycetes that produce various bioactive natural products including antibiotics, are being used as pharmaceuticals and agrochemicals [15, 22,34].

The similar to the work of was obtained by [34], who reported 4 out of 10 actinomycete strains isolated being active against fungal pathogens in vitro. In the current study, strain KEH23 that seem to be strong fungal inhibitor, showed antibiosis against all highly resistant fungal species (example, F. oxysporum, A. niger, A.alternata, Trichoderma hamatum, C. oxysporum, Penicillium spp . [17].

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