Antifungal activity of agent produced by *Streptomyces*spp. isolated from soil samples

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Abstract:

Thirty soil samples were collected from Hilla City. Thirteen actinomycetes isolates was isolated. Antifungal activity of these isolates were tested against *C. albicans* . Results showed that actinomycetes. 4 was given higher inhibition zone compared with other isolates. Actinomycetes. 4 isolate was selected for extraction of antifungal agent. According to cultural and biochemical tests of Actinomyces. 4 these isolates was belong to *Streptomyces* spp. *Streptomyces* spp.4 isolate was grey aerial mycelium, and yellow-green substrate mycelium , no melanin production and no diffusible pigment, able to ferment sugars, negative for catalase and H2S and positive for citrate utilization. Crude extract of *Streptomyces* spp.4 was active against *C.albicans* and *A.niger* with inhibition zone (22mm) against *C. albicans* and (16 mm) against *A. niger*. MIC of *Streptomyces* spp.4 antifungal agent showed that MIC values against *C. albicans*, *A. niger* were 22, 38 µg/ ml respectively. UV spectrum of absorption for antifungal agent showed that it have a single peak with maximum absorption (λ max) 293 nm.

Key words: *Streptomyces*, antifungal activity, extraction of antifungal agent.

Microbiology Classification QR1 – 74.5

Introduction:

broad host range are limited. The enzymatic and antifungal activites of *Streptomyces rochei* against *Aspergillus fumigatus* was reported [18].The polyenes antibiotic derived from *Streptomyces* sp. have a broad *in vitro* spectrum of activity against a wide range of fungi including the *Aspergillus* sp. and *Candida* spp.[15] Fungi are eukaryotic and have machinery for protein and nucleic acid synthesis similar to that of higher animals. It is, therefore, very difficult to find out compounds that selectively inhibit fungal metabolism without exhibiting any toxicity to humans [13,6]. Actinomycetes have been recognized as the potential producers of metabolites such as antibiotics, growth promoting substances for plants and animals, immunomodifiers, enzyme inhibitors and many other compounds of use to man. They have provided about two-thirds (more than 4000) of the naturally occurring antibiotics discovered, including many of those

Sterptomyces are member actinomycetes that proved are richest sources of antibiotics . *Streptomyces* species generally synthesize a sizeable number of diverse natural secondary metabolites, the best known of which are antibiotics currently used worldwide as veterinary and pharmaceutical industry [21]. *Streptomycetes,* the gram positive filamentous bacteria, are widely living in natural and manmade environments, constituting a significant component of the microbial population in most soils [2]. Its belong to the family streptomycteaceae and its predominant genera comprsing about 37% of total soil actinobacteria [10]. Antifungal activity of extracellular metabolites from *Streptomyces* against pathogenic fungi was previously reported [10, 12, 21, 22, 24]. The antagonistic ability of the extracellular metabolites of *Streptomyces* strains for suppressing growth of the fungal pathogens such as *C. gloeosporioides* and *S. rolfsii* having a

The study aimed to isolation of a new *Streptomyces* in local soil sample which produce antifungal agent and extraction of antifungal agent.

Materials and methods:

Sample collection:

Thirty soil samples were collected from different places in Hilla city , and stored in sterile plastic bags.

Actinomycetes isolation:

Soil samples were suspended in 25 ml of basal salt solution contains on (5.0 g/l KH2PO4 and 5.0 g/l NaCl) and shaken in a rotary shaker (150 rpm) at 28 °C for 30 min. Soil suspension was diluted and heated at 50 °C for 6 min. 0.1 ml of diluted soil suspension was spread onto starch casein agar plates (soluble starch 10.0 g/l; casein 0.3 g/l; KNO3 2.0 g/l; NaCl 2.0 g/l; gSO4.7H2O 0.05 g/l; CaCO3 0.02 g/l; FeSO4.H2O 0.01 g/l; KH2PO4 2.0 g/l; and agar 18.0 g/l) and incubated at 28 °C for 14 days. Actinomycetes colony on the agar plates were picked and purified on ISP-2 (Yeast malt dextrose agar) agar. For inducing sporulation, purified colonies were sub cultured onto ISP-3 [22].

Characteristics of actinomycetes isolates:

Cultural characteristics of actinomycetes were recorded on YMD (Yeast malt dextrose agar) agar which includes color of aerial mycelium, color of substrate mycelium and pigmentation of the selected actinomycete isolates medium. The morphological characteristics of actinomycete isolates were examined by slide culture method . Utilization of carbon sources and melanin pigments for isolate was carried. The ability of actinomycetes for producing H2S, urea, citrate utilization , catalase production was tested [5,22] .

Production of antifungal metabolite:

The actinomycete metabolites produced by isolates were extracted by the method of [11]. The purified culture of actinomycete was transferred into the YMD broth. After 24 h of incubation, the seed culture at a rate of 10% was inoculated into the production medium of the same composition. Fermentation was carried out at (28 ± 2 °C) for 1 week under agitation at 250 rpm. The flasks were harvested and the biomass was separated from the broth after 24 h interval. The culture filtrates were extracted twice with ethyl acetate and the pooled solvent extracts were evaporated to dryness under vacuum to yield a crude residue. The extraction of secondary metabolites, was followed for the active isolate. The residue was then dissolved in dimethyl sulphoxide (DMSO) and the extracts thus obtained were used for antifungal activity against the test fungi such as *Aspergillus niger* and *Candida albicans* .

Antimicrobial activity of culture filtrate of actinomycetes :

The culture filtrate were screened for antibacterial activity against *Candida albicans* by well diffusion method. 100 µl of the crude was placed in wells made on Muller Hinton agar plates seeded with the test bacterial pathogen cultures. The plates were incubated at 37°C and observed for inhibition zone after 24 h. [20].

Activity of agent produced by *Streptomyces* spp.4:

Antifungal activity of agent was tested by using agar well diffusion method. Mueller Hinton agar plates were prepared and solvent extracts dissolved in DMSO at a concentration of 1000 µg/ml were added to each well using DMSO as a negative control. The plates were incubated at 37oC for 48 - 72 h and the diameter of the inhibition zones of the test fungi around each well was measured [8].

Minimum inhibitory concentration (MIC) of antifungal agent:

Minimum inhibitory concentration (MIC) of the antifungal agent were determined by broth tube dilution procedure using two-fold dilution in Sabouraud dextrose broth at 28°C [7].

important in medicine, such as aminoglycosides, anthracyclines, chloramphenicol, β- lactams, macrolides, tetracyclines etc. [14].

UV/VIS spectrophotometer. One milligram of sample was dissolved in 10 ml water and the spectrum was recorded at 200–400 nm range [3].

Ultra- violet of spectrum of antifungal metabolite :

The absorption spectrum of agent was determined in UV region by using

Results and Discussion:

compared with other isolates (Table 1).Based on the antifungal activity of actinomyces 4 isolate. These isolate was selected for antibiotic production, because it has broad antifungal spectrum. *Streptomyces hygroscopicus* BS-112 showed broad-spectrum antifungal and antibacterial activities [26].

Actinomycetes isolation and activity:

Out of 30 soil samples, 13 actinomyces isolates was isolated , the actinomyces isolates was tested for antifungal activity against *Candida albicans* , and the result showed that 4 isolates were able to inhibit fungal culture . Out of 4 isolates the Actinomyces.4 isolates have most effective with 15 mm inhibition zone

Table (1): Antifungal activity of actinomycetes isolates against *Candida albicans*

Actinomyces isolates	Inhibition zone(mm)
Actinomyces. 1	0
Actinomyces. 2	0
Actinomyces. 3	11
Actinomyces. 4	15
Actinomyces. 5	0
Actinomyces. 6	0
Actinomyces. 7	0
Actinomyces. 8	0
Actinomyces. 9	0
Actinomyces. 10	12
Actinomyces. 11	0
Actinomyces .12	0
Actinomyces .13	10

yellow-green substrate mycelium , no melanin production on tyrosine broth and no diffusible pigment (Table 2).

Table(2):Cultural characterization of Actinomyces. 4 isolate

Characterization of actinomyces. 4 isolate:

Actinomyces. 4 was identified and diagnosed by culturing on yeast –malt extract agar and the result showed that the isolate had a grey aerial mycelium, and

Isolate	character
Gram stain	positive
Aerial mycelium	grey
Substrate mycelium	Yellow-green
Melanin production	negative
Diffusible pigment	negative

Streptomycetes are gram-positive aerobic members of the order *Actinomycetales* within the class *Actinobacteria* and they

Streptomyces are the group of gram positive filamentous bacteria which are ubiquitous various natural environment [6].

Actinomyces. 4 was able to ferment glucose, mannitol, fructose xylose and mannose only negative for H2S production , catalase, urea and positive for citrate utilization.

are living in various natural environment [23, 6].

Biochemical tests of actinomycetes. 4 isolate :

Table(2):Biochemical test of actinomycetes. 4 isolate

Biochemical test	Results
Sugar fermentation: glucose	+
fructose	+
mannitol	+
Xylose	+
mannose	+
Lactose	-
H2S production	-
Catalase	-
citrate utilization	+
urea test	-

According to morphological and biochemical tests these isolate was belong to *Streptomyces* spp.4.

Antifungal activity of metabolite produced by *Streptomyces* spp.4 :

isolated mainly from *Streptomyces cinnamonensis* were recorded by [19].

Minimum inhibitory concentration:

The result showed that MIC of *Streptomyces* spp.4 antifungal agent showed that MIC values against *C. albicans*, *A. niger* were 22,38 µg/ ml respectively. These results is similar to findings obtained by [4] who showed that biological activities (MIC) produced by *Streptomyces* spp. was active against *A. niger* and *C. albicans*, equals to 20,40 µg ml .

Ultra-violet of antifungal agent produced by *Streptomyces* spp.4 isolate:

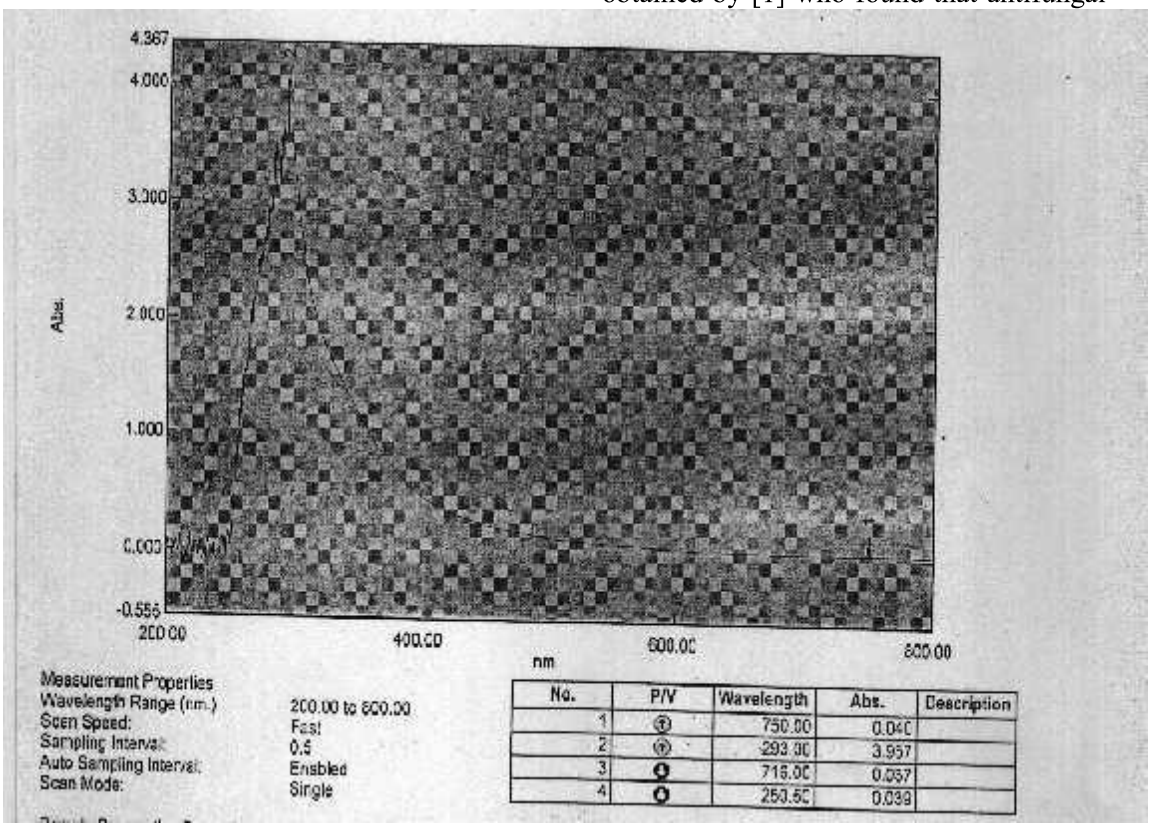
UV spectrum antifungal agent produced by *Streptomyces* spp.4 was measured by

Streptomyces spp.4 metabolite was tested for antifungal activity against *Candida albicans* and *Aspergilus niger* and the results showed a high activity against fungal pathogens with inhibition zone 22mm against *C. albicans* and 16 mm against *A. niger*. Our results agreed with results obtained by [17] who showed that the growth of *C. albicans* , *A. niger* was inhibited by a metabolite produced by *Streptomyces* spp..*S. rochei* metabolites showed strong antifungal activity against *Candida albicans* followed by *Aspergillus niger* [16].

The antifungal activity of Lasalocid and Monensin which belong to the family of the polycyclic carboxylic polyethers

Agent produced by *Streptomyces olivaceiscleroticus*, AZ-SH514 have a maximum absorption peak at 225 and 321 nm.

UV/VIS spectrophotometer .The result showed that it have a single peak with maximum absorption (λ max) 293 nm. (Figure 1). This result is similar to results obtained by [1] who found that antifungal



Figure(1): Ultra violet spectrum of antifungal metabolite produce by *Streptomyces* spp.4 isolate

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الفعالية المضادة الفطرية للعامل المنتج بواسطة السبرتومايسس المعزولة من عينات تربة

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الخلاصة:

جمعت ثلاثون عينة تربة من مدينة الحلة.عزلت ثلاثة عشر عزلة من الاكتينومايسيتات.فحصت فعالية هذه العزلات ضد الكانديدا . اظهرت النتائج ان عزلة الاكتينومايستس4 اعطت اعلى قطر تثبيط مقارنة مع العزلات الاخرى.اختيرت العزلة 4 لاستخلاص العامل المضاد.اظهرت الخصائص الزرعية والفحوصات البايوكيمياوية ان العزلة الاكتينومايسيتس 4 تعود الى الستربتومايسس ولها مايسليم رصاصي اللون مع مادة اساس صفراء -خضراء وليس لها القابيلة على انتاج الميلانين والصبغات المنتشرة وكانت قادرة على تخمير بعض السكريات وكانت سالبة لانتاج الكتليز وانتاج كبريتيد الهيدروجين وموجبة لاستهلاك السترات. ان المستخلص الخام للعزلة4 فعال ضد الكانديدا والاسبرجلس مع قطر تثبيط 22 و16 ملم بالتتابع. اظهر التركيز المثبط الادنى للعامل المضاد للعزلة 4 نتائج 38و 22مايكرو غرام ضد الكانديدا والاسبرجلس بالتتابع. اظهر طيف امتصاص الاشعة فوق البنفسجية للعامل المضاد الفطري اعلى قمة امتصاص عند 293 نانوميتر.

الكلمات المفتاحية: ستربتومايسس, الفعالية المضادة الفطرية,استخلاص العامل المضاد الفطري