

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

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Abstract:

Twenty two soil samples were collected from Hilla City. Twelve isolates of *Actinomycetes* were found. Eight isolates of *Streptomyces* spp. were detected according to morphological test on yeast malt extract agar. These isolates are positive for gram stain and having grey aerial mycelium and yellow-brown substrate mycelium. Activity of *Streptomyces* spp. isolates were tested against *S.aureus*. *Streptomyces* spp.6 was given higher inhibition zones (20)mm compared with low inhibition zone by other *Streptomyces* spp. isolates. *Streptomyces*6 was chosen for agent extraction. *Streptomyces* spp.6 having grey aerial mycelium and yellow-brown substrate mycelium, unable for melanin production and able to ferment glucose, mannitol, sucrose, and negative for urea and catalase test. Agent was extracted from *Streptomyces* spp.6. Activity of agent was examined against clinical bacterial isolates. The results showed that this agent having antimicrobial activity against clinical isolates with inhibition zone about (12- 22 mm).

Microbiology Classification QR 75-99.5

Key word: *Streptomyces* isolation, antimicrobial activity

Introduction:

Streptomyces are gram positive bacteria which growth aerobically. Its mainly distributed in soil environments. It have a big important role for generating half of antimicrobial employed [19]. These bacteria are thread like and having higher G+C content which represent about 60-70 % of molecular weight percentages. More than five hundred species of *Streptomyces* characterized by aerial mycelium and spore formation on solid media. The diagnosis of *Streptomyces* depended on color different, dryness and wrinkles for colonies. It having cell wall type 1 and coming back to *Streptomyceceae* family which represent a member of Actinomycetales order with very complex in life pattern [20]. They considered as opulent source for production of a big number of biologically active compounds which used in agrochemicals and pharmaceuticals manufacturing [14].

Many antibiotics that employed in medicinal use are produced by *Streptomyces*. The biological buffering of soil are caused by soil *Streptomyces*. *Streptomyces* having a big role in breaking down of organic compound also it responsible for generating for many antibiotics and having inhibitory effects on fungi and bacteria [3,4,7].

Large number of antibiotics which produced by *Streptomyces* such as Monensin, Lasalocid which having antimicrobial activity [9]. Other Bioactive secondary metabolite was detected which produced by *Streptomyces parvulus* RSPSN2. isolated from marine soil sediment [17]. This study aimed to isolation of *Streptomyces* with antimicrobial activity

and extraction of antimicrobial compound.

Materials and Methods

Streptomyces isolation:

Twenty two soil samples were collected from Hilla city. At first these samples were treated with CaCO₃ and placed in oven at 45 °C for One hour in order to minimizing occurrence of other bacteria and fungi. The soil samples was diluted by soil dilution plate methods and the samples was cultured on yeast malt dextrose agar after pH controlled at 7.2 and incubated for 10 days at 30 °C. Sugar fermentation test was examined for glucose, mannose ,xylose ,Mannitol ,sucrose ,fructose ,and Urea and Catalase test were made , production of melanin was tested for *Streptomyces* spp. by culturing on tyrosine broth medium [18].

Isolation and clinical test of pathogens:

Twenty clinical samples were collected from Maternity and Pediatrics Hospital of Hilla City. These samples included (10 urine samples and 10 blood samples). Samples cultured on blood and MacConky agar for 24 hr at 37 °C. The growth colonies were identified according to [8].

Activity of Streptomyces spp. isolates:

Agar pieces with cylindrical form are made from *Streptomyces* spp. grown on solid media. These pieces are placed in Petri dish covered by test bacteria (*S.aureus*) at (10⁸ cell/ml). Inhibition zone (mm) was read after incubation for 24 hour at 37 °C [12].

Extraction of antimicrobial agent:

Streptomyces spp.6 inoculate on yeast malt extract broth and incubated in shaker incubator at 28 °C for 7 days. Mycelium cake was removed and the filtrate was centrifuged at 8000 rpm for 17 minutes. The supernatant was

employed and mixed with equal volume of ethyl acetate. The extraction was made by ethyl acetate extraction methods [5].

Antimicrobial activity:

Activity of *Streptomyces* spp.6 agent was examined against clinical isolates by using well diffusion plate methods. About 100 μ of agent was placed in well made on Muller Hinton agar seeded with clinical bacterial isolates. The result was noted after 24 hr incubation at 37 °C. Diameter of inhibition zone was recorded [11].

Results and Discussion:

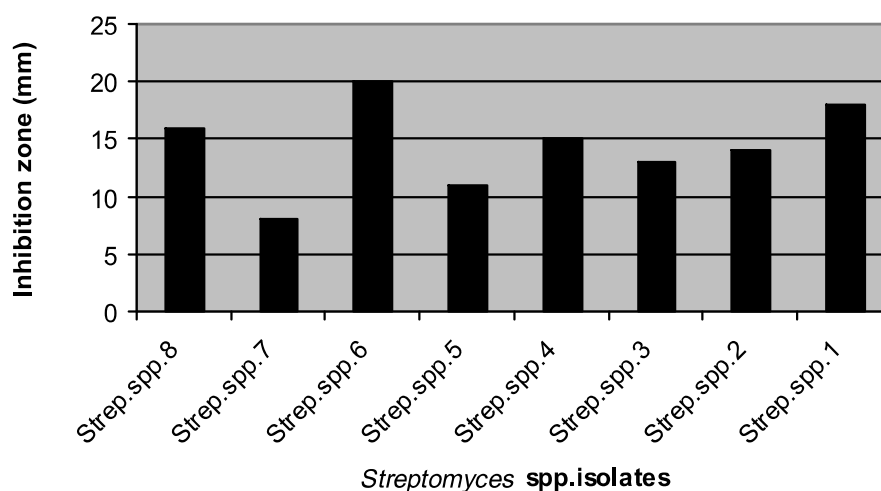
Isolation of *Streptomyces* spp.:

Twenty two soil samples were collected from Hilla City.(12) isolates of

Actinomycetes were detected. Morphological test was made and results found that (8) were belong to *Streptomyces* spp. All isolates were positive for gram stain and having grey aerial mycelium , and yellow- brown substrate mycelium. These isolates having earthy odor.

ibacterial activity of *Streptomyces* spp. isolates:

Activity of *Streptomyces* spp. isolates were examined against *S.aureus*. Higher inhibition zone (20)mm against test pathogen was recorded by *Streptomyces* spp.6 compared with other isolates (Figure 1).*Streptomyces*.6 was selected for agent extraction.



Std.Error=1.34878

Figure(1): Activity of *Streptomyces*spp. isolates against *S.aureus*

Characteristic of *Streptomyces* spp.6:

Characteristics of *Streptomyces* spp.6 was noted. The Morphological tests ,

melanin production and sugar

fermentation were recorded (Table 1).

Table (1): Characteristics of *Streptomyces* spp.6 isolate

<i>Streptomyces</i> spp.6	Results
Gram stain	positive
Aerial mycelium color	grey
Substrate mycelium color	Yellow-green
Melanin production	negative
Fermentation of Sugars:	
glucose	+
mannose	-
xylose	-
mannitol	+
sucrose	+
fructose	-
Catalase	-
Urea test	-

Activity of agent against clinical test isolates:

The agent was extracted from *Streptomyces* spp.6. Activity of agent was examined against clinical bacterial pathogens after culturing of clinical samples on blood and MacConkey agar, these isolates includes (2) *S.aureus* isolates , (1) *S.epidermids* isolate ,(3) *E.coli* isolates,(2) *C.albicans* isolates and (1) *Klebsiella* isolate. The results showed that this agent having antimicrobial activity against clinical isolates with inhibition zone about (12-22 mm) (Figure 2). Our results was agreed with results obtained by [13] who found that the extract produced by *Streptomyces* spp soil isolate was active against *S.aureus* with (19)mm inhibition zone. [15] found that *Streptomyces*

isolates having ability for inhibition Coagulase negative *S.aureus* with inhibition zone ≥ 20 mm.

Also our results united with findings obtained by [2] who found that the soil *Streptomyces* were active against *E.coli* with inhibition zone 16mm.[1] found that soil actinobacteria having activity against multi drug resistant bacteria which includes *K.pneumoniae* and *E.coli*.

A metabolite produced by *Streptomyces* isolates having antifungal activity against *C.albicans* [6,15]. Anti-candidial metabolites was produced by *Streptomyces griseolus* strain SY1 isolated from soil samples [16]. Antimicrobial activity of *Streptomyces* isolates was recorded by many researchers [10,15].

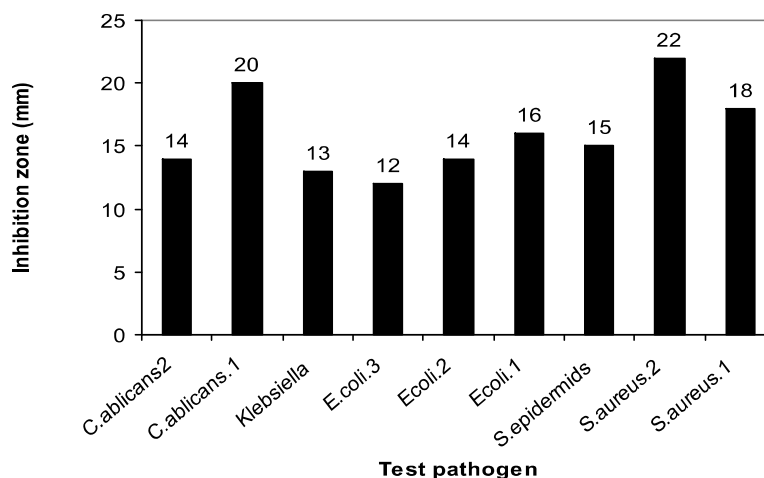


Figure (2):Activity of *Streptomyces*spp.6 Agent against Clinical isolates

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الفعالية المضاد للميكروبات للعامل المنتج بواسطة الستربتومايسيس المعزولة من عينات تربة

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الخلاصة:

جمعت اثنتان وعشرون نموذج تربة من مدينة الحلة. وجدت اثنتا عشر عزلة من الاكتينومايسيتات. اكتشفت ثمانية عزلات من الستربتومايسيس حسب الخصائص الشكالية على وسط مستخلص الخميرة والشعير. كانت هذه العزلات موجبة لصبغة غرام وتمتلك مايسليم هوائي رصاصي اللون ومايسليم اساس اصفر-بنّي. فحصت فعالية هذه العزلات ضد البكتريا العنقودية الذهبية. اعطت العزلة الستربتومايسيس 6 فعالية عالية للتثبيط مع قطر تثبيط (20 ملم) مقارنة بالعزلات الاخرى التي اعطت أقطار تثبيط اقل. اختيرت العزلة الستربتومايسيس 6 لاستخلاص العامل المضاد. امتلكت عزلة الستربتومايسيس 6 مايسليم هوائي رصاصي اللون مع مايسليم اساس اصفر-بنّي اللون. وكانت غير قادرة على انتاج الميلانين، وقادرة على تخمير الكلوكوز والمانيتول والسكرورز. وكانت سالبة لفحص الكتليز واليوريا. استخلص العامل المضاد من عزلة الستربتومايسيس 6 وفحصت قابليته المضادة ضد العزلات البكتيرية المرضية. اظهرت النتائج ان العامل المضاد يمتلك فعالية مضادة ضد العزلات السريرية مع اقطار تثبيط تتراوح من 12-22 ملم.

الكلمات المفتاحية: ستربتومايسيس, عزل , الفعالية المضادة للميكروبات