

The Isolation and Study of Morphological Characterization of *Streptomyces* Isolated From the Soil as a Source of Active Antibiotic

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Abstract:

The study involved the isolation of (5)isolates belonging to the Genus *Streptomyces* from(10)soil samples collected from fields and house gardenal from the (rhizosphere) in Mosul city, The isolates were morphologically distinct on the basis of spore color, aerial and substrate mycelium formation and production of diffusible pigment, Isolates were tested under a microscope by using slide culture technique, The results indicated that isolates had antibacterial activity against test organism including *Staphylococcus aureus*,. *Bacillus cereus*, *B subtilis*, *Pseudomonas aeruginosa* and isolates also showed antifungal activity against *Candida albicans* and *Aspergillus niger*, The results indicates that the soil of this region is source of *Streptomyces* having antibacterial and antifungal activity and thus enable us using microorganisms as biological control agents.

عزل ودراسة مظهرية لبكتيريا الـ *Streptomyces* المعزولة من التربة كمصدر للمضادات الحيوية

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ملخص البحث:

تضمنت الدراسة عزل (5) عزلات تابعة الى جنس الـ *Streptomyces* من (10) عينات تربة جمعت من حقول وحدائق منزلية من منطقة الرايزوسفير في مدينة الموصل ،درست العزلات مظهريا على اساس الوان السبورات والغزل الأرضي والهوائي وإنتاج الصبغات فحصت العزلات بالمجهر بوساطة تقنية زراعة الشريحة الزجاجية ،دلت النتائج على امتلاك العزلات فعالية مضادة للبكتيريا *Staphylococcus aureus* و *Bacillus cereus* و *B. subtilis* و *Pseudomonas*

Candida albicans كما أظهرت العزلات فعالية مضادة للفطريات و *aeruginosa* و *Aspergillus niger* وأشارت النتائج بان تربة الموصل تعد مصدرا غنيا بالـ *Streptomyces* التي تمتلك فعالية مضادة للبكتيريا والفطريات وهكذا يمكن استخدامها في السيطرة الحيوية.

Introduction:

Streptomyces species was considered as a major producers of bioactive compounds for the biotechnology industry, They are the source of most clinically used antibiotics, as well as of several widely used drugs against common diseases.

Antibiotics are the best known products of Actinomycete. Over 5,000 antibiotics have been identified from the cultures of Gram positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases (Demain *et al.*, 1999).

The genus, *Streptomyces*, is responsible for the formation of more than 60 % of known antibiotics while a further 15 % are Made by anumber of related Actinomycete, *Micromonospora*, *Actinomadura*, *Streptoverticillium* and *Thermoactinomyces*. (Waksman, 1954), Actinomycetes are the dominant group of soil population together with bacteria and fungi and are originally considered as an intermediate group between them, They are free living saprophytic bacteria and a major source for production of antibiotics(Unaoguet *et al.*, 1994),They play a major role in recycling of organic matter ,production of novel pharmaceuticals, nutritional materials, enzymes, antitumor agents, enzyme inhibitors, immune-modifiers and vitamins(Wellington, 1992), *Streptomyces* gram-positive, filamentous bacteria capable for secondary metabolite production such as antibiotics and antifungal compounds.

The study aimed at isolation and characterization of *Streptomyces* collected from soil samples of agricultural soil (rhizosphere of plant)and its ability for antibiotic production.

Keywords: *Streptomyces*, Isolation, Characterization, Antibacterial activity, Antifungal activity.

Material and Methods:

Isolation of microorganisms

Agricultural soil samples were collected from the (rhizosphere of plant), The samples were taken up from a depth of 20 cm after removing approximately 3 cm of the soil surface, The samples were placed in sterile polyethylene bags, closed tightly and stored in a refrigerator.

The soil was pretreated with CaCO₃ (10:1 gm soil / CaCO₃) and incubated at 37°C for 4 days,In conventional dilution plate technique(first tube containing 1 gm of soil sample suspended in 9 ml of sterile Ringer

solution and successive dilutions were prepared upto 10⁻⁴. An aliquot (0.5 ml) of suspension from the last dilution test tube was spread over(*Pepton-Glycerol-Yeast extract agar medium*) and incubated for 7 days at 28°C (Balagurunathan, 2001). After incubation period, the plates were examined for typical colonies of *Streptomyces*, The typical round, small, opaque, compact, frequently pigmented, earthy odor, chalky appearance, colonies were examined by using slide culture technique examined under a light microscope testing for substrate and aerial mycelium, . The colonies that bear typical *Streptomyces* morphology were purified and sub-cultured on Glycerol asparagine agar medium plates were stored for further assay (Bernard, 2007,2008، سليمان).

Screening isolates for Antimicrobial activity producing Antimicrobial (Agar streak method)

Nutrient agar medium(Oxoid), Potato dextrose agar(Booth,1971) plates were prepared and inoculated with *Streptomyces* isolate by a single streak of inoculum in the center of the petri dish. After 7days of incubation at 28°C the plates were seeded with test organisms by a single streak at an angle of 90° to the *Streptomyces* strains without touching each other, and incubated at 37 °C for 24 h in the case of bacteria and 28 °C for 48 h in the case of fungi ,Microbial interactions were analyzed by the determination of the size of the inhibition zone (Madigan *et al.*2003).

Test microorganisms

Six bacteria, including three Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, and one Gram negative *Pseudomonas aeruginosa* and two fungi- *Candida albicans* and *Aspergillus niger*, Test organisms were obtained from Science college/Biology department in Mosul University.

Culture media

1. Pepton- Glycerol-Yeast extract agar medium(Oskay *et al.*,2004) Used for isolation and purification of *Streptomyces*.
2. Glycerol asparagines agar medium (Williams,Cross,1971)Used for isolation and purification of *Streptomyces*.
3. Starch mineral salt agar medium(Williams *et al.*,1983)
Used to detect colors of substrate and aerial mycelium.(figure 1)
4. Nutrient agar medium(Oxoid)
5. Potato dextrose agar(Booth,1971).
- 6- Tyrosine agar(Lennette *et al.*,1985)

Table(1) Antimicrobial activity of *Streptomyces* isolates

Microorganism Isolates no.	Diameter of zone of Inhibition (mm)					
	S.aureus	B.cereus	B.subtilus	P.aeruginosa	A.niger	C.albicans
S1	14	14	12	12	15	14
S2	11	14	12	/	17	/
S3	12	/	13	12	15	15
S4	14	15	/	/	/	15
S5	14	15	13	13	14	14

Table(2) Growth Characteristics of *Streptomyces*
(Incubation period:14days,Temp: 28 °C)

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Glycerol asparagines agar	Good	Gray,white	White	None
Starch mineral salt agar	Good	Green,red,gray, white	None	None
Pepton-Glycerol-Yeast extract agar	Moderate	Red	None	Brown
Tyrosine agar	Moderate	Gray,white	Brown,white	Brown

to raise the value of hydrogen power which limit the growth of most fungi and increase growth of Actinomycetes (نهر واخرون، 1997). In study of (Sahin, Ugur, 2003) isolates of *Streptomyces* showed moderate effect against *Staphylococcus aureus* and *Bacillus subtilis* which is similar to this study while there is no effect against *Pseudomonas aeruginosa* which is different from this study, Also in other study (السماك، 2006) isolates of *Streptomyces* gave moderate to low effect antifungal activity, Which is similar to this study.



Figure (2) Agar streak method

Conclusion

The isolated microorganism was identified as a member of *Streptomyces* genus using the slide culture technique which was the best method for recognizing, isolates of *Streptomyces* having antibacterial and antifungal activity from soils of fertile areas particularly in rhizosphere of plants. This indicates that the soils of this region may be an interesting source of new antibiotics, Extensive study will be carried out in the future to explore more bioactive compounds from this source of bioactive microbes.

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