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.

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**المعززة من التربة كمصادر *Streptomyces* للمضادات الحيوية**

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ملخص البحث:

تضمنت الدراسة عزل (5) عزلات تابعة إلى جنس الـ *Streptomyces* من (10) عينات تربة جمعت من حقول وحدائق منزلية من منطقة الرايروسفي في مدينة الموصل، درست العزلات مظهرياً على أساس الوان السبورات والغزل الأرضي والبهائي، وإنتاج الصبغات، فحصت العزلات بالمجهر بوساطة تقنية زراعة الشريحة الزجاجية. بدأت النتائج على المواد والمضادات، *Pseudomonas* و *B. subtilis* و *Bacillus cereus* و *Staphylococcus aureus* للبكتيريا.
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 Actinomycte, *Micromonospora, Actinomadura, Streptoverticillium and Thermoactinomycetes*. (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 up to 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 asparagagine agar medium plates were stored for further assay (Bernard, 2007, 2008).

**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 7 days 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

<table>
<thead>
<tr>
<th>Isolates no.</th>
<th>S. aureus</th>
<th>B. cereus</th>
<th>B. subtilus</th>
<th>P. aeruginosa</th>
<th>A. niger</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>S2</td>
<td>11</td>
<td>14</td>
<td>12</td>
<td>/</td>
<td>17</td>
<td>/</td>
</tr>
<tr>
<td>S3</td>
<td>12</td>
<td>/</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>S4</td>
<td>14</td>
<td>15</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>14</td>
<td>15</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Substrate mycelium</th>
<th>Diffusible pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol asparagines agar</td>
<td>Good</td>
<td>Gray, white</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Starch mineral salt agar</td>
<td>Good</td>
<td>Green, red, gray, white</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Pepton-Glycerol-Yeast extract agar</td>
<td>Moderate</td>
<td>Red</td>
<td>None</td>
<td>Brown</td>
</tr>
<tr>
<td>Tyrosine agar</td>
<td>Moderate</td>
<td>Gray, white</td>
<td>Brown, white</td>
<td>Brown</td>
</tr>
</tbody>
</table>
Figure( 1) :*Streptomyces sp.* grown on Starch mineral salt agar media after 14 days.

**Results and Discussion**

The study included the isolation of (5) isolates belonging to the Genus *Streptomyces* from (10) soil samples collected from agricultural soil (rhizosphere of plant). Isolates of *Streptomyces* were identified on the basis of chalky appearance of colonies and production of moist earthy odor then confirmed by using slide culture technique. All of the isolates were tested for their ability to produce inhibitory activity against six test microorganisms. The test microorganisms includes 3 Gram positive bacteria, 1 Gram negative bacteria and 2 fungi, *Streptomyces* isolates showed antimicrobial activity against the test organisms,(Table 1)(figure2). Morphological examination of these isolates, indicates that these belong to the genus *Streptomyces* (Waksman 1961) .They showed good sporulation with compact, chalk-like colonies of different colors and characteristic earthy odor. The Genus formed branched substrate and aerial mycelium which on maturation differentiated into spiral spore chains. The mycelia growth as well as development of spiral spore chains were studied microscopically under a light microscoape,Cultural characteristicsm (Table 2) were determined in Pepton- Glycerol-Yeast extract agar medium ,Starch mineral salt agar medium, Glycerol asparagines agar medium and Tyrosine agar, The addition of CaCO3 and heat treatment lead
to raise the value of hydrogen power which limit the growth of most fungi and increase growth of Actinomycetes (نهـر واطـخـرون،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 particulary 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.
Reference
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   Streptomyces Lavendule