

## Antagonistic determination of *Streptomyces spp* bacteria against fungi

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### Abstract

In attempt to find new organisms that have the ability to produce antibiotics for fungi biological control usage, in this study used isolated bacteria which belongs to *Streptomyces spp* from cultivated soil which collected from plant root region in Tobruk district, the soil samples were Dark brown and black, the soil was sandy clay loam with proximately pH 7.13- 7.29. The physiological and morphological characterise for *Streptomyces* isolated samples were studied According to (Sharling & Gottlieb 1966), where isolates showed colour properties to areal mycelia and the colours were grey 40%, white 20%, yellow 20% and green 20%. The most of isolates showed inability to produce soluble pigment, the isolates were tested to show the ability to produce antifungals to prevent the growth of unicellular fungi and filament fungi as *Candida*, *Rhizopus*, *Mucor*, *Phoma*, *Aspergillus*, and *Trichoderma*.

The results showed the ability of *Streptomyces* isolates to produce anti-material against tested fungi samples which used in this study.

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### Introduction

Fungi is grown in nature like huge mycelia which cannot be imagined, where found fungal colony belongs to *Armillaria bulbosa* that covered about 30 acre in Michigan jungle in USA, thulls estimated weight of about 10 tons...(6)

Fungi play an important role in the environment and our daily life which is useful as the decomposition of dead organisms also the extraction of many materials that use in Food and pharmaceutical industries and harmful roles as the Corruption of wood products, leather, cloth and Food in addition it produces mycotoxins that causes poisoning to human .....(9)

Some fungi can cause diseases as Tinea which is caused by *Epidermophyton*, *Microsporus*, *trichophyton*, *floccosum*, some of fungi attacks the epidermis like *Candidiobolus*, *Basidiobolus* and some of them afflict the lung as *Aspergillus fumigatus*.....(6)

*Streptomyces species* have metabolically divers and have the ability to consume the most of sugars compound, alcohols, amino acids, organic acids, Aromatic compound, this as rustle of its ability to produce Extracellular hydrolytic enzymes for that the bacteria known as bioremediation because its ability to produce different antibiotics as Chloramphenicol, Streptomycin, Neomycin and Tetracycline against pathogenic bacteria and fungi .....(11)

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*Streptomyces spp* used in biological control to protect many of agricultural crops from fungi for that it uses in many researches in USA and it is considered to be EPA-registered.....(10)

### Material and Methods.

The soil samples were collected from cultivated land near to roots after removing the outer layer , the samples were taking from depth 3-5 cm and kept in plastic bags then it taked to the laboratory where the sample turned to powder by using pestle mortar , soil suspension was prepared by taken ( 1 soil :5 DW) and the pH was determined , 1\1000,000 diluted solution of soil samples was prepared, then 0.1 ml of it was taken to be put on 4 plates of Inorganic salts starch agar media which is selective media for *Streptomyces* bacteria the plate was incubated on 28 C° for 3-4 days to identify *Streptomyces* colonies according to Bregey's manual book and Sharling and Gottlieb 1966 after identification of *Streptomyces* colonies the pure colonies were growing on anther plates from the same media by streaking method .

The pure colonies of *Streptomyces* were growing on Oatmeal agar media to study the colour characters and physiological characters according to Sharling and Gottlieb 1966 method.

The pour *Streptomyces* colonies were growing on starch casein agar media , starch casein broth media and nutrient agar media individually , the agar media was incubated in incubator whereas the broth media was incubated on shaker on 125rpm for 3-4 days to determine the effectiveness antagonistic of *Streptomyces spp* against fungi . Antagonism experiments were used by diffusion technique in different two ways ( agar disc antagonistic and agar well antagonistic). (Egrov 1985)

The media plates with tasted fungi were incubated on 28-30 C° for 24-72hr, inhibition zone was observed and diameter of it was determined as (++) active and anti-produced, (+) anti-produced, (±) weak ,(-) non anti-produced.

### Result and Discussion

the samples were described according to standard to soil ,where the soil samples which collected were brown to black , sandy clay loam and dark brown , sandy clay with ph between 7.13- 7.29 as shown in table (1).

According to Sharling & Gottlieb (1966) the plate that contain 50-100 colonies were chosen and the *Streptomyces spp* were isolated based on external characters which is whole colony , colony edge ,colony surface, then five isolates were chosen and were pureed as shown in Table (2)

Table (3) show the morphological characteristics study was according to Sharling & Gottlieb (1966), whereas the areal mycelia was white 20%, yellow 20%, green 20% and grey 40% . Substrata mycelium was dark brown, yellow, light brown, also was noticed that some isolates were produced to soluble pigment.

Antagonistic of *Streptomyces spp* isolates which grown on starch casein agar and tasted by using agar disc method presented effective on tested fungi and were

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more effective on *Aspergillus sulph*, *Candida* as illustrated in Table (4), we find the *Streptomyces spp* isolates which grown in Nutrient broth more effective than isolates in starch casein broth ,recorded a higher results on *Candida* , *Mucor* as shown in Table (6).if we compared with the results in Table (5).

## Conclusion

Through this study some effective strains of *Streptomyces* were isolated, these strains have the ability to produce anti-substance which can be used to develop new and effective antibiotics especially that fungi day by day became more resident to antibiotics. We recommended to go ahead in these study to develop a new effective substance against fungi.

Table (1) Soil sample characteristic.

| Soil sample | Colour         |                 | pH   |
|-------------|----------------|-----------------|------|
| 1           | Brown to Black | Sandy clay loam | 7.13 |
| 2           | Dark brown     | Sandy clay      | 7.29 |

Table (2) *Streptomyces spp* isolates.

| Soil sample | Number of isolates | Strain series number     |
|-------------|--------------------|--------------------------|
| 1           | 5                  | 1-1A,1-2A,1-2B,1-4B,1-3C |
| 2           | 5                  | 2-1,2-2,2-3,2-4,2-1+     |

Table (3) Morphological characteristics of *Streptomyces spp* isolates which grown on Oatmeal agar media.

| Streptomyces spp isolates | Spore colour | Substart mycelium colour | Soluble pigment      |
|---------------------------|--------------|--------------------------|----------------------|
| 1-1A                      | Grey -brown  | Dark brown               | Light brown produced |
| 1-2A                      | Grey         | Light brown              | No- pigment          |
| 1-2B                      | White        | Light yellow             | No- pigment          |
| 1-4B                      | Yellow-green | Light brown              | No-pigment           |
| 1-3C                      | Yellow-green | Dark brown               | Light brown produced |

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Table (4) Antagonistic of *Streptomyces spp* which grown on Starch Casein Agar against tested fungi.

| Streptomyces isolates | Tested fungi |                |              |                 |                          |                          |                              |                    |
|-----------------------|--------------|----------------|--------------|-----------------|--------------------------|--------------------------|------------------------------|--------------------|
|                       | <i>Mucor</i> | <i>Candida</i> | <i>Phoma</i> | <i>Rhizopus</i> | <i>Aspergillus niger</i> | <i>Aspergillus sulph</i> | <i>Aspergillus ochraceus</i> | <i>Trichoderma</i> |
| 1-1A                  | -            | ±              | +            | -               | -                        | +                        | -                            | -                  |
| 1-2A                  | -            | +              | -            | ±               | -                        | +                        | -                            | -                  |
| 1-2B                  | -            | +              | +            | -               | -                        | +                        | -                            | -                  |
| 1-4B                  | +            | +              | -            | +               | -                        | +                        | +                            | -                  |
| 1-3C                  | -            | -              | +            | -               | -                        | -                        | -                            | -                  |
| 2-1                   | -            | +              | -            | -               | -                        | +                        | -                            | -                  |
| 2-2                   | -            | ±              | -            | -               | -                        | +                        | -                            | -                  |
| 2-4                   | -            | -              | ±            | -               | -                        | -                        | -                            | -                  |

Table (5) Antagonistic of *Streptomyces spp* which grown on Starch Casein Broth against tested fungi.

| Streptomyces isolates | Tested fungi |                |              |                 |                          |                          |                              |                    |
|-----------------------|--------------|----------------|--------------|-----------------|--------------------------|--------------------------|------------------------------|--------------------|
|                       | <i>Mucor</i> | <i>Candida</i> | <i>Phoma</i> | <i>Rhizopus</i> | <i>Aspergillus niger</i> | <i>Aspergillus sulph</i> | <i>Aspergillus ochraceus</i> | <i>Trichoderma</i> |
| 1-1A                  | -            | +              | -            | -               | -                        | -                        | +                            | -                  |
| 1-2A                  | -            | ++             | -            | +               | -                        | -                        | -                            | -                  |
| 1-2B                  | -            | ++             | -            | -               | -                        | +                        | -                            | ±                  |
| 1-4B                  | -            | -              | -            | +               | -                        | -                        | -                            | -                  |
| 1-3C                  | -            | +              | +            | -               | -                        | -                        | -                            | -                  |
| 2-1                   | -            | ++             | -            | -               | -                        | -                        | ±                            | -                  |
| 2-2                   | +            | +              | +            | -               | -                        | -                        | +                            | -                  |
| 2-4                   | +            | ±              | +            | -               | -                        | -                        | ±                            | -                  |

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Table (6) Antagonistic of Streptomyces spp which grown in Nutrient Broth against tested fungi.

| Streptomyces isolates | Tested fungi |                |              |                 |                          |                          |                              |                    |
|-----------------------|--------------|----------------|--------------|-----------------|--------------------------|--------------------------|------------------------------|--------------------|
|                       | <i>Mucor</i> | <i>Candida</i> | <i>Phoma</i> | <i>Rhizopus</i> | <i>Aspergillus niger</i> | <i>Aspergillus sulph</i> | <i>Aspergillus ochraceus</i> | <i>Trichoderma</i> |
| 1-1A                  | -            | +              | -            | -               | -                        | -                        | -                            | -                  |
| 1-2A                  | -            | -              | -            | -               | -                        | -                        | -                            | -                  |
| 1-2B                  | -            | -              | -            | -               | -                        | ±                        | -                            | -                  |
| 1-4B                  | ±            | ±              | -            | -               | -                        | -                        | -                            | -                  |
| 1-3C                  | -            | +              | ±            | -               | -                        | -                        | -                            | -                  |
| 2-1                   | -            | +              | -            | -               | -                        | -                        | -                            | ±                  |
| 2-2                   | ±            | +              | -            | -               | -                        | -                        | -                            | -                  |
| 2-4                   | ±            | ±              | -            | -               | -                        | -                        | -                            | -                  |

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