

Isolation and Screening of Antibiotics Producing Streptomycetes from Western Region Soils of Saudi Arabia

A. A. MALIBARI

*Department of Biological Sciences, Faculty of Science,
King Abdulaziz University, Jeddah, Saudi Arabia*

ABSTRACT. Streptomycetes group was studied in twenty-three soil samples of western region. The highest density of this group was found in 3 soil samples collected from Al-Madinah Al-Munawwarah. Six *Streptomyces* series were observed. Gray series represented the most dominant isolates. The highest antimicrobial activity was recorded in the case of gray and red series. *Streptomyces* MR13 (red series) had a broad spectrum against test organisms. *Streptomyces* MY18 (gray series) showed a high antimicrobial effect against *Staph. aureus*, *Escherichia coli*, *P. mirabilis* and *B. subtilis*. Some antibiotics excreted by *Streptomyces* cultures had a very narrow spectrum against test organisms. On the other hand, *B. subtilis* was inhibited by 14 *Streptomyces* cultures followed, in descending order, by *E. Coli* and *Staph. aureus*. *Candida albicans* and *Penicillium* sp. were only sensitive to only one *Streptomyces* isolate.

Introduction

Actinomycetes group is a large and complex one containing many genera which are distinguished primarily by their structural and developmental properties. Gray and Williams^[1] and Hawker and Linton^[2] mentioned that large numbers of actinomycetes, as many as millions per gram, are present in dry worm soils and the large counts on dilution plates suggest that this group is present very largely as spores. Williams^[3], Singal *et al.*^[4], Alexander^[5] and Pelczar *et al.*^[6] reported that the most predominant genera of this group are *Streptomyces*, *Streptosporangium*, *Micromonospora* and *Nocardia*. These organisms are responsible for the characteristic musty or earthy

odor of a freshly ploughed field being attributable to volatile substances which they produce.

Streptomycetes are capable of degrading many complex organic substances and consequently play an important role in building soil fertility. They are also noted for their ability to synthesize and excrete antibiotics. Some *Streptomyces* species have a broad antimicrobial spectrum inhibitory to the growth of the test organisms representing bacteria, yeast and fungi. Kalyuzhnaya *et al.*^[7] observed that 70-90%, 23-24% and 52-62% of actinomycetes cultures inhibited Gram-positive bacteria, *E. coli* and *Enterococci* respectively.

The objective of this investigation was to study the density of Streptomycetes and especially *Streptomyces* series in different localities of western region soils. The potentiality of these organisms to excrete antibiotics was also observed.

Material and Methods

Soil Samples

In this investigation twenty-three samples were taken from different localities of western region soils of Saudi Arabia. These localities included Al-Madinah Al-Munawwarah (7 samples), Taif (8 samples), Heda-Alsham (3 samples), Khulais (1 sample) and Al-Jumoum (4 samples). Soil samples from each site were taken from the top layer of cultivated soils (0-30 cm depth) and directly transferred into polyethylene bags to minimize moisture losses during transportation. These samples were subjected to bacteriological analysis.

Bacteriological Analysis

Total bacteria and streptomycetes counts were determined in different soil samples using the dilution plate technique. Soil extract agar medium was used for counting of total bacteria^[8]. Jensen's medium^[9] and glycerol casein medium^[10] were used to determine the counts of streptomycetes.

Isolation of Streptomycetes Organisms

The developed colonies on the suitable plates of actinomycetes enumeration were picked up and transferred to glycerol casein agar slants.

Isolation of Some Bacterial Groups

From plates of bacterial counts, 21 different colonies were picked up at random and transferred into nutrient glucose agar slants^[11]. These cultures were screened according to their morphology and Gram-staining into spore-forming Gram-positive bacilli, short rods Gram-negative and Gram-positive cocci. Three cultures each of these groups were chosen at random to study their sensitivity to streptomycetes producing antibiotics as isolates from the soil under investigation.

Morphological Properties of Streptomyces Isolates

All streptomyces cultures isolated from different localities were examined to determine the color of (substrate mycelium) conidiospore and diffusible soluble pigments. These isolates were divided into different series according to Bergey's Manual of Determinative Bacteriology^[12].

Test Organisms

Bacillus subtilis ATCC 6633, *Staphylococcus aureus* 209P, *Escherichia coli* S15, *Proteus mirabilis* 1287, *Azotobacter chroococcum* AS1, G +ve *Bacillus* isolate No. 1, G -ve rods isolate No. 2, G +ve coccus isolate No. 3, *Saccharomyces cerevisiae* G104, *Candida albicans* R17, *Aspergillus niger* AN20, *Penicillium* sp PS15 and *Trichoderma viride* TV25.

These cultures were obtained from the Microbial Cultures Collection of the Biological Sciences Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

Sensitivity Tests

To select the most promising antibiotic producing streptomyces, diffusion plate method was used, using the two following techniques :

A. Disk Method. In this method; the specific medium for each test organism was poured in petri-dishes and inoculated with one ml of bacterial, yeast or fungal spore suspension (test organisms). This standard inoculum contained $10^4 - 10^5$ cells or spores/ml. Then, the inoculation was distributed by a sterile glass rod (L-shape) on the surface of the medium and left for 5 minutes in laminar flow cabinet to dry the surface of inoculated medium. Nutrient agar medium was used for cultivation of *Bacillus subtilis* ATCC 6633, *Escherichia coli* S15 and *Proteus mirabilis* 1287 and local isolates (No. 1, 2 and 3). *Staphylococcus* medium^[11] was used for cultivation of *Staphylococcus aureus* 209P. *Azotobacter chroococcum* AS1 was grown on modified ashby's medium^[13]. Yeast and fungal strains were grown on Sabouraud Dextrose medium^[11]. Disks (10 mm diameter) from the cultures of streptomyces isolates (10 days old-grown on glycerol casein agar) were picked up and placed on the surface of medium seeded with test organisms. The plates were refrigerated at 5°C for 30 minutes for complete diffusion of antibiotics, thereafter they were incubated for 2-5 days at 30°C for all test organisms except *E. coli*, *Staph. aureus*, *Proteus mirabilis* were 37°C was used. The size of inhibition zone (area of visible inhibited growth) was used as an indicator of effectiveness. A comparison of inhibition zone measurement showed the relative effectiveness on various test organisms^[14].

B. Culture Filtrate Method. Conical flasks (100 ml vol.) containing 25 ml glycerol casein medium were inoculated with streptomyces isolates and were shaken on a reciprocal shaker (120 stroke/min) for 10 days.

Four disks (10 mm diam.) were removed from each plate containing medium seeded with the test organism and 0.1 ml of each streptomyces filtrate culture was

transferred to the place of disks and left for 20-30 minutes at 5°C until the diffusion of culture filtrate was completed. The plates were incubated and examined as mentioned before.

Results and Discussion

Streptomycetes and Total Bacterial Counts in Different Soil Samples

Densities of streptomycetes and total bacterial counts are presented in Table 1. Results clearly showed that the counts of both groups varied widely from one soil sample to another. Soil samples 8T, 9T and 10T contained the highest density of total bacterial counts being 9.15×10^6 , 10.11×10^6 and 12.70×10^6 cells/gram dry weight soil respectively (L.S.D. at 5% and 1% = 1.45×10^6 and 1.97×10^6 respectively). It seems that conditions in the soils of these three samples were favourable for bacterial proliferation, since these soils contained the lowest E.C., Mg^{2+} , Na^+ , HCO_3^- and Cl^- [15]. In addition, the regular cultivation of the soil supplies the microflora with organic materials which support the bacterial growth. On the contrary, the highest densities of streptomycetes were observed in soil samples 5M, 6M and 7M collected from Al-Madinah where counts were 9.44×10^5 , $6.21 \times 10^{8.41 \times 10^5}$ spores/gram dry soil using Jensen's medium (L.S.D. at 5% and 1% = 1.12×10^5 and 1.52×10^5 , respectively). The density of this group in these soil samples also showed the same trend when glycerol casein medium was used where the correlation coefficient was positively high being 0.9396. The lowest numbers of total bacterial flora were recorded in the soil sample number 5M collected from Al-Madinah (0.08×10^6 cells/g) whereas the lowest streptomycetes numbers were noticed in soil samples number 9T and 10T collected from Taif.

With respect to the correlation coefficient (R) between counts of total bacteria and streptomycetes counts, it was found that (R) was negative either on Jensen's medium or glycerol casein medium being -0.4409 and -0.3062 respectively. It means that the high density of streptomycetes led to decrease in total bacterial counts in soil samples under investigation.

Generally, it could be concluded that the counts of total bacterial flora were higher than actinomycetes. Counts of the first group ranged from 0.08×10^6 to 12.70×10^6 whereas the counts of second group ranged from 0.03×10^5 to 9.44×10^5 on Jensen's medium and from 0.01×10^5 to 8.99×10^5 on glycerol casein medium. This result confirms the results of many investigators^[16] who observed that the population of bacteria exceeds the population of all other groups.

The occurrence of high densities of total bacterial flora in soil samples collected from different localities cultivated with alfalfa plants, as compared with other plants, is expected. This result is in line with many investigators^[5] who stated that the metabolic products of root nodules of leguminous plants are secreted in the soil causing such high densities.

TABLE 1. Counts of total bacterial flora and streptomycetes in different soil samples collected from Western Region, Saudi Arabia.

| Soil Sample | Cultivar | Site | Moisture | Total bacterial count × 10 ⁶ /g dried soil | Streptomycetes count × 10 ⁵ /g dried soil | |
|-------------|--------------|--------------------------|----------|---|--|------------|
| | | | | | medium - 1 | medium - 2 |
| 1M | Alfalfa | Al-Madinah Al-Munawwarah | 9.1 | 2.11 | 0.71 | 0.32 |
| 2M | Alfalfa | Al-Madinah Al-Munawwarah | 12.1 | 4.23 | 0.89 | 0.45 |
| 3M | Alfalfa | Al-Madinah Al-Munawwarah | 11.2 | 2.71 | 4.66 | 3.41 |
| 4M | Alfalfa | Al-Madinah Al-Munawwarah | 9.4 | 3.65 | 5.32 | 4.21 |
| 5M | Sorghum | Al-Madinah Al-Munawwarah | 15.6 | 0.08 | 9.44 | 8.71 |
| 6M | Sorghum | Al-Madinah Al-Munawwarah | 14.2 | 0.16 | 6.21 | 7.21 |
| 7M | Sorghum | Al-Madinah Al-Munawwarah | 13.1 | 0.23 | 8.41 | 8.99 |
| 8T | Alfalfa | Taif | 12.1 | 9.15 | 0.21 | 2.11 |
| 9T | Alfalfa | Taif | 13.2 | 10.11 | 0.05 | 0.03 |
| 10T | Alfalfa | Taif | 10.4 | 12.70 | 0.03 | 0.01 |
| 11T | Millet | Taif | 16.1 | 1.11 | 1.21 | 0.07 |
| 12T | Squash | Taif | 15.0 | 0.50 | 1.50 | 1.01 |
| 13T | Tomato | Taif | 17.0 | 0.32 | 0.40 | 0.42 |
| 14T | Maize | Taif | 10.1 | 1.17 | 2.11 | 1.12 |
| 15T | Pepper | Taif | 13.2 | 0.15 | 1.11 | 0.05 |
| 16H | Alfalfa | Hada-Alsham | 11.1 | 2.07 | 2.13 | 0.67 |
| 17H | Alfalfa | Hada-Alsham | 8.4 | 2.13 | 2.11 | 1.85 |
| 18H | Sudana Grass | Hada-Alsham | 9.6 | 0.77 | 3.21 | 1.21 |
| 19K | Alfalfa | Kulais | 8.5 | 2.21 | 4.21 | 2.31 |
| 20A | Alfalfa | Aljumoom | 10.2 | 3.31 | 3.44 | 2.54 |
| 21A | Alfalfa | Aljumoom | 7.9 | 2.21 | 2.50 | 0.72 |
| 22A | Alfalfa | Aljumoom | 9.3 | 2.23 | 1.30 | 0.75 |
| 23A | Alfalfa | Aljumoom | 8.4 | 1.42 | 1.20 | 0.92 |

Medium - 1 Jensen's medium

Medium - 2 Glycerol Casein Medium

- A** L.S.D. for
- | | at 5% | at 1% |
|------------------------------|--------------------|-------------------|
| 1. Total bacterial counts | 1.45×10^6 | $.97 \times 10^6$ |
| 2. Streptomycetes counts | | |
| a) On Jensen's medium | 1.12×10^5 | $.52 \times 10^5$ |
| b) On glycerol casein medium | 1.16×10^5 | $.58 \times 10^5$ |
- B** Regression analysis for counts of :
- Total bacteria and Streptomycetes (med. 1) counts
 $Y = 0.34 X + 3.671$
Correlation coefficient (R) = -0.4409
 - Total bacteria and streptomycetes (med. 2) counts :
 $Y = -0.2455 X + 2.825$
Correlation coefficient (R) = -0.3062
 - Streptomycetes counts on med. 1 and med. 2
 $Y = 0.9739 X - 0.506$
Correlation coefficient (R) = 0.9396

***Streptomyces* Groups**

In this investigation 508 cultures of streptomycetes were isolated from different soil samples during actinomycetes counting. These isolates are characterized by the formation of mycelial filaments which tend to remain intact and not fragment and abundant aerial mycelium and long spore chains. These isolates did not form sporangium-like vesicles. Aerial spores (conidiospores) were non-motile and were not borne on verticillate sporophores. Colonies of these isolates were small discrete and lichenoid, leathery or butyrous; initially relatively smooth surfaced but later develop a weft of aerial mycelium that may appear granular, powdery, velvety or floccose. These isolates produced a wide variety of pigments responsible for colors of vegetative mycelium, aerial mycelium and substrate. According to Bergey's Manual of Determinative Bacteriology^[12], these isolates are considered to be different species of the genus *Streptomyces*. These isolates were classified into six series according to the color of mature sporulated aerial mycelium.

With respect to *Streptomyces* series, six series were recorded being gray, white, red, yellow, blue and green series. Gray series represented the highest isolates comparing with other series being 237 out of 508 *Streptomyces* isolates (46.66%). The gray series were also found in all soil samples under investigation where their percentages ranged from 25% to 100%. This series was followed by the white series where 144 out of 508 isolates were recorded (28.35%). This series was also recorded in all soil samples except sample 6M collected from Al-Madinah. Blue and green series were the least abundant representing 2.76% and 1.57% respectively where they were observed in few soil samples (6M and 9M). This result was in agreement with those observed by Osman^[17] and Al-Garni^[18].

Antimicrobial Spectrum of Different *Streptomyces* Isolates

1. Disk Method

In this experiment, 13 test organisms representing different microbial groups (fungi, yeast and bacteria) were used to study their sensitivity to antibiotics excreted by *Streptomyces* isolates during their propagation of glycerol casein agar medium. It was found that 145 out of 508 *Streptomyces* isolates (28.54%) had an antagonistic effect. Gray and red series comprised higher number of antagonistic isolates (being 50 and 45 isolates respectively) than other series. Blue and green isolates did not exhibit any activity test organisms.

The degree of antagonistic effect as indicated by the inhibition zone for all tested *Streptomyces* isolates are shown in Tables 2, 3, 4, and 5. Results in Table 2 indicated that *B. subtilis*, *E. coli* and *P. mirabilis* were the most sensitive test organisms to gray isolates. The antagonistic effect was also noticed against *Staph. aureus*, *Az. chroococcum*, G +ve Bacillus (isolate No. 1) G -ve short rod (isolate No. 2) and G +ve coccus (isolate No. 3). The highest inhibition zone (20-24 mm) was obtained from 19 *Streptomyces* isolates of gray series against all bacterial test organisms except *Staph. aureus* and G +ve cocci (isolate No. 3). No activity was detected on yeast and fungal test organisms.

TABLE 2. Effect of gray series of *Streptomyces* isolates on test organisms as indicated by zone of inhibition.

| Test Organisms | No response | Number of antagonistic isolates (inhibition zone range – mm) | | | | Total | Percentage of antagonistic isolates |
|---------------------------|-------------|--|---------|---------|---------|-------|-------------------------------------|
| | | 25 - 30 | 20 - 24 | 15 - 19 | 11 - 14 | | |
| <i>Bacillus subtilis</i> | 200 | 5 | 5 | 20 | 7 | 37 | 15.61 |
| <i>Staph. aureus</i> | 232 | 2 | – | – | 3 | 5 | 2.11 |
| <i>Escherichia coli</i> | 207 | 4 | 2 | 6 | 18 | 30 | 12.66 |
| <i>Proteus mirabilis</i> | 207 | 2 | 3 | 20 | 5 | 30 | 12.66 |
| <i>Az. chroococcum</i> | 217 | – | 5 | 5 | 10 | 20 | 8.44 |
| G +ve Bacillus (No. 1) | 210 | – | 3 | 10 | 14 | 27 | 11.39 |
| G –ve short rod (No. 2) | 215 | – | 1 | 6 | 15 | 22 | 9.28 |
| G +ve Coccus (No. 3) | 234 | – | – | 2 | 1 | 3 | 1.27 |
| <i>Sacch. cerevisiae</i> | 237 | – | – | – | – | – | – |
| <i>Candida albicans</i> | 237 | – | – | – | – | – | – |
| <i>Aspergillus niger</i> | 237 | – | – | – | – | – | – |
| <i>Penicillium</i> sp. | 237 | – | – | – | – | – | – |
| <i>Trichoderma viride</i> | 237 | – | – | – | – | – | – |
| Total | | 13 | 19 | 69 | 73 | 174 | – |

White series of *Streptomyces* isolates showed inhibition zones in the case of *E. coli*, *P. mirabilis*, *Az. chroococcum* and G –ve short rod (isolate No. 2). *Escherichia coli* and Gram-negative short rods (isolate No. 2) were more sensitive to 14 and 5 *Streptomyces* isolates respectively (Table 3), where 20-24 mm diameter of inhibition zones

TABLE 3. Effect of white series of *Streptomyces* isolates on test organisms as indicated by zone of inhibition.

| Test Organisms | No response | Number of antagonistic isolates (inhibition zone range – mm) | | | | Total | Percentage of antagonistic isolates |
|---------------------------|-------------|--|---------|---------|---------|-------|-------------------------------------|
| | | 25 - 30 | 20 - 24 | 15 - 19 | 11 - 14 | | |
| <i>Bacillus subtilis</i> | 144 | – | – | – | – | – | – |
| <i>Staph. aureus</i> | 144 | – | – | – | – | – | – |
| <i>Escherichia coli</i> | 120 | – | 14 | 10 | – | 24 | 16.67 |
| <i>Proteus mirabilis</i> | 130 | – | – | 10 | 4 | 14 | 9.72 |
| <i>Az. chroococcum</i> | 140 | – | – | – | 4 | 4 | 2.78 |
| G +ve Bacillus (No. 1) | 144 | – | – | – | – | – | – |
| G –ve short rod (No. 2) | 115 | – | 5 | 10 | 14 | 29 | 20.14 |
| G +ve Coccus (No. 3) | 144 | – | – | – | – | – | – |
| <i>Sacch. cerevisiae</i> | 144 | – | – | – | – | – | – |
| <i>Candida albicans</i> | 144 | – | – | – | – | – | – |
| <i>Aspergillus niger</i> | 144 | – | – | – | – | – | – |
| <i>Penicillium</i> sp. | 144 | – | – | – | – | – | – |
| <i>Trichoderma viride</i> | 144 | – | – | – | – | – | – |
| Total | 144 | – | 19 | 30 | 22 | 71 | – |

were recorded. The white series of isolates was also uheffective against some bacterial strains (*B. subtilis*, *Staph. aureus*, G +ve Bacillus No. 1, G +ve coccus No. 3) and all yeast and fungal test organisms.

The response of test organisms to red series of *Streptomyces* isolates is shown in Table 4. Results indicated that all test organisms were sensitive to the antibiotic excreted by some red series except *Sacch. cerevesiae*, *Candida albicans* and *Trichoderma viride*. The highest inhibition zone was noticed with *B. subtilis* (25-30 mm diameter). Red series isolates exhibited the highest antagonistic effect against *E. coli*; being 60.60% of red isolates.

TABLE 4. Effect of red series of *Streptomyces* isolates on test organisms as indicated by zone of inhibition.

| Test Organisms | No response | Number of antagonistic isolates (inhibition zone range - mm) | | | | Total | Percentage of antagonistic isolates |
|---------------------------|-------------|--|-------|-------|-------|-------|-------------------------------------|
| | | 25-30 | 20-24 | 15-19 | 11-14 | | |
| <i>Bacillus subtilis</i> | 30 | 5 | 1 | 10 | 20 | 36 | 54.55 |
| <i>Staph. aureus</i> | 40 | - | - | - | 26 | 26 | 39.39 |
| <i>Escherichia coli</i> | 26 | - | 15 | 15 | 10 | 40 | 60.60 |
| <i>Proteus mirabilis</i> | 39 | - | 1 | 10 | 16 | 27 | 40.91 |
| <i>Az. chroococcum</i> | 64 | - | - | 2 | - | 2 | 3.03 |
| G +ve Bacillus (No. 1) | 46 | - | 5 | 15 | - | 20 | 30.30 |
| G -ve short rod (No. 2) | 56 | - | 1 | 4 | 5 | 10 | 15.15 |
| G +ve Coccus (No. 3) | 46 | - | - | - | 20 | 20 | 30.30 |
| <i>Sacch. cerevesiae</i> | 66 | - | - | - | - | - | - |
| <i>Candida albicans</i> | 66 | - | - | - | - | - | - |
| <i>Aspergillus niger</i> | 46 | - | - | - | 20 | 20 | 30.30 |
| <i>Penicillium</i> sp. | 57 | - | - | - | 9 | 9 | 13.64 |
| <i>Trichoderma viride</i> | 66 | - | - | - | - | - | - |
| Total | 66 | 5 | 23 | 56 | 126 | 210 | - |

With respect to the yellow series (39 isolates), Table 5 showed that the effectiveness of these organisms was observed against all test organisms except *Sacch. cerevisiae* and *Penicillium* sp. Three isolates showed 25-30 mm inhibition zone against *E. coli* while seven isolates showed the same inhibition zone against *P. mirabilis* where the highest response percentage 56.41% was noticed.

Blue and green isolates did not show any activity against test organisms. It means that these isolates had no inhibitory effect against bacterial test organisms either G +ve, G -ve or fungal isolates.

Generally, it could be concluded that some gray, red and yellow *Streptomyces* series showed higher activity in the inhibition of test organisms than white series.

2. Culture Filtrate Method. The most active *Streptomyces* isolates which showed the highest antimicrobial effect were used throughout this investigation. These or-

TABLE 5. Effect of yellow series of *Streptomyces* isolates on test organisms as indicated by zone of inhibition..

| Test Organisms | No response | Number of antagonistic isolates (inhibition zone range - mm) | | | | Total | Percentage of antagonistic isolates |
|---------------------------|-------------|--|---------|---------|---------|-------|-------------------------------------|
| | | 25 - 30 | 20 - 24 | 15 - 19 | 11 - 14 | | |
| <i>Bacillus subtilis</i> | 20 | — | 5 | 4 | 10 | 19 | 48.72 |
| <i>Staph. aureus</i> | 25 | — | 2 | 3 | 9 | 14 | 35.90 |
| <i>Escherichia coli</i> | 25 | 3 | 7 | — | 4 | 14 | 35.90 |
| <i>Proteus mirabilis</i> | 17 | 7 | — | — | 15 | 22 | 56.41 |
| <i>Az. chroococcum</i> | 23 | — | — | 7 | 9 | 16 | 41.03 |
| G +ve Bacillus (No. 1) | 25 | — | — | 2 | 12 | 14 | 35.90 |
| G -ve short rod (No. 2) | 26 | — | — | 6 | 7 | 13 | 33.33 |
| G +ve Coccus (No. 3) | 30 | — | — | 4 | 5 | 9 | 23.08 |
| <i>Sacch. cerevisiae</i> | 39 | — | — | — | — | — | — |
| <i>Candida albicans</i> | 30 | — | — | — | 9 | 9 | 23.08 |
| <i>Aspergillus niger</i> | 35 | — | — | — | 4 | 4 | 10.26 |
| <i>Penicillium</i> sp. | 39 | — | — | — | — | — | — |
| <i>Trichoderma viride</i> | 27 | — | — | — | 12 | 12 | 30.77 |
| Total | 39 | 10 | 14 | 26 | 96 | 146 | |

ganisms were 6 gray series (HG1, HG4, MY18, TG5, TG6, and TG23), 5 red series (TR8, MR10, KR11, MR12 and MR13), 7 yellow series (KY15, MY16, TG70, KY19, MY22, KY24 and MY25) and one white series (HW20).

These organisms were grown in shake flasks and the culture filtrate after 10 days incubation was tested for the presence of antibiotics.

Results in Table 6 clearly indicated that *Streptomyces* MR13 (red series) showed a broad spectrum where the excreted antibiotic was effective against 10 test organisms out of 13 (76.92%). This was followed by *Streptomyces* MR10 and *Streptomyces* TR8 where 8 and 7 test organisms were sensitive. The highest inhibition zone was recorded in the case of *Streptomyces* MY18. The culture filtrate of this isolate gave inhibition zone ranging from 17 to 30 mm. This organism also had an antimicrobial effect against *B. subtilis*, *Staph. aureus*, *E. coli*, *P. mirabilis*, G +ve Bacillus (No. 1) and G -ve short rods (No. 2). Some antibiotic excreted by *Streptomyces* isolates had a very narrow spectrum against test organisms such as *Streptomyces* HG1, *Streptomyces* HG4, *Streptomyces* HW20, *Streptomyces* TG23 and *Streptomyces* MY25.

With respect to the number of *Streptomyces* which had an antimicrobial effect for each test organism, it could be stated that *B. subtilis* was inhibited by 14 *Streptomyces* isolates. This was followed in descending order by *E. coli* and *Staph. aureus*. *Candida albicans* and *Penicillium* sp. were only sensitive to one *Streptomyces* isolate.

It could be concluded that *Streptomyces* MY18 (gray series) was highly active against bacterial test organisms specially against some pathogenic bacteria (*Staph. aureus*, *Proteus mirabilis* and *E. coli*) in addition to some microorganisms isolated

TABLE 6. The antagonistic spectra of antibiotic producing *Streptomyces* series.

| Test organisms | Antagonistic effect of <i>Streptomyces</i> series (inh. zone - mm) | | | | | | | | | | | | | | | | | | Antagonistic isolates | |
|---------------------------|--|------|-------|-------|------|-------|---------|-------|-------|-------|-------|-------|------------------|-------|-------|-------|-------|------|-----------------------|-------|
| | ← Gray → | | | | | | ← Red → | | | | | | ← Yellow → White | | | | | | No. | % |
| | HG1 | HG4 | TG5 | TG6 | TG23 | MY18 | TR8 | MR10 | KR11 | MR12 | MR13 | KY15 | MY16 | TG70 | KY19 | KY24 | MY22 | MY25 | | |
| <i>Bacillus subtilis</i> | - | 11 | 22 | 18 | - | 24 | 15 | 15 | 19 | 14 | 25 | 11 | 13 | 22 | 15 | - | 12 | - | 14 | 73.68 |
| <i>Staph. aureus</i> | - | - | - | - | - | 24 | 15 | 14 | 11 | - | 12 | 11 | 11 | 16 | 16 | - | - | - | 9 | 47.37 |
| <i>Escherichia coli</i> | 12 | - | 19 | - | - | 30 | 12 | 15 | 14 | - | 20 | - | - | 15 | 20 | - | - | - | 20 | 52.63 |
| <i>Proteus mirabilis</i> | - | - | 15 | - | 11 | 28 | - | - | - | - | 15 | - | 14 | - | - | - | 11 | - | 6 | 31.58 |
| <i>Az. chroococcum</i> | - | - | 20 | - | - | - | - | 15 | - | 15 | - | - | - | - | - | - | - | 11 | 4 | 21.05 |
| G +ve Bacillus (No. 1) | - | - | - | 12 | - | 17 | 13 | 12 | - | 13 | 16 | - | - | 16 | - | - | - | - | 7 | 36.84 |
| G -ve short rod (No. 2) | - | - | - | 11 | - | 18 | 12 | 14 | - | 11 | 13 | - | - | 15 | - | - | - | - | 7 | 36.84 |
| G +ve Coccus (No. 3) | - | - | - | 12 | - | - | 13 | 17 | - | 16 | 14 | - | - | 2 | - | - | - | - | 6 | 31.58 |
| <i>Sacch. cerevisiae</i> | - | - | - | - | - | - | 12 | 13 | - | 12 | - | - | - | - | - | 11 | - | - | 4 | 21.05 |
| <i>Candida albicans</i> | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 14 | - | - | 1 | 5.26 |
| <i>Aspergillus niger</i> | - | - | - | - | - | - | - | - | - | 12 | - | - | 11 | - | - | - | - | - | 2 | 10.53 |
| <i>Penicillium</i> sp. | - | - | - | - | - | - | - | - | - | 11 | - | - | - | - | - | - | - | - | 1 | 5.26 |
| <i>Trichoderma viride</i> | - | - | - | - | - | - | - | - | - | - | - | - | 11 | - | - | 18 | - | - | 2 | 10.53 |
| Antagonistic No. isolates | 1 | 1 | 4 | 4 | 1 | 6 | 7 | 8 | 3 | 5 | 10 | 2 | 5 | 6 | 3 | 3 | 2 | 1 | 1 | 73 |
| % | 7.69 | 7.69 | 30.77 | 30.77 | 7.69 | 46.15 | 53.85 | 61.54 | 23.08 | 38.46 | 76.92 | 15.38 | 38.46 | 46.15 | 23.08 | 23.08 | 15.38 | 7.69 | 7.69 | |

from soil. *Streptomyces* MR13 (red series) showed a high broad spectrum as compared with other isolates. Another three isolates i.e. *Streptomyces* MR10, MY16 and KY24 also showed a considerable antimicrobial effect. On the other hand, high density of gray series in different soil samples may play an important role in the excretion of antibiotics or other inhibitory substances which affect the normal growth processes or survival of other organisms. The production of antibiotics by this group of microorganisms in soil may enable them to thrive successfully in a competitive environment. The existence of streptomycetes in different soil samples under investigation confirms their potentiality to produce antimicrobial agents. In vitro, 145 out of 508 *Streptomyces* isolates had an antagonistic effect.

References

- [1] Gray, T.R.G. and Williams, S.T., Microbial productivity in soil, In: Hughes, D.E. and Rose, S.H. (ed.), *Microbes and Biological Productivity*, Eds. D.E. Hughes and S.H. Rose, *Symp. Soc. Gen. Microbiol.* **21**: 255, (1971).
- [2] Hawker, E.L. and Einton, H.A., *Microorganisms. Function from Environment*, 2nd ed., Edward Arnold Publ. Limited, London, (1979).
- [3] Williams, S.T., The role of actinomycetes in biodeterioration, *Int. Biodeterioration Bull.* **2**: 125, (1966).
- [4] Singal, E.M., Mismustina, I.E., Rudaya, S.M. and Soloveno, N.K., Actinomycetes population in some soils of equatorial tropical zones of the Indian Ocean. *Antibiotiki*, **18** (7): 605-608, (1973).
- [5] Alexander, M., *Introduction to Soil Microbiology*, 2nd ed., John Wiley & Sons, Inc., New York, 573 p., (1982).
- [6] Pelczar, M.J., Chan, E.C.S. and Krieg, N.R., *Microbiology*, 5th ed., McGraw-Hill Book Company, New York, 917, (1986).
- [7] Kalyuzhnaya, L.D., Bryanskaya, E.T., Litovchenko, E.T., Likach, I.G., Lysento, S.A., Maiko, I.I. and Protinnov, S.M., Isolation and study of actinomycetes having antagonistic properties from soil in several oblasts of Ukraine, *Microbiologia*, **31** (4): 654-661, (1961).
- [8] Holm, E. and Jensen, V., Aerobic chemoorgano-trophic bacteria in a Danish beech forest, *Microbiology of a Danish beech forest, I. Oikos* **23**: 248-260, (1971).
- [9] Allen, O.N., *Experiments in soil microbiology*, Burgess Publ. Co., Minnesota, 255 p., (1961).
- [10] Kuster, E. and Williams, S.T., Selection of media for isolation of streptomycetes. *Nature*, **202**: 928, (1964).
- [11] *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures*, 9th ed. Difco Laboratories Incorporated, Detroit, Michigan, (1977).
- [12] Buchanan, R.E. and Gibbons, N.E., *Bergey's Manual of Determinative Bacteriology*, 8th ed. Williams and Wilkins Co., Baltimore, 599-881, (1974).
- [13] Abdel-Malek, Y. and Ishac, Y.Z., Evaluation of methods used in counting Azotobacters, *J. Appl. Bact.* **31**, 267-275, (1968).
- [14] Gottfried, S.S. and Kelly, J.L., *Fundamentals in laboratory microbiology*, The Iowa State Univ. Press, Ames, Iowa, (1984).
- [15] Malibari, A.A., Al-Fassi, F.A. and Ramadan, E.M., Occurrence of Vesicular-Arbuscular Mycorrhizas in soils of western region and their role in plant nutrition (1989) (under publication).
- [16] Burges, A., *Microorganisms in the soil*, Hutchinson, London, (1958).
- [17] Osman, M.K.A., Role of actinomycetes in Egyptian soil with special reference to antibiotic production. Ph.D. thesis, Fac. of Agric., Ain Shams Univ., Cairo, Egypt, (1982).
- [18] Al-Garni, S.M.S., *Microflora of soil and rhizosphere of natural vegetation in Al-Baha with special reference to antibiotic producing actinomycetes*, M.Sc. thesis, Fac. of Science, King Abdulaziz Univ., Jeddah, Saudi Arabia.

عزل وانتقاء الاستربتوميستات المنتجة للمضادات الحيوية من تربة المنطقة الغربية - المملكة العربية السعودية

عباس أحمد مليباري

قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز

جدة ، المملكة العربية السعودية

المستخلص . درست مجموعة الاستربتوميستات في ثلاثة وعشرين عينة تربة من المنطقة الغربية . وقد وجدت أعلى كثافة من هذه المجموعة في ثلاث عينات تربة تم جمعها من تربة المدينة المنورة . كما لوحظ وجود ست سلاسل لونية من الاستربتوميستات . وكانت السلسلة الرمادية اللون من أكثر العزلات السائدة . وقد سجلت السلسلتان الرمادية والحمراء أعلى نشاط مضاد للميكروبات . وكان لميكروب سترتوميستات إم . آر ١٣ (سلسلة حمراء) تأثير ذو مجال واسع ضد الميكروبات المختبرة . وأظهر سترتوميستات إم . واي ١٨ (سلسلة رمادية) أعلى تأثير مضاد لميكروبات ستافيلوكوكس أيريس ، وإيشيريشيا كولاي ، بروتيس ميرابيليس ، باسلس سبتيلس . كما أن بعض مزارع سترتوميستات قد أظهرت تأثيراً مضاداً محدوداً ضد الميكروبات المختبرة . ومن ناحية أخرى وجد أن ميكروب باسلس سبتيلس قد حدث له تثبيط بواسطة أربع عشرة مزرعة من الاستربتوميستات تلاها في ترتيب تنازلي كل من إيشيريشيا كولاي و ستافيلوكوكس أيريس . وكانت خميرة كانديدا البيكنس وفطيرة البنسليوم حساستين لمزرعة واحدة فقط من الاستربتوميستات .