CHAPTER IV

ISOLATION AND PRELIMINARY SCREENING OF
ANTAGONISTIC MICROORGANISMS

METHODS FOR PRELIMINARY ISOLATION

A number of methods and media were adopted for the isolation of microorganisms from the soil samples. It is a well-known fact that in the study of microbiology the artificial media, which are used for isolating different microorganisms, are usually elective in nature. In view of this no single medium and technique is sufficient to disclose all the organisms that might be present in a given soil sample. Consequently, the validity of results and conclusions derived from such a data depends very greatly upon the techniques used. Further, the antagonistic activity of an organism and the nature of the substances it produces depend largely upon the type of nutrition and the environment provided. It becomes necessary, therefore, to employ a number of methods with various media and other conditions if a true picture of the nature of the organisms is to be gathered.

The methods which were used for the preliminary isolations could be grouped mainly under two headings. In the first, these methods are included which are popularly used for the isolation of various organisms from the soil and which are the modifications of the soil or dilution plates. In these the
organisms were picked out randomly from the isolation plates, cultivated in culture and then screened for antagonism. In the second group of methods, some test organism was added either by a sprayer or in the medium as such to test its reaction with the organisms in the plate. The details of these methods are described below.

The routine soil plate methods:

1. Soil dilution plate: The method and the details of the procedure are similar to those described by Johnson et al. (1959). The soil water suspension (1:20) was shaken on a mechanical shaker (wrist action) at about 200 oscillations per minute for 30 minutes. From this, suitable dilutions were made and plated. The following media were used: for the isolation of fungi - potato dextrose agar and synthetic acid agar; for the isolation of bacteria and actinomycetes - glucose-asparagine agar and soil extract agar.

The inoculated plates were incubated at 25°C. When colonies appeared, usually after 5-7 days for fungi and bacteria and 6-14 days for actinomycetes, they were transferred and isolated for further study. Even in these preliminary isolations some inhibitory zones could be observed due to the antagonism among organisms forming colonies. These were specially picked up.

2. Method for actinomycetes (Lawrence, 1956): For the isolation of actinomycetes the soil was treated with phenol to reduce bacterial and fungal contaminants. Two drops of a 1:20
soil suspension were added to 10 ml. of a 1:140 dilution of phenol. After 10 minutes one drop of the phenol soil dilution was placed in 12 ml. of melted and cooled agar medium in a Petri-dish. The dishes were rotated by hand in a broad swirling motion so that the diluted soil is dispersed in the agar uniformly. The media used were soil extract agar and glucose-asparagine agar. The plates were incubated at 25\degree C. and the isolates obtained in this way were cultured and tested.

3. *Warnup's soil plate:* This well-known method by Warnup (1960) was used with the same media as given above in the case of dilution plates.

4. *Couch's mycelium isolation method* (Lechevalier et al., 1968): Soil actinomycetes were isolated by employing this technique. The method is as follows:

Green grass leaves were taken and boiled for a few minutes in a beaker of water. 5-10 g. soil was taken in each sterile Petri-dish and was flooded with sterile water. The grass leaves were kept floating on the surface of water. The plates were incubated at room temperature (20-25\degree C.). After 6-14 days of incubation, colonies developed on the green grass leaves were transferred, purified and stored for further study.

**Special methods:**

5. *The spray technique:* The sprayer used was that described by Wilska (1947) and was found to be extremely useful in these studies. It produces a fine mist by a spray which settles on the plated dish. The apparatus is described here.
(Plate I A). It consists of a conical flask whose entire bottom has been cut away. The diameter of this hole was slightly smaller than that of a 100 mm. diameter Petri-dish. The sprayer was fitted snugly in two holed rubber stopper and adjusted properly. The stopper was then fitted into the neck of the flask. Air is blown by means of a rubber bulb through the cotton wool A into the tube B, the lower part of which is drawn into a jet. Another tube C, sealed at the top of the air tube is drawn out into a fine point so that the surface tension will prevent the few drops of microbial suspension which are placed in it, from running out by gravity. This tube C is aligned with the air tube B so that a nebulous spray free from coarse droplets is obtained. The apparatus is sterilized before use.

A few drops of suspension of each of the test organisms *B. subtilis* and *Curvularia lunata* were placed in tube C and the flask was placed on the Petri-dish with upper lid removed. The Petri-dish having the organisms to be tested were sprayed with each of the test organisms.

The apparatus was found to be of a great use in testing experiments. It was also found useful for seeding plates with microorganisms for the purpose of assaying by cup or cylinder plate methods.

The sprayed plates were incubated at 28°C. for 24-48 hours. Organisms showing the inhibition zones (Plate I B & C) were detected and transferred. They were further tested for
antagonistic effects by cross-streak and other assay methods.

This method has several advantages over other methods of preliminary isolation: 1. Proper time interval was given to the antagonist for growth and production of antibiotic substance before inoculating with the test organisms. 2. The antagonistic organisms can be readily detected by the inhibition zones. 3. Any isolation soil plate in one of the earlier experiments containing the organisms could be tested readily.

6. Bacterial agar plate (Waksman, 1947): This is the method which was followed by earlier workers. This is not as convenient as the spray technique described above. The earlier workers used mostly bacterial test organisms. In the present study, both R. subtilis and C. lunata were used as the test organisms.

Soil dilutions were prepared and 1 ml. portions were transferred to sterile Petri-dishes. To this was poured 10 ml. glucose phosphate agar seeded with the test organism. The seeded agar was prepared as follows: Culture tubes containing 10 ml. of agar were taken and cooled to 47°C. To these 0.06 ml. of cell suspension of R. subtilis or 0.1 ml. of spore suspension of C. lunata was added and mixed thoroughly.

The plates were incubated at 28°C. Organisms showing the inhibition zones were transferred and further tested by cross-streak and other assay methods.
Isolates obtained by any of the above described preliminary screening methods were separated, purified and kept as stock cultures for further study. Proper marks for the identification of bacterial, actinomycete and fungal species and strains were kept on the isolates. In case of all organisms, different strains of the species were kept separately for test as it is now well-known that the different strains often differ in their capacities for producing antibiotic substances.

ASSAY IN FUSE CULTURE

By the preliminary testing a very large number of organisms were isolated. In all 106 isolates of actinomycetes were picked up for further study and similarly 52 isolates of bacteria and 104 of fungi were taken (Table - I). These organisms were then subjected to more rigorous tests by the following assaying methods. The actinomycetes were experimented against 12 test organisms, which consisted of 3 bacterial plant pathogens i.e. Acroboterium tumefaciens, Pseudomonas solanacearum and Xanthomonas malvacearum; 2 human pathogens i.e. Escherichia coli and S. aureus; 2 other bacterial species i.e. B. subtilis and Bacillus cereus. They were also tested against 4 plant pathogenic fungi: Glomerella cingulata, C. lunata, Phytophthora infestans and Myroctonia solani Strain I and one saprophytic fungus, Cunninghamella verticillata (Table - III page 33 ). It may be noted here that the bacterial species were so selected that they belonged to both gram positive and gram negative groups.
The fungal and bacterial species were tested against only 5 test organisms vis. _B. subtilis_, _P. acnesvarum_, _G. lunata_, _G. ginsulata_ and _Streptomyces erythrasa_ A-10. Actinomycetes being a more promising group was paid greater attention in these studies.

As the activity of an organism and the nature of the substance produced depend greatly upon the type of nutrition and environment, various types of media and test methods were employed so as to get a correct picture of the antagonistic activity and the substances produced.

**Test methods**

Four methods were used to measure the selective nature of the antagonistic action and to get some quantitative information concerning the intensity of this activity. In these methods the parasite or any other organism, whose susceptibility is to be tested is referred to as the test organism and the organism being so tested for antagonism as the antagonist. These methods described below were found to be very convenient and effective in experimenting with the agar media.

1. The antagonist and the test organism were placed on media in the form of a streak or spot opposite each other near the periphery of the Petri-dishes.
   a. Test organism and antagonist were applied on the same day.
   b. Test organism was applied 2 days after the antagonist.
   c. Test organism was applied 3-4 days after the antagonist.
d. Test organism was applied 5-6 days after the antagonist.

e. Antagonist was applied 2 days after the test organism.

The manipulation of the timings eliminates the anomalies due to great differences in the growth rates and also rates of diffusion of the substances produced. The slow growing species were planted earlier and various times were tried with respect to the nature of the interacting species.

2. The antagonist was placed on media in the form of a streak or spot. The test organism was placed 4.0 cm. from the antagonist.

   a. Test organism and the antagonist were applied on the same day.
   b. Antagonist was applied 3 days after the test organism.
   c. Test organism was applied 5-6 days after the antagonist.

3. The antagonist was streaked on agar near the periphery of the Petri-dish. The test organism was streaked at right angles to the original streak of the antagonist.

   a. Test organism was applied 2 days after the antagonist.
   b. Test organism was applied 3-4 days after the antagonist.
   c. Test organism was applied 5-6 days after the antagonist.

This method which is often called the "cross-streak assay test" was found to be extremely efficient and handy and was used for confirmation whenever found necessary.

4. Cellophane discs were cut to measure and fit in the Petri-dishes (9 cm. in diameter), boiled, sterilized and were laid on the surface of the agar. The antagonist was streaked over it near the periphery of the dish. After 4 days the
cellophane was removed and the test organisms were cross-streaked at right angles.

This method was employed to eliminate difficulty when the antagonist is fast growing but its active substance is slow to diffuse so that, if the experiment is done ordinarily the antagonist grows very fast near the test organism without showing effect.

In all experiments control plates of test organisms were run side by side without the inoculation of the antagonist.

All these tests were performed on solid agar media in Petri-dishes (100 x 10 mm.). The media used were Emerson's medium, glucose peptone agar A, glucose peptone agar B (Waksman, 1930) and meat infusion yeast extract agar (Dawson, 1937). 20 ml. of the medium was used in each plate (because the plates were under observation for long time), uniformly plated and incubated for 24 hours before use.

Both antagonists and the test organisms were inoculated as cell or spore suspensions. Only some times hyphal tips were used for this purpose. The cultures for inoculation were taken from well growing culture tubes. Actinomycetes and a few fungi were grown on oatmeal yeast extract agar; other fungi on potato dextrose agar; and bacteria on nutrient agar. Actinomycetes and fungi were incubated at 26°C. for 6-14 days and bacteria at 37°C. for 24 hours. Spore or cell suspensions were prepared in sterile distilled water and applied to the dishes with the help of a 3 mm. loop.
All the plates were incubated at 28°C. in inverted position. After 1-5 days of incubation the plates were observed for the antagonistic activity of the organisms under test and the results were recorded.

**CRITERIA FOR ANTAGONISM**

The following criteria were taken to indicate antagonism.

1. When zone of inhibition developed between the antagonist and the test organism.

2. When the hyphae of the fungal test organism or bacterial cells were killed or lysed after meeting of both antagonist and test organism.

3. When there was actual parasitism of fungal hyphae by the antagonist as in the case of some strains of *Trichoderma viride*.

4. When the colony of test organism was flattened on the margins nearest to the antagonist.

5. When there was distinct general stunting of the test organism as compared with that on control plates in which the test organism was grown alone.

**RECORD OF DATA**

The total number of organisms isolated after the preliminary screening was 262, out of which 106 were actinomycetes, 52 were bacteria and 104 were fungal isolates (Table -I page 30).
These organisms were then used for detailed tests against a number of test organisms and the results were recorded as follows:

**Actinomycetes**: The 106 isolates were tested against 12 selected test organisms which included organisms of various kinds. The results are given in the table - III (Plate I D) with the amount of activity shown against each case by the number of '+' signs. Those isolates which did not show any activity against any of the test organisms or those which appeared to be merely duplicates by general characteristics and antibiotic activity were also eliminated from this list. In this way, 18 isolates of actinomycetes which showed antibiotic activities and were found to be distinct species or strains were selected for further studies.

Since this group is of particular interest for this type of study and secondly because the identification of these species is extremely difficult and needs biochemical and physiological tests a more detailed data was recorded on these isolates giving information on morphology, pigments etc. so that the duplicates could be eliminated at this stage. This information is recorded in Table - II (pages 31 & 32).

**Bacterial cultures**: The initial isolates of bacteria were 52 which were tested against 5 test organisms. Not much of antagonistic activity could be noted in these species and most of them were rejected. Only 3 bacterial isolates were selected for further work. The details of their behaviour are given in Table - IV S.No. 1-3 (Page 34).
**Fungi**: In all 104 isolates of fungi were selected after preliminary screening. These were tested in dishes against 5 test organisms (as in case of bacteria). 17 fungal isolates were found to show antagonistic activity and were selected out for further study. The details of their behaviour are given in Table - IV S.No. 4-20 (Plate I E).

**CONCLUSIONS AND GENERAL REMARKS**

In this chapter the details of collection of soil samples and their preliminary screening for antagonistic organisms have been detailed and discussed. In the preliminary screening, spray technique method was found to be most convenient and efficient, though other methods like soil plates of various types were also used. From the preliminary screening 262 isolates comprising 106 of actinomycetes, 58 of bacteria and 104 of fungi were selected for further assaying.

These organisms were then tested under pure culture conditions against 12 test organisms which comprised of species of actinomycetes, bacteria, both gram positive and gram negative, and fungi. Among these methods of assaying cross-streak method was found to be very efficacious though other methods were also tried. This led to further selection and screening. It was further revealed that the antagonistic activity of these organisms was varied and selective in their nature. Certain organisms were effective against both bacteria and fungi; some were active against both gram positive and gram negative bacteria and others were found effective only against one variety of
## Table I.

Table showing the number of microorganisms isolated and tested.

<table>
<thead>
<tr>
<th>Group of organisms</th>
<th>No. of cultures tested</th>
<th>No. of cultures which showed activity after detailed assaying</th>
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</thead>
<tbody>
<tr>
<td>Actinomycetes</td>
<td>106</td>
<td>18</td>
</tr>
<tr>
<td>Bacteria</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>Fungi</td>
<td>104</td>
<td>17</td>
</tr>
<tr>
<td>S.No.</td>
<td>Isolate No.</td>
<td>Name of the organism</td>
</tr>
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</tr>
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<td>2.</td>
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<td>-</td>
</tr>
<tr>
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<td>9.</td>
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<td>-</td>
</tr>
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<tr>
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</tr>
<tr>
<td>12.</td>
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<td>-</td>
</tr>
<tr>
<td>25.</td>
<td>A-25</td>
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<td>A-26</td>
<td><em>Streptomyces bikiniensis</em></td>
<td>Aerial mycelium white; amber brown pigment. +</td>
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<td>A-27</td>
<td>-</td>
<td>Colony folded; brown pigment. -</td>
</tr>
<tr>
<td>A-28</td>
<td>-</td>
<td>Greenish yellow colonies. -</td>
</tr>
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<td>A-29</td>
<td>-</td>
<td>Growth white; light brown soluble pigment. (+)</td>
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<tr>
<td>A-30</td>
<td><em>Streptomyces sp.</em></td>
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</tr>
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<td><em>Streptomyces bikiniensis</em></td>
<td>Brownish growth. +</td>
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<tr>
<td>A-32</td>
<td>-</td>
<td>Soluble pigment brown. -</td>
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<td>Soluble pigment light brown. -</td>
</tr>
<tr>
<td>A-34</td>
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<tr>
<td>A-35</td>
<td>-</td>
<td>Brown growth. (+)</td>
</tr>
<tr>
<td>A-36</td>
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<td>Brown growth. -</td>
</tr>
<tr>
<td>A-37</td>
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<td>White growth; no pigment. -</td>
</tr>
<tr>
<td>A-38</td>
<td>-</td>
<td>Colonies white; reverse pink. -</td>
</tr>
<tr>
<td>A-39</td>
<td>-</td>
<td>Colonies white to grey. -</td>
</tr>
<tr>
<td>A-40</td>
<td>-</td>
<td>Colonies dull white. (+)</td>
</tr>
<tr>
<td>A-41</td>
<td>-</td>
<td>Colonies light brown. -</td>
</tr>
<tr>
<td>A-42</td>
<td>-</td>
<td>Colonies brown. -</td>
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<tr>
<td>A-43</td>
<td>-</td>
<td>Colonies dull white, lobed. (+)</td>
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<td><em>Streptomyces flavovius</em></td>
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<td>-</td>
<td>Grey colonies, white margins. -</td>
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<td>A-46</td>
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<td>Colonies wrinkled, dull brown. -</td>
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<tr>
<td>A-47</td>
<td><em>Streptomyces sp.</em></td>
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<td>-</td>
<td>Colonies grey. -</td>
</tr>
<tr>
<td>A-49</td>
<td>-</td>
<td>Colonies light yellow. -</td>
</tr>
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<td>A-50</td>
<td>-</td>
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<td>A-51</td>
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<td>Brown soluble pigment. (+)</td>
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</tr>
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<td>-</td>
<td>Colonies dull white, wrinkled. -</td>
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F.T.O.
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<td>Lawrence (1956) soil extract agar</td>
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<td>B.G.&amp;M.F.</td>
<td>White colonies.</td>
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<td>Couch’s azygoid isolation method</td>
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</tr>
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<td>66</td>
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<td>-do-</td>
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<td>-do-</td>
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<td>-do-</td>
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<td>-do-</td>
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<td>-do-</td>
<td>B.G.</td>
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<td>B.G.</td>
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<tr>
<td>79</td>
<td>A-79</td>
<td>-</td>
<td>-do-</td>
<td>B.G.</td>
<td>Yellow green growth.</td>
<td>-</td>
</tr>
<tr>
<td>80</td>
<td>A-80</td>
<td>-</td>
<td>-do-</td>
<td>B.G.</td>
<td>Yellow green growth; brown pigment.</td>
<td>-</td>
</tr>
<tr>
<td>81</td>
<td>A-81</td>
<td>Streptomyces hikiniensis</td>
<td>Lawrence (1956) spray technique</td>
<td>S.F.</td>
<td>Amber brown pigment.</td>
<td>+</td>
</tr>
<tr>
<td>82</td>
<td>A-82</td>
<td>Lawrence (1956) soil extract agar</td>
<td>S.F.</td>
<td>Blue growth.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>A-5</td>
<td><strong>Streptomyces lividans</strong></td>
<td>Lawrence (1956) spray technique</td>
<td>S.F.</td>
<td>Amber brown pigment.</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>--------------------------</td>
<td>---------------------------------</td>
<td>-----</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>A-82</td>
<td>-</td>
<td>Lawrence (1956) soil extract agar</td>
<td>S.F.</td>
<td>Blue growth.</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>A-83</td>
<td>-</td>
<td>-</td>
<td>S.F.</td>
<td>Brown growth.</td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>A-84</td>
<td>-</td>
<td>-</td>
<td>S.F.</td>
<td>Grey mycelium; red pigment.</td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>A-85</td>
<td>-</td>
<td>-</td>
<td>S.F.</td>
<td>Brown growth; wrinkled.</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>A-86</td>
<td>-</td>
<td>-</td>
<td>S.F.</td>
<td>No aerial mycelium; brown growth.</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>CA-1</td>
<td><strong>Streptomyces flavovirens</strong></td>
<td>-</td>
<td>A.P.</td>
<td>Grey aerial mycelium.</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>CA-2</td>
<td><strong>Streptomyces globisporus</strong></td>
<td>-</td>
<td>A.P.</td>
<td>Colourless to yellowish growth.</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>CA-3</td>
<td><strong>Streptomyces oyaneus</strong></td>
<td>-</td>
<td>A.P.</td>
<td>Greenish yellow growth; Blue soluble pigment.</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>CA-4</td>
<td>-</td>
<td>A.P.</td>
<td>Light yellow growth.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>CA-5</td>
<td><strong>Streptomyces sp.</strong></td>
<td>-</td>
<td>A.P.</td>
<td>Pink growth.</td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>CA-6</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Light pink growth.</td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>CA-7</td>
<td><strong>Streptomyces sp.</strong></td>
<td>Dilution plate, soil extract agar</td>
<td>A.P.</td>
<td>Blue green growth.</td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>CA-8</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Aerial mycelium grey.</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>CA-9</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Grey green growth.</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>CA-10</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Aerial mycelium grey.</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>CA-11</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Aerial mycelium grey with yellow tinge.</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>CA-12</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>White mycelium; brown pigment.</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>CA-13</td>
<td>-</td>
<td>Lawrence (1956) soil extract agar</td>
<td>A.P.</td>
<td>White mycelium; no pigment.</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>CA-14</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Blue green growth.</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>CA-16</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>White aerial mycelium.</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>CA-17</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Colourless growth.</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>CA-18</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Yellowish growth.</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>CA-19</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Light pink growth.</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>CA-20</td>
<td>-</td>
<td>-</td>
<td>A.P.</td>
<td>Pink growth.</td>
<td></td>
</tr>
</tbody>
</table>

* = R.F. = Babatgarh Forest  
B.G. = Botanical Garden (Old site)  
M.F. = Makronia Fields  
S.F. = Sugarcane Fields (Agricultural farm)  
A.P. = Andhra Pradesh (Collapropu)

** = Duplicate culture.  
♀ = See Chapter XVIII, page 203.
Table - III.

Table showing the antimicrobial activity of actinomycete cultures.
(Only the activity of the selected isolates is given)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antagonist</th>
<th>A. baumannii</th>
<th>E.coli</th>
<th>E. faecalis</th>
<th>E. coli &amp; faecalis</th>
<th>L. monocytogenes</th>
<th>L. infantis</th>
<th>S. aureus</th>
<th>S. typhymurium</th>
<th>E. faecalis</th>
<th>E. coli &amp; faecalis</th>
<th>S. aureus</th>
<th>L. monocytogenes</th>
<th>L. infantis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>S. viridatus</em> A-1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td><em>Streptomyces</em> sp. A-10</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Isolate No. A-19</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td><em>S. erythraeus</em> A-15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td><em>S. bikiniensis</em> A-26</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td><em>Streptomyces</em> sp. A-30</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td><em>S. bikiniensis</em> A-31</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td><em>S. flavovirga</em> A-44</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td><em>Streptomyces</em> sp. A-47</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Isolate No. A-54</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td><em>S. globisporus</em> A-69</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>12.</td>
<td><em>Streptomyces</em> sp. A-70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td><em>S. anulatus</em> A-71</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>14.</td>
<td><em>S. bikiniensis</em> A-81</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td><em>S. flavovirga</em> CA-1</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>16.</td>
<td><em>S. globisporus</em> CA-2</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>17.</td>
<td><em>S. venenata</em> GA-3</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td><em>Streptomyces</em> sp. GA-5</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table - IV.

Antimicrobial activity of selected bacteria and fungi.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antagonist</th>
<th>V. unavus</th>
<th>V. alginosa</th>
<th>S. clavata</th>
<th>S. esterhusi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bacillus cereus</em> var. <em>wuxiaiensis</em> B-44</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2.</td>
<td>Isolate No. B-35</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><em>Bacillus</em> sp. B-40</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td><em>Trichoderma viride</em> T-10</td>
<td>++</td>
<td>=</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td><em>L. viride</em> T-13</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td><em>Mucor indicus</em></td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td><em>Chaetomium</em> sp. F-78</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td><em>Chaetomium trilaterale</em> F-75</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>9.</td>
<td><em>Aspergillus terreus</em> var. <em>Aureus</em> F-79</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td><em>A. flavus</em></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td><em>A. candidus</em> F-81</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>12.</td>
<td><em>A. niger</em></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td><em>A. sydowi</em></td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td><em>A. chevalieri</em></td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>15.</td>
<td><em>A. flavus</em> F-63</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td><em>A. terreus</em></td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td><em>Penicillium nigricans</em></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td><em>P. herbarum</em></td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td><em>Penicillium</em> sp.</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20.</td>
<td><em>Penicillium flavescens</em> F-82</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
organisms. Further, it was observed that organisms which were active against gram negative bacteria were also found to be active against gram positive ones but the reverse was not true. Maximum activity was recorded in the case of actinomycetes and then in fungi, and bacteria were least effective in this respect.

The antagonistic activity when observed on different media generally corresponded according to organisms, though it could be said that Emerson's medium gave the best results and was closely followed by glucose peptone agar A, glucose peptone agar B and meat infusion yeast extract agar in this respect. The results of these experiments are given in Tables I to IV pages 30-34.

On the basis of these data 13 organisms were finally selected for the next stage of this study i.e. the determination of the antimicrobial spectra against 25 test organisms. The selection was based upon the intensity and variety of their antagonistic activity as detailed in the aforementioned pages.