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# PRIMARY STUDIES ON ANTIMICROBIAL ASSAY OF FIRE ANT HILL SOIL ACTINOMYCETES BY SCREENING METHODS

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## ABSTRACT

Multi drug resistance among pathogenic bacteria is the key problem and also challengeable for every microbiologist in order to find out novel antibiotics to overcome this situation. Therefore, our present research work is aimed to catch better antimicrobial compounds from unique soil actinomycetes isolated from dissimilar environment i.e., Fire Ant hill soil. During the study period, a total of 43 isolates of actinomycetes were isolated from the fire ant hill soil samples of Dr. APJ Abdul Kalam Eco garden of our KMGIPSR campus, Puducherry. Among the 43 isolates, 25 isolates produced pigments on the PDA media. All the isolates were subjected to anti-bacterial and anti-candida activity. Among the actinomycetes, isolates like A6, A8, A31, A33 and A35 showed good antimicrobial activities against the MTCC cultures viz., *Vibrio cholera* (MTCC-3906), *S. epidermidis* (MTCC-435), *Bacillus subtilis* (MTCC- 1755), *Pseudomonas aeruginosa (*MTCC-424), *Candida albicans* (MTCC- 439). 12% of all the actinomycetes isolates showed good antimicrobial activity against all the bacteria and *Candida albicans* while 46% showed the least activity with pigmentation but 42% were not able to show any pigmentation during their growth on different media on laboratory conditions.

**KEYWORDS:** Antimicrobial assay, Fire ant hill soil actinomycetes, screening methods, MTCC cultures, Pathogenic bacteria, *Candida albicans.*

## INTRODUCTION

Actinomycetes are considered as one of the toughest antagonists among microorganism and ruled over time immemorial. The antibiotic materials explained by their antifungal, antiviral, antibacterial, anticancer and antiprotozoal properties. Out of the total identified antibiotics produced by microbes, 70% are of actinomycetes origin; of them, the genus *Streptomyces* accounts for two thirds.[1] Actinomycetes are known to be the potent sources of antibiotics, too with enzymes and vitamins and such antagonistic actinomycetes of marine derivation are being frequently informed by varied authors for a time record.[2,3,4,5] Some of the reports disclose that soil is the major source of actinomycetes.[6,7,8,9] The search of new and novel antibiotics and other bioactive microbial metabolites is more significant to fight against new developing pathogens which are not able to be prohibited by the prevailing drugs in the market.[10,11,12,13] It is very significant to segregate and count of microbes from different environmental bases of highest potency to act as antibiotics.[14,15,16] Moreover, isolation of actinomycetes from unique unexplored natural habitats is of interest to avoid re-isolation of strains that harvest known bioactive metabolites.[17,18] Wild and natural habitats are evidencing to be a good source of novel actinomycetes as well as bioactive compounds. The present study aims at finding better antibacterial composite for controlling the bacterial diseases with the help of bio-compounds extracted from the actinomycetes isolated from Fire Ant Hill soil collected from Dr. APJ Abdul Kalam Eco Garden of our K. M. Govt. Institute for Postgraduate Studies and Research campus, Puducherry, India.

## MATERIALS AND METHODS

Fire ant hill soil samples were collected from Dr. APJ Abdul Kalam Eco-garden of our K. M. Govt. Institute for Postgraduate Studies and Research campus, Puducherry, India and the soil samples were transported by the sterile polythene bags and brought into the Microbiology Laboratory of our Department of Botany for further study (Plate 1). The pH of the fresh soil sample was determined.[14] Later on, the soil samples were grounded and sieved to remove the debris.

**Isolation of Fire anthill actinomycetes**

One gram soil sample was mixed into 10ml of sterile distilled water ($10^{-1}$) and serially diluted up to $10^{-5}$. For the isolation of the actinomycetes, $10^{-5}$ dilution was taken and pour plate technique was done[15] using Starch Casein Agar[16] which was supplemented with Cycloheximide 75µg/ml and Nalidixic acid 75µg/ml to avoid the microbes of non-actinomycetes colonies. All the plates were incubated at room temperatures for 15-30 days and the plates were examined for appearance of actinomycetes colonies. Selected colonies were sub cultured and maintained in SCA and PDA for their routine checkup for viability and additional study viz., morphological, biochemical and molecular identification.

**Plate 1: Fire Anthill soil in Dr. Abdul Kalam Eco-garden, KMGIPSR, Puducherry.**

**Preparation of Test bacteria**

Test bacteria such as *Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Vibrio cholerae* and *Candida albicans* were procured from Microbial type culture collection (MTCC), IMTECH, Chandigarh, India and were subjected to antimicrobial assay with the actinomycetes extracts in the present study. Test bacteria were cultured in Nutrient broth at pH-7 and stored in refrigerator at 4°c. 24 hours culture was used for antibacterial activity for better results.[2]

**Screening Test For Antibacterial Assay**

**Primary screening by cross streak method**

All the actinomycetes were primarily screened by cross streak method.[17] A loop of actinomycetes colonies were streaked as a thin line on SCA medium and allowed to grow for 10 days for better antibiotic production. After incubation, 24hours bacterial cultures were streaked perpendicular to the line of grown actinomycetes and incubated for 24 hours at 30° c. After incubation, the zone of inhibition was measured and recorded.

**Secondary screening by Agar well diffusion method**

Based on the result of primary screening, five active isolates were selected and subjected to agar well diffusion method. All the selected colonies were cultured in Potato Dextrose Broth and incubated for 15 days at room temperature. After incubation, medium was filtered using Whatman No.1 filter paper. Nutrient agar plates of pH-7 were prepared and surface inoculated by test bacterial culture. Six mm wells were created in medium using sterile cork borer. Each well was loaded with 50µl of culture filtrate and the plates were incubated at room temperature for 24hours. After incubation, zone of inhibition was measured and the antimicrobial activity was determined.

## RESULTS AND DISCUSSION

During the study period, fire ant hill soil samples were inoculated on SCA, PDA, AIA media plates by serial dilution-pour plate technique. Altogether, 43 actinomycetes colonies were isolated, pure cultured and maintained on SCA and PDA for future use. Out of the total actinomycetes isolates, 25 isolates showed the development of pigmentation during their pure culture in the laboratory conditions. Twenty isolates could not show their potential in order to prevent the growth of any pathogenic bacteria and *Candida albicans* procured from MTCC culture collection center, IMTECH, Chandigarh, India. The great majority of antibiotics that have been isolated in numerous screening curricula worried with the search for new therapeutic agents have been tested primarily for their activity against diverse bacterial strains.[18] Five of the actinomycetes showed their efficacy towards the MTCC bacteria and *Candida albicans* in our study. They are named as Ahs-06, Ahs-08, Ahs-31, Ahs-33, Ahs-35 isolates of Ant hill soil actinomycetes. The pure culture of these Ant Hill Soil actinomycetes is shown in Fig 1.

**Table 1: Antimicrobial activity of actinomycetes isolated from fire anthill soil.**

| Sl. No. | Isolates of Anthill Actinomycetes | Zone of inhibition in mm (*Mean of 3 replicates) | | | | |
|---|---|---|---|---|---|---|
| | | ***S. epidermidis*** | ***V. cholerae*** | ***B. subtilis*** | ***P. aeruginosa*** | ***C. albicans*** |
| 1. | Ahs-06 | 18±0.4* | 17±0.5 | 14±0.3 | 18±0.5 | 25±0.6 |
| 2. | Ahs-08 | 16±1.0 | 15±0.2 | 15±0.1 | 20±0.6 | 18±0.3 |
| 3. | Ahs-31 | - | 12±0.3 | - | - | 15±0.4 |
| 4. | Ahs-33 | - | - | 10±0.1 | 15±0.4 | 14±0.7 |
| 5. | Ahs-35 | 10±1.0 | - | 11±0.2 | - | 17±0.3 |

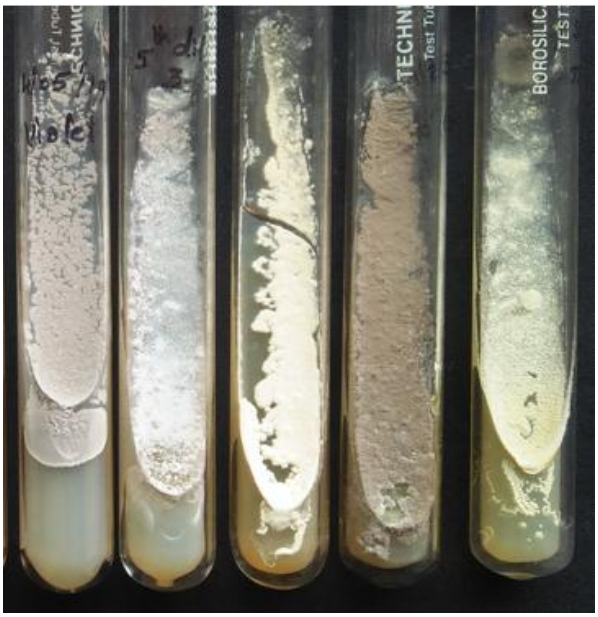
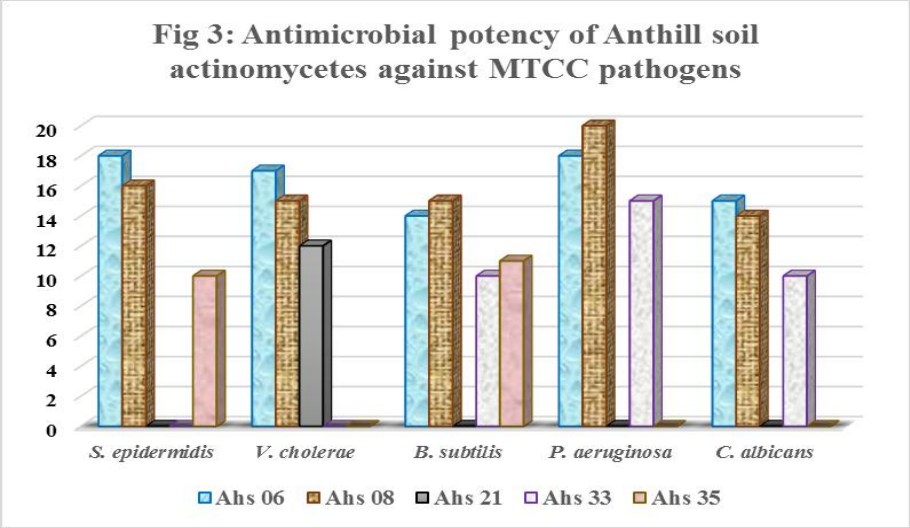


**Fig. 1: 15 Day pure culture of active actinomycetes of antibiotic potential isolated from Fire anthill garden soil.**




**Antibacterial assay of Fire Anthill soil actinomycetes**

During the present study of antibacterial activity of anthill soil actinomycetes, few actinomycetes were confirmed for their antagonistic behavior against the MTCC cultures of pathogenic microbes viz., *Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Vibrio cholerae* and *Candida albicans* based on cross streak method. Five of the anthill soil actinomycetes isolates were found active *against Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Vibrio cholerae* and *Candida albicans* (Fig 2 & 3). Table 1 shows the antimicrobial activity of actinomycetes isolated from fire anthill soil collected from garden environments. Out of the twenty five isolates, five isolates were selected for secondary screening done by Agar well diffusion method due to their strong inhibitory activity in primary screening (Table 1). In order to obtain better antibiotic production, liquid medium such as PDB was used. Active isolates, Ahs-06 and Ahs-08 showed strong inhibition from both primary and secondary screening. Antibacterial activity of actinomycetes by secondary screening (Agar well diffusion assay) is given in Table 1, which depicted the antibacterial potency of the two best actinomycetes strains showing good activities against five pathogens. *Candida albicans* was the most inhibited (25±0.6) pathogenic fungus by the actinomycetes extracts of Ahs-06 followed by the pathogenic bacteria i.e., *Staphylococcus epidermidis* (18±0.4 and *Pseudomonas aeruginosa* (18±0.5) (Table 1). In the case of Ahs-08, it inhibited the growth of *Pseudomonas aeruginosa* (20±0.6) at the maximum followed by *Candida albicans* (18±0.3) and *Staphylococcus epidermidis* (16±1.0). Extracts of other three actinomycetes showed a marginal inhibitory zones against the studied bacteria and *Candida albicans* in our present work (Fig 2 and 3). Janaki et al[6] explained about their work on Antibacterial activity of soil actinomycetes from the rhizosphere zone of the mangrove plant, *Avicennia marina*. During their work, they found that a total of 20 (80%) of actinomycetes

showed antibacterial activity towards any one of the tested bacteria, 5 (20%) actinomycetes showed no antagonistic activity. Nayak et al[4] worked on the antimicrobial potency of the mangrove plant leaf extracts of *Avicennia marina* against selected bacteria and fungi. Among the extracts, Ethyl alcohol extract (50µl) showed wide inhibition against all the fungi used for the susceptibility test and showed inhibition against *P. aeruginosa* (18) and *Bacillus subtilis* (6). *Staphylococcus aureus* found to be resistant against all the 4 solvent extracts used in the study. Thiruvarasan et al[19] worked on the antibacterial study of actinomycetes on MTCC pathogens and opined that ethyl alcohol extract showed wide inhibition against all the fungi used for the susceptibility test and showed inhibition against *P. aeruginosa* (18mm) and *Bacillus subtilis* (9mm). *Staphylococcus aureus* found to be resistant against all the 4 solvent extracts used in their study. The present work is agreed with other workers on the antibacterial properties of actinomycetes[2,3,13,14] but our work pertaining to the isolation of actinomycetes from fire anthill soil is itself is unique and new in its work.

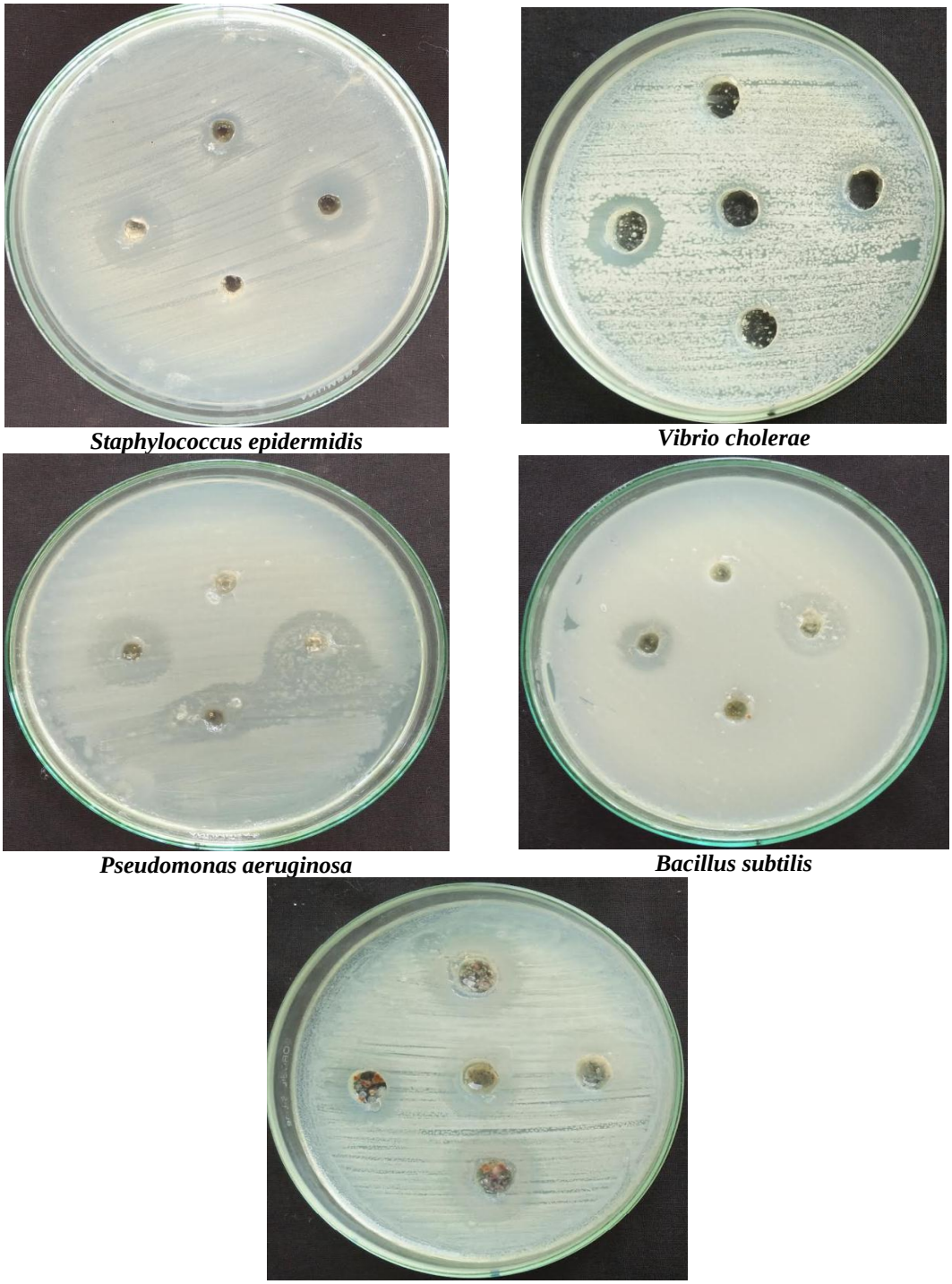



**Fig. 2: Antimicrobial activity of anthill soil actinomycetes by well diffusion method.**

## CONCLUSION

Resistance to multi drugs is a global challenge in the treatment of infectious diseases and to counteract to this situation is very hard and problematic too. The present study was a purpose to overcome this situation by generating new bio-compounds from the actinomycetes isolated from an untouched environmental habitat viz., Fire Ant Hill soil which was yet to be known as a rich source of actinomycetes and in the production of decent metabolites of inhibitory compounds against bacterial pathogens. In our present study, 12% of all the actinomycetes isolates showed good antimicrobial activity against all the bacteria and *Candida albicans* while 46% showed least activity but 42% were not able to show any pigmentation during their growth on different media on laboratory conditions. Since the Fire Ant Hill soil actinomycetes are probable source for producing antibiotics for pathogenic bacteria and *Candida albicans*, these would be used in the pharmaceutical field to find novel drugs for bacterial and fungal infections.

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