

## STUDIES ON ANTIBACTERIAL ACTIVITY OF TEMPLE TANK ACTINOMYCETES AGAINST MTCC BACTERIAL STRAINS BY SCREENING METHODS

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Received on: 23/10/2020

Revised on: 13/11/2020

Accepted on: 03/12/2020

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### ABSTRACT

During this study period, actinomycetes were isolated from karaikal region temple tank Actinomycetes by serial dilution pour plate method. All the actinomycetes were curtaired for their antagonistic properties against pathogenic bacterial strains procured from **IMTECH**, Chandigarh, India. Initially the actinomycetes were primary screened by cross streak method and only two potential actinomycetes were tested subjected to secondary screening which was done by agar well diffusion method. Most of the isolates were found active against *Bacillus subtilis*, *staphylococcus aureus*, *shawanella putrefaciens*, *vibrio cholera*, and *E.coli*. Most of the actinomycetes (around 80%) showed good antagonistic effect against most of the tested pathogens and 20% of the total actinomycetes were found non-effective against the test pathogens.

**KEYWORDS:** Antibacterial activity, Temple tank actinomycetes , Well diffusion method, cross streak method, MTCC. Strains.

### INTRODUCTION

Actinomycetes are gram-positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants .from the 22,500 biologically active compounds that have obtained from microbes,45% are produced by actinomycetes, 38% by fungi, and 17% by unicellular bacteria.<sup>[1]</sup> The species belong to the genus *streptomyces* constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immunomodulators, anticancer drugs, antiviral drugs ,herbicides, and insecticides.<sup>[2-4]</sup> Actinomycetes are known to be the potent sources of antibiotics, too with vitamins and enzymes And such of antagonistic actinomycetes of marine origin are being regularly reported by varied authors for a time record.<sup>[5,6,7,8,9]</sup> Some of the reports reveal that soil is the major source of actinomycetes.<sup>[10,11,12,13]</sup> The search of new and novel antibiotics and other bioactive microbial metabolites is more important for the fight against new emerging pathogens which are not able to be prevented by the existing drugs in the market.<sup>[14,15,16,17]</sup> It is very important to isolate and enumerate of microbes from different environmental sources of highest potency to act as antibiotics. Further, isolation of actinomycetes from unique unexplored natural habitats is of interest to avoid re-isolation of strains that produce known bioactive metabolites. The present investigation aims at finding better antibacterial compound for controlling the bacterial diseases with the help of bio-compounds

extracted from the actinomycetes isolated from temple tank soil collected from Sri Aayiram Kalamman temple, Karaikal, Puducherry, India.

### MATERIALS AND METHODS

#### Collection of Temple Tank soil

Temple tank soil was collected from Sri Aayiram Kalamman Temple Karaikal, Puducherry, India (Fig. 1) and the soil samples were transferred to sterile polythene bag and brought into laboratory. The pH of the fresh soil sample was determined.<sup>[18]</sup> Further , the soil samples were grounded and sieved to remove the debris.



**Fig. 1: Sri Aayiram Kaliyamman Temple tank, Karaikal.**

### Isolation of Temple tank actinomycetes

One gm of soil sample was poured into 9 ml sterile water, ( $10^{-1}$  distilled water and serially diluted up to  $10^{-5}$ ). For the isolation of the actinomycetes,  $10^{-5}$  dilution was taken and pour plate technique was done,<sup>[19]</sup> Using Starch Casein Agar<sup>[20]</sup> which was supplemented with cycloheximides 75  $\mu\text{g/ml}$  to avoid non-actinomycetes colonies. All the plates were incubated at Room temperature 15-30 days and the plates were examined for appearance of actinomycetes colonies. Selected colonies were sub cultured and maintained in SCA and PDA medium.

### Preparation of Test bacteria

Test bacteria were procured from Microbial type culture collection (MTCC), Chandigarh. Selected pathogens such as *Bacillus subtilis*, *staphylococcus aureus*, *shawanella putrefaciens*, *vibrio cholera*, and *E.coli*. 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.

### Screening test for antibacterial assay

#### Primary screening by cross streak method

All the actinomycetes were primarily screened by cross streak method.<sup>[21]</sup> A loop full 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, 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, all active isolates were taken 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 pH-7 were prepared and surface inoculated by test bacterial culture. Six mm wells were created in medium using sterile corkborer. Each well was loaded with 100  $\mu\text{l}$  of culture filtrate and the plates were incubated at room

temperature for 24 hours. After incubation, zone of inhibition was measured and the antibacterial activity was determined.

## RESULTS AND DISCUSSIONS

### Isolation and maintenance of Temple tank soil actinomycetes

Temple tank soil was collected and the soil samples were inoculated on SCA, PDA, AIA media plates by serial dilution-pour plate technique. 15 actinomycetes colonies were isolated and pure cultured and maintained on SCA and PDA for future use. The great majority of antibiotics that have been isolated in numerous screening programs concerned with the search for new therapeutic agents tested primarily for their activity against different bacteria.<sup>[22]</sup> All the actinomycetes were subjected for screening test using MTCC bacterial cultures.

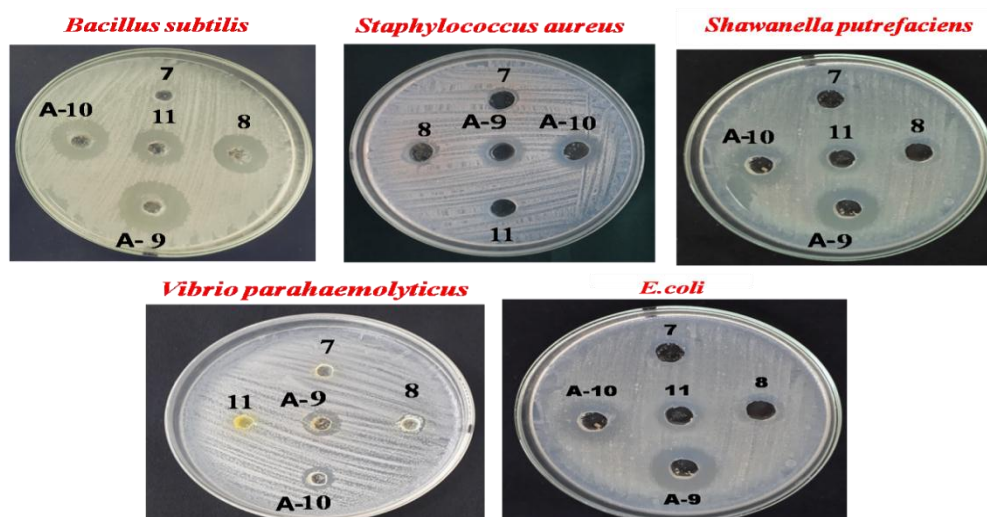
### Antibacterial assay of temple tank soil actinomycetes

For the analysis of antibacterial activity of 15 actinomycetes, secondary screening was done by Agar well diffusion assay and its results are plotted in Table 1. Most of the isolates were against *Bacillus subtilis*, *staphylococcus aureus*, *shawanella putrefaciens*, *vibrio cholera*, and *E.coli*. Out of 15 isolates, two isolates were showed good zone of inhibition for their strong inhibitory activity in secondary screening. In order to obtain better antibiotic production, liquid medium such as PDB was used. Active isolates A-9 and A-10 showed strong inhibition from both primary and secondary screening. Antibacterial activity of actinomycetes by secondary screening (Agar well diffusion assay) is given which depicted the antibacterial potency of the two best actinomycetes strains showing good activities against five pathogens. Antibacterial activity of both A-9 and A-10 isolates were done by Agar well diffusion method and is given in Fig. 2, which shows the morphological view of the antagonistic behaviour of actinomycetes against bacterial pathogens. The present work is agreed with other workers on the antibacterial properties of actinomycetes<sup>[6,7,17,18]</sup> but our work pertaining to the

isolation of actinomycetes from temple tank soil is itself in Karaikal region unique and new in its work.

**Table 1: Secondary screening of antibacterial activity of actinomycetes isolated from temple tank soil by Agar well diffusion method.**

Sl. No.	Identification code of the isolate	Zone of inhibition in mm				
		<i>S.aureus</i>	<i>Vibrio cholera</i>	<i>B. Subtilis</i>	<i>Shawanella putrefaciens</i>	<i>E.coli</i>
1	A-1	12	12	12	17	05
2	A-2	11	11	11	14	13
3	A-3	10	11	10	11	04
4	A-4	04	18	19	13	04
5	A-5	12	12	11	16	13
6	A-6	13	14	14	12	02
7	A-7	-	12	11	12	04
8	A-8	10	10	10	11	08
9	A-9	19	17	20	18	16
10	A-10	18	18	18	16	18
11	A-11	04	13	13	12	10
12	A-12	05	14	10	10	02
13	A-13	12	12	04	06	03
14	A-14	10	14	08	11	08
15	A-15	08	10	12	10	06



**Fig. 2: Antibacterial activity of A-9 and A-10 actinomycetes by secondary screening (Agar well diffusion assay).**

## CONCLUSION

Bacterial resistance to multidrug is a global problem in the treatment of infectious diseases and to counteract to this situation is very tough and critical. The present study was an aim to overcome this situation by producing new bio-compounds from the actinomycetes isolated from an untouched environmental sources viz., Temple tank soil which was found as a rich source of actinomycetes and produced good metabolites inhibitory compounds against bacterial pathogens. Mostly 80% of all the actinomycetes isolates showed good antibacterial activity against all the bacteria while 20% showed least activity. Since the temple tank soil actinomycetes are potential source for producing antibiotics for bacteria, these can be used in the pharmaceutical field to find novel drugs for bacterial infections.

## ACKNOWLEDGEMENT

The research workers and authors of this manuscript sincerely acknowledge to **KMGIPSR**, Puducherry for providing the facilities to complete the work in time.

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