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ANTIMICROBIAL ACTIVITY OF SOIL ACTINOMYCETES FROM THE TEMPLE TANK OF KARAIKAL REGION

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ABSTRACT

The search for potential bioactive compounds from the natural environment had rapidly been gaining momentum with the increase in the multi-drug resistant (MDR) pathogens. In the present study, the antimicrobial potential of actinomycetes has been evaluated by initial screening of one soil samples. Secondary screening was performed against *Bacillus subtilis*, *Staphylococcus aureus, Vibrio chlorae, Shawanella putrefaciens Escherichia coli, and Candida albicans* and bacterial and fungal test strains, twenty active isolates were selected for further study. Microbial strains were identified on the basis of growth conditions and other biochemical characters. In conclusion, we study the isolation and bacterial strains of actinomycetes for producing potential compounds having antibacterial and antifungal activities from the Temple tank - actinomycetes from the Karaikal region.

KEYWORDS: Antimicrobial activity, Temple tank actinomycetes, Well diffusion 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.[1] They show marked chemical and morphological diversity and form a distinct evolutionary line of organisms.[2] The species belong to the genus constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immuno modulators, anticancer drugs, antiviral drugs, herbicides, and insecticides.[3-5] Additionally nitrogen fixing actinomycetes of the genus frankia have one of the broadest host ranges known, forming root nodule symbiosis in more than 200 species of flowering plants.[6] Streptomyces species are gram positive ,aerobic microorganisms with high DNA G+C contents and produce about half of all known antibiotics from microorganisms. In fact, Streptomyces species are the resource of 75% of commercially produced and medically useful antibiotics.[7] Although thousands of antibiotics have been isolated from Streptomyces, these represent only a small fraction of the repertoire of bioactive compounds produced.[5,7,8] Therefore, isolation of new Streptomyces from natural resources and characterization of their secondary metabolites is a valuable endeavour.

Karaikal is a Town of the Indian union Territory of Puducherry. Small coastal enclave which was formerly part of French India. It has a Tropical wet and dry climate characterised by heavy seasonal rainfall, high temperatures, and humidity. The Alluvial soil of Karaikal is highly fertile. Studies using Karaikal temple tank soils to screen for new actinomycetes for new bioactive compounds.

This report describes the isolation of actinomycetes strains producing antimicrobial secondary metabolites from soil samples collected from Ganapathy temple tank around Karaikal region, Puducherry state.

MATERIALS AND METHODS

Collection of Temple Tank soil

Soil samples were collected from the Ganapathy Temple Tank place of Karaikal district, Puducherry. Samples were collected from various depth of the earth surface, ranging from temple tank just beneath the upper surface to 1 meter depth. They were collected in the sterile small plastic tubes and properly labelled with the date of collection. Soil samples were collected January 2018. The collected soil samples were dried in a hot air oven at 60-65 ºC for 3 hours and stored in 4 ºC until examined.

Fig. 1: Ganapathy Temple Tank – Karaikal.

Table 1: Collection site and depth of Temple Tank soil from where the isolates were collected using starch casein–agar media.

Date Of Collection	Collection Site	Number of actinomycetes in each gram of soil (c.f.u/gm of dried weight soil)	Isolates
28/01/2018	Ganapathy Temple tank	1.46×10^{-4}	GT 1-20

Table 2: Color grouping of the isolates.

Color series	Isolate	Color of aerial mycelia	Color of substrate mycelia	Diffusible pigment
Gray series	GT-1	Light gray	Light gray	ND*
	GT-3	Light brownish gray	Dark yellowish brown	ND
	GT-6	Light brownish gray	Grayish red	ND
	GT-8	Pinkish gray	Dark reddish brown	ND
	GT-2	Light brownish gray	Grayish yellow	ND
	GT-7	Light grey	Grayish yellow	ND
	GT-5	Light bluish gray	Dusky red	ND
	GT-9	Light gray	Light gray	ND
	GT-10	Ash greyish	Light gray	ND
	GT-18	Very Light Gray	Grayish yellow	ND
White Series	**GT-4**	**White**	**Grayish yellow**	**yellow**
	GT-14	White	White	ND
	GT-16	White	Grayish	ND
	GT-12	White	Moderate yellow	ND
	GT-11	**White**	**Blackish white**	**Light black**
Red Series	GT-17	Pale Red	Moderate yellow	ND
	GT-19	Grayish red	Moderate yellow	ND
	GT-15	Grayish red	Moderate yellow	ND
Orange Series	GT-13	Pale yellowish orange	Grayish orange	ND
Brown Series	GT-20	Pale Brown	Yellowish Gray	ND

ND: Not detectable.

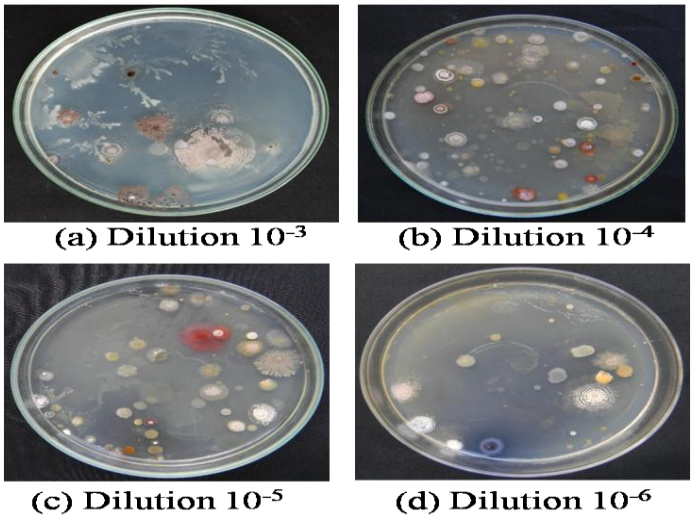

Fig. 2: Colonies of actinomycetes appeared on the dilution plates using the soil samples collected from Ganapathy Temple Tank.

Isolation of Pure Culture of Actinomycetes

Twenty actinomycetes strains were isolated and obtained as pure culture by using standard microbiological method. From one soil sample, 1 gm of dried soil was suspended in 9 ml sterile water, and successive serial dilutions were made by transferring 1 mL of aliquots to 2nd test tube containing 9 mL of sterile water, and in this way dilutions up to 10^{-4} to 10^{-6} were prepared. Each time the contents were vortexes to form uniform suspension. An aliquot of 0.1 mL of each dilution was taken and spread evenly over the pteridishes and pour the starch-casein agar medium supplemented with cycloheximide (100µg/mL) on 16 cm petridishes. Plates were incubated at 36ºC and monitored for 7 days. The colonies were carefully counted by visual observation and c.f.u per gram of soil was determined. Plates those have 10-20 colonies were chosen for further isolation in pure culture. Suitable colonies those showed *streptomyces* like appearance under light microscope were recultivated several times for purity. The purified actinomycetes were preserved on starch casein agar slants at 4 ºC for two months and at -20 ºC in the presence of glycerol (15%v/v) for longer periods.

Color Grouping of the Isolates

The color of the aerial mycelia and pigment production by the isolates were determined on starch casein agar plates after 7 days of incubation at 36 ºC. The color of the substrate mycelia and those of the soluble pigment were determined according to the National Bureau Standards color chart.[11]

Screening of Antimicrobial Activities of pure Isolates

Screening for antimicrobial activity of the isolates was done by using agar well diffusion method on potato dextrose agar medium. The microbes were grown in nutrient broth for 12 hour well was created on potato dextrose agar well was loaded with 50 µl of different culture filtrate and incubated. The plates were then incubated for 24 hours ,which appeared as clear area around the wells inhibition zone of diameter was measured in mm by the using a millimetre sale.[12]

Test Organisms

Six test organisms were used to test the antimicrobial activity of the isolates. Two of them were gram positive bacteria and three gram-negative bacteria and one unicellular fungus. Gram positive species were *staphylococcus aureus* MTCC-96, *Bacillus subtilis* MTCC-441, Gram negative strains were *Shawanella putrefaciens* MTCC- 8104, *Escherichia coli* MTCC-1687, *vibrio cholera* MTCC- 3906, and one fungus *Candida albicans* MTCC-183. They were maintained in nutrient agar slants at 4 ºC.

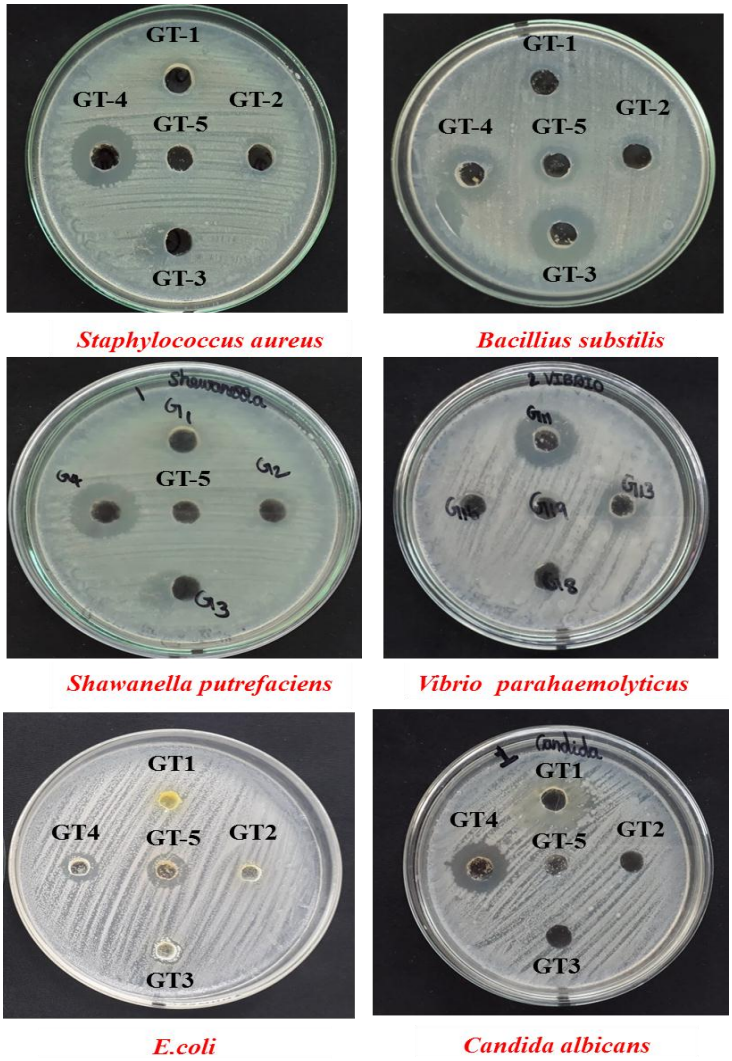

Fig. 3: Antimicrobial activity of GT-4 and GT-11 actinomycetes by secondary screening (Agar well diffusion assay).

(a) GT-4 **(b) GT-11**

Fig. 4: Representative isolates of pure culture on selected isolates were grown on Starch casein Agar for 7 days at 32 ºC.

RESULTS AND DISCUSSIONS

This study was performed with an aim of isolating actinomycetes strains with antimicrobial activities using the selective isolation media. Twenty different actinomycetes strains were isolated from one soil samples collected from Karaikal region (Table. 1) in the year of 2018. All of the strains were collected by using starch casein agar media supplemented with cycloheximide (100 µg/mL) to inhibit fungal growth. The colony forming units (c.f.u) were determined by counting the colonies on the dilution plates (Fig. 2). Maximum number of colonies (1.46×10^4 c.f.u/gm of soil) were obtained in the soil collected from Ganapathy temple tank site study.

All purified isolates grew on yeast–extract-glucose- agar media showing morphology of typical *Streptomyces;* the colonies were slow growing, aerobic, glabrous or chalky, folded, and with aerial and substrate mycelia of different colors.[13] In addition, all colonies possessed an earthy odour. All of the strains were acid fast negative and gram positive and fitted to the description of genus *streptomyces* in Bergey's Manual of systemic Bacteriology. The isolates were categorized into five color series according to their color of the mature sporulated substrate mycelium. The gray series were predominant (33.3% of the total isolates). Out of 20 isolates, two isolates produce soluble pigments in the media (Table. 2).

All the isolated actinomycetes strains were screened for their antimicrobial activity on potato dextrose agar medium using agar well diffusion method. A broad spectrum of antibacterial activity was observed in 53.3 % (11 out of 20) of the pure isolates. Percentages of active isolates in between the series were different. About 80.0% of the grey series, 28.0% of brown series, 100.0% of white series, 25.0% orange series, and 66.0% of red series isolates were active against the test bacteria, (Table.3).

Most of the isolates were active against Staphylococcus aureus, *Bacillus subtilis, Vibrio cholera, Shawanella putrefaciens, Candida albicans and E.coli.* Out of 20 isolates, two isolates were **GT-4** and **GT-11** showed strong inhibition from secondary screening (Agar well diffusion assay) is given in Table 3, which depicted the antimicrobial potency of the two best actinomycetes strains showing good activities against six pathogens. Antimicrobial activity of both GT-4 and GT-11 isolates were done by Agar well diffusion Assay is given in Fig 4. The present work is agreed with other workers on the antimicrobial activity of actinomycetes but our work pertaining to the isolation of actinomycetes from Ganapathy temple tank soil is itself unique and new in its work.

Table 3: Secondary screening of antibacterial activity of actinomycetes isolated from temple tank by agar well diffusion method.

SL. No.	Identification code of the Isolates	*Zone of Inhibition in mm*					
		S. Aureus	***B. Subtilis***	***Vibrio cholera***	***Shawanella putrefaciens***	***E.coli***	***Candida albicans***
1	GT-1	17	12	18	16	12	19
2	GT-2	10	14	16	15	8	12
3	GT-3	10	17	16	14	10	04
4	**GT-4**	**20**	**16**	**18**	**19**	**13**	**20**
5	GT-5	08	10	14	04	14	06
6	GT-6	06	05	12	04	02	04
7	GT-7	08	06	06	04	02	18
8	GT-8	06	05	06	05	02	06
9	GT-9	10	08	05	14	04	08
10	GT-10	08	06	06	14	02	12
11	**GT-11**	**19**	**16**	**24**	**17**	**14**	**20**
12	GT-12	08	06	07	08	04	16
13	GT-13	09	07	08	06	02	06
14	GT-14	10	08	06	08	03	06
15	GT-15	16	12	14	12	10	17
16	GT-16	08	06	07	10	04	06
17	GT-17	04	03	05	03	01	04
18	GT-18	06	05	11	08	04	13
19	GT-19	10	08	13	13	04	06
20	GT-20	11	08	10	07	03	12

CONCLUSION

In the study, from the soil samples of Karaikal regions of Puducherry, about 20 actinomycetes of different genera were isolated and screened for antibacterial activity. In their screening work, they found that 20 isolates were active against test organisms. In another study, 356 *streptomyces* isolates were obtained from soils in the Aegan and East Black Sea regions of turkey, and 36% of the isolates were found to be active against tested microorganisms.[14] In a recent study performed in 2010 by Dehand et al.[15] The antibacterial activity of *streptomyces* isolates from soil samples of West of Iran was investigated. Out of 150 actinomycetes, only 20 isolates (13.30%) showed activity against the test bacteria.

Comparing the above mentioned results with this study, we can conclude that the soil samples of Karaikal are rich source of actinomycetes which produce metabolites inhibitory to bacterial pathogens. We found that 53.3% of the isolated colonies were active against the test

bacteria. **GT-4** and **GT-11** were very active and showed very large zone of inhibition. Future studies will be done to identify the active isolates up to the species level. The type of antimicrobial agents produced by these isolates will be investigated as well.

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