*Research Article*

# Diversity of antidermatophytic *Streptomyces* in the coastal region of Chennai, Tamil Nadu, India

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**ABSTRACT**

The present study was designed to screen the antidermatophytic activity of marine *Streptomyces* isolated from the aquatic environments of the coastal region of Chennai, Tamil Nadu, India. Actinobacteria exhibiting antidermatophytic activity against *Trichophyton rubrum* were identified among 100 isolates by cross streak method. Among them only 2 isolates, namely DKD 6 and DKD 7 exhibiting potential antidermatophytic activity were further characterized for cultural conditions and morphology. Based on these observations the two strains were assigned to the genus *Streptomyces*. The soil samples collected were also analyzed for various physico-chemical parameters and correlated with the total actinomycetes population (TAP) using SPSS package, the results of which indicated the absence of correlation between the parameters and TAP.

**Key words:** Actinobacteria; *Streptomyces; Trichophyton rubrum*; Cross streak method.

## INTRODUCTION

Natural organic compounds produced by microorganisms are an important screening target for a variety of bioactive substances. Compounds of actinomycetal origin, in particular, have been valuable in the field of bioactives (1). About 70% of the earth surface is covered with water, an inexhaustible potential source of many biologically active compounds which needs to be properly unraveled (2). Most actinomycetes were believed to be terrestrial, however, some strains have also been found in marine environments, though no marine actinomycetes with special characteristics have been isolated (1). The search for novel antibiotics and other bioactive microbial metabolites for potential agricultural, pharmaceutical and industrial applications is of current importance (3-4).

Actinobacteria, the filamentous, Gram positive and the most attractive bacteria are the widely distributed groups of microorganisms in nature (5). Screening of marine actinobacteria will lead to the discovery and identification of novel antibiotics from either novel *Streptomyces* spp or from the existing strains (6). Members of the actinomycete genus *Streptomyces* have long been recognized as prolific producers of useful bioactive compounds (7), providing more than half of the naturally occurring antibiotics discovered to date and continuing to be a major source of new bioactive metabolites (8). About 75- 80% of the commercially available and medicinally important antibiotics are produced by *Streptomyces* spp (9) and has the ability to produce at least 1, 00,000 new compounds of biological interest (10).

Dermatophyte infections are one of the earliest known fungal infections of mankind and are very common throughout the world. There are three genera of dermatophytes, *Trichophyton, Microsporum* and *Epidermophyton* (11). Dermatophytoses are world wide in distribution with high prevalence in tropical and sub-tropical countries due to the hot and humid climate which favours their growth (12). As the dermatophytes have developed resistance to antimycotic drugs and due to a lack of safe and effective antifungal antibiotics, there is an urgent need for nontoxic, safe and cost effective antifungal antibiotics (13). The present study was undertaken with the aim of determining the antidermatophytic activity of marine *Streptomyces*.

## MATERIALS AND METHODS

### Sample collection

Marine soil samples were collected from the coastal region of Chennai (13.04 N, 80.17 E.), Tamil Nadu. The samples were brought to the laboratory under aseptic conditions and stored at 4°C for further analysis.

### Isolation of actinobacteria from the soil sample

Soil samples (1gm) were serially diluted up to $10^{-6}$ dilution and 1ml of each dilution were plated on Starch Caesin agar by pour plate technique (14). The plates were incubated at 30°C for 7-10 days. The colonies obtained after incubation were purified by streak plate technique.

### Screening of actinobacteria for antidermatophytic activity

The pure cultures of actinobacteria from the two samples were then screened for their antidermatophytic activity by cross streak method. Starch Caesin agar plates were inoculated with pure cultures of actinobacteria and incubated at 30°C for 7-10 days. After considerable growth of the isolates the test organism, *Trichophyton rubrum* was inoculated perpendicular to the streak line of the isolates. The plates were then incubated at 30°C for a period of 7-10 days.

### Identification of the potential antidermatophytic actinobacteria

The isolates which showed potential antidermatophytic activity were identified using morphological and cultural characteristics as described in the International Streptomyces Project (ISP) (15). The morphology of aerial mycelium and hyphae bearing the spores were examined using the slide culture technique (16).

### Physico-chemical characterization of soil samples

Soil samples were also analyzed for various physico-chemical parameters like nitrate, phosphorus, potassium, calcium, magnesium, sulphur, sodium, zinc, manganese, iron, copper, boron, pH, electrical conductivity, organic matter, CEC, potassium, magnesium, sodium and calcium saturation. These parameters were correlated with the total actinomycetes population (TAP) in the collected marine soil samples using SPSS software.

## RESULTS AND DISCUSSION

Chennai is located on the southeast coast of India in the northeast of Tamil Nadu with an average elevation around 6.7 meters (20 ft) (17) and its highest point is 60 m (18). The Marina beach runs for 12 km along the shoreline of the city. The soil texture of this city is mostly clay, shale and sandstone (19). Sandy areas are found along the river banks and coasts.

About 100 strains were isolated from the marine soil samples collected from the coastal region of Chennai. The colonies were designated as DKD1-DKD100 based on their colony morphology observed on the master plate. To study the colony morphology the cultures were streaked on Starch casein agar plates and observed for the colony size, shape, texture, colour, margin and pigmentation. All the isolates grew well on starch casein agar. The isolates were small to medium sized, creamish white to pure white in colour, round, powdery, with regular margin and non-pigmented. Similar studies were carried out with different media like Actinobacteria agar, Streptomyces agar and ISP-2, 6 and 7 media (20). Actinobacteria exhibits different colony morphology on different media which is evident from the work of Stefka et al (21) where cultivation on ISP-6 and ISP-7 enhanced the production of melanin.

The soil samples collected were subjected to physico-chemical analysis. A correlation between the physico-chemical parameters and the actinobacteria population (TAP) obtained from the respective soil samples was calculated to determine the parameter that most influenced the growth of actinobacteria (Table 1). The correlation coefficient analysis revealed that none of the physico-chemical parameters influenced the total actinobacteria population (TAP). Similar type of study was reported by Mansour (22); Lakshmanaperumalsamy et al (23); Jiang and Xu (24); Saadoun and Al-Momoni (25) where the parameters such as pH, moisture, organic matter, nitrogen and phosphorous content of the soils were correlated with actinomycetes population. The correlation between salinity, pH and organic content of marine sediments and actinomycetes population has been reported by several workers (26).

In the present study all the 100 isolates obtained were screened for antidermatophytic activity by cross streak method and among them only 2 isolates (DKD6 and DKD7) exhibited the ability to produce antidermatophytic secondary metabolite. In the cross streak method, across the streak lines of DKD6 and DKD7, there is no growth of the test organism, *T.rubrum* (Fig.1).

The isolates DKD6 and DKD7 which showed activity against *T.rubrum* were characterized morphologically and culturally. Both the cultures showed similarity in colony morphology to *Streptomyces*. The colonies on SCA were white in colour, slow growing, powdery and possessed an earthy odour characteristic of *Streptomyces* as described by Locci (27). In addition to the above mentioned characteristics, the isolate DKD6 showed reverse side yellowish brown colour pigmentation (Fig.2). It was observed that the isolates DKD6 and DKD7 microscopically exhibited the typical spiral and bent sporophores (Fig. 3A and B).

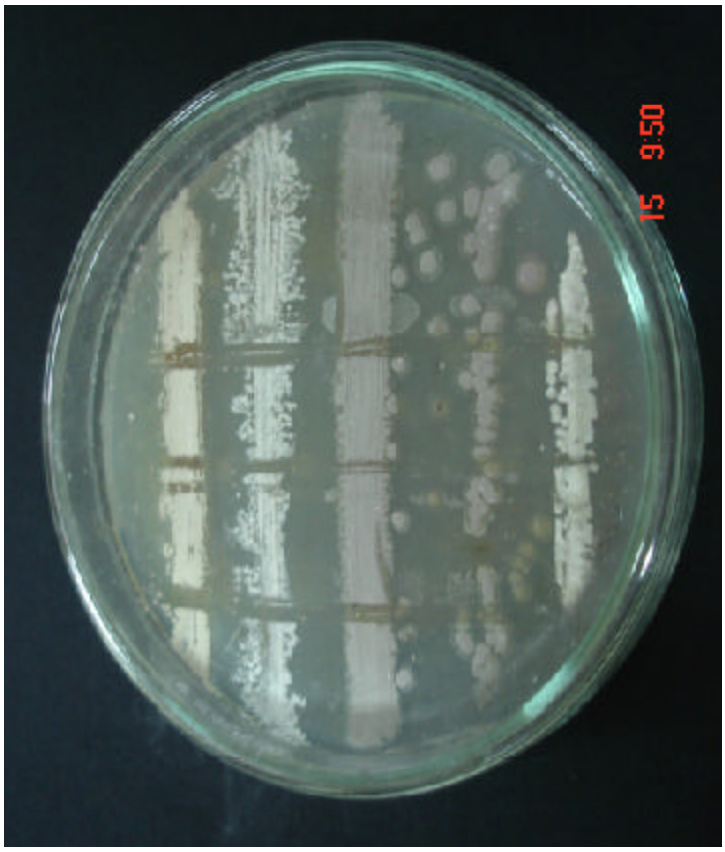

**Fig.1. Antidermatophytic activity exhibited by DKD6 and DKD7 by cross streak method. *Streptomyces,* DKD6 and DKD7 inhibit the growth of *T.rubrum* is clearly seen across the streak lines**

**Fig.2. Aerial spore mass and reverse side pigment produced by DKD6 on Starch Casein agar and the aerial spore mass produced by *Streptomyces,* DKD6 was shown after complete growth of the organism. The photograph shows the reverse side yellowish brown pigmentation production by DKD6.**

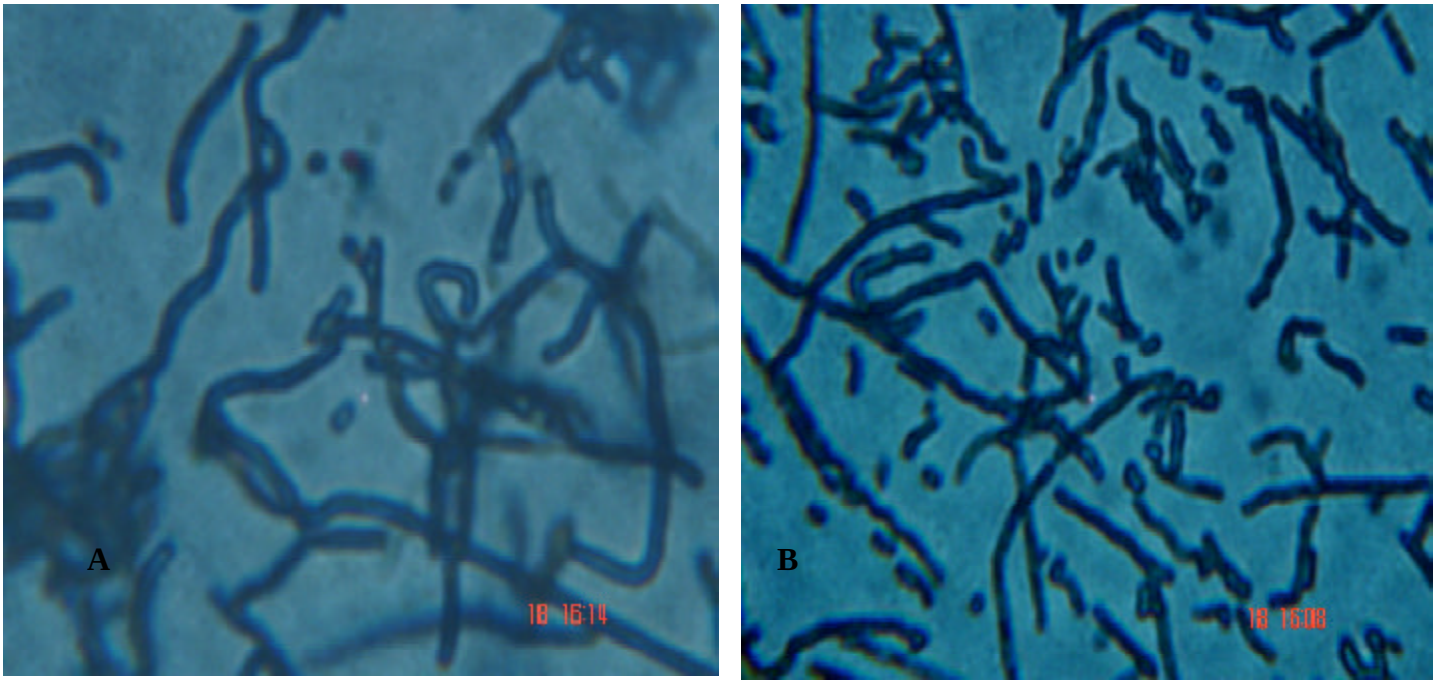


**Fig.3. The *Streptomyces,* DKD6 (A) and DKD7 (B) shows typical spiral and bent sporophores under light microscopic examination (100x magnification).**

**Table.1 Correlation co-efficient between physico-chemical properties of marine sediments and total actinomycetes population**

| | *N* | *P* | *K* | *Ca* | *Mg* | *S* | *Na* | *Zn* | *Mn* | *Fe* | *Cu* | *Br* | *pH* | *EC* | *OM* | *CEC* | *TAP* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 1 | | | | | | | | | | | | | | | | |
| P | -0.17 | 1 | | | | | | | | | | | | | | | |
| K | 0.508 | 0.072 | 1 | | | | | | | | | | | | | | |
| Ca | 0.579 | 0.243 | 0.882* | 1 | | | | | | | | | | | | | |
| Mg | 0.448 | 0.072 | 0.982** | 0.872* | 1 | | | | | | | | | | | | |
| S | 0.417 | 0.207 | 0.966** | 0.922** | 0.957** | 1 | | | | | | | | | | | |
| Na | -0.195 | 0.058 | 0.407 | 0.354 | 0.538 | 0.466 | 1 | | | | | | | | | | |
| Zn | 0.031 | 0.752* | 0.476 | 0.596 | 0.546 | 0.588 | 0.635 | 1 | | | | | | | | | |
| Mn | 0.736* | -0.145 | 0.924** | 0.789* | 0.873* | 0.825* | 0.204 | 0.237 | 1 | | | | | | | | |
| Fe | 0.007 | 0.61 | 0.56 | 0.677 | 0.558 | 0.704 | 0.206 | 0.622 | 0.275 | 1 | | | | | | | |
| Cu | -0.074 | 0.762* | 0.207 | 0.508 | 0.223 | 0.411 | 0.371 | 0.792* | -0.01 | 0.652 | 1 | | | | | | |
| Br | 0.558 | -0.022 | 0.812* | 0.669 | 0.767 | 0.701 | 0.28 | 0.32 | 0.883* | 0.1 | 0.054 | 1 | | | | | |
| pH | 0.727* | -0.012 | 0.62 | 0.575 | 0.557 | 0.564 | -0.234 | 0.071 | 0.714 | 0.32 | -0.091 | 0.464 | 1 | | | | |
| EC | -0.002 | 0.213 | 0.646 | 0.609 | 0.753* | 0.702 | 0.938** | 0.775* | 0.422 | 0.431 | 0.464 | 0.47 | -0.013 | 1 | | | |
| OC | 0.675 | 0.042 | 0.947** | 0.895* | 0.904** | 0.909** | 0.326 | 0.438 | 0.961** | 0.423 | 0.259 | 0.874* | 0.642 | 0.551 | 1 | | |
| CEC | 0.432 | 0.19 | 0.912** | 0.949** | 0.943** | 0.948** | 0.616 | 0.681 | 0.774* | 0.626 | 0.48 | 0.691 | 0.445 | 0.817* | 0.881* | 1 | |
| TAP | -0.051 | -0.051 | -0.05 | -0.221 | 0.042 | -0.049 | 0.531 | 0.265 | -0.03 | -0.326 | -0.055 | 0.005 | -0.142 | 0.389 | -0.03 | 0.003 | 1 |

Significance * $P<0.05$; ** $P<0.01$

The morphology and cultural characteristics were determined according to the International Streptomycetes Project (15). The results of the present investigation revealed that the isolates DKD6 and DKD7 belonged to the genus *Streptomyces* and that both the isolates showed potential antidermatophytic activity. The present study also supports the claim that marine actinobacteria are a potential source of novel secondary metabolites. Further studies on molecular taxonomical characterization of the potential organisms and purification and characterization of antidermatophytic compounds are under progress in the investigators laboratory

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