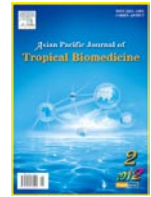




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## Actinomycetes from Western Ghats of Tamil Nadu with its antimicrobial properties

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### ABSTRACT

**Objective:** To isolate the actinomycetes from Western Ghats of Tamil Nadu with its antimicrobial properties. **Methods:** Starch casein agar medium supplemented with actidione and nalidixic acid was used to isolate actinomycetes from Western Ghates region of Kanyakumari, Thirunelveli, Dindigul and Nilgiri districts. Modified nutrient medium was used as the base for screening actinomycetes against pathogenic Gram positive, Gram negative and filamentous fungi. **Results:** Among 367 actinomycetes; 17.71% showed activity against both bacteria and fungi. The highest antibacterial activity was observed against *B. subtilis*, 140 isolates (38.1%), *S. aureus* 128 (34.9%); *S. epidermidis* 123 (33.5%); *P. aeruginosa* 105 (28.6%); *K. pneumoniae* 88 (24%); *Xanthomonas* sp 62 (16.9%). Less number of actinomycetes showed activity against *Erwinia*, *S. typhi*, *V. fischeri* and *P. vulgaris*. Hundred and three isolates showed activity against *B. cinerea* and *A. niger*. Twenty five isolates revealed activity against *T. simii*. **Conclusions:** Present investigation concludes that Western Ghats region of Tamil Nadu is the potential place for actinomycetes diversity. Further studying about these medically important strains from this region can be useful in identification of valuable bio-molecules.

## 1. Introduction

Numerous antibiotics have been isolated from a variety of microorganisms; however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria. The number and species of microbes in soil vary directly in response to environmental conditions such as nutrient availability, soil texture, and type of vegetation cover<sup>[1]</sup>. Filamentous actinomycetes are known to have the ability to produce a wide variety of secondary metabolites. Indeed, each strain of actinomycetes likely has the genetic potential for the production of 10 to 20 secondary metabolites<sup>[2,3]</sup>. Actinomycetes are useful biological tools for the production of antimicrobials against fungi and bacteria<sup>[4]</sup>. In general, Streptomyces are saprophytic and are commonly associated with soils, where they contribute significantly to the turnover of complex biopolymers and antibiotics<sup>[5]</sup>. However, in the past two decades there has

been a decline in the discovery of new lead compounds from common soil-derived actinomycetes. As a result, the cultivation of various taxa of actinomycetes has become a major focus in the search for the next generation of pharmaceutical agents<sup>[6]</sup>.

Interestingly, the majority of these antibiotic-producing actinomycetes are found among these Streptomyces which lead to a growing economic importance of this group of organisms<sup>[7–9]</sup>. Streptomyces are characterized by a complex morphological differentiation cycle accompanied by the production of numerous extracellular enzymes as well as, many kinds of bioactive secondary metabolites having great structural and functional diversity including antibiotics, anticancer, immunosuppressant agents, insecticides, and herbicides<sup>[10,11]</sup>. Actinomycetes are filamentous, aerobic, spore forming, multicellular and Gram-positive soil bacteria which belong to the order actinomycetales<sup>[12]</sup>. The soil habitat is very challenging for microorganisms, and Streptomyces as an important member of this ecosystem which has evolved complex morphological and physiological adaptations to survive in this environment. Actinomycetes are found in virtually every natural substrate such as soils, composts, fresh water basins, food stuffs, and marine and in the atmosphere. The wide range of climatic

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conditions is the reason for Western Ghats richest variety of forest and generation of medicinal plants. Dead plant tissue is rich in carbon but concentrations of N and P nutrients are less than optimal for building microbial flora. Bacteria, actinomycetes and fungi can take up the exogenous inorganic N and P to supplement the nutrients in litter being decomposed. Nutrients are washed into forest soil by rainfall leading to the availability of more microorganisms' growth. Due to large geographical variation, there is large variation in soil type and their contents in Western Ghats and hence it is quite likely that the distribution of antibiotic producing actinomycetes is also variable. This article describes actinomycetes from Western Ghats of Tamil Nadu with its antimicrobial properties.

## 2. Materials and methods

### 2.1. Samples collection

Soil samples were collected from Western Ghats of Tamil Nadu; it includes four main districts such as Kanyakumari, Thirunelveli, Dindigul and Nilgiri (Figure 1 & 2)). The soils were collected from rhizosphere of plants, vegetative soils and soils under rock. The collected samples were transported to laboratory and stored at 4 °C in order to isolate the microorganisms. Kanyakumari is located at the southern tip of the Indian subcontinent, with an area of 1 672 km<sup>2</sup>. It occupies 1.29% of the total extent of Tamil Nadu. The Forests are verdant, virgin and said to be 75 million years old. The district lies between 77 ° 15' and 77 ° 36' of the eastern longitudes and 8 ° 03' and 8 ° 35' of the northern Latitudes. Thirunelveli district lies between 8 ° 05' and 9 ° 30' of the Northern latitude and 77 ° 05' and 78 ° 25' of Eastern longitude. Dindigul is located at 10 ° 21' N 77 ° 57' E 10.35 ° N 77.95 ° E. It has an average elevation of 268 metres (879 feet). Nilgiri located at (Latitude: 11 ° 08' to 11 ° 37' N) by 185 km (Longitude: 76 ° 27' E to 77 ° 4' E).

### 2.2. Isolation and purification of actinomycetes

One gram of soil sample was shade dried and added into 100 mL of sterile distilled water and mixed thoroughly in a shaker for 30 min at 150 rpm at room temperature. The suspension was serially diluted; 0.1 mL of each dilution was spread plated on Oat meal Agar and Starch Casein Agar plates in triplicates and incubated at 28°C for 7 d. Agar plates were supplemented with actidione and nalidixic acid to inhibit common contaminants fungi and bacteria, respectively. After the incubation period plates were examined for the presence of actinomycetes colony. The suspected actinomycetes colony was picked up and purified on ISP-2 (International Streptomyces Project-2) media and incubated at room temperature for about 4 d. The pure cultures were stored in ISP-2 slants at 4 °C for further identification and antimicrobial screening study. The cultures were stored in 15% glycerol at -20 °C for long term preservation<sup>[13]</sup>.

### 2.3. Antibacterial activity

Preliminary screening of antibacterial activity was done on Modified Nutrient Glucose Agar (MNGA) medium. Spore suspension of actinomycetes were inoculated in straight line on plates and incubated at 30 °C for 4 d. The selected reference microorganisms like Gram positive (*Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615) and *Enterococcus faecalis* (ATCC 29212)) and Gram negative (*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 15380), *Erwinia* (MTCC 2760), *Salmonella typhi* (MTCC 733), *Vibrio fischeri* and *Proteus vulgaris* (MTCC 733)) bacteria were streaked perpendicular ('T'- streak) to the actinomycetes<sup>[14]</sup>. After 24 h of incubation at 37 °C, antibacterial activity was evaluated by measuring the distance of inhibition (in mm) between target microorganisms and actinomycetes colony margin.

### 2.4. Antifungal activity

Antifungal activity was evaluated on Modified Nutrient Glucose Agar (MNGA) medium against *Epidermophyton floccosum* 73/01, *Trichophyton rubrum* (MTCC 296), *Trichophyton simii* 110/02, *Candida albicans* (MTCC 227), *Curvularia lunata* (46/01), *Aspergillus niger* (MTCC 1344) and *Botrytis cinerea*. Spore suspension of actinomycetes were inoculated in straight line on plates and incubated at 30 °C for 4 d. Target fungal spores were spotted perpendicular to the streaked line. After 96 to 120 h of incubation at 28 °C, the inhibition of fungi was recorded.

## 3. Results

The pharmaceutically useful actinomycetes were screened for their antibacterial and antifungal property from Western Ghats ecosystems of Tamil Nadu. Eighteen different soil samples had been collected from different parts of Western Ghats (Table 1). A total number of 367 different actinomycetes were isolated from different places, different soil samples.

### 3.1. Antibacterial and antifungal activity

All the 367 actinomycetes isolates were evaluated for their antibacterial and antifungal activity. The detailed activity was given in (Table 2). Most of the actinomycetes showed activity against bacteria (Figure 3). Among the isolates 65 (17.71%) exhibited activity towards both bacteria and fungi (Figure 4). The highest antibacterial activity was observed against *B. subtilis*, 140 isolates (38.1%), *S. aureus* 128 (34.9%); *S. epidermidis* 123 (33.5%); *P. aeruginosa* 105 (28.6%); *K. pneumoniae* 88 (24%); *Xanthomonas* sp 62 (16.9%). Less number of actinomycetes showed activity against *Erwinia*, *S. typhi*, *V. fischeri* and *P. vulgaris*. Hundred and three isolates (28.1%) revealed activity against *B. cinerea*, 97 (26.4%), *A. niger* and 25 (6.8%) isolates showed activity against *T. simii*.

#### 3.1.1. Antimicrobial potential of strains from Kanyakumari

**Table 1.**  
Soil samples name and sampling area.

No	Sample Code	Local area name of sampling	Place of sampling	District
1	ATT	Guest House	Arukani	Kanyakumari
2	ATRS	16th kooopu	Arukani	Kanyakumari
3	ERIAS	Orunuram vail	Arukani	Kanyakumari
4	AMR	Kila malai	Arukani	Kanyakumari
5	ATW	Kila malai	Arukani	Kanyakumari
6	AMW	Vannathiparai	Arukani	Kanyakumari
7	ELW	Power house	Kothayaroo	Kanyakumari
8	ELS	Power house	Kothayaroo	Kanyakumari
9	GTW	Theku malai	Kothayaroo	Kanyakumari
10	ATS1	Keral border line	Arukani	Kanyakumari
11	ERI	Chempagaparai	Arukani	Kanyakumari
12	BTS	Mancholai	Koutallam	Thirunelveli
13	BMS	Manthoppu	Koutallam	Thirunelveli
14	BMW	Lower agastiar malai	Koutallam	Thirunelveli
15	CBR	Guna cave	Kodaikanal	Dindugal
16	DTR	Pykkara	Odagamandalam	Nilgiri
17	DTW	Avilanchi	Odagamandalam	Nilgiri
18	D52	Avilanchi	Odagamandalam	Nilgiri

**Table 2.**  
Number of actinomycetes inhibited the growth of bacteria and fungi.

Origin	Total strains	Total number of active isolates against bacteria										Total number of active isolates against fungi									
		B.s	S.a	S.e	E.f	E.c	P.a	K.p	X.sp	E.sp	S.t	V.f	P.v	C.a	A.n	C.l	B.c	E.f	T.m	T.s	
ATT	23	9	9	8	8	4	5	5	3	3	4	5	5	6	10	10	10	9	7	3	
ATRS	16	8	8	8	6	6	4	4	3	2	1	1	0	5	10	9	9	8	5	2	
ERIAS	26	11	9	9	7	7	4	3	3	5	3	5	3	4	10	10	9	8	6	2	
AMR	27	12	10	10	10	9	8	5	4	3	3	2	2	6	8	10	10	10	8	4	
ATW	30	13	12	12	12	11	6	4	4	5	4	4	4	6	12	12	13	12	8	4	
AMW	30	12	11	11	9	7	5	1	1	1	1	1	1	2	12	1	12	2	8	5	
ELW	8	2	2	2	1	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	
ELS	10	1	1	1	1	1	1	0	0	0	0	0	0	0	0	2	1	1	1	0	
GTW	20	5	5	5	4	3	3	0	0	1	2	2	1	0	2	2	2	2	1	0	
ATS1	17	8	7	6	7	4	1	4	3	5	4	3	4	0	1	9	2	2	1	0	
ERI	73	27	25	24	20	19	17	15	13	8	5	4	3	9	8	5	9	6	5	4	
BTS	23	8	8	8	4	3	3	2	1	1	0	0	0	0	3	5	5	4	2	0	
BMS	13	5	4	4	4	4	1	1	0	1	1	1	0	3	5	3	5	5	2	1	
BMW	8	4	4	4	3	2	1	0	0	0	0	0	0	1	3	3	3	3	2	0	
CBR	23	9	8	6	4	2	2	1	1	1	0	0	0	4	8	8	8	8	1	0	
DTR	5	2	2	2	2	1	0	0	0	0	0	0	0	0	2	2	2	2	1	0	
DTW	5	1	1	1	1	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	
D52	10	3	2	2	2	2	0	0	0	0	0	0	0	1	2	2	2	2	0	0	
Total	367	140	128	123	105	88	62	46	37	37	28	28	23	47	97	94	103	85	58	25	

B.s – *B. subtilis*; S.a – *S. aureus*; S.e – *S. epidermidis*; E.f – *E. faecalis*; E.c – *E. coli*; P.a – *P. aeruginosa*; K.p – *K. pneumoniae*; X.sp – *Xanthomonas* sp.; E.sp – *Erwinia*; S.t – *S. typhi*; V.f – *V. fischeri*; P.v – *P. vulgaris*; C.a – *C. albicans*; A.n – *A. niger*; C.l – *C. lunata*; B.c – *B. cinerea*; E.f – *E. floccosum*; T.m – *T. rubrum*; T.s – *T. simii*.

### district

280 actinomycetes strains were isolated from different parts of Western Ghats in Kanyakumari District were screened for antibacterial and antifungal activity on MNG agar medium. Among them, 126 strains (45%) showed activity against both Gram-positive and Gram-negative bacteria. Seventy six (27.1%) actinomycetes inhibited growth of the tested fungal strains. 108 (38.57%) isolates inhibited the growth of *B. subtilis* and 99 (35.35%) showed activity against *S. aureus*, other tested bacteria revealed activity as follows *S. epidermidis* (34.28), *E. faecalis* (30.35%), *P. aeruginosa* (25.71%), *K. pneumoniae* (19.28%), *Xanthomonas* sp. (14.64%), *Erwinia*

(12.14%), *S. typhi* (11.78%), *V. fischeri* (9.64%) and *P. vulgaris* (8.21%). Out of all the tested bacteria *P. vulgaris* growth was retarded by less number of actinomycetes isolates (23). In the case of antifungal activity, 38 (13.57%) actinomycetes showed antifungal activity against *C. albicans*. Maximum activity (27.85%) was found against *B. cinerea* followed by *A. niger* (26.42%) and anti dermatophytic activity was found only in 8.6% isolates against *T. simii*. The actinomycetes, ERI-1 and ERI-3 showed strong activity against all the tested microbes. ERI-26 was found to be active against most of the tested bacteria and fungi.

**Table 4.**

Preliminary screening of actinomycetes for antibacterial and antifungal property isolated from Western Ghats of Thirunelveli District.

Culture No.	Antimicrobial activity (mm)																		
	Bacteria												Fungi						
	B.s	S.a	S.e	E.f	E.c	P.a	K.p	X.sp	E.sp	S.t	V.f	P.v	C.a	A.n	C.l	B.c	E.f	T.m	T.s
BTS-1	10	11	8	7	12	12	10	12	9	-	-	-	-	+	+	+	-	-	-
BTS-5	10	9	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-8	12	10	17	10	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BTS-11	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BTS-12	12	14	15	16	19	12	12	-	-	-	-	-	-	+	+	+	-	-	-
BTS-14	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BTS-16	15	19	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-17	5	13	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-20	15	21	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-22	10	11	10	9	10	8	11	-	-	-	-	-	-	+	+	+	+	+	-
BMS-4	6	-	12	13	12	23	10	-	-	-	-	-	10	+	+	+	+	-	-
BMS-5	18	12	15	13	10	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMS-6	12	12	9	14	13	-	-	-	-	-	-	-	15	-	-	-	-	-	-
BMS-7	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BMS-8	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMS-9	-	-	-	-	-	-	-	-	22	16	10	-	-	-	-	-	-	-	-
BMS-10	-	-	-	-	-	-	-	-	-	-	-	-	10	+	+	+	+	+	+
BMS-11	14	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMS-12	15	10	12	14	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BMW-2	5	13	15	10	12	15	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-4	12	15	10	11	12	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMW-7	10	15	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-8	10	11	10	10	-	-	-	-	-	-	-	-	10	+	+	+	+	+	-

- No activity; + activity, B.s - *B. subtilis*; S.a - *S. aureus*; S.e - *S. epidermidis*; E.f - *E. faecalis*; E.c - *E. coli*; P.a - *P. aeruginosa*; K.p - *K. pneumoniae*; X.sp - *Xanthomonas* sp.; E.sp - *Erwinia*; S.t - *S. typhi*; V.f - *V. fischeri*; P.v - *P. vulgaris*; C.a - *C. albicans*; A.n - *A. niger*; C.l - *C. lunata*; B.c - *B. cinerea*; E.f - *E. floccosum*; T.m - *T. rubrum*; T.s - *T. simii*.

**Table 4.**

Preliminary screening of actinomycetes for antibacterial and antifungal property isolated from Western Ghats of Thirunelveli District.

Culture No.	Antimicrobial activity (mm)																		
	Bacteria												Fungi						
	B.s	S.a	S.e	E.f	E.c	P.a	K.p	X.sp	E.sp	S.t	V.f	P.v	C.a	A.n	C.l	B.c	E.f	T.m	T.s
BTS-1	10	11	8	7	12	12	10	12	9	-	-	-	-	+	+	+	-	-	-
BTS-5	10	9	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-8	12	10	17	10	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BTS-11	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BTS-12	12	14	15	16	19	12	12	-	-	-	-	-	-	+	+	+	-	-	-
BTS-14	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BTS-16	15	19	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-17	5	13	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-20	15	21	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BTS-22	10	11	10	9	10	8	11	-	-	-	-	-	-	+	+	+	+	+	-
BMS-4	6	-	12	13	12	23	10	-	-	-	-	-	10	+	+	+	+	-	-
BMS-5	18	12	15	13	10	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMS-6	12	12	9	14	13	-	-	-	-	-	-	-	15	-	-	-	-	-	-
BMS-7	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BMS-8	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMS-9	-	-	-	-	-	-	-	-	22	16	10	-	-	-	-	-	-	-	-
BMS-10	-	-	-	-	-	-	-	-	-	-	-	-	10	+	+	+	+	+	+
BMS-11	14	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMS-12	15	10	12	14	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
BMW-2	5	13	15	10	12	15	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-4	12	15	10	11	12	-	-	-	-	-	-	-	-	+	+	+	+	-	-
BMW-7	10	15	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BMW-8	10	11	10	10	-	-	-	-	-	-	-	-	10	+	+	+	+	+	-

- No activity; + activity, B.s - *B. subtilis*; S.a - *S. aureus*; S.e - *S. epidermidis*; E.f - *E. faecalis*; E.c - *E. coli*; P.a - *P. aeruginosa*; K.p - *K. pneumoniae*; X.sp - *Xanthomonas* sp.; E.sp - *Erwinia*; S.t - *S. typhi*; V.f - *V. fischeri*; P.v - *P. vulgaris*; C.a - *C. albicans*; A.n - *A. niger*; C.l - *C. lunata*; B.c - *B. cinerea*; E.f - *E. floccosum*; T.m - *T. rubrum*; T.s - *T. simii*.

**Table 5.** Screening of actinomycetes from Dindigul District against bacteria and fungi.

Culture No	Inhibition zone																		
	Bacteria									Fungi									
	B.s	S.a	S.e	E.f	E.c	P.a	K.p	X.sp	E.sp	S.t	V.f	P.v	C.a	A.n	C.l	B.c	E.f	T.m	T.s
CBR-1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
CBR-2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
CBR-3	5	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CBR-4	8	11	10	11	9	10	8	10	-	-	-	-	-	-	-	-	-	-	-
CBR-5	10	11	14	11	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
CBR-6	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
CBR-10	15	12	12	17	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
CBR-15	8	12	10	11	10	9	-	-	-	-	-	-	12	+	+	+	+	-	-
CBR-18	10	10	-	-	-	-	-	-	-	-	-	-	11	+	+	+	+	-	-
CBR-20	15	15	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CBR-21	12	23	21	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-
CBR-22	-	-	-	-	-	-	-	-	-	-	-	-	10	+	+	+	+	-	-

- No activity; + activity; B.s - *B. subtilis*; S.a - *S. aureus*; S.e - *S. epidermidis*; E.f - *E. faecalis*; E.c - *E. coli*; P.a - *P. aeruginosa*; K.p - *K. pneumoniae*; X.sp - *Xanthomonas* sp.; E.sp - *Erwinia*; S.t - *S. typhi*; V.f - *V. fischeri*; P.v - *P. vulgaris*; C.a - *C. albicans*; A.n - *A. niger*; C.l - *C. lunata*; B.c - *B. cinerea*; E.f - *E. floccosum*; T.m - *T. rubrum*; T.s - *T. simii*.

**Table 6.** Screening of actinomycetes from Nilgiri District against bacteria and fungi.

Culture NO	Inhibition zone																		
	Bacteria									Fungi									
	B.s	S.a	S.e	E.f	E.c	P.a	K.p	X.sp	E.sp	S.t	V.f	P.v	C.a	A.n	C.l	B.c	E.f	T.m	T.s
DTR -3	5	12	15	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DTR -4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
DTR -5	13	10	12	12	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
DTW -1	18	10	12	10	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DTW -2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
DTW -3	-	-	-	-	12	10	12	12	16	-	-	-	-	-	-	-	-	-	-
DTW -5	-	-	-	-	-	-	-	-	-	-	-	-	10	+	+	+	+	-	-
D5S-4	5	15	12	15	15	10	-	-	-	-	-	-	-	+	+	+	+	-	-
D5S-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D5S-6	5	5	5	12	15	10	-	-	-	-	-	-	-	-	-	-	-	-	-
D5S-10	5	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- No activity; + activity; B.s - *B. subtilis*; S.a - *S. aureus*; S.e - *S. epidermidis*; E.f - *E. faecalis*; E.c - *E. coli*; P.a - *P. aeruginosa*; K.p - *K. pneumoniae*; X.sp - *Xanthomonas* sp.; E.sp - *Erwinia*; S.t - *S. typhi*; V.f - *V. fischeri*; P.v - *P. vulgaris*; C.a - *C. albicans*; A.n - *A. niger*; C.l - *C. lunata*; B.c - *B. cinerea*; E.f - *E. floccosum*; T.m - *T. rubrum*; T.s - *T. simii*.

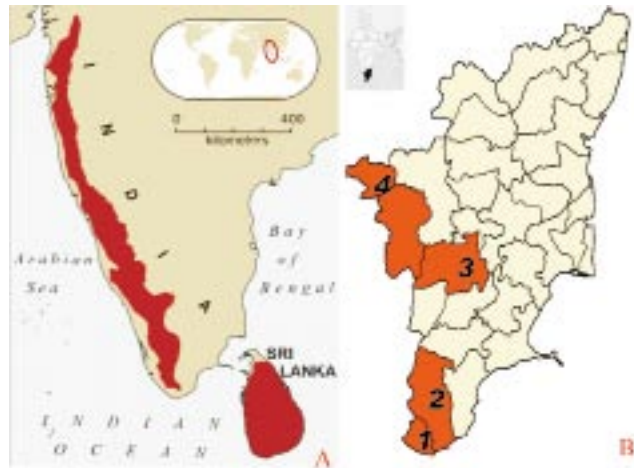
**Table 7.** Number of actinomycetes from different soils and its activity towards antibacterial and antifungal activities.

No	Origin	Total strains isolated	No. of active isolates against bacteria (%)	No. of active isolates against Fungi (%)	ABA+ AFA (%)
1	ATT	23	12 (52.1)	11 (47.8)	7 (30.4)
2	ATRS	16	9 (56.2)	9 (56.2)	5 (31.2)
3	ERIAS	26	11 (42.3)	11 (42.3)	8 (30.7)
4	AMR	27	13 (48.8)	6 (22.2)	4 (14.8)
5	ATW	30	14 (46.6)	14 (46.6)	6 (20.0)
6	AMW	30	13 (43.3)	12 (40.0)	10 (33.3)
7	ELW	8	2 (25.0)	1 (12.5)	1 (12.5)
8	ELS	10	1 (10.0)	1 (10.0)	0 (0.0)
9	GTW	20	5 (25.0)	1 (10.0)	1 (5.0)
10	ATS1	17	9 (52.9)	2 (11.8)	2 (23.6)
11	ERI	73	37 (50.6)	8 (10.9)	6 (8.2)
12	BTS	23	8 (34.7)	4 (17.4)	3 (13.0)
13	BMS	13	6 (46.2)	6 (46.2)	2 (16.4)
14	BMW	8	4 (50.0)	3 (37.5)	2 (25.0)
15	CBR	23	9 (39.1)	9 (39.1)	6 (26.1)
16	DTR	5	3 (60.0)	2 (40.0)	1 (20.0)
17	DTW	5	2 (40.0)	2 (40.0)	0 (0.0)
18	D52	10	3 (30.0)	2 (40.0)	1 (10.0)

ABA: Antibacterial activity; AFA: Antifungal activity.

### 3.1.2. Antimicrobial potential of strains from Thirunelveli district

Only forty four actinomycetes cultures having antimicrobial activity were isolated from the soil sample belonged to Thirunelveli District. Seventeen isolates (38.63%) showed activity against *B. subtilis* and 16 isolates (36.36%) were active against *S. aureus*. None of the isolates showed activity against *P. vulgaris* (Table 3). Among the 44 isolates, thirteen (29.5%) inhibited the growth of *B. cinerea* followed by *E. floccosum* (27.2%). 25% of the actinomycetes isolates revealed activity against *A. niger*. A less number of isolates, 4 (9.0%) and 1 (2.2%) showed inhibitory activity against *C. albicans* and *T. simii*, respectively.



**Figure 1.** Map of region.

A – Map showing the Western Ghats Region in India. B – Map showing the sampling site region of Western Ghats of Tamil Nadu. 1: Kanyakumari District; 2: Thirunelveli District; 3: Dindigul District; 4: Nilgiri District.

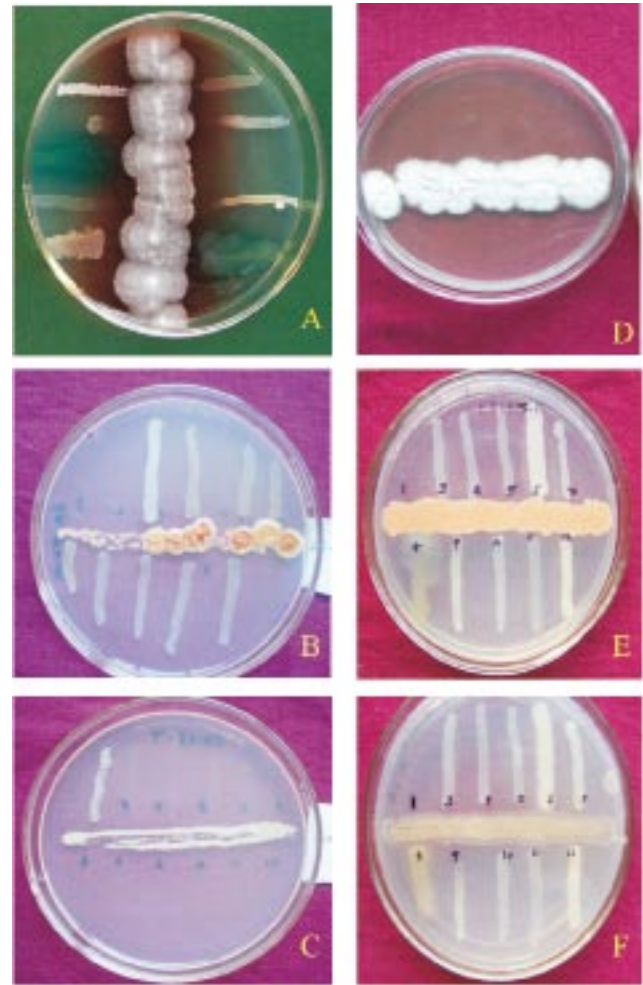


**Figure 2.** Soil samples were collected from Western Ghats of Tamil Nadu.

### 3.1.3. Antimicrobial potential of strains from Dindigul district

### district

Twenty three potential actinomycetes isolates were isolated from the Western Ghats forest soil samples of Kodaikanal (Table 4). All the strains were evaluated for their antimicrobial properties. Nine isolates (39.13%) showed significant activity against *B. subtilis*. *S. aureus*, *S. epidermidis*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae*, *Xanthomonas sp.* and *Erwinia* in decending order. *S. typhi*, *V. fischeri* and *P. vulgaris* growth was not affected by many of the isolates from Kodaikanal. Thirty five percent of actinomycetes from this area showed antifungal activity against *A. niger*, *C. lunata*, *B. cinerea* and *E. floccosum*. None of the actinomycetes were showed activity against *T. simii*



**Figure 3.** Antibacterial activity of actinomycetes.

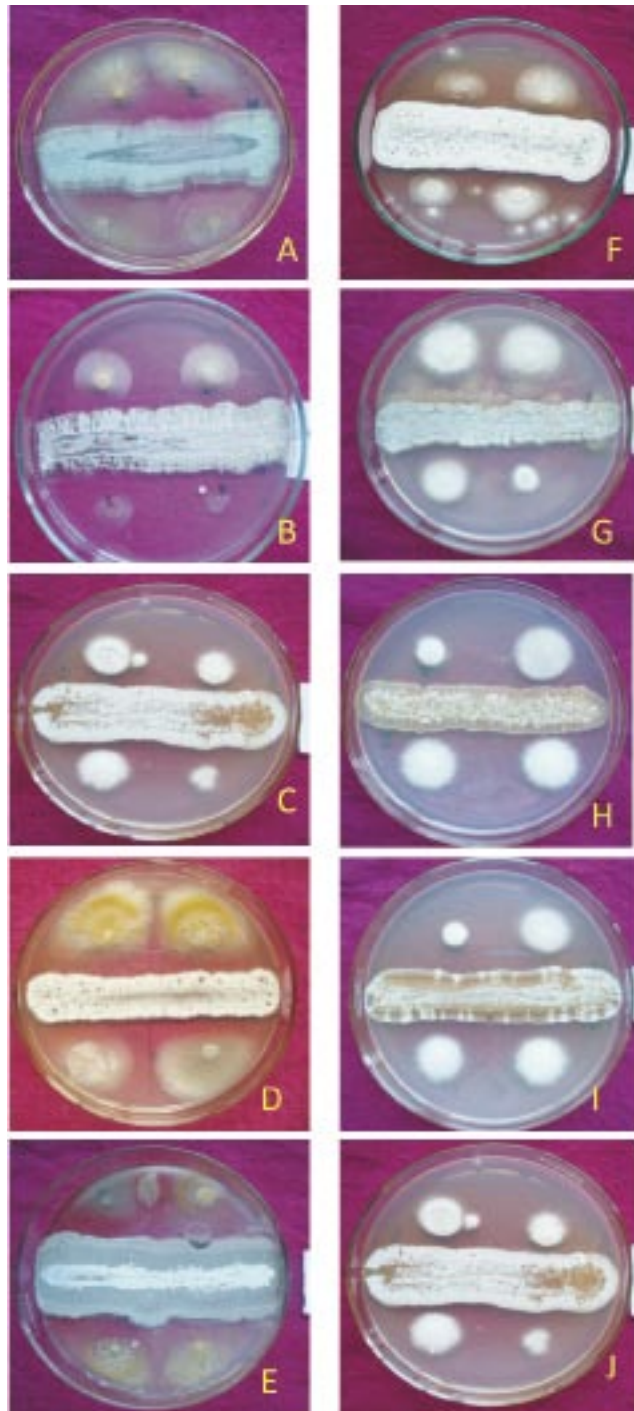
A– ERIAS–2, B– ATW–4, C– AMR–19, D– ERI–26, E– ELS–7, F– ATW–19.

### 3.1.4. Antimicrobial potential of strains from Nilgiri district

Twenty promising actinomycetes were isolated from Western Ghats forest soil samples of Nilgiri District. Thirty percent of isolates exhibited antibacterial activity against *B. subtilis*. Twenty five isolates showed activity against *S. aureus*, *S. epidermidis*, *E. faecalis* and *P. aeruginosa*. None of the isolates showed activity against *S. typhi*, *V. fischeri* and *P. vulgaris* (Table 5). Comparatively less number of isolates showed antifungal activity against *A. niger*, *C. lunata*, *B. cinerea* and *E. floccosum*. *C. albicans* was inhibited by five

percent of the isolates. The isolates from this region did not show anti-dermatophytic activity.

Out of 367, hundred and forty actinomycetes were inhibited the growth of *B. subtilis* and 128 isolates revealed activity against *S. aureus*. *V. fischeri* and *P. vulgaris* growth was inhibited by minimum number of actinomycetes. Eighty eight actinomycetes showed activity against *E. coli*. *B. cinerea* growth was inhibited by hundred and three actinomycetes. 148 actinomycetes showed activity against bacteria and hundred and four actinomycetes inhibited the growth of fungi (Table 6 and Figure 5). Fifty five isolates exhibited activity against both bacteria and fungi.



**Figure 4.** Antifungal activity of actinomycetes. A-ATSI-5, B-CTW-12, C-ERI-1, D-ATW-13, E-AMW-25, F-CBR-2, G-CBR-20, H-ERIAS-17, I-ERIAS-16, J-AMR-20.

#### 4. Discussion

In the present study, 367 actinomycetes were isolated from different parts of Western Ghats forest soil of Tamil Nadu. All the isolates were evaluated for their potential activity against bacteria (Gram-positive and Gram-negative) and fungi. All the 367 actinomycetes were evaluated for their antibacterial and antifungal activity. Most of the actinomycetes exhibited activity towards Gram-positive bacteria (38.15%) when compared with Gram-negative bacteria. Rajendran *et al*[15] isolated *Streptomyces aurantiacus* AAA5 strain showed activity against *S. aureus* and *V. anguillarum*; none of the isolates showed activity against Gram-negative bacteria and fungi. Subramani and Narayanasamy[16] isolated 288 *Streptomyces* strains, 111 isolates exhibited antimicrobial activity against human pathogens, and 151 showed antifungal activity against two plant pathogens. Two hundred and eighty actinomycetes were isolated from Kanyakumari exhibited good antibacterial and anti fungal activity. Among the isolates ERI-1, ERI-3 and ERI-26 showed significant activity against both Gram-positive and Gram-negative bacteria. These isolates were also exhibited good antifungal activity. Jeffrey[17] observed four strains with antimicrobial property out of 61 isolates. Yedir *et al*[18] reported 320 strains of actinobacteria; only thirty two isolates showed strong activity against yeast, moulds and bacteria. Dhevendaran and Praseetha[19] studied the effect of pigment producing *Streptomyces* against six bacterial species; only 10% isolates showed moderate antagonistic activity against all the tested pathogens.

Most of the soil actinomycetes from Western Ghats of Tamil Nadu showed more activity against Gram-positive bacteria (*B. subtilis*) than the Gram-negative bacteria (*E. coli*), which corroborated with previous observations[20,21]. Basilio and co-workers[22] observed that the activities against Gram-negative bacteria were less frequent than the Gram-positive bacteria. These findings could be attributed to the difference in the sensitivities of Gram-positive and Gram-negative bacteria to the inhibiting effects of antibiotics and the differences in the structure and composition of their cell walls. Gram-positive bacteria have cell walls consisting of several layers of peptidoglycan. In contrast, Gram-negative bacteria possess a unique outer membrane, a thinner peptidoglycan layer, and a periplasmic space between the cell wall and the cell membrane. In general, Gram-negative bacteria showed low levels of activity or resistance to antibiotics; it may be due to its outer membrane, which consists of lipopolysaccharides (LPS). This may block certain antibiotics and protecting the sensitive inner membrane and cell wall[23].

A total 367 actinomycetes were isolated from different parts of the forest soil samples. Actinomycetes recovered from Kanyakumari showed some significant activity against most of the tested bacteria and fungi. Hundred and forty actinomycetes exhibited activity against *B. subtilis* and hundred and twenty eight actinomycetes inhibited the

growth of *S. aureus*. Hundred and three actinomycetes showed activity against *B. cinareae*. ERI–1, ERI–3 and ERI–26 isolates from Western Ghats of Kanyakumari District revealed significant activity against all the tested bacteria and fungi. Further studies are focused on standardization of fermentation conditions for ERI–1, ERI–3 and ERI–26 to improve the effective antimicrobial production in liquid medium and isolation of the active molecules

### Conflict of interest statement

We declare that we don't have conflict of interest.

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