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Isolation, Identification and Antimicrobial Activity of Marine Actinomycetes Isolated from Parangipettai

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Abstract

Five Actinomycetes strains were isolated from soil collected in two different regions of parangipattai. The physico-chemical characteristics of soil samples are analysed. Morphological studies indicated that the strains belonged to the genera *Streptomyces spectabilis, Actinomadura roseale, Streptomyces platensis, Streptomyces kavamyceticus* and *Streptomyces citricolor.* Out of five isolated Actinomycetes species, one was selected for antimicrobial activity against five human pathogens. *Streptomyces citricolor* showed the best level of antibacterial effect against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Vibrio cholerae* whereas did not showed any antifungal effect against *Aspergillus niger, Fusarium oxysporum,Rhizoctonia solani, Candida albicans* and *Penicillium citrinum.*

Key Words: Physico-chemical characteristics, Actinomycetes, Human pathogens and Antimicrobial activity

Introduction

Actinomycetes are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds viz., substrate mycelium and aerial mycelium. Among actinomycetes, the streptomycetes are the dominant. The non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera. Members of the actinomycetes, which live in marine environment, are poorly understood and only few reports are available pertaining to actinomycetes from mangroves.

Actinomycetes are numerous and widely distributed microorganisms in nature. They are the best common source of novel antibiotics (Okami and Hotta, 1988). In the last decades actinomycetes became almost the fruitful source for antibiotics. In the 60's and 70's of the 20th century 75 to 85% of all discovered antibiotics derived from the order Actinomycetales, mainly from *Streptomyces* species. In the 70's and 80's the ratio and significant of the other, non *Streptomycete* actinomycetes (so called rare actinomycetes) increased upto 20% of cell microbial antibiotics, and 30-35% of Actinomycetales species.

Streptomyces is the most common actinomycete genus in soils, which forms 90% of the populations. However, new approaches for the isolation of soil actinomycetes have revealed that other genera are also significant and many new species have been isolated. Most of them have been demonstrated to produce novel secondary metabolites. Although the first antibiotic from actinomycetes has been reported more than 50 years ago (Schatz *et al*., 1944). Since more than 4000 new bioactive compounds have been obtained, the search for new actinomycetes of interest to biotechnology is still important.

Most of the bioactive microbial metabolites were isolated from actinobacteria especially from *Streptomycetes* and also from some rare actinomycetes. During the last 20-30 years, the interest in the marine microflora increased due to the investigation of novel bioactive compounds especially antibiotics and enzymes. As the frequency of novel bioactive compounds obtained from terrestrial actinobacteria decreases with time, actinobacteria from diverse environments have been increasingly screened for their ability to produce new secondary metabolites. It has been emphasized that actinobacteria from marine sediments may be valuable for the isolation of novel strains which could potentially yield a broad spectrum of secondary metabolites (Ismet,2004) .there has been a worldwide search for antibiotics from various terrestrial substrates and geographic regions (Hockenhull, 1963 and Podogi, 1984).

The genus *Streptomyces* was classified in the family Streptomycetaceae on the basis of morphology and cell-wall chemo type. Streptomycetes are Gram-positive, aerobic micro-organisms with DNA G + C contents of 69 – 78 mol% (Wendish & Kutzner, 1992); they produce extensive branching substrate and aerial mycelia that develop into chains of spores by the formation of cross-walls in the multinucleate aerial filaments (Anderson & Wellington, 2001). The Streptomycetes are widely used in industry because of their ability to produce numerous chemical compounds, including enzymes, antitumour agents and antibiotics (Berdy, 1995).

Materials and Methods

Collection of Soil Samples

The soil samples were collected from two regions of Parangipettai. Soil samples were air-dried under room

temperature for about 30 days before isolation .A second set used air-dried soils stored at room temperature over a long period (9-11 months). Soil pretreatment was required for inhibiting or eliminating unwanted microorganisms. Moist heat treatment (Water Bath, Bibby Sterllin) had been employed to select various actinomycetes groups.

Physico-chemical analysis of soil

The physical and chemical analyses of the soil samples were carried out by using standard methods (APHA, 1975).

Isolation of Actinomycetes

Isolation of actinomycetes was performed by plating technique using Starch casein agar medium (Kuster and Williams, 1964). The medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 minutes. Then it was supplemented with pinch amount of streptomycin and griseofulvin to prevent the bacterial and fungal growth. The medium was poured into the sterile petriplates. The collected soil samples were diluted up to 10^{-6} and 0.1ml of the diluted samples was spread over the agar plates. The inoculated plates were incubated at 28 ± 2°C for 7-10days. After incubation the actinomycetes colonies were observed, and used for further investigation.

Biochemical characterization

The Biochemical characterization of actinomycetes sp. was carried out by using standard methods (Shrilling and Gittlieb., 1966).

Utilization of carbon sources

Kuster's agar medium was prepared with carbon sources like xylose, inositol, sucrose, mannitol and Fructose separately. Actinomycetes cultures were streaked into the medium. The plates were incubated at 28+ 2Oc for 4-7 days, after which the tubes were examined for the growth pattern.

Culture Preservation

Nutrient agar slants were used for short time preservation of purified actinomycetes. The actinomycetes were inoculated in nutrient agar slant using a sterile loop and incubated at 25°C for 2 or 3 days. The one drum vial containing actinomycetes were kept in refrigerator at 4°C for short time storage. For longterm preservation, the expected bacterial growth were collected from subculture or purification plate by sterile inoculating loop and adequately mixed by vortex mixer. The isolates were kept at -40ºC in 10% glycerol broth.

Test microorganisms

Antimicrobial activity of the selected isolates was tested against some bacterial and fungal organisms such *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae, Aspergillus niger, Fusarium oxysporum,Rhizoctonia solani, Candida albicans* and *Penicillium citrinum.*

Assay of Antibacterial activity

Streptomyces citricolor was grown on starch casein broth and incubated for 7 days at 28°C. After incubation, the culture was centrifuged at 1500 rpm for 20 minutes. The sterile nutrient agar medium was poured into each sterile petriplates and allowed to solidify. Using a sterile cotton swabs, fresh bacterial cultures with known population count was speared over the plates by following speared plate technique. The well-cut prepared are placed over the media inoculated with appropriate bacteria, then supernatant of these actinomycetes cultures is added in to the well.all the plates were incubated at 37º C for 24-48 hours. After the incubation period the results were observed and measured the diameter of clear zone was recorded after 3 days.

Assay of Antifungal activity

In the freshly prepared and sterilized potato dextrose agar medium, a pinch of streptomycin was added and mixed well. Then it was poured into each petriplate and allowed to solidify. Using sterile cotton swabs freshly fungal culture was spread over the plates. The well-cut prepared are placed over the media inoculated with appropriate fungi, then supernatant of these actinomycetes culture is added into the well. All the plates were incubated at 27ºC for 48-72 hours. The diameter of inhibition zone was measured.

Results and Discussion

The physico – chemical analysis of the soil samples from two regions of parangipettai, was indicated in Table-1. Two soil samples showed the alkaline pH. The values of Turbidity, TDS, Conductivity and DO of two samples were distinguished.

The soil sample was collected from parangipettai of Tamilnadu. Five actinomycetes isolates were obtained in pure form. Out of five actinomycetes the dominant one was analysed for their antimicrobial activities. The pure cultures were maintained on the same medium that was used for isolation and preserved at 4º C.

Coastal soils and the oceans could be precious resource of both novel actinomycetes taxa and novel bioactive compounds. Goodfellow and Haynes (1984) reviewed the literature on isolation of actinomycetes from marine sediments and suggested that this source may be valuable for the isolation of novel actinomycetes with the potential to yield useful new products, and the taxonomic work was used to direct studies for natural product discovery.

The five isolates were identified up to the species level and found to be four of *Streptomyces* sp. and one was *Actinomadura* based on the morphological, physiological and biochemical properities of isolates was provided in Table 2 while the utilization of different carbon sources was showed in Table 3.

The antimicrobial activities of culture filtrates of *Streptomyces citricolor* were determined against five pathogenic bacteria and fungi and the results were given in Table 4. The culture filtrate showed significant antibacterial activity. The maximum zone of inhibition was noted in *Klebsiella pneumoniae* of 22mm followed by *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Solmonella typhi* of 14mm, 12mm, 13mm and 20mm respectively.

In the present investigation, it has been observed that, compared to other actinomycetes, *Streptomyces citricolor* showed efficient growth in starch casein broth. Thus, this isolate was selected for the antimicrobial activity. There were five pathogenic bacteria tested for antibacterial activity. Of these, *Klebsiella pneumoniae* was highly inhibited with culture filtrate of *Streptomycetes citricolor*.

Normally, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Salmonella typhi* are even capable of growth in some antibiotics and their resistance to more antibiotics has also

been medical concern (Tortora, *et al*., 2000).In this study, *Streptomyces citricolor* is found to exhibit a broad spectrum of activity against *Klebsiella pneumoniae* and *Salmonella typhi.*

Eighty three percent of actinomycetes isolated from Sagamy Bay were found to be antifungal (Okami and Okazaki, 1972).But in the present study, *Streptomyces citricolor* failed to inhibit the test pathogenic fungi. Recent investigations indicate that tremendous potential of marine actinomycetes, particularly *Streptomyces* sp as a useful and sustainable source of new bioactive natural products.

The culture filtrate of *Streptomyces citricolor* did not show any activity on pathogenic fungi were given in table 5

Table 1: Physico-chemical characteristics of soil samples

Name of samples	pH	Turbidity (NTU)	Salinity (ppt)	TDS (mg/l)	Conductivity (ms)	DO (mg/l)
parangipattai	8.07	2.44	4.5	3.83	5.91	1.6
Salt pan	8.00	3.16	2.1	1.91	3.07	2.4

Table 2: Biochemical characteristics of Actinomycetes

Test	*S.spectabilis*	*A.roseole*	*S.platensis*	*S.kavamyceticus*	*S.citricolor*
Indole	-	-	+	-	+
MR	+	-	-	+	-
VP	-	-	-	-	-
Urease	+	+	+	+	-
Citrate	+	+	-	+	-
Nitrate reduction	-	+	-	-	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+

\+ → Positive
\- → Negative

Table 3: Carbohydrate Utilization Test

Carbon Sources	*S.spectabilis*	*A.roseole*	*S.platensis*	*S.kavamyceticus*	*S.citricolor*
Xylose	+	+	+	-	+
Inositol	+	+	+	+	-
Sucrose	+	+	+	+	+
Mannitol	+	-	+	±	-
Fructose	+	-	+	+	-

\+ → Positive
\- → Negative
± → Moderate

Table 4: Antibacterial activity of *Streptomyces citricolor*

S.No.	Bacterial Pathogens	Zone of inhibition (mm)
1.	*Pseudomonas aeruginosa*	12
2.	*Escherichia coli*	13
3.	*Klebsiella pneumonia*	22
4.	*Salmonella typhi*	20
5.	*Vibrio cholera*	14

Table 5: Antifungal activity of *Streptomyces citricolor*

S.No.	Fungal Pathogens	Zone of inhibition (mm)
1.	*Aspergillus niger*	-
2.	*Rhizoctonia solani*	-
3.	*Candida albicans*	-
4.	*Fusarium oxysporum*	-
5.	*Penicillium citrinum*	-

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