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ACTINOMYCETES DIVERSITY IN FIVE FRESH WATER SYSTEMS OF PUDUKKOTTAI, TAMIL NADU AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Actinomycetes are excellent elaborators of biotechnological products such as antibiotics, industrial enzymes and other bioactive compounds. Emergence of multiple drug resistant pathogens implies the need to search for new and novel antimicrobials and the discovery of new molecules from actinomycetes has marked an epoch in antibiotic research and subsequent developments in antibiotic chemotherapy. Sediment samples were collected from five different ponds of Kottaipattinam region, Pudukkottai District situated near the Bay of Bengal. Actinomycetes strains were isolated from the air-dried sediment samples by applying serial dilution and spread plate technique on three different media such as Glycerol-Arginine Agar, Starch-Casein Agar, and Starch-Nitrate Agar. Different media were used in order to compare the recovery on different media as well as to obtain maximum diversity of actinomycetes strains. The test organisms used were bacteria such as Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus, Vibrio cholerae, Salmonella paratyphi and Staphylococcus epidermidis. The test fungus/yeast used were Aspergillus niger and Candida albicans. The test organisms were collected from Government Hospital, Tiruchirappalli, Tamil Nadu. In the present work, the antibacterial activity of actinomycetes strains were checked by using three different media (such as Glycerol Argine Agar (GAA), Starch-Casein Agar (SCA) and Starch Nitrate Agar (SNA) and three methods (like cross streak method, spectra-plate method and well diffusion method). However, the strains showed antibacterial activity only by well diffusion method on Glycerol-Yeast Extract Agar for bacteria and Potato Dextrose Agar for fungi. Thus, it is concluded that the substances repressing these strains were accumulated only in the mycelium and not in the medium.

KEY WORDS

Actinomycetes, Freshwater ponds, Sediment, Medium, Antibacterial activity

INTRODUCTION

Actinomycetes, a group of gram-positive filamentous free-living saprophytic bacteria with true aerial hyphae widely distributed in soil and colonizing plant, are well known as a good source of microbial secondary metabolite producer in drug discovery programmes. They are excellent elaborators of biotechnological products such as antibiotics, industrial enzymes and other bioactive compounds (Goodfellow *et al*., 1998; Lam, 2006; Ndonde and Semu, 2000; Sacramento *et al.*, 2004). Among the genus of actinomycetes group, *Streptomyces* species account for more than 70 per cent of the total antibiotic production (Ningthoujam *et al.*, 2009). Emergence of multiple drug resistant pathogens implies the need to search for new and novel antimicrobials (Wise, 2008; Demain and Sanchez, 2009) and the discovery of new molecules from actinomycetes has marked an epoch in antibiotic research and

subsequent developments in antibiotic chemotherapy (George *et al*., 2012). The rise of antibiotic resistant pathogenic strains dictates an increasing need for the survey of unexplored and under explored niche habitats for novel antibiotic producing actinomycetes strains.

Further, literature regarding fresh water actinomycetes appears to be scarce and hence the present study was attempted at isolating promising actinomycetes strains and screening them for antibiotic activities from freshwater bodies which are numerous in this part of the globe.

MATERIALS AND METHODS

Sediment sampling and pre-treatment

Sediment samples were collected from five different ponds of Kottaipattinam region, Pudukkottai District (Lat. 10.38° N, long. 78.82° E) situated near the Bay of Bengal. Each collection was made from a 10-15 cm depth of soil sediment. The samples were then air dried for 10 days (Williams *et al.*, 1972), crushed and sieved. The sieved sediment soils were then used for actinomycetes isolation.

Isolation of Actinomyctes from Samples

Actinomycetes strains were isolated from the air-dried sediment samples by applying serial dilution and spread plate technique on three different media such as Glycerol-Arginine Agar (El-Nakeeb and Lechevalier, 1963), Starch-Casein Agar, and Starch-Nitrate Agar (Cochrane, 1961). Different media were used in order to compare the recovery on different media as well as to obtain maximum diversity of actinomycetes strains. Plates were incubated at room temperature for 2 - 3 weeks. After incubation, typical actinomycetes colonies were selected on morphological basis (Shirling and Gottlieb, 1966) and were transferred to Glycerol-Arginine Agar and maintained at room temperature. The plates were incubated at 28 - 30°C for 2 - 3 weeks. Isolated colonies were further subcultured on GA plates (Saadoun *et al*., 1999; Williams and Cross, 1971). Selected pure isolates were then used for antimicoribal assay.

Test Organisms

The test organisms used were bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus, Vibrio cholerae, Salmonella paratyphi* and *Staphylococcus epidermidis*. The test fungus/yeast used were *Aspergillus niger* and *Candida albicans*. The test organisms were collected from Government Hospital, Tiruchirappalli, Tamil Nadu.

Antimicrobial Activity

Velvety actinomycetes isolates were examined microscopically to determine if they are gram-positive, filamentous or had long cells. Mycellium structure, arrangement of conidospore and arthrospore on the mycelium was observed microscopically (Olympus, 201, Japan) using oil immersion (100X) objective. Observed structure was compared with Berger's Manual of Determinative Bacteriology and the organism identified. Morphologically distinct actinomycetes isolates were selected for antimicrobial activity screening against the pathogenic test organisms. This was done by using Kirby-Bauer method (Bauer *et al*., 1966).

Test group isolates were grown on antibiotic producing medium (GS medium) in an orbital shaker (150 rpm ambient temperature 28 °C). The fermentation broths were centrifuged after third, fifth and seventh days of incubation and the supernatant used as crude antibiotic extract of the isolates.

Test pathogens were spread on the test plates - Nutrient Agar (NA) for bacteria and Sebouround agar (SA) for yeast and fungi. Sterile antibiotic discs (Himedia, 5 mm diameter) impregnated with the crude antibiotic extract of the test isolates were transferred to the test plates and incubated for 24 - 48 h. The zone of inhibition (in mm diameter) were read and taken as the activity against the test pathogen.

RESULTS AND DISCUSSION

Actinomycetes isolates were classified into 5 different groups based on visual observations (Table 1). Actinomycetes with green aerial mycelium and yellow substrate mycelium were placed under **A_1**, those with white aerial mycelium and brown substrate mycelium as **A_2**, actinomycetes with white aerial mycelium and yellow substrate mycelium were considered as **A_3**, those with aerial mycelium and black substrate mycelium as **A_4** and actinomycetes with white aerial mycelium and pink substrate mycelium as **A_5**.

The actinomycetes load in the five different fresh water pond sediments are shown in Table 2. As seen from the table, ponds-5 and 3 (P 5 and P 3) sediment samples recorded the maximum (18-19 $\times$ 10^7 cfu/gm) and pond 4 (P 4) the minimum (2 $\times$ 10^7 cfu/gm) load. Among the various nutrient medium used, the maximum load was found to occur in Glycerol Arginine Agar (19 $\times$ 10^7

cfu/gm) medium and the lowest in Starch Nitrate Agar Medium (2×10^7 cfu/gm).

The percentage occurrence of the different actinomycetes group in the different ponds are presented in Table-3. As evident from the table, group **A_1** recorded the highest count in pond-5 (55.4%) and the least in pond-3 (32.3%). The group **A_2** recorded the highest percentage in pond-1 (26.5%) and the lowest in pond (20.2%); the group **A_3** recorded the highest percentage in pond-3 (23.7%) and the lowest in pond-1 (3.3%); the group **A_4** recorded the highest percentage in pond-4 (10.8%) and the least in pond-3 (8.4%) and **A_5** recorded maximum in pond-3 (11.6%) and the lowest in pond-4 (8.2%). Thus, among the various group of actinomycetes, **A_1** recorded the highest percentage (46.64%) and **A_4**, the least percentage (9.7%).

The antimicrobial activity using the different groups of actinomycetes are presented in Table 4. While **A_1** recorded the maximum antimicrobial activity against *Klebsiella pneumoniae* and *Escherichia coli*, **A_3** showed highest activity against *Salmonella typhi, Bacillus cereus, Virio cholerae, Salmonella paratyphi* and *Aspergillus niger* and **A_5** recorded the highest activity against *Staphyloccoccus epidermidis* and *Candida albicans*; **A_2** and **A_4** recorded intermittent activity. Thus, each group recorded differential antimicrobial activity. Nevertheless, among the different groups of actinomycetes, **A_3** appeared to be the best candidate as it showed the highest antimicrobial activity (both bacteria and fungi) against five of the nine pathogens examined.

Optimization of the conditions for antibiotic synthesis of the strains as well as establishing a suitable media for antibiotic production is essential to determine their activities. In the present work, the antibacterial activity of actinomycetes strains were checked by using three different media (such as Glycerol Argine Agar (GAA), Starch- Casein Agar (SCA) and Starch Nitrate Agar (SNA) and three methods (like cross streak method, spectra-plate method and well diffusion method). However, the strains showed antibacterial activity only by well diffusion method on Glycerol-Yeast Extract Agar for bacteria and Potato Dextrose Agar for fungi. Thus, it is concluded that the substances repressing these strains were accumulated only in the mycelium and not in the medium as also observed by Moncheva *et al*. (2002) and George *et al.* (2011).

The search to discover a novel compound of pharmaceutical interest requires the isolation of a large number of isolates and will be more promising if diverse actinomycetes are sampled and screened (Oskay *et al*., 2004). Such an endeavour can lead to discoveries and new uses of secondary metabolites which can be used in other areas which will undoubtedly help mankind.

Table-1: Actinomycetes in the sediment samples from five different freshwater ponds

Sample	Medium	Actinomycetes Load
Pond-1	Glycerol Argine Agar (GAA)	3×10^7
	Starch Casein Agar (SCA)	16×10^7
	Starch Nitrate Agar (SNA)	4×10^7
Pond-2	Glycerol Argine Agar (GAA)	8×10^7
	Starch Casein Agar (SCA)	3×10^7
	Starch Nitrate Agar (SNA)	4×10^7
Pond-3	Glycerol Argine Agar (GAA)	18×10^7
	Starch Casein Agar (SCA)	4×10^7
	Starch Nitrate Agar (SNA)	7×10^7
Pond-4	Glycerol Argine Agar (GAA)	16×10^7
	Starch Casein Agar (SCA)	14×10^7
	Starch Nitrate Agar (SNA)	2×10^7
Pond-5	Glycerol Argine Agar (GAA)	19×10^7

Starch Casein Agar (SCA)	8×10^7
Starch Nitrate Agar (SNA)	4×10^7

Where,

A_1 : Actinomycetes with Green aerial mycelium and Yellow substrate mycelium
A_2 : Actinomycetes with White aerial mycelium and Brown substrate mycelium
A_3 : Actinomycetes with White aerial mycelium and Yellow substrate mycelium
A_4 : Actinomycetes with White aerial mycelium and Black substrate mycelium
A_5 : Actinomycetes with White aerial mycelium and Pink substrate mycelium

Table-2: Percentage occurrence of Actinomycetes in the pond system

	Pond-1 (P_1)	**Pond-2 (P_2)**	**Pond-3 (P_3)**	**Pond-4 (P_4)**	**Pond-5 (P_5)**
A_1	50.2	42.1	32.3	53.3	46.64
A_2	26.5	24.2	24.0	20.2	23.06
A_3	3.3	13.7	23.7	6.5	10.28
A_4	9.4	9.4	8.4	10.8	9.68
A_5	10.6	10.6	11.6	8.2	10.12

Where,

A_1 : Actinomycetes with Green aerial mycelium and Yellow substrate mycelium
A_2 : Actinomycetes with White aerial mycelium and Brown substrate mycelium
A_3 : Actinomycetes with White aerial mycelium and Yellow substrate mycelium
A_4 : Actinomycetes with White aerial mycelium and Black substrate mycelium
A_5 : Actinomycetes with White aerial mycelium and Pink substrate mycelium

Table-3: Antimicrobial activity of Actinomycetes against microbial pathogens

		Pond-1 (P_1)	**Pond-2 (P_2)**	**Pond-3 (P_3)**	**Pond-4 (P_4)**	**Pond-5 (P_5)**
1.	*Escherichia coli*	18	16	17	9	10
2.	*Klebsiella pneumoniae*	20	17	15	12	12
3.	*Salmonella typhi*	12	10	22	21	18
4.	*Bacillus cereus*	12	15	18	15	15
5.	*Vibrio cholerae*	16	13	20	17	16
6.	*Salmonella paratyphi*	13	10	16	9	11
7.	*Staphylococcus epidermidis*	10	11	14	11	17
8.	*Aspergillus niger*	8	12	16	13	12
9.	*Candida albicans*	9	10	14	12	16

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