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STUDIES ON FRESH WATER ACTINOMYCETES DIVERSITY OF MAYANUR DAM, TAMIL NADU, INDIA

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ABSTRACT

Actinomycetes are a group of physiologically versatile Gram-positive filamentous bacteria found in most environments including terrestrial and aquatic habitats. In India, studies on fresh water actinomycetes are rare. Hence, the present study was attempted to study their diversity in a fresh water system located in Karur District, Tamil Nadu, India. Fresh water sediment samples were collected from three different locations of Mayanur Dam, Karur District, Tamil Nadu. The Actinomycetes loads in the sediments of the three different sites in the Mayanur Check Dam are presented. The occurrence and distribution of different genera of actinomycetes in the sediments of different sites are a total of 11 actinomycetes species could be isolated from the three sites. Site-2 recorded the presence of 9 species of actinomycetes while Site-2 recorded the presence of 11 species and Site-3 recorded the presence of 8 species. While *Streptomyces* (pure-white) and *Streptomyces* (dark ash) were absent in Site-1. *Streptomyces* (Blue ash), *Microtospora* sp. and *Nocardiopsis* sp. were absent in Site-3 and Site-2 recorded all the 11 species of actinomycetes. Thus Site-2 recorded maximum actinomycetes diversity followed by Site-1 and 2. The percentage frequency of the identified genera of actinomycetes was found to fluctuate. The frequency of the genus *Streptomyces* was found to be 52.6% followed by *Saccharopolyspora* (16.6%) and *Actinopolyspora* (12.8%). *Actinomyces* sp. was the actinomycetes that appeared in the least frequency (1.2%). In the present study, the actinomycetes population was found to vary in the three sites that were examined. This is probably the reason why actinomycetes showed a higher diversity in Site-2 when compared with Site-1 and Site-3.

Keywords: Fresh water sediment, Actinomycetes diversity, Colonies, Frequency

INTRODUCTION

Actinomycetes are a group of physiologically versatile Gram-positive filamentous bacteria found in most environments including terrestrial and aquatic habitats (Strelczyk *et al.*, 1969) due to their metabolic potential (Navami *et al*., 2015; Buedenbender *et al*., 2017). In aquatic habitats, actinomycetes plays a great role in carbon cycle due to their ability to grow at low concentrations of carbonaceous substances and to degrade recalcitrant organic matter (Kuznetsov, 1970). Even though they make up a large part of the microbial population in aquatic systems (Lacey, 1973; Nakayama, 1981; Goodfellow and Williams, 1983), diversity and bioprospecting studies on actinomycetes are mainly focused on terrestrial and marine ecosystems and less on fresh water systems (Radhika *et al*., 2011; Saravanan *et al*., 2015). In India, studies on fresh water actinomycetes are rare. Hence, the present study was attempted to study their diversity in a fresh water system located in Karur District, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of Soil Samples

Fresh water sediment samples were collected from three different locations of Mayanur Dam, Karur District, Tamil Nadu during the year 2018-2019.

Sample Treatment

Heat treatment was performed by holding the sediment samples in a water bath at 50 °C for 60 min. for prevention of other bacterial flora. All samples were diluted (up to 10^{-6}) with sterile 0.5% saline prior to inoculation into the isolation plates (Takizawa *et al*., 1993).

Starch casein agar medium (Difco Chemicals) (g/l: starch 10, casein 0.3, KNO3 2, NaCl2, K2HPO4 2, MgSO4.7H2O 0.05, CaCO3 0.02, FeSO47H2O 0.01 and agar 18; and supplemented with Griseofulvin and Cycloheximide (Himedia, Mumbai) 25 and 10 mg/ml) was used for the isolation of actinomycetes and enumeration was done as per Kuster and Williams (1964). The diluted sediment samples (0.1 ml) were spread over the medium with a sterilized bent (L) rod and plate spinner. The inoculated plates were incubated at 30 °C for seven to ten days. After incubation, colonies were purified using streak plate technique and maintained for further investigation.

Purified isolates of actinomycetes were identified using morphological and cultural characteristics by the methods as described in the International Streptomyces Project

(ISP) (Shirling and Gottlieb, 1966). The morphology of the spore bearing hyphae with the entire spore chain, the structure and arrangement of the spore chain with the substrate and aerial mycelium of the actinomycetes were examined using slide culture technique and identified (Williams *et al.*, 1989). After growth, the slide cultures were examined under light microscope. Color of spore mass was visually estimated by using the colour chart (Pridham, 1965).

RESULTS AND DISCUSSION

The Actinomycetes load in the sediments of the three different sites in the Mayanur Check Dam are presented in Tables 8-10 and the cultural characteristics of the actinomycetes isolates are presented in Tables 8 & 9 (Plate-7). The occurrence and distribution of different genera of actinomycetes in the sediments of different sites are presented in Table 9. As evident from Tables 9 & 10, a total of 11 actinomycetes species could be isolated from the three sites. Site-2 recorded the presence of 9 species of actinomycetes while Site-2 recorded the presence of 11 species and Site-3 recorded the presence of 8 species. While *Streptomyces* (pure-white) and *Streptomyces* (dark ash) were absent in Site-1. *Streptomyces* (Blue ash), *Microtospora* sp. and *Nocardiopsis* sp. were absent in Site-3 and Site-2 recorded all the 11 species of actinomycetes. Thus Site-2 recorded maximum actinomycetes diversity followed by Site-1 and 2.

The percentage frequency of the identified genera of actinomycetes was found to fluctuate. The frequency of the genus *Streptomyces* was found to be 52.6% followed by *Saccharopolyspora* (16.6%) and *Actinopolyspora* (12.8%). *Actinomyces* sp. was the actinomycetes that appeared in the least frequency (1.2%).

A perusal of literature reveals that the dominance of *Streptomyces* among the actinomycetes especially in the sediments have been documented by large number of workers (Pisano *et al*., 1986; Balagurunathan *et al.*, 1996; Ravel *et al*., 1998; Moncheva *et al*., 2002; Mansour, 2003; Vijayakumar *et al*., 2007; Dhanasekaran *et al*., 2012; Priyadharsini *et al.*, 2013). The other actinomycetes that commonly occurred were *Saccharoplyspora, Actinopolyspora* and *Microbispora*. A similar result was also reported earlier by Vijayakumar *et al.,* (2007) while studying the biodiversity of actinomycetes in the Palk Strait region in the Bay of Bengal.

In the present study, the actinomycetes population was found to vary in the three sites that were examined. A perusal of literature reveals that the actinomycetes population was dependent on the local physico-chemical variables in the sites like temperature, pH, electrical conductivity, moisture, organic matter, nitrogen and phosphorous content in the soil (Jiang and Xu, 1990; Jensen *et al*., 1991; Saadoun and Al-Momani, 1996; Ndonde and Semu, 2000; Mansour, 2003; Vijayakumar *et al.*, 2007; Dhanasekaran *et al*., 2009; Priyadharsini and Dhanasekaran 2015). In the present study also there was a strong positive correlation temperature (0.52), pH (0.24), nitrate (0.22) and phosphate (0.96) content as opined by others. Thus, it can be suggested that even though the actinomycetes have a wide distribution in various media, their population dynamics are influenced by the physico-chemical variables as well as the nutrient availability at that regions. This is probably the reason why actinomycetes showed a higher diversity in Site-2 when compared with Site-1 and Site-3.

Table-1: Actinomycetes in the sediment samples from 3 sites of Check Dam, Mayanur (cfu/g sediment)

S. No.	Medium	Actinomycetes load	Percentage Occurrences of Actinomycetes in this system
Site-1			
1.	Glycerol Argine Agar (GAA)	$4x10^6$	
2.	Starch Casein Agar (SCA)	$18x10^6$	28.54
3.	Starch Nitrate Agar (SNA)	$5x10^6$	
Site-2			
1.	Glycerol Argine Agar (GAA)	$5x10^7$	
2.	Starch Casein Agar (SCA)	$12x10^7$	42.26
3.	Starch Nitrate Agar (SNA)	$6x10^7$	
Site-3			
1.	Glycerol Argine Agar (GAA)	$7x10^6$	
2.	Starch Casein Agar (SCA)	$14x10^6$	39.54
3.	Starch Nitrate Agar (SNA)	$8x10^6$	

Table-2: Actinomycetes diversity of Mayanur Dam

S. No.	Name of the Actinomycetes (sp.)	Site-1	Site-2	Site-3
1.	*Micromonospora*	+	+	+
2.	*Saccharopolyspora*	+	+	+
3.	*Streptomyces*	+	+	–
4.	*Streptomyces*	–	+	+
5.	*Streptomyces*	–	+	+
6.	*Microbispora*	+	+	–
7.	*Actinopolyspora*	+	+	+
8.	*Actinomadura*	+	+	+
9.	*Nocardiopsis*	+	+	–
10.	*Actinomyces*	+	+	+
11.	*Actinoplolyspora*	–	+	+

Table-3: Genera of Actinomycetes isolates and its culture characteristics

S. No.	Name of the Actinomycetes (sp.)	Aerial mycelium colour	Reverse side colour	Colony size (mm)	% of Frequency
1.	*Micromonospora*	Dark ash	Brown	6.3	4.5
2.	*Saccharopolyspora*	Ash	Light black	2.2	16.6
3.	*Streptomyces*	Bluish ash	Colourless	4.0	52.6
4.	*Streptomyces*	Pure white	Dark yellow	8.5	52.6
5.	*Streptomyces*	Dark ash	Dark ash	3.0	52.6
6.	*Microbispora*	Ash	Dull yellow	4.0	8.9
7.	*Actinopolyspora*	Dull white	Yellow	4.2	12.8
8.	*Actinomadura*	Blue	Pink	2.5	3.6
9.	*Nocardiopsis*	Light green	Yellow	3.2	3.1
10.	*Actinomyces*	Light rose	Light orange	2.0	1.2
11.	*Actinoplolyspora*	Ash	Dull black	2.4	1.4

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