TBR
Trends in Biotechnology Research
An International Peer-Reviewed Journal
Online and Print Version
www.sciencejournal.in

SEARCHING THE SUBMERGED: A REPORT ON PREVALENCE OF ACTINOMYCETES IN SEDIMENTS OF RIVER GODAVARI AND OPTIMIZED STRATEGY FOR THEIR ISOLATION

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ABSTRACT
The present work aims to serve as a contribution to the knowledge on microbiology of aquatic habitats in India. River sediments were collected using a cylindrical coring device, specially designed for the purpose using a PVC pipe (5ftX6Cm) with sharply filed ends to facilitate penetration into the sediments and could be capped from both the ends to contain the sample within. The sediment sample was subject to two pretreatments (heat pre treatment and drying for a week) and the fresh wet sediment used as a control. The chosen set of media was Actinomycetes Isolation Agar (AIA); Starch Casein Agar (SCA), Kuster's Agar (KA) and Water Yeast Extract Agar (WYEA). Each of these media was supplemented to yield modified forms by addition of the following two- 50 µg/l Potassium Dichromate and a combination of 10µg/mL Nalidixic Acid and 10µg/mL Cycloheximide to reduce bacterial and fungal contamination, with unsupplemented plates being the control. Each of these modifications was further supplemented with 25% sediment extracts in an attempt to make the media 'habitat-based', the control in this case being the modified & unmodified medium, without the sediment extracts. Modified media and controls thus obtained were used in three sets - one each for the pre treated samples and third for fresh wet sediment, making a total of 72 combinations. A combination of Actinomycetes Isolation Agar and Starch Casein Agar supplemented with 10µg/mL Nalidixic Acid and 10µg/mL Cycloheximide and 25% sediment extracts gave a high yield of actinomycetes.

KEY WORDS: Aquatic, Actinomycetes, habitat-based media, sediment extracts.

INTRODUCTION

Searching unexplored habitats with modified sampling methods, 'habitat-based media' and better understanding of microbial ecology of studied habitats constitute the future strategies in biodiscoveries (Kurtböke, 2012). This realization, combined with a perception that environmental stresses may lead to a novel chemistry through natural selection and exhaustion of terrestrial habitat as a source bioactive microbes leading to rediscovery of already known compounds, has perhaps been the driving force behind of focus shift from terrestrial to other habitats for search of bioactive actinomycetes in the recent years (Ningthoujam, Sanasam, & Nimaichand, 2009). Extensive research through these shifts of habitat points to a promising future, but there is a habitat that has remained relatively 'neglected' as recognized commonly- The aquatic habitat. Actinomycetes and bioactive compounds in general, from aquatic habitats have been relatively neglected (Kümmerer, 2009; Ningthoujam et al, 2011; Rifaat, 2003); however potential value of these habitats as source of actinomycetes that produce useful products has been demonstrated by recent researches (Benimeli, Castro, Chaile, & Amoroso, 2007; Cwala, Igbinosa, & Okoh, 2011; Fuentes, Benimeli, Cuozzo, & Amoroso, 2010; Hahn et al., 2003; Jiang & Xu, 1996; Mane & Deshmukh, 2009; Ningthoujam et al., 2009; Rifaat, 2003). River Godavari is the second largest river in India next to the Holy Ganga. A very little study on microbiology especially with reference to Actinomycetes of Godavari River has been recorded. The present investigation was designed to reveal the presence of actinomycetes in the sediments of this river.

MATERIALS AND METHODS

Site selection and sampling station

The site studied was the Godavari river stretch at Nagina Ghat, between Goverdhan Ghat and Old Mondha Flyovers of Nanded City. The site was chosen after carefully assessing it in terms of ease of reach, sampling, safety and easy identification (viz. between the two mentioned flyovers) which made the site reproachable for further field replication.

Designing Equipment

Difficulties in sample collection by earlier reported methods (Arifuzzaman, Khatun, & Rahman, 2010; Mohan, Bhasker, & Charya, 2012; Saurav & Kannabiran, 2010; Vonothini, Murugan, Sivakumar, & Sudha, 2008) led us to device a modified sediment coring device. This was done, using a simple PVC pipe purchased from a local hardware supplier. The specifications are: 6 cm diameter and 5 ft height. The height could vary depending upon the depth of sampling. The open ends of the pipe were filed sharp to facilitate penetration into the sediments, and these ends could be sealed using 'caps'. The whole assembly so obtained, the 'corer', was very affordable, light weight, manageable under waters, easy to clean and sanitize and was reusable.

Sediment Collection

The filed end of the corer, with the cap on was lowered into the water and cap removed thereafter, and lowered into the water until it hit the sediment. It was now pushed with application of downward force, into the sediment, until it was approximately 10 cm into it. The corer was then twisted in clockwise and anticlockwise direction to separate the core from the sediment and pulled out. The open end, thus pulled out, was capped immediately and the 'sediment core' thus obtained, was safely transferred to the laboratory. A schematic representation of the coring process and dimensions of the corer is presented in figure 1.

Media and culture conditions

The top 3 cm portion from the extruded core was collected; a part preserved as field duplicate. From the remaining part three aliquots were made, one aliquot was kept for air drying for one week (serially diluted and spread thereafter). Another was serially diluted and heat treated (55^0 Celsius, 6 minutes) (Mincer, Jensen, Kauffman, & Fenical, 2002) whereas the last aliquot was serially diluted and spread directly, serving as a control for heat treatment and air drying.
The isolation media used were *Actinomycetes Isolation Agar* **(**HiMedia, Mumbai India); *Starch Casein Agar* **(**HiMedia, Mumbai, India); *Water Yeast* Extract *Agar* **(**Per liter of distilled water: Yeast Extract, 0.25g; Dipotassium Phosphate, 0.5g; Agar, 18g.) (HiMedia, Mumbai); *Kuster's Agar* **(**Per liter of distilled water: Glycerol, 10mL; Casein, 0.3g, Potassium Nitrate, 3g; Dipotassium Phosphate, 2g; Sodium Chloride, 2g; Magnesium Sulfate, 0.05g; Calcium Carbonate, 0.02g; Ferrous Sulfate, 0.01g; Agar, 16g) (HiMedia, Mumbai). Each of these four media was prepared in three sets: one for untreated samples which served as a control for pretreatment methods, second for heat treated samples and third for air dried (one week) sample. Each of these three sets consisted of un-modified medium plates, modified media plates by addition of each of 50µg/L Potassium Dichromate (Zhang, Ye, & Tang, 2011) and combination of 10µg/mL Nalidixic Acid and 10µg/mL Cycloheximide (NA/C) (Takizawa, Colwell, & Hill, 1993) and sediment extracts (Saurav & Kannabiran, 2010). That made 18 forms of each of the used medium making a total of 72 isolation combinations. The un-modified plates inoculated with fresh wet sediment served as control for all mentioned modifications and pretreatments. The plates were incubated at 30^0 Celsius for 21 days, with monitoring at every 24 hours. Colonies showing typical actinomycetes morphology were picked up as and when they appeared and maintained on the slants of the same medium (supplement free) on which they were isolated.

RESULTS AND DISCUSSION

Media: Actinomycetes Isolation Agar (AIA) and Starch Casein Agar (SCA) yielded most number of actinomycetes colonies. There could not be one best suited medium, because AIA better supported growth of pigmented actinomycetes, whereas SCA yielded a good number of *Streptomycetes spp* colonies, and no combination could yield both *Streptomycetes spp* on SCA and the pigmented actinomycetes on AIA together (data not shown).

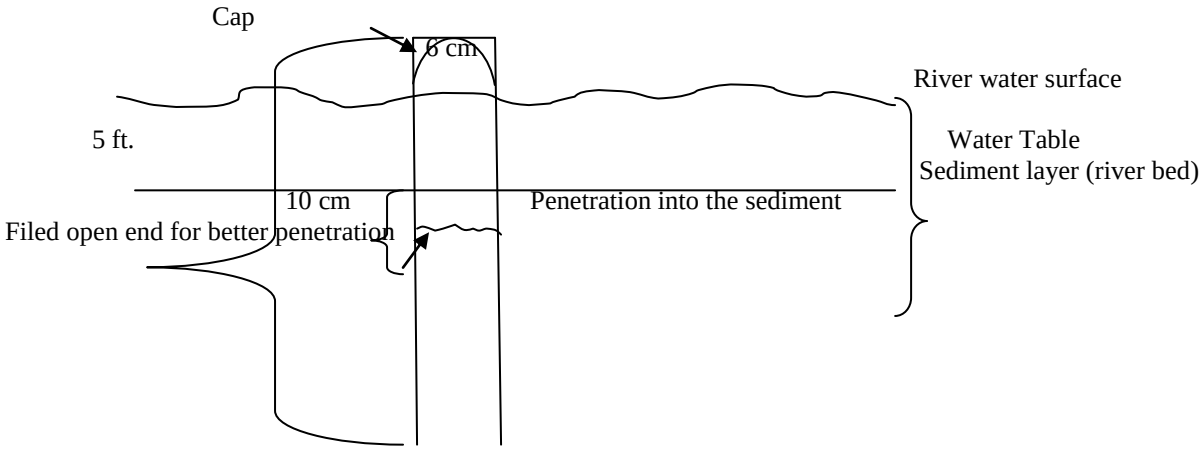


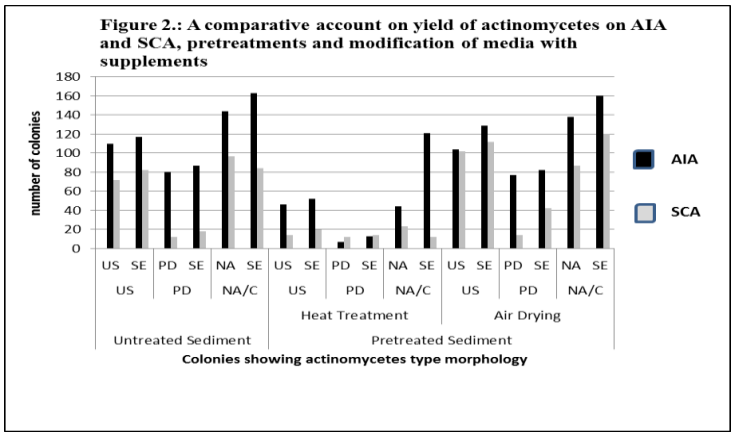
Figure 1. A schematic representation of sampling with a corer. Also shown are the dimensions of the corer

Pretreatments: Both untreated and air dried sediment samples gave a similar yield of actinomycetes on AIA, the only difference being, reduced bacterial contamination in case of air drying. On SCA the number of *Streptomycetes spp* increased when the air dried sample was used. Results with heat treatment have been interesting, though it reduced the yield of actinomycetes, and this finding being consistent with the findings of a study reported earlier (Takizawa et al., 1993)**,** there was a striking contrast observed on AIA supplemented with Sediment Extracts, where heat treated sample gave a very good yield of actinomycetes. *Selective inhibitory agents:* Potassium dichromate successfully lowered the fungal contamination as compared to unsupplemented plates; however the yield of actinomycetes was also reduced.

NA/C combination on the other hand successfully reduced the fungal and non-target bacteria without affecting the yield of actinomycetes. Excessive fungal contamination was observed on unmodified plates (without supplements) of all the tested media along with a poor yield of actinomycetes.

Table 1: Number of colonies with actinomycetes morphology recorded on the two high yielding media. US: Un supplemented (un-modified) medium; PD: Potassium Dichromate; NA/C: Nalidixic Acid and Cycloheximide; S: Sediment Extract.

Medium	Untreated Sediment						Pretreated Sediment											
							Heat Treatment						Air Drying					
	US		PD		NA/C		US		PD		NA/C		US		PD		NA/C	
	US	SE	PD	SE	NA /C	SE	US	SE	PD	SE	NA /C	SE	US	SE	PD	SE	NA /C	SE
Actinomycetes Isolation Agar (AIA)	110	117	80	87	144	**163**	46	52	7	13	44	121	104	129	77	82	138	160
Starch Casein Agar (SCA)	72	82	12	18	97	84	14	20	12	14	23	12	102	112	14	42	87	**120**



AIA: Actinomycetes Islation Agar; SCA: Starch Casein Agar; US: Un supplemented (un-modified) medium; PD: Potassium Dichromate; NA/C: Nalidixic Acid and Cycloheximide; S: Sediment Extract.

Sediment extracts: addition of sediment extracts increased the yield of actinomycetes in every combination tested. As already mentioned, in contrast to deleterious effect of heat on growth of actinomycetes on other tested combinations, heat pretreated samples gave an exceptionally good yield of actinomycetes on AIA supplemented with sediment extracts. Hence AIA and SCA supplemented with NA/C and sediment extracts, with use of air dried samples for SCA, were used for further isolation procedures. Expanding horizons of knowledge in microbiology and bioactive potential of microbes from Indian rivers will unravel the best kept bioactive secrets of these habitats. Studies on diversity and bioactive potential of the isolated actinomycetes reported in this research are currently underway.

ACKNOWLEDGEMENT

The first author is thankful to the University Grants Commission, Government of India for financial support through the UGC-Maulana Azad National Fellowship Scheme and the Hon. Vice Chancellor of SRTM University, Nanded for providing necessary facilities required for the research

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