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Isolation and Identification of Potential Marine Actinomycetes Isolates along the Coast of Bay of Bengal, Visakhapatnam

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ABSTRACT

Till now many antibiotics are available, but some pathogenic organisms are showing resistance to existing antibiotics. So it is mandatory to search new antibiotics for resisting pathogenic organisms. Actinomycetes are very good resource for producing secondary metabolites that is antibiotics; they are Streptomycin, Erythromycin, and Tetracycline. Actinomycetes are available in both marine and terrestrial ecosystem. From terrestrial ecosystem isolation of new strain actinomycetes is very difficult compared with marine ecosystem. Marine ecosystem is a best resource for many strains of actinomycetes, because it is unexplored ecosystem having millions of different types of microbes. Up to 1% of marine actinomycetes were isolated, but still we have to explore more for discovering new microbes. Most of the isolated marine microbes are from Streptomyces genus (70%), marine fungi (20%), Bacillus spp. (7%) and other bacteria (1%-2%). Present study deals with the collection of soil sample from the coast of Bay of Bengal near Sagar Nagar, Visakhapatnam. This article provides you the future scope.

Keywords: Actinomycetes, Antibiotics; Marine ecosystem.

INTRODUCTION

Actinomycetes are described by the arrangement of spreading strings or poles, as often as possible offering ascend to an average mycelium which is unicellular particularly during the beginning phases of development. The hyphae are commonly non septate, under certain extraordinary conditions, septa might be seen in certain structures. The mycelium is either vegetative or filling in substrate, or airborne, where an exceptional mycelium is created over the vegetative development A large number of Streptomyces spp. have been isolated and screened from soil in the past several decades [1,2]. Actinomycetes comprise about 10% of the bacteria colonizing marine aggregates and can be isolated from marine sediments [3].

Actinomycetes repeat through exceptional sporulating bodies or from part of the vegetative mycelium. The spore bearing hyphae are delivered on the mycelium either separately and Monopodially, or in a brush like or bunch like developments, or in verticillate like tufts or whorls upon the mycelium. The sporophores fluctuate from Long to short structures. The spores may likewise be created independently on tips of side branches or in chains appended straightforwardly to the mycelium. The spores are framed either by the discontinuity of the plasma inside the spore bearing elevated mycelium, or by the cycle of division or division of the mycelium into regenerative cells by methods for cross dividers, in a way like oidium development. The sporulating mycelium might be stretching or non-expanding, straight or winding formed. The spores are circular, barrel shaped or oval; they grow by the arrangement of 1 to 3 tubes. Inspite of improvements being made in the cultural methods for the isolation of rare marine actinomycetes, many of these organisms still remain unculturable and have to be detected by using molecular techniques [4,5].

MATERIALS AND METHODS

Sample collection

Two marine samples were collected in different regions along the coast of Bay of Bengal at Sagar Nagar, Visakhapatnam at a depth of 1 meter. All the samples were collected in bottles, maintained with sea water and transported to the laboratory for the isolation of Marine Actinomycetes (**Table 1**).

Table 1: Source, place and description of marine sediment samples.

Sample	Place	Sediment texture and character
1	Sagar Nagar	Fine powdered sample

Isolation of marine actinomycetes

To 90 ml of sterilized distilled water taken separately in 250 ml conical flasks, add 10 g each of the sediment samples and incubate in a shaker at 28°C, 150 rpm for 24 h to separate

filamentous Actinomycetes and also for detachment of spores. After 24 h of incubation and agitation, 2 ml of top suspension of the samples were collected and centrifuged at 1000 rpm for 10 minutes. The supernatants were collected and used for isolation by Pour plate method. Starch Casein Agar (SCA) medium was supplemented with Rifampicin 5 µg/ml and Fluka 25 µg/ml to inhibit bacterial and fungal contamination (**Table 2**).

Table 2: Composition of SCA medium.

Component	Gram/litre
Soluble starch	10
Casein	0.3
KNO_3	2
NaCl	2
K_2HPO_4	2
$MgSO_4$ $7H_2O$	0.05
$CaCO_3$	0.02
$FeSO_4$ $7H_2O$	0.01
Agar	20
Distilled water	500 ml
50% filter sterile sea water	500 ml
pH	7.2

Pour plate method

1 ml of each of the centrifuged extract of the samples was serially diluted to 10^{-10} with sterilized water. 0.1 ml serially distilled water from (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9}) was mixed with molten SCA medium and poured into petri plates. The plates were allowed to solidify and incubated at 28°C for two weeks for the growth of Actinomycetes. After two weeks, the selected individual colonies were streaked on SCA plates until pure cultures were obtained.

Maintenance of pure cultures

The Actinomycetes colonies which appeared different from one another to the naked eye (surface texture), were transferred to SCA petri dishes and incubated at 28°C for two weeks. The isolates which appear identical to the naked eye in respect of color of aerial mycelium, reverse color, soluble pigment and colony texture were eliminated. 12 actinomycetes isolates were isolated from Sagar Nagar sample.

Pure cultures were maintained on SCA medium in a petri dish for better sporulation. The pure cultures from petri dishes with SCA medium were inoculated into slants, incubated at 28°C for seven to ten days until good growth was observed and stored in refrigerator at 4°C. The cultures were sub cultured for every 4 weeks.

RESULTS AND DISCUSSIONS

Sample collection

One marine sediment sample was collected in the coast of Bay of Bengal at Sagar Nagar, Visakhapatnam at a depth of I meter (**Table 3**).

Table 3: Characteristics of marine sediment sample.

Sample	Color	Texture
Sagar Nagar	Brown	Soft and sandy

The marine sediment samples collected from Sagar Nagar, Visakhapatnam were selected for isolation.

Isolation of actinomycetes

Isolated samples were pour plated on a SCA medium in a laminar air flow. After pour plating was done, petri dishes were incubated in an orbital shaker at 28°C for 14 days. After 14 days, actinomycetes colonies were carefully isolated from different plates avoiding any bacterial or fungal contamination. Actinomycetes colonies can easily be distinguished on the plate from those of fungi and through bacteria. They are often compact, leathery giving a conical appearance and have a dry surface. The actinomycetes colonies which appeared different from one another to the naked eye in respect of colour of aerial mycelium, reverse colour, soluble pigment and colony texture were transferred and incubated at 28°C for 2 weeks and maintained on starch casein. About 40 actinomycetes isolates were obtained from the samples (**Table 4**) (**Figure 1**).

Table 4: Number of isolates obtained from marine sediment samples.

Sample	Pour plate
Sagar Nagar	40

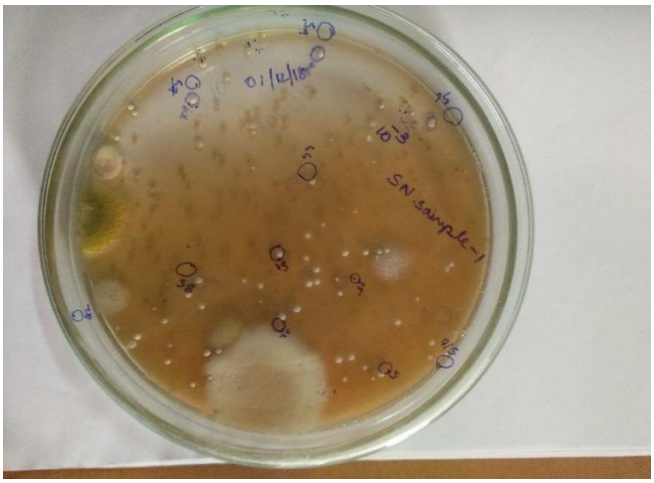

Figure 1: Isolates of actinomycetes obtained from marine sediment sample.

Pure culture preparation

Individual colonies or mixed colonies from the isolation plates were picked up and streaked on SCA medium. Mixed colonies containing more than one culture were distinguished on the pure culture plates and incubated for 14 days at 28°C and stored in a refrigerator. They can be used as master cultures (**Figure 2**).

Figure 2: Streaking of colonies on SCA medium.

CONCLUSION

Present study involved isolation of marine actinomycetes from the sample collected from the coast of Bay of Bengal near Sagar Nagar, Visakhapatnam. About 40 isolates were obtained from the marine samples. We have selected 8 isolates among them and named them as S1-S8. This study concludes that the marine environment is really a good source for isolation of Actinomycetes.

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