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Screening of rare actinomycetes diversity in marine sample from Atharampattinam village by adopting modified conventional methods

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ABSTRACT
Recovery of rare actinomycetes from ecological sample is a key for discovery of novel biomedical drugs. Isolation of undiscovered actinomycetes from marine environment switched over by recovering non Streptomyces colonies. Rare genera of actinomycetes were isolated from marine sediments by selective isolation procedures.Totally95 actinomycetes colonies were isolated and 11 of them were isolated by conventional dilution method. Among the isolates,72.7% of them belong to Streptomycesspand 27.2% of them non Streptomyces in nature. Maximum recovery (87.5%) of genus Streptomycesspwas isolated from the sample treated 0.1% phenol. Isolation of nonstreptomyces colonies were also recovered by different adopted isolation methods such as, 0.5Msucrose gradient centrifugation (80%) followed bydry heat treatment (64%). The non Streptomyces colonies were identified as Nocardiasp, Planomonosporasp, Micropolysporasp, Streptosporangium sp and Micromonospora sp.The research confirms application of heat treatment and sucrose gradient centrifugation promotes the selective isolation of non Streptomyces from environmental samples.
Key words: nonstrptomyces; Planomonospora; Sucrose solution; SDS; Phenol

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INTRODUCTION

Actinomycetes are widely distributed in natural environment and play an important role in antibiotics production [1]. Marine-derived actinomycetes are rich sources of novel secondary metabolites which harbour unique structures and have diverse antimicrobial activity. Marine microorganisms widely distributed on our oceans of the earth and emerging as the great source for antibacterial antifungal, antiinfective, enzyme inhibitors and antiviral. Most isolates reported predominantly produced by the Genera of *Streptomyces* sp[2]. Rare *Actinomycetes* are usually regarded as strains whose isolation frequency much lower than those strains isolated by conventional methods. Efforts on isolation of rare Actinomycetes have discovered some genera, such as *Actinomadura*, *Actinoplanes*, *Micromonospora*, and *Microtetraspora* that had been recovered from many soil samples[3].When conventional isolation techniques were applied, most of the isolates recovered on agar plates have been identified as genus *Streptomyces*, which are the dominant actinomycetes in soil [4]. For the purpose of screening novel bioactive molecules, several factors must be considered: choice of screening source, pretreatment, selective medium, culture condition, and recognition of candidate colonies on a primary isolation plate[5]. The role of rare actinomycetes as bioactive molecule sources became apparent as these organisms provided about 25% of the antibiotics of actinomycete origin reported during 1975 to 1980 [6] . Rare actinomycetes have usually been regarded as strains of actinomycetes whose isolation frequency by conventional methods is much lower than that of *Streptomycete* strains. Consequently basic knowledge of the habitat, physiology and productivity of molecules of rare actinomycetes gradually increased. An alternative approach was to make the isolation procedure more selective by adding chemicals such as phenol to the soil suspension [7]. Specialized growth media were developed to isolate specific actinomycete genera [8].Choice of natural materials like soils in researches is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore hopefully, novel metabolites as a result of the geographical variation[9] . Besides, the important approaches helpful in discovering new microbial species or unknown bioactive substances include

isolation and characterization of microorganisms from the most extreme habitations and relatively unknown or unstudied areas [10] .

MATERIAL AND METHOD

Sampling and study area

Marine sediments were collected Atharampattinam sea shore. Samples were collected 10 cm depth in zizag manner between 100 meter distances. 10 sampling sites were chosen. All the samples collected aseptically in a sterile container. One part of (1g) of each sample mixed together in a sterile container and used for isolation

Heat treatment:

About 10 gm of air dried soil sample was heated at 60° C for 30 min and then serially diluted up to 10^{-7} by normal saline. One ml of 10^{-6} was poured on sterile plates and overlaid with Actinomycetes isolation agar. All the plates were incubated at 28° C for 15 days.

Phenol treatment

1gm of soil was mixed with 10 ml of 0.1% phenol and stirred well for 30 min. Then the aliquot was serially diluted up to 10^{-7} and one ml of 10^{-6} was poured on sterile plates and overlaid with Actinomycetes isolation agar. All the plates were incubated at 28° C for 15 days.

SDS treatment

1gm of soil was mixed with 10 ml of 1% SDS and stirred well for 30 min. Then the aliquots was serially diluted up to 10^{-8} using 1% SDS and one ml of 10^{-6} was poured on sterile plates and overlaid with Actinomycetes isolation agar. All the plates were incubated at 28° C for 15 days

Sucrose gradient centrifugation

Soil sample was serially diluted with 0.2, 0.4, 0.6, 0.8 and 1M sucrose of sucrose solution and centrifuged at 10,000RPM for 30 minutes. One ml of 10^{-6} aqueous suspension of different concentration was poured on sterile plates and overlaid with Actinomycetes isolation agar. All the plates were incubated at 28° C for 15 days.

Identification by Spore and mycelium[11]

Spore ornamentation was observed by a Nikonphoto microscope. Mycelia production was identified by Slide culture method and the nature of mycelium was determined by staining with Sudan black.

RESULTS AND DISCUSSION

Differences among diversity of actinomycetes by sample treatment procedure were studied by basic conventional isolation procedure with slight modification. Fine powdery, granular and leathery colonies were isolated from marine sediment and identified based on their spore and mycelia study. Initially the aerial mycelia were white and turns to brown, ash, gray, red, chalky white in nature. Substrate mycelium is pale yellow to brownish black. Studies shows untreated sample shows recovery of 11 actinobacterial colonies contain *Streptomyces* sp with spiral chain of retractile spores was most frequently isolated followed by *Micromonospora*sp with monospore on substrate mycelium. Besides the treated sediments, phenol treatment significantly found to be selective for isolation of *Streptomyces* sp given 87.5 % of *Streptomyces*sp out of 16 isolates. Among the nine different selective isolation methods it was observed that the heat treated and sucrose gradient centrifugation are found to be most effective methods for selective isolation of nonstreptomyces. The frequency of isolated actinomycetes was given in table1. Treatment of sample with SDS showed maximum colony forming unit of actinomycetes than untreated soil and also enhanced the recovery of *Micromonospora* sp. Similarly least number of actinomycetes was observed in chlorinated sediments. Based on spore morphology the isolates were identified as *Streptomyces*sp, *Micromonospora*sp, *Micropolyspora*sp, *Nocardia*sp, *Streptosporangium* sp and *Planomonospora*sp. The frequency of non Streptomyces was given in table 2. Soil sample treated at 60°C showed reduction on recovery of *Streptomyces* sp.Soil suspension treated at 70°C for 15 min inhibited the fungal and bacterial colonies, thus the recovery of actinomycetes, specifically, rare actinomycetes, increased up to 50% of the total microorganisms. Phenol treatment of soil suspension lowered the number of fungi and other bacteria, but the actinomycetes were less affected, thus 65% of the colonies belonged to rare actinomycete s[12-13].The maximum frequency of *Micromonospora*sp (70%) and *Nocardia*sp was 20 % were isolated from heat treated soil. Other non streptomyces like *Micropolyspora*sp and *Planomonospora* sp were predominant in 1M sucrose solution treated sediments. Sample treated with 5% sodium chloride showed the isolation of *Streptosporangium* sp. The significant interest of search for rare and new actinomycetes is a key for drug discovery due to a growing need for the development of new and potent therapeutic agents. Characteristics of the spore bearing hyphae and spore chains was determined by phase contrast microscopy using slide culture techniques [14]. Though there are many methods available to isolate Actionomycetes application of pre-treated soil is adopted for better recovery

[15].Rare actinomycetes are more difficult to cultivate than *Streptomyces* species due to the requirement of appropriate isolation procedures [16] and optimized selection methods. Selective isolation of genera *Herbidospora*, *Microbispora, Microtetraspora* and *Streptosporangium* can be done by chloramines treatment as chlorination is known to suppress growth of contaminant bacteria and promotes the growth of these rare actinomycetes [17].

Table 1: Frequency of isolated actinomycetes from soil sample

S.No	Sample	No. of. Streptomyces	Streptomyces (%)	No. of. Non Streptomyces	Total
1.	Untreated soil	8	72.7	3	11
2.	Heat treated	0	0	6	6
3.	Phenol treated (0.1%)	14	87.5	2	16
4.	Chlorinated sample (10 ppm)	1	33.33	2	3
5.	SDS (5%)	7	63.63	4	11
6.	Sucrose Gradient centrifugation (0.25M)	6	54.54	5	11
7.	Sucrose Gradient centrifugation (0.5M)	2	13.33	12	15
8.	Sucrose Gradient centrifugation (0.75M)	1	7.69	12	13
9.	Sucrose Gradient centrifugation (1M)	1	11.11	8	9

Table 2: Frequency of non Streptomyces isolates from soil

S.No	Sample	Genus name	Frequency percentage
1.	Untreated soil	*Micromonosporasp*	27.2
2.	Heat treated	*Micromonosporasp* *Nocardiasp* *Micropolysporasp*	70 20 10
3.	Phenol treated (0.1%)	*Micromonosporasp*	12.5
4.	Na Cl treatment (5%)	*Streptosporangiumsp*	66.66
5.	SDS (5%)	*Micromonosporasp*	36.36
6.	Sucrose Gradient centrifugation (0.25M)	*Micromonosporasp* *Planomonosporasp*	27.27 18.18
7.	Sucrose Gradient centrifugation (0.5M)	*Micromonosporasp* *Micropolysporasp* *Nocardiasp* *Planomonosporasp*	20 20 20 26.6
8.	Sucrose Gradient centrifugation (0.75M%)	*Micromonosporasp* *Micropolysporasp* *Planomonosporasp*	38.46 23.07 30.76
9.	Sucrose Gradient centrifugation (1M)	*Micropolysporasp* *Planomonosporasp*	33.33 55.55

CONCLUSION

We have evaluated the different isolation methods for selective isolation of rare actinomycetes. Other than Streptomyces all other are sensitive to phenol. Chlorine, SDS ,sucrose reduced the frequency of Streptomyces. Isolation of actinomycetes followed by the treatment of sucrose gradient centrifugation was a fast and suitable method for the recovery of nonstreptomyces. Similarly, elimination of unwanted bacteria by heat, chlorine and SDS treatment also enhanced the recovery of nonstreptomyces colonies.

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