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Isolation of Marine Organisms and their Antifungal Studies

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Marine organisms are a good source of new secondary metabolites that possess many biological activities including antibacterial, antifungal, anticancer, insecticidal and enzyme inhibition. Actinomycetes are known to produce bioactive substances, especially antibiotics that are effective against phytopathogenic fungi. Biocontrol with beneficial bacteria is one promising alternative to fungicides. Hydrolases such as chitinase contribute to degradation of fungal cell walls . Chitin is the second most abundant polysaccharide in nature and a major component of fungal walls, ,insect exoskeletons and crustacean shells.Chitinase secreted by a BCA is likely to be effective against pathogenic fungi, the cell walls of which are mainly Chitin. Several species have been isolated and screened from the soil in the past decades. Many of these secondary metabolites possess biological activities and have the potential to be developed as therapeutic agents. Bio-active compounds from marine organisms possess distinct chemical structures that are used in the synthesis of new drugs that could be used against pathogens. In this paper isolation of marine organism samples are collected at different marine environments and habitats and their antifungal studies are described.

Key words: Secondary metabolites, Bio-active compounds, Antifungal activity.

Certain marine organisms are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds *viz.*, substrate mycelium and aerial mycelium. Among several marine organisms, many actinomycetes and the Streptomycetes are the dominant. The non-Streptomycetes, are called rare actinomycetes, comprising approximately 100 genera. Members of the actinomycetes, which live in marine environment, are poorly understood and only few reports are available pertaining to actinomycetes from mangroves[1-2]. Terrestrial actinomycetes are soil organisms which have characteristics common to bacterial and fungi and yet possess sufficient distinctive features to delimit them into a distinct category. . On agar plates, they can easily be distinguished from true bacteria. Unlike slimy distinct colonies of true bacteria which grow quickly. Actinomycetes colonies appear slowly, show powdery consistency and stick firmly to agar surface. A closer look at a colony under the compound microscope reveals slender unicellular branched mycelium (diameter of the hyphaerarely exceeding one micron) forming asexual spores for propagation. The above marine organisms are proved to be producing bio active substances. Literature studies clearly indicate antibiotics are effective against phytopathogenic fungi[3-4].

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MATERIALS AND METHOD

Identification Methods

Numerical Taxonomic Approach

Numerical taxonomy involves examining many strains for a large number of characters prior to assigning the test organism to a cluster based on shared features. The numerically defined taxa are polythetic; so, no single property is either indispensable or sufficient to entitle an organism for membership of a group. Once classification has been achieved, cluster-specific or predictive characters can be selected for identification[5]

Numerical taxonomy was first applied to Streptomyces by[6].The numerical taxonomic study of the genus Streptomyces by Williams et al. (1983).[7-8] Involves determination of 139 unit characters for 394 type cultures of Streptomyces; and the former co-efficient is being used to define the clusters. His study includes 23 major, 20 minor and 25 single member clusters. Thus, numerical taxonomy provides us with an invaluable framework for streptomyces taxonomy, including identification of species[9].

Secondary metabolites

The marine habitat is particularly important for discovery of novel bioactive products. Although major attention has been dedicated to the study of shallow and deep water sediments, microbial associates of marine molluscs have proven to be a rich source of biologically active substances such as antimicrobial and antitumor compounds[10-11]. Shellfish molluscs are known to be natural accumulators of microorganisms due to their filter-feeding habit. Their internal tissues are enriched by nutrient compounds and adhesive substances which provide a unique set of conditions for microbial association, quite different from those in the surrounding seawater or sediment[12]. Value of shellfish microbial associates include the metabolic removal of waste products, protecting their hosts by chemically mediated defense mechanisms from dangers like predation or pathogen, and production of bioactive metabolites of pharmaceutical application[13].

Actinomycetes, including the common soil genus Streptomyces, are proven source of structurally diverse natural products, possessing broad ranges of biological activities[14]. Examples include antibiotic (erythromycin and tetracycline), anticancer (mitomycin and daunomycin), immunosuppressant (rapamycin and FK506), and veterinary (thisostrepton and monensin) agents. While terrestrial strains have been actively isolated and screened for decades in academia and industry, Streptomycetes isolated from the marine environment have been largely ignored until recently. Early reports suggested that marine actinomycetes were derived from terrestrial sources and that they existed as metabolically inactive spores[15].

Isolation of marine actinomycetes

Sample of each sediment was first mixed, suspended in sterile distilled water as described below. 5g of soil sample was mixed within 45 ml of sterile distilled water blank. This suspension was serially diluted up to 10^{-2}. One ml of the diluted sample was taken from 10^{-2}dilution and the samples were pour plated on Starch Casein Agar (SCA) medium and they were incubated at 37°C for 7 days.

Composition of Starch Casein Agar (g/L) [Kuster and Williams, (1964)]

Starch	10
Casein	0.3
CaCOƒ	0.02
Feƒ SO„ .	0.001
KNOƒ	2
K_2HPO_4	2
MgSO„	0.005
Nacl	2
Distilled water	1000
Agar	20
pH	7.0 ± 0

Quadrant Streak Technique

In order to obtain well- isolated discrete colonies, the quadrant streak should be used. As the original sample is diluted by streaking it over successive quadrant, the number of organism decreases. Usually by the third or fourth quadrant only a few organisms are transferred on the inoculating loop and these produce a few isolated colonies.

The quadrant streak technique is described below,

1. Flame the inoculating loop until it is red hot and then allows it to cool. Remove a small amount of actinomycetes growth with the sterile inoculating loop.
2. Immediately streak the inoculation loop very gently over a quarter of the plate using a

back and forth motion

3. Flame the loop again and allow it cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2).
4. Flame the loop again and allow it cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3)
5. Flame the loop again and allow it cool. Going back to the area that you just streaked (area 3), extend the streaks into the third quarter of the plate (area 4).

Fungal cultures

Fungal phytopathogen *Rhizoctoniasolani* which causes sheath blight in rice. They were grown on Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (50¼g/ml) antibiotic to avoid the bacterial contamination. They were preserved in PDA slants at 4ÚC.

Composition of Potato Dextrose Agar (g/L)

Potato	200
Dextrose	20
Agar	20
pH	6.5 ± 0.2

Table 1. Secondary metabolites produced by actinomycetes:

S.No	Compound	Source	activity
1	Erythromycin	*Saccharopolyspora erythrae*	Antibacterial
2	Kanamycin	*Streptomyces kanamyceticus*	Antibacterial
3	Rapamycin	*Streptomyces hygroscopicus*	Antifungal
4	Himastatin	*Streptomyces hygroscopicus*	Antitumor
5	Amphotericin	*Streptomyces nodosus*	Antifungal
6	Tylosin	*Streptomyces fradiae*	Antibacterial
7	Urdamycin A	*Streptomyces fradiae*	Antitumor
8	Fosfomycin	*Streptomyces fradiae*	Antibacterial
9	Streptomycin	*Streptomyces griseus*	Antibacterial
10	Valinomycin	*Streptomyces griseus*	Mitochondrial toxin
11	Chloramphenicol	*Streptomyces venezuelae*	Antibacterial
12	Rifamycin	*Amycolatopsis mediterranei* U32	Antibacterial
13	Amythiamicins	*Amycolatopsis* sp.	Antibacterial
14	Clorobiocin	*Streptomyces coelicolor*	Inhibitor of bacterial gyrase
15	Meilingmycin	*Streptomyces nanchangensis*	Antiparasitic
16	Nanchangmycin	*Streptomyces nanchangensis*	Insecticidal
17	Nikkomycins	*Streptomyces ansochromogenus*	Antifungal
18	TubelactomycinA	*Nocardia* sp.	Antibacterial
19	Spiramycin	*Streptomyces ambofaciens*	Antibacterial
20	Nogalamycin	*Streptomyces nogalater*	Antibacterial
21	Pristinamycin	*Streptomyces pristinaespiralis*	Antibacterial
22	Oligomycin	*Streptomyces avermililis*	Cell growth inhibitor
23	Actinomycin	*Streptomyces chrysomallus*	Antitumor
24	Dunamycin	*Streptomyces* sp.	Antitumor
25	Resormycin	*Streptomyces platensis*	Herbicidal, antifungal
26	Ileumycin	*Streptomyces lavendulae*	Antifungal
27	Mitomycin	*Streptomyces lavendulae*	Antitumor
28	Lomofungin	*Streptomyces lomodensis*	Antifungal, antibacterial
29	Axenomycins	*Streptomyces lisandri* nov.sp.	Antiprotozoal, Antifungal
30	Tetracycline	*Streptomyces aureofaciens*	Antibacterial
31	Saptomycins	*Streptomyces* sp.HP 530	Antitumor, Antimicrobial
32	Fattiviracin A1	*Streptomyces microflavus*	Antiviral
33	FK 506	*Streptomyces tsukubaensis*	Antiviral
34	Retamycin	*Streptomyces olindensis*	Antitumor
35	Apramycin	*Streptomyces tenebrabrius*	Antibacterial

RESULTS AND DISCUSSION

Isolation of actinomycetes

A total of 16 actinomycetes isolates (Fig. 1) were obtained from marine sediments collected from Kanathur East coast area, Chennai, India. The pure cultures of actinomycetes were maintained on starch casein agar (SCA) slants at -20°C.
Primary screening of culture filtrates of marine actinomycetes against plant pathogens:

Table 2. Primary screening against plant pathogen

S. No.	Isolate code	Antifungal activity	Zone of Inhibition (mm)
1	P-304	+	3
2	P-309	+	2
3	P-311	+	2
4	P-312	+	2

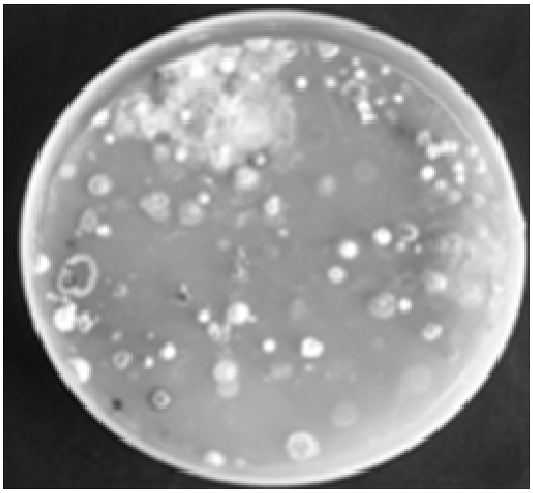

Plate showing actinomycetes colonies in sample A dilution 10^{-1}

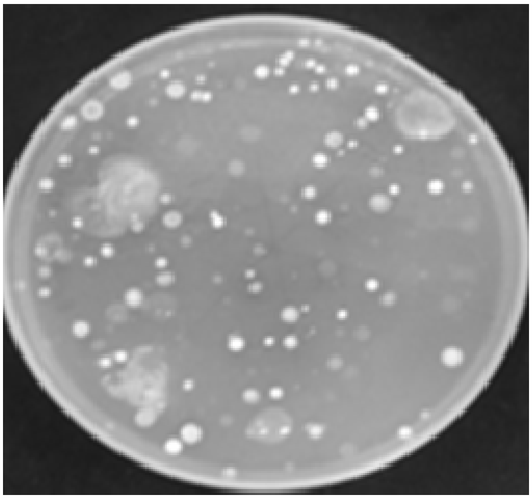

Plate showing actinomycetes colonies in sample A dilution 10^{-3}

Fig. 1. Isolation of acitnomycetes

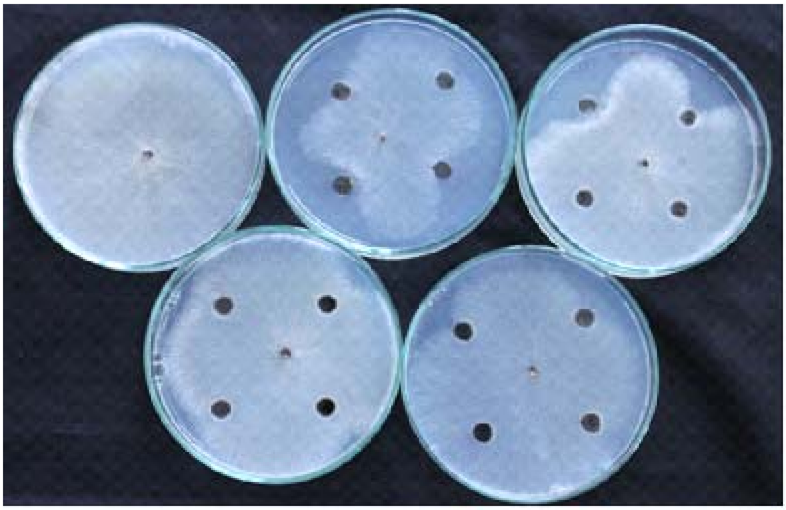

Fig. 2. Screening the culture filtrates of marine actinomycetes for antifungal activity

Among 16 isolates, four isolates showed antifungal activity against *Rhizoctoniasolani*. The four isolates, p- 304, p- 309, p- 311,p- 312 showed antifungal activity with a inhibition of 3 mm followed by 2 mm respectively. (Fig 2, Table 2).

CONCLUSION

Among the 16 isolates of marine actinomytes that were collected from the marine sediments, only four potential strains were isolated. These four potential strains showed high antifungal activity.

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