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Isolation of Marine Actinomycetes and Screening its Antibacterial Potential

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Abstract: ***Antibacterial and pharmaceutical potential of marine isolates are considered as a significant objective in the present research. Marine actinomycetes were isolated and screened for its antibacterial activity. Five isolates showing chalky white and whitish grey colour colonies were isolated and designated as ACT_1, ACT_2, ACT_3, ACT_4 and ACT_5. ACT_2 exhibited the inhibitory zones of 20mm, 19mm, 19mm and 22mm respectively against Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Bacillus subtilis (ATCC 6051) and Staphylococcus aureus (ATCC 9144). ACT_5 showed maximum inhibitory zones of 22mm, 18mm, 21mm and 24mm against the respective test organisms. Thus, the obtained results in the present research revealed the pharmaceutical applications of marine metabolites. The types of antibiotics and its structures synthesized by the marine isolates shall be studied as future study.***

Keywords: Actinomycetes, Antibacterial activity, Pathogens, Antibiotics, Pharmaceutical

I. INTRODUCTION

Marine actinomycetes produce unique secondary metabolites for their successful surveillance in the high salinity of the sea water. They have greatest potential to produce different types of bioactive compounds which can be used for drug development and therapeutic applications (Thangapandian et al., 2007). Secondary metabolites from actinomycetes exhibit tremendous novelty. Actinomycetes are Gram-positive filamentous spore formers with high G+C (>55%) content of DNA (Cwala et al., 2011). Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and actinomycetes alone produce 10,000 of these compounds (Vimal et al., 2009). Many of these secondary metabolites are potent antibiotics, which has made actinomycetes the primary antibiotic-producing organisms exploited by the pharmaceutical industry (Jensen et al., 2007).

Repeated use of antibacterial drugs had resulted in the development of resistance against them. In particular, multiple drugs resistance is one of the major problem and new bioactive compounds are required to overcome this multi-drug resistance (Black et al., 1982). Antibiotic resistance is a complex, continually evolving problem which is often difficult to put into perspective (Levy, 2002). The resistance problem is due to increased use of antibiotic and the presence resistance gene in the microbial strains. Hence there is a need for development of new drugs that act on new targets and those that block resistance mechanisms (Levy, 1994).

Considering the rise of resistant pathogens that ruin current antimicrobial therapeutic options, the necessity to explore the unexplored areas in an ecofriendly, and safe manner to isolate and to identify potent compounds is very crucial. In the present study, the antimicrobial potential of culture filtrates of actinomycete species was studied against medically important fungal and bacterial pathogens.

II. MATERIALS AND METHODS

A. Sample Collection and isolation of actinomycetes (Korn-Wendisch and Kutzner, 1992)

Marine sediment samples were collected at different locations from the southeast coast of Bay of Bengal, India at a depth of 50mts. The sediment samples were dried and used for the isolation of actinomycetes. Sediment samples were serially diluted and plated on to Actinomycete isolation agar supplemented with cycloheximide (25 mg/ml) and nalidixic acid (25 mg/ ml). All the inoculated plates were incubated at 28°C for 7-14 days to obtain white chalky colonies.

B. Screening Antibiotic Producing Actinomycetes

Actinomycete isolated from the marine samples was screened for its antibiotic production ability. Cell free extracts of each isolates were tested for antibacterial activity against four bacterial pathogens by well diffusion method. About 15-20ml of Muller Hinton Agar was poured on sterile plates. The test bacterial cultures of *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051) *and Staphylococcus aureus* (ATCC 9144) were swabbed on the surface of the agar plates using sterilized swabs. Wells of 4mm diameter were made onto the agar plates using sterile cork-borer. The cell-free supernatant of Actinomycetes broth was loaded into the well. All the plates were incubated at 37ºc for 24 hours to observe the zone of inhibition around each well.

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C. Mass Culturing of Isolates Using Standard Production Conditions

Spores of each marine isolate (10^5/ml) was used to inoculate 1000ml Erlenmeyer flasks containing 250ml of Actinomycetes broth supplemented with 1% (w/v) of glucose and magnesium. After incubation at 30°C for 48h in an orbital incubator shaker at 200rpm, the pre-culture (10% v/v) was used to inoculate a total volume of 15L culture medium. After six days of incubation the culture broth was filtered to separate mycelium and supernatant.

D. Solvent Extraction of Extracellular Metabolite

The supernatant was extracted twice with equal volume of ethyl acetate. The ratio of filtrate and solvents 1:1 (v/v) was mixed and shaken vigorously. The organic layer was collected and the solvents were evaporated using fume hood. Similar procedure was carried out for the other solvent, methanol.

E. Antibacterial Activity of Solvent Extracts of Marine Isolates

Antibacterial activity of crude solvent extracts was evaluated using standard well diffusion method described earlier. About 15-20ml of Muller Hinton Agar was poured on sterile plates. The test bacterial cultures of *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051) *and Staphylococcus aureus* (ATCC 9144) were swabbed on the surface of the agar plates using sterilized swabs. Wells of 4mm diameter were made onto the agar plates using sterile cork-borer. The solvent extracts of Actinomycetes broth was loaded into the well. All the plates were incubated at 37ºc for 24 hours to observe the zone of inhibition around each well.

III. RESULTS

A. Isolation of actinomycetes

White powdery and dried colonies were observed after the incubation period. Five different colonies showing chalky white and whitish grey colours suspected for actinomycetes were subcultured on AIA agar. The selected actinomycetes were designated as ACT_1, ACT_2, ACT_3, ACT_4 and ACT_5.

B. Screening Antibiotic Producing actinomycetes

Table-1: Antibacterial activity of cell free extracts of marine isolates

S. No	Cell free extracts of marine isolates	Test pathogens (Zone of inhibition in mm)			
		1	2	3	4
1	ACT_1	10	8	9	12
2	ACT_2	18	17	15	17
3	ACT_3	11	9	10	9
4	ACT_4	12	10	9	12
5	ACT_5	19	18	15	16

1: *Pseudomonas aeruginosa* (ATCC 27853), 2: *Escherichia coli* (ATCC 25922), 3: *Bacillus subtilis* (ATCC 6051) and 4: *Staphylococcus aureus* (ATCC 9144)

The selected isolates were investigated for antibacterial activity against drug resistant bacterial pathogens. The screening methods employed showed variable efficacy in detecting antibiotic producers. Among the five isolates, the cell free extracts of ACT_2 and ACT_5 showed good antibacterial efficacy against all the test pathogens. In Table-1, the antibacterial activity of all the five isolates against the pathogens was expressed qualitatively as inhibitory zones. ACT_2 exhibited the inhibitory zones of 18mm, 17mm, 15mm and 17mm respectively against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051) *and Staphylococcus aureus* (ATCC 9144). Similarly, another isolate ACT_5 also showed maximum inhibitory zones against the test organisms. Other isolates ACT_1, ACT_3 and ACT_4 exhibited varied inhibitory zones ranging from 8mm to 14mm which was found not significant comparatively. Hence, the isolates ACT_2 and ACT_5 were further subjected to mass culturing and extraction of extracellular metabolites under laboratory conditions.

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C. Antibacterial activity of ACT_2 and ACT_5 extracts

Table-2: Antibacterial activity of ethyl acetate and methanol extracts of marine isolates

S. No	Cell free extracts of marine isolates	Test pathogens (Zone of inhibition in mm)			
		1	2	3	4
1	ACT_2	20	19	19	22
2	ACT_5	22	18	21	24

1: *Pseudomonas aeruginosa* (ATCC 27853), 2: *Escherichia coli* (ATCC 25922), 3: *Bacillus subtilis* (ATCC 6051) and 4: *Staphylococcus aureus* (ATCC 9144)

Extracellular metabolites of ACT_2 and ACT_5 from the production media was extracted separately using two solvents, ethyl acetate and methanol. Antibacterial activity of the crude extracts exhibited potent antibacterial activity against the test organisms. Both solvent extracts showed affordable inhibitory zones ranging from 18mm to 24mm. In Table-2, the antibacterial activity of the isolate extracts was presented. ACT_2 exhibited the inhibitory zones of 20mm, 19mm, 19mm and 22mm respectively against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6051) *and Staphylococcus aureus* (ATCC 9144). ACT_5 also showed maximum inhibitory zones of 22mm, 18mm, 21mm and 24mm against the respective test organisms.

IV. DISCUSSION

Natural product drug discovery using marine microbes prevailed for many years in pharmaceutical industry. Among the diverse marine microbial communities, actinomycetes have occupied a prominent and significant position as potential producers of structurally complex and unique metabolites (Abirami et al., 2013). Actinomycetes are diverse group of Gram positive bacteria that usually grow by filament formation. They are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents and enzymes (Ravikumar *et al*., 2011). The genus *Streptomyces* has long been recognized as a rich source of useful secondary metabolites and continues to be a major source of new bioactive molecules (Miyadoh 1993).

Based on these biological properties of the actinomycete, in the present research five different organisms were isolated from the marine source. Among the five, two isolates ACT_2 and ACT_5 showed promising antibacterial potential actions against the drug resistant bacterial pathogens. Two solvents were used to extract the metabolite components of ACT_2 and ACT_5. Both ethyl acetate and methanol extracts of isolates showed good antibacterial activity which revealed the presence of secondary metabolite drugs. The mode of action of extracellular solvent extracts on the test organisms was reported earlier in the literature review.

The molecular basis of this action is classified by the interaction of antibiotics targeting essential cellular functions to inhibit bacterial growth. Mode of action of antibiotics synthesized by actinomycetes is a complex process that starts with the physical interaction of the molecule and its specific targets on the bacterial cell components. This involves biochemical, molecular, and structural changes, acting on multiple cellular targets such as DNA replication, RNA synthesis, cell wall synthesis, and protein synthesis (Kohanski et al., 2010).

DNA gyrase (topoisomerase) controls the topology of the DNA by catalyzing the cleavage pattern and DNA binding. This reaction is important for DNA synthesis and mRNA transcription, and the complex antibiotic-topoisomerase prevent replication, leading to death of the bacteria (Chopra et al., 2002).

The enzymatic process is essential for cell growth, making it an attractive target for antibiotics, which inhibits the synthesis of RNA by using a stable connection with high affinity to the β-subunit in the RNA/DNA channel, separating the active site by inhibiting the initiation of transcription and blocking the path of ribonucleotide chain growth (Brötz-Oesterhelt and Brunner, 2008).

The ribosome is composed of two subunits (50S and 30S), which are targets of the main antibiotic that inhibits protein synthesis. Metabolites of actinomycetes act by blocking the 50S subunit, preventing the formation of the peptide chain. And in the 30S subunit it blocks the access of the aminoacyl tRNA-ribosome (Nikaido, 2009).

Antibiotics get inserted into the cytoplasmic membrane of bacteria in a calcium-dependent fashion, forming ion channels, triggering the release of intracellular potassium. Several antibiotics can cause disruption of the membrane. These agents can be divided into cationic, anionic, and neutral agents (Wang et al., 2006).

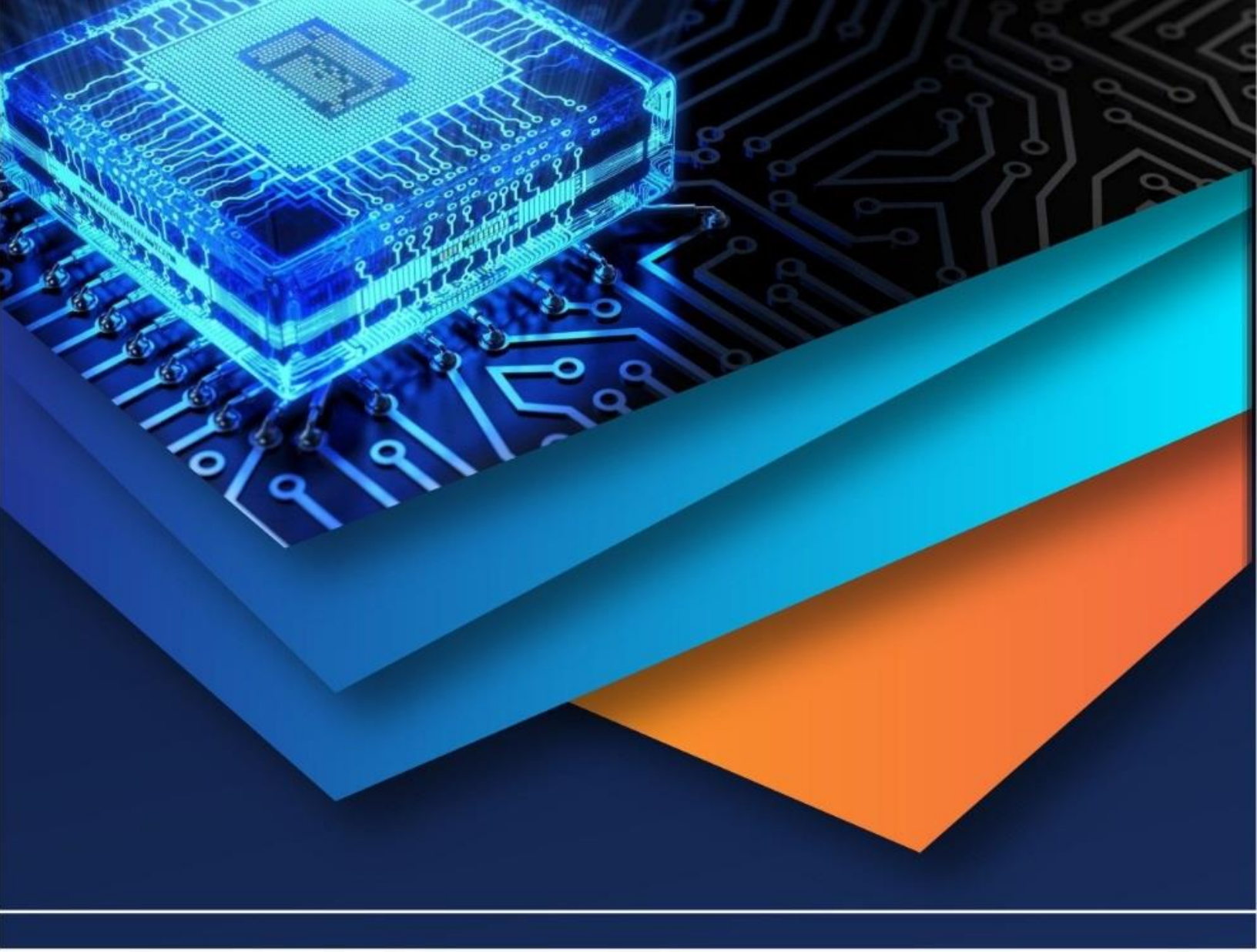
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Actinomycetes were isolated from marine soil samples and screened for antibacterial activity against bacterial pathogens. Out of five isolates, the isolate ACT_2 and ACT_5 showed significant antibacterial activity against all the test organisms. The ethyl acetate and methanol extracts of the marine isolates revealed the presence of different secondary metabolites. The antibacterial potential of the marine metabolites were found to be target specific mode of action on the cell components of bacteria. Mode of action of antibiotics involves biochemical, molecular, and structural changes, acting on multiple cellular targets such as DNA replication, RNA synthesis, cell wall synthesis, and protein synthesis. Thus, the obtained results in the present research revealed the pharmaceutical applications of marine metabolites. The types of antibiotics and its structures synthesized by the marine isolates shall be studied as future study.

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