



**Screening and characterization of selected marine Actinomycetes
from Nagapattinam District**

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Abstract

As marine environmental conditions are extremely different from terrestrial ones, it is surmised that marine actinomycetes might produce novel bioactive compounds. Hence marine sediments such as sea shore obtained from Nagapattinam dist., Tamilnadu. The Actinomycetes isolated from this ecosystem are capable of producing antibiotics that strongly inhibit the growth of gram positive and gram negative bacteria. A total of 10 isolates representing the range of morphological diversity obtained from each sample were obtained in pure culture. However of the 10, two were found to produce antibiotics substances NgpSS -1 and NgpSS-2 exhibited higher activity and was, therefore selected for further studies. Antimicrobial activities of isolates were also tested against various bacterial pathogens. Out of 10 isolates, 4 isolates had antimicrobial activity, with 2 isolates showing broad spectrum of antimicrobial activity.

Keywords: Marine Actinomycetes, Isolation, biochemical characterization, antimicrobial activity.

Introduction

The biodiversity of marine environment proved to be an important resource for isolation of potent microorganisms to produce biologically active secondary metabolites [Bhaskaran R *et al.*, 2011; Ramesh S *et al.*, 2009]. Actinomycetes are a group of bacteria which possess many important and interesting features (Hirsch *et al.*, 1983). It is the best known for their ability to produce antibiotics and is gram positive bacteria which branching unicellular microorganism. Actinomycetes of about 100 genera exist in soil (Yokota *et al.*, 1997). Actinomycetes which is prolific products of antibiotics and important supplier to the pharmaceutical industry.

Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. The researches have been remarkably successful and approximately. Two thirds of naturally occurring antibiotics, including many of medical

importance, have been isolated from actinomycetes, (Okami *et al.*, 1988). Recent days the discovery of known metabolites and actinomycetes are increasing due to the exploitation of natural ecosystems. Exploitation of less and unexplored ecosystems for actinomycetes is highly necessary for the discovery of novel bioactive metabolites.

Actinomycetes are important sources of new bioactive compounds such as antibiotics and *et al.*, 1992) which have diverse clinical effects and are active against many organisms (Bacteria, Fungi, Parasites etc.) Hence the present study made an attempt to estimate the Actinomycetes population in different marine soil, as to screen for their antimicrobial properties. Further, the identified antagonistic Actinomycetes were characterized based on morphological, biochemical, cultural and physiological characteristic.

Materials and Methods

Isolation of Actinomycetes

The actinomycetes strain was isolated from the marine soils from Nagapattinam, Tamilnadu. Isolation of actinomycetes was carried out by Serial dilution and spread plate technique (Collins *et al.*, 1989) in starch Casein agar.

Identification of Actinomycetes

Colony morphology of actinomycetes was noted with respect to colour, mycelium and pigmentation. Spore chain morphology was identified by cover slip culture technique actinomycetes isolate where biochemically characterized.

Screening of antimicrobial activity of Actinomycetes

Primary screening: Antimicrobial activity of actinomycetes was subjected to primary screening by cross streaked plate technique straight line inoculation of the actinomycetes isolates were made on modified nutrient agar medium and incubated at 28°C for 4 to 5 days.

Secondary screening: secondary screening of promising isolates was done by submerged fermentation. slant cultures of mature actinomycetes strains were inoculated in the medium containing soybean meal 20g, glucose 20g, NaCl 4 g, K_2HPO_4 0.05g, $MgSO_4$ 0.05g and $CaCO_3$ 5g, for 1000ml and maintained at pH 7.2. The cultures were incubated in a rotary shaker (180 rev/min) at 27°C for 7 days and the

fermented broth was centrifuged at 10,000rpm at 4°C for 20 min. The supernatant was filtered using 0.45µm pore size membrane filter (Millipore). (Ruan *et al.*, 1977). The clear supernatant samples were tested for their antimicrobial activity by Agar well diffusion method. To determine the pathogenic bacteria cultured on nutrient broth at 37°C for 24 hrs. The cultures were swabbed on nutrient agar media the relative activities based on the diameter of zones of inhibition formed.

Results and Discussion

The selective isolation process resulted in isolation of 10 actinomycetes strains from marine samples Collected from Nagapattinam district. Among 10 Actinomycetes colonies isolated, 2 isolates were morphologically distinct; both 2 isolates exhibited the antimicrobial activity in the primary screening test against bacterial pathogens whereas, in the secondary screening test out of 2 active isolates NgpSS - 2 Isolate showed the potent activity against bacterial pathogens.

Hence, this strain has been taken for further character analysis. The Zone of inhibition of all 2 Isolates were shown in Table (3). The morphological and cultural characteristic of most active isolate were studied on various media (Table 1). Gram staining reveals that the active isolate was Gram positive. Assessment of Physiological characteristics of the strain revealed that they could be grow well at 25 °C and 40°C temperature, pH 7-10 respectively. However the strain had maximum growth rate at a NaCl Concentration of 2-7%. In this study various biochemical test were performed and result were Shown in (Table 2).

Table 1: Cultural characteristics of isolate NgpSS-2

Name of the medium	Growth	Aerial mycelium	Reverse colour	Soluble pigment
Oat meal agar	Moderate ,spreading, powdery	Abundant, powdery, grey	Pale yellow	None
Glycerol Asparagine agar	Good, spreading, powdery	Abundant, powdery ,grey	Yellow	None
Nutrient agar	Moderate, spreading, powdery	Moderate, powdery, grey	Pale yellow	None
Starch casein agar	Abundant, spreading, powdery	Abundant, powdery, grey	Pale yellow	None
Bennetts agar	Good, spreading, powdery	Moderate, powdery, grey	Yellow	None

Table 2 Biochemical characteristics of isolate NgpSS- 2

Reaction	Observation	Results
Indole Production	Negative	Negative
Methyl red	Negative	Negative
VogesProskauer	Negative	Negative
Citrate utilization	Positive	Positive
H ₂ S Production	Negative	Negative
Urease	Positive	Positive
Oxidase	Negative	Negative
Catalase	Positive	Positive
NaCl Tolerance	2% to 7%	
Gram staining	Stained to violet colour	Positive
Spore staining	No stained observed	Negative
Chemical tolerance(pH)	6.5 to10	

Table 3:Antimicrobial activity of selected isolates

Test organisms	NgpSS 1	NgpSS 2
<i>Bacillus subtilis</i>	11	12.4
<i>Staphylococcus aureus</i>	13	17.2
<i>Escherichia coli</i>	12	13.5
<i>Pseudomonas aeruginosa</i>	10.3	12.2
<i>Enterobacter spp</i>	6.5	8.3

Conclusion

The study has showed that Starch-casein agar was found to be suitable for isolating actinomycetes from marine soils, which were collected from the coastal areas of Nagapattinam District Tamil nadu. Of ten isolates only two exhibited significant antibacterial activity. The cultural and taxonomical studies which confirmed the typical biochemical behaviors resembling actinomycetes. Starch-casein broth, temperature of 28°C and pH of 7 were found to be suitable for the fermentation of NGPSS2, the most active isolate. The exploitation of marine actinomycetes as a source for novel secondary metabolites production is in its infancy. However, the discovery rate of novel secondary metabolites from marine actinomycetes has recently surpassed that of their terrestrial counterparts. In this context, ours is a small but an honest effort directed towards isolating antibiotic producing marine actinomycetes. But future success relies on the ability to isolate novel actinomycetes from the marine environments. Further development work in improving isolation strategies in the recovery of marine actinomycetes is of utmost importance for ensuring success in this area.

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