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Isolation and Identification of Marine Actinomycetes and their Potential in Antimicrobial Activity

[1]N.K. Asha Devi, [1]M. Jeyarani and [2]K. Balakrishnan
[1]Department of Zoology and Microbiology, Thiagarajar College, Madurai-9, India
[2]Department of Biotechnology, Bharathidasan University, Trichy

Abstract: The objective of the present study was to isolate and identify high antimicrobial activity producing actinomycetes strains from marine habitat, to study antimicrobial production to determine their for the inhibition of the growth of the common human pathogens. Marine actinomycetes strains were isolated from coastal water of Dhanushkodi, Ramanathapuram District, India. Out of 10 isolated actinomycetes species 3 were identified and selected for antimicrobial activity. Out of the 3 actinomycetes species, *Streptomyces* sp. showed the best level of antibacterial and antifungal effect against selected human pathogens of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Klebsiella* sp. and *Aspergillus niger*. So, *Streptomyces* of actinomycetes may offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites. Hence, it is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics and novel species of marine actinomycetes.

Key words: Human pathogens, actinomycetes, antimicrobial activity

INTRODUCTION

Many natural products have been isolated from marine environments. However, only a small fraction of them was derived from marine microorganisms (Munro *et al*., 1999; Pomponi, 1999). Most secondary metabolites from marine microorganisms found so far were isolated from *Streptomyces* and *Alteromonas* sp. (Wagner *et al*., 2002) Marine actinomycetes are of considerable value as antibiotic producers and other therapeutically useful compounds with diverse biological activities (Bernan *et al*., 1997; Sharma and Pant, 2001). In the present investigation, it has been observed that compared to other actinomycetes, *Streptomyces* showed efficient antagonistic activity. Only very few reports are available on the occurrence and distribution of antagonistic *Streptomyces* in the marine environment (Weyland, 1969; Okazaki and Okami, 1972; Postmaster and Frietas, 1975; Lakshmanaperumalsamy *et al*., 1994) The objective of the present study was to isolate and identify high antimicrobial activity producing actinomycetes strains from marine habitat, to study antimicrobial production to determine their for the inhibition of the growth of the common human pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Klebsiella* sp. and *Aspergillus niger*.

MATERIALS AND METHODS

The water samples were collected in clean, sanitized and autoclaved bottles and transported to the laboratory for further analysis. The medium used for the isolation and cultivation of actinomycetes was CSPY-ME medium (g L^{-1}): K_2HPO_4, 0.5 g; Casein, 3.0 g; Maize starch, 10.0 g; Peptone, 1.0 g; Malt extract, 10.0 g; Agar, 15.0 g and Marine water, 1.0 L) The pH of the medium was adjusted to 7.5. The petriplates were incubated at 37°C for 7 days. The isolated actinomycetes were identified based on the colony morphology and gram staining (Holt *et al*., 1989).

Identified actinomycetes colonies were incubated at 30± 2°C on rotary shaker at 220 rev/min for 10 days for the enrichment of secondary metabolite producers. Then the culture was collected and centrifuged at 6000 rpm for 15 min and the supernatant immediately transferred and it was filtered through Millipore filter (0.45 μm) to get cell free extract. Antimicrobial activities were assayed in

Corresponding Author: N.K. Asha Devi, Department of Zoology and Microbiology, Thiagarajar College, Madurai-625009, Tamil Nadu, India

duplicate, using 50 μL of marine bacterial samples in well diffusion assay (Schillinger and Lucke, 1989) and the test organisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Klebsiella* sp. and *Aspergillus niger*.

RESULTS AND DISCUSSION

Three actinomycetes species were identified by morphological characteristics and by gram staining. Based on these characteristics actinomycetes were identified as *Actinopolyspora* sp. *Nocardia* sp. and *Streptomyces* sp. (Table 1).

Out of three selected and identified actinomycetes, *Streptomyces* sp. showed significant antimicrobial activity against human pathogens. In the present investigation, zone of inhibition of 57 mm was the highest antimicrobial activity showed by *Streptomyces* sp. against *Pseudomonas aeruginosa*. Other two actinomycetes of *Actinopolyspora* sp. and *Nocardia* sp. showed antifungal activity only (Table 2).

Based on several studies, among bacteria, the actinomycetes are noteworthy as antibiotic producers, making three quarters of all known products; the *Streptomyces* are especially prolific (Waksman, 1961; Lachevalier, 1989; Locci, 1989; Saadown and Gharaibeh, 2003). According to Kokare *et al.* (2004) during the screening of the novel secondary metabolite, actinomycetes isolates are often encountered which show more active antimicrobial activity against gram positive bacteria than gram negative bacteria. But from the observed results, only *Streptomyces* sp. showed significant antibacterial activity against *Staphylococcus aureus* next to *Pseudomonas aeruginosa*.

Normally, *Pseudomonas aeruginosa*, *Klebsiella* sp. and *Salmonella typhi* are even capable of growth in some antibiotics and their resistance to more antibiotics has also been medical concern (Tortora *et al*., 2000). Marine *Streptomyces* sp. exhibited the highest antibacterial activity against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*, *Klebsiella* sp. and *Salmonella typhi*. It is interesting to note that this response represents an antibiotic potential competing microorganism against *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella* sp. in the marine environment.

Eighty three percent of actinomycetes isolated from Sagamy Bay were found to be antifungal (Okami and Okazaki, 1972). Many marine microorganisms showed antifungal activity against *Aspergillus niger* but not against *Candida albicans* (Kokare *et al*., 2004). As *Aspergillus niger* is such a common contaminant, highly effective antibiotics are required. Hundred percent of the tested actinomycetes exhibited antifungal activity. Among the tested isolates *Streptomyces* sp. showed strong antifungal activity. Among microorganisms, actinomycetes particularly, *Streptomyces* will proved to be as fruitful as their counter parts isolated from terrestrial habitat (Pisano *et al*., 1989). However, the marine *Streptomyces* have not received much attention. Recent investigations indicate that the tremendous potential of marine actinomycetes, particularly *Streptomyces* sp. as a useful and sustainable source of new bioactive natural products. Thus, the results of the present investigation reveal that the marine actinomycetes from coastal environment are a potent source of novel antibiotics. It is anticipated that the isolation, characterization and the study of actinomycetes can be useful in the discovery of antibiotics and novel species of actinomycetes.

Table 1: Identification of Actinomycetes

Organisms	Morphology	Gram staining+ve/-ve	Microscopical structure
Actinopolyspora sp.	Smooth, whitish, wrinkled colonies, hardy in nature Colonies have a mat	+	Short rod (chain form)
Nocardia sp.	appearance, irregular wrinkled, yellow pigments produced	+	Rod shaped
Streptomyces sp.	Initially leathery colonies, later developing powdery in nature, pink colour colonies produced	+	Coccus (Chain like)

(+) sign indicates Gram positive actinomycetes

Table 2: Antimicrobial activity of marine actinomycetes against human pathogens

	Diameter of zone of inhibition in mm		
Human pathagences	*Actinopolyspora* sp.	*Nocardia* sp.	*Streptomyces* sp.
Staphylococcus aureus	_	_	43
*Pseudomonas aeruginosa*_	_	57	
Salmonella typhi	_	_	37
Vibrio cholerae	_	_	_
Klebsiella sp.	_	_	37
Aspergillus niger	15	15	25

(-) sign indicates no zone formation (no antimicrobial activity); where numbers indicates marked inhibition zone

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REFERENCES

Bernan, V.S., M. Greenstein and W.M. Maiese, 1997. Marine microorganisms as a source of new natural products. Adv. Applied Microbiol., 4357-4390.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and Williams, S.T., 1994. Bergey's Manual of Determinative Bacteriology, 9th Edn., Williams and Wilkins, Baltimore, pp: 518-537.

Kokare, C.R., K.R. Mahadik and S.S. Kadam, 2004. Isolation of bioactive marine actinomycetes from sediments isolated from Goa and Maharashtra coast lines (West Coast of India). Ind. J. Marine Sci., 33: 248-256.

Lachevalier, H.A.,1989. The Actinomycetes III, A Practical Guide to Generic Identification of Actinomycetes. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins Company, Baltimore, 4, pp: 2344-2347.

Lakshmanaperumalsamy, D., D. Chandramohan and R. Natarajan, 1994. Antibacterial and antifungal activity of *Streptomycetes.* Isolated from portonova costal environment. Ind, J. Marine Sci., 23:15-24.

Locci, R., 1989. *Streptomyces* and related Genera. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins Company, Baltimore, 4, pp: 2541-2508.

Munro, M.H.G., J.W. Blunt, E.J. Dumdei, S.J.H. Hickford, R.E. Lill, S. Li, C.N. Battershill and A.R. Duckworth, 1999. The discovery and development of marine compounds with pharmaceutical potential. J. Biotechnol., 70:15-25.

Okazaki, T. and Y. Okami, 1972. Studies on marine microorganisms II. Actinomycetes in Sagami Bay and their antibiotic substances. J. Antibiotics, 25: 461-466.

Okami, Y. and T. Okazaki, 1972. Studies on marine microorganisms (1) Isolation from the sea J. Antibiot., 25: 456-460.

Pisano, M.A., M.J. Sommer and L. Brancacci, 1989. Isolation of bioactive actinomycetes from marine sediments using rifampicin. Applied. Biotechnol., 31: 609-612.

Pomponi, S.A., 1999. The Bioprocess Technological Potential of the Sea. J. Biotechnol., 70:5-13.

Postmaster, C. and Y.M. Frietas, 1975. Biomedical aspects of actinomycetes. Hindustan Antibio. Bull., 17: 118.

Saadown, I. and R. Gharaibeh, 2003. The *Streptomyces* flora of Badia region of Jordon and its potential as a source of antibiotic resistant bacteria. J. Arid. Environ., 53: 365-371.

Schillinger, U. and F. Lucke, 1989. Antibacterial activity of *Lactobacillus* strain isolated from meat. Applied Environ. Microbiol., 55:1901-1906.

Sharma, S.L. and A. Pant, 2001. Crude oil degradation by a marine actinomycetes *Rhodococcus* sp. Indian. J. Mar. Sci., 30:146-150.

Tortora, G.J., B.R. Funke and C.L Case, 2000. Microbiolgy, 6th Edn., The Benjamin/Cummings publishing company Inc., pp: 300, 659-668.

Wagner, D.I., W. Beil, S. Lang, M. Meiners and H. Latch, 2002. Integrated approach to explore the potential of marine microorganisms for the production of bioactive metabolites. Adv. Biochem. Eng. Biotechnol., 74:207-238.

Weyland, H., 1969. Actinomycetes in North Sea and Atlantic Ocean Sediments Nature, London, 223: 853.

Waksman, S.A.,1961. The Actinomycetes, Classification, Identification and Description of Genera and Species. Baltimore. The Williams and Wilkins Company, 2: 61-292.