Indian Journal of Marine Sciences
Vol. 30(4), December 2001, pp. 264-267

Short Communication

Control of bacterial pathogens, associated with fish diseases, by antagonistic marine actinomycetes isolated from marine sediments

R Patil, G Jeyasekaran*, S A Shanmugam & R Jeya Shakila
Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Tuticorin 628 008, Tamil Nadu, India
[E.mail: fishco@vsnl.com]

Received 20 February 2001, revised 13 August 2001

Actinomycetes were isolated from different marine samples collected from various stations along the Tuticorin coast. About 133 cultures of actinomycetes were isolated from 129 marine samples. Of the 104 isolates of actinomycetes screened for their inhibitory activity against the bacterial pathogens associated with fish diseases viz. *Aeromonas hydrophila*, *A. sobria* and *Edwardsiella tarda*, 77 isolates were found to be inhibitory to at least one of the pathogens. The highest incidence of inhibitory isolates was from the sediment samples. All the isolates of antagonistic marine actinomycetes were identified to be *Streptomyces*. The findings suggest that the antagonistic marine *Streptomyces* isolates or the antibacterial substances produced by them could be used as antibiotics, which might have a future application in aquaculture systems.

Occurrence of diseases in fish culture systems pose a great threat as fish farmers suffer from heavy losses. Indiscriminate use of antibacterial compounds has led to the development of resistant strains of fish bacterial pathogens[1] and the fish farmers are facing lot of problems in controlling fish diseases caused by these resistant strains. Hence, the need of the hour is a search for novel antibacterial compounds with therapeutic potential, for which the pathogens may not have resistance. Most of the commonly used antibiotics are from bacteria belonging to one taxonomic group, the Actinomycetales[2]. Only a few workers have reported the occurrence of the antagonistic actinomycetes in marine sediment[3,4]. Novel antimicrobial compounds can be extracted from these marine actinomycetes and used for controlling bacterial diseases in fish culture systems. Present study was undertaken with the aim of isolating marine actinomycetes from marine environment, which are antagonistic to bacterial pathogens associated with fish diseases.

Marine sediment samples were collected from five sampling stations viz. mangrove swamp, Roche Park, Thermal Beach, Hare Island and inshore area of Tuticorin in the State of Tamil Nadu along the southeast coast of India. The sediment samples were collected in an aseptic manner in sterile polypropylene bags and brought to the laboratory immediately. The samples were air dried for 4-5 days and used for the isolation of actinomycetes. Some wet sediment samples were used for the enumeration of culturable bacterial load and actinomycetes population. Sediment samples were serially diluted using aged seawater diluent and inoculated onto Starch-Casein Agar (SCA) plates[5], with the anti-fungal agents, cycloheximide and ketoconazole at 50 µg/ml. The plates were then incubated at room temperature (30±2°C) for 5-7 days. Culturable bacterial load of sediment samples was estimated. Colonies of actinomycetes were recognized by their characteristic chalky to leathery appearance. Both the culturable bacterial load and actinomycetes population were enumerated and expressed as cfu per g of sediment.

Air-dried sediment samples were used for the isolation of actinomycetes, as this inhibits most of the bacteria. Colonies of actinomycetes were picked-up and purified onto Yeast extract Malt extract Agar (YMA) plates. The isolates were screened by light microscopy for confirmation of actinomycetes based on the filamentous nature and the width of the hyphae. Confirmed actinomycete isolates were subcultured and maintained on YMA slants for further use. The cross-streak method was used to detect the inhibitory strains among the isolates of actinomycetes[6] . Actinomycete strains were streaked

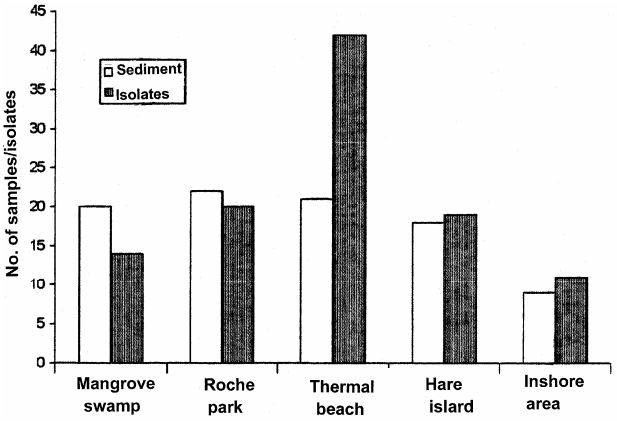
*Corresponding author

across the diameter onto Soyabean Yeast extract Glucose Agar (SYGA) plates with a width of the streak being 8-10 mm. After incubation at room temperature for a period of 5-7 days, young culture of each test organism (such as *Aeromonas hydrophila, A. sobria* and *Edwardsiella tarda*) was streaked horizontally to the central strip of the actinomycete culture, 1-2 mm apart from the central streak. The plates were re-incubated at room temperature for 24 h. The inhibitory activity of the actinomycete isolates was indicated by absence of growth near the central strip. A control plate was maintained with only the test organisms.

The antibacterial spectrum of antagonistic marine actinomycetes against bacterial pathogens associated with fish diseases viz. *Aeromonas hydrophila, A. sobria* and *Edwardsiella tarda* was determined and the zone of inhibition was measured and expressed in mm. Marine actinomycetes having inhibitory activity were identified up to the generic level by following the standard taxonomic methods[7,8]. Correlation coefficient was estimated between the average total bacterial load and the average actinomycete population in the marine sediment samples. Rank correlation coefficient was also estimated between the antibacterial activity of the two highly antagonistic strains of actinomycetes[9].

A total of 106 actinomycete cultures were isolated from 90 marine sediment samples (Fig.1). The incidence of actinomycetes out of the average culturable bacterial load ranged from 2.35% to 23.19% in the marine sediment samples analyzed. As few reports have described the occurrence of actinomycetes in the sediment of marine and estuarine environments[3,10] , in the present study also, high number of actinomycetes were isolated from the marine sediments collected from the Thermal Beach. On an average, the actinomycetes were found to constitute about 11.33% of the average culturable bacterial population (log counts per g) in the marine sediment samples (Fig. 2). It has been reported that actinomycetes represent only a small fraction of the bacterial population enumerated from the marine environment[11].

Of the 106 actinomycete cultures isolated, only 84 isolates were tested for their activity against the bacterial pathogens associated with fish diseases viz., *Aeromonas hydrophila, A. sobria* and *Edwardsiella tarda*, because, only these grew on the medium which



ig.1—Occurrence of actinomycetes (no.) in marine sediments (no.) of different sampling stations of Tuticorin coast

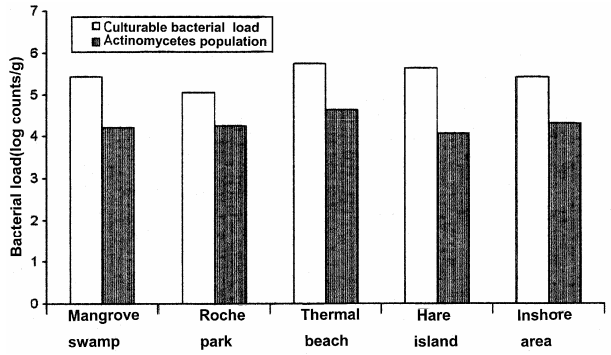


Fig.2—Comparison of average culturable bacterial load with the actinomycetes population

supported the growth of the test pathogens. Among the 84 isolates tested, 63 (74.97%) isolates were found to be inhibitory to one or more bacterial fish pathogens at varying levels. Earlier, a similar result was observed, in which it has been found that about 75% of their actinomycete isolates were inhibitory[12]. However, in another study, it has been reported that only 27% of actinomycete cultures isolated from marine sediments of Sagami Bay were antagonistic to various microorganisms[4]. The samples from the Roche Park yielded high number of inhibitory actinomycetes, followed by Mangrove swamp, Thermal Beach, Inshore Area and Hare Island. The high incidence of antagonistic actinomycetes in the Roche Park sediment samples could be attributed to the possible effect of pollution of the area due to the discharge of domestic sewage by the fishermen community living nearby. On the contrary, Hare island sediment samples recorded less number of inhibitory actinomycetes and this may be due to the

Table 1—Antagonistic activity of *Streptomyces* isolates against fish bacterial pathogens

Fish bacterial pathogens	*Streptomyces* isolates (zone of inhibition, in mm)				
	A_{30}	A_{36}	A_{47}	A_{63}	A_{103}
A	11	10	20	15	6
B	12	20	15	22	26
C	20	20	10	6	8

A-Aeromonas hydrophila; B- *A. sobria*; C- *Edwardsiella tarda*

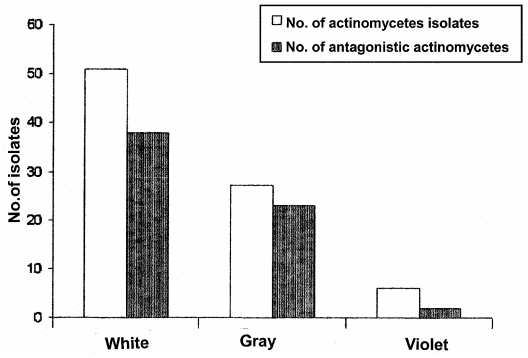


Fig.3—Colour series of antagonistic Streptomyces isolated from marine sediment samples

fact that this area is relatively unpolluted. The relationship between the high incidence of the antagonistic actinomycetes and the polluted environments has been well established[13].

Of the 63 antagonistic actinomycetes, 5 isolates showed strong antagonism against all the test pathogens (Table 1). The rest of the isolates showed weak inhibitory activity against one or more test pathogens. In another study, it has been observed that the isolates of *Streptomyces* from marine sediments were inhibitory to *Bacillus cereus, B. subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*[3]. It has also been reported that the actinomycetes from marine sediments inhibited many bacterial pathogens[4].

All the antagonistic actinomycetes isolated in this study were identified as *Streptomyces*. The results of the present study are in agreement with the earlier findings, in which it has been reported that *Streptomyces* are mainly found in shelf and shallow areas, when compared to other genera of actinomycetes[14]. Streptomycetes exhibiting antibacterial activity have also been isolated from decaying materials found in the littoral zone[15] . Majority of the isolates of the *Streptomyces* were found to be from white color series (60.58%), followed by gray (32.69%) and violet color series (6.70%) (Fig. 3). However, 85.2% of the gray color series exhibited antagonistic properties as compared to 74.5% of white and 33.3% of violet color series. These findings are in agreement with earlier results, in which it has been reported that a majority of the antagonistic *Streptomyces* isolates belong to gray series[16].

A positive correlation (p>0.05) was observed between the actinomycetes population and the average culturable bacterial load in the marine sediment samples tested. Insignificant rank correlation was also obtained between the antibacterial activities of the two highly antagonistic cultures of *Streptomyces* isolated from marine sediment samples against fish bacterial pathogens (p>0.05). The results of the present study clearly suggest that the antagonistic marine actinomycetes could be used to produce antibiotics. Further studies are continued to isolate, purify and characterise the antibacterial compounds from these antagonistic marine actinomycetes and to test their possible use as the alternative chemotherapeutic drugs on a commercial scale.

Financial support extended to the first author by the ICAR, Government of India, New Delhi for this study is gratefully acknowledged.

References

1 Karunasagar I, Pai R, Malathi G R & Karunasagar I, Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection, *Aquaculture,* 128 (1994) 203-209.

2 Sanglier J J, Haag H, Huck T A & Fehr T, Review of actinomycetes compounds 1990-1995, *Exp Opin Invest Drugs*, 5 (1996) 207-223.

3 Grein A & Meyers S P, Growth characteristics and antibiotic production of actinomycetes isolated from littoral sediments and materials suspended in seawater, *J Bacteriol*, 76 (1958) 454-463.

4 Okazaki T & Okami Y, Studies on marine microorganisms:II-Actinomycetes in Sagami Bay and their antibiotic substances, *J Antibiot*, 25 (1972) 461-466.

5 Kuster E & Williams S T, Selective media for the isolation of streptomycetes, *Nature*, 202 (1964) 928-929.

6 Lemos M L, Toranzo A E & Barja J L, Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds, *Microbial Ecol*, 11 (1985) 149-163.

7 Berd D, Laboratory identification of clinically important aerobic actinomycetes, *Appl Microbiol*, 25 (1973) 665-681.

8 Korn- Wendisch F & Kutzner H J, The family streptromycetaceae, in *The Prokaryotes, Vol.1*, edited by A

Balows, H G Truper, M Dworkin, W Harder & K H Schleifer, (Springer-Verlag, New York) 1992, pp. 921-995.

9 Snedecor G W & Cochran W G, *Statistical methods*, (Oxford and IBH Publishing Company, Calcutta) 1962, pp 379-380.

10 Jensen P R, Dwight R & Fenical W, Distribution of actinomycetes in near-shore tropical marine sediments, *Appl Environ Microbiol*, 57 (1991) 1102-1108.

11 Goodfellow M & Williams S T, 1983. Ecology of actinomycetes, *Ann Rev Microbiol*, 37 (1983) 189-216.

12 Vanajakumar S, Selvakumar N & Natarajan R, Antagonistic properties of actinomycetes isolated from molluscs of the Porto Novo region, South India, in *Bioactive compounds from marine organisms*, edited by M F Thompson, R Sarojini & R Nagabhushanam, (Oxford and IBH Publishing Co., New Delhi) 1991, pp. 267-274.

13 Walker J D & Colwell R R, Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and South Eastern Atlantic Ocean sediments, *Mar Biol*, 30 (1975) 193-201.

14 Weyland H & Helmke E, Actinomycetes in the marine environment, in *Biology of actinomycetes*, edited by Y Okami, T Beppu & H Ogawara, (Japan Scientific Societies Press, Tokyo) 1988, pp. 294 -299.

15 Chandramohan D, Ramu S & Natarajan R, Cellulolytic activity of marine streptomycetes, *Curr Sci*, 41 (1972) 245-246.

16 Hatano K, Actinomycete population in mangrove rhizospheres. *IFO Res Commun*, 18 (1997) 26-31.