BVG Trust

J. Sci. Trans. Environ. Technov. 2014, 8(1) : 27-32

Scientific Transactions in Environment and Technovation

Diversity of bioactive compound producing actinobacteria isolated from natural disaster affected marine environments

R .Vijayakumar[1*], A. Panneerselvam[2] and N. Thajuddin[3]

[1]Department of Microbiology, Bharathidasan University Constituent College, Kurumbalur – 621 107, Perambalur, Tamilnadu, India. [2]Post Graduate and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur, Tamil Nadu, India. [3]Department of Microbiology, Bharathidasan University, Tiruchirappalli - 620 024 Tamil Nadu, India.

Abstract

Populations of marine actinobacteria were surveyed from the coastal soils of Tamilnadu (India) after Indian Ocean tsunami occurred on December 26, 2004. Totally 72 marine sediment samples were collected. The isolated actinobacteria were characterized on the basis of the colour of spore mass, reverse side colour, aerial and substrate mycelia formation, production of diffusible pigment and ssporophore morphology, and screened for their antimicrobial activity. Totally, 272 actinobacterial colonies were isolated, among them, 102 isolates were morphologically distinct, which belonged to 9 different genera. From these, 98 isolates with aerial mycelia, 98 isolates with substrate mycelia and 91 isolates had both aerial and substrate mycelia. Among the 102 isolates, 68 and 34 isolates were isolated during pre-and post-tsunami periods respectively. Only 36.8% isolates of pre tsunami period showed antimicrobial activity, whereas 58.8% isolates of post-tsunami period showed antimicrobial activity The sediments of Palk Strait region of Bay of Bengal has a huge diversity of bioactive compound producing marine actinobacteria with pharmaceutical value, which was modified to a great extent by the natural calamities like tsunami.

Key words: actinobacteria, antimicrobial activity diversity, Palk Strait of Bay of Bengal, Tsunami

INTRODUCTION

Generally, coastal environment plays a vital role in the economy of a nation by virtue of the resources, productive habitats and rich biodiversity. The oceans cover more than 70% of the earth's surface, and little is known about the microbial diversity of marine sediments, which is an inexhaustible resource that has not been properly exploited. India has a long coastal line of over 7,500 km an area of 2.02 million m^2. The coastline of Tamilnadu has a length of about 1076 km, which constitutes about 15% of the total coastal length of India and stretches along the Bay of Bengal, Indian Ocean and Arabian Sea. The Palk Strait region of Bay of Bengal has diverse marine habitats such as seashore, hyper saline lakes, estuaries, saltpans and a variety of soil habitats (Vijayakumar *et al.*, 2007).

Tsunami, the natural disaster, which occurred on December 26, 2004 in the Indian Ocean, caused severe damage to mankind in the coastal areas. Tsunami is a series of waves created when a body of water, such as an ocean is rapidly displaced. Earthquakes, mass movements above or below water, some volcanic eruptions and other underwater explosions, landslides, underwater earthquakes, large asteroid impacts and testing with nuclear weapons. The effect of tsunami can be devastating due to the immense volumes of water and energy involved.. Most severe damage was observed in Nagapattinam beach, Nabiyarnagar, Vellaipalayam, and the Nagapattinam Port of Nagapattinam district on the east coast and Keelamanakudy Village of Kanyakumari district on the west coast of Tamilnadu. These earthquake and subsequent tsunami reportedly killed over 220,000 people around the rim of the Indian Ocean. The tsunami devastated the Coromendel coast, killed many and swept away many coastal communities. In this context, it was justifiably anticipated that it could have had its impact on the microbial community also. The major ecological changes in the seashore area of the south east coast of Bay of Bengal have been reported by many workers (Chandrasekaran *et al.*, 2005; Chaudhary *et al.*, 2006; Ramesh *et al.*, 2006).

The potentiality of the actinobacteria to produce bioactive compound has been well established. Nevertheless there is no study on the diversity variation of bioactive compound producing actinobacteria from the sediments of Bay of Bengal with particular reference to tsunami.

A research work was carried out in the Palk Strait regions of Bay of Bengal in relation to diversity of marine actinobacteia and their antimicrobial potentialities by Vijayakumar (2006) during tsunami 2004 to 2006. The present investigation deals with actinbacteria isolated from various marine habitats of Palk Strait regions of Bay of Bengal and their ability to produce antimicrobial bioactive compounds.

MATERIALS AND METHODS

Collection of soil samples

A study was carried out to understand the changes in the marine actinobacterial ecology and diversity at Palk Strait coast of Bay of Bengal due to tsunami that hit the

*Corresponding author :
e-mail address: rvijayakumar1979@gmail.com

Indian coast on 26th December, 2004. A total of 72 marine sediment samples were collected from nine locations, Chola lighthouse, Old lighthouse and New lighthouse [near Point Calimere, Nagapattinam District, Lat. 10°18′ N and Long. 79° 51′ E (sea shore)], Muniyappan Lake (near Vedaranyam, Nagapattinam District), Muthupet [(Lat. 10° 20′ N and 79° 8′ E) (mangrove)] and Vedaranyam [(LT. 10° 22′ N and Long. 79° 51′ E (salt pan)], Thondi [(Lat. 9° 45′ N and Long. 79° 3′ E) (salt pan)] and Tuticorin [(Lat. 8° 72′ N and Long. 78° 12′ E) (salt pan)] (Fig. 2) representing three different environments found in Palk Strait region of south east coast of India. Samples were collected at random from the top layer of each location and brought to the laboratory and stored for further study.

Sample treatment

Heat treatment was performed by holding the soil and sediment samples in a water bath at 50°C for 60 min for prevention of other bacterial flora. All samples were diluted (upto 10^{-6}) with sterile 0.5% saline prior to inoculation into the isolation plates.

Media and culture conditions

Starch casein agar (SCA) medium (Himedia, Mumbai, India) [g/l: starch 10, casein 0.3, KNO_3 2, NaCl 2, K_2HPO_4 2, MgSO4. $7H_2O$ 0.05, CaCO3, 0.02, $FeSO_4$ $7H_2O$ 0.01 and agar 18; and supplemented with Griseofulvin and Cycloheximide (Himedia, Mumbai, India) 25 and 10 mg/ml] was used for the isolation of actinobacteria and its enumeration (Kuster and Williams, 1964). The diluted sediment samples (0.1 ml) were spread over the medium with a sterilized bent (L) rod and plate spinner. The inoculated plates were incubated at 30°C for seven to ten days. After incubation, colonies were purified using streak plate technique and maintained for further investigation.

Identification of actionbacterial isolates

Purified isolates of actinobacteria were characterized by using morphological and cultural properties as methods described in International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1964). The morphology of the spore bearing hyphae with spore chain, the structure and arrangement of the spore chain with substrate and aerial mycelia of the isolates were examined under phase contrast microscope using slide culture technique.

Evaluation of antimicrobial activity of the actinobacterial isolates

The actinobacterial isolates were screened to determine their ability to produce antimicrobial compounds against *Escherichia coli, Bacillus subtilis* and *Candida albicans* by cross streak method. The method of antimicrobial activity carried out was already described in our earlier studies (Vijayakumar *et al.,* 2010; 2012a; b).

RESULTS AND DISCUSSION

The Palk Strait region of Bay of Bengal Coast is one of the hot biodiversity spot of the globe, it has high range (45-90‰) of salinities, from seashore, estuaries, mangrove and hyper saline saltpan environments. The coast is generally low and punctuated by deltas of several large rivers, including Cauvery, Vaigai, Palar and Pennar which rise in the highlands of the Western Ghats and flow across the Deccan Plateau to drain into the Bay of Bengal. The alluvial plains created by these rivers are fertile and favour agriculture. In addition, a large amount of soil carrying spores of terrestrial micro flora, especially actinobacteria, enters the heads of the Bay through backwater. Observations made at the field and analysis of collected soil samples after the tsunami showed that there was deposition of salts on the surface in all the areas where sea water intrusion took place during tsunami. However, in the coastal soils of Tamilnadu, the tsunami caused three kinds of major damage: (i) deposition of slushy grayish-brown clay material, (ii) deposition of sand, and (iii) sea water intrusion, which receded leaving salts in the soil. Deposition of slushy grayish-brown clay was exclusively noticed at Vedharanyam and Point Calimere area, where the thickness of deposits varied from 5-30 cm under wet condition, the sea sediment with high salt concentration (17.6 dsm^{-1}). A thousand years old Chola Light House (built by Chola's King) was partially demolished and thrown a distance of 100 meters away, a portion of *Sweda* shrub field was completely inundated with seawater and sand and destroyed; sea bang near Old Light House of Point Calimere was corroded and trees were uprooted (Fig. 3). Effect of tsunami on coastal crop husbandry in Nagapattinam district of Tamilnadu has been already reported (Chandrasekaran *et al.,* 2005; Chaudhary *et al.,* 2006).

In the present study, discrimination of actinobacterial populations and their potentiality of the production of antimicrobial compounds against some human pathogens have been investigated. Heat treatment, which was often used as a pre-treatment of marine sediments, reduced the numbers of Gram-negative bacteria that commonly occur in marine samples and often overrunning the isolation media (Jensen *et al.* 1991).Of the 272 colonies isolated, 102 were morphologically discriminated. Among the 102 isolates, 68 isolates were isolated during pre-tsunami period, and 34 were isolated during post-tsunami period. A maximum number of actinobacterial isolates were recorded in mangrove sediments during both pre- (28) and post-tsunami (14) era, followed by 21 and 11 isolates from seashore and 19 and 9 isolates from saltpan environments during pre- and post tsunami periods respectively. On the whole (pre- and post-

Table 1: Occurrence and identification of actinobacteria in different marine sediment samples

Sampling environment and stations	No. of samples	Total no. of colonies/isolates	No. of actinobacterial isolates								
			Streptomyces	Actinopolyspora	Saccharopolyspor	Actinomadura	Nocardiopsis	Micromonospora	Microbispora	Actinomyces	Actinoplanes
Pre-tsunami – Seashore											
Chola Light House	4	12/8	6	-	1	-	1	-	-	-	-
Old Light House	4	15/7	2	3	1	1	-	-	-	-	-
New Light House	4	16/6	4	-	1	-	1	-	-	-	-
Mangrove											
Muniyappan Lake	4	22/12	5	-	2	2	-	2	-	1	-
Muthupet	4	25/8	4	2	1	-	-	-	-	-	1
Mimisal	4	27/8	5	2	-	1	-	-	-	-	-
Saltpan											
Vedaranyam	4	29/7	5	1	1	-	-	-	-	-	-
Thondi	4	18/5	3	2	-	-	-	-	-	-	-
Tuticorin	4	28/7	5	-	-	-	1	-	1	-	-
Pre-tsunami – Seashore											
Chola Light House	4	12/5	2	-	1	-	1	-	1	-	-
Old Light House	4	15/5	2	1	1	1	-	-	-	-	-
New Light House	4	6/1	-	-	1	-	-	-	-	-	-
Mangrove											
Muniyappan Lake	4	10/5	-	-	1	2	-	2	-	-	-
Muthupet	4	8/4	2	1	1	-	-	-	-	-	-
Mimisal	4	7/5	1	2	-	1	-	-	1	-	-
Saltpan											
Vedaranyam	4	9/3	1	1	1	-	-	-	-	-	-
Thondi	4	10/2	-	1	1	-	-	-	-	-	-
Tuticorin	4	8/4	2	-	-	-	1	-	-	1	-
Total	**72**	**272/102**	**49**	**16**	**14**	**8**	**5**	**4**	**3**	**2**	**1**

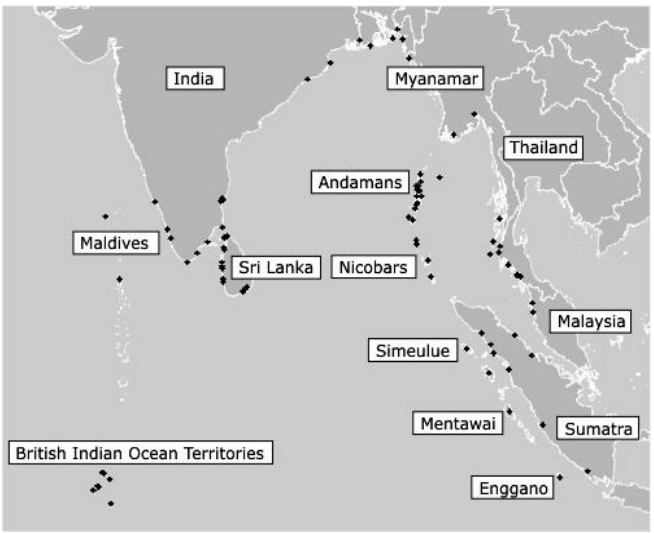

Fig. 1. Map showing the regions affected by tsunami 2004

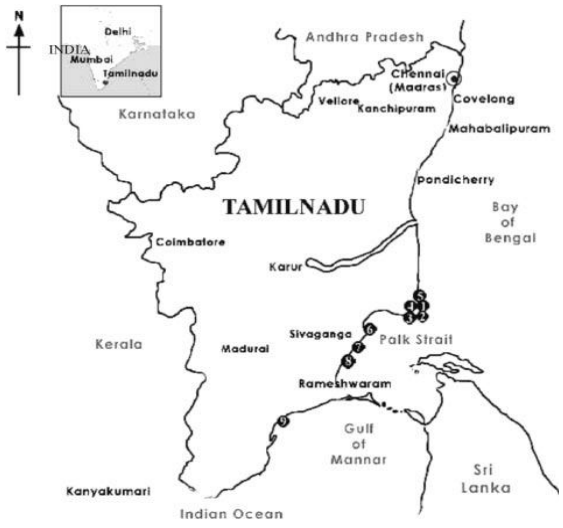

Fig. 2. Map showing the sampling sites - 1. Chola lighthouse, 2. Old lighthouse; 3. New lighthouse; 4. Muniyappan lake; 5. Vedaranyam; 6. Muthupet; 7. Mimisal; 8. Thondi; 9. Tuticorin.

Fig. 3. Portraits of the sampling sites (a) Chola Light House at Point Calimere Reserve Forest - pre-tsunami (b) post-tsunami; (c) broken piece of Chola Light House thrown over 100 meters away; (d) Sweda shrub field pre-tsunami; (e) wide view of the completely inundated/destroyed Sweda shrub field covered with seawater and sand; (f) The corroded sea bang of the Old Light House area with uprooted trees post-tsunami.

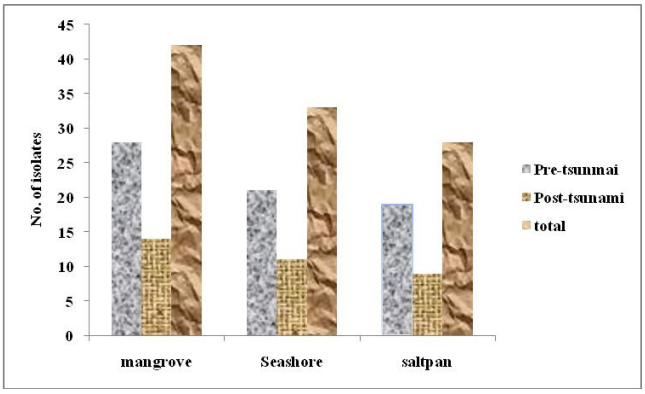

Fig. 4. Actinobacterial population in sampling environment wise

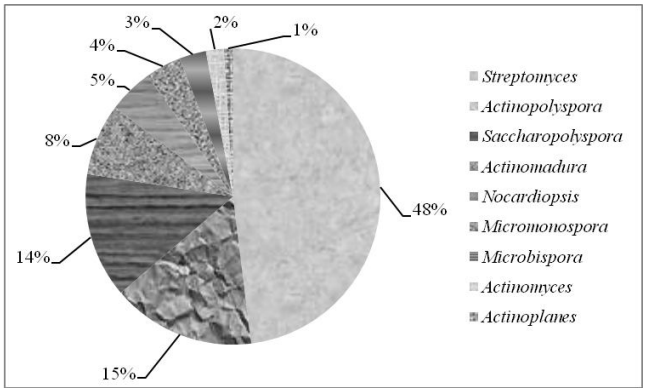

Fig. 5. Total percentage frequency of actinobactreia genera

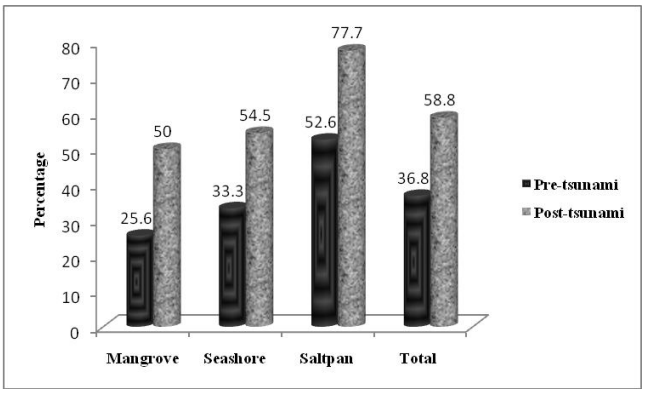

Fig. 6. Percentage of antimicrobial isolates from station wise during tsunami era

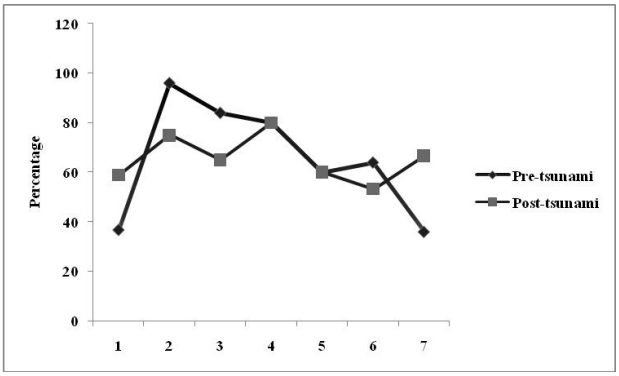

Fig. 7. Antimicrobial activity pattern of actinomycetes isolates. 1. Total antagonist; 2. antibacterial; 3. antifungal; 4. both antibacterial and antifungal; 5. against Gram-positive bacteria; 6. against Gram-negative bacteria; 7. against both Gram-positive and Gram-negative bacteria.

tsunami), mangrove environment has contributed maximum 42 (41%) actinobacterial isolates, followed by seashore 33 (32.2%) and saltpan 28 (27.4%) (Table 1; Fig. 4). Similarly, mangrove sediments were recorded a maximum of 8 different actinobacterial genera, followed by saltpan 7 genera and seashore 6 genera (Table 1). The actinobactreial isolates of saltpan were considered as halophilic, since they could adopt the higher salinities (75 to 90‰ in laboratory condition) and the isolates from mangroves and seashore sediments were halotolerant forms. Among the 102 isolates, 91 isolates produced both aerial and substrate mycelia, 98 isolates with aerial mycelium, 98 isolates with substrate mycelium. All these 102 isolates were belonged to streptomycetes, nocardiofarm, actinoplanetes, maduromycetes and thermomonospora groups. The occurrence and distribution of different genera of actinobacteria in different marine sediment samples in station wise are presented in Table 1. Out of 102 isolates, 49 isolates were identified as genus *Streptomyces* (spore chain with coiling, spiral and looped), 16 as *Actinopolyspora* (long chains of spores on aerial mycelium), 14 as *Saccharopolyspora* (very long chains of conidia on the aerial mycelium), 8 as *Actinomadura* (spore chains are straight and open hooked), 5 as *Nocardiopsis* (aerial mycelium totally sporulated), 4 as *Micromonospora* (clusters of single conidia on substrate mycelium), 3 as *Microbispora* (pairs of spores on aerial mycelium), and 2 as *Actinomyces* (branching vegetative mycelium) and 1 as *Actinoplanes* (spores on sporangia spherical shaped). The identity of the actinobacterial isolates were confirmed with Bergey's Manual of Systematic Bacteriology. Actinobacteria, especially *Streptomyces*, have been reported from the marine sediments and also from almost all parts of the world (Peela *et al.*, 2005; Vijayakumar *et al.*, 2007; Remya and Vijayakumar, 2008) dye polluted soil (Vijayakumar and Malathy, 2014) and terrestrial soils (Vijayakumar *et al.*, 2009; Cholarajan and Vijayakumar, 2006).

Frequencies of actinobacterial isolates in different sampling stations varied. The overall (pre- and post-tsunami) frequency of the genus *Streptomyces* was 48% followed by *Actinopolyspora* (15.7%), *Saccharopolyspora* (13.7%), *Actinomadura* (7.8%), *Nocardiopsis* (4.9%), *Micromonospora* (3.9%), *Microbispora* (2.9%), *Actinomyces* (1.9%) and *Actinoplanes* (0.98%). Fortunately, the genus *Streptomyces* was recorded from all the nine sampling stations during pre-tsunami period, but not recorded from all the stations during post-tsunami period. All the isolates were recorded during both pre- and post-tsunami period, whereas *Actinoplanes* was not recorded during post-tsunami period, it was recorded only at Muthupet mangrove sediments during pre-tsunami (Table 1; Fig. 5). The dominance of *Streptomyces* among the actinobacteria in marine soils has also been reported by many workers (Vijayakumar *et al.*, 2007; Remya and Vijayakumar, 2008; You *et al.*, 2005). Moreover, *Actinopolyspora, Saccharopolyspora, Actinomadura, Nocardiopsis* and *Micromonospora* were frequently isolated on culture media (Waksman, 1967).

Among the 68 isolates of actinobacteria recorded during pre tsunami 25 (36.8%) isolates showed variations in antimicrobial activity against various human pathogens tested. The antibacterial (against Gram-positive, Gram-negative both), antifungal and both antibacterial and antifungal activities of the isolates were already recorded during pre-tsunami[Vijayakumar,2006; Ramya and vijayakumar, 2008]. Location wise, maximum antimicrobial compound producing isolates (52.6%; n=10) were documented at saltpan sediments, followed by (33.3%; n=7) and (25.6%; n=8) from seashore and mangrove sediments respectively. During post-tsunami, out of 34 isolates, 20 (58.8%) isolates possessed antimicrobial activity. Location wise, greatest antimicrobial compound producing isolates (77.7%; n=7) were contributed at saltpan sediments, followed by (57.5.3%; n=6) and (50%; n=7) from seashore and mangrove

sediments respectively (Fig. 6). From the 20 antimicrobial compound producing isolates, 15 (75%) had antibacterial activity, 13 (65%) had antifungal activity, and 16 (80%) with both antifungal and antibacterial activities. Nine (60%) out of 15 isolates inhibited the growth of Gram-positive bacteria, 8 (53.3%) inhibited Gram-negative bacteria, and 10 (66.7%) inhibited both Gram-positive and Gram-negative bacteria (Fig. 7). Even though, the marine sediments contributed less actinobacterial population during the post-tsunami period, considerable percentage was increased in the antimicrobial compound production from all the three locations, particularly in saltpan sediments. Nevertheless, when compared to other stations the sediments of saltpan not only superior in antimicrobial compound production (52.6% and 77.7%) during the pre- and post-tsunami periods but also exhibited higher percentage of activity against bacteria, fungi, both bacteria and fungi, Gram-positive bacteria, Gram-negative bacteria and both Gram-positive and Gram-negative bacteria (Fig. 6 and 7). It is concluded that the indigenous actinobacterial population density was greatly influenced by sudden changes like tsunami in their habitats, and possibly novel actinobacterial populations have been introduced to seashore area from benthic and deep sea area. However, there was notable increase in the diversity of antimicrobial compound producing actinobacteria in all the sampling stations.

Acknowledgement

We thank the Management of A.V.V.M. Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur, Tamil Nadu, India for providing necessary facilities.

References

Chandrasekaran, H., Singh Rao, V., Nagarajan, M. and Chandrasekaran, B. 2005. Effect of tsunami on coastal crop husbandry imparts of Nagapattinam district, Tamilnadu. *Curr. Sci.,* 89: 30-32.

Chaudhary, D.R., Ghoshand, A. and Patolia, J.S. 2006. Characterization of soils in the tsunami-affected coastal areas of Tamilnadu for agronomic rehabilitation. *Curr. Sci.*, 91: 99-104.

Cholarajan, A. and Vijayakumar, R. 2013. Isolation, identification, characterization and screening of antibiotic-producing actinobacteria from the crop fields of Thanjavur district, Tamilnadu, India. *Int. J. Recent Sci. Res.,* 4: 55-60.

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

Kuster, E. and Williams, S.T. 1964. Selection of media for isolation of streptomycetes. *Nature*, 202: 928–929.

Peela, S., Bapiraju Kurada, V.V.S.N. and Terli, R. 2005. Studies on antagonistic marine actinomycetes from the Bay of Bengal. *World J. Microbiol. Biotechnol.,* 21: 583-585.

Ramesh, S., Jayprakashvel, M. and Mathivanan, N. 2006. Microbial status in seawater and coastal sediments during pre- and post-tsunami periods in the Bay of Bengal, India. *Mar. Ecol.,* 27: 198–203.

Remya, M. and Vijayakumar, R. 2008. Isolation and characterization of marine antagonistic actinomycetes from West Coast of India. *Facta Universitatis: Med Biol.*, 15: 1513-1519.

Shirling, E.B. and Gottlieb, D. 1964. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.*, 16: 312–340.

Vijayakumar, R., 2006. Studies on actinomycetes from Palk Strait region of Tamil Nadu coast with reference to antibiotic production. Ph.D. Thesis, Bharathidasan University, Tamilnadu, India, p. 72.

Vijayakumar, R., Seethalakshmi, V., Anitha, S. and. Saravanamuthu, R., 2009. Isolation and characterization of antagonistic actinomycetes from Coimbatore soils, Tamilnadu, India. *J. Sci. Trans. Environ. Technov.,* 2: 191-201.

Vijayakumar, R., Murugesan, S. and Panneerselvam, A. 2010. Isolation, characterization and antimicrobial activity of actinobacteria from Point Calimere coastal region, East Coast of India. *Int. Res. J. Pharma.*, 1: 358-365.

Vijayakumar, R., Muthukumar, C., Thajuddin, N., Panneerselvam, A. and Saravanamuthu, R. 2007. Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. *Actinomycetologica*, 21: 59-65.

Vijayakumar, R., Panneer Selvam, K., Muthukumar, C., Thajuddin, N., Panneerselvam, A. and Saravanamuthu, R. 2012a. Optimization of antimicrobial production by the marine actinomycetes *Streptomyces afghaniensis* VPTS3-1 isolated from Palk Strait, East Coast of India. *Indian J. Microbiol.,* 52: 230–239.

Vijayakumar, R., Panneer Selvam, K., Muthukumar, C., Thajuddin, N., Panneerselvam, A. and Saravanamuthu, R. 2012b. Characterization and antimicrobial potentiality of *Streptomyces griseus* - a halophilic strain isolated from saltpan environment of Vedaranyam, India. *Ann. Microbiol.,* 62: 1039-1047.

Vijayakumar, R. and Malathi, R. 2014. Isolation, characterization and antibacterial activity of actinobacteria from dye polluted soils of Tirupur. *Facta Universitatis Med. Biol.,* 16: 43-48.

Waksman, S.A., 1967. The genus *Actinomyces*. In: The actinomycetes. A summary of current knowledge, The Ronald Press Company, New York, p. 75-80.

You, J.L., Cao, L.X., Liu, G.F., Zhou, S.N., Tan, H.M. and Liu, Y.C. 2005. Isolation and characterization of actinomycetes antagonistic to pathogenic *Vibrio* spp. from nearshore marine sediments. *World J. Microbiol. Biotechnol.,* 21: 679-682.