Malaysian Journal of Microbiology, Vol 18(6) 2022, pp. 677-682
DOI: http://dx.doi.org/10.21161/mjm.221509

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

SHORT COMMUNICATION

Marine actinomycetes from the Kerala coastal region as a potential expedient for the natural drug discovery

Deepa Mathew Pandipurathu and Valsalam Robin Perinba Smith*

Department of Zoology, Scott Christian College, Nagercoil, Kanyakumari District, 629003, Tamilnadu, India.
Email: deepamathew1947@gmail.com

Received 13 April 2022; Received in revised form 2 November 2022; Accepted 5 November 2022

ABSTRACT

Aims: The marine actinomycetes are a rich source of novel bioactive molecules. Especially the exotic tropical marine habitat of the Kerala coastal region favours the actinomycete diversity. The present study focuses on the isolation, purification and morphological characterization of marine actinomycetes for the discovery of new bioactive compounds.
Methodology and results: A total of 280 morphologically distinct actinomycetes were isolated from marine soil and sediments of 10 different isolation sites located along the coastal region of Thiruvananthapuram district, Kerala, India using standard microbiological techniques. The physicochemical analysis of the soil samples collected from different stations was also done.
Conclusion, significance and impact of study: Even though the soil/sediment samples were collected from geographically nearby places, the physicochemical parameters showed a significant variation. This may be one of the factors which may trigger the actinomycete diversity in these regions. The diversity of actinomycetes prevalent in this region could serve as a potential source for the discovery of novel biomolecules.

Keywords: Actinomycete, biomolecules, marine, physicochemical, soil

INTRODUCTION

More than 70% of our planet's surface is covered by oceans and life on earth originated from the sea. In some marine ecosystems, such as the deep-sea floor and coral reefs, experts estimate that the biological diversity is higher than in tropical rainforests (Edward, 2006). As marine environmental conditions are highly different from terrestrial ones, it is inferred that marine actinomycetes have different characteristics from those of terrestrial counterparts and therefore, might produce different types of bioactive compounds (Fenical *et al*., 1999; Gesheva *et al*., 2005). From the ecological point of view, several species of actinomycetes (*i.e.*, *Streptomyces* and *Nocardiopsis*) are frequently distributed in marine environments such as oceans, rivers and seas. They dwell in sponges, marine sediments, sea sands and water (Goodfellow and Williams,1983). These bacteria are an important group of microorganisms due to their ability to produce a wide array of antimicrobial metabolites, notably antibiotics, anti-parasitic agents, antitumor agents, enzymes, cosmetics, secondary metabolites, glycopeptides, beta-lactams, aminoglycosides, polyenes, polyketides, macrolides, actinomycins and tetracycline, nutritional materials immunosuppressive agents, vitamins, pesticides and herbicides (Imada, 2005; Atta *et al*., 2009; Valli *et al.*, 2012). It has been reported that marine actinomycetes have not only several new species but also have plenty of novel structures with potent bioactivities (Subramani and Sipkema, 2019). Many researchers have isolated novel antibiotics from the marine environment, such as polyketide antibiotic SBR-22, rabelomycin (1), fridamycin D (2b), N-benzylacetamide and N-(2'phenylethyl) acetamide, two new anthracycline antibiotics designated as himalomycin A (2c) and B (2d) (Biabani *et al*., 1997; Maskey *et al*., 2003; Charan *et al*., 2004; Li *et al*., 2005; Sujatha *et al*., 2005).

Actinomycetes are Gram-positive bacteria with high G+C content (Barka *et al*., 2015), which are free-living, saprophytic, filamentous bacteria and are a significant source for the production of antibiotics (Valli *et al*., 2012). Actinomycetes are described by the arrangement of spreading strings or poles, as often as possible, offering to ascend to an average mycelium which is unicellular, particularly during the beginning phases of development. The hyphae are commonly non-septate; under certain extraordinary conditions, septa might be seen in specific structures. Actinomycetes comprise about 10% of the

***Corresponding author**

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(6) 2022, pp. 677-682
DOI: http://dx.doi.org/10.21161/mjm.221509

Table 1: Methods used in Pedagogical Analysis.

	Factor	Method
1	pH	Potentiometry in a 1:2.5 soil:water suspension using pH meter
2	EC	Using a conductivity meter in a 1:2.5 soil water suspension
3	OC	Walkely- Black acid digestion method (Gelman *et al*., 2012)
4	P	Bray and Kurtz method using a spectrophotometer (Irving and McLaughlin, 1990)
5	K	Flame photometrically using 1 N ammonium acetate as extractant
6	Ca	Flame photometrically using 1 N ammonium acetate as an extractant
7	S	Using a spectrophotometer with sodium acetate buffer as an extractant
8	Boron	Using a spectrophotometer with Azomethine-H

bacteria colonizing marine aggregates and can be isolated from marine sediments (Ward and Bora, 2006). *Streptomyces* is the largest genus of Actinobacteria proposed by Waksman and Henrici in 1943 and more than 500 species of this genus have been reported by Euzeby (2008). Most of the antibiotics in clinical use are direct natural products or semi-synthetic derivatives from actinomycetes.

MATERIALS AND METHODS

Marine sediments and seashore soils were collected from different sampling sites and processed according to standard protocols (Pisano *et al*., 1989). The samples were collected from 10 different locations along the coastal regions of the Arabian Sea, Thiruvananthapuram District, Kerala, India (Figure 1). The marine actinomycetes were isolated from the samples using standard microbiological isolation methods with actinomycete isolation agar and casein starch agar (Kuster and Williams, 1964). The physiochemical parameters of the samples were analyzed at Central Soil Analytical Laboratory Thiruvananthapuram following standard protocols (Table 1). The actinomycete load in each sample was calculated by the standard method and expressed as CFU/mL using the mathematical expression given below.

The actinomycete load in each sample = (Average number of colonies × Dilution factor)/Volume of sample

Morphologically distinct actinomycetes colonies were selected for further studies based on their macroscopic qualities such as colony size, shape, margin, elevation, pigmentation etc. Selected actinomycetes isolates were microbiologically purified by repeated sub-culturing on starch casein agar and the purified isolates were stored at four degree Celsius in starch casein agar slants for further studies.

RESULTS AND DISCUSSION

In the present study, we have done sampling at the post-monsoon season. From the literatures it is evident that the actinomycete diversity is rich in the post-monsoon season. During post-monsoon season, the seawater will be rich in micro and macronutrients, which trigger the growth and metabolism of marine actinomycetes.

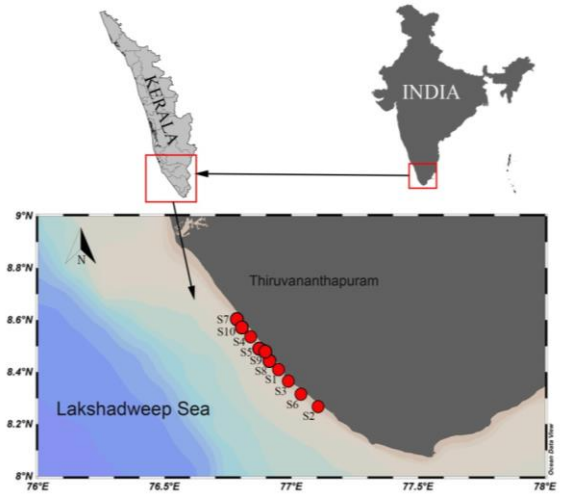

Figure 1: Location map of the study area. S1-Kovalam, S2-Poovar, S3-Vizhinjam, S4-Veli, S5-Shanghumugam, S6-Azhimala, S7-Perumathura, S8-Poonthura, S9-Valiyathura, S10-Menamkulam.

Rathode *et al*. (2019) reported that they observed most morphotypes of actinomycetes during the post-monsoon season than during monsoon and pre-monsoon season.

The results showed that the marine habitats of Kerala coastal regions are rich in diverse actinomycetes. The morphologically distinct isolates were selected for downstream studies. From the sediment samples collected from 10 different sites, we obtained 156 morphologically distinct isolates (SD 1-SD 156). While from seashore samples, 124 morphologically distinct isolates were obtained (V 1-V 124). All these results show that the marine habitat of the Kerala coastal region is rich in actinomycete diversity.

The actinomycete load in different samples was also analyzed using CFU method. In order to statistically signify the results, the experiments were done in triplicates and average values were obtained (Table 2). The physiochemical parameters of the samples were analysed at Central Soil Analytical Laboratory. The results showed that, in general, the soil/sediment of the Kerala coastal region is slightly alkaline in nature. Organic C, P and K were found to be low, while S 100-300 ppm and Ca 100-800 ppm were found. The available B was found in the range between 0.5 to 2.6 ppm. The availability of

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(6) 2022, pp. 677-682
DOI: http://dx.doi.org/10.21161/mjm.221509

Table 2: Average colony forming units from each of the samples CFU/mL.

	Station	Cite	Sample	Geographical Coordinates	Average CFU/mL (done in triplicate)
1	Kovalam	I	Sediment 1	8.3841 N 76.9793 E	1.8×10^3
			Sediment 2	8.3851 N 76.9783 E	2.1×10^3
			Shore soil	8.3988 N 76.9820 E	9.0×10^2
		II	Sediment 1	8.3831 N 76.9563 E	4.0×10^2
			Sediment 2	8.3877 N 76.9767 E	2.5×10^3
			Shore soil	8.3878 N 76.9720 E	3.1×10^3
		III	Sediment 1	8.3831 N 76.9693 E	1.2×10^3
			Sediment 2	8.3834 N 76.9763 E	1.1×10^3
			Shore soil	8.3868 N 76.9556 E	2.5×10^3
2	Poovar	I	Sediment 1	8.3114 N 77.0723 E	1.2×10^3
			Sediment 2	8.3154 N 77.1703 E	9.0×10^2
			Shore soil	8.3123 N 77.0704 E	3.0×10^2
		II	Sediment 1	8.3214 N 77.4723 E	2.1×10^3
			Sediment 2	8.3204 N 77.0783 E	3.3×10^3
			Shore soil	8.7123 N 77.1764 E	1.0×10^3
		III	Sediment 1	8.3114 N 77.0723 E	9.0×10^2
			Sediment 2	8.3104 N 77.0703 E	1.1×10^3
			Shore soil	8.3134 N 77.0698 E	1.3×10^3
3	Vizhinjam	I	Sediment 1	8.3759 N 76.9933 E	2.2×10^3
			Sediment 2	8.3756 N 76.9940 E	1.9×10^3
			Shore soil	8.3766 N 76.9942 E	3.1×10^3
		II	Sediment 1	8.3659 N 76.9263 E	2.2×10^3
			Sediment 2	8.3816 N 76.9658 E	9.0×10^3
			Shore soil	8.3758 N 76.9957 E	8.0×10^3
		III	Sediment 1	8.3714 N 76.9943 E	1.8×10^3
			Sediment 2	8.3656 N 76.9910 E	3.2×10^3
			Shore soil	8.3756 N 76.9946 E	1.1×10^3
4	Veli	I	Sediment 1	8.5187 N 76.8758 E	2.0×10^3
			Sediment 2	8.5287 N 76.8658 E	1.2×10^3
			Shore soil	8.5145 N 76.8815 E	2.5×10^3
		II	Sediment 1	8.5170 N 76.8771 E	1.4×10^3
			Sediment 2	8.5130 N 76.8800 E	2.0×10^3
			Shore soil	8.5113 N 76.8770 E	9.0×10^3
		III	Sediment 1	8.5156 N 76.8671 E	3.1×10^3
			Sediment 2	8.5220 N 76.8700 E	5.0×10^3
			Shore soil	8.5213 N 76.8760 E	2.4×10^3
5	Shangumugham	I	Sediment 1	8.4780 N 76.9113 E	8.0×10^3
			Sediment 2	8.4800 N 76.9196 E	6.0×10^3
			Shore soil	8.4807 N 76.9101 E	1.6×10^3
		II	Sediment 1	8.4680 N 76.9123 E	3.0×10^3
			Sediment 2	8.4890 N 76.9296 E	5.0×10^3
			Shore soil	8.4794 N 76.9111 E	1.6×10^3
		III	Sediment 1	8.4580 N 76.9143 E	2.0×10^3
			Sediment 2	8.4790 N 76.9396 E	4.0×10^3
			Shore soil	8.4784 N 76.9121 E	1.1×10^3
6	Azhimala	I	Sediment 1	8.3558 N 77.0108 E	1.6×10^3
			Sediment 2	8.3545 N 77.0092 E	1.4×10^3
			Shore soil	8.3570 N 77.0105 E	2.2×10^3
		II	Sediment 1	8.3508 N 77.0118 E	3.0×10^3
			Sediment 2	8.3575 N 77.0492 E	2.4×10^3
			Shore soil	8.3583 N 77.0090 E	5.0×10^3
		III	Sediment 1	8.3608 N 77.1118 E	8.0×10^3
			Sediment 2	8.3075 N 77.0452 E	1.4×10^3
			Shore soil	8.3556 N 77.0144 E	2.1×10^3

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(6) 2022, pp. 677-682
DOI: http://dx.doi.org/10.21161/mjm.221509

(Continued)

7	Perumathura	I	Sediment 1	8.6346 N 76.7843 E	3.0×10^3
			Sediment 2	8.6349 N 76.7842 E	2.1×10^3
			Shore soil	8.6351 N 76.7845 E	1.2×10^3
		II	Sediment 1	8.6335 N 76.7850 E	1.0×10^3
			Sediment 2	8.6351 N 76.7840 E	3.0×10^3
			Shore soil	8.6355 N 76.7842 E	1.8×10^3
		III	Sediment 1	8.6235 N 76.7830 E	1.4×10^3
			Sediment 2	8.6151 N 76.7849 E	1.6×10^3
			Shore soil	8.6455 N 76.7742 E	1.1×10^3
8	Poonthura	I	Sediment 1	8.4428 N 76.9424 E	9.0×10^3
			Sediment 2	8.4328 N 76.9524 E	5.0×10^3
			Shore soil	8.4423 N 76.9431 E	1.5×10^3
		II	Sediment 1	8.4458 N 76.9324 E	6.0×10^3
			Sediment 2	8.4628 N 76.9124 E	2.1×10^3
			Shore soil	8.4431 N 76.9420 E	2.0×10^3
		III	Sediment 1	8.4428 N 76.9424 E	9.0×10^3
			Sediment 2	8.4381 N 76.9554 E	3.0×10^3
			Shore soil	8.4440 N 76.9417 E	9.0×10^2
9	Valiyathura	I	Sediment 1	8.4644 N 76.9245 E	11×10^2
			Sediment 2	8.4641 N 76.9242 E	23×10^2
			Shore soil	8.7644 N 76.9246 E	19×10^2
		II	Sediment 1	8.4634 N 76.9215 E	15×10^2
			Sediment 2	8.4541 N 76.9142 E	29×10^2
			Shore soil	8.4641 N 76.9249 E	18×10^2
		III	Sediment 1	8.4656 N 76.9248 E	5×10^2
			Sediment 2	8.4569 N 76.9102 E	14×10^2
			Shore soil	8.4639 N 76.9250 E	25×10^2
10	Menamkulam	I	Sediment 1	8.5548 N 76.8489 E	11×10^2
			Sediment 2	8.554. N 76.8492 E	3×10^2
			Shore soil	8.5546 N 76.8496 E	9×10^2
		II	Sediment 1	8.5508 N 76.8419 E	4×10^2
			Sediment 2	8.504. N 76.8192 E	16×10^2
			Shore soil	8.5537 N 76.8502 E	13×10^2
		III	Sediment 1	8.5547 N 76.8493 E	3×10^2
			Sediment 2	8.5543 N 76.8899 E	5×10^2
			Shore soil	8.5552 N 76.8490 E	10×10^2

these mineral nutrients as well as the salinity and pH, must have a direct role in determining the actinomycete diversity of these regions. Nutrients are considered as one of the most critical parameters in the marine environment influencing the growth, reproduction and metabolic activities of biotic components (Saravanakumar *et al.*, 2008). Due to global warming and climate change, the pH of the seas is changing to acidic. In our study also, many of the soil/sediment samples were showed neutral pH, which is an indicator of the pH shift of the marine environment from alkaline to acidic. Similar studies were done along the coast of Tamilnadu by Manikandan and Vijayakumar (2016). They observed that the pH of many of the soil/sediment samples collected along the Palk Strait region was acidic in nature, which substantiates the acidification of oceans.

The samples from Kovalam and Shangumugham showed less salinity compared to other samples. The variation in these sampling sites may affect the actinomycete diversity pattern also. The highest organic C content was reported from the soil samples of Poovar and Perumathura. Available K, B, S and Ca also showed an increased range of variation among the soil samples. The highest K was reported from the samples collected from Veli and Perumathura. Other parameters like B, S and Ca showed a wide variation among samples.

Identification and characterization of novel molecules from natural sources is a continuous process to cope with the demand for the treatment of various infectious diseases. Many molecules from various biological sources have been used to treat medically associated infectious diseases. The marine members of the order Actinomycetales contain innumerable diversity in both phenotypic and genomic characteristics and utility in various fields. In the medical field, actinomycetes play roles as sources of secondary metabolites that function as antibiotics, antifungals, antihelmintic and antitumor agents (Barka *et al*., 2015). Few reports claimed that the actinomycetes from marine origin were ideal sources of novel secondary molecules with diverse chemical diversity (Prabhavathy *et al*., 2006; Almalki, 2020). Many reports suggested that the actinomycetes could be isolated using starch casein agar medium supplemented with some antibiotics such as actidione and nalidixic acids

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

(Sujatha *et al*., 2005; Al-Dhabi *et al*., 2019). We expect that looking to these marine actinomycetes will likely become more commonplace in the search for new antibiotics. In the present study, a total of 280 actinomycete isolates with distinct morphological differences were obtained from the marine samples. The result clearly indicates the unique actinomycete diversity present in the tropical seas.

CONCLUSION

The marine actinomycetes are a potential source of several bioactive molecules. The results of this study showed that the marine soil/sediments of Kerala regions harbour a diverse group of actinomycetes. Further characterization is needed to elucidate its full biological potential. The characteristic features unique to the coastal areas of Kerala may be one of the contributing factors to the actinomycete richness we observed in this research.

REFERENCES

Al-Dhabi, N. A., Ghilan, A. M., Esmail, G. A., Arasu, M. V., Duraipandiyan, V. and Ponmurugan, K. (2019). Environmental friendly synthesis of silver nanomaterials from the promising *Streptomyces parvus* strain Al-Dhabi-91 recovered from the Saudi Arabian marine regions for antimicrobial and antioxidant properties. *Journal of Photochemistry and Photobiology B: Biology* **197, 111529.**

Almalki, M. A. (2020). Isolation and characterization of polyketide drug molecule from *Streptomyces* species with antimicrobial activity against clinical pathogens. *Journal of Infection and Public Health* **13(1), 125-130.**

Atta, H. M., Dabour, S. M. and Desoukey, S. G. (2009). Sparsomycin antibiotic production by *Streptomyces* sp. AZ-NIOFD1: Taxonomy, fermentation, purification and biological activities. *American-Eurasian Journal of Agriculture and Environmental Science* **5(3), 368-377.**

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., Klenk, H., Clément, C., Ouhdouch, Y. and van Wezel, G. P. (2015). Taxonomy, physiology and natural products of actinobacteria. *Microbiology Molecular Biology Reviews* **80(1), 1-43.**

Biabani, M. A., Laatsch, H., Helmke, E. and Weyland, H. (1997). Delta-Indomycinone: A new member of pluramycin class of antibiotics isolated from marine *Streptomyces* sp. *Journal of Antibiotics* **50(10), 874-877.**

Charan, R. D., Schlingmann, G., Janso, J., Bernan, V., Feng, X. and Carter, G. T. (2004). Diazepinomicin, a new antimicrobial alkaloid from a marine *Micromonospora* sp. *Journal of Natural Products* **67, 1431-1433.**

Edward, G. (2006). Bergey's Manual of Derminative Bacteriology, 9th edition. WMC Brown Publishers, USA. **pp. 712-829.**

Euzeby, J. P. (2008). Genus *Streptomyces*. List of prokaryotic names with standing in nomenclature. http://www.bacterio.cict.fr/s/streptomycesa.html

Fenical, W., Baden, D., Burg, M., Goyet, C.V., Grimes, J. D., Katz, M., Marcus, N. H., Pomponi, S., Rhines, P., Tester, P. and Vena, J. (1999). Marine derived pharmaceuticals and related bioactive compounds, Understanding the Ocean's role in human health. *National Academies Press Washingtom* **71-86.**

Gelman, F., Binstock, R. and Halicz, L. (2012). Application of the Walkley–Black titration for the organic carbon quantification in organic rich sedimentary rocks. *Fuel* **96, 608-610.**

Gesheva, V., Ivanova, V. and Gesheva, R. (2005). Effects of nutrients on the production of AK-111-81 macrolide antibiotic by Streptomyces hygroscopicus. *Microbiological Research* **160(3),243-8.**

Goodfellow, M. and Williams, S. T. (1983). Ecology of actinomycetes. *Annual Review of Microbiology* **37(1), 189-216.**

Imada, C. (2005). Enzyme inhibitors and other bioactive compounds from marine actinomycetes. *Antonie Van Leeuwenhoek* **87(1), 59-63.**

Irving, G. C. and McLaughlin, M. J. (1990). A rapid and simple field test for phosphorus in Olsen and Bray No. 1 extracts of soil. *Communications in Soil Science and Plant Analysis* **21(19-20), 2245-2255.**

Kuster, E. and Williams, S. T. (1964). Selection of media for isolation of *Streptomycetes*. *Nature* **202(4935), 928-929.**

Li, F., Maskey, R. P., Qin, S., Sattler, I., Fiebig, H. H., Maier, A., Zeeck, A. and Laatsch, H. (2005). Chinikomycins A and B: Isolation, structure elucidation and biological activity of novel antibiotics from a marine *Streptomyces* sp. isolate M045. *Journal of Natural Products* **68(3), 349-353.**

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1989). Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. **pp. 10.51-10.67.**

Manikandan, R. and Vijayakumar, R. (2016). Physico-chemical analysis of marine soils from different ecosystem Palk Strait coastal regions of Tamilnadu, India. *Journal of Marine Science: Research and Development* **6, 208.**

Maskey, R. P., Helmke, E. and Laastsch, H. (2003). Himalomycin A and B: Isolation and structure elucidation of new fridamycin type antibiotics from a marine streptomyces isolate. *Journal of Antibiotics* **56(11), 942-949.**

Pisano, M. A., Sommer, M. J. and Brancaccio, L. (1989). Isolation of bioactive actinomycetes from marine sediments using rifampicin. *Applied Microbiology and Biotechnology* **31(5), 609-612.**

Prabavathy, V. R., Mathivanan, N. and Murugesan, K. (2006). Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5. *Biological Control* **39(3), 313-319.**

Rathore, D. S., Sheikh, M., Gohel, S. and Singh, S. P. (2019). Isolation strategies, abundance and characteristics of the marine actinomycetes of Kachhighadi, Gujarat, India. *Journal of the Marine Biological Association of India* **61(1), 71-78.**

Saravanakumar, A., Rajkumar, M., Sesh Serebiah, J. and Thivakaran, G. A. (2008). Seasonal variations in physico-chemical characteristics of water, sediment and soil texture in arid zone mangroves of Kachchh-Gujarat. *Journal of Environmental Biology* **29(5), 725-732.**

Subramani, R. and Sipkema, D. (2019). Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products. *Marine Drugs* **17(5), 249.**

Sujatha, P., Raju, K. B. and Ramana, T. (2005). Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*. *Microbiological Research* **160(2), 119-126.**

Valli, S., Suvathi, S. S., Aysha, O. S., Nirmala, P., Vinoth, K. P. and Reena, A. (2012). Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pacific Journal of Tropical Biomedicine* **2(6), 469-473.**

Ward, A. C. and Bora, N. (2006). Diversity and biogeography of marine actinobacteria. *Current Opinion in Microbiology* **9(3), 279-286.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538