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

Streptomycetes associated with marine sponges collected from Andaman Sea and its antibacterial activity

Ram Baskaran[1]*, Subramanian Thenmozhi[1], K. Vinod Kumar[2], P.M. Mohan[1], Ramasamy Vijayakumar[3]

[1]Department of Ocean Studies and Marine Biology, Pondicherry University, Brookshabad Campus, Port Blair - 744 112, Andaman Island, India.
[2]Regional Medical Research Centre (ICMR), WHO Collaborating Centre for Diagnosis, Reference, research and training in leptospirosis, Port Blair -744101, Andaman and Nicobar Islands, India.
[3]Department of Microbiology, Bharathidasan University Constituent College, Kurumbalur - 621 107, Perambalur Dt, Tamilnadu, India.
Email: sivan.thamilan@gmail.com

Received 6 January 2016; Received in revised form 7 January 2017; Accepted 23 March 2017

ABSTRACT

Aims: Sponges harbour diverse prokaryotic and eukaryotic microbes. However, diversity of sponge-derived *Actinomycetes* is of high interest because of its bioactive compounds. In the present study, diversity of *Streptomycetes* associated with marine sponges collected from Pongibalu (south Andaman) was investigated.
Methodology and results: Sponges samples were collected by underwater SCUBA diver and Kuster's agar media was used for isolation of actinobacteria. Colony morphology and 16s rRNA were studied for identification of isolates and phylogenetic tree was constructed using MEGA.6. A total of ten *Streptomyces* species were identified based on 16S rRNA gene sequencing from three sponge species (*Hemiasterella* spp. *Tentorium* spp. and *Tethyopsis* spp.). The organic extracts of these ten isolates revealed bioactivity against tow Gram positive and eight Gram negative pathogenic bacteria.
Conclusion, significance and impact of study: This study suggests prospects and potentials of the diverse population of *Streptomyces* with bioactivity in marine sponges. It would be a potential source in the pharmaceutical industries. As well as actinobacteria associated with sponge may prevent the sponge from pathogenic bacteria, fungi, virus and other microflora by secretion of secondary metabolites on surface and inside. To understand the sponge and actinobacteria association and its bioactivity, a profound study need to be conducted.

Keywords: Marine sponges, *Streptomyces*, diversity, bioactivity, Andaman Islands

INTRODUCTION

Sponges (*Porifera*) are the ancient multicellular phylum with a fossil record dating back to Precambrian times (Hentschel *et al*., 2012). Sponges populate tropical reefs in great abundance (Schmitt *et al*., 2012), and are commonly known to harbour diverse microbes (Hentschel *et al*., 2006; Wang *et al*., 2006). Studies show these are phylogenetically diverse, yet highly sponge-specific, prokaryotic microbial groups. Actinomycetes have been cultivated from the marine environment including sea water (Zhang *et al*., 2012), marine snow, and marine sediments (Becerril-Espinosa *et al*., 2013). So far, at least 32 bacterial phyla and candidate phyla were described from marine sponges by both cultivation-dependent and cultivation-independent techniques; with the most common phyla being *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadetes*, *Nitrospira*, Planctomycetes, *Proteobacteria*, (Alpha, Delta, Gamma subclasses) and Spirochaetes (Hentschel *et al*., 2012; Schmitt *et al*., 2012). *Actinomycetes* have also been cultivated from different marine invertebrates (Abdelmohsen *et al*., 2010; Sun *et al*., 2012; Wyche *et al*., 2012), with the majority being isolated from sponges (Zhang *et al*., 2006a; Xi *et al*., 2012). Marine *actinomycetes* produced a multitude of novel lead compounds with biotechnological and pharmaceutical applications.

The genus *Streptomyces* of the family *Streptomycetaceae* contains the largest number of species among the genera of the *Actinomycetales* (Goodfellow *et al*., 1992). Because of the importance of the *Streptomyces* as a source of novel bioactive compounds, more than 3000 *Streptomycetes* species have been proposed. Biological activities such as antibacterial, antifungal, antiparasitic, antimalarial, immunomodulatory, anti-inflammatory, antioxidant, and anticancer activities were reported from sponge-

*Corresponding author

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

associated *actinomycetes* (Bull and Stach, 2007; Pimentel-Elardo *et al*., 2010; Abdelmohsen *et al*., 2012; Blunt *et al*., 2013). Actinomycetes account for approximately half of the natural products [MarinLit database 2013 (John Blunt, MarinLit, University of Canterbury, New Zealand)] (Lam, 2006; Thomas *et al*., 2010). A total of 411 natural products from marine *Actinomycetes* were reported in the Marin Lit database in 2013 (J. Blunt, Marin Lit, University of Canterbury, New Zealand) of which 22% were obtained from marine sponge-associated *Actinomycetes* (Abdelmohsen *et al*., 2014). At present, the finding of rare or novel marine *Actinomycetes* has become a major focus in the search for the next generation of pharmaceutical agents (Bull *et al*., 2000). Andaman and Nicobar islands are a rich source for flora and fauna diversity, a total of 88 species of sponges was reported from Andaman and Nicobar islands. But very few reports are available pertaining to sponge associated bacteria especially actinobacteria from Andaman and Nicobar Islands. In this study, *Streptomycetes* were isolated from marine sponge species collected from the Andaman Islands. The obtained *Streptomyces* were phylogenetically characterized based on 16S rRNA gene sequencing and their biological activities against pathogenic bacteria are reported.

MATERIALS AND METHODS

Sponge collection

Three sponge species (*Hemiasterella* sp., *Tentorium* sp. and *Tethyopsis* sp.) were collected by SCUBA diving at depths of 5-10 m in Pongibalu, south Andaman Island. Sponges were transferred to plastic bags containing sterile sea water and transported to the laboratory. Sponge specimens were rinsed in steriled sea water and ddH_2O, cut into pieces of 1 cm^3 and thoroughly homogenized in a sterile mortar with 10 volumes of sterile sea water. The supernatant was diluted in 10-fold series and subsequently plated out on agar plates.

Streptomycetes isolation and identification

Kusters agar and actinomycetes agar was supplemented with 30 µg/mL of the fungicide cyclohexamide and 20 µg/mL nalidixic acid to facilitate the isolation of actinobacteria and deter the growth of sponge associated fungi and gram negative bacteria on the plates. Aliquots of 100 µL were transferred from each sponge homogenate dilution to the two selective media in triplicate. The plates were incubated at 28 °C for 3-4 weeks until actinomycetes were observed growing on the plates. Individual colonies were isolated and plated on new agar plates with the same media that was used for their initial growth. Following this procedure, actinomycetes were isolated and identified through sequencing of the 16S rRNA gene.

Isolation of DNA and PCR amplification

Streptomycetes isolates were cultured in Kuster's agar. The genomic DNA was extracted following the procedure described by Ausubel *et al*. Polymerase chain reaction (PCR) was performed using 16S rRNA primers Forward 5'- AGA GTT TGA TCM TGG CTC AG -3', Reverse 5'- ACG GCT ACC TTG TTA CGA CTT -3' following the standard protocol (Lane *et al*., 1985). The amplified products were sequenced using DNA analyzer 3730 (Applied Biosystems, CA, USA). The partial 16S rRNA gene sequences and sequences of closely related strains in the NCBI database were retrieved and assembled using MEGA 5 software (Tamura *et al*., 2011) and the unknown sequences were identified using the CLUSTALW alignment tool of MEGA 5.

Test bacterial strains for bioactivity

Gram positive bacteria *(Bacillus* spp. MTCC-3133 and *Staphylococcus aureus* MTCC-3160) and Gram negative bacteria (*Klebsiella pneumoniae* subs MTCC-3040, *Salmonella infantis* MTCC-1167, *Loctococcus lactis* sup spp. 1-440, *Escherichia coli* (Electro aggregative) ICMR, *Vibrio cholerae* 01 Inaba- ICMR, *Pseudomonas* spp. (KU), *Proteuse* sp. MTCC- 425, *Citrobacter* spp. (KU) and *Shigella flexneri*.

Screening for bioactivity

The *Streptomyces* spp. were inoculated into a 5 L Halfman flask containing 2.5 L production media broth (dextrose- 20 g, soya bean- 20 g, soluble starch-5 g, peptone- 5 g, $(NH_4)_2SO_4$- 2.5 g, $MgSO_4.7H_2O$- 0.25 g, K_2HPO_4- 0.02 g, NaCl- 4 g, $CaCo_3$- 2 g, seawater- 500 mL, ddH_2O- 500 mL and pH 7±0.2), and incubated at 28±2 °C on a rotary shaker at 250 rpm for seven days. The fermented broth was centrifuged at 10000 rpm at 4 °C for 20 min and the supernatant was filtered using 0.45 µm filter (Millipore). An equal volume (1:1) of the organic solvent (ethyl acetate) was added to the cell free culture filtrates and mixed on a shaker for 12 h, the solvent was separated and the extract was evaporated using a rotary evaporator and the crude powder was collected as antimicrobial compound. The crude powder was mixed with DMSO and used to test the activity against tested pathogens as well as DMSO alone was maintained as a control.

RESULTS

Isolation and phylogenetic identification

A total of 37 actinobacteria were isolated from three sponges in two used media, among them 16, 12 and 9 were observed in *Hemiasterella* sp., *Tentorium* sp. and *Tethyopsis* sp. respectively. Off 37 only ten isolates were found to be distinguished on the colony morphology and aerial and substrate spore color. Among the ten 4, 3 and 3 were obtained from *Hemiasterella* sp., *Tentorium* sp. and

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

Tethyopsis sp. respectively. These *Streptomycetes* were observed under light microscope (400×) for its confirmation before 16S rRNA analysis. The microscopic observation proved distinguished morphology among them. Distinguished *Streptomyces* were depicted with Gene bank accession number in Table 1.

DNA based molecular methods have been used for species differentiation and the identification of Streptomycetes. Significance of phylogenetic studies based on 16S rDNA sequences enhanced the knowledge on the systematics of actinobacteria (Yokota, 1997). Sequences of 16S rDNA have provided actinobacteriologists with a phylogenetic tree that allowed the investigations on the evolution of actinobacteria and also provides the basis for identification. In the present study, variations in the 16S rDNA sequences of ten sponge associated different *Streptomyces* spp. were observed. The phylogenetic analysis of partial 16S rDNA sequence showed maximum similarity with *Streptomyces* and no similarity among the sponge isolated *Streptomyces* genus. The same was analysed with various genus of actinobacteria and other Gram positive and negative bacteria, it was distinguished from the Gram negative and positive bacteria when it shows maximum similarity with *Streptomyces* and moderately with other actinobacterial species (Figure 1). It shows different species of *Streptomyces* associated with marine sponges.

Antibacterial activity of ethyl acetate extract of *Streptomycetes*

All the morphologically distinguished strains were tested against two Gram positive and eight Gram negative pathogenic bacteria. The organic solvent extract of the *Streptomyces* spp. showed activity against most of the tested pathogens. Among the isolates, *Streptomyces* sp.-DOSMB SP10 showed maximum activity in range of 14.33mm to 40mm against all the test pathogens except *S. infantis*. SP3, SP5, SO7, SP9, SP11, SP20, SP23, SP24 also showed activity against most of the tested pathogens (Table 2). Almost all isolate showed activity against both Gram positive and negative bacteria.

Table 1: Identification of the sponge associated bacterial isolates by alignment with 16S rRNA gene sequences of published bacterial species in the NCBI database.

Strain ID	Host sponge spp.	Sequence length (bp)	Closest published database accession	Proposed identity	Accession number
DOSMB-SP9	*Hemiasterella* spp.	565	KF477115	*Streptomyces xiamenensis*	KJ933452
DOSMB-SP7	*Hemiasterella* spp.	772	NR109175	*Streptomyces abyssalis*	KJ933451
DOSMB-SP6	*Hemiasterella* spp.	753	KT163794	*Streptomyces thermocarboxydus*	KJ933450
DOSMB-SP24	*Hemiasterella* spp.	694	NR044534	*Streptomyces nanshensis*	KJ933449
DOSMB-SP5	*Tentorium* spp.	534	EU158356	*Streptomyces* spp.	KJ933448
DOSMB-SP10	*Tentorium* spp.	766	AB498661	*Streptomyces* spp.	KJ933447
DOSMB-SP20	*Tentorium* spp.	694	KF444511	*Streptomyces plicatus*	KJ933446
DOSMB-SP23	*Tethyopsis* spp.	653	HQ238361	*Streptomyces sindenensis*	KJ933445
DOSMB-SP11	*Tethyopsis* spp.	723	KM215732	*Streptomyces cyaneus*	KJ933444
DOSMB-SP3	*Tethyopsis* spp.	863	KJ002642	*Streptomyces gancidicus*	KJ933435

DISCUSSION

Marine ecosystems consist of taxonomically and biologically diverse macro- and microorganisms which exhibit unique physiological and structural features enabling them to survive under the extremes of pressure, salinity and temperature. Many marine organisms are further endowed with the ability to produce novel molecules with interesting therapeutic applications not observed in their terrestrial counterparts (Lane, 2008; Li and Vederas, 2009; Rateb and Ebel, 2011).

Andaman and Nicobar Islands are the one of the most biodiverse environment in Indian Ocean. .

However, few studies have been carried out so far to investigate actinobacterial communities from sponges of Andaman Islands. *Actinomycetes* are known for their unprecedented ability to produce novel lead compounds of clinical and pharmaceutical importance. The most powerful approach to solve the taxonomic problems of actinobacteria is the study of nucleic acids. Comparison of nucleic acids yields considerable information on the true relatedness. Molecular systematic which includes both classification and identification, has its origin in the early nucleic acid hybridization studies has achieved a new status following the identification of nucleic acid sequences through sequencing techniques (Brenner *et al*., 2001).

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

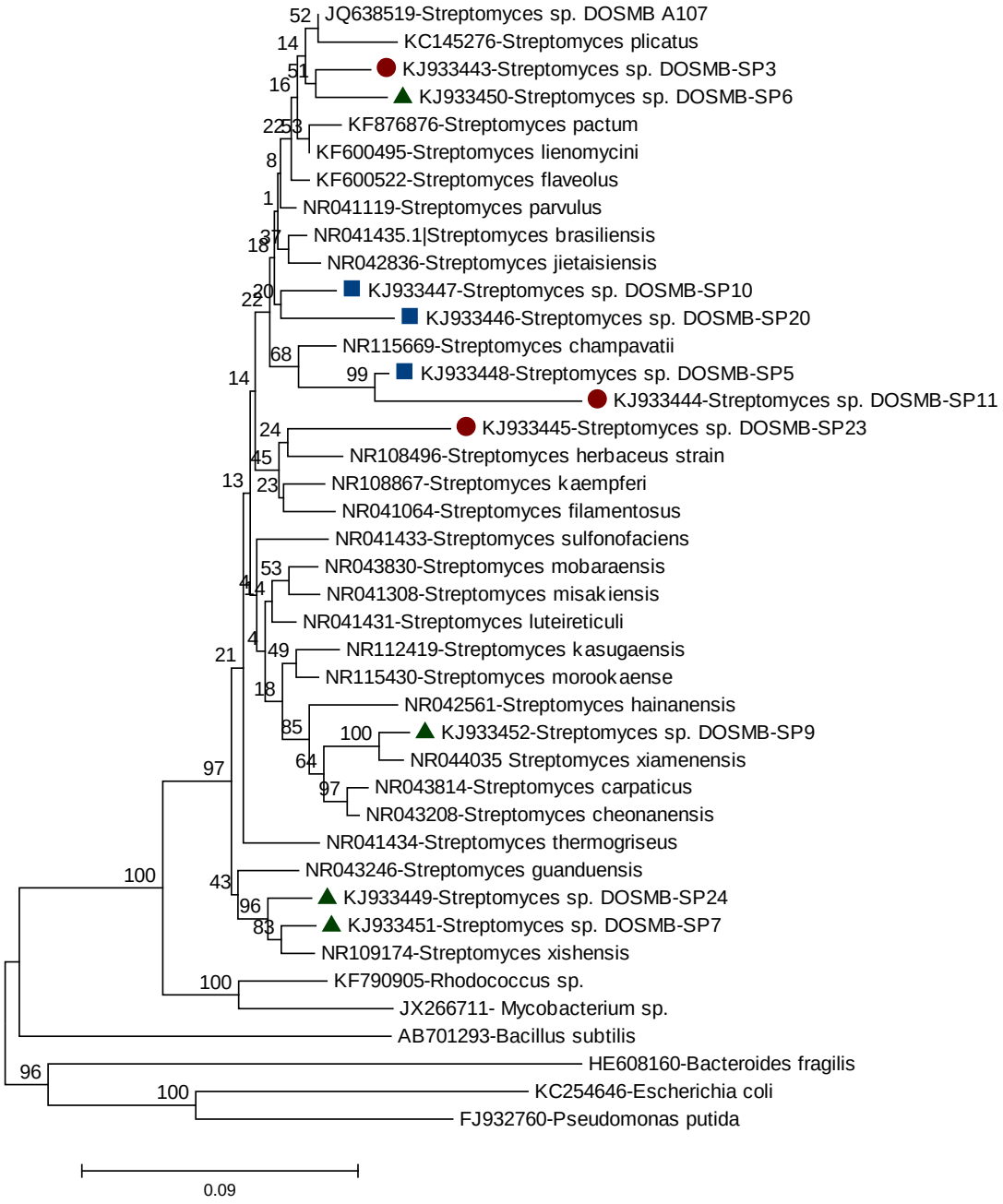

Figure 1: The inferred phylogenetic tree showing the diversity of 10 sponge associated *actinomycetes* isolates, including 31 reference strains. Evolutionary distances were determined with pairwise dissimilarities of the 16S rRNA gene sequences and the dendrogram was generated using the neighbor-joining algorithm (MEGA5). Closed sphere closed triangle, closed square indicate isolated from *Hemiasterella* spp., *Tentorium* spp. and *Tethyopsis* spp. respectively.

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

Table 2: Antimicrobial activity of organic solvent extract of *Streptomyces* spp.

Test pathogens	*Streptomyces* spp. Mean values are presented (Zone of inhibition mm)									
	SP3	SP5	SP6	SP7	SP9	SP10	SP11	SP20	SP23	SP24
Gram positive										
S. aureus	17.33 ±0.19	22±0 0	14.33± 0.19	15.66± 0.19	22.66± 0.19	30.33± 0.33	14.66±0. 38	18.33± 0.19	-	17.33± 0.19
Bacillus spp.	-	21.66 ±0.19	12.33± 0.19	13±0.3 3	12.33± 0.19	14.33± 0.38	13.33±0. 19	13.33± 0.19	7.33±0. 19	-
Gram negative										
K. pneumoniae	17.66 ±0.19	24.66 ±0.9	9.33±0. 19	-	22.66± 0.19	25 ±0.33	8.33±0.1 9	13.33± 0.19	16.66± 0.19	17.66± 0.19
S. infantis	15.33 ±0.19	-	11.66+ 0.19	13±00	20.66± 0.19	-	18.33±0. 38	17.33± 0.19	10.66± 0.19	15.33± 0.19
E. coli	14.66 ±.19	22.66 ±0.50	14.66± 0.19	11±0.3 3	17.66±. 0.19	29.33± 0.38	12±0.00	11.33± 0.19	12.33± 0.38	-
V. cholerae	-	23.66 ±.19	18.66± 0.19	22±033	9±0	24.66± 0.50	-	20.66± 0.19	18.33± 0.38	18.66±. 19
S. flexneri	16.66 ±.0.1 9	25±0. 33	-	22.33± 0.19	7.33±0. 19	22±00	9.33±0.1 9	18.66± 0.19	20.33± 0.38	16.66±. 0.19
Pseudomonas spp.	13.66 ±0.19	14.33 ±.38	12.66± 0.19	17.33± 0.19	16±0	40±0.3 3	11.33±0. 19	11.66± 0.19	-	13.66± 0.19
Proteus spp.	8.66± 0.19	20.33 ±0.19	11.66± 0.19	-	20.66± 0.19	24.66± 0.19	15.66±0. 19	10.33± 0.19	12.33± 0.19	8.66±0. 19
Citrobacter spp.	11.33 ±0.19	15.33 ±0.19	13.66± 0.19	10.66± 0.19	14.66± 0.19	18.33± 0.19	11.66±0. 19	-	11.33± 0.19	11.33± 0.19
Bacillus spp.	-	21.66 ±0.19	12.33± 0.19	13±0.3 3	12.33± 0.19	14.33± 0.38	13.33±0. 19	13.33± 0.19	7.33±0. 19	-

A total of 10400 actinomycete 16S rRNA gene sequences were thus far obtained by isolation from marine sources. This compares to about 36000 16S rRNA gene sequences from terrestrial *Actinomycetes*, which, however, have a much longer history of exploration. *Actinomycetes* were found in association with different marine invertebrates such as so corals, tunicates and fish (Bull *et al*., 2005; Abdelmohsen *et al*., 2010; Sun *et al*., 2012; Wyche *et al*., 2012) but the majority was isolated from sponges (Hentschel *et al*., 2003; Zhang *et al*., 2006a; Selvin *et al*., 2010).

It has long been known that actinomycetes can be cultured from marine sources (Grein and Meyers, 1958) yet it was not clear whether these typically soil-derived bacteria should be considered as terrestrial “contaminants” or as true components of the marine ecosystem (Weyland, 1969). Early evidence for the existence of indigenous marine actinomycete populations came from the description of the first marine species, *Rhodococcus marinonascene* in 1984 (Helmke and Weyland, 1984), the metabolic activity of some *Streptomyces* strains in marine sediments (Moran *et al*.,

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

1995) and the isolation of obligate marine strains (Jensen *et al*., 1991).

In the present study, we have isolated ten phylogenetically distinguished *Streptomyces* species from three sponges of Andaman Islands with antibacterial properties. Similarly, Abdelmohsen *et al*. (2014) has reported the diverse actinobacteria associated with marine sponges of Red Sea. During the investigation of actinobacterial diversity from intertidal marine sponges, a novel actinobacterium, *Actinoalloteichus hymeniacidonis* was isolated from *Hymeniacidon perleve* (Zhang *et al*., 2006b). Similarly, novel species such as *Streptomyces haliclonae*, *S. marinus*, and *S. tateyamensis* were isolated from the marine sponge, *Haliclona* spp. (Khan *et al*., 2011). However, very few reports were available for actinobacteria associated with *Hemiasterella* spp. and *Tentorium* spp. and *Tethyopsis* spp.

Abdelmohsen *et al*., (2014) and others had also been reported bioactivity of sponge associated actinomycetes against variety of Gram positive and negative bacteria, fungi, human parasites and virus. Understandable in the present study all the ten *Streptomycetes* isolates showed activity against least of eight out of ten test pathogen used. Pimentel-Elardo *et al*. (2011) obtained tetromycins 1-4 with antibacterial potential from marine sponge *Axinella polypoides* associated *Streptomyces axinellae* Pol001T. Similarly, lobophorin C and D with cancer activity was purified from *Streptomyces carnosus* strain AZS17 associated with marine sponge *Hymeniacidon* spp. (Wei *et al*., 2011). Mayamycin with cytotoxicity and antibacterial activity was isolated from *Streptomyces* sp. strain HB202 associated with marine sponge *Halichondria panacea* (Schneemann *et al*., 2010). Gao *et al*. (2010) obtained *Streptomyces* sp. DA18 with antimicrobial activity from marine sponge. Likewise *Salinispora* sp. strain M403 with antibacterial activity was isolated from marine sponge *Pseudoceratina clavata* (Kim *et al*., 2006), *Brevibacterium* sp. KMD 003 with antibacterial potential was isolated from marine sponge *Callyspongia* spp. (Simmons *et al*., 2011). As well as Karuppiah *et al*. (2015) reported that sponge-associated actinobacteria are the largest source of natural *Callyspongia* spp. (Simmons *et al*., 2011) products, which account for about 56% followed by mollusk and mangrove-derived actinobacteria. In comparison to actinomycetes from other environmental sources, the sponge associated actinomycetes are remarkably rich, with higher chances of finding new chemotypes and fewer problems with rediscovery (Abdelmohsen *et al*., 2014).

CONCLUSION

In conclusion, this study on streptomycetes associated with three marine sponges from the Andaman Islands has revealed the future prospect of finding diverse microbiota in this unexplored ecosystem. Present and previous studies explored the diversity of actinobacteria with potential bioactivity in the marine fauna (Sponge). However, to understand the biodiversity of marine flora and fauna associated actinobacteria with special reference to industrial application has to be explored for novel secondary metabolites.

ACKNOWLEDGEMENT

Authors thank the University Grants Commission (UGC) for providing with a Research Fellowship in Science to Meritorious Student (RFSMS) to R. Baskaran. Authors are also thankful to the Central Instrumentation Facility section (CIF), Pondicherry University.

REFERENCES

Abdelmohsen, U. R., Pimentel-Elardo, S. M., Hanora, A., Radwan, M., Abou-El-Ela, S. H., Ahmed, S. and Hentschel, U. (2010). Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes. *Marine Drugs* **8, 399-412.**

Abdelmohsen, U. R., Szesny, M., Othman, E. M., Schirmeister, T., Grond, S., Stopper, H. and Hentschel, U. (2012). Antioxidant and anti-protease activities of Diazepinomicin from the Sponge-associated Micromonospora strain RV115. *Marine Drugs* **10, 2208-2221.**

Abdelmohsen, U. R., Yang, C., Horn, H., Hajjar, D., Ravasi, T. and Hentschel, U. (2014). Actinomycetes from Red Sea sponges: Sources for chemical and phylogenetic diversity. *Marine Drugs* **12, 2771-2789.**

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman. J. G., Smith, J. A. and Struhl, K. (1999). Current Protocols in Molecular Biology. John Wiley & Sons Inc. New York. **pp. 2.1.1-2.1.7.**

Becerril-Espinosa, A., Freel, K. C., Jensen, P. R. and Soria-Mercado, I. E. (2013). Marine actinobacteria from the gulf of California: Diversity, abundance and secondary metabolite biosynthetic potential. *Antonie van Leeuwenhoek* **103, 809-819.**

Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. and Prinsep, M. R. (2013). Marine natural products. *Natural Product Reports* **30, 237-323.**

Brenner, D. J., Staley, J. T. and Krieg, N. R. (2001). Classification of prokaryotic organisms and the concept of bacterial speciation. *In*: Bergey's Manual of Systematic Bacteriology. 2nd edition. Boone, D. R. and Castenholz, R. W. (eds.). Springer-Verlag, New York Berlin, Heidelberg. **pp. 27-48.**

Bull, A. T. and Stach, J. E. (2007). Marine actinobacteria: New opportunities for natural product search and discovery *Trends in Microbiology* **15, 491-499.**

Bull, A. T., Stach, J. E., Ward, A. C. and Goodfellow, M. (2005). Marine actinobacteria: Perspectives, challenges, future directions. *Antonie van Leeuwenhoek* **87, 65-79.**

Bull, A. T., Ward, A. C. and Goodfellow, M. (2000). Search and discovery strategies for biotechnology: The paradigm shift. *Microbiology and Molecular Biology Reviews* **64, 573-606.**

Gao, Y., Yu, L., Peng, C., Li, Z. and Guo, Y. (2010). Diketopiperazines from two strains of South China

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

Sea sponge-associated microorganisms. *Biochemical Systematics and Ecology* **38, 931-934.**

Goodfellow, M., Ferguson, E. V. and Sanglier, J. J. (1992). Numerical classification and identification of *Streptomyces* species : A review. *Gene* **115, 225-233.**

Grein, A. and Meyers, S. P. (1958). Growth characteristics and antibiotic production of *Actinomycetes* isolated from littoral sediments and materials suspended in sea water. *Journal of Bacteriology* **76, 457-463.**

Helmke, E. and Weyland, H. (1984). *Rhodococcus marinonascens* sp. nov., an *Actinomycete* from the sea. *International Journal of Systematic Bacteriology* **34, 127-138.**

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J. and Horn, M. (2003). Microbial diversity of marine Sponges. *In*: Sponges (Porifera), Book 37. Müller, W. G. (ed.). Springer, Berlin, Heidelberg. **pp. 365-372.**

Hentschel, U., Piel, J., Degnan, S. M. and Taylor, M. W. (2012). Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology* **10, 641-654.**

Hentschel, U., Usher, K. M. and Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS Microbiology Ecology* **55, 167-177.**

Jensen, P. R., Dwight, R. and Fenical, W. (1991). Distribution of *Actinomycetes* in near-shore tropical marine sediments. *Applied Environmental Microbiolology* **57, 1102-1108.**

Karuppiah, V., Li, Y., Sun, W., Feng, G. and Li, Z. (2015). Functional gene-based discovery of phenazines from the actinobacteria associated with marine sponges in the South China Sea. *Applied Microbiology and Biotechnology* **99, 5939-5950.**

Khan, S. T., Komaki, H., Motohashi, K., Kozone, I., Mukai, A., Takagi. M. and Shin-ya, K. (2011). *Streptomyces* associated with a marine sponge *Haliclona* sp.; biosynthetic genes for secondary metabolites and products. *Environmental Microbiology* **13, 391-403.**

Kim, T. K., Hewavitharana, A. K., Shaw, P. N. and Fuerst, J. A. (2006). Discovery of a new source of rifamycin antibiotics in marine sponge actinobacteria by phylogenetic prediction. *Applied Environmental Microbiology* **72, 2118-2125.**

Lam, K. S. (2006). Discovery of novel metabolites from marine actinomycetes. *Current Opinion in Microbiology* **9, 245-251.**

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. and Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceding of the Natural Academy of Science* USA **82, 6955-6959.**

Lane, N. (2008). Marine microbiology: Origins of death. *Nature* **453, 583-585.**

Li, J. W. and Vederas, J. C. (2009). Drug discovery and natural products: End of an era or an endless frontier? *Science* **325, 161-165.**

Moran, M. A., Rutherford, L. T. and Hodson, R. E. (1995). Evidence for indigenous *Streptomyces* populations in a marine environment determined with a 16S rRNA probe. *Applied Environmental Microbiology* **61, 3695-3700.**

Pimentel-Elardo, S. M., Buback, V., Gulder, T. A. M., Bugni, T. S., Reppart, J., Bringmann, G., Ireland, C. M., Schirmeister, T. and Hentschel, U. (2011). New Tetromycin derivatives with anti-Trypanosomal and protease inhibitory activities. *Marine Drugs* **9, 1682-1697.**

Pimentel-Elardo, S. M., Kozytska, S., Bugni, T. S., Ireland, C. M., Moll, H. and Hentschel, U. (2010). Anti-parasitic compounds from *Streptomyces* sp. strains isolated from Mediterranean sponges. *Marine Drugs* **8, 373-380.**

Rateb, M. E. and Ebel, R. (2011). Secondary metabolites of fungi from marine habitats. *Natural Product Reports* **28, 290-344.**

Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., Perez, T., Rodrigo, A., Schupp, P. J., Vacelet, J., Webster, N., Hentschel, U. and Taylor, M. W. (2012). Assessing the complex sponge microbiota: Core, variable and species-specific bacterial communities in marine sponges. *International Society for Microbial Ecology* **6, 564-576.**

Schneemann, I., Kajahn, I., Ohlendorf, B., Zinecker, H., Erhard, A., Nagel, K., Wiese, J. and Imhoff, J. F. (2010). Mayamycin, a cytotoxic polyketide from a marine *Streptomyces* strain isolated from the marine sponge Halichondria panicea. *Journal of Natural Products* **73, 1309-1312.**

Selvin, J., Ninawe, A. S., Seghal Kiran, G. and Lipton, A. P. (2010). Sponge-microbial interactions: Ecological implications and bioprospecting avenues. *Critical Reviews in Microbiology* **36, 82-90.**

Simmons, L., Kaufmann, K., Garcia, R., Schwar, G., Huch, V. and Muller, R. (2011). Bendigoles D-F, bioactive sterols from the marine sponge-derived Actinomadura sp. SBMs009. *Bioorganic and Medicinal Chemistry* **19, 6570-6575.**

Sun, W., Peng, C., Zhao, Y. and Li, Z. (2012). Functional gene-guided discovery of type II polyketides from culturable *Actinomycetes* associated with soft coral Scleronephthya sp. *PloS One* **7, e42847.**

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* **28, 2731-2739.**

Thomas, T. R. A., Kavlekar, D. P. and LokaBharathi, P. A. (2010). Marine drugs from sponge-microbe association-a review. *Marine Drugs* **8, 1417-1468.**

Wang, L., Huang, Y., Liu, Z., Goodfellow, M. and Rodriguez, C. (2006). *Streptacidiphilus oryzae* sp. nov., an *Actinomycete* isolated from rice-field soil in Thailand. *International Journal of Systematic and Evolutionary Microbiology* **56, 1257-1261.**

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

Wei, R. B., Xi, T., Li, J., Wang, P., Li, F. C., Lin, Y. C. and Qin, S. (2011). Lobophorin C and D, new kijanimicin derivatives from a marine sponge-associated actinomycetal strain AZS17. *Marine Drugs* **9(3), 359-368.**

Weyland, H. (1969). *Actinomycetes* in North Sea and Atlantic Ocean sediments. *Nature* **223, 858-858.**

Wyche, T. P., Hou, Y., Vazquez-Rivera, E., Braun, D. and Bugni, T. S. (2012). Peptidolipins B-F, antibacterial lipopeptides from an ascidian-derived *Nocardia* sp. *Journal of Natural Products* **75, 735-740.**

Xi, L., Ruan, J. and Huang, Y. (2012). Diversity and biosynthetic potential of culturable *Actinomycetes* associated with marine sponges in the China seas. *International Journal of Molecular Science* **13, 5917-5932.**

Yokota, A. (1997). Phylogenetic relationship of actinomycetes. *In*: Atlas of Actinomycetes. Miyadoh, S. and Gakka, N. H. (eds.). Asakura Publishing Co. Ltd., Japan. **pp. 194-197.**

Zhang, H., Lee, Y. K., Zhang, W. and Lee, H. K. (2006a). Culturable actinobacteria from the marine sponge *Hymeniacidon perleve*: Isolation and phylogenetic diversity by 16S rRNA gene-RFLP analysis. *Antonie van Leeuwenhoek* **90, 159-169.**

Zhang, H., Zheng, W., Huang, J., Luo, H., Jin, Y., Zhang, W., Liu, Z. and Huang, Y. (2006b). *Actinoalloteichus hymeniacidonis* sp. nov., an *Actinomycete* isolated from the marine sponge *Hymeniacidon perleve*. *International Journal of Systematic and Evolutionary Microbiology* **56, 2309-2312.**

Zhang, L., Xi, L., Ruan, J. and Huang, Y. (2012). Microbacterium *marinum* sp. nov., isolated from deep-sea water. *Systematic and Applied Microbiology* **35, 81-85.**

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