CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1041770

Available online at: http://www.iajps.com Research Article

ANTIBACTERIAL ACTIVITY OF SECONDARY METABOLITES FROM MARINE SPONGE (*CLATHRIA INDICA*) ASSOCIATED BACTERIA

G. Kalaivani, N. Siddharthan, G. Thangadurai, E. Poongothai, R. Thilagam and N. Hemalatha*

Department of Microbiology,Periyar University, Palkalai Nagar,Salem – 636011, Tamil Nadu, India.

Abstract:

Objective: *To isolate the potential bioactive secondary metabolites producing bacteria associated with Clathria indica from Thondi, Tamil Nadu.*

Methods*: Ethyl acetate extraction method was used to extract the secondary metabolites and antimicrobial assays was carried out.*

Results: *Three potential strains were highly active in test pathogens. The extracted secondary metabolites were used to MIC and MBC test. Conclusion: Based on the present study MSB11 secondary metabolites can be effective bioactive drug against clinical pathogens.*

Keywords: *secondary metabolites, Clathria indica, MSB11 strain, clinical pathogens, MIC and MBC*

Corresponding author:

N. Hemalatha*

Department of Microbiology,
Periyar University, Palkalai Nagar,
Salem – 636011, Tamil Nadu, India.
kalaivani.microbio90@gmail.com

QR code

Please cite this article in press as N. Hemalatha et al , ***Antibacterial Activity of Secondary Metabolites from Marine Sponge (Clathria Indica) Associated Bacteria****, Indo Am. J. P. Sci, 2017; 4(11).*

INTRODUCTION:

Considering 70 % of the earth surface is covered by oceans providing the habitat for rich biodiversity, The diversity of marine organisms and habitats, marine natural products encompass a wide variety of chemical classes such as terpenes, polyketides, acetogenins, peptides and alkaloids of varying structures representing biosynthetic schemes of stunning variety [1] (Wright, 1998). Over the past 30–40 years marine organisms have been the focus of a worldwide effort for the discovery of novel natural products. A small number of marine plants, animals and microbes have already yielded more than 12,000 novel chemicals with hundreds of new compounds still being discovered every year [2] (Donia and Hamann, 2003). The use of cultured sponges as alternative materials, however, requires careful content analysis because natural sponges and cultured sponges could produce different substances. Sponges which belong to the same species may have different secondary metabolites as they live in different places [3] (Boobathy et al., 2009). One possible alternative for acquiring sufficient amount of secondary metabolites is by cultivating sponges. Sponge and other reef organism cultivation has much been developed lately, especially through transplantation method. Coral transplantation method is relatively easy to be carried out and its production can be customized in terms of quantity and time. In addition, these unique ecological niches have copious amount of particulate organic matter [3,4] (Vogel, 1977 and Wehrl *et al.*, 2007). A single sponge host can be inhabited by diverse bacteria. Bacteria which constitute 40% of the sponge [5] (Wilkinson, 1978) produce extracellular hydrolytic enzymes that facilitate the metabolism of complex organic matter thereby assisting the host in nutrition and various metabolic processes. Bacterial enzymes provide a greater diversity of catalytic activity and can be produced economically. Currently bacteria from terrestrial sources are employed for industrial production of enzymes. Sponges are considered as microbial fermenters that provide exciting new avenues in marine microbiology and biotechnology ([6] Hentschel *et al.*, 2006). Although, the potentialities of hydrolytic enzymes from marine bacteria have been recognized, studies on enzymes from bacteria associated within the microhabitats of sponge for biotechnological application are rare [7] (Wang, 2006).The emergence of infectious diseases and drug resistance mechanism developed by infectious microorganisms makes the natural product scientist to find effective molecules from marine environment to treat the disease accurately. The pathogens developed drug resistance mechanism progressively to the exposed therapeutic agents and caused remerging of infection. In the present study, an attempt has been made to investigate the antibacterial activity of marine sponge *Clathria Indica* associated bacterial secondary metabolites.

MATERIALS AND METHODS:

Collection of Marine Sponge

Thondi is a small village situated in the Palk Strait region of Tamil Nadu. The study area lies in the latitude of 99°44”N and 79 10’ 45” E. The rainfalls in Thondi region are mainly due to north east and south west monsoon. Thondi coast has a very minimal wave action. Turbidity of the seawater is moderately low and also they are rich in nutrients hence, it serves as a treasure house for valuable marine resources like sea grass, seaweeds, and invertebrates like coelenterates, echinoderms and shell fishes. The major occupation of the people is fishing. *Clathria indica* species of Sea sponges were collected during monsoon month (January) for the isolation of endophytic THB strains from Thondi Palk Strait.

Isolation of Sponges Associated Bacteria

1 gm of fresh sea sponges species were aseptically weighed and were washed thrice with sterilized distilled water at different time intervals (1hr, 30 min and 15 min.) After that, the samples were ground well by using mortar and pestle with the addition of 1 ml of sterilized distilled water. Crushed samples were serially diluted with sterilized 50% aged seawater and were plated with the Zobell marine agar medium. One milliliter of the serially diluted sample was pipetted out into sterile Petri dishes. The sterile Zobell marine agar medium was poured into Petri dishes aseptically and swirled for thorough mixing. After solidification, the plates were incubated in an inverted position for 24 hrs at 37 ± 2 ºC. All the determinations were carried out in triplicates.

Screening of Bioactive Efficacy against Pathogens

Collection of Clinical Pathogens

The clinical pathogens were collected from Applied Microbiology Laboratory from department of Microbiology, Periyar University, Salem, Tamil Nadu. The Test pathogens were Gram positive organisms like *Staphylococcus aures, Streptococcus pyogens, Bacillus cereus* and Gram negative organisms like *E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, and Salmonella typhi.*

Cross Streak Assay of Isolates Against Pathogenic bacteria

The antagonistic activity was tested by following cross streak assay method. Single streak (4-6mm in diameter) of the isolated strains were streaked on the

surface of Muller Hinton Agar plates. On obtaining a ribbon-like growth, the overnight culture of antibiotic resistant were streaked at perpendicular to the original streak of isolates and incubated at 37±2°C. The inhibition was measured after 24 hours in the case of bacteria. A control plate was also maintained without inoculating isolates to assess the normal growth of bacteria.

Mass Cultivation of Potential Isolate

Five isolated strains, which showed higher promising antagonistic activity, were selected for mass cultivation for the extraction of antimicrobial metabolites. A loopful of chosen isolated strains were further inoculated into 1000ml conical flask containing 300ml of nutrient broth and kept at 28°C for 72 hours with continuous shaking. The volume was made up to 1litre using distilled water pH of the medium was adjusted 7.2±0.2.

Extraction of Bioactive Compounds

The mass cultivated broth was filtered by using filter paper. 300ml of filtrate was mixed with 300ml of ethyl acetate in separating funnel to extract bioactive compounds. After removing the lower aqueous phase, the upper solvent phase was concentrated in a vacuum evaporator at room temperature for 24 hours and crude extract was obtained. This crude extract was used for further secondary screening studies against human pathogens.

MIC and MBC of Bioactive Compounds

For MIC determination 0.5 ml of various concentration of extracts (32, 64, 125, 250, 500, 1000, 1500, 2000µg) and mixed with 0.5ml of nutrient broth. 50µl of bacterial inoculums serves as positive control. Nutrient Broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 48 hours. The MIC was the lowest concentration of the extract that did not permit any visible growth after 24 hours of incubation and it was examined on the basis of turbidity.

The above selected serial dilutions are plated out on the homologous medium. The MBC are determined by sub culturing the above (MIC) serial dilutions after 48hours in NB plates using 0.01ml loop and incubating at 37ºC for 24 hours. MBC was regard as the lowest concentration that prevents the growth of bacterial colony on the solid media.

RESULTS AND DISCUSSION:

Isolation of Sponges Associated Bacteria

The marine sponge specimen *Clathria indica* (Fig.1) was collected thondi is a small village situated in the Palk Strait region of Tamil Nadu. Totally 14 different bacterial colonies were isolated and purified. The isolated colonies were marked as MSB1 to MSB14. Six out of 14 strains were pigmented like yellow, brown, pale yellow in color. All the 14 isolates for used to screening the bioactive compounds producing ability. Prem Anand *et al*., 2005 [8], reported the 75 different strains isolated from four different species of sponges. The marine sponge specimen *S. inconstans* was collected from intertidal zone of Palk Bay region and 14 different bacterial colonies were purified [9] (Bharathiraja *et al*., 2014).

Fig.1: Marine Sponge (*Clathria indica*)

Screening of Bioactive Efficacy against Pathogens

The collected test pathogens, Gram positive organisms like *Staphylococcus aures, Streptococcus pyogens, Bacillus cereus* and Gram negative organisms like *E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* were used to screening of antibacterial activity (Fig.2). The antibacterial profile was tabulated in Table. 1. Among the 14 isolated bacterial strains only three strains were highly active in test pathogens. The three strains were used to further analysis. Matabole *et al*., 2017 reported the screening of 29 microbial isolates; only six isolates showed and induced antibacterial activities. It has also been suggested that some of bacteria chemically defend the host against microbial infection [10] (Engel *et al*., 2002). From the study they have ascertained that the genera *Vibrio, Pseudomonas/Marinobacter* and *Bacillus* are dominantly represented [11] (West and Colwell, 1984).

Fig.2: Antibacterial Activity (Cross Streak Assay)

Table.1: Antibacterial activity of Bacterial isolates

Bacterial Isolates	Test Pathogens						
	Staphylococcus aures	***Streptococcus pyogens***	***Bacillus cereus***	***E. coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Proteus mirabilis***
MSB1	+	-	-	+	-	+	+
MSB2	+	-	+	+	+	-	+
MSB3	-	-	+	+	+	-	-
MSB4	-	-	-	+	-	+	-
MSB5	+	+	-	+	-	+	+
MSB6	-	-	-	-	-	-	-
MSB7	+	+	-	+	-	-	+
MSB8	+	-	-	+	-	-	-
MSB9	-	-	+	-	+	+	-
MSB10	+	-	-	+	-	-	+
MSB11	+	-	+	+	+	+	+
MSB12	-	+	+	-	-	-	-
MSB13	-	-	-	-	-	-	-
MSB14	+	-	+	+	-	+	+

(+) presence of activity and (-) absence of activity

Extraction of Bioactive Compounds

The isolated three potential strains were used to mass cultivation on nutrient broth at 28°C for 72 hours with continuous shaking. After incubation periods, filtered the culture broth. The supernatant was added to equal volume of ethyl acetate to separate the bio active compounds by using separating flask. And the separated compounds were evaporated and obtained the crude compounds (Fig.3). A range of bioactive metabolites has been found in about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce powerful anti-cancer, anti-inflammatory agents, but their cultivation has not been studied [12,13](Bhimba *et al*., 2013 and Lopez *et al.*, 2006).

Fig.3: Extraction of Crude Compounds

MIC and MBC of Bioactive Compounds

The extracted crude compounds were used to MIC (Fig.4) and MBC (Fig.5) of test pathogens. The isolated Bacterial strain MSB2 was produced activity against 250 µg of *S. aures*, 250 µg of *B. cereus*, 125 µg of *E. coli*, 500 µg of *P. aeruginosa* and 250 µg of *P. mirabilis*. MSB5 was produced activity against 500 µg of *S. aures*, 250 µg of *E. coli*, 500 µg of *P. aeruginosa*, 500 µg and 250 µg of *P. mirabilis*. MSB11 was produced activity against 125 µg of *S. aures*, 500 µg of *B. cereus*, 125 µg of *E. coli*, 125 µg of *P. aeruginosa*, 250 µg of *K. pneumonia* and 250 µg of *P. mirabilis* (Table.2). In a previous study on antibiotic production in marine bacteria, Bernen *et al*. (1997)[12] have reported that 36% of the strains were Gram-negative rods. In our study, Gram-positive as well as Gram-negative bacteria were more or less equally represented in the producers encountered. We have isolated that the most active strain MSB11. This strain was physiologically and biochemically characterized so that this information will help in future for the optimization of media and physical factors to achieve maximum antibiotic production.

Table.2: Minimum Inhibitory Concentration of potential isolates

Bacterial Isolates	Concentration of compounds	Test Pathogens						
		S. aures	***S. pyogens***	***B. cereus***	***E. coli***	***P. aeruginosa***	***K. pneumonia***	***P. mirabilis***
MSB2	125µg	-	-	-	+	-	-	-
	250 µg	+	-	+	+	-	-	+
	500 µg	+	-	+	+	+	-	+
	1000 µg	+	-	+	+	+	+	+
MSB5	125µg	-	-	-	-	-	-	-
	250 µg	-	-	-	+	-	-	+
	500 µg	+	-	-	+	+	+	+
	1000 µg	+	+	-	+	+	+	+
MSB11	125µg	+	-	-	+	+	-	-
	250 µg	+	-	-	+	+	+	+
	500 µg	+	-	+	+	+	+	+
	1000 µg	+	+	+	+	+	+	+

(+) presence of activity and (-) absence of activity

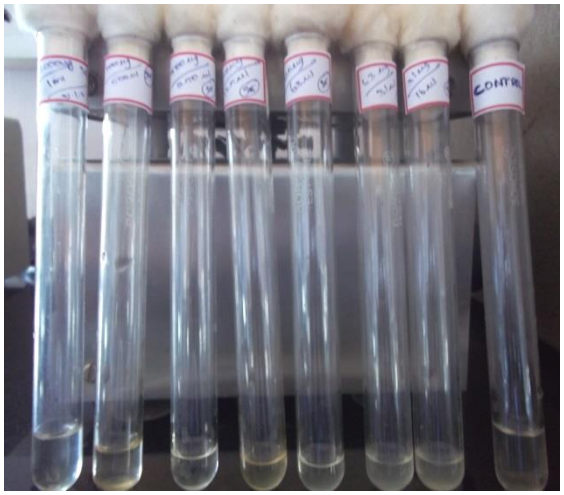

Fig.4: MIC of Test Pathogen

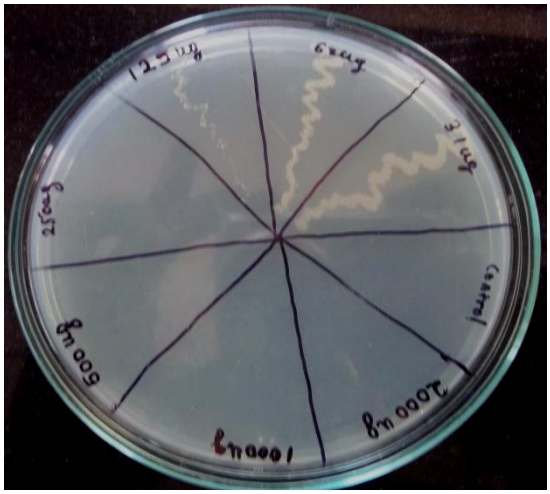

Fig.5: MBC of Test Pathogen

CONCLUSION:

The marine sponge specimen *Clathria indica* was collected Thondi, Tamil Nadu. Totally 14 different bacterial colonies were isolated and purified. Out of the 14 isolated bacterial strains only three strains were highly active in test pathogens. We have isolated that the most active strain MSB11 from the three potential isolates. The isolated MSB11 strain was produced activity against 125 µg of *S. aures*, 500 µg of *B. cereus*, 125 µg of *E. coli*, 125 µg of *P. aeruginosa*, 250 µg of *K. pneumonia* and 250 µg of *P. mirabilis.* This strain was characterized so that this information will help in future for the optimization of media and physical factors to achieve maximum antibiotic production and purification.

ACKNOWLEDGEMENT:

The authors are thankful to the Department of Microbiology, Periyar University, Salem, Tamilnadu, India, for providing laboratory facility to carry out the entire research. The instrumental support under DST-FIST (Ref No. SR/FST/LSI-640/2015(c)3Dt.30/05/2016) to the Department of Microbiology, Periyar University is duly acknowledged.

REFERENCES:

1.Wright, A.E., (1998) Isolation of marine natural products. In: Cannell, R.J.P. (Ed.), Methods in Biotechnology, vol. **4**. Humana Press, New Jersey, USA, pp. 365–408.

2.Donia, M., Hamann, M.T., Marine natural products and their potential applications as anti-infective agents. *Lancet* ,2003;**3**: 338–348.

3.Vogel, S. Current-induced flow through living sponges in nature. *Proceedings of the National Academy of Sciences of the United States of America*, 1977;**74(5)**: 2069- 2071.

4.Wehrl, M., Steinert, M. and Hentschel, U.Bacterial uptake by the marine sponge. *Aplysina aerophoba*. *Microbial Ecology*, 2007;**53(2)**:355-365.

5.Wilkinson, C.R. Microbial associations in sponges. II. Numerical analysis of sponge and water bacterial populations. *Marine Biology*, 1978;**49(2)**, 169-176.

6.Hentschel, U., Usher, K.M. and Taylor, M.W. Marine sponges as microbial fermenters. *FEMS Microbiology Ecology*, 2006;**55(2)**: 167-177.

7.Wang, G.Y.Diversity and biotechnological potential of the sponge-associated microbial consortia. *Journal of Industrial Microbiology and Biotechnology*, 2006;**33(7)**: 545-551.

8.Prem Ananda T, Abdul Wajid Bhata, Yogesh S. Shoucheb, Upal Royb, Jay Siddharthb and Siddhartha P. Sarmaa, Antimicrobial activity of marine bacteria associated with sponges from the waters off the coast of South East India, *Microbiological Research,2006;* **161**: 252—262.

9.Bharathiraja S, Rajasekaran R and Suriya J Antibacterial activity of marine bacteria isolated from sponge *Spirastrella inconstans, Journal of Chemical and Pharmaceutical Research*,2014; **6(11),** 395-398.

10.Engel, S., Jensen, P.R., Fenical, W.,Chemical ecology of marine microbial defence. *J. Chem. Ecol.* **2002;28 (10):** 1971–1985.

11.West, P.A., Colwell, R.R., (1984) Identification and classification of Vibrionaceae – an overview. In: Colwell, R.R. (Ed.), Vibrios in the Environment. Wiley, New York, pp. 285–363.

12.Bernen, V.S., Greenstein, M., Maiese, W.M., (1997) Marine Microorganisms as a Source of New Natural Products. *Advances in Applied Microbiology*, vol. **43**. Academic Press, New York, pp. 57–90.

13.Lopez, E. Tuido, A.L.Aler, C. Martin, C. Castro, M.Ranirez, E. Canton, C. Almeida and EA. Murtin Mazuelos.Evaluation of disk diffusion method for determing posaconazole susceptibility of filamentous fungi. *Antimicrob. Agents Chaemother*, **2006;50**: 1108-1111.