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Characterization of Sponge associated Antimicrobial Endosymbiotic Bacteria

Vimala[1], B. Xavier Innocent[2], G. Prabhakaran[1] and Huxley V. A. J.[*1]

[1]Biotech Research Laboratory, Department of Zoology, Thiru. Vi. Ka. Govt. Arts College Tiruvarur – 3, Tamilnadu, India
[2]PG and Research Department of Zoology, St. Xavier's College (Autonomous), PalayamKottai, Thirunelveli, Tamilnadu, India
[*]– Corresponding author E. mail: aldobiotech@yahoo.com

Abstract

Antimicrobial substance producing endosymbionts were isolated from three marine sponges collected from south peninsular coast of India. There efficacy were checked with ten potential human and shrimp pathogens. Among that ESB3 strain produced vast antmicrobial activity over 80% of tested pathogens. The growth pattern of ESB3 was also studied.

Key words: Antimicrobial activity, Sponge endosymbionts, Growth pattern.

Introduction

Marine invertebrates have developed highly specific relationships with numerous associated microorganisms and these associations are recognized as ecological and biological importance (Osinga *et al.,* 2001). The ratio of microorganisms with antimicrobial activity from invertebrates was higher than from other sources (Taylor *et al*., 2007). Invertebrate-associated microorganisms plays a major roles in the production of novel biologically active secondary metabolites. (Laport *et al.,* 2009). Bioactive substances from sponges or associated microorganisms have shown anticancer, antibacterial, antifungal, antiviral, antiprotozoal, anthelmintic, anti-inflammatory, immunosuppressive, neurosuppressive, and antifouling activities. Manoalide is one of the first sesterterpenoids to be isolated from a marine sponge (*Luffariella variabilis*), was found to be an antibiotic. This is the only example of antibiotic seterterpenoid discovered so far. In the present study, the antimicrobial secondary metabolites were isolated from marine sponge associated bacteria, collected from south peninsular coast of India.

Materials and Methods

Collection and Identification of Sponges

Three different marine sponges (MS1, MS2 and MS3) were collected as 'bycatch' from Arokyapuram coast of south peninsular region in Nov 2010. Collected specimens were identified with the help of Dr. P.A. Thomas, Emeritus Scientist CMFRI, Vizhinjam.

Isolation of Antibiotic Producing Endosymbiotic Bacteria

Endosymbiontic bacteria were isolated with the help of three different media like Nutrient agar, Zobell marine agar and Zobell marine agar + sponge extracts.

Quantitative Analysis of Endosymbionts

A piece of 1c.m^2 area of collected sponges MS1, MS2 and MS3 aseptically removed and were cultured in both Nutrient agar + sponge extracts and Zobell marine agar + sponge extracts. The No of colonies produced in 1c.m^2 area of sponge is calculated.

Antibacterial activity of endosymbiont against Human pathogen, and Shrimp pathogen

The antibacterial activity of endosymbiont was determined against 10 pathogenic bacteria of Human and Shrimp (*Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Stophytococcus aureus* and *Staphylococcus eoderods, E.coli, Proteus valgaris, Psudomonas aeruginasa, Vibrio alginolyticus* and *V. fischeri).*

Growth curve of ESB3

The growth of ESB 3 was determined at different intervals and the data were ploted in a graph.

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Results

Three different types of sponges were collected from Arokiapuram coast and identified in the present study are given Table 1.

Table1. Collection and Identification of Sponges

Sl.No.	Sp.	Colour	Code
1	*Sigmadocia carnosa*	Brown	MS1
2	*Ircinia fasciculate*	Brownish yellow	MS2
3	*Callyspongia diffusa*	Yellow	MS3

Isolation of Antibiotic Producing Endosymbiotic Bacteria

Different type of media were used to isolate the sponge endosymbionts. The combination of zobell marine agar and sponge extract supplement produced more number of bacterial groth that tha other medium (table -2). Among the total 13 bacteria isolated from *Sigmadocia* more than 61% of bacteria were isolated by sponge extract supplement.

Table 2. Isolation of endosymbiotic bacteria from marine Sponges

Sponge sp.	No of colonies produced in various media			
	Nutrient agar	Zobell marine agar	Zobell marine agar+sponge extracts	Total
Sigmadocia carnosa	**2**	**3**	**8**	**13**
Ircinia fasciculata	**2**	**3**	**4**	**9**
Callyspongia diffusa	**6**	**6**	**8**	**20**

Quantitative analysis of endosybionts

The results of quantitative analysis indicated that the sponges contain a considerable amount of bacteria in *Sigmadocia, Callyspongia* and *Axinella* contains bacterial population. The results are also shown in Table 3.

Table 3.Quantitative Analysis of Endosymbionts

Sponge	No of colonies produced in 1c.m^2 area of sponge	
	Nutrient agar + sponge extracts	Zobell marine agar + sponge extracts
Sigmadocia carnosa	**7**	**9**
Ircinia fasciculate	**5**	**5**
Callyspongia diffusa	**4**	**6**

The antibacterial activity of endosymbionts (ESB) were determined against 10 pathogenic bacteria of Human and Shrimp. The 10 pathogens used are very commonly seen pathogens. Details are given in the Table. 4.

Table 4. Antibacterial activity of endosymbiont against Human pathogen, and Shrimp pathogen

Bacteria	ANTIBACTERIAL ACTIVITY									
	GRAM POSITIVE					GRAM NEGATIVE				
	Microcom Luteus	*Bacillus cereus*	*Bacillus subtilis*	*Stophyto coccus aureus*	*Staphyio-coccis eoder,ods*	*E.coli*	*Proteus valgaris*	*P.aeruginasa*	*V. algindyticus*	*V. fischmri*
ESB – 1	-	+	++	-	+++	++	+	-	+	++++
ESB – 2	++	++++	-	+++	+	-	++	+	+++	-
ESB – **3**	**+++**	**++**	**++**	**++**	**++**	**-**	**++++**	**-**	**+**	**++**
ESB – 4	-	++	+	-	+	+++	-	++	-	-
ESB – 5	+	-	+++	++	-	+	-	-	-	+
ESB – 6	++	+	-	-	+++	-	+	++++	-	-
ESB – 7	++	++	++++	++	+	+	-	-	-	+
ESB – 8	++++	-	+	+	-	++	-	+	+++	+
ESB - 9	-	+	++	++	-	-	+	++	-	+
ESB - 10	++	+	-	+	++	+++	-	-	+	-
ESB - 11	-	++	+	-	-	-	+	++	++	+++
ESB - 12	-	-	-	++++	+	+	++	+++	-	-
ESB - 13	++	++++	+	-	-	+	+++	++	-	+

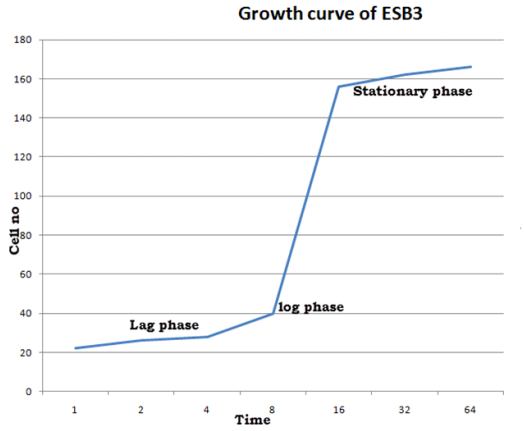
++++=30; +++=20-30mm; ++=10-20mm; +=1-10mm
- = No Activity

Based on the results ESB 3 was considered as potent antimicrobial strain. The growth of ESB 3 was determined and plotted on the Table 6 and Fig 1. Based on the result, the log phase starts after 4th hour and it was attain a stable phase at 18th to 24hours

Table 6. Results of growth curve of ESB3

Time (hrs)	CFU/ml
0 (inoculation)	20
1	22
2	26
4	28
8	40
16	156
32	162
64	166

Fig. 1. Growth pattern of potent antimicrobial endosymbiont



Discussion

The marine sponges are collected from the Indian coast have particularly proved to be very productive as for as secondary metabolites are concerned. They are a part of the benthic fauna and live in all areas of the marine world, from the shallow coastal seas to the deepest oceans (Soltwedel and Vopel, 2001). Since sponges have a wide range of biosynthetic capabilities, they are the dominant source of these compounds (Taylor *et al.*, 2007). The sponge class Demospongiae is known to produce the largest number and diversity of secondary metabolites isolated from marine invertebrates (Sara, 1971).The focus of this review is to provide information on the biological activities of the marine sponge *Axinella*.

Several demosponges are reported from southern peninsular coast of India. In the present study, three demosponges were collected from southern peninsular coast of India. The sponges *Sigmadocia carnosa, Ircinia fasciculate* and *Callyspongia diffusa* are collected from Vizhinjam, Mangano *et al.*, (2007) studied that the sponge *Ectyobatsella enigmatica* has bactericidal activity. It is screened against clinical isolates of bacteria effective against *Streptococcus pyogenes* and it is ineffective against *Pseudomonas aeruginosa* and *Klebsiella* sp. Sponge-associated antogonistic actinomycetes (Rohilla *et al.,* 2010), were isolated from two marine sponges reported for potent biological activity, using modified Emerson agar enriched with respective host sponge extract.

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* Author for correspondence