Microbial & Biochemical Technology

Kadiri et al., J Microbial Biochem Technol 2014, S8
http://dx.doi.org/10.4172/1948-5948.S8-003

Research Article Open Access

Screening and Isolation of Antagonistic Actinobacteria Associated With Marine Sponges from Indian Coast

Sunanda Kumari Kadiri[1]*, Nagendra Sastry Yarla[2] and Siddaiah Vidavalur[2]

[1]Department of Microbiology, College of Science and Technology, Andhra University Visakhapatnam, India
[2]Department of Organic Chemistry, College of Science and Technology, Andhra University Visakhapatnam, India

Abstract

Sponges are host organisms for various symbiotic microorganisms such as Archea, Bacteria, Cyanobacteria and Microalgae. Sponges associated microorganisms are sources of wide variety of useful natural products like antibiotics, anti-inflammatory, antioxidant, antiviral, antifouling and cytotoxic compounds. Nearly 60 isolates of action bacteria were found to be associated with 6 species of marine sponges collected at various locations. The isolates were screened for antimicrobial activity against 6 pathogenic bacteria and 4 pathogenic fungi. Among 60 isolates, 15 isolates showed antibacterial activity and 6 isolates showed antifungal activity. Among active isolates, isolate no.42 showed highest antimicrobial activity against all the pathogenic bacteria and fungi studied and it was identified as streptomyces species.

Keywords: Marine sponges; Actinomycetes; Streptomyces; Antimicrobial activity

Introduction

Marine sponges are a rich source of structurally unique natural compounds possessing a wide range of biological activities [1]. Recently several studies proved that bioactive natural products, initially isolated from marine sponges, are produced by microorganisms, which are associated commensally or symbiotically with marine invertebrates [2]. It is generally assumed that the interior of the sponge body is continuously oxygenated, due to the efficient pumping of water through the aquiferous system [2,3]. Hence sponges are not likely to harbour anaerobic microorganisms. However, the presence of facultative anaerobic bacteria in sponges has been demonstrated [4,5] and the recent discovery of sulfate reducing bacteria [6,7] and other symbiotic archea in sponges show that anaerobic microbial process may take place in sponges tissues. Actinomycetes have been traditionally a rich source for biologically active metabolites [8]. They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Almost 80% of the world's antibiotics are known to come from Actinomycetes, mostly from the genera streptomyces [9]. Thus in the present study, an attempt was made on isolation of streptomyces strains associated with marine sponges along the Indian Coast.

Materials and Methods

Collection of sponges

Sponges were collected from the Indian coast (17° 43' N, 83° 18' E and 16.93° N, 82.22° E). Samples were collected in sterile polythene bags containing sterile sea water and kept on ice. Then the samples were transported to the laboratory with minimum possible time to avoid the external microbial contamination and stored at 4°C.

Isolation of actinobacteria from Marine sponges

Sponge specimens were rinsed in sterile sea water, cut into pieces of 1 cm^3 with a help of a sterile knife. Further, homogenized in a sterile mortar with 10 volumes of sterile sea water. The supernatant was diluted in ten-fold series and subsequently plated out on various media like Actinomycetes isolation agar, starch casein, Glycerol asparagine agar, M1 medium, ISP 2 medium and R2A agar for isolating actinobacteria [10,11]. All the media were supplemented with 0.2 µm pore size filtered cycloheximide (100 µg/ml), nystatin (25 µg/ml) and nalidixic acid (25 µg/ml) to facilitate the isolation of slow-growing actinobacteria and to inhibit fungal contamination. The inoculated plates were incubated in inverted position for 1-8 weeks at [28+2°C].

Enumeration and maintenance of cultures

In six selective media the number of Actinobacterial colonies found was sub-cultured on starch-casein agar. Later they were kept in refrigerator (4°C) till further analysis was to be carried out [12].

Characterization of the isolates

The isolates were characterized upto genus level by observing the spore bearing hyphae, structure of spore chain, color of the spore, aerial mass color and color of substrate mycelia as described by Bergey's manual [13] and International Streptomyces Project [13,14].

Screening of actinobacteria for antibiotic compounds

Initial screening of actinobacteria for antibiotic production was performed by cross streak method. The isolates having the activity were cultured in 100 ml of production medium (0.8 g NaCl, 1 g NH_4Cl, 0.1 g KH_2PO_4, 0.2 g $MgSO_4$. $7H_2O$, 0.04 g $CaCl_2$, $2H_2O$, 2 g glucose, 3 g yeast extract in 1 l of distilled water, pH 7.3). These cultures were grown in a rotary shaker at 200 rpm, 28°C for 120 hrs under the standard conditions of aeration and agitation. The resultant cultures were centrifuged for 15 min.

The clear supernatant samples were tested for antimicrobial

***Corresponding author:** Dr. Kadiri Sunanda Kumari, Principle Investigator, DST Project –Young Scientist, Dept. of Microbiology, Andhra University Visakhapatnam -530 003, India, Tel: 2844683/+91-9441571261; Fax: 0891-2713813; E-mail: alakkrburagohain@gmail.com

Received September 08, 2014; **Accepted** September 27, 2014; **Published** October 03, 2014

Citation: Kadiri SK, Yarla NS, Vidavalur S (2014) Screening and Isolation of Antagonistic Actinobacteria Associated With Marine Sponges from Indian Coast. J Microb Biochem Technol S8: 003. doi:10.4172/1948-5948.S8-003

activities by agar well diffusion method [15]. The Muller Hinton agar plates were seeded with *S. aureus* MTCC 7443, *B. sublitis* MTCC 8141, *P. vulgaris* NCIM 2813, *E. coli* MTCC 6365 for antibacterial activity. Potato dextrose agar plates were seeded with *S. cerevisiae* MTCC 463, *C. albicans* MTCC 1346, *A. niger* MTCC 6484 for antifungal activity. Wells of 6 mm diameter were prepared in the plates, filled with 50 µl of crude culture supernatant samples and the diameter of inhibition zones were measured after incubation at 37°C for 24 hours for antibacterial activity and 28°C for 72 hours for antifungal activity. Ciprofloxacin (for bacteria) and Griseofulvin (for fungi) were used as positive control (10 µg).

Results and Discussion

Isolation of actinobacteria from sponges

In the present study a total of six species of sponges were collected along the Indian Coast. Six species of sponges were identified as:

i. *Phycopsis sps,*

ii. *Axinella sps,*

iii. *Halichondria sps,*

iv. *unknown Demospongiae,*

v. *Petrosia sps,*

vi. *unknown Demospongiae*

A total of 60 actinomycetes were isolated from these sponges. Out of the 60 isolates of actinomycetes, 4 isolates were identified as genus streptomyces (spore chain was coiling spiral and looped), 10 as Micromonospora, (clusters of single conidia on substrate mycelium), 8 as Nocardia (conidia on powdery appearance aerial hyphae, carotenoid like pigments) and 2 as Streptoverticillium (whorls of straight chain of conidia formed) (Table 1).

Characterization of the isolates

The cultural characteristics of actinobacteria were presented in Table 2. Out of 60 isolates, 42 isolates showed pigment production. 25 isolates produced melanin, 17 isolates showed distinctive revere side pigment and 20 morphology of spore-bearing hyphae, 20 isolates show (33.3%) flexous sporophores followed by 15 isolates show (25%) spiral sporophores, 12 isolates show (20%) retinaculum apertum sporophores, 9 isolates show rectus (15%) and 4 isolates show monoverticillus (6.7%).

Sampling sponges	Total No. of actinobacteria isolated	Actinobacterial species			
		Streptomycetes	Micromonospora	Nocardia	Streptoverticillium
1	15	10	2	2	1
2	6	4	1	1	-
3	9	6	1	2	-
4	13	9	2	2	-
5	8	4	2	1	1
6	9	7	2	-	-
Total	60	40	10	8	2

Table 1: Distribution of actinobacteria in different sampling stations of Indian Sea Coast.

Character	No. of isolates	%
Spore morphology		
Flexous	20	33.3
Spiral	15	25
Retinaculumapertum	12	20
Rectus	9	15
Monoverticillus	4	6.7
Pigment Productions		
Melanin	25	42
Reverse colour	17	28
Soluble colour	20	33
Isolates producing pigment	42	70

Table 2: Cultural characteristics of actinobacteria.

Antimicrobial activity of selective isolates

The mean diameter of inhibition zones was taken as the degree of antimicrobial activity of the isolates. In the present study, the zone of inhibitions is the mean of triplicates. As shown in Table 3, out of the 60 isolates 9 isolates were active against the pathogenic bacteria. Out of 9 isolates, 4 isolates [MB 31, MB 33, MB 42 and MB 60] showed excellent activity against the pathogenic bacteria. Whereas 2 isolates [MB 15 and MB 54] inhibited the growth of only gram positive bacteria and did not exhibit any activity against gram negative bacteria.

Isolates No.	Name of the Test Organism [Inhibition Zone diameter in mm]			
	E.coli MTCC 6365	*S.aurens* MTCC 7443	*P.vulgaris* NCIM 2813	*B. subtilis* MTCC 8141
MB-06	13	10	10	18
MB-15	8	13	8	11
MB-24	15	10	13	11
MB-31	20	21	20	19
MB-33	21	20	22	21
MB-42	22	21	22	21
MB-51	18	12	15	12
MB-54	9	12	9	13
MB-60	20	21	20	18
Ciprofloxacin	22	25	26	28

Table 3: Antagonistic activity of active Marine actinobacteria against gram-positive and gram-negative bacteria.

Isolates No.	Name of the Test Organism [Inhibition Zone diameter in mm]			
	A. niger MTCC 6484	*A. awamori* MTCC 7711	*C. albicans* MTCC 1346	*S. cerevisiae* MTCC 463
MB-06	10	10	8	19
MB-21	11	12	13	9
MB-33	9	13	13	11
MB-42	18	19	18	20
MB-45	14	11	10	9
MB-60	20	19	20	19
Griseofulvin	26	25	24	26

Table 4: Antagonistic activity of active Marine actinobacteria against pathogenic fungi.

As shown in Table 4, out of the 60 isolates 6 isolates were active against pathogenic fungi. Out of the 6 isolates, 2 isolates [MB 42 and MB 60] showed excellent activity. Whereas, the remaining 4 isolates were active against only three fungal species. The antimicrobial studies revealed that isolate MB 42 and MB 60, showed excellent antagonistic activities against both the bacterial and fungal species under study.

Conclusion

The main focus of the study reveals that the marine sponges act as the potential source for the development of new active compounds in the development of drugs. However, further studies are needed in the characterization of the isolates, to identify the chemical nature of the active compound.

Acknowledgement

Author (Sunanda Kumari Kadiri) wish to express immense gratitude to Department of Science and Technology [DST], Government of India for sponsoring a project under the Fast Track–Young Scientist Scheme with File no. SB/FT/LS-247/2012 Dated: 2.5.2013.

References

1. De Rosa S, Mitova M, Tommonaro G (2003) Marine bacteria associated with sponge as source of cyclic peptides. Biomol Eng 20: 311-316.
2. Proksch P, Edrada RA, Ebel R (2002) Drugs from the seas-current status and microbiological implications. Appl Microbiol Biotechnol 59: 125-134.
3. Müller WE, Zahn RK, Kurelec B, Lucu C, Müller I, et al. (1981) Lectin, a possible basis for symbiosis between bacteria and sponges. J Bacteriol 145: 548-558.
4. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59: 143-169.
5. Wilkinson CR (1983) Net primary productivity in coral reef sponges. Science 219: 410-412.
6. Schumann-kindel G, Bergbauer M, Manz W, Szewzky U, Reitner J (1997) Aerobic and anaerobic microorganisms in modern sponges: a possible relationship to fossilization processes. Facies 36: 268-272.
7. Manz W, Arp G, Schumann-Kindel G, Szewzyk U, Reitner J (2000) Widefield deconvolution epifluorescence microscopy combined with fluorescence in situ hybridization reveals the spatial arrangement of bacteria in sponge tissue. J Microbiol Methods 40: 125-134.
8. Oskay M, Tamer AU, Azeri C (2004) Antibacterial activities of some actinomycetes isolated from farming soils of Turkey. African J Biotechnol 3: 441-446.
9. Pandey B, Ghimire P, Agarwal VP (2004) International Conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Organised by Kathmandu University and the Aquatic Ecosystem Health and Management Society, Canada.
10. Zhang YQ, Chen J, Liu HY, Zhang YQ, Li WJ, et al. (2011) Geodermatophilus ruber sp. nov., isolated from rhizosphere soil of a medicinal plant. Int J Syst Evol Microbiol 61: 190-193.
11. Shirling EB, Gottlieb D (1966) Methods for characterization of Streptomyces species. Int J Syst Bacteriol 16: 313-340.
12. Pridham TG, Lyons AJ Jr (1961) Streptomyces albus (Rossi-Doria) Waksman et Henrici: taxonomic study of strains labeled Streptomyces albus. J Bacteriol 81: 431-441.
13. Bergy DH, (1989) Bergy's Manual of Systematic Bacteriology, Vol. 4. Baltimore, USA: Williams and Wilkins Company. ISBN 0–683-09061-5.
14. Pridham TG (1965) Color and Streptomycetes. Report of an International Workshop on Determination of Color of Streptomycetes. Appl Microbiol 13: 43-61.
15. Barry AL, Thornsberry C (1985) Susceptibility tests: Diffusion Test Procedure. In Manual of Clinical Midrobiology, 4th Edition., editors.Ballows EA, HawslerWJ Jr, Shadomy HI 978 -987. Washington DC: American Society of Microbiology. ISBN 0-914826-65-4.

Citation: Kadiri SK, Yarla NS, Vidavalur S (2014) Screening and Isolation of Antagonistic Actinobacteria Associated With Marine Sponges from Indian Coast. J Microb Biochem Technol S8: 003. doi:10.4172/1948-5948.S8-003

This article was originally published in a special issue, **Biomaterials: Down Stream Processing** handled by Editor. Dr. Peter Kilonzo, University of Western Ontario, Canada