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Isolation and characterization of Polychaetes associated Marine bacteria

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Abstract

The ocean is the largest home for all kinds of creatures. It ranges from microscopic bacteria, algae and fungi to the largest animals in the world. More than 2,50,000 marine species have been discovered in the world. Benthic habitat is important for many reasons. The benthic ecosystems in the east and near the coast support a plethora of marine life by providing spawning habitat and feeding grounds for fins and shellfish. Benthic organisms contribute to the sedimentation, decomposition and turnover of organic matter on the seafloor, and help to circulate nutrients to the overlying water column. Polychaete is the most important group in the benthic community, accounting for about 75% of the total number of macrobenthic communities. Their diet includes microorganisms, namely bacteria, microalgae, protists and fungi. The distribution of marine bacteria in different seasons.

Keywords: Polychaete, Marine Bacteria, density, Antibacterial activity, Phylogenetic tree.

Introduction

Marine environments are complex ecosystems that often contain a variety of closely intertwined organisms. Among them, eukaryotic communicative microorganisms have received much attention in the past decade (Egan et al., 2008). The surface of all marine eukaryotes is covered with microbes that inhabit diverse communities and often integrate into substrates to form biofilms (Perez-Matos et al., 2007). In addition, host specificity has been demonstrated by studies showing unique and stable populations of individuals of geographically distant species (Webster and Bourne, 2007). The natural microbial products of the sea have not yet been fully studied. The marine environment consists of many unique microorganisms that produce biologically active compounds to adapt to specific environmental conditions. For example, surface microbes are a rich source of novel biological activity, as chemicals must be developed to protect crops from fierce competition between surface microbes in the marine environment. Marine eukaryotes (Penesyan et al., 2010).

The number of natural products found in a variety of organisms, including plants, animals, and microorganisms, currently exceeds one million (Berdy, 2005), and more (40-60%) are derived from terrestrial plants. 20-25% of these natural products have some biologically active properties, particularly antibacterial, antifungal, antiprotozoal, antihemolytic, anticancer, antiviral and anti-inflammatory properties. Plants and plant extracts have been used to treat human ailments for thousands of years, and their use is documented in the oldest archaeological sources. In contrast, the discovery of microorganisms as manufacturers of therapeutics did not begin until the 20th century (Monaghan and Tkacz, 1990). Bacteria have been shown to be associated with all organisms. The main attraction of the sea is Verongia sp. 50% of bacterial biomass. Cyanobacteria and oxychlorobacteria are abundant in many sponges. Many eukaryotic microbes are also known to coexist. For example, Dinophyceae sponges, various invertebrates, algae, fungi and algae (Taylor et al., 2007). Polychaetes or polychaetes are a group of annelids. Each part of the body has many hairs called cheetahs and a pair of fleshy projections called parapods made of chitin. Many spiny-bound microbes have not been studied much. There are many benefits to using microorganisms as a source of bioactive compounds. Therefore, in this study, the antibacterial activity of some fungi was investigated. This study provides details on the important activity of bacteria associated with marine invertebrates.

Materials and Methods

Isolation of marine bacteria from polychaetes

Polychaetes were collected from the Uppanar estuary in Cuddalore, India, stored in plastic bags and transported to a cooler laboratory where they were stored at 40 °C. Approximately 1 g of total folic acid tissue was triturated and diluted with 10 ml of PBS (phosphate buffer). Up to 105 using this as a thinner. Diluted samples were plated on marine agar and incubated at room temperature for 48 hours. Bacterial colonies were identified based on significant evidence of determinative bacteriology.

Antibacterial activity

The antibacterial activity of the culture filtrate against bacterial clinical pathogens was tested according to the well assay. After Swapping pathogens from the Mueller-Hinton agar plate, 0.1 ml of a cell-free culture solution filtered at 10,000 rpm was added to the well for 20 minutes, and the plate was incubated at 370° C. for 48 hours. Filtration of bacterial cultures that inhibit the growth of pathogens around the wells was evaluated from the zone of inhibition around the wells after 2 days.

Statistical Analysis

Data analysis was carried out with SPSS, Inc. software (version 10.0). One-way ANOVA was used to study any significant difference between means with a significant level of $P<0.05$. Critical difference values were used to perform multiple comparisons between means. All data are expressed as the mean ± standard deviation.

Results

Annual bacterial densities associated with Polychaetes were observed in samples from January 2020 to December 2020. Simultaneous bacterial spread was observed at various times of the year. They were present in the monsoon and summer seasons with densities of 1.8×10^5 and 6.0×10^5 CFU/g, respectively. During the work, the Zobel Marine Marine Egger was used. Bacterial transmission during the study period was up to 6.0 x 10^5 CFU/g for *Perineris cultifera* species in the Uppanar estuary in summer. The highest bacterial load was recorded in the Uppanar estuary during the summer and in the year when the maximum load was found prior to rainfall, with a minimum of 1.8 x 105 CFU/g in the Uppanar estuary during the monsoon season (Table 1).

Table. 1 Seasonal variation of Bacterial density in different Polychaetes of Uppanar estuary.

S. No	Polychaetes of Uppanar estuary	Seasonal variation of Bacterial density (CFU/g)			
		Post monsoon	Summer	Pre monsoon	Monsoon
1	*Arenicola* sp.	2.5×10^5	5.1×10^5	3.6×10^5	2.7×10^5
2	*Ancistrosyllis parva*	-	5.0×10^5	3.5×10^5	-
3	*Arabella mutans*	2.7×10^5	-	-	2.1×10^5
4	*Armandia longicaudata*	2.8×10^5	4.7×10^5	3.7×10^5	2.8×10^5
5	*Armandia intermedia*	-	4.3×10^5	3.9×10^5	-
6	*Capitella capitata*	2.4×10^5	4.2×10^5	3.2×10^5	3.0×10^5
7	*Chaetopterus* sp.	2.5×10^5	3.9×10^5	3.3×10^5	2.2×10^5
8	*Cirratulus chrysoderma*	2.9×10^5	4.1×10^5	3.3×10^5	-
9	*Cirratulus* sp.	-	4.4×10^5	3.7×10^5	2.6×10^5
10	*Cossura coasta*	-	4.5×10^5	4.2×10^5	-
11	*Dodecaceria* sp.	2.6×10^5	4.2×10^5	3.5×10^5	2.9×10^5
12	*Dorvillea gardineri*	2.9×10^5	-	3.7×10^5	2.0×10^5
13	*Euchone rosea*	-	4.6×10^5	-	-
14	*Euchone tentaculata*	-	5.0×10^5	3.7×10^5	-
15	*Fabricia filamentosa*	3.7×10^5	5.5×10^5	-	2.3×10^5
16	*Goniada emeriti*	3.4×10^5	4.2×10^5	3.9×10^5	2.8×10^5
17	*Glycera alba*	3.5×10^5	5.9×10^5	3.5×10^5	2.6×10^5
18	*Glycera* sp.	3.3×10^5	5.5×10^5	3.2×10^5	2.7×10^5
19	*Lumbrineris heteropoda*	-	-	3.4×10^5	-
20	*Lumbrineris aberrans*	2.9×10^5	4.1×10^5	3.3×10^5	2.9×10^5
21	*Lumbrineris brevicirra*	-	4.4×10^5	3.7×10^5	-
22	*Maldane sarsi*	-	4.5×10^5	4.2×10^5	-
23	*Nephtys dibranchis*	2.6×10^5	4.2×10^5	3.5×10^5	2.9×10^5
24	*Nephtys hombergi*	2.9×10^5	4.2×10^5	3.7×10^5	2.0×10^5
25	*Nephtys* sp.	3.4×10^5	4.6×10^5	3.6×10^5	1.8×10^5
26	*Notocirrus australis*	-	5.0×10^5	-	-
27	*Nereis capensis*	3.5×10^5	5.1×10^5	3.6×10^5	2.7×10^5
28	*Nereis virens*	-	5.0×10^5	3.5×10^5	-
29	*Nereis* sp.	3.3×10^5	5.5×10^5	-	2.1×10^5
30	*Perinereis cultrifera*	2.9×10^5	6.0×10^5	3.7×10^5	2.8×10^5
31	*Scololepsis squamata*	-	4.3×10^5	3.9×10^5	-
32	*Notomastus aberans*	2.7×10^5	4.7×10^5	3.2×10^5	3.0×10^5
33	*Notomastus* sp.	2.7×10^5	3.9×10^5	3.3×10^5	2.2×10^5
34	*Polydora ciliata*	-	4.1×10^5	-	-
35	*Pisione indica*	2.5×10^5	4.4×10^5	3.7×10^5	2.6×10^5
36	*Onupis* sp.	-	5.1×10^5	3.6×10^5	2.7×10^5
Average		**3.7×10^5**	**2.8×10^5**	**4.5×10^5**	**3.7×10^5**

A total of 140 strains were isolated during the 1-year sampling period. Of these, only 15 were selected for further investigation due to their antimicrobial activity against clinical pathogens (Table 2). In this study, we selected the power of bacteria to produce more bacteriocins. Of the 15 strains tested for clinical pathogens, BSJ2 was significant against *Staphylococcus aureus* (31.31 ± 0.02), *Klebsiella pneumoniae* (18.00±0.007), *Pseudomonas aeruginosa* (33.21 ± 0.014), and *E. coli.*

Table.2 Antibacterial activity by marine bacterial filtrates

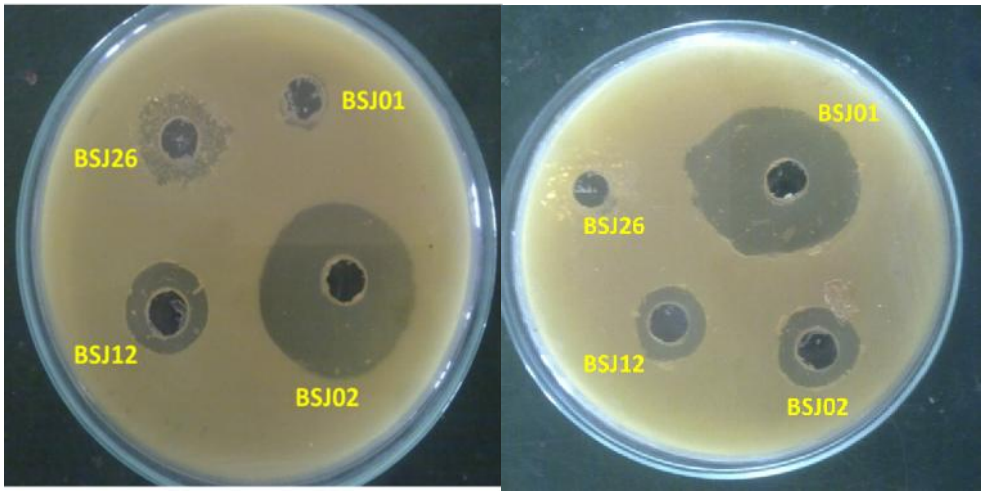
Organism	*Staphylococcus aureus(mm)*	*Klebsiella pneumoniae (mm)*	*Pseudomonas aeruginosa (mm)*	*E. coli (mm)*
BSJ01	2.07 ±0.14	1.03 ±0.07	1.61 ±0.11	1.38 ±0.09
BSJ02	**15.31 ±0.02**	**18.00 ±0.007**	**33.21 ±0.014**	**15.09 ± 0.006**
BSJ 12	0.21 ±0.014	0.02 ± 0.003	0.10 ±0.007	0.06 ±0.004
BSJ 26	1.89 ±0.13	1.11 ±0.10	1.64 ±0.11	1.52 ±0.106
BSJ 39	8.19 ±0.57	2.32 ±0.30	5.91 ±0.41	3.20 ±0.22
BSJ 41	9.63±1.37	8.57±0.59	9.65±0.67	8.56±0.07
BSJ 53	2.92 ±0.20	2.41 ±0.16	2.10 ± 0. 14	2.71 ±0.18
BSJ 60	3.46 ±0.24	2.62 ±0.18	2.32 ±0.16	2.89 ±0.20
BSJ 72	7.35±0.12	1.27±0.15	2.72±0.19	5.00±0.35
BSJ 81	13.35±0.49	4.53±0.42	7.81±0.49	6.75±0.45
BSJ 93	2.1 ±0.15	1.3 ±0.15	2.92 ±0.06	0.4 ±0.06
BSJ112	5±0.12	11±0.25	10±0.92	9±0.13
BSJ 104	2.07 ±0.14	1.03 ±0.07	1.61 ±0.11	1.38 ±0.09
BSJ 126	0.31 ±0.02	0.08 ±0.007	0.21 ±0.014	0.09 ± 0.006
BSJ 135	0.21 ±0.014	0.02 ± 0.003	0.10 ±0.007	0.06 ±0.004

Among the collected samples, BSJ2 showed the highest antibacterial activity. Based on morphological, physiological and biochemical characteristics, it was identified as *Lysinibacillus sphaericus*, named *L. sphaericus*, and classified as BSJ2 species (Table 3). This strain was also confirmed by 16S rDNA sequencing. Further sequencing was performed for analysis using BLAST software. Based on the results shown in Figure 1, the BSJ2 strain was identified as a member of the genus Bacillus. In addition, the similarity between the BSJ2 strain and the *Lysinibacillus sphaericus* strain was 99%, and based on this similarity, it was identified as *Lysinibacillus sphaericus*. This sequence was sent to GenBank (accession number: KF781636).

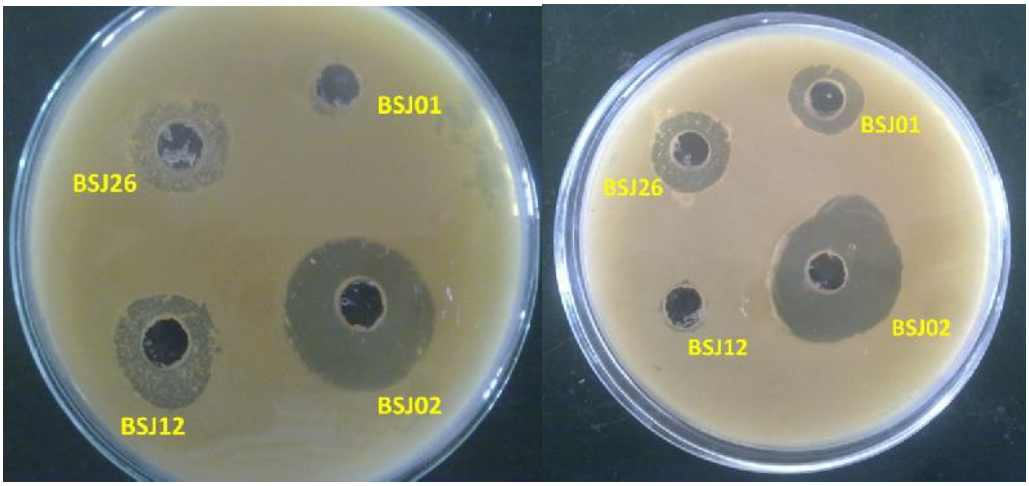
Table.3 Biochemical identification of BSJ02 strain

S. No	Biochemical Tests	Result
1	Gram stainig	**+ (Rod)**
2	Oxidase Reaction	+
3	Catalase Reaction	_
4	Growth at 42°C	+
5	Production of fluorescent pigment	_
6	Indole production on tryptophan	_
7	Urease	_
8	Esculin hydrolisis	_
9	Casein hydrolisis	+
10	Tween 20 hydrolisis	+
11	β –Galactosidase	_
12	N-Acetyl-D-glucosamine	_
13	Maltose	_
14	Gluconate	_
15	L-Malate	_
16	Citrate	+

Key, - = negative, + =Positive,



Staphylococcus aureus *Klebsiella pneumoniae*



Pseudomonos aeruginosa *Escherichia coli*

Fig. 1 Antibacterial activity of BSJ01 marine bacterial filtrates.

Fig. 1 Phylogenetic tree of BSJ02 strain

Discussion

This study showed that the bacterial densities in vertebrates were on the order of 1.8 x 10^5 and 6.0 x 10^5 CFU/g. Bacterial contamination was observed in almost all samples throughout the year. This may be due to the way the sediment is settling, as it is already rich in nutrients and therefore contaminated with bacteria. Rajendran and Nagatomo (1999) and Rooney-Varga et al. (1997) observed seasonal changes in microbial community composition in coastal seabed sediments using phospholipid esters and related fatty acids in genetic analysis. In temperate estuaries, temperature is an important regulator of seasonal changes in the microbial community (Heil, 2011), suggesting that it may be responsible for seasonal effects on the bacterial composition of the gut lumen. The screening method used in this study provides a rich source of microorganisms with the desired properties. We performed a preliminary evaluation of antimicrobial activity against clinical bacterial pathogens such as *Staphylococcus aureus*, *Pneumococcus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Chemical interactions between different types of bacteria can influence the production and secretion of secondary antimicrobial metabolites (Patterson and Bolis, 1997). In this study, 140 spinal cords were isolated from the Obner Estuary. Several studies of the prevalence of bacterial forms in marine environments (Fenical, 1993) and similar studies of antibiotic production in marine bacteria (Bernen, 1997) have been identified. 36% of antibiotic producers were Gram-negative bacilli. In this study, gram-positive and gram-negative bacteria were isolated from midges (data not shown). Since only 15 species of midges have been isolated, more accurate culture methods can reveal the true diversity of many related bacteria. Different types of bacteria that produce marine antibiotics (Hentschel, et al., 2000).

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