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ISOLATION AND SCREENING OF MARINE BACTERIA PRODUCING ANTIBIOTICS AGAINST HUMAN PATHOGENS

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ABSTRACT

The need for the development of new antibiotics to counter drug resistance in bacterial pathogens has been stressed by various researchers worldwide. As the discovery of novel chemical classes have been in decline for the past two decades, the need to exploit new resources in search for effective chemicals with novel mechanism of actions is imperative. Marine bacteria are such a resource yet to be tapped, and the potential it offers is vast. The principal objective of this present investigation was to isolate and screen marine bacteria for the production of bioactive metabolites. Associated bacterial strains were isolated from 10 species of sponges, 12 species of algae, 4 species of crabs, 3 species of ascidians, sediment, one species each of sea cucumber, sea urchin and jellyfish. Gut microflora was isolated from 3 species of mollusc, biofilm bacteria from dead corals and dead oyster shell and egg bacterial symbionts from crab eggs and eggs of molluscs. A total of 633 strains were isolated during a period of two years. In the antibiotic production test 170 strains were found to be active against the five human pathogens *(E.coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillis substilis and Candida albicans)*.In the genus level identification of the potential strains, *Alteromonas sp.* was found to be the dominant antibiotic producer.

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KEYWORDS

marine bacteria, antibacterial, antifungal, Tuticorin coast

INTRODUCTION

The use of antimicrobial drugs to control infectious diseases must be among the greatest achievements of medicine in this century[1]. Waksman started the screening of antibacterial substances produced by soil actinomycete and discovered the actinomycins of which some are used as antitumor agents. After many clinically useful antibiotics like streptomycin, chloramphenicol, chlortetracycline, neomycin, oxytetracycline, erythromycin etc. were discovered and there by most bacterial infections seemed to be conquered. However about 10 years after the spread of antibiotic therapy a number of species of Staphylococcus, Mycobacterium and Gram negative enteric bacteria had developed resistance to antibiotics[3].

In the past 20 years the pharmaceutical industry has been relatively successful in containing problems due to single resistance determinants. However the advent of multiple resistance mechanism has severely limited the effective use of many major classes of drugs. The development and spread of resistance to any new antimicrobial agent is probably inevitable, however new drug classes with novel mechanism of action will create effective therapy at least for a time[4].Marine microorganisms encompass a complex and diverse assemblage of microscopic life forms and occur throughout the oceans including environments of extreme pressure, salinity and temperature. Marine microorganisms have developed unique metabolic and physiological capabilities that not only ensure survival in a great variety of extreme habitats but also offer the potential for the production of metabolites, which would not be observed, from terrestrial microorganisms[5]. Early indications that marine microorganisms represent are source for biomedically relevant compounds came from the work of Rosenfeld and Zobell and Grein and Meyers [6, 7]. It is now known that marine microorganisms are capable of producing unusual natural products that are not observed from terrestrial sources and many of these compounds have antibiotic and other biological activities.

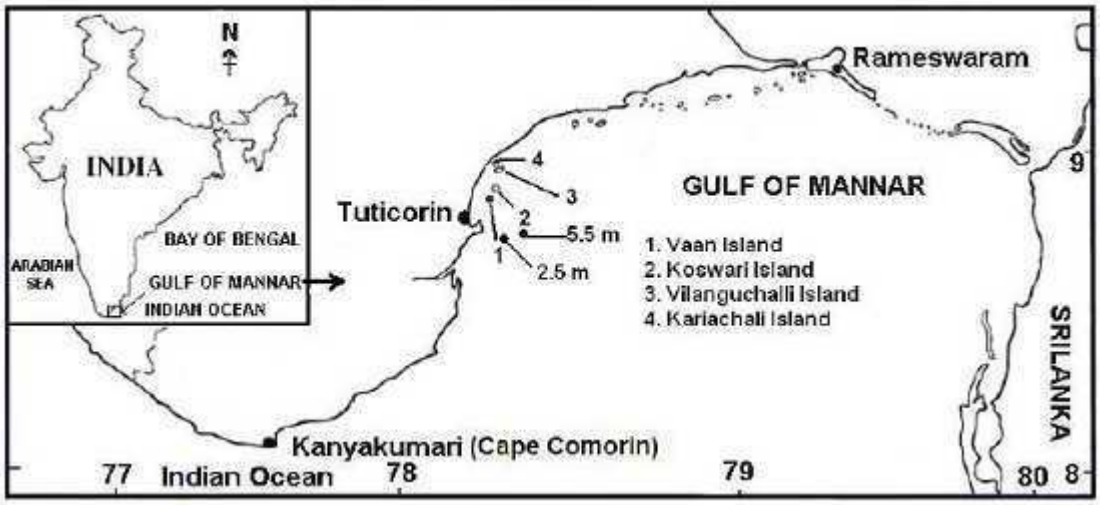
Overall the distributions of marine bacteria are poorly known. Gram-negative genuses like Vibrio are found in abundance in seawater. The remainders are gram-positive forms of a variety of taxonomic affiliations including representatives of the genus Bacillus. The other important microhabitats for marine bacteria are the sediments, animate and inanimate surfaces and internal spaces of invertebrate animals. Marine plants and animals are well known to have developed symbiotic relationships with numerous microorganisms. This is particularly true of the bacteria which are widely distributed on the surfaces and within the tissue of marine plants and animals. The importance of bacterial symbiosis is growing in recognition that it may be the true producers of many compounds isolated from sponges, ascidians and other marine invertebrates[7].

In the present investigation bacteria were isolated from various Marine sources and screened for antibiotic production against 5 potential human pathogens.

MATERIAL AND METHODS

Different marine samples such as Sponges (10 species), Seaweeds (12 species), Crabs (3 species), Biofilm (3 substrates - dead pearl oyster shells and dead corals), Invertebrate eggs (4 species), Ascidians (3 species), Corals (3 species), Gastropods (gut associated bacteria)(3 species), Sediment (1 sample), Ophisthobranch (surface associated bacteria) (2 species), Jelly fish (1 species), Sea Urchin (1 species) and Sea cucumber (1 species) were collected from Tuticorin coastal waters (Fig.1) and transferred to the laboratory aseptically for the isolation of bacteria.

Fig 1
Study area



Isolation of marine bacteria associated with seaweeds

Seaweeds were collected from Hare Island (Tuticorin coast) and transferred to the laboratory in sterile plastic bags aseptically. The seaweed samples were rinsed with sterile seawater to remove the non-attached bacteria[9].Then 1 g of the sea weed sample was homogenized with sterile seawater using a mortar and pestle andthe bacterial strains were isolated employing the conventional pour plate technique using Zobell marine agar 2216 (Himedia, Bombay, India). Each dilution wasplated in triplicates and incubated at room temperature (27° C) for 7 days and isolation of bacteria was carried out from the seventh day onwards.

Isolation of marine bacteria associated with sponges

Sponge associated bacteria were isolated by following the method outlined by Santavy *et al.,* 1990. Sponge samples were collected near Koswari Island from a depth of 10 m by SCUBA diving. The samples were collected and brought to the laboratory in an icebox aseptically. The samples were washed with jets of filtered autoclaved seawater until they were visibly free of debris. Then the sponge surface was sterilized by a rapid wash of 70% ethanol and immediately immersed in autoclaved, filtered seawater and aspirated. One gram of sponge tissue was

removed from the calcarious skeleton with a sterile scalpel. The tissue was immediately transferred to 99 ml sponge dissociation medium (2.7% NaCl, 0.008% KCl, 0.01% Na_2So_4, pH 8.0). The samples were soaked for 20 mins. The tissue and diluents were macerated and the homogenate was plated using a dilution series of 10"[5] employing the conventional pour plate technique using Zobell marine agar 2216. (Himedia, Bombay, India). Each dilution was plated in triplicates.

Isolation of marine bacteria associated with ascidians, sea cucumber, jellyfish and crabs

All samples were collected along Tuticorin coast and transferred to the lab aseptically. From each organism one gram of tissue was taken and homogenized in sterile seawater. The associated bacteria were isolated employing the conventional pour plate technique using Zobell marine agar 2216 (Himedia, Bombay, India).

Isolation of marine bacteria associated with crab, gastropod and cephalopod eggs

Berried crabs were collected from Vellapatti fishing village and the eggs were cut and removed from the crabs using sterile scissors and forceps. The eggs were then surface sterilized by swabbing with a sterile swab dipped in 70% ethanol. 1 g of the egg sample was cut from inside the egg mass and homogenized using a mortar and pestle in sterile seawater and the homogenate was plated in Zobell marine agar 2216 from gastropod and cephalopod eggs, 1 ml of perivitalline fluid was taken using a disposable sterile syringe and plated on Zobell marine agar 2216.

Isolation of marine bacteria from sediments, biofilm and ophisthobranch surface

Sediment sample was collected at a depth of 10 m and bacteria were isolated on Zobell marine agar 2216. For isolation of biofilm bacteria, the dead oyster shells and dead corals were collected and brought to the laboratory aseptically and the biofilm was scraped using a sterile spatula and one gram was transferred to 99 ml of sterile seawater. Then biofilm bacteria were isolated in Zobell marine agar 2216. From ophisthobranch surface, bacteria were isolated using a sterile swab by swabbing the dorsal surface in an area of $1cm^2$ and applying the swab on a prepoured Zobell marine agar plate. The bacterial strains from all the samples with different colony characteristics were further isolated by repeated streaking and stored at 4°C in Zobell marine agar slants for further studies.

Screening for antibiotic production by marine bacteria

The screening of antimicrobial substances was carried out following the methods by Spragg *et al.*[10].In the first method, antibiograms of marine strains were streaked onto TSA plates (Tryptone Soya Agar + 1 % Nad) and incubated at room temperature for 5 days. Test strains of human pathogens such as *E.coli, Pseudpmonas aeroginosa, Bacillus substilis, Vibrio cholerae* and *Candida albicans* were then streaked perpendicular to the marine strains in the agar and incubated overnight. Inhibitory activity was indicated by inhibited growth of pathogenic strain on the agar as compared to a control plate showing healthy growth of the test strains.

In the second method all the isolated strains were inoculated into 5 ml Zobell marine broth and shaken at 290 rpm for 5 days at room temperature. The cultures were then centrifuged at 5000 rpm for 5 mins

and the supernatant was discarded. Sterile seawater (200 µl) was added to the cell pellet and resuspended. The resuspended cell material was further incubated for 24 hrs. Concentrated supernatant (100 µl) was added onto sterile paper discs (Whatman, 6mm diameter) and antibacterial activity was assayed following the disc-diffusion assay[11].

In the third method, all the marine strains were inoculated onto 100ml of Zobell marine broth individually. The inoculated strains were broth cultured in a shaker at 290 rpm for 5-7 days at room temperature and then the broth culture was extracted employing liquid-liquid extraction[12]. Equal volume of ethyl acetate was added to the broth and stirred for 30 mins. using a magnetic stirrer. The two phases were then separated in a separating funnel and the solvent phase was concentrated by evaporation. The concentrate (crude extract) was then impregnated on to sterile Whatman 6 mm disc and antibacterial activity was assayed following the disc-diffusion assay. The inhibition zone was measured from the border of the disc to the edge of the clear zone. The potential strains were identified up to genus level using the biochemical methods outlined in Bergey's manual of systematic bacteriology[13, 14]

.

RESULTS

In sponge samples, the total count ranged from a highest of 3×10^5 to a lowest of 3×10^3CFU/g. The highest count was obtained in sponge *Damirina papillata,* but the highest numbers of strains were obtained from sponges *Echinodictyum longistylum* and *Carmia sulevoidea.* In algal samples, the total count ranged from a highest of 8×10^4 to a lowest of 5×10^3. The highest numbers of strains were obtained from *Gracillaria crassa.* From the three crab samples, the highest count (30×10^5), and the highest number of strains were observed and isolated from *Portunes pelagicus.* In biofilm-associated bacteria, the highest numbers of strains were obtained from Pearl oyster biofilm and dead coral biofilm and the total count varied from a highest of 60×10^4 to a lowest of 3×10^3. In sea cucumber, sea urchin and jellyfish, the total count were 8×10^4, 12×10^4 and 22×10^4 respectively and out of them the highest numbers of strains were obtained from sea cucumber. For gut microflora, the total count ranged from a highest of 20×10^4 in *Cypraea errones* to a lowest of 7×10^4in *Babylonia spirata.* In ascidians, the total count ranged from highest of 150×10^4 to a minimum of 23×10^4 and the highest numbers of strains were obtained from *Didemnum pssamathodes.* In egg-associated bacteria, the total count ranged from a highest of 10×10^4 in crab egg *(Portunes pelagicus)* and lowest count in squid egg (4.5×10^3 and the highest numbers of strains were obtained from crab egg *(Portunes pelagicus).* For corals, the total count ranged from a highest of 25×10^5 to a lowest of 46×10^3 and the highest numbers of strains were from soft coral. From ophisthobranch surface, a total of 32 strains were isolated. Antibacterial activity (inhibition zone) against the five pathogens by the antibiotic producers is given in tables 1-10.

Table 1
Antimicrobial activity of sponge associated bacteriaagainst human pathogens

Sponge strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S.aureu*	*P.*	*B.substili*	*C.albican*
SA3	5	-	-	-	-
SA6	2	-	-	-	-
SB3	5	-	-	5.5	-
SB5	-	-	-	7	6
SC1	6	-	5.5	-	-
SC2	2	1		7.5	
SC3	7	-	2	-	1
SE2	-	-	-	6.5	-
SE3	-	-	-	2	-
SE5	-	-	7	-	-
SE11	2.5	-	7	7	-
SF5	3	-	4.5	9	-
SF6	4	-	6	9	-
SG5	-	-	6	-	-
SH2	6.5	-	-	-	-
SH3	7	1	-	1	-
SH5	-	-	-	3	-
SH6	-	-	-	-	7.5
SH8	-	-	-	6.5	-
SJ2	t	-	1	2	-
SJ4	-	-	3	5	-
SJ7	-	-	-	6	-

Inhibition zone (mm) - From the edge of the disc to the edge of the clear t - trace.

Table 2
Antimicrobial activity of algae associated bacteria against human pathogens

Algal strains	Pathogens (Inhibition zone in mm)				
	E.coll	*S. aureus*	*P. aeruginosa*	*B. substilis*	*C.albicans*
AA2	2	2	-	t	-
AA5	-	7	1	-	-
AA7	-	-	5	-	-
AB3	-	-	8.5	-	-

AB6	-	-	8	3	-
AB9	-	-	5	-	-
AB19	7	-	t	-	-
AB21	2	1	8.5	-	-
AC3	4	2	5	2	-
AC6	-	-	-	2	-
AD3	-	-	5.5	5	-
AD7	-	-	-	6.5	-
AD9	-	-	-	6	-
AD15	-	-	-	7	6
AD16	-	-	2	t	-
AD18	-	-	-	5	-
AE2	-	-	-	2.5	-
AE3	-	-	-	t	-
AE4	-	-	t	2	-
AE7	-	-	-	2.5	-
AE8	t	-	-	2	-
AF2	3.5	-	3	2	-
AF3	7		5	2	
AF5	2	2	t	-	-
AF12	5	-	7	-	-
AF15	-	1	3	-	t
AF16	6	t	-	-	-
AG1	3	-	8.5	2	-
AG2	7	t	2	5	-
AG4	5	3	t	-	t
AG11	2	-	7	-	-
AG12	t	5	-	t	-
AH4	8.5	3	2	-	-
AH7	3	t	4.5	9	-
AH13	5	2	1	t	-
AI4	t	1	7	5	-
AI9	-	3	-	-	-
AM 1	t	-	-	7	-
AI13	7.5	-	6	-	-
AJ3	5	-	-	2	-
AJ10	1	-	4	-	t
AK4	5	3.5	-	2	-
AK6	-	2	-	7	-
AK10	8	5	-	t	-
AK14	3	-	t	1	-
AK17	6.5	4	5	-	-
AL3	-	2	-	t	-
AL7	2	-	-	3	-
AL11	8	1	7.5	-	t

Table 3

Antimicrobial activity of crab associated bacteria against human pathogens

Crab strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P.aeruginosa*	*B.substilis*	*C. albicans*
CA4	3	-	7	-	10
CA7	3.5	-	t	2	-
CB2	3.5	-	5	-	-
CB3	-	-	4	3	-
CB7	2	2	15	-	-
CB9	3.5	-	5	3	-
CC1	2	t	-	-	-
CC2	-	-	2	-	-
CC6	-	t	4	-	t
CC7	t	-	2	-	-
CC10	-	-	-	5	-

Table 4

Antimicrobial activity of bio-film bacteria against human pathogens

Bio-film strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P. aeruginosa*	*B. substilis*	*C.albicans*
BFA7	t	-	7	-	t
BFA8	2	t	2.5	-	-
BFA9	-	-	8.5	3	-
BFA18	3.5	1	t	-	-
BFA19	t	2	-	1.5	-
BFB6	-	1	-	-	-
BFB12	1.5	-	5	-	-
BFB13	-	2	2	-	-
BFC2	-	2	1	1	-
BFC3	-	-	7.5	-	-
BFC7	1	-	-	t	-
BFC15	-	5.5	-	t	-
BFC16	t	-	1	1	-
SM1	-	7.5	-	1	-
SM2	-	3.5	-	5	-
SM6	t	t	-	1	-

SM10	-	-	6		

Table 5

Antimicrobial activity of sea cucumber, sea urchin and jellyfish associated

Strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P. aeruginosa*	*B. substilis*	*C. albicans*
SCU2	5	t	-	2	-
SCU7	-	-	t	1	-
SUR3	6.5	-	-	2	-
SUR4	-	1	-	-	-
SUR7	t	t	t	1	-
JF2	t			2	
JF4	8	8	2	3	7

Table 6

Antimicrobial activity of gut microflora of gastropods against human pathogens

Gut microflora strains	Pathogens (Inhibition zone in mm)				
	E.coli	*5. aureus*	*P. aeruginosa*	*B. substilis*	*C. albicans*
GMA3	8	-	t	-	-
GMA4	-	t	1	2	-
GMA8	-	t	4.5	7	-
GMB2	1	3.5	-	7.5	-
GMB3	-	t	-	t	-
GMB8	t	-	1	2	-
GMC1	-	6.5	-	-	-
GMC4	-	1	3.5	5	-
GMC8	1	-	7	-	-
GMC9	-	-	2	t	-
GMC10	-	-	1	-	-

Table 7

Antimicrobial activity of ascidian associated bacteria against human

Ascidian strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P. aeruginosa*	*B.substilis*	*C.albicans*
ASA2	3.5	7	5	3	7
ASA3	t	-	-	5	-
ASA4	-	-	1	-	-
ASA7	5	-	2.5	-	-
ASB2	13	-	-	-	10
ASC1	13	11.5	10	12	15
ASC3	1	-	2	-	13

ASC5	-	-	1	t	-

Table 8

Antimicrobial activity of cephalopod andgastropod egg associated bacteria against human pathogens

Egg strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P. aeruginosa*	*B. substilis*	*C. albicans*
CE4	2	1	t	-	-
CE5	6	7	2	7	3
CE11	4.5	t	6	-	-
CE12	t	-	1.5	-	t
GE3	5	t	2.5	3	5
GE4	1	2	-	2	-
GE6	7	-	-	5	-
GE10	3	-	-	4	-
GE11	10	6	-	-	-
SPE2	1	2.5	-	t	-
SPE3	5	t	6.5	-	-
SPE4	-	-	7	-	-
SPE5	t	-	t	2.5	-
SPE9	-	1	t	-	-
SQE1	5	2	3	8.5	-
SQE2	-	1.5	-	2	-
SQE3	-	-	1	-	-
SQE4	6.5	-	4	-	-
SQE5	-	2.5	t	-	-
SQE6	7	-	6.5	9.5	-
SQE8	t	t	1	-	-

Table 9

Antimicrobial activity of coral associatedbacteria against human pathogens

Coral strains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P.*	*B.substilis*	*C.*
BCL5	6	-	4	7	-
BCL7	2	5	-	-	-
SCL2	5	5	-	-	-
SCL3	20	-	22	19	-
SCL9	2	-	t	-	-
SCL10	2	1	3.5	-	-
SCL12	1	-	-	t	2.5
STCL4	9	6	30	-	-
STCL5	-	-	5	t	-
STCL9	1	t	-	3	-
STCL13	3	-	6	8	-
STCL14	7	-	2	-	-
STCL15	1	-	2.5	2	t

STCL17	t	1	-	-	t

Table 10

Antimicrobial activity of nudibranch surfacebacteria against human pathogens

Nudibranch surfacestrains	Pathogens (Inhibition zone in mm)				
	E.coli	*S. aureus*	*P. aeruginosa*	*B.substili*	*C.albican*
NBSA1	-	-	-	2	t
NBSA2	7	1.5		3	-
NBSA9	t	-	3	t	-
NBSA12	2	7	5	5	-
NBSA18	1	-	t	5	t
NBSB2	8	-	7	-	t
NBSB3	1.5	2	-	t	-
NBSB4	5.5	-	1	t	-
NBSB5	7.5	-	3	-	-
NBSB9	1	3	t	-	-

Out of the total 633,[Delete punctuation] isolates 170 were found to be antibiotic producers. The highest percentage occurrence of antibiotic producers was found in cephalopod egg. The highest number of antibiotic producing strains were isolated form algae and then from sponge and eggs respectively. In antibiotic producing strains the number of pigmented colonies varied and the highest numbers of pigmented colonies were isolated from algae followed by sponges. Antibacterial activity exhibited against human pathogens was observed to be broad spectral as well as species specific. 14% of the antibiotic producers exhibited broad spectral activity and 86% exhibited species-specific activity. In the genus level identification of the potential strains, *Alteromonas sp.* was found to be the dominant antibiotic producers against human pathogen in this study (Table 11).

Table 11

Genus level identification of potential strains exhibiting activity

S.No.	Strains	Genus	S.No.	Strains	Genus
1	AA5	*Alteromonas sp.*	22	GMB2	*Vibrio sp.*
2	AB19	*Bacillus sp.*	23	OBSA2	*Alteromonas sp.*
3	AB21	*Bacillus sp.*	24	ASC1	*Vibrio sp.*
4	AD15	*Vibrio sp.*	25	CE5	*Alcaligenes sp.*
5	API 2	*Bacillus sp.*	26	SPE4	*Streptomyces sp.*
6	AG1	*Alteromonas sp.*	27	STCL14	*Bacillus sp.*
7	AG11	*Pseudomonas sp.*	28	AL11	*Micrococcus sp.*
8	AI13	*Alteromonas sp.*	29	AH4	*Micrococcus sp.*
9	AK6	*Alteromonas sp.*	30	AI11	*Bacillus sp.*

10	AK10	*Alteromonas sp.*	31	CA4	*Alteromonas sp.*
11	SC3	*Vibrio sp.*	32	CB7	*Flavobacterium sp.*
12	SE5	*Cytophaga sp.*	33	ASA2	*Alteromonas sp.*
13	SE11	*Alteromonas sp.*	34	ASB2	*Bacillus sp.*
14	SF5	*Micrococcus sp.*	35	GE6	*Alteromonas sp.*
15	SF6	*Alteromonas sp.*	36	GE11	*Vibrio sp.*
16	SH3	*Micrococcus sp.*	37	SQE1	*Alteromonas sp.*
7	BFA7	*Flavobacterium sp.*	38	SQE6	*Unidentified*
18	BFA9	*Vibrio sp.*	39	BCL5	*Streptomyces sp.*
19	BFC3	*Bacillus sp.*	40	SCL3	*Alteromonas sp.*
20	SM1	*Alteromonas sp.*	41	STCL4	*Flavobacterium sp.*
21	JF4	*Vibrio sp.*	42	STCL13	*Micrococcus sp.*

DISCUSSION

Initial antibiotic production by marine bacteria was screened using three methods. Out of the 633 isolates, 170 strains (26.8%) were found to be antibiotic producers. Lemos[15] isolated 224 epiphytic bacterial strains from intertidal seaweeds, out of which 38 strains (16.9%) displayed antibacterial activity. Spragg[16] isolated 51 strains from marine algae *Fucus resiculosus,* and in that, 13 (25%) exhibited activity against Methicillin resistant *Staphylococcus aureus,* (MRSA). Out of that 13 strains, only one exhibited good activity against MRSA. Jayanth[17] isolated 166 pigmented marine bacteria from different sources like seawater, sediment, algae (2 species) and bivalves (2 species) and found that 62 bacterial strains exhibited antibacterial activity (37.35%). In this present study, the total percentages of antibiotic producers were 26.8% and the highest numbers of antibiotic produces were obtained from algae (49 strains out of 170 strains). Jayanth[17] also reported similar results in their study on antagonistic marine bacteria against human pathogens.

Large numbers of heterotrophic bacteria of diverse morphology are reported from marine sponges[16-19]. In ultra structural studies, it has been shown that often bacteriaoccupymore sponge volume than do the sponge cells, even upto 60% of the mesohyl region[20-23]. In this presentstudy a total of 97 including 22 antibiotic producers strains were isolated from 10species of sponges. Sponges have provided more natural products than any otherphylum of marine invertebrates. A microbial origin has been proposed for a number of sponge metabolites but only in a few instances have these hypotheses been tested and proved. The total number of strains isolated from sponges was poor incomparison to their bacterial richness and this may be due to the unculturability ofmajority of the bacterial sponge symbionts. Santavy[20]reported that only3 to 11% of the total bacteria inhabiting the sponge are culturable with the existing conventional culture techniques and also the other limitation may be that only a single medium (Zobell marine agar) was used for the isolation of bacteria. As Zobellmarine agar is a nutrient rich medium, bacteria requiring low nutrient concentrations (oligotrophs) may not grow in this medium.

Gil-Turnes and Fenical[24] observed that the eggs of estuarine shrimp *Palaemon macrodactylus* possess significant bacterial epibionts which protected the

eggs from the pathogenic fungi *Lagondium callinectus.* Keeping this in mind egg associated bacteria was isolated from crab, gastropod and cephalopod. A total of 58 strains were isolated from eggs and among that 21(43.75 %) were found to be antibiotic producers. The higher percentage of bacterial symbionts producing antibiotics from eggs of marine animals may be due to the protective function that these symbionts play in nature to safeguard the eggs.

From the ascidians, 18 strains were isolated, of which, 8 strains were found to be antibiotic producers. Ascidians are already reported to be rich source of nitrogen compounds with a wide range of biological activities[25]. James[26] has reported the isolation of a marine bacterium from a tunicate, producing novel antibacterial protein. The low number of strain yield may be due to the unculturability of the symbionts. The total count of *Phalusia nigra* was very high (150×10^4) but only 4 different strains were isolated from the ascidian.

In the present study, 52 bacterial strains were also isolated from 3 species of out of which 14 strains were found to be antibiotic producers. Coral mucus have been reported to harbour rich bacterial diversity[27]. Fenical[28] reported the isolation of an unidentified *Streptomyces* from the surface of a gorgonian coral *(Pacifigorgia sp)* from the Gulf of California, Mexico.

Sea cucumber, sea urchin and jellyfish yielded 32 strains, including 7 antibiotic producers. Jellyfish yielded a potent strain (JF4), which exhibited activity against all the 5 human pathogens. Fenical[8] reported actinomycetes identified as a *Streptomyces sp* (CNB - 091) from the surface of an undescribed jellyfish from the Florida Keys and extracts of this culture were found to possess significant antibiotic activity.

Guts of aquatic animals may be intermittently or permanently populated by many groups of microorganisms that could be beneficial or pathogenic depending on their influence. Gut microflora also plays an important role in the digestive process, growth and disease susceptibility of marine deposit feeders[27-28]. Gut microflora (37 strains) were isolated from three species of gastropods and out of which 11 strains were found to be antibiotic producers. Only2 strains were potential and they exhibited higher activity against *Bacillus substilis* .Among the 38 isolates from 3 species of crabs 11 strains were found to be antibiotic producers. Potent activity was exhibited by two strains against *Pseudomonas aeruginosa.* 92 strains were isolated from 3 biofilm samples and one sediment sample with 17 antibiotic producers. Potential activity was exhibited by 5 strains, 4 against *P. aeruginos* and 1 against *Staphylococcus aureus.*

Epiphytic bacteria from algae were intensively screened for the production of antibiotics[8-9]. In the present investigation, bacteria present in the dorsal surface of 2 ophisthobranch species were screened for antibiotic production. A total of 32 strains were isolated and 10 were found to be antibiotic producers. Four potential strains were found to exhibit higher activity against *E.coli, S. aureus* and *P. aeruginosa.*

In the genus level identification of the potential strains, *Alteromonas sp.* Was found to be the dominant antibiotic producers against human pathogen in this study (Fig 5). Jayanth[16] reported that in the antagonistic marine bacterial strains they isolated highest numbers of strains represented this species. Till 1999 around 63 bioactive metabolites have been reported from Alteromonas *sp*[29].Out of 170 strains exhibiting antibacterial activity against human pathogens, 24 strains exhibited broad spectral antibacterial activity. Further

studies dealing with isolation and purification of the active compounds may yield potent novel antibiotics.

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