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Full Length Research Paper

Bioactive potential of symbiotic bacteria and fungi from marine sponges

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Marine sponges are rich in microbial biota. In this study, totally four sponges namely *Callyspongia diffusa, Hyattella Cribriformis*, *Sigmadocia carnosa, Spongia officininalis* Var *ceylonensis* were collected and their associated bacteria and fungi were isolated. Among the microbes isolated, *Pseudomonas fluorescens* and *Penicillium citrinum* were isolated from *C. diffusa* which showed broad range of activity against tested pathogens. This study demonstrates that the culturable fraction of bacteria and fungi from the sponges were diverse and appears to possess much potential as a source for the discovery of new medically relevant biological active agents.

Key words: Sponges, antibacterial activity, antifungal activity.

INTRODUCTION

Coral reefs are the most diverse marine ecosystems. Marine sponges are benthic animals found in the wide range of marine environments. The species diversity of sponges is superior in the tropical coral reef environments, while the sponges would be one of the most to-be-studied groups among reef fauna. Indeed, the sponges are often ignored by divers and naturalists. Encountering mobile animals, such as fish, turtles, mammals, rays, and even sharks, and looking at colorful corals draw much of their attraction, but sponges are quite interesting animals, in that the origins of their diversity in species, and the morphologies on the ecological significance as members of tropical coral reef habitats can be questioned.

Many marine sponges contain dense, highly diverse microbial communities. More than 10 bacterial phyla (including *Proteobacteria, Actinobacteria, Nitrospira, Chloroflexi, lanctomycetes, Cyanobacteria, Acidobacteri*a) have been found in sponges, as well as both major lineages of Archaea and a range of unicellular eukaryotes such as diatoms and dinoflagellates. Collectively, these organisms exhibit an enormous diversity of metabolic traits of potential use to the host, including nitrification, photosynthesis, anaerobic metabolism and secondary metabolite production.

However, in most cases the exact nature of the interactions between sponges and microbes remains a mystery, and to an individual sponge a given microorganism could represent a potential food source, a pathogen, a parasite or a symbiont.

Microbial associates of sponges gained significance as source of bioactive compounds. Remarkable similarity was found between those compounds isolated predominantly from sponges and those found in terrestrial organism of entirely different taxa. It is hypothesized that symbiotic marine microorganism harbored by sponges are the original producers of these bioactive compounds (Proksch et al., 2002; Zhang et al., 2005).

The bacterial association with marine sponges has been well known for a long time and several investigations have explored this association using different approaches. It has been reported that in some sponge species as much as 40% of animal biomass is attributed to bacteria, which exceeds the bacterial population of seawater by 2 orders of magnitude.

Sponges are, however, not only rich in bacteria but also known to harbor fungi irrespective of the nature of sponge-fungi associations. Sponge-derived fungal cultures have repeatedly been shown to be interesting sources of new bioactive secondary metabolites previously unknown from terrestrial strains of the same species. Unusual fungal metabolites include hortein, a new polyketide from the fungus *Hortaea werneckii* isolated from the sponge *Aplysina aerophoba*, new

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A- *Callyspongia diffusa* B- *Sigmadocia carnosa*

C- *Spongia officinalis var. ceylonensis* D- *Hyattella cribriformis*

Figure1. Sponges from the gulf of mannar, Southeast coast of India analyzed in this study.

anthraquinone and betaenone derivatives as well as new ç-pyrones from the fungus *Microsphaeropsis* sp., also obtained from *A. aerophoba*, new spiciferone derivatives from the fungus *Drechslera hawaiiensis* derived from the sponge *Callyspongia aerizusa* (Jadulco et al., 2001) and new xestodecalactones produced by the fungus *Penicillium, montanense*, which was isolated from the sponge *Xestospongia exigua* (Proksch et al., 2002; Bringmann and Lang, 2003).

Bioactive substances from sponge associated microorganisms have shown anticancer, antibacterial, antifungal, antiviral, antiprotozoal, anthelmintic, anti-inflammatory, imunosuppressive, neuro suppressive, and antifouling activities.

In this research, sponge associated bacteria, fungi and their anti microbial potential has been studied.

MATERIALS AND METHODS

Collection of samples

Sponges were collected from the gulf of mannar, Southeast coast of India (Lat 9°5' N; Long 79°5' E).The sponge sample soon after collection was transferred to a sterile polyethylene bag and transported under frozen condition to the laboratory for the isolation of associated microbes. On reaching the laboratory, the

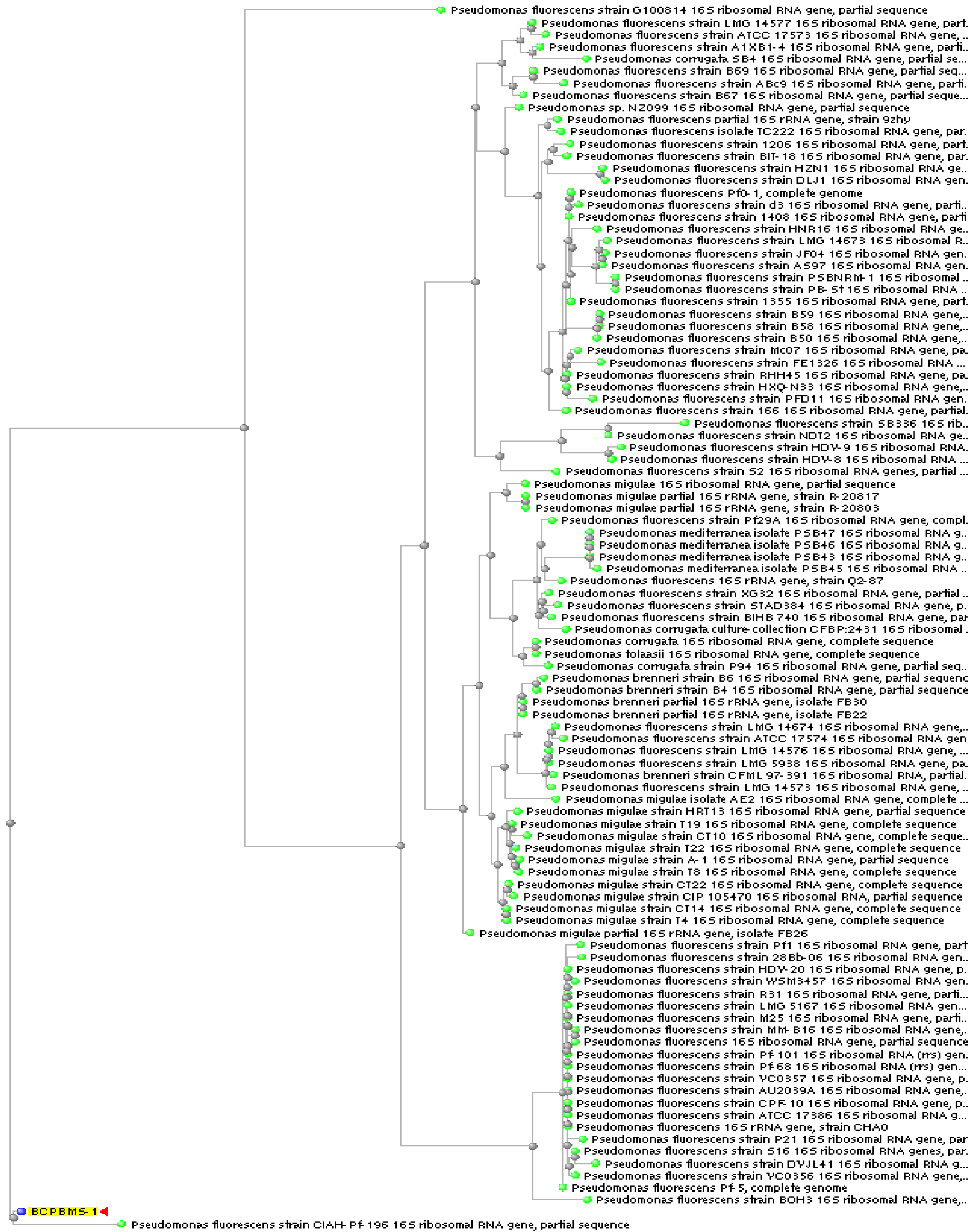

Figure 2. Phylogenetic tree view of *P. fluroscence*- BCPBMS-1.

invertebrate was thawed and cut aseptically into small pieces (2 × 2 cm) using a sterile scalpel. The pieces were freed from adhering particles by vortexing twice for 20 seconds with 2 ml of sterile seawater. The seawater was decanted, which was once again

replaced with sterile seawater with continued vortexing between washings.

Isolation of bacteria associated with marine sponge

The sponge sample soon after collection was transferred to a sterile polyethylene bag and transported at 4°C to the laboratory for the isolation of associated microbes. On reaching the laboratory, the invertebrate was brought to room temperature and cut aseptically into small pieces (2 × 2 cm) using a sterile dissection razor. The pieces were freed from adhering particles by vortexing twice for 20 sec with 2 ml of sterile seawater. The seawater was decanted, which was once again replaced with sterile seawater with continued vortexing between washings.

Finally, sample in sterile seawater was homogenized using sterilized mortar and pestle in a Laminar flow hood. The homogenate was serially diluted up to 10^{-6} dilutions and then spread plated on 50% Nutrient agar plates. The plates were incubated at room temperature for 24 to 48 h.

The media composition is as follows: Peptone, 5.00 g; sodium chloride, 5.00 g; beef extract, 1.50 g; yeast extract, 1.50 g; agar, 15.00 g; 50% of seawater, 1000 ml; pH, 7.0 to 7.4.

Colonies were selected on the basis of varying colony morphology and pure cultures were maintained in the same medium in slants at 4°C for further study.

Isolation of fungi associated with marine sponges

One gram of sponge sample was mixed in 9 and 99 ml sterile water blank, respectively. This suspension was serially diluted up to 10^{-4}. 1 ml of the diluted sample was taken from 10^{-3} and 10^{-4} dilutions and was pour plated with 15 to 20 ml potato dextrose agar (PDA) and incubated at room temperature (28 ± 2°C) for 5 days.

The PDA composition is as follows: Potato infusion, 200 g; dextrose, 20 g; agar, 15 g; 50% of seawater, 1000 ml; pH, 7.0 to 7.2.

To eliminate the bacterial contamination of 8 ml of 1%, streptomycin was added to 1 L of the sterilized medium.

Cultivation of bacterial isolates for screening

The isolated bacteria were sub cultured on nutrient agar plates and incubated at 28 ± 2°C for two days. A loopfull of the bacterial culture was transferred in to Nutrient broth and incubated on a shaker at 30°C for 48 h. At the end of incubation period, broth cultures were used for screening.

Cultivation of fungi isolates for screening

The isolated fungi were sub cultured on Potato dextrose agar plates and incubated at 28 ± 2°C for two days. A loopfull of the fungal culture from the plate was inoculated into 10 ml of potato dextrose broth prepared in sterile 50% aged sea water and incubated on a shaker at 30°C for 2 to 4 days. At the end of incubation period, broth cultures were used for screening.

Screening for antimicrobial activity

Antagonistic assay for bacterial and fungi against bacterial pathogens

Antagonistic assay was done by an agar-well diffusion method under aerobic conditions. Isolated bacterial and fungal strains were tested for the antibacterial activity. Bacterial pathogens such as *Escherichia coli*, *Proteus mirabilus*, *Salmonella typhi*, *Salmonella paratyphi*, *Vibrio cholera, Klebsiella oxytoca*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Lactobacillus vulgaris* and spreaded on Muller Hinton agar plates. Then wells were made and 50 µl culture of each strain was inoculated in to separate well. Antagonistic activity was detected after an incubation of 24 to 48 h at 35°C. The presence of zone of clearance on agar plates was used as an indicator for the antibacterial activity. The strain which showed the maximum zone of clearance was chosen for further study. The presence of zone of clearance on agar plates was used as an indicator of bioactive potential of the strain (Portrait et al., 1999).

Antagonistic assay for bacterial and fungal strains against fungal pathogens

Antagonistic activity of isolated bacterial and fungal strains were tested for their anti fungal activity (Geels and Schipper, 1983) against selected fungal pathogens such as *Alternaria alternata, Botrytis cinerea, Cercospora theae, Fusarium udum, Fusarium xysporum*, *Macrophomina phaseolina* , *Poria hypolateritia, Phomopsis thae* and *Rhizoctonia solani*, Initial screening for *in vitro* antagonistic activity was tested against fungal strains on PDA agar plates. Wells were made and 50 µl culture of each strain was inoculated in to separate well. Antagonistic activity was detected after an incubation of 24 to 48 h at 35°C. The presence of zone of clearance on agar plates was used as an indicator of bioactive nature of the strain.

Identification of bacteria

All associated bacterial strains which were selected based on morphology were identified biochemically. For the most potential strain in addition to biochemical study, 16S Ribosomal ribonucleic acid rRNA partial sequencing also done. Morphological characters were observed under a phase contrast microscope and all the organisms were biochemically identified up to the species level by following bergey's manual of determinative bacteriology (Buchanan et al., 1974).

Identification of bacteria by 16S rRNA partial sequencing

The genomic DNA extracted from the marine sponges associated potent strain was PCR amplified for 16S rRNA genes using the universal bacterial primers Eubac 27F (5' - AGA GTT TGA TCM TGG CTC AG - 3') and 1492R (5' - GGT TAC CTT GTT ACG ACT T-3'). This primer combination amplifies a 1500 bp 16S rDNA fragment (Weisburg et al., 1991).

Amplification reaction was performed in a 0.2 ml optical-grade PCR tube. 50 nanogram of DNA extract was added to a final volume of 50 µl of PCR reaction mixture containing 1.5 mM $MgCl_2$, 1X Reaction buffer (without $MgCl_2$) (Fermentas), 200 µM of each dNTPs (Fermentas), 100 pM of each primer and 1.25U Taq DNA polymerase (Fermentas). PCR was performed in an automated thermal cycler (Lark Research Model L125 +, India) with an initial denaturation at 95°C for 5 min. followed by 30 cycles of 95°C for 30 s (denaturation), 52°C for 45 s (annealing), 72°C for 90 s (extension) and 72°C for 10 min (final extension). Polymerase chain reaction (PCR) product was run on 1% agarose in TAE buffer [40 mM Tris, 20 mM Acetic acid, 1mM EDTA (pH8.0)] to confirm that the right product (1500 bp) was formed.

The PCR product was purified using the QIAGEN PCR purification kit for sequencing and further analysis. The partial 16S rRNA gene sequencing was done using Perkin Elmer Applied biosystems (ABI) and ABI Prism software was used to align the

sequence and compare the sequences retrieved by the queries generated by BLAST of GenBank database. Phylogenetic analysis was performed with the MEGA 4.0 program (molecular evolutionary genetics analysis, version 4.0).

The tree topologies were evaluated by bootstrap analyses based on 1,000 replicates and phylogenetic trees were inferred using the neighbour-joining method and submitted to NCBI GenBank accession number: 1428145 HQ907732.

Identification of fungi

The sponge associated fungi were identified up to species level by referring standard mycological books and manuals (Gilman, 1959, 1998; Ellis, 1971, 1976; Subramanian, 1971; Kohlmeyer and Kohlmeyer, 1979; Ellis and Ellis, 1985). Fungal pathogens were gotten from CAS in botany, Madras University, Chennai.

RESULTS

Density of microbes associated with sponges

Bacterial density

The sponges viz., *C. diffusa, H. cribriformis, S. carnosa* and *S. officininalis Var ceylonensis* were analysed for associated bacterial and fungal population. In *C. diffusa*, bacterial density was in the range of 7.68×10^3 to 1.1×10^7 CFU/g, whereas in the other three species, which are *H. Cribriformis, S. carnosa* and *S. officininalis Var ceylonensis*, 3 the bacterial density was found to be 13×10^3 to 1.6×10^7 CFU/g, 6.77×10^3 to 1.5×10^7 CFU/g and 2.69×10^3 to 1.4×0^7 CFU/g, respectively (Figure1).

Fungal density

In the sponge, *C. diffusa* fungal density was in the range of 1.6×10^2 to 6.1×10^3 CFU/g, whereas 1.5×10^2 to 8.2×10^3 CFU/g of fungal count was observed in *H. Cribriformis*. In *S. carnosa,* it was 1.8×10^2 to 5.0×10^3 CFU/g and in *S. officininalis Var ceylonensis* it varied from 1.9×10^2 to 7.0×10^3 CFU/g and 2×10^2 to 6.2×10^3 CFU/g (Figure1).

Identification of potential bacterial strains strain by 16 s r – DNA sequencing

In this study, phylogenetic tree revealed that *P. fluroscence*- BCPBMS-1 (bioactive compound producing bacteria from marine sponge) was isolated from marine sponge *C. diffusa* (Figure2).

Anti bacterial activity of sponge associated bacteria

In this study, among the *C.diffusa associated* bacteria, maximum (17 mm) anti bacterial activity was observed against *S. paratyphi* by *P. fluorescens* (Table 1). Among the *H. crobriformis* associated bacteria, maximum anti bacterial activity were observed with *C. freundi* (11mm) against *P. mirabilis, N. mucosa* (11 mm)*, S. aureus and L. plantrum* (11mm), *S. paratyphi* with 11 mm zone of clearance, respectively (Table 2).

Among *S. carnosa* associated bacteria, maximum activity was observed with *B. marscencs* against *S. typhi* of about 13 mm (Table 3). *B. subtilis* which was associated with S. *officininalis Var ceylonensis* showed maximum of about 13 mm activity against *P. mirabilis* (Table 4).

Anti fungal activity of sponge associated bacteria

In this study, among the *C. diffusa* associated bacteria, maximum anti fungal activity was observed against *P. hypolateritia* (14mm) by *P. fluorescens* (Table 5). Among the *H. crobriformis* associated bacteria, maximum anti fungal activity was observed with *C. frundi* (15mm) against *F. oxysporum* (Table 6).

Among *S. carnosa* associated bacteria, maximum activity was observed with *B. marscencs* against *F. udum* of about 13 mm (Table 7). *B.subtilis* which was associated with S. *officininalis Var ceylonensis* showed maximum of about 13 mm activity against *P. thae* (Table 8).

Antibacterial activity of sponge associated fungi

In this study, among the *C. diffusa* associated fungi, maximum (18 mm) anti bacterial activity was observed against *K. pnemoniae* by *P. citrinum* (Table 9). Among the *H. crobriformis* associated fungi, maximum anti bacterial activity were observed with *P. citrinum* (11 mm) against *V.cholareae* (Table 10).

Among *S. carnosa* associated bacteria, maximum activity was observed with *A. niger* against *S. typhi* of about 6 mm (Table 11). *Penicilllium* spp. which was associated with S. *officininalis Var ceylonensis* showed maximum of about 13 mm activity against *S. aureus* (Table 12).

Anti fungal activity of sponge associated fungi

In this study, among the *C. diffusa* associated fungi, maximum (11 mm) anti fungal activity was observed against *P. hypolateritia* (14 mm) by *P. citrinum* (Table 13). Among the *H. crobriformis* associated bacteria, maximum anti fungal activity was observed with *P. citrinum* (12 mm) against *R. solani* (Table 14).

Among *S. carnosa* associated bacteria, maximum activity was observed with *A. flavus* against *M. phaseolina* of about 6 mm (Table 15). *A. niger* which was associated with *S. officininalis Var ceylonensis* showed maximum of about 15 mm activity against *M. phaseolina* (Table 16).

Table 1. Antibacterial activity of *C. diffusa* associated bacteria.

Associated bacteria	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***	***A. tumefaciens***
A. faecalis	-	4	1	5	-	3	3	5	1	1
A. hydrophila	8	5	3	-	-	-	5	3	6	2
B. licheniformis	5	5	7	1	4	6	6	3	5	-
B. subtilis	2	5	5	3	4	7	6	7	9	8
P. aeruginosa	12	11	11	7	8	4	4	-	2	12
P.fluroscence	10	11	17	9	12	5	16	11	8	14
P. putida	10	6	6	8	9	-	5	6	6	8

Table 2. Anti bacterial activity of associated bacteria from *H. cribriformis.*

Associated bacteria	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. Typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***	***A. tumefaciens***
B. cereus	-	-	4	5	3	3	3	2	2	6
B. megaterium	5	5	7	3	2	2	1	-	-	-
B. subtilis	4	-	6	6	5	4	-	-	2	5
C. freundii	4	2	2	2	1	11	6	6	-	4
N. mucosa	11	5	-	-	-	4	1	-	1	2
P. putida	10	4	3	3	7	8	5	6	7	9
L. plantarum	2	-	11	5	4	-	-	9	-	-

Table 3. Anti bacterial activity of associated bacteria of *S. carnosa.*

Associated bacteria	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. Typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumonia***	***V. cholerae***	***A. tumefaciens***
B. cereus	10	2	-	-	4	4	5	3	2	1
B. macerans	4	13	11	5	-	11	3	-	3	-
B. megaterium	8	5	3	-	-	-	5	3	6	
B. brivis	5	3	3	2	3	1	1	-	-	-
L. casei	6	3	9	5	4	9	1	1	3	1
S. marcescens	1	2	7	6	4	-	-	-	9	7
P. aeruginosa	11	6	2	-	4	10	3	11	2	8
P. putida	7	2	3	6	6	-	3	10	7	2

DISCUSSION

Marine bacteria have been recognized as an important and untapped resource for novel bioactive compounds. The chemical compounds of marine microorganisms are not well known as terrestrial counterparts. However, in the last decade several bioactive compounds have been isolated from marine bacteria and are new resources for the development of medically useful compounds (Donia and Haman, 2003; Anand et al., 2006). Antibacterial activity among marine bacteria is a well-known phenomenon and has been demonstrated in a number of studies (Isnansetyo and Kamei, 2003; Uzair et al., 2006).

In this study among the associated strains of different sponges *P. fluorescens* and *Penicillium cirtinum* which were isolated from *C. diffusa showed* activity against all

Table 4. Antibacterial activity of *S. officininalis Var ceylonensis* associated bacteria.

Associated bacteria	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***	***A. tumefaciens***
B. brevis	8	5	3	-	-	-	5	3	6	-
B. subtilis	1	12	11	3	3	13	12	-	5	2
B. megaterium	3	3	*4*	*11*	*3*	*12*	*4*	*7*	*3*	-
V. parahaemolyticus	3	3	1	4	-	-	5	3	-	-
E. coli	-	-	-	7	-	6	1	1	-	-

Table 5. Antifungal activity of *C. diffusa* associated bacteria.

Associated bactria	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	***B. cinerea***	***A. alternata***	***R. solani***	***F. udam***	***M. phaseolina***	***P. hypolateritia***	***C. theae***	***P thae***
A. faecalis	11	5	7	9	10	16	6	7	9
A. hydrophila	12	11	8	-	8	9	10	4	5
B. licheniformis	8	8	1	-	-	11	10	9	11
B. subtilis	-	7	9	9	7	-	-	-	-
P. aeruginosa	5	9	12	7	13	3	-	-	-
P. fluroscence	16	11	11	6	9	9	14	6	6
P. putida	14	11	1	-	8	4	7	-	-

Table 6. Anti fungal activity of associated bacteria from *H. Cribriformis.*

Associated bacteria	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	***B. cinerea***	**A. alternata**	***R. solani***	***F. udam***	***M. phaseolina***	***P. hypolateritia***	***C. theae***	***P. thae***
B. cereus	-	2	-	-	-	-	2	-	-
B. megaterium	12	11	7	9	-	11	3	-	8
B. subtilis	14	10	10	-	-	-	7	3	-
C. freundii	15	11	10	7	5	9	-	-	-
N. mucosa	-	-	2	2	-	7	-	-	-
P. putida	-	-	-	7	-	4	3	-	2
L. plantarum	6	-	-	-	-	-	-	-	-
V. cholarea	-	-	8	-	-	-	3	-	-

Table 7. Anti fungal activity of associated bacteria from *S. carnosa.*

Associated bacteria	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	***B. cinerea***	***A. alternata***	***R. solani***	***F. udam***	***M. phaseolina***	***P. hypolateritia***	***C. theae***	***P. thae***
B. cereus	3	4	-	9	-	-	8	-	-
B. macerans	7	-	-	-	13	-	5	-	-
B. megaterium	1	1	6	-	-	-	-	7	7
B. brivis	-	-	-	-	-	-	5	9	6
L. acidophilus	-	-	-	-	-	6	9	-	-
S. marcescens	*2*	*5*	3	-	*1*	-	-	-	-
P. aeruginosa	-	2	-	-	-	-	-	1	-
P. putida	-	3	-	-	-	-	*4*	-	-

Table 8. Antifungal activity of *S. officininalis Var ceylonensis* associated bacteria.

Associated bacteria	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	***B. cinerea***	***A. alternata***	***R. solani***	***F. udam***	***M. phaseolina***	***P. hypolateritia***	***C. theae***	***P. thae***
B. brevis	7	-	4	8	8	1	-	-	3
B. subtilis	11	1	10	2	-	-	7	-	13
B. megaterium	2	-	3	7	9	4	-	-	-
V. parahaemolyticus	-	-	1	-	-	3	-	-	-
E. coli	-	-	-	-	-	-	1	-	-
L. fermentum	12	9	-	-	-	6	-	5	1

Table 9. Antibacterial activity of *C. diffusa* associated Fungi.

Associated fungi	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumonia***	***V. cholerae***	***A. tumefaciens***
A. flavus	1	1	1	-	4	-	-	1	-	-
A. flavipes	4	6	-	-	-	-	-	-	-	-
A. niger	8	-	6	5	4	1	1	3	2	-
P. citrinum	13	2	7	12	5	5	2	18	5	3
Trichoderma sp.	3	1	5	-	-	-	-	-	-	-

Table 10. Anti bacterial activity of associated fungi from *H. cribriformi.*

Associated fungi	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***	***A. tumefaciens***
A. flavus	-	1	-	-	-	-	3	-	-	-
A. fumigatus	-	-	-	-	-	7	-	-	-	1
A. niger	3	4	-	5	4	-	-	-	-	-
A. terreus	5	-	-	-	4	-	-	-	-	-
P. citrinum	10	7	3	5	2	7	1	7	11	1
Trichoderma sp.	-	4	-	-	-	3	-	-	3	2

bacterial and fungal pathogens tested. Hence, they were selected for further study.

In recent years, fluorescent Pseudomonades have drawn attention worldwide because of the production of secondary metabolites such as antibiotics, enzymes and phyto hormones (Isnansetyo and Kamei, 2003). The extract of *Pseudomonas* sp. PB2 associated with a sponge, *S. domuncula*, exhibited anti-angiogenic, hemolytic, antimicrobial, and cytotoxic activities (Thakur et al., 2001).

Marine isolates of *Pseudomonas* spp. are found in diverse ecosystems, including coastal regions, the deep sea, and also in extreme environments like halophilic and thermophilic conditions. Marine *Pseudomonas* spp. were also reported in bacterioplankton in seawater, in association with other marine organisms, and in sea sediment. The production of marine secondary meta-bolites can be viewed in an ecological context (Engel et al., 2002). Thus, the diversity of *Pseudomonas* isolated from a wide range of marine ecosystems suggested that these organisms may produce novel and diverse bioactive substances.

Thakur et al. (2001) isolated two marine *Pseudomonas* sp. (strains PB1 and PB2) from a *S. domuncula* sponge which exhibited anti bacterial activity. Anand et al. (2006) also screened for antibiotic-producing marine bacteria associated with sponges from the coastal waters of southeast India and isolated a bacterium, strain SC11, which was closely related to *Pseudomonas* based on the 16S rDNA sequences. There are only few reports of marine *Pseudomonas* sp. compared to terrestrial species that produce bioactive metabolites (Kaneko et al., 2000).

Table 11. Anti bacterial activity of associated fungi from *S. carnosa*.

Associated fungi	Pathogens tested (zone of clearance in mm)								
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***
A. niger	-	6	-	-	-	-	2	1	-
A. flavus	4	1	-	-	-	-	-	-	-
Fusarium sp.	-	-	2	-	4	-	5	-	-

Table 12. Antibacterial of *S. officininalis Var ceylonensis* associated fungi.

Associated fungi	Pathogens tested (zone of clearance in mm)									
	S. aureus	***S. typhi***	***S. paratyphi***	***K. oxytoca***	***E. coli***	***P. mirabilis***	***L. vulgaris***	***K. pneumoniae***	***V. cholerae***	***A. tumefaciens***
A. fumigatus	-	-	-	4	-	-	3	-	2	-
A. niger	7	-	3	-	-	-	-	-	-	-
A. terreus	10	3	-	5	8	1	1	-	-	-
Penicillium spp.	13	9	5	-	-	7	11	3	7	1
T. viride	-	-	5	-	1	-	-	-	-	-

Table 13. Antifungal activity of *C. diffusa* associated fungi.

Associated fungi	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	***B. cinerea***	**A. alternata**	***R. solani***	***F. udam***	***M. phaseolina***	***P. hypolateritia***	***C. theae***	***P. thae***
A. flavus	9	8	7	-	-	7	6	-	9
A. niger	3	-	2	3	-	8	2	-	1
P. citrinum	4	8	6	8	7	6	11	2	1
Trichoderma sp.	9	8	6	7	-	-	6	-	-

However, some bioactive substances with novel biological activities and mechanisms have been extracted from marine isolates of *Pseudomonas*, and some of these metabolites have antimicrobial properties.

Freiberg et al. (2006) evaluated the *in vivo* efficiency of Moiramide B and some of its synthetic derivatives using a *S. aureus* sepsis model in mice. These pyrrolidinedione derivatives exhibited antibacterial activity with minimum inhibitory concentrations (MICs) of 0.01 to 8, 0.25 to 32, and 16 to 64 µg/ml against *S. aureus* 133, *S. pneumoniae* G9A, and *E. coli,* respectively. When evaluated in a murine model of *S. aureus* sepsis, two of the moiramide B derivatives also showed *in vivo* activity comparable to linezolid, an antibiotic that is used currently. These reports indicate that antibiotics produced by marine isolates of *P. fluroscence* may be potential lead compounds in the search for new classes of antibiotics to treat bacterial infections.

Pseudomonas sp. 1531-E7 isolated from a sponge, *Homophymia* sp., produced quinolones (2-undecyl-4-quinolone, 2-undecen-18-yl-4-quinolone, 2-nonyl-4-quinolone and 2-onyl-4-hydroxyquinoline *N*-oxide) anti-*Plasmodium falsifarum* activity was exhibited by2-undecyl-4-quinolone, 2-undecen-18-yl-4-quinolone, and2-nonyl-4-quinolone. Cytotoxicity to KB cells is noticedfor 2-undecen-18-yl-4-quinolone and 2-nonyl-4- hydroxyquinoline *N*-oxide. In addition, 2-undecyl-4-quinolonev and 2-nonyl-4-hydroxyquinoline *N*-oxide are active against HIV-1 and *S. aureus* (Bultel et al., 1999).

Wratten et al. (1977) isolated the antibiotic-producing *Pseudomonas* sp. 102-3 from a seawater sample from a La Jolla, California tide pool. The bacterium inhibits the growth of *Vibrio anguillarum*, *V. harveyi, S. aureus*, and *C. albicans* and produced three antibacterial compounds namely 4-hydroxybenzaldehyde, 2-*n*-heptyl-4-quinolinol, and 2-*n*-pentyl-4-quinolinol.

Uzair et al. (2006) reported a marine *Pseudomonas* sp. CMG1030 which had antimicrobial activity. This organism was originally identified as *P. aeruginosa*, but the strain identification was revised as *P. stutzeri* CMG1030, which produced a novel antibacterial compound zafrin (Uzair et al., 2008).

Kim et al. (2007) reported that *P. fluroscence* HAK-13, has algal-lytic activity against *Heterosigma akashiwo*

Table 14. Anti fungal activity of associated fungi from *H. cribriformis.*

Associated fungi	Pathogens tested (zone of clearance in mm)								
	F. oxysporum	*B. cinerea*	A. alternata	*R. solani*	*F. udam*	*M. phaseolina*	*P. hypolateritia*	*C. theae*	*P. thae*
Acremonium sp.	-	-	-	-	-	7	2	-	-
A. alternata	5	5	-	-	-	3	2	1	8
A. flavus	4	1	3	2	-	3	1	-	-
A. fumigatus	3	1	6	-	-	1	1	-	1
A. niger	-	-	-	3	2	-	3	2	-
A. terreus	-	-	-	-	-	-	-	-	-
P. citrinum	6	11	3	12	5	7	8	1	8
Trichoderma sp.	10	3	8	-	4	2	-	-	-

Table 16. Antifungal activity of *S. officininalis Var ceylonensis* associated fungi.

Associated fungi	Pathogens tested (zone of clearance in mm)									
	F. oxysporum	*B. cinerea*	A alternata	*R. solani*	*F. udam*	*M. phaseolina*	*P. hypolateritia*	*C. theae*	*P. thae*	*F. oxysporum*
A. fumigatus	2	1	7	8	1	-	7	4	1	-
A. niger	2	7	11	10	9	15	-	-	-	-
A. terreus	-	-	-	1	4	1	9	2	1	-
Penicillium spp.	-	-	9	-	3	-	11	3	1	-
T. viride	2	1	7	-	5	8	11	-	6	-

(Raphidophyceae), *Alexandrium tamarense and Cochlodinium polykrikoides*, but it was inactive against *Gymnodinium catenatum*. The substance responsible for the activity was proteinaceous compound that localizes to the cytoplasmic membrane of the bacterium. Barotolerant marine *Pseudomonas* sp. BT1 was isolated in deep water (4,418 m) in a Japanese ocean trench (Kaneko et al., 2000).

Marine *Pseudomonas* strain I to 2, isolated from estuary water, had anti-*Vibrio* activity (Chythanya et al., 2002).The antibacterial activity was evaluated against the following shrimp pathogenic vibrios like *V. harveyi*, *V. fluvialis*, *V. parahaemolyticus*, *V. damsel* and *V. vulnificus*. The active substance was found to be non-proteinaceous substance that was soluble in chloroform. The chloroform extract from the bacterium was active against *V. harveyi* at the concentration 20 µg/ml, but there was no toxic effect found on shrimp larvae even up to 50 µg/ml. This suggested that the substance can be used to control pathogenic marine *Vibrio*. However, *Pseudomonas* sp. I to 2 was non-pathogenic to shrimp larvae, the bacterium could be used as a biocontrol agent against vibriosis in marine aquaculture.

The fungal kingdom includes many species with unique and unusual biochemical pathways (Keller et al., 2005). The production of secondary metabolites in fungi is a complex process often coupled with morphological development (Calvo et al., 2002). Secondary metabolites often have obscure or unknown functions in organisms but have considerable importance for mankind due to their broad range of useful antibiotic, pharmaceutical as well as toxic activities (Yu and Keller, 2005). The products of these pathways include important pharmaceuticals such as penicillin (Pelaez, 2005), cyclosporin (Bentley et al., 1997) and statins (Demain, 1999; Demain, 2006), as well as potent poisons including aflatoxins (example, aflatoxin B1) and trichothecenes (Keller et al., 2005).

Citrinin (Calvo et al., 2002) was first isolated from *Penicillium citrinum* before world war II; subsequently, it was identified in over a dozen species of *Penicillium* and several species of *Aspergillus* (Bennett and Klich, 2003). Citrinin had also been isolated from *Monascus ruber* and *Monascus purpureus,* that is, industrial species used to produce red pigments (Blanc et al., 1995).

Citrinin is bactericidal against Gram-positive bacteria (Vilar et al., 1999). *Aspergillus* isolates showed an inhibitory activity mainly against *S. aureus,* one *E. coli* isolate and *S. albus*, while *Penicillium* isolates were effective mainly against *B. subtilis*, *S. albus*, *S. aureus* and *S. pyogenes*. Previously, *Penicillium* isolates from dry-cured ham had shown wide antibiotic effects when tested against both bacteria and yeast, similarly they observed a high sensitivity in *E. coli*, *B. subtilis* and *S. aureus* isolates, while *S. marcescens* displayed a weak sensitivity (Huerta et al., 1987). Larrondo and Calvo (1990) observed that P. *oxalicum* was having a broader spectrum of activity against *S. aureus, B. subtilis, B.*

cereus, P. mirabilis and *Candida albicans.*

P. chrysogenum E01-10/3 strain was cultured from a sample of the Mediterranean sponge *Ircinia fasciculate* was shown to be capable for production of polyketides with pharmacologically interesting features (Bringmann and Lang, 2003).

Sponge derived fungi only recently received broader interest in the natural products chemists' community as producers of new and biologically active metabolites (Konig and Wright, 1996; Pietra, 1997; Biabani and Laatsch, 1998). *Penicillium* is a large anamorphic (asexual state) ascomycetous fungal genus with wide-spread occurrence in most of the environments. This genus comprises more than 200 described species and many are common soil inhabitants, as well as food borne contaminants or food ingredients used in the preparation of cheese and sausages (Pitt, 2000 and Frisvad and Samson, 2004).

Penicillium species produce a much diversified array of active secondary metabolites, including antibacterial (Rancic et al., 2006; Lucas et al., 2007), antifungal substances (Nicoletti et al., 2007), immunosuppressants, cholesterol-lowering agents (Kwon et al., 2002), and also potent mycotoxins (Frisvad and Samson, 2004).

Thousands of *Penicillium* isolates have probably been screened in bio prospecting programs since the discovery of penicillin, and new bioactive metabolites continue to be discovered from these fungi nowadays (Larsen et al., 2007; Ge et al., 2008; Takahashi and Lucas, 2008), indicating their current importance as sources of high amounts of novel bioactive molecules to be used by pharmaceutical industry.

P. cirtinum produces a variety of beneficial metabolites act against certain pathogens. It is already known for producing mycotoxin citrinin and cellulose digesting enzymes like cellulase and endoglucanase, as well as xylanase.

Citrinin is produced by some *Penicillium*, *Aspergillus* and *Monascus* species. Pitt (2002) indicated that production of citrinin has been reported from at least 22 *Penicillium* species. Citrinin producing strains include *P. citrinum*, *P. verrucosum* (Frisvad and Thrane, 2002; Pitt and Hocking, 1997; Pitt, 2002), *P. expansum* (Vinas et al., 1993), *A. terreus* (Frisvad and Thrane, 2002), *Monascus ruber* and *M. purpureus* (Blanc et al., 1995; Hajjaj et al., 1999; Xu et al., 1999). El-Kassas and Khairy, (2009) reported the biological control of opportunistic pathogen ic marine fungi *Fusarium solani* by C. *salina.* Diby et al. (2005) concluded that *P. pseudoalcaligenes* MSP-538 which was obtained from the salty soils of the coastal agricultural belt of south coast of India, was an effective biocontrol agent against *X. oryzae* which is the bacterial blight pathogen of rice.

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