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Characterization and Partial Purification of Bioactive Compounds Produced from Marine Microbes

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ABSTRACT

Marine water samples were collected from a coastal region of Pushpavanam, Nagapattinam District, Tamil Nadu, India. The Physico – Chemical parameters such as Nitrogen, Phosphorus, Calcium, Magnesium, Manganese, Zinc, Copper, Iron, Organic carbon were analyzed using standard methods. Marine samples were subjected to serial dilution and the organisms was counted by measuring plate counting techniques. The isolated marine organisms were grown in suitable medium. In this study, Marine organisms were Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus subtilis , Proteus vulgaris, Aspergillus niger, Trichoderma, Streptomyces and Nocardia were identified by cultural, morphological and biochemical characteristics. The identify marine organisms were maintained with suitable broth medium. The molecular weight of the bioactive protein was determined by SDS – PAGE and the bioactive protein produced by marine species was purified using HPLC method. In this study, antibacterial activity was studied using test pathogens were obtained from PG and Research Department of Microbiology , STET Women's College , Mannargudi. The maximum antibacterial and antifungal protein activity was noted in Bacteria, Bacillus subtilis (21.5mm) against Escherichia coli, for fungi, Aspergillus niger (18mm) against Fusarium. For Actinomycetes, Streptomyces (16mm) against Bacillus subtilis. The minimum antibacterial and antifungal protein activity was recorded in Bacteria, Pseudomonas fluorescens (12 mm) against Enterobacter aerogenes, for fungi, Trichoderma (12 mm) against Penicillium, for Actinomycetes, Nocardia (10 mm) against Escherichia coli. Finally, concluded that the isolated marine species such as , Pseudomonas fluorescens, Bacillus subtilis, Aspergillus niger , Trichoderma , Streptomyces and Nocardia were highly recommended to be potential resources of natural antibiotic compounds.

Keywords: *Antibacterial Activity, Sodium Dodecyl Sulfate – Poly Acrylamide Gel Electrophoresis, High Performance Liquid Chromatography, Antibiotic Compounds.*

1. INTRODUCTION

The marine surface environment is a site of intense competition for living space by a wide variety of organisms. Bacteria are generally recognized as primary colonizers of this habitat.[1] In last few years, marine microorganisms emerged as a new field for the discovery of novel biologically active compounds.[2] Marine natural products have an exceedingly bright future in the discovery of life saving drugs . The first antibiotic from marine bacterium was identified and characterized.[3]

Bacteria are generally recognized as primary colonizers of this habitat[1] and are able to rapidly form biofilms over freshly exposed surfaces. The prominence of bacteria during early colonization events and their almost universal presence on marine surfaces, including those of benthic marine invertebrates and algae.[4,5]

Thousands of marine Bacilli are known to contain antibiotic substance and less than 1% has been examined for their pharmaceutical activity. For example, Bacillus silvestris, Bacillus cereus, Bacillus marinus, Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens and Bacillus pumilus are known to produce bioactive substances in the marine environment even if they are specifically antibiotic producers. Bacteria exhibiting antibacterial activities have been isolated from various water and soil samples. In recent years, marine microorganisms have become important in the study of novel microbial products exhibiting antibacterial, antiviral, antitumour as well as anticoagulant and cardio active properties.

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis was performed in vertical gels by standard protocols using Tris – glycine buffer[6]. Duplicate samples of purified antibacterial substance and molecular mass markers were subjected to 15% SDS-PAGE. Molecular weight markers for peptides ranging from 10 to 225 kDa were used as a molecular marker standard. After electrophoresis, the gel was sliced vertically. The first part, consisting of samples of purified antibacterial substance and protein standards, was stained with Coomassie Brilliant blue R-250. The other part of the gel was assayed for direct detection of antibacterial substance activity according to Cherif *method* [7].

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

In the present study was carried out to isolate the marine microbial species from coastal locations of Pushpavanam. The selected isolates were subjected to screen the production of bioactive protein antibacterial compounds against selected pathogens.

2. MATERIALS AND METHODS

Sample Collection

Marine water samples were collected from a coastal region of Pushpavanam, Nagapattinam District, Tamil Nadu, and India. The water samples were collected in sterile container and transferred into laboratory by aseptic condition.

Physico – Chemical Parameter Analysis

Physico - chemical parameters like Nitrogen, Phosphorus, Calcium, Manganese, Magnesium, Zinc, Copper, Iron, Organic Carbon were analyzed in water sample using standard methods[8]

Enumeration of Total viable count

Total viable count (TVC) of aquatic samples were calculated by following pour plate technique. Each sample was serially dilluted with dilutions ranging from 10^{-1} - 10^{-9} and 1ml of each dilution were poured on the nutrient agar plates. Plates were incubated at 37ºC for 48 hours in an inverted position and the colonies were counted. The count were expressed as the number of colony forming units in 1 ml of the original sample. The colony count below 30 is indicated as TFTC (Too few to count) and above 300 as TNTC (Too number to count)[9] .

Isolation of Marine Bacteria

The samples (0.5ml) were triturated , suspended with sterile distilled water and spreaded on the entire surface of 1/10 Complex Marine Growth Medium. After incubation at 37ºC for 2 days, all colonies were observed.[10]

Isolation of Marine Fungi

The samples were diluted at 10 and 100 fold with autoclaved filtered seawater (0.22µm). Aliquots of 200 ml from each water sample were spreaded onto Sabouraud Dextrose Agar Medium. The antibiotic streptomycin was added to each agar plate to inhibit the growth of bacteria. The plates were incubated at ^{25} ºC for 7 days. After incubation, the fungal colonies were observed from further isolation and purification11

Isolation of Marine Actinomycetes

Starch casein agar medium was prepared and sterilized at 121ºC at 15 min and supplemented with streptomycin to prevent the fungal and bacterial growth respectively. The collected water samples were diluted up to 10^{-6} and 0.1 ml of the diluted samples were spreaded over the agar plates and triplicates were maintained. The inoculated plates were incubated at 28 ± 28ºC for seven to ten days. After incubation, the actinomyces colonies were observed, purified and maintained in SCA medium for further investigation.[12]

Preparation of bacterial supernantant

100 ml of Luria – Bertani broth was prepared (Luria – Bertani broth – 15 g, Distilled water – 100 ml). The marine bacteria were cultured in 100 ml of Luria - Bertani broth in 500ml of conical flask for the production[13].

Preparation of fungal supernantant

100 ml of Sabouraud Dextrose broth was prepared (Peptone – 0.05 g, Dextrose - 0.10 g, Distilled water – 100 ml)**.** The marine fungal were cultured in 100 ml of Sabouard Dextrose broth in 500ml of conical flask for the production.14

Preparation of Actinomycetes supernantant

100 ml of Starch Casein broth was prepared (Starch Soluble – 10 g, Casein – 5 g, Distilled water – 100 ml). The marine actinomycetes were cultured in 100 ml of Starch Casein broth in 500ml of conical flask for the production.15

Characterization of bioactive protein band by native gel electrophoresis

Glass plates, spacers and comb were cleaned thoroughly and dried. Glass plates were sealed using Vaseline and assembled using clips. 12% separating gel was prepared and poured inside a chamber between the glass plates without any air bubbles and allowed to polymerize for 30 – 60 minutes. After polymerization, 3% stacking gel was prepared and poured over the resolving gel. Comb was inserted between the glass plate and the gel was allowed for polymerization. After polymerization of stacking gel, comb was removed gently without destroying the shape of the wells. The spacer at the bottom of the glass plate was removed and the plate was fixed in the electrophoretic apparatus. Running buffer was poured into the electrophoretic tank without any air bubbles. The sample to be loaded was prepared by mixing sample and loading dye in 1: 2 concentrations. Marker was also prepared in the same way. It was then denatured for 3 minutes and loaded in the wells. Marker was added in the first well and power supply was on. Once the bromophenol blue dye was reaches 1cm above the bottom of the gel, current was put off. The gel was then carefully removed for staining .[16]

Antibacterial protein purification by High Performance Liquid Chromatography

High – Performance Liquid Chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir, mobile phase, a pump, an injector, a separationcolumn and a detector compounds are separated by injecting a plug of the sample mixture onto the column.[17]

Antibacterial activity of bioactive compounds of marine bacteria

Marine bacteria were screened for antibacterial activity , using terrestrial microbe including *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes* and *Streptococcus mutans* were obtained from PG and Research Department of Microbiology , STET Women□s College, Sundarakkottai . Antibacterial activity was assayed in duplicate using a standard paper disc assay[18]. The dried crude extracts were dissolved in EtOAc to a concentration of 100 mg ml^{-1}. The samples (20µl) were used to saturate the antibacterial assay paper disks (6mm) with a period of drying between each application. The disks were placed onto the agar surface containing the test microorganisms and incubated at 37 °C for 24 hours after a diffusion process for 10 hours at 8℃ . The diameters of any inhibition zones formed around the paper discs were measured.

Antifungal activity of bioactive compounds of marine fungi

Antifungal activity of fungal extracts was tested by using paper disc diffusion method14. 10µl of crude extract was measured and the stock solution was added into sterile filter paper disc (5mm in diameter) and allowed to dry. Final concentration of crude extract is 100 µg / disc. The crude extract impregnated discs were placed over sabouraud dextrose agar plates inoculated at test organisms were *Aspergillus niger , Fusarium , Trichoderma* and *Penicillium* were obtained from PG and Research Department of Microbiology, STET Women's College, Sundarakkottai. All the plates were incubated at 37℃ for 24 hours. The zone of inhibition was measured and expressed in diameter.

Antibacterial activity of bioactive compounds of marine actinomycetes

The antibacterial activity was studied preliminarily by well diffusion method [19] against the pathogenic bacteria and fungi. The test pathogenic organisms were *Bacillus subtilis, Staphylococcus aureus , Escherichia coli , Pseudomonas aeruginosa , Aspergillus niger* were obtained from PG and Research Department of Microbiology , STET Women's College, Sundarakkottai. The selected isolates were inoculated on Starch Casein Broth, and shaken at 28 ℃ for seven to ten days. After incubation, the substances were filtered through the whatmann filter paper. The filtrate was transferred aseptically into the conical flask and stored at 4℃ for further assay. An equal volume of five different solvents (alcohol, chloroform, distilled water, ethyl acetate and methanol) were separately added to the cell free culture filtrates and shaken for 2 hours and the antibacterial compounds were extracted by the diffusion method[20]. After 24 – 72 hours, the zone of inhibition was measured and expressed in diameter.

3. RESULT AND DISCUSSION

In the present study, marine water samples were collected from Pushpavanam coastal areas of Nagapattinam District, TamilNadu , India.

The physico - chemical parameters of the Pushpavanam water samples were analyzed using the standard methods. The chemical factors of water sample such as Nitrogen (kg) 4.7, Phosphorus (ppm) 4.6 , Calcium (ppm) 4.4 , Carbon (%) 4.8 , Zinc (ppm) 5.4 , Copper (ppm) 5.2 , Iron (ppm) 5.5 and Manganese (ppm) 5.6 were recorded. The physico - chemical parameters of the marine water samples collected from coastal area. The parameters such as P^H , temperature, electrical conductivity, dissolved solids, salinity, zinc, copper, iron, nickel, cobalt, total mercury, total cyanide, total lead, selenium, total silver, nitrate, nitrite, ammonia, inorganic sulphide and sulphate were analyzed using the standard methods[8].

Enumeration of Total Viable Count

The samples were subjected to serial dilution and the bacteria, fungi, and actinomycetes count was made in the plate counting techniques. The bacteria, fungi, and actinomycetes loads, obtained were enumerated from coastal area (Pushpavanam). Some colonies were counted particularly in the dilution rate of 10^{-4} - 10^{-6}. The number of bacteria in Pushpavanam water 130 x 10^{-4} CFU / ml . The total fungal colonies of Pushpavanam water was 100 x 10^{-5} CFU /ml and the number of colonies in actinomycetes for Pushpavanam water was 125 x 10^{-6} CFU/ml recorded.

The direct viable count, a microscopic method was used for enumeration of viable bacteria. The modified direct viable count will be useful in growth and survival studies of bacterial cells in marine water samples [21].

Isolation and Identification of Marine Microorganisms

Marine Bacteria

The marine bacterial species were identified by gram staining, motility and biochemical tests. The isolated marine bacterial species such as *Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus subtilis, Proteus vulgaris.* All the isolates showed positive and negative results for gram staining and motility tests (Table - 1) .

The samples (approximately 4g each) were collected using some clean, dry and sterile polythene bag along with sterile spatula. All the samples were transferred to lab under sterile conditions, 1g of each samples was added to 5ml of nutrient broth and incubated at 35° C for 24 hours. After the incubation period, 0.1 ml of the supernatant of each tube containing suspension of soil and culture media were inoculated in nutrient agar plates by streaking at 30°C for 24 hours. After that, the plates were examined and the suspected colonies were stained by Gram staining method. The Gram – positive, rod – shaped, spore forming *bacilli* were selected for additional identification tests. Subsequent identification tests including susceptibility test to Penicillin, Citrate Hydrolysis, Motility, Voges – Proskauer, Indole Production, Catalase, Nitrate Reduction and production of H_2S were performed[22].

TABLE 1
Biochemical Characteristics of Selected Marine Isolates

S.No	Biochemical Charactersitics	*P. aeruginosa*	*P. fluorescens*	*B. subtilis*	*Proteus vulgaris*
1	Indole	-	-	-	-
2	Methyl Red test	+	+	+	+
3	Voges proskauer test	+	+	+	+
4	Citrate utilization test	-	+	+	+
5	Catalase test	+	+	+	+
6	Oxidase test	+	-	-	-
7	Urease test	-	-	-	-

(+) Indicates positive reaction

(-) Indicates negative reaction

Marine Fungi

The marine fungal species were isolated from Pushpavanam coastal area. The marine fungi were isolated using Sabouraud Dextrose Agar Medium. The isolated fungal colonies were identified by cultural, morphological characteristics and the results were showed in Table - 2.

The marine fungal species were identified by Lactophenol Cotton Blue Staining. The isolated marine fungal species such as *Aspergillus niger and Trichoderma* .

Micromorphology was studied using Lactophenol cotton blue staining. All isolates were determined at genus level, based on mycelia morphology[23]. The effects of salt concentration on the growth of isolated fungi were determined[24].

TABLE – 2
Morphological characteristics of Selected Marine Isolates

S. No:	Organisms	Morphological Characteristics		
		Staining / Lactophenol Cotton Blue	Motility	Shape
1	*Aspergillus niger*	+	-	Spherical
2	*Trichoderma*	+	-	Conidia

(+) indicates positive reaction

(-) indicates negative reaction

Marine Actinomycetes

The marine actinomycetes species were identified by gram staining, motility and biochemical tests. The isolated marine actinomycetes species such as *Streptomyces and Nocardia*. All the isolates showed positive and negative results for gram staining and motility tests (Table - 3).

Isolation and identification of actinomycetes were performed by dilution plate technique using Glycerol-Yeast Extract Agar[25]. One gram of dried soil was taken in 9 ml of distilled water, agitated vigorously and preheated at 50^{o}C for 0.5 hours. Different aqueous dilutions, 10^{-3}, 10^{-5} and 10^{-7} of the suspension were applied onto plates and 20 ml of melted medium at around 50^{o} C was added to it. After gently rotating, the plates were incubated at 27^{o}C for 7 to14 days. Selected colonies (rough, chalky) of actinomycetes were transferred from mixed culture of the plates onto respective agar plates and incubated at 27^{0} C for 7 days. Plates containing pure cultures were stored at 40℃ until further examinations.

TABLE 3
Biochemical Characteristics of Selected Marine Isolates

S.No	Biochemical Charactersitics	*Streptomyces*	*Nocardia*
1	Indole	+	+
2	Methyl Red test	+	-
3	Voges proskauer test	+	+
4	Citrate utilization test	-	-
5	Catalase test	+	+
6	Oxidase test	+	-
7	Urease test	-	+

(+) Indicates positive reaction

(-) Indicates negative reaction

Preparation of Bacterial, Fungal and Actinomycetes Cultures

The identification of marine bacteria, fungi and actinomycetes species such as Pseudomonas *aeruginosa, Pseudomonas fluorescens, Bacillus subtilis, Proteus vulgaris, Aspergillus niger , Trichoderma , Streptomyces* and *Nocardia* were cultured in the each 100 ml of Luria Bertani broth for bacteria, Sabouraud Dextrose broth for fungi and Starch casein broth for actinomycetes in each 100 ml of conical flask for the production.

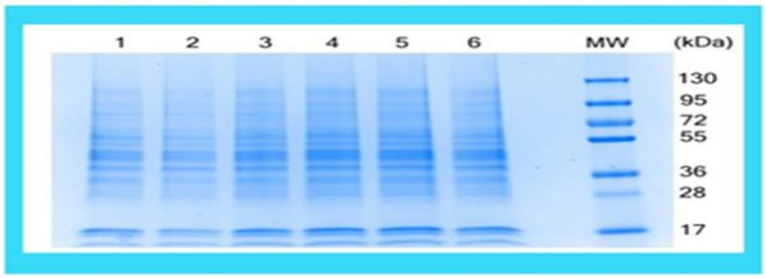
Characterization of bioactive protein by native gel electrophoresis

Protein was determined by SDS – PAGE which indicated, the molecular mass of protein was approximately 130 kDa when determined by size exclusion chromatography. The highly purified antibacterial protein gives an apparent molecular mass of 95 kDa for *Pseudomonas aeruginosa*, 72 kDa for *Pseudomonas fluorescens*, 55 kDa for *Bacillus subtilis*, 36 kDa for *Proteus vulgaris*, 28 kDa for *Aspergillus niger*, and 17 kDa for *Streptomyces* isolates. The results were showed in Plate 1.

Protein homogeneity was determined by SDS – PAGE which indicated a purity superior to 95 % and an apparent mass of 87 kDa. The molecular mass of protein was approximately 280 kDa when determined by size exclusion chromatography. This suggest that in aqueous solution, the antimicrobial protein may form trimers . Such a characteristic is similar to the oligomeric structure of the antibacterial protein purified form the marine bacterium[26].

Plate 1

Characterization of bioactive compounds by SDS - PAGE

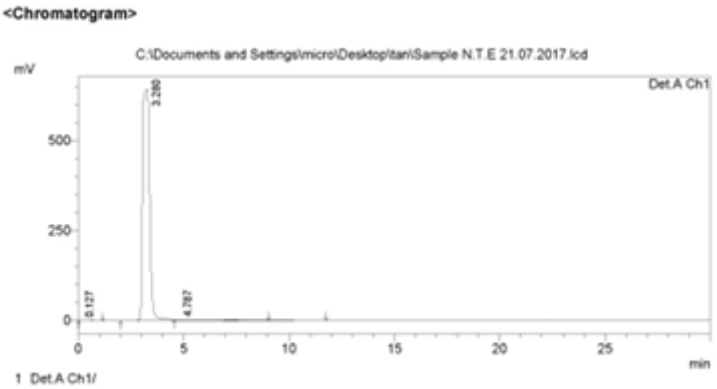


1. *Pseudomonas aeruginosa*
2. *Pseudomonas fluorescens*
3. *Bacillus subtilis*
4. *Proteus vulgaris*
5. *Aspergillus niger*
6. *Streptomyces*

Antibacterial protein purification by High Performance Liquid Chromatography

The purified antibacterial compound displayed a single protein peak at 500 nm in the HPLC (High - Performance Liquid Chromatography) method. The result were recorded two characteristic absorption bands at 4,787 and 3,280 cm-1.

The purified antimicrobial compound displayed a single protein peak at 225 nm in the UV spectrum, and the infrared spectroscopy showed two characteristic absorption bands at 1,650 and 1,075 cm $^{-1}$, which correspond to peptide bonds and polysaccharide residues, respectively[27]. A wide band indicative of the presence of polar groups in the molecule appeared at 3,350 cm^{-1}.



Antibacterial Protein activity of bioactive compounds from marine bacteria

Antibacterial Protein activity of zone of inhibition were tested for their activity against the terrestrial pathogens namely *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes* and *Streptococcus mutans*. The crude extract were tested for antibacterial activity by well diffusion method. Antibacterial Protein activity of bacterial species namely *Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus subtilis* and *Proteus vulgaris* against terrestrial pathogens were observed (Plate - 2).

Among the above results, the maximum antibacterial activity was noted in *Bacillus subtilis* (21.5mm) against *Escherichia coli.* The minimum antibacterial activity was recorded in Pseudomonas *fluorescens* (12 mm) against *Enterobacter aerogenes*. The results were showed in Table - 4.

The antimicrobial efficacy of the isolates was tested by using different solvent extracts and ethyl acetate extract produced the maximum inhibitory zones against all the pathogens tested followed by chloroform and methanol extracts. Ethyl acetate extract of the strain *Streptomyces hygroscopicus* BDUS 49 showed maximum activity against *S. aureus* (35mm) followed by *Saccharomyces cerevisiae* (28mm) *B. subtilis* (25mm) *A. niger*(23mm) *E.coli* (20mm). Similary, various solvent were used for the extraction of antibiotics from actinomycetes by many workers using ethyl acetate and methanol[28,29,30].

TABLE 4

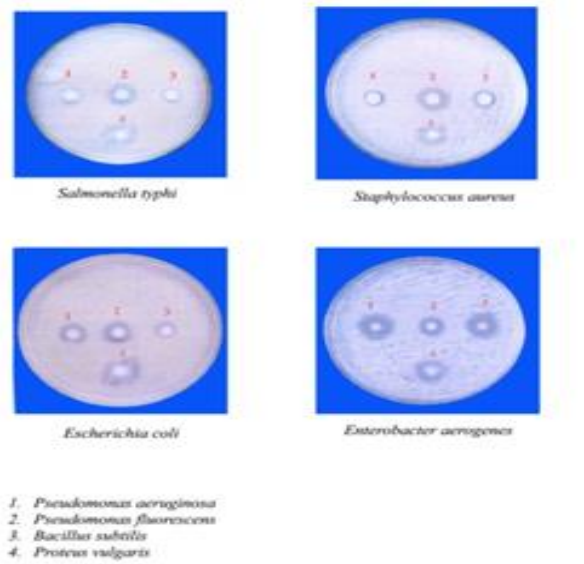
Antibacterial Protein activity of isolated Marine Bacterial Isolates

S.No	Bacterial Strains	Zone of inhibition (mm in diameter)			
		Pseudomonas aeruginosa	***Pseudomonas fluorescens***	***Bacillus subtilis***	***Proteus vulgaris***
1	*Salmonella typhi*	16 ±4.32	13.75±2.21	19±2.94	14.5±5.08
2	*Staphylococcus aureus*	18.5±3.51	12.25±2.21	19±2.58	12.25±2.21
3	*Escherichia coli*	16±1.82	13±2.16	21.5±3.87	14.25±6.02
4	*Enterobacter aerogenes*	16.5±3.10	12±1.82	18.5±4.04	16.5±1.29

Values are expressed as Mean ± Standard Deviation

Plate 2

Antibacterial activity of Marine Bacterial Isolates against terrestrial pathogens



Antifungal Protein activity of bioactive compounds from marine fungi

Antifungal Protein activity of zone of inhibition were tested for their activity against the terrestrial pathogens namely *Fusarium, Penicillum, Cephalosporium, Alternaria.* The crude extract were tested for antifungal activity by well diffusion method. Antifungal Protein activity of bacterial species namely *Aspergillus niger and Trichoderma* against terrestrial pathogens were observed (Plate - 3).

Among the above results, the maximum antifungal activity was noted in *Aspergillus niger* (18mm) against *Fusarium*. The minimum antifungal activity was recorded in *Trichoderma* (12 mm) against *Penicillium* . The results were showed in Table - 5.

Antifungal Protein activity of fungal extracts was tested by using paper disc diffusion method14. 10 g of crude extract was measured and the stock solution was added into sterile filter paper disc (5mm in diameter) and allowed todry. Final concentration of crude extract is 100 µg / disc. The crude extract impregnated discs were placed over Sabouraud dextrose agar plates inoculated at test organisms were *Aspergillus niger, Fusarium , Trichoderma* and *Penicillium* were obtained from PG and Research Department of Microbiology, STET Women' s College, Sundarakkottai. All the plates were incubated at 37℃ for 24 hours. The zone of inhibition was measured and expressed in diameter.

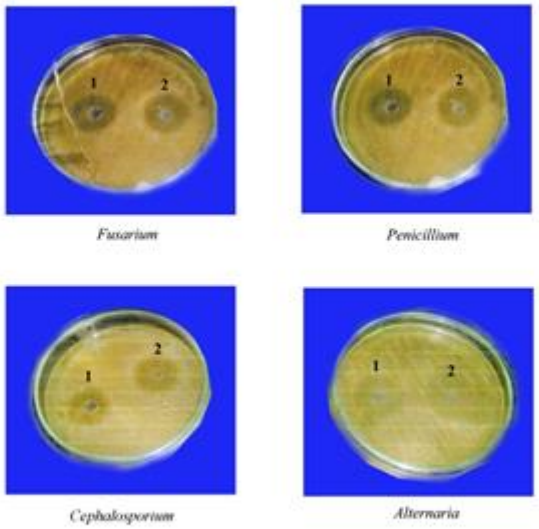
TABLE 5
Antifungal Protein activity of isolated Marine Fungal Isolates

S.NO	Fungal Strains	Zone of inhibition (mm in diameter)	
		Aspergillus niger	***Trichoderma***
1	*Fusarium*	18 ±1.72	13 ± 2.21
2	*Penicillium*	17 ±1.31	12±2.21
3	*Cephalosporium*	16±0.173	14±2.16
4	*Alternaria*	15±0.84	15±1.82

Values are expressed as Mean ± Standard Deviation

Plate 3

Antifungal activity of Marine Fungal Isolates against terrestrial pathogens



1. *Aspergillus niger*
2. *Trichoderma*

Antibacterial Protein activity of bioactive compounds from marine actinomycetes

Antibacterial Protein activity of zone of inhibition were tested for their activity against the terrestrial pathogens namely *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus.*. The crude extract were tested for antifungal activity by well diffusion method. Antibacterial Protein activity of bacterial species namely *Streptomyces and Nocardia* against terrestrial pathogens were observed (Plate - 4).

Among the above results, the maximum antibacterial activity was noted in *Streptomyces* (16mm) against *Bacillus subtilis*. The minimum antibacterial activity was recorded in Nocardia (10 mm) against *Escherichia coli*. The results were showed in Table - 6.

The antibacterial protein activity was studied preliminarily by well diffusion method[19]against the pathogenic bacteria and fungi. The test pathogenic organisms were *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger* were obtained from PG and Research Department of Microbiology , STET Women s College, Sundarakkottai. The selected isolates were inoculated on Starch Casein Broth, and shaken at 28 ℃ for seven to ten days . After incubation, the substances were filtered through the whatmann filter paper. The filtrate was transferred aseptically into the conical flask and stored at 4℃ for further assay. An equal volume of five different solvents (alcohol, chloroform, distilled water, ethyl acetate and methanol) were separately added to the cell free culture filtrates and shaken for 2 hours and the antibacterial compounds were extracted by the diffusion method[20].

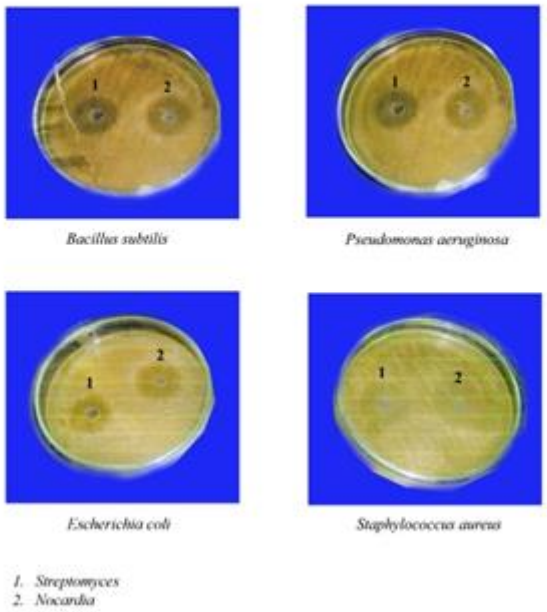
TABLE 6
Antibacterial Protein activity of isolated Marine Actinomycetes Isolates

S.No	Bacterial Strains	Zone of inhibition (mm in diameter)	
		Streptomyces	***Nocardia***
1	*Bacillus subtilis*	16 ±0.8	13±0.29
2	*Pseudomonas aeruginosa*	15 ±0.81	15±0.61
3	*Escherichia coli*	14 ±0.93	10±2.16
4	*Staphylococcus aureus*	13 ±0.81	14±1.23

Values are expressed as Mean ± Standard Deviation

Plate 4

Antibacterial activity of Marine Actinomycetes Isolates against terrestrial pathogens



1. *Streptomyces*
2. *Nocardia*

4. CONCLUSION

Screening the marine species from the coastal regions were taken upto evaluate their antimicrobial potential against *Salmonella typhi, Staphylococcus aureus , Escherichia coli, Enterobacter aerogenes, Streptococcus mutans, Fusarium, Penicillium, Cephalosporium, Alternaria, Bacillus subtilis , Staphylococcus aureus , Escherichia coli , Pseudomonas aeruginosa , Aspergillus niger*. Marine species such as Pseudomonas *aeruginosa, Pseudomonas fluorescens, Bacillus subtilis, Proteus vulgaris, Aspergillus niger , Trichoderma , Streptomyces* and *Nocardia* isolated from coastal region of Pushpavanam water sample showed antibacterial and antifungal activity against terrestrial pathogens. Finally, it was concluded that the isolated marine species such as Pseudomonas *aeruginosa, Pseudomonas fluorescens, Bacillus subtilis, Proteus vulgaris, Aspergillus niger , Trichoderma , Streptomyces* and *Nocardia* were highly recommended for antibiotic production in the pharmaceutical field.

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