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Antibacterial Activity of Seagrass Species Against Biofilm Forming Bacteria

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Abstract: The present study was carried out on antimicrobial properties of seagrass species against biofilm forming bacteria's from boat hull during the period April 2008 to March 2009. Seagrass species have a very potential groups were producing several secondary metabolites. The bioactive potential of two different seagrass species viz., *Cymodocea serrulata* and *Syringodium isoetifolium* occurring commonly along the Tuticorin coastal area were selected and preliminary effort has been made against the marine biofilm forming bacteria's *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus vulgaris*, *P. mirabilis*, *E. coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococus aureus* and *Vibrio paraheamolyticus*, which also the human pathogens. The seagrasses of *C. serrulata* and *S. isoetifolium* were extracted with four different solvents such as ethanol, methanol, acetone and dichloroethane. Ethanol and methanol extracts of *S. isoetifolium* was inhibited the biofilm forming bacteria such as *E. coli* (14 mm), *P. aeruginosa* (8 mm) and *V. paraheamolyticus* (7 mm) and it showing Minimum activity against *S. aureus* (2 mm). The crude extract of ethanol and methanol of *C. serrulata* was inhibited the growth of all the 9 species of the biofilm forming microbes. The results of present study were concluded that seagrasses have potential bioactivity against marine biofilm forming microorganisms.

Key words: Biofilm, Biofouling, *Cymodocea serrulata*, *Syringodium isoetifolium*

INTRODUCTION

Fouling is major technical as well as economic problem in submerged ocean. Biofouling is refers the undesirable accumulation of living organisms on hard surfaces. Commonly fouling occurs in two types of organisms such as microfoulers (bacteria, algae and protozoa) and macrofoulers (barnacles, bryozoans and tube worms). World wide over 400 marine organisms are causing fouling problems. The microfouling organisms are commonly known as biofilm which occur every where in natural and industrial environments where the surfaces are exposed to water (Costerton, 2007). Biofoulers accumulate on the ship hulls to increase the drag and surface corrosion and thus severely causing the caring capacity and increase the fuel consumption up to 40% (Champ, 2000; Chambers *et al*., 2006). To prevent and controlling marine fouling mechanism the US government spent around $ 6.5 billion per year (Bhadury and Wright, 2004).

Biofouling control is world wide problem in marine system. TBT (Tributyrain), a biocide used in antifouling application (Paints), is considered as the most successful antifouling

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substances. It is also most toxic compounds ever deliberately introduced into the marine environment. The TBT is the endocrine disrupter in marine mollusk and cause imposex (super imposition to male sexual characters on female) in gastropod mollusk. The TBT is toxic to many untargeted species such as shell fish and variety of marine species also humans (Alzieu, 2000; Konstantinu and Albanis, 2004). The International Marine Organization (IMO), considering the threat to the marine environment, proposed phasing out of TBT based antifouling by 2003 followed by a complete ban by 2008. An efficient alternative on TBT is not available currently, therefore nontoxic alternates are urgently needed the eco-friendly compounds.

Many researchers have been carried out the natural product with antifouling properties in algae, corals, ascidians and bacteria (Zobell and Allen, 1933; Stockers, 1980; Clare *et al*., 1992; Clark, 1996; Dworjanyn *et al*., 2006; Qi *et al*., 2008). Seagrass have proved to be an excellent source of bioactive compounds. Todd *et al*. (1993), who isolated B-(Sulphoxy) cinnamic acid from the seagrass *Zostera marina* (eelgrass) and found to prevent attachment of marine bacteria and barnacles to artificial surfaces at non-toxic concentrations. Hence the present investigation has been made explore the bioactive potential of seagrasses; two different species viz., *Cymodocea serrulata* and *Syringodium isoetifolium* occurring commonly along the Tuticorin coastal area were selected and tested for their ability of antifouling properties.

MATERIALS AND METHODS

Isolation of Biofilm Bacteria from Boat Hull

The present study monthly samples were collected from the study area during the period of April 2008 to March 2009. The bottom of the boat was gently swabbed with a sterile cotton swab, placed in tubes containing 10 mL sterile water. Then they were inoculated in specific media for the isolation of microbes. The biofilm bacterial strains used in the antibacterial assay were isolated by the pour plate technique (Whal, 1995).

Enumeration of Total Viable Count (TVC)

Zobell Marine Agar (Himedia, Mumbai) was used for the enumeration Total Viable Count (TVC). The 0.1 mL of the samples was inoculated on the medium and it was spread uniformly with sterile glass spreaders. It was left at room temperature for about 30 min till the sample is completely absorbed by the medium. The dishes were inverted and stacked in lots. It was wrapped in paper and incubated at room temperature (27°C) for 48 h. The colonies were counted and expressed as cfu mL^{-1}. For isolation, the suspected colonies were repeatedly streaked on nutrient agar medium and then the pure cultures were maintained in nutrient broth with added supplement salt and stored in a refrigerator at 4°C.

Preparation of Seagrass Extracts

Organic solvents like diethyl ether, acetone and methanol were used to prepare different extracts. All these solvents used were of analytical grade.

Dry Extraction Method

Fresh samples of seagrasses *C. serrulata* and *S. isoetifolium* were collected from the Tuticorin coast (Lat 8°45'N; Long 78°10'E). The present study following method of Rao (1995) was used for dry extraction. The dried seagrass samples were ground to coarse powder and packed in Soxhlet apparatus and extracted successively with diethyl ether,

acetone and methanol for 36 to 48 h at a room temperature of 50-55°C. The extracts were concentrated and dried under reduced pressure in a rotary evaporator and kept in deep freezer until its utilization in antibacterial assay.

Antibacterial Assay

Antibacterial activity of the sea grasses extracts were assessed using the standard agar diffusion method with 6 mm diameter whatman No.1 filter paper discs (Becerro *et al*., 1994). Zobell marine agar was used for the antimicrobial test. Before the antibacterial assay, the biofilm forming bacteria (*P. aeruginosa*, *B. cereus*, *P. vulgaris*, *P. mirabilis*, *E. coli*, *L. monocytogenes*, *S. enteritidis*, *S. aureus* and *V. paraheamolyticus*) were inoculated into the Zobell marine agar plates and incubated at 27°C for 24 h. The paper disc of 6 mm diameter soaked in 6 µL of had solidified, standard antibiotic disc used for control. Inhibition of zone was measured after 24-48 h of incubation.

RESULTS

Total Viable Count

The incidence of total bacterial population was increase during every month intervals on surface of boat hull. The count varied between 12×10^6 and 45×10^6 cfu mL^{-1}.

Identification of Biofilm Microorganisms

The biofilm forming bacteria's (*P. aeruginosa*, *B. cereus*, *P. vulgaris*, *P. mirabilis*, *E. coli*, *L. monocytogenes*, *S. enteritidis*, *S. aureus* and *V. paraheamolyticus*) were isolated from the boat hull and identified using standard Berghuis manual.

Antimicrobial Activity

Syringodium Isoetifolium

The ethanol extract was showed the maximum activity against *E. coli* (14 mm), *P. aeruginosa* (8 mm) and *V. paraheamolyticus* (7 mm) and it showed the minimum activity against *S. aureus* (2 mm), *P. vulgaris* and *P. mirabilis* (3 mm).

The methanol extract of *S. isoetifolium* was showed a maximum activity against *E. coli* (9 mm) and minimum activity against *S. aureus* (1 mm) and *P. vulgaris* (1 mm) (Table 1). The acetone extracts of *S. isoetifolium* showed a maximum activity against pathogen *E. coli* (7 mm) and *P. aeruginosa* (7 mm) and it was showing a minimum activity against the *B. cereus* (2 mm) and *V. paraheamolyticus* (4 mm) and also observed that it showed no activity against *P. vulgaris* and *L. monocytogenes*.

Table 1: Antibacterial activity Syringodium isoetifolium against biofilm forming bacteria

	Solvent used (Zone of inhibition mm in diameter)			
Pathogens	Ethanol	Methanol	Acetone	Dichloroethane
Pseudomonas aeruginosa	8	6	7	3
Bacillus cereus	4	2	2	-
Proteus vulgaris	3	1	-	-
Proteus mirabilis	3	T	1	-
E. coli	14	9	7	5
Listeria monocytogenes	T	T	-	-
Salmonella enteritidis	4	2	T	-
Staphylococus aureus	2	1	T	-
Vibrio paraheamolyticus	7	6	4	1

T: Trace

Table 2: Antibacterial activity against Cymodocea serrulata biofilm forming bacteria

	Solvent used (Zone of inhibition mm in diameter)			
Pathogens	Ethanol	Methano	Acetone	Dichloroethane
Pseudomonas aeruginosa	12	8	6	3
Bacillus cereus	10	7	5	2
Proteus vulgaris	11	9	7	3
Proteus mirabilis	13	10	6	2
E. coli	16	14	8	4
Listeria monocytogenes	6	3	1	T
Salmonella enteritidis	9	5	2	T
Staphylococus aureus	7	2	T	-
Vibrio paraheamolyticus	8	3	5	1

T: Trace

Dichloroethane extracts of *S. isoetifolium* was showed the maximum activity against *E. coli* and it showed minimum activity against *V. paraheamolyticus* (1 mm) and *P. aeruginosa* (3 mm) where as it showed no activity against *B. cereus*, *Proteus vulgaris*, *P. mirabilis*, *L. monocytogenes*, *S. enteritidis* and *S. aureus.*

Cymodocea serrulata

The ethanol extracts of *C. serrulata* maximum activity was recorded among the biofilm forming bacteria's followed by *E. coli* (16 mm), *P. mirabilis* (13 mm), *P. aeruginosa* (12 mm), *P. vulgaris* (11 mm) and *B. cereus* (10 mm) and the crude extract showed the minimum activity against the bacteria *L. monocytogenes* (6 mm).

The methanol extracts of *C. serrulata* was showed a maximum activity against *E. coli* (14 mm) and *P. mirabilis* (10 mm) and the minimum activity was recorded against *S. aureus* (1 mm), *L. monocytogenes* (2 mm) and *V. paraheamolyticus* (2 mm) (Table 2).

Acetone extract of sea grass species *C. serrulata* was showed the maximum activity against *E. coli* (8 mm), *P. vulgaris* (7 mm) and *P. aeruginosa* (6 mm) and it was showed minimum activity against, *L. monocytogenes* (1 mm) and *S. enteritidis* (2 mm).

The Dichloroethane extract of *C. serrulata* was showed a maximum activity against pathogens *E. coli* (4 mm). Whereas, the minimum activity was recorded against *V. paraheamolyticus* (1 mm) (Table 2).

DISCUSSION

In the present study, ethanol and methanol extracts of *C. serrulata* and *S. isoetifolium* was showed broad spectrum of antibacterial activity (Table 1, 2). Similar results were reported in *Cypraea errones* which exhibited antibacterial and antifungal activity by Anand *et al*. (2002). The present investigation was showed that two seagrass species were effectively inhibited the biofilm forming bacteria's. Comparatively Ioanna *et al*. (2008) was isolated a new metabolite from *Cymodocea nodosa*, which shows antibacterial activity against multi-drug resistant pathogens. In a work executed on in-vitro antimicrobial susceptibility test of the red, green and brown macro algae showed that the methanolic extracts were efficient in their action (Gonzalez-del-val *et al*., 2001). Smith *et al*. (1997) has dealt with the analysis of novel metabolites including sulfated flavonoids in seagrass, *Halophila johnsonii* that showed good antibacterial activity. The present work suggested that novel metabolite which supports the hypothesis that polarity seems to influence the in-vitro antibacterial activity that previously reported in the literature.

The antibacterial activity was observed against marine bacteria's (Table 1) it indicates that seagrasses were having some metabolite or phenolic compounds that will show bioactivity against biofilm forming bacteria's. The similar results was supported by Todd *et al.* (1993), who isolated B-(Sulphoxy) cinnamic acid from the seagrass *Zostera marina* (eelgrass) and found to prevent attachment of marine bacteria and barnacles to artificial surfaces at non-toxic concentrations. Dumay *et al*. (2004) revealed that variation of phenolic compounds in *Posidonia oceanica* under competitive condition. They have also postulated that the occurrence of secondary metabolites in seagrasses may result of physiological adaptation due to environmental conditions.

CONCLUSION

In present investigation has been concluded that the eco-friendly and effective antifouling agents, which were isolated from the two marine seagrass species. The ethanolic and methanolic extracts of *C. serrulata* and *S. isoetifolium* was inhibited the growth of all nine species of biofilm forming bacteria's. However through purification and characterization of the active components will reveal the exact nature of the active principles involved in the antibacterial assay.

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