Journal of Biological Research **4**: 181 – 188, 2005
J. Biol. Res. is available online at http://www.jbr.gr

Screening of medicinal plant extracts for eco-friendly antimicrofouling compounds

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Received: 2 April 2005 Accepted after revision: 15 July 2005

The diversified biofouling organisms cause damage to fishing vessels including accessory materials in marine environment. Their occurrence and diversity are influenced by environmental variables. In the harbor environment, their growth is favored by pollutants from anthropogenic activities, a fact deserving critical investigation. The site selected for the present study is Chinnamuttom fisheries harbor in southeast coast of India. Four different substrata (panels), i.e. wood, FRP, stainless steel and carbon steel were selected and exposed to seawater of fisheries harbor for 72 h. From these panels, the biofilm was scrapped out at an interval of 24 h for a period of 72 h and then the total viable count (TVC) of the biofilm was enumerated. It showed a gradual increase of TVC with increase in time. Among the tested substrata, stainless steel showed the highest (30.0-85.30 × 10^4 CFU ml^{-1}) bacterial load against the lowest (10.3-19.6 × 10^4 CFU ml^{-1}) in carbon steel. In total, eight bacterial species were isolated and identified from all panels, with varying population diversity. The daily variations in physico-chemical parameters of the source water showed little fluctuation. Further, the methanolic extracts of 10 selected medicinal plants were screened for antimicrofouling properties against the identified bacterial species. Among the medicinal plants screened, *Phyllanthus niruri* exhibited the highest inhibitory activity against all tested bacterial species (12.0-14.0 mm). *Lawsonia innermis* (10.0-12.5 mm), *Azadirachta indica* (9.5-11.5 mm), and *Aloe vera* (8.5-10.5 mm) exhibited considerable antimicrofouling property. Plant extracts with their active principle compounds were evidenced to be essential components for the preparation of biofilm repellents.

Key words: Biofilm, wood, FRP, carbon steel, stainless steel, panels.

INTRODUCTION

Marine biofouling is a result of growth of microbes, algae and sessile invertebrates on submerged surfaces, both natural and man-made. Despite being a natural process, fouling is one of the most important problems marine technology faces (Callow, 1986; Gerhart *et al*., 1988). Fouling organisms may colonize man-made structures, creating problems such as inadequate surface aeration (Davis *et al*., 1989; Safriel *et al*., 1993), speed reduction and increase of fuel consumption of boats and ships (Champ & Lowenstein, 1992). The competition for living space is intense in the marine environment and hence all submerged surfaces in the sea are innate or susceptible to fouling (Wahl *et al*., 1994; Steinberg *et al*., 1997). Fouling of ship hulls increases the hydrodynamic drag, resulting in a significant increase of the operational and maintenance cost. It was found that 5% fouling on the hull of a tanker may increase fuel cost by 17% and according to Lewis (2001), 1 mm thick layer of slime may cause a 15% loss of ship speed.

The biological fouling can be subdivided into three main stages, whilst it now seems that some of these stages can overlap with each other or occur in parallel. They are: conditioning film formation, microfouling or biofilm formation and macrofouling. Conditioning film formation is an initial event in the formation of a biofilm. When a solid surface is ex-

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posed to seawater, it adsorbs dissolved organic materials such as proteins, polysaccharides, humic acids and small hydrocarbons and a thin molecular conditioning film is formed (Marshall *et al*., 1994; Callow & Callow, 2002).

Formation of a conditioning film on a substratum encourages the next sequential process called biofilm formation or microfouling. The predominant organisms in the microfouling process are bacteria, algae, fungi, protozoa and diatoms. After adhesion, the bacteria not only reproduce, but also extrude a mucous material (Costerton *et al*., 1978, 1985) composed of polysaccharides with variable amounts of proteins. Adsorption of bacteria to a surface may be completed within 10 min (Wiencek & Fletcher, 1995). The formation of an initial biofilm is closely followed by the attachment of larger organisms, such as diatoms and ciliated stalked protozoa.

Corpe (1973) has reported the initial bacterial colonizers to be pseudomonads, principally species of *Pseudomonas*, *Flavobacterium* and *Alcaligens*. Avelin *et al*. (1984) have isolated 18 biofilm bacteria derived from five genera *viz. Aeromonas*, *Alcaligens*, *Flavobacterium*, *Pseudomonas* and *Vibrio*. Bacteria in the biofilm, such as those of the genus *Vibrio*, have been found to be effective in inducing settlement and metamorphosis of the coelenterates, like *Cassiopea andromeda* and *Hydractina echiata* (Hofmann *et al*., 1978; Newmann, 1979). Whether the inductive is within the biofilm is poorly understood (Johnson *et al*., 1991), however it is clear that bacteria can stimulate settlement of hydroids (Spindler & Müller, 1972), polychaetes (Kirchman *et al*., 1982), gastropods (Morse & Morse, 1984), bivalves (Fitt *et al*., 1990), barnacles (Maki *et al*., 1990), etc. Often, the settlement of macrofoulers, such as blue mussels and barnacles, causes a serious damage to ship hulls, cooling systems of power stations, aquaculture systems, fishing nets, pipelines and other marine infrastructure.

Hence, there is a need for proper fouling control strategy for all surface materials that come into contact with the seawater. TBT, a biocide used in antifouling applications, is considered as the most successful antifouling substance. TBT-based antifouling paints maintain efficiency for five or more years and save the shipping industry (2.5 billions of US$ per year in fuel, dry docking and associated costs). It is the most toxic compound ever deliberately introduced to marine environment without any comprehensive environmental risk assessment. The TBT released from antifouling paints is now known to adversely affect the marine environment and numerous states have regulated its use (Lewis, 2001). TBT is toxic to a variety of marine species, particularly to shellfish (Baumann, 1991; Matthiessen & Gibbs, 1998). The most sensitive invertebrate species, including dog whelk, *Nucella rapillus*, exhibit imposex (imposition of male sexual characters on the female) at a concentration < 1 ng l^{-1}.

The TBT from the ship paints becomes adsorbed by the surrounding seawater and accumulates in the sediment around harbors and also in shipping lanes (Stewart & Thompson, 1997; Hashimoto *et al*., 1998). It becomes absorbed by animals and accumulates in the food chain. In some countries, the seafood samples were found to contain TBT above the tolerable average residue levels (TARL) (Belfroid *et al*., 2000). To avoid such conditions, several scientists are still trying to formulate harmless, nontoxic and eco-friendly antifouling agents based on natural products in the form of extracts from marine and terrestrial organisms.

Considering the threat to the marine environment, the International Maritime Organization (IMO) proposed a phasing out of TBT-based antifoulants by 2003, followed by a complete ban by 2008 (Lewis, 2001). Therefore, there has been considerable interest regarding the way marine plants and animals resist this assault and also whether there are any "natural" mechanisms that could protect artificial surfaces. To combat this problem, there is an urgent need to identify eco-friendly antimicrofouling compounds other than synthetic chemicals. The natural antimicrobial compounds originating from medicinal plants can be used as a replacement for the chemicals commonly used in antifouling coatings. In view of this, the present study was undertaken in order to find out suitable antimicrofouling compounds from selected medicinal plants.

MATERIALS AND METHODS

Description of the study

Four different panels such as wood, fiber glass reinforced plastic (FRP), stainless steel (316L) and carbon steel, which are widely used to construct ship hulls, boats and other structures, were used as fouling patterns. All the panels were of the same size, i.e. 15 cm length × 10 cm breadth × 2 mm width. Each panel was exposed to a depth of 1 m from an anchored boat in Chinnamuttom fisheries harbor (8° 6′

12″ N, 7° 34′ 09″ E) at the southeast coast of India. The panels were assessed every 24 h up to 72 h and the biofilms were swabbed using sterile cotton swabs (Wahl *et al*., 1994) and transported to the laboratory in aseptic conditions on ice. Simultaneously, water samples were also collected from the study area to determine the physico-chemical properties of the water. Water temperature was recorded using a thermometer (Hermes, India; sensitivity ± 1°C). Salinity and pH of the water samples were recorded using a salinity refractometer (New S-100, Tanaka Sanjiro, Japan; sensitivity ± 1 ppt) and a pH meter (Elico, India). Other chemical parameters, such as dissolved oxygen, silicate, nitrate, total phosphorous, phosphate and ammonia were analyzed based on APHA (1985).

Isolation of biofilm bacteria

The collected swabs were transferred to test tubes individually containing 10 ml of sterile seawater. From each tube, 1 ml of the sample was taken and serially diluted up to 10^{-4} dilution. A volume of 0.1 ml of the 10^{-4} dilution was plated on a Petri dish containing Zobel marine agar (2216) and incubated at 30°C for 48 h. The mean total bacterial population was estimated and recorded in each plate. Pure bacterial colonies were isolated and identified to species by Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The individual biofilm colonies were re-streaked in Zobel marine agar and kept as a stock.

Selection of medicinal plants

Ten medicinal plants (*Lawsonia innermis*, *Zingiber officinalis*, *Phyllanthus niruri*, *Curcuma longa*, *Cynodon dactylon*, *Solanum xanthocarpum*, *Calotropis gigantia*, *Ricinus communis*, *Azadirachta indica* and *Aloe vera*) having antibacterial properties were selected for the present study.

Extract preparation

The above plants were washed thoroughly, air-dried in shade and macerated with a mixer grinder. The powdered material was extracted in a Soxhlet apparatus using methanol as a solvent. Extracts were filtered with Whatman No. 1 filter paper, evaporated and concentrated. The crude extracts were used for the antibacterial assay.

Antibacterial assay

The individual biofilm colonies were seeded in Muller Hinton agar plates. The antibacterial assay was determined using the standard disc diffusion method (Kirby Bauer method, Bauer *et al*., 1966). Sterile Whatman No. 1 filter paper discs of 5 mm diameter were impregnated with 25 mg/disc of the crude extract, air-dried and placed on Muller Hinton agar plates seeded with individual organisms and incubated for 24 h at 30°C. The assay was carried out in triplicate. The zone of inhibition was measured in mm from the centre of the disc and the results were recorded.

Statistical analysis

The results obtained were analysed through the two-way ANOVA test according to Zar (1974).

RESULTS

Physico-chemical parameters of water samples of Chinnamuttom fisheries harbor

During the three days of study, the physical and chemical parameters of the seawater of Chinnamuttom fisheries harbor showed little fluctuation (Table 1). The salinity was normal (35-36 ppt). Similarly, the levels of temperature, pH and dissolved O_2 (DO_2) did not vary significantly (26-28°C, 8.0-8.2 pH and 5.1-5.3 mg l^{-1} DO_2). Other parameters, such as silicate, nitrate, nitrite, total phosphorous, phosphate and ammonia levels were marginally variable, i.e. 0.133-0.319, 2.06-3.66, 0.155-0.278, 17.75-21.30, 14.2 -21.30 and 0.011-0.035 µmol, respectively.

Bacterial density of biofilm on experimental panels

The variation in Total Viable Count (TVC) of biofilm during 24, 48 and 72 h exposure of panels in Chinnamuttom fisheries harbor water is shown in Fig. 1. The TVC of biofilm was found to increase with time in all tested panels. For instance, the TVC of biofilm samples of the wooden panel within 24 h was 25 ± 3.26 × 10^4 CFU ml^{-1}. This level increased subsequently during 48 h (31.3 ± 2.05 × 10^4 CFU ml^{-1}) and 72 h (40.0 ± 2.44 × 10^4 CFU ml^{-1}).

The bacterial density on the stainless steel panel exposed to seawater during 24 h was 30.0 ± 2.97 × 10^4 CFU ml^{-1}. This density increased to 40.37 ± 3.8 and 85.3 ± 9.03 × 10^4 CFU ml^{-1} during 48 and 72 h, respectively. Likewise, the bacterial population on

TABLE 1. Physico-chemical parameters recorded in the seawater of the study area in Chinnamuttom fisheries harbor

Days	Salinity (ppt)	Temperature (°C)	Dissolved oxygen (mg l^{-1})	pH	Silicate (µmol)	Nitrate (µmol)	Nitrite (µmol)	Total phosphorus (µmol)	Phosphate (µmol)	Ammonia (µmol)
1	35.0 ± 0.0	26.0 ± 0.0	5.10 ± 0.10	8.0 ± 0.0	0.159 ± 0.003	3.666 ± 0.012	0.278 ± 0.002	21.30 ± 1.10	17.75 ± 1.50	0.035 ± 0.0
2	36.0 ± 0.0	27.50 ± 0.0	5.30 ± 0.15	8.20 ± 0.0	0.319 ± 0.003	3.147 ± 0.016	0.207 ± 0.004	17.75 ± 1.50	21.30 ± 1.40	0.011 ± 0.0
3	36.0 ± 0.0	28.0 ± 0.0	5.20 ± 0.20	8.0 ± 0.0	0.133 ± 0.001	2.065 ± 0.008	0.155 ± 0.003	18.87 ± 1.20	14.20 ± 0.80	0.031 ± 0.0

Each value is a mean of three replicates (± SD)

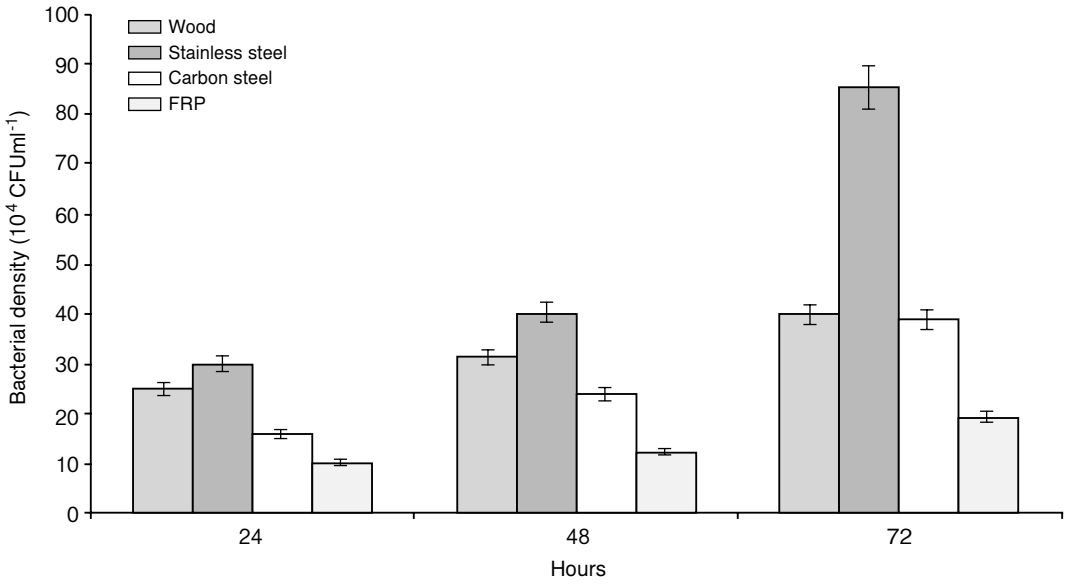

FIG. 1. Bacterial density (10^4 CFU ml^{-1}) recorded in the panels exposed to seawater of Chinnamuttom fisheries harbor at different time intervals.

the carbon steel panel was 16.0 ± 2.94, 24.0 ± 3.74 and 39.0 ± 3.26 × 10^4 CFU ml^{-1} during time intervals of 24, 48 and 72 h, respectively. The bacterial density on the FRP panel in the exposure durations of 24, 48 and 72 h had a marginal increase of 10.3 ± 2.05, 12.6 ± 2.49 and 19.6 ± 1.24 × 10^4 CFU ml^{-1}, respectively.

The bacterial density in the tested panels was in the following order: stainless steel panel > wooden panel > carbon steel panel > FRP panel. The statistical analysis on bacterial density in the panels revealed that the variation among the panels (P) as well as among the time duration (T) were statistically significant ($F_P = 5.97$ and $F_T = 5.68$, $p < 0.05$).

Diversity of bacterial strains on biofilm of tested panels

The bacterial strains isolated from different panels were subjected to Gram staining, motility test as well as biochemical and physiological tests for species level identification (Table 2). In total, eight bacterial strains were isolated and identified from all tested panels. They were *Pseudomonas aeruginosa*, *Holomonas aquamarina*, *Vibrio alginolyticus*, *Enterobacter agglomerans*, *Serratia marcescens*, *Vibrio fischeri*, *Vibrio parahaemolyticus* and *Serratia liquifaciens*. The diversities of bacterial strains at 24, 48 and 72 h were pooled together and produced as a mean value. In all panels, *P. aeruginosa* ranged from 29 ± 0.81 to 35 ± 1.63%, followed by *H. aquamarina* with a range between 21 ± 0.81 and 29 ± 1.63%. *Vibrio alginolyticus* was maximum (21 ± 1.24%) in the wooden panel and minimum (13 ± 1.63%) in the carbon steel panel. *Serratia marcescens* was found to be more abundant in the wooden panel (12 ± 0.81%) than in the other tested panels (5-6%). *Enterobacter agglomerans* and *V. fischeri* were found within a range of 5 to 8% in all tested panels. *Vibrio parahaemolyticus* and *S. liquifaciens* were found in smaller proportions (1-4%).

Antimicrofouling activity of medicinal plant extracts

The antimicrofouling activity of the methanolic extracts of the selected medicinal plants was screened against the eight isolated biofilm bacterial strains. Among the 10 tested plants, four (*P. niruri*, *L. innermis*, *A. indica* and *A. vera*) showed a better bacterial growth inhibitory effect. Among these, *P. niruri* exhibited the highest inhibitory activity by forming a zone of inhibition with a range from 12 to 15 mm against all bacterial strains. Next to this, *L. innermis* recorded a zone of inhibition between 10.0 and 12.5 mm against all bacterial strains. *Azadirachta indica* showed a zone of inhibition with a range of 9.5 to 11.5 mm and *A. vera* with a range of 8.5 to 10.5 mm. The least active plant *C. dactylon* also exerted an inhibitory effect on two bacterial strains, *H. aquamarina* (6.5 mm) and *S. marcescens* (4 mm) (Table 3).

DISCUSSION

In general, the potential of materials exposed to natural seawater can be influenced by several abiotic factors and may result in adhesion of biotic factors including bacteria and other unicellular microorganisms creating a biofilm (Palanichamy *et al*., 2002). In the present study, four different panels (substrata) were exposed to seawater in order to determine the potential quantity and diversity of microfoulers in the panels along the Chinnamuttom fisheries harbor, at the southeast coast of India. During the experimental period, the microbial load (TVC) was found to increase gradually, i.e. from 25 to 40, from 30 to 85.3, from 16 to 39 and from 10.3 to 19.6 × 10^4 CFU ml^{-1} in the wooden, stainless steel, carbon steel and FRP panels, respectively from 24 to 72 h intervals. The observed increase in the microfouling biomass was due to enhanced settlement and growth of already colonized microbes on the panel surface (Jordan & Staley, 1976; Gerchakov *et al*., 1976; Wiencek & Fletcher, 1995).

In the present study, out of four substrata used, stainless steel panel showed the highest bacterial load, whereas the lowest bacterial population was observed on the FRP panel. The highest population

TABLE 2. Percentage diversity of bacterial strains recorded in different panels exposed to seawater in the study area of Chinnamuttom fisheries harbor

Bacterial strains	% diversity in tested panels			
	Wood	Stainless steel	Carbon steel	FRP
Pseudomonas aeruginosa	29 ± 0.81	33 ± 2.49	35 ± 1.63	31 ± 1.24
Halomonas aquamarina	23 ± 1.63	21 ± 0.81	27 ± 0.81	29 ± 1.63
Vibrio alginolyticus	21 ± 1.24	19 ± 1.63	13 ± 1.63	15 ± 0.81
Enterobacter agglomerans	5 ± 1.63	8 ± 1.24	5 ± 0.81	7 ± 1.63
Serratia marcescens	12 ± 0.81	6 ± 1.63	6 ± 1.24	5 ± 1.24
Vibrio fischeri	5 ± 1.24	6 ± 0.81	8 ± 0.81	6.5 ± 0.62
Vibrio parahaemolyticus	4 ± 2.49	3 ± 0.81	2 ± 1.63	3 ± 1.63
Serratia liquifaciens	1 ± 0.81	4 ± 1.24	4 ± 1.24	3.5 ± 1.24

Each value is a mean of three replicates (± SD)

TABLE 3. Antimicrobial activity of methanolic extracts of medicinal plants against the microfouling bacterial strains isolated from the test panels exposed to seawater in the study area of Chinnamuttom fisheries harbor

Bacterial strains	Zone of inhibition (mm)/methanolic extracts of plants									
	1	2	3	4	5	6	7	8	9	10
Pseudomonas aeruginosa	10.0 ± 0.23	7.0 ± 0.40	13.0 ± 0.62	8.0 ± 0.44	R	8.0 ± 0.44	R	7.5 ± 0.40	10.0 ± 0.23	9.5 ± 0.23
Vibrio fischeri	11.0 ± 0.23	7.5 ± 0.40	13.0 ± 0.23	7.5 ± 0.40	R	8.5 ± 0.40	6.0 ± 0.40	R	10.5 ± 0.23	10.5 ± 0.40
Halomonas aquamarina	10.5 ± 0.40	7.0 ± 0.23	14.0 ± 0.23	7.5 ± 0.23	6.5 ± 0.44	7.5 ± 0.40	5.5 ± 0.44	7.0 ± 0.23	10.0 ± 0.11	10.0 ± 0.23
Serratia liquifaciens	12.5 ± 0.44	7.5 ± 0.23	15.0 ± 0.23	8.5 ± 0.62	R	7.0 ± 0.23	R	6.5 ± 0.23	11.0 ± 0.44	9.0 ± 0.40
Vibrio parahaemolyticus	11.5 ± 0.44	8.0 ± 0.62	12.0 ± 0.23	8.0 ± 0.23	R	6.5 ± 0.44	6.0 ± 0.44	R	9.5 ± 0.44	8.5 ± 0.44
Vibrio alginolyticus	11.0 ± 0.62	7.5 ± 0.62	13.0 ± 0.43	7.0 ± 0.40	R	6.0 ± 0.23	R	7.5 ± 0.40	11.5 ± 0.44	9.0 ± 0.44
Serratia marcescens	10.5 ± 0.23	7.5 ± 0.23	12.0 ± 0.44	7.5 ± 0.44	4.0 ± 0.23	7.0 ± 0.23	6.5 ± 0.40	R	10.0 ± 0.62	9.5 ± 0.62
Enterobacter agglomerans	11.0 ± 0.23	7.5 ± 0.44	14.0 ± 0.40	7.5 ± 0.44	R	6.0 ± 0.23	R	7.5 ± 0.40	11.5 ± 0.23	9.0 ± 0.62

1: *Lawsonia innermis*, 2: *Zingiber officinalis*, 3: *Phyllanthus niruri*, 4: *Curcuma longa*, 5: *Cynodon dactylon*, 6: *Solanum xanthocarpum*, 7: *Calotropis gigantia*, 8: *Ricinus communis*, 9: *Azadirachta indica*, 10: *Aloe vera*
Each value is a mean of three replicates (± SD)
R: Resistant

on the stainless steel may be due to its surface nature. Similarly, in the case of carbon steel, the bacterial population was lower than that of the wooden panel, but the difference was not large. The variation in bacterial population on different substrata may be due to the surface and/or the nature of the substratum used. Little & Wagner (1997) have reported that the rate of initial bacterial colonization is substratum-dependent, i.e. not all surfaces are colonized at the same rate.

It has been suggested that effective inhibition of biofilm formation at the early stage would lead to a surface that lacks the necessary characteristics to permit the settlement of larvae of macrofoulers (Satuito *et al*., 1997; Wieczorek & Todd, 1997). Therefore, the application of paints with antibacterial activity may disrupt the early stages of biofilm development and provide an antifouling coating for the

protection of marine structures. The study of natural product antifoulants has included the assessment of the activity of crude extracts or isolated metabolites in antifouling assays (Armstrong *et al*., 2000a, b).

Methanolic extracts of 37 marine organisms including marine plants and animals were screened for antibacterial properties against five biofilm bacterial strains. Among these, 10 plant and nine animal extracts exhibited good antibacterial activity against one bacterial strain. The extracts of three species of marine plants [*Stocheospermum marginatum* (brown alga), *Cymodocea rotundata* (seagrass), *Padina tetrastromatica* (brown alga)] and three species of marine animals [*Petrosia* sp., *Psammaplysilla purpurea* (sponge), *Cassiopeia* sp. (jelly fish)] were active against all tested strains (9-11 mm) (Bhosale *et al*., 2002). In view of this, methanolic extracts of 10 medicinal plants were also screened in the present study for antimicrofouling activity against eight biofilm bacterial strains isolated from different substrata. Among the tested plants, *P. niruri*, *L. innermis*, *A. indica* and *A. vera* showed a great inhibition effect against all tested microfoulers. The zone of inhibition ranged from 8.5 to 15.0 mm in diameter.

From the present study, it may be inferred that plant species with active principle compounds could be used as components for preparation of antimicrofouling substances (antifouling paints) as an ecofriendly alternative solution against biofouling.

REFERENCES

APHA, 1985. *Standard methods for the examination of water and wastewater*. American Public Health Association, Washington DC.

Armstrong E, Boyd KG, Burgess JG, 2000a. Prevention of marine biofouling using natural compounds from marine organisms. *Biotechnology annual review*, 6: 221-241.

Armstrong E, Boyd KG, Pisacane A, Peppiatt CJ, Burgess JG, 2000b. Marine microbial natural products in antifouling coatings. *Biofouling*, 16: 215-224.

Avelin M, Mary V, Sarojini R, Nagabhushanam R, 1984. Bacteriostatic compounds in extracts of marine animals from the Indian Ocean. In: Thompson MF, Nagabhushanam R, Sarojini R, Fingerman M, eds. *Recent developments in biofouling control*. Oxford and IBH Publishing, New Delhi.

Bauer AW, Kirby WMM, Sherris JC, Tvrok M, 1966. Antibiotic susceptibility testing by standardized single disc method. *American journal of clinical pathology*, 45: 493-496.

Baumann SB, 1991. The vibration probe as a sensitive toxicology assay for the effects of tributyltin on ionic currents in *Catabulasia mediterranea*. *Toxicology in vitro*, 5: 103-107.

Belfroid AC, Purperhart M, Ariese F, 2000. Organotin levels in seafood. *Marine pollution bulletin*, 40: 226-232.

Bhosale SH, Nagle VL, Jagtap TG, 2002. Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas*. *Marine biotechnology*, 4: 111-118.

Callow ME, 1986. Fouling algae from in service ships. *Botanica marina*, 29: 351-357.

Callow ME, Callow JA, 2002. Marine biofouling: a sticky problem. *The biologist*, 49: 10-14.

Champ MA, Lowenstein FL, 1992. TBT: the dilemma of high technology antifouling paints. *Oceanus*, 35: 69-77.

Corpe WA, 1973. Microfouling: the role of primary film forming marine bacteria. In: Acker RF, Brown BF, De Palma JR, Iverson WP, eds. *Proceedings of the 3rd international congress on marine corrosion and fouling*. Northwestern University Press, New York: 598-609.

Costerton JW, Geesey GG, Cheng KJ, 1978. How bacteria stick. *Scientific american*, 238: 86-95.

Costerton JW, Marrie TJ, Cheng KJ, 1985. Phenomenon of bacterial adhesion. In: Savage DC, Fletcher M, eds. *Bacterial adhesion – mechanisms and physiological significance*. Plenum Press, New York: 3-43.

Davis CM, Targett NM, McConnell OJ, Young CM, 1989. Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and overgrowth. In: Scheuer PJ, ed. *Bioorganic marine chemistry*. Springer-Verlag, Heidelberg: 85-114.

Fitt WK, Coon SL, Walch M, Weiner RM, Colwell RR, Bonar DB, 1990. Settlement behaviour and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Marine biology*, 106: 389-394.

Gerchakov SM, Marzalek DS, Roth IJ, Ude LR, 1976. Succession of periphytic microorganisms on metal and glass surfaces in natural seawater. In: *Proceedings of the 4th international congress on marine corrosion and fouling*. Juan-les-Prins, France.

Gerhart DJ, Rittschof D, Mayos SW, 1988. Chemical ecology and the search for marine antifoulants. Studies of a predator-prey symbiosis. *Journal of chemical ecology*, 14: 1905-1917.

Hashimoto S, Watanabe M, Noda Y, Hayashi T, Kurita Y, Takusa Y, Otsuki A, 1998. Concentration and distribution of butyltin compounds in a heavy tanker route in the Strait of Malacca and in Tokyo Bay. *Marine environmental research*, 45: 169-177.

Hofmann DK, Newmann R, Henne K, 1978. Strobilation, hudding and initiation of Scyphistoma morphogen-

esis in the rhizostome *Cassiopea andromeda* (Cnidaria: Scynhozoa). *Marine biology*, 47: 161-176.

Holt JG, Krieg NR, Shiath PHA, Stanley JT, Williams SH, 1994. *Bergey's manual of determinative bacteriology*. Wilkins and Wilkins, Baltimore.

Johnson CR, Muir DG, Reysenbach AL, 1991. Characteristics of bacteria associated with surfaces of coralline algae; a hypothesis for bacterial induction of marine invertebrate algae. *Marine ecology progress series*, 74: 281-294.

Jordan TL, Staley JT, 1976. Electron microscopic study of succession in the periphyton community of lake Washington. *Microbiology and ecology*, 2: 241-245.

Kirchman D, Graham S, Reish D, Mitchell R, 1982. Bacteria induce settlement and metamorphosis of *Janua* (*Dexiospira*) *brasiliensis* Grube (Polychaeta: Spirobidae). *Journal of experimental marine biology and ecology*, 56: 153-163.

Lewis, 2001. *National shipping industry conference*, 8-9 March, Sydney.

Little BJ, Wagner PA, 1997. Succession in microfouling. In: Nagabhusnan R, Thomson F, eds. *Fouling organisms of the Indian Ocean. Biology and control technology*. Oxford and IBH Publishing, New Delhi: 105-134.

Maki JS, Rittschof D, Samuelson MO, Szewzyk U, Yule AB, Kjelleberg S, Costlow JD, Mitchell R, 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bulletin of marine science*, 46: 499-511.

Marshall K, Power KN, Angles ML, Schneider RP, Goodman AE, 1994. Analysis of bacterial behaviour during biofouling of surfaces. In: Geesey GG, Lewandowski Z, Flemming HC, eds. *Biofouling and biocorrosion in industrial water systems*. CRC Press, Boca Raton, Florida: 15-26.

Matthiessen P, Gibbs PE, 1998. Critical appraisal of the evidence for tributyltin mediated endocrine disruption in mollusks. *Environment and toxicological chemistry*, 17: 37-43.

Morse ANC, Morse DE, 1984. Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surface of crustose red algae. *Journal of experimental marine biology and ecology*, 75: 191-215.

Newmann R, 1979. Bacterial induction of settlement and metamorphosis in the planula larva of *Cassiopea andromeda* (Cnidaria: Scyphozoa, Rhizosoomeae). *Marine ecology progress series*, 1: 21-28.

Palanichamy S, Maruthamuthu S, Manickam ST, Rajendran A, 2002. Microfouling of manganese-oxidizing bacteria in Tuticorin harbour waters. *Current science*, 82: 865-868.

Safriel UN, Cohen Y, Erez N, Gateno D, Keasar T, Doley Y, 1993. Biological control of marine biofouling. *Oebalia*, 19: 193-199.

Satuito CG, Shimizu K, Fusetani N, 1997. Studies on the factors influencing larval settlement in *Balanus amphitrite* and *Mytilus galloprovincialis*. *Hydrobiologia*, 358: 275-280.

Spindler KD, Müller WA, 1972. Induction of metamorphosis by bacteria and by a lithium pulse in the larvae of *Hydractina echinata* (Hydrozoa). *Wilhelm Roux's archives of developmental biology*, 169: 271-280.

Steinberg PD, Schneider R, Kjelleberg S, 1997. Chemical defenses of seaweed against microbial colonization. *Biodegradation*, 8: 211-220.

Stewart C, Thompson JAJ, 1997. Vertical distribution of butyltin residues in sediments of British Columbia harbours. *Environmental technology*, 18: 175-202.

Wahl M, Jensen PR, Fenical W, 1994. Chemical control of bacterial epibiosis on ascidians. *Marine ecology progress series*, 110: 45-57.

Wieczorek SK, Todd CD, 1997. Inhibition of bryozoan and ascidian settlement by natural multispecies biofilms: effects of film age and the roles of active and passive algal attachment. *Marine biology*, 128: 463-473.

Wiencek KM, Fletcher M, 1995. Bacterial adhesion to hydroxyl-a methyl-terminated alkanethiol self-assembled monolayers. *Journal of bacteriology*, 177: 1959-1966.

Zar JE, 1974. *Biostatistical analysis*. Prentice-Hall, New Jersey.