Biocatalysis and Agricultural Biotechnology 4 (2015) 114–121

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Original Research Paper

Antimicrofouling properties of chosen marine plants: An eco-friendly approach to restrain marine microfoulers

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ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form
7 November 2014
Accepted 11 November 2014
Available online 15 November 2014

Keywords:
Marine biofilm bacteria
Seaweeds
Seagrasses
Antimicrofouling
Cytotoxicity
Antifouling

ABSTRACT

Biofouling is a panic issue in the marine environment where the major perpetrator is the biofilm forming microbial cells like bacterial groups. Hence, the present study was focused to study the diversity and density of marine biofilm forming bacteria on different experimental panels immersed in Palk Bay region. The results are inferred that the density and distribution of biofilm forming bacterial groups were significantly ($P < 0.05$) varied, whereas the *Pseudomonas* spp. (15.78–22.22%) had maximum distribution in the immersed all the test panels. The current antifouling paints create toxic effects on non-target organisms. Of late, natural products were replaced with current toxic antifouling problems. In the present study, the four marine plants *viz*. two seaweeds (*Sarconema furcellatum, Sargassum wightii*) species and two seagrasses (*Syringodium isoetifolium, Cymodocea serrulata*) species were selected to screen their antimicrofouling activity. From this, the crude acetone extract of *S. furcellatum* exhibited the good antimicrofouling activity over the other marine plant extracts against test microfoulers; antibacterial (7 ± 0.16 to 13 ± 0.26 mm) with least concentration of MIC and MBC values (12.5–25 μg/ml and 25–50 μg/ml), antimicroalgal (50–300 μg/ml) and *Artemia* cytotoxicity (LC_{50} 133.88 μg/ml; $P < 0.001$) and anticrustacen activity was significantly ($P < 0.05$) increased mortality with increasing test concentrations of crude extracts. Also, phytochemical studies of the four marine plants revealed the presence of chemical constituents such as flavanoids, alkaloids, phenols and sugars. Further studies on the purification and identification of active compounds from *S. furcellatum* might help to characterize the nature of eco-friendly antifouling compounds filed study.

1. Introduction

The undesirable formation of biotic deposits on artificial or natural surface systems immersed in seawater is called biofouling, and this is currently one of the most imperative tribulations in the offshore marine establishment (Callow, 1986; Gerhart et al., 1988). Chief fouling communities which play a significant role in the fouling cycle are aquatic bacteria, unicellular microalgae such as diatoms and cyanobacteria (Qian et al., 2007). These ubiquitous microfouling members attach to the immersed surface by complex biochemical glue called Extracellular Polysaccharide Substances (EPS) (Salta et al., 2013). Microfouling plays a critical role in the settlement of macrofoulers like macroalgae larvae of barnacle, mollusks, bryozoans, polychaetes, tunicates, coelenterates etc. The formation and attachment of micro and macrofouling communities are influenced by environmental factors and surface nature of immersed substrata (Immanuel et al., 2005).

Biofouling creates problems such as surface alternation, speed reduction and increase in fuel consumption of ships, corrosion, weight increase and distortion of the initial configuration of submerged man-made structures (Schultz, 2007), damage the aquaculture equipments and cause disease in fish and shell fishes communities (Fitridge et al., 2012). All these problems lead to a huge economic loss. As a result, commercial antifoulants came in to market to manage the problems of fouling. But, they are found to affect the nontarget aquatic organisms (Konstantinou and Albanis, 2004; Zhou et al., 2006) and it has also been recognized as the global environmental problem since they cause marine pollution. For

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http://dx.doi.org/10.1016/j.bcab.2014.11.002
1878-8181/

instance, the copper based antifoulant, tributyltin (TBT) have been banned by IMO (International Maritime Organisation) (Yebra et al., 2004).

Hence, there is a need for the development of effective and novel environmentally compatible antifouling compounds from the natural resources to control fouling cycle. The natural antifouling compounds can be one of the best replacement options for the most successful antifouling technology currently available (Raveendran and Limna Mol, 2009). Recently, the marine natural products (such as secondary metabolites) found to exhibit strong antifouling properties which have been separated and identified from number of marine organisms including seaweeds (Manilal et al., 2010; Goecke et al., 2012; Ramirez et al., 2012), seagrasses (Xu et al., 2005; Mayavu et al., 2009; Prabhakaran et al., 2012; Iyapparaj et al., 2013, 2014), sponges, ascidians, bryozoans, and gorgonians (Clare, 1996) from different coastal regions. Information's available on biofouling cycling and biological properties of marine plants from Thondi coastal region is very scanty. Therefore, the present investigation was made to understand the dynamics of biofilm forming bacterial communities on different immersed substrata and to screen antimicrofouling properties of marine plants.

2. Material and methods

2.1. Biofilm samples collection

The present investigation was carried out in Thondi coastal water, southeast coast of Tamil Nadu, India (Latitude: 9° 44′N and Longitude: 79° 00′E). Three different experimental panels such as PVC (15×6 cm^2), wood (*Artocarpus hirsutus*) (15×6 cm^2) and titanium (15×3 cm^2) were immersed to a depth of one meter below the surface water, using wooden raft in Thondi coast for a period of four days. To enumerate biofilm forming bacteria, the biofilm slime samples from each test panels were collected up to 96 h with a time interval of 24 h using sterile cotton swabs and transported to the laboratory (Wahl et al., 1994). Physico-chemical parameters such as temperature, salinity and pH of the water samples were also measured in the field itself with the help of a thermometer, refractometer (Agato, Japan) and pH pen (pHep, Henna instrument Pvt. Ltd., Portugal) respectively. The level of dissolved oxygen, nitrite, nitrate, inorganic phosphate, total phosphorus, and ammonia in the water sample collected from the study area was estimated as per the procedure given by Grasshoff et al. (1983) and APHA (1985).

2.2. Isolation and identification of biofilm forming bacterial strains

The biofilm slime samples collected from all the experimental panels were serially diluted up to 10^5 using filter sterilized seawater and 100 μl of each diluents was spread on individual Zobell Marine Agar (2216E) plates and incubated at 37 °C for 48 h. The bacterial colonies developed on the plates were counted and their population density was expressed as CFU/ml. The morphologically distinct biofilm bacterial colonies were purified and identified up to genus level by Bergey's Manual of Determinative Bacteriology (Holt et al., 1996). The pure individual bacterial colonies were maintained on slants for further study.

2.3. Generic level confirmation on selected biofilm film bacterial strains

The predominant biofilm bacterial strains were identified and confirmed up to genus level using Probabilistic Identification of Bacteria (PIB) software package (Bryant, 1995), an implementation of Bayes' theorem by West et al. (1986). An identification score, as the Wilcox probability (P) was calculated for identification thresholds of $P > 0.99$ for all the isolated biofilm bacterial strains. As a result, 10 biofilm film forming bacterial strains such as *Pseudomonas* sp., ($P > 0.987$), *Vibrio* sp., ($P > 0.993$), *Proteus* sp., ($P > 0.978$), *Salmonella* sp., ($P > 0.998$), *Serratia* sp., ($P > 0.986$), *Escherichia* sp., ($P > 0.994$), *Morganella* sp., ($P > 0.988$), *Staphylococcus* sp., ($P > 0.997$), *Bacillus* sp., ($P > 0.999$) and *Micrococcus* sp., ($P > 0.995$) were identified.

2.4. Antimicrofouling activities of marine plants

The young and healthy seaweeds (*Sarconema furcellatum*, *Sargassum wightii* and seagrass species (*Syringodium isoetifolium*, *Cymodocea serrulata*) were collected from the present study area. The samples were identified by referring to the keys methodology as described by Umamaheswara Rao (1987) and Kuo and den Hartog (2001). The collected samples were thoroughly washed with running tap water to remove associated debris and shade dried for 3–5 days. The dried samples were powdered, weighed and subjected to cold percolation by soaking them individually in two different organic solvents *i.e.* acetone and diethyl ether (HPLC grade) at room temperature for a week. Then the crude extracts were obtained by concentrating them under vacuum at 37 °C and stored for further studies.

2.4.1. Antibacterial properties of chosen marine plants

The antibacterial activity of the crude extracts obtained from marine plants species was tested against biofilm bacterial strains using an agar well diffusion method (Perez et al., 1990). Each well was loaded with 100 μl of DMSO containing 500 μg/ml of crude extract and incubated for 24 h at 37 °C. 100 μl of DMSO without extract was maintained as negative control. The zone of inhibition was measured from the edge of the well to the clear zone in millimeter (mm). Based on the results, all the acetone extracts were chosen for further research.

2.4.2. Bacteriostaic (MIC) and bactericidal concentration (MBC) of crude extract of marine plants

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were screened by the macrotube dilution method (Trampuz et al., 2007). Each strains of biofilm forming bacteria (0.01 ml) with the cell density of 2×10^8 cell/ml (Amsterdam, 1996) were added to series of tubes containing 0.5 ml of varying concentrations of crude acetone extract of marine plants (12.5, 25, 50, 75, 100, 150, 200, 250 and 300 μg/ml). Then, the total volume was made up to 1 ml by sterile Zobell marine broth and incubated at 37 °C for 24 h in thermostat shaker. The tubes were examined for microbial growth by turbidity observation. To determine the MIC and MBC, a loopful of inoculum was streaked onto Zobell marine agar plates and incubated at 37 °C for 24 h. The concentration which inhibits the bacterial growth was recorded as MIC value and at which there is no visible bacterial growth on ZMA plates as MBC.

2.4.3. Antimicroalgal activity

The crude acetone extract of marine plants was tested against fouling microalgal strains by following the method of Thabard et al. (2009). The two brown marine microalgae namely *Chaetoceros* sp., *Pavlora* sp., and four green marine microalgae *Nannochloropsis* sp., *Dunaliella* sp., *Chorella* sp., and *Tetraselmis* sp. were obtained from marine algal culture unit, CMFRI (Central Marine Fisheries Research Institute), Tuticorin, Tamil Nadu, India. The individual seed culture was maintained in Conway medium at 20 °C in 12 h light periods for 7 days. Different concentrations (25–300 μg/ml) of crude extracts in the carrier solvent of acetone: methanol (1:3) were coated in the 96 well flat bottom microplates (Polystyrene). Each microalgal strain of 100 μl volume with a initial density of 1×10^5 cells/ml was loaded in the extract coated plates and incubated at