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Effects of certain seaweed extracts on the primary biofilm forming bacteria

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Abstract

The present investigation is an attempt to bring out the specific antifouling properties of extracts of five species of marine algae collected from the Gulf of Mannar area of Tuticorin coast. The extracts from seaweeds namely, *Ulva lactuca*, *Caulerpa scalpelliformis*, *Padina boergesenii*, *Caulerpa* sp. and *Chaetomorpha linoides* were tested against biofilm forming bacteria namely, *Micrococci* sp., *Aeromonas* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Cytophaga* sp. and *Enterobacter* sp. The disc diffusion method was used to find out the inhibition of biofilm forming bacteria through zone of inhibition. Four species were found to have antagonistic activity against biofilm forming bacterial isolates. We conclude that the broad antifouling properties of the crude extracts inhibit the growth of biofilm forming bacteria.

Keywords: Seaweeds, antifouling, biofilm bacteria

Introduction

Marine biofouling is a phenomenon causing large penalties to engineered structures such as ships and offshore platforms by way of increased use of manpower, fuel, material and dry-docking time (Chambers *et al*., 2006). Materials immersed in seawater are acted upon by a series of physical, chemical and biological events which result in the formation of a biofilm complex, including a layer of attached organisms like bacteria, fungi, algae, diatoms and barnacles and the colonization of biofoulants depends on polluted nature of the environment (Karande, 1968, 1978; Charaklis, 1981; Karande and Srivastava, 1984; Srivastava *et al*., 1990; Abarzua and Jakubowski, 1995). Immediately after immersion surfaces are coated with a glycoproteinaceous film which favors the colonisation by bacteria, fungi, diatom, protozoa and other microorganisms. Biofilms are clusters of microbial cells that are attached to a surface. They occur in nearly every moist environment where sufficient nutrient flow is available and surface attachment can be achieved. A biofilm can be formed by a single bacterial species, although they also consist of many species of bacteria, fungi, algae and protozoa. Antifouling compounds produced by bacteria are the main components of biofilms in a marine environment and possibly show important effects on biofouling (Maki, 2002). Bacteria in a biofilm can also affect the growth of other bacteria in the same biofilm (Burgess *et al*., 1999). For example, the presence of ''resident'' bacterial strains on particles either increases or decreases the colonisation rate of ''newcomer'' strains (Grossart *et al*., 2003). Macrophytes and invertebrates such as barnacles and blue mussels subsequently attach to this film (Davis *et al.*, 1989). The control of biofouling is of particular concern in modern marine engineering and shipping operations and is one of the most important problems currently facing marine technology (Hattori and Shizui, 1996). Uncontrolled settlement and adhesion of marine invertebrates and algae to the hulls of ships increase frictional drag, with a corresponding decrease in speed, maneuverability and fuel efficiency.

Marine algae play an important role in the fouling

of a wide range of immersed artificial substrata, particularly in shallow water where there is sufficient light to permit the active growth of algae. Although there are many diverse phyla of marine algae, only relatively small numbers are considered to be important as fouling organisms. The three most economically important phyla are the Chlorophyta, Heteroconta and Rhodopyta; Cyanophyta are also frequently reported particularly as primary colonizers (Fletcher, 1980). Microalgae and macro algae such as *Enteromorpha intestinalis* and *Ulothrix zonata* settle after development of a primary film comprising bacteria, with a clear quantitative domain of benthic diatoms (Abarzua and Jakubowski, 1995). Uncontrolled adhesion of fouling by macroalgae such as *Ulva* sp. and *Enteromorpha* sp. cause serious problems by settling on ships, hulls and power plant cooling system (Fletcher, 1980; Miki *et al*., 1996). Macroalgae are a rich source of natural bioactive products although little has been done to define an ecological role for these compounds. It is therefore possible that they possess chemical defenses to prevent the colonization of the surfaces.

Many substances obtained from marine algae such as alginate, carrageen and agar as phycocolliods have been used for decades (Taskin *et al*., 2001). Since algae have been used in traditional medicine for a long time (Fitton, 2006) and also some algal substances have bacteriostatic and bactericidal activity, they have been extensively studied by several researchers (Ghosh *et al*., 2004; Freile - Pelegri´n and Morales, 2004; Salvador *et al*., 2007). Seaweeds are the major principle producers of oceanic plant community, distributed widely and indigenously and are recognised for their elaborate chemical defense characteristics against many biotic and abiotic factors (Aseer *et al*., 2010). A variety of seaweed biogenic compounds exhibiting antifouling activity which belongs to the group of fatty acids, lipopeptides, amides, alkaloids, terpenoids, lactones, pyrroles and sterols have been discovered (Bhadury and Wright, 2004). The aim of this work is to investigate the antifouling activity of seaweeds collected from Tuticorin coast against the biofilm forming bacteria. Most of the earlier studies are on the antibacterial activity of seaweed extracts against pathogenic bacteria. Studies on the antifouling activity of seaweed extract against biofilm forming bacteria from India are rare. The use of marine natural products that are capable of inhibiting one or several steps in fouling may provide a solution to the marine biofouling. Considering the significance of the issue, five macroalgae were screened for their antifouling activity against biofilm bacteria.

Material and Methods

Collection of seaweeds: Live and healthy samples of the seaweeds *Ulva lactuca, Caulerpa scalpelliformis, Padina boergesenii, Caulerpa* sp. and *Chaetomorpha linoides* were collected by handpicking during low tide from Hare Island in the Gulf of Mannar of Tuticorin coast (08°46′25.15″ N lat., 78°11′16.05″ E long.).

Preparation of seaweed samples: Seaweeds were thoroughly washed with seawater to remove all epiphytes, shells, barnacles etc. and again washed with freshwater to remove the surface salts, sand particles if any and allowed to air dry at room temperature for 3 to 4 days. Air drying will retain certain amount of moisture; it can be dried in hot air oven at 45-50°C and then pulverised.

Extraction of bioactive principles from seaweeds: For the extraction of crude bioactivies**,** 20 g of seaweed powder was taken in a 250 ml conical flask. About the same volume of solvents (v/v) like di-ethyl ether (DEE), chloroform and methanol were added to get the natural concentrations of seaweeds and they were extracted by cold steep method at -10°C (Wright, 1998).

Enumeration of bacteria in biofilm: Biofilm generated on the PVC coupons exposed to natural seawater was scrapped using sterile brush and immediately transferred to sterile saline water and serially diluted using sterile seawater. Samples were inoculated by spread plating method on Zobell Marine Agar to enumerate total aerobic heterotrophic bacteria (THB). The pure cultures were maintained in agar slant at 4°C for bacterial characterisation. The isolate was characterised using various morphological, biochemical and molecular characters. Gram staining and motility tests were performed for preliminary identification of the isolate (Allegrucci and Sauer, 2007). Morphological

parameters include colony color, size, shape and margin studied by microscopic observation. Biochemical tests such as MR-VP, indole, oxidase, catalase, sugar fermentation and nitrate reduction test were carried out (Dalton *et al*., 1994).

Antibacterial assay: Disc diffusion method (Bauer *et al.*, 1966) was employed for the study of antifouling activity of seaweed extract. Sterile nutrient agar plates were prepared for the purpose. Cotton swabs were autoclaved and used to inoculate the culture on the dried surface of agar plates to ensure the even distribution on the medium. The isolated pure cultures of *Micrococci* sp., *Aeromonas* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Cytophaga* sp. and *Enterobacter* sp. from the biofilm were swabbed on to separate agar plates. After 24–36 hr of incubation at 37°C, the maximum inhibition zone area was 8 mm and the minimum was 1 mm.

Results

The results in terms of inhibition zone are given in Fig. 1 to 5. Among the five extracts of seaweeds tested, methanolic extract of *Ulva lactuca* showed considerable antibacterial activity against the *Micrococci* sp. and the maximum zone of inhibition was 8 mm and the minimum zone (3 mm) was observed in *Cytophaga* sp. The chloroform extract of *Ulva lactuca* showed a maximum zone of inhibition (6 mm) in *Flavobacterium* sp. and *Aeromonas* sp. exhibited a minimal zone of clearance (4 mm). The DEE extract of *U. lactuca* was found to be quite ineffective against most of the biofilm bacteria. However, *Pseudomonas* sp. was found to be sensitive (6.0 mm) while the inhibitory zone was in trace (<1 mm) amount for *Cytophaga* sp (Fig. 1).

The methanolic extracts of *Caulerpa scalpelliformis* showed the maximum antibacterial activity against the *Micrococci* sp. (7 mm) and the minimum zone of clearance was observed in *Flavobacteria* sp. (2 mm). The chloroform extracts of *C. scalpelliformis* showed a maximal zone of inhibition (6 mm) against *Cytophaga* sp. while *Aeromonas* sp. was found to be rather tolerant, which exhibited only a minimum zone of clearance (1 mm). As observed for *Ulva lactuca*, the DEE extracts of *C. scalpelliformi* were found to be quite ineffective

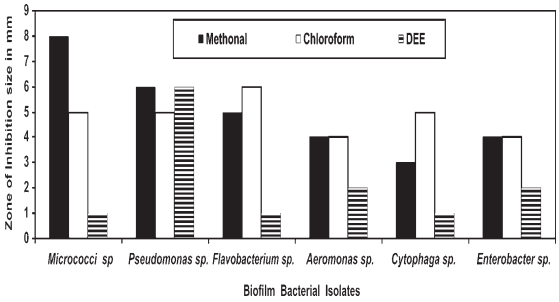

Fig. 1. Antibacterial activity of the marine alga *Ulva lactuca*

against most of the biofilm bacteria. However, *Cytophaga* sp. was found to be rather sensitive (3 mm) while the inhibitory zone was less (1 mm) for all the other bacterial strains (Fig. 2).

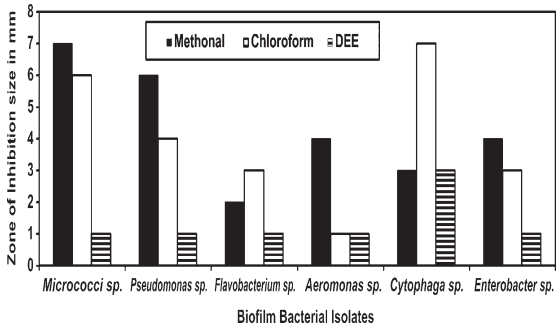

Fig. 2. Antibacterial activity of the marine alga *Caulerpa scalpelliformis*

The methanolic extract of *Padina boergesenii* showed the maximum antibacterial activity against the *Micrococci* sp. (8 mm) and the minimum zone of clearance was in *Flavobacterium* sp. (<1 mm). The chloroform extracts of *P. boergesenii* showed a maximal zone of inhibition (7 mm) in *Psuedomonas* sp. while *Micrococci* sp. was found to be tolerant (1 mm). As noted for the other seaweeds, the DEE extracts of *P. boergesenii* were found to be quite ineffective against most of the biofilm bacteria. However, *Pseudomonas* sp. was found to be sensitive (5 mm), while inhibitory zone was in trace amount (<1 mm) in all the others (Fig. 3).

The methanolic extract of *Caulerpa* sp. showed the maximum antibacterial activity against *Micrococci* sp., *Psuedomonas* sp. and *Enteriobacter*

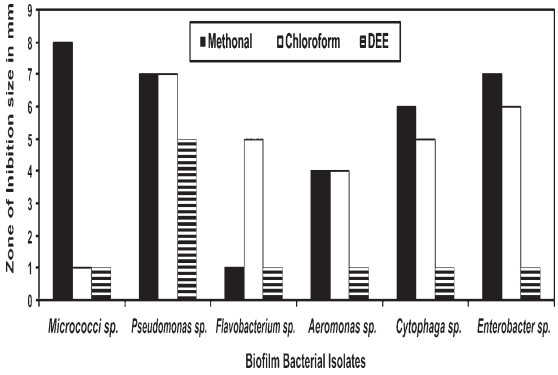

Fig. 3. Antibacterial activity of the marine alga *Padina boergesenii*

sp. (8 mm), while the zone of clearance was found to be in traces for *Flavobacterium* sp. (1mm). The chloroform extracts of *Caulerpa* sp. showed a maximal zone of inhibition of 7mm for *Pseudomonas* sp. and *Cytophaga* sp., while *Aeromonas* sp. and *Flavobacterium* sp. exhibited a minimal zone of inhibition of 4 mm. The DEE extracts of *Caulerpa* sp. were found to be ineffective against most of the biofilm bacteria. However, *Pseudomonas* sp. was found to be sensitive (7 mm), while *Flavobacterium* sp. and *Aeromonas* sp. showed only a minimal zone of 3 mm inhibition (Fig. 4).

The methanolic extract of *Chaetomorpha linoides* showed the highest antibacterial activity against the *Flavobacterium* sp. (10 mm), while the minimal zone of clearance was observed in *Enterobacter* sp. (4 mm). Similarly, the chloroform extracts of *C. linoides*

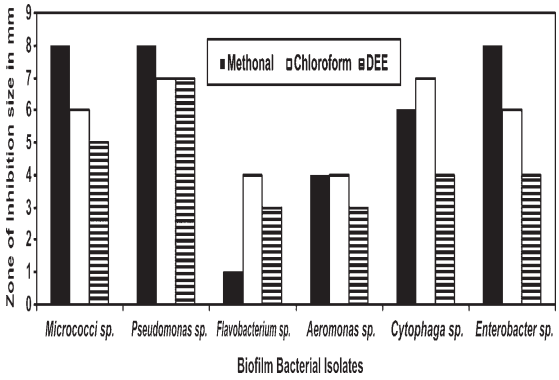

Fig. 4. Antibacterial activity of the marine alga *Caulerpa* sp.

showed an appreciable level of inhibition zone against *Flavobacterium* sp. (9 mm) while *Micrococci* sp. was found to be rather tolerant (1 mm). The DEE extracts of *C. linoides* showed no activity against most of the biofilm bacteria. However, *Flavobacterium* sp. was found to be sensitive (5 mm), while *Micrococcus* sp., *Cytophaga* sp., *Aeromonas* sp. and *Enterobacter* sp. were found to have traces (<1 mm) of inhibitory zone (Fig. 5).

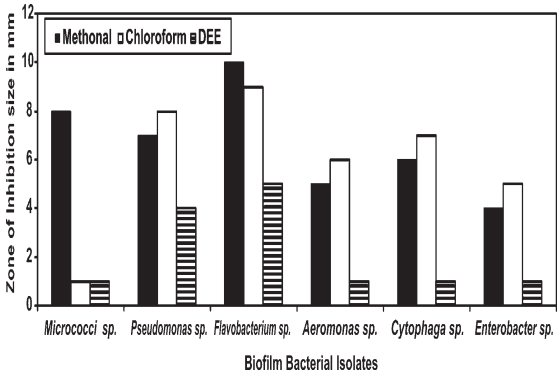

Fig. 5. Antibacterial activity of the marine alga *Chaetomorpha linoides*

Discussion

The primary biofilm provides a supporting substrate for subsequent attachment of other fouling organisms. The deterrence of the formation of this layer is fundamental to effective control of further large scale biofouling. The marine macroalgae are well documented as possessing generalised antibacterial and antifungal activity (Caccamese *et al*., 1980, 1985; Naqvi *et al*., 1980; Padma Sridher *et al*., 1984; Pesando and Caram 1984; Bernard and Pesando 1989; Vlachos *et al*., 1996). Pholorotannins from brown algae have been implicated in antifouling (Sieburth and Conover, 1965; McLachlan and Craigie, 1966; Langlosis, 1975; Fletcher, 1989). Philipps and Towers (1982) suggest that the red alga *Rhodomela larix* may act as an antifoulant. De Nys *et al*. (1995) showed that the red alga *Delisea pulchra* produced a suite of unique secondary metabolites, furanones, that inhibit surface colonization traits in epiphytic marine bacteria without toxicity.

Among the tested biofilm bacteria, *Micrococci*

sp. and *Pseudomonas* sp. were found to be sensitive when compared to all the other heterotrophs. *Flavobacterium* sp. and *Cytophaga* sp. showed moderate sensitivity, while *Aeromonas* sp. and *Enterobacter* sp. were found to be resistant in some cases. However, from the present study, it is evident that all the five seaweeds possess antifouling compounds. *Ulva* sp., and *Caulerpa* spp. were found to have remarkable activity, while *Padina* sp. showed mild activity. The degree of antibacterial activities of the seaweed extracts was found to be solvent specific. The algae extracted in methanol and chloroform was found to show considerable antifouling activity, while DEE exhibited poor activity against biofilm bacteria. Sastry and Rao (1994) showed antibacterial activity against both gram positive and gram negative pathogenic bacteria after successive extraction with benzene, chloroform and methanol. Similarly, Mahasneh *et al*. (1995) have shown antibacterial activity in organic extracts of six species of marine algae against multi-antibiotic resistant bacteria.

In the present study, almost all the algal extracts showed antifouling activity against most of the biofilm bacteria tested indicating that these algae are potential sources of antifouling compounds. This may be further investigated with various fractions of the extracts. Caccamese *et al*. (1985) reported that brown and red algal extract showed higher antibacterial activity against human pathogenic bacteria like *Bacillus* sp. and *E. coli*. Similarly, Padmini (1991) reported that red and brown algal extracts showed greater antibacterial activity than green algae. On the contrary, in the present study, green algae were found to be more effective against biofilm forming bacteria compared to brown algae (*Padina* sp.). The variation in the effects of algal extracts suggests that they are not simply functioning as broad spectrum toxins against marine biofilm bacteria; rather they appear to have specific activities against one or several biofilm bacteria.

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