Surface associated bacteria of marine algae in kovalam beach, Chennai, had screened for its antifouling activity

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Surface associated bacteria of marine algae have a role to play in antifouling strategy for marine algae. In the present study, totally 15 epiphytic bacterial isolates were obtained from two marine algae of Kovalam Beach, Chennai, India, using specially designed media. Among 15, 2 epiphytic bacterial isolates (SC2 and SC3) from Stoechospermum polypodioides showed maximum zone of inhibition against fouling bacteria in the antibacterial well diffusion assay method. Attempt was made to identify positive antifoulers by their morphological, biochemical and molecular characteristics. Results of the present study revealed that the positive antifoulers were Vibrio sp. and Exiguobacterium sp. and may have a role to play as antifouling agent for Stoechospermum polypodioides.

[Keywords: Epiphytic bacteria, Stoechospermum polypodioides, Exiguobactrium sp., Vibrio sp, Antifouling]

Introduction

Biofouling is divided into microfouling and macrofouling, where microfouling involves a biofilm formation and bacterial adhesion and macrofouling involves attachment of larger organisms, of which the culprits are barnacles, mussels, polychaete worms and bryozoans. So far, the most effective methods of biofouling control are based on the application of toxic substances like tributyl tin (TBT), copper or organic compounds and are found as pollutants in aquatic environment. Therefore, there is clear need for the development of “environmental-friendly” non-toxic antifoulants. Where this compound should have repellent or settlement inhibition property without being biocide is most recommended. Antifouling agents has been isolated from various sources such as sponges, corals, mollusks, echinodermata, seagrasses, cyanobacteria, algae and bacteria. Bacteria have little advantage to sound them as a better source than the others. Such as, it can be cultured quickly by means of fermentation, molecular alteration will be easy for better need, product yield and production will be quick and cost effective for better marketing. But only a countable works had been tried and reported with bacteria.

Nature has always guided us to find the better solution, which seems true with this too; many marine organisms have evolved efficient strategies to combat epibiosis. Where marine algae employ a number of physical and chemical defense systems to prevent fouling, such as the shedding of outer layers of cells or production of inhibitory compounds. However antifouling agent is costly for developing from this means. It has been hypothesized that the marine algae Ulva lactuca which has neither physical nor chemical defenses, relies on microbial defense. It has been already reported that marine algal surface are covered by bacterial biofilms were they have high competitive environment in which space and access to nutrients are limited. The distribution of bacterial populations on algal surface suggests that it get benefited from algae in the form of nutrients and in other hand colonization is not simply a fouling process for algae, but a process mediated by algae to protect it from advance fouling. Bacteria which take advantage of such interactions and secure a place on the algal surface may well provide them with an edge over other bacteria. This algae-bacterial relationship is symbiotic in most cases and these surface associated bacteria play a protective role for algae by releasing bioactive compound into surrounding seawater that help in preventing subsequent colonization by micro and macro-organisms. The strategy of the above has been mentioned in Fig. 1. Therefore, these surface associated bacteria are attracting attention as a source of new natural product. So an attempt had taken in this present study to screen out the bacteria which might be playing a role of antifouling agent for available marine algae of Kovalam Beach, Chennai, India.
Materials and Methods

Available marine algae was collected from the rocky shores of Kovalam Beach, \(12°50'N, 79°45'E\) Chennai, Tamil Nadu, during the month of December and was used as a source of epiphytic bacteria. The collected marine algae were brought to the laboratory in sterile bags for further investigation. The collected marine algae were identified as *Stoechospermum polypodioides* and *Ulva lactuca* based on their vegetative and morphological characteristics.

Algae surfaces were thoroughly washed with sterile sea water to get rid of the unbound bacteria, the unwashed or the strongly bound bacteria with the marine algae were considered as the epiphytic bacterial sample. Algae fronds made into 2 cm and directly placed over designed algae incubated sea water agar (AISA) plates to obtain the epiphytic bacteria. Plates were incubated at 28±2°C for 24 to 48 h. Colonies were sub cultured by repeated streaking to isolate the pure culture.

The sea water was sterilized by autoclaving at 121°C for 15 min and the sterilized sea water was transferred to the marine algae and incubated at ambient temperature for 24 h. Water was filtered through filter paper (Whatman No 1), and added with 5% of agar and NaCl-2.4 g per 100 mL. Fouling bacteria were isolated from the rock and boat surface in the intertidal zone of Kovalam Beach, Chennai after washing their surface with sterile seawater. The Zobell Marine agar was used for the cultivation of fouling bacteria. The plates were incubated at 28±2°C for 24 to 48 h.

The antibacterial well diffusion assay was carried out according to the method of Burgess *et al.* (2003) & Francois *et al.* 2009 as follows. A single colony of isolated epiphytic bacteria were used to inoculate in 5 mL Zobell marine broth and incubated at 28°C for 24 h. From this, 25 µL of culture was added to the well made on Zobell marine agar plate which had been freshly swabbed with 10 µL liquid cultures of the fouling bacteria. Plates were incubated overnight at ambient temperature. Zone of inhibition around the well was considered as evidence of antifouling activity of the epiphytic bacteria.

The epiphytic bacteria which showed zone of inhibition were characterized using various morphological characteristics (nature of the colony, Gram staining, motility and shape) and biochemical tests (Indole, methyl red, Voges proskauer, citrate utilization tests, urease and sugar fermentation tests). All the above mentioned biochemical tests were performed by following standard methodology given in the microbiological laboratory manual by cappuccino.

The genomic DNA was isolated from the positive antifoulers and subsequently amplified by PCR using the universal primers 5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAA GGAGG TGATC CAGCC-3' respectively. Amplified PCR products were then analyzed in a 1.0% (w/v) agarose gel. The DNA band was excised from the gel and sequenced for 16S-rDNA using the ABI 3130 Genetic Analyzer. Nucleotide sequence obtained was compared with the known bacterial sequences available in the National centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). Secondary structure of the bacterial 16S rDNA was predicted using GENBEE software. Restriction site present on the bacterial 16S rDNA was predicted using the NEB Cutter program.

Results

In the present study, 15 epiphytic bacteria were isolated from the marine algae and named as SC1, SC2, SC3, SC8, SC9, SC11 and SC12 from *Stoechospermum polypodioides* and SC4, SC5, SC6, SC7, SC10, SC11, SC13, SC14 and SC15 from *Ulva lactuca*. Fouling bacteria RF1, RF2, RF3 and RF4 were isolated from rock surfaces and BF1, BF2 and BF3 were isolated from boat according to the method followed by 15. Among 15 isolates, SC2 and SC3 from *Stoechospermum polypodioides* showed the maximum zone of inhibition 12 mm and 8 mm respectively against BF2 fouling bacteria and minimum zone of inhibition was found against other
fouling bacteria in the antibacterial well diffusion assay (Figs 2 and 3 and Table 1). Bacterial isolate SC2 was observed as creamy white, smooth colonies on algae incubated seawater agar medium. Gram staining report showed that the SC2 isolate is gram negative, rod shaped and motile. Isolate SC3 was observed as orange pigmented colonies on algae incubated seawater agar medium and the Gram staining report showed, it is gram positive, rod shaped and motile. Various biochemical characteristics were used for the identification of the epiphytic bacterial isolates SC2 and SC3. The isolate SC2 was found to be positive for Indole, Methyl red, Voges Proskauer and citrate test. It can ferment glucose, lactose and sucrose. Isolate SC3 was found to be positive for Methyl red and citrate test. It can ferment glucose, lactose and sucrose. The 16S rDNA sequence showed, the isolates SC2 and SC3 were identified as *Vibrio* sp. and *Exiguobacterium* sp. respectively. BLAST result showed that both the isolate SC2 had 99% similarity with *Vibrio* sp (G0907039), And SC3 had 99% similarity with *Exiguobacterium* sp (EU073122.1). The 16S rDNA sequences were submitted to the GenBank database (NCBI) and assigned the accession No HM016871 for *Vibrio* sp. and HM016870 for *Exiguobacterium* sp. Secondary structure of *Vibrio* sp. 16S rDNA showed 23 loops and 57 stems with an overall free energy of -227.0 kkal/mol (Fig. 4). 16S rDNA of *Exiguobacterium* sp. showed 23 loops and 51 stems with an overall free energy of -279.6 kkal/mol (Fig. 5). Restriction sites present on 16S rDNA of *Vibrio* sp. showed 48 restriction sites and 54% GC and 46% AT contents (Fig. 6) and 16S rDNA of *Exiguobacterium* sp. showed 48 restriction sites and 57% GC and 43% AT contents (Fig. 7), respectively.

<table>
<thead>
<tr>
<th>Fouling bacteria</th>
<th>SC2</th>
<th>SC3</th>
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<tbody>
<tr>
<td>RF1</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>RF2</td>
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<td>1.3</td>
</tr>
<tr>
<td>RF3</td>
<td>3.8</td>
<td>-</td>
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<tr>
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<tr>
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<td>8</td>
</tr>
<tr>
<td>BF3</td>
<td>1.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table 1—Screening result of antifouling activity by SC2 and SC3 against fouling bacteria.
Discussion

Little is known about the establishment and ecological role of microbial communities on the surface of marine algae. However, the abundance of bacteria that produce extracellular inhibitory compounds on the surface of the marine algae *Ulva australis* has prompted speculation that these organisms may protect the alga against fouling\(^{19-21}\). It has been proved that *pseudoalteromonas tunicate* an epiphytic bacteria of *Ulva lactuca* play role of antifouling by producing 190-kDa protein (ALpP) inhibitory compound\(^{19,22}\). In this study we explored some of the common available algae from Kovalam beach for which antifouling strategy played by its surface associated bacteria. Interestingly in our initial screening we found bacteria isolated from surface of *Stoechospermum polypodioides* inhibiting the fouling bacteria, which were isolated from boats of same Kovalam Beach. So here, it might be like algae and its associated bacteria *Vibrio sp* and *Exiguobacterium sp* have a strong interaction and these bacteria might help algae from not getting major fouled.

Conclusion

Bacteria may have highly specific, symbiotic relationships with their “hosts” and important source of antifouling compounds. These bacterial candidates may be a promising source of biotechnologically interesting substances for “environmentally-friendly” antifouling application\(^{23}\). Though a huge research is going on worldwide for the detection of a novel green antifouling agent from various sources.

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References