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Antagonistic Effect of Epiphytic Bacteria from Marine Algae, Southeastern India

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Abstract: Aim of this study was to evaluate the antagonistic potential of epibiotic bacteria from seaweeds, *Ulva lactuca, Dictyota dichotoma* and *Padina tetrastromatica* against some potent human pathogens. The epibiotic bacteria of *Ulva lactuca* shows higher level of inhibition properties than the other species. The strain UL1 shows broad spectrum inhibitory activity against 7 pathogens. The inhibitory level of epibiotic bacteria ranged from low to moderate activity. The present investigation suggests that the epibiotic bacteria are good source for the isolation of antibacterial compounds of biomedical importance. The compounds can further be purified and can used to save mankind from dreadful diseases.

Key words: Epibiotic bacteria, antagonistic effect, human pathogens, tuticorin coastal waters

INTRODUCTION

Ocean covers approximately 70% of the earth's surface. Marine plants, especially seaweeds are potential resources in the marine environment. They divided into green, brown, red and blue-green algae. About 271 genera and 1153 sp of marine algae have been reported from Indian coast (Kaliaperumal, 2006). The use of seaweeds as manure in coastal area is very common, they contains macro nutrients, trace elements, organic substances like amino acid, plant growth regulators suitable for root crop because of their potash content (Chapman, 1980). Epiphytic microbes on marine plants can be in various statistics and species which are available in sea water and Gauthier (1977) have reported the isolation of antibiotic producing marine bacteria from algae. Isolation of marine bacteria from marine plants and other organisms showed a series of notable bioactive properties (Chellaram and Anand, 2011, Chellaram and Edward, 2009). Initially number of important antibiotics from marine resources were identified and characterized by Burkholder *et al.* (1960). Provided higher ratio of antibiotic producing bacteria are maximum available in the biofilm of the marine organisms (Chellaram *et al*., 2012a; Lemos *et al*., 1985). There are certain epiphytic bacteria attached with rich nutrient marine plants and invertebrates which also produces potential secondary metabolites and it would inhibit the deposition of potential competitors (Bernan *et al*., 1997; Chellaram *et al*., 2012b).

The multi-drug resistance bacterial strains are becoming a nuisance to humans and a constant lookout is necessary to combat this problem. Also, the advancement of human race is associated with new diseases. So new drugs are necessary for human survival and new sources are being assessed for drug leads. The isolated marine bacteria from sea weeds, mollusk etc., contains metabolites which has biomedical importance. The surface associated bacteria have been demonstrated to possess potential bioactive substances. So it has been planned to isolate the epibacteria from the surface of *Ulva lactuca, Dictyota dichotoma* and *Padina tetrastromatica.*

MATERIALS AND METHODS

Study area: The seaweeds were collected from Tuticorin coast (Lat. 8°45'N; Long. 78°10'E) of Gulf of Mannar, Southeast Coast of India. Gulf of Mannar, a marine biosphere reserve established in 1989, harbours biodiversity of global significance and is unique for coral reef, seaweed and sea grass ecosystems.

Isolation of epiphytic bacteria: The screening of seaweed associated bacteria for anti microbial defense was carried

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out by following the method outlined by Boyd *et al.* (1999). The seaweeds, *Ulva lactuca, Dictyota dichotoma* and *Padina tetrastromatica* collected from intertidal area of Tuticorin coast, brought to laboratory and rinsed with sterile seawater to remove loosed attached bacteria. A sterile plastic film with 1 cm^2 hole was placed on the surface of the seaweed. The area within the hole was swabbed with sterile cotton swab, placed in tubes containing 9 mL sterile seawater and serially diluted up to 10^{-6} dilution and plated on Zobell marine agar (Himedia, Mumbai) using pour plate technique. The plates were incubated at room temperature for 48 hours. The colonies were counted and expressed as Colony Forming Unit (CFU) per cm^2. Colonies with different morphology were selected and tested for antibacterial assay. Fifteen epiphytic bacterial strains from seaweed were selected for further study. The designated codes such as UL, DD and PT were given for strains from *Ulva lactuca*, *Dictyota dichotama* and *Padina tetrastromatica* respectively. The isolated epiphytic bacterial strains were stored in Zobell marine agar slant at 4°C for further assay.

Antagonistic assay: The antibacterial activity was determined against the ten human pathogens such as *Staphylococcus* sp., *Streptococcus* sp., *Bacillus subtillis*, *Escherichia coli*, *Pseudomonas* sp., *Shigella* sp., *Proteus* sp., *Salmonella* sp., *Bacillus cereus* and *Bordetella* sp.

The antibacterial assay was carried out by the method of Lima-Filho *et al.* (2002) with modification of the expression of the results in percentage of inhibition. The isolated epibacterial genera were individually applied as a single streak on the Zobell marine agar (Himedia, Mumbai) plates and incubated at room temperature for 48 h. Then the test bacterial strains (human pathogens) were applied as a single streak perpendicular to the epibacterial streak without touching the epibacterial strain streak. The plates were incubated for another 24 h and the inhibition zones were compared and scored with that of control as high (81-100% inhibition), moderate (21-80% inhibition), low (1-20% inhibition) and nil (no activity).

RESULTS

The bacterial load in the surface of the seaweed was represented in Table 1. The population load was high on *Ulva lactuca* followed by *Padina tetrastromatica* and *Dictyota dichotoma* with 85×10^2, 78×10^2 and 65×10^2 CFU cm^{-2}, respectively. In the present study three seaweeds were screened and a totally 45 different bacterial strain were isolated, purified and preserved. All of the isolated bacterial strains were tested for their antimicrobial activity by cross streak method. Antimicrobial activity was assessed against 10 different human pathogens. Epiphytic isolates which shown clear zone of inhibition against at least one of the pathogenic strains was tabulated. The activity against the human pathogen was low to moderate in the present study. The epibacterial strain UL1 and DD2 were shown to produce high level activity in the present study. There was no activity against *Pseudomonas* sp. which indicated that they are resistant to all the epibacterial strain in this study. The activity against *Bacillus subtilis* and *Shigella* sp. were relatively low with all the epibacterial strain. The percentage of inhibition against human pathogens was tabulated in Table 2.

Table 1: Percentage of epiphytic bacterial active strains

Name of the algae	No.of strains isolated	No.of active strain	Percentage active strains
Ulva lactuca	15	1	6.7
Dictyota dichotoma	15	2	13.3
Padina tetrastromatica	15	1	6.7

Table 2: Antibacterial activity of epibacteria isolated from the seaweeds

	Zone of inhibition (%)			
Humanpathogens	UL1	DD1	DD2	PT1
Staphylococcus sp.	25	20	20	-
Streptococcus sp.	30	5	-	-
Bacillus subtilis	5	-	10	-
Escherichia coli	-	-	15	-
Pseudomonas sp.	-	-	-	-
Shigella sp.	-	-	5	-
Proteus sp.	10	10	15	-
Salmonella sp.	5	-	10	15
Bacillus cereus	15	-	-	-
Bordetella sp.	20	-	30	-

[No activity (-), High activity (81-100%), Moderate activity (21-80%), Low activity (1-20%)]

DISCUSSION

Beneficial association between surface bacteria and their host have previously been reported. The epibacteria on the larvae of some crustaceans produce antimicrobial compounds and protect the host from fungal infection (Gil-Turnes *et al.*, 1989). The epibacteria isolated from a tunicate inhibited the settlement of barnacle and tunicate larvae (Holmstrom *et al.*, 1996). Epibacteria, due to their ecological significance and evolution produce novel chemical substances so, they may form the basis of new drug leads.

The highest epibacterial load observed in the surface of *Ulva lactuca* was (85×102 CFU mL^{-1}) is very high when compared to the work of Premila *et al.* (1996) who reported 16×10^1 CFU cm^{-2} load on the same seaweed. Also was found to be high than the *Chaetomorpha linoides* (15×10^1 CFU cm^{-2}) by Shiba and Taga (1980). The low level of epibacteria on the surface of seaweed indicated that the seaweed possess some inhibitory substance. However the presence of epibacteria could be attributed to its selective inhibition. The less number of epibacteria could also be attributed to the surface characteristics of the seaweed which plays a key role in controlling the epibacterial population density (Conover and Sieburth, 1964). The presence could also be due to suppression of inductive strains and enhancing the growth of non-inductive strains (De Nys *et al*., 1995). Laycock (1974) postulated the requirement of surface bacteria for the proper development of the seaweed.

The screening of human pathogens against the macro algal epibacteria in the present study indicated that the epibacteria possess inhibitory activity. This is in line with the concept demonstrated by Lemos *et al*. (1985). In this context, the marine bacterium offers much scope, though in many instances the compounds are produced by non culturable bacteria (Armstrong *et al*., 2001). The present study suggests that the possibility of epibacterial strains may produce antimicrobial substances of biomedical importance. The epibacteria could be further explored as a potential source of novel antimicrobial compounds against human pathogenic bacteria.

CONCLUSION

This study showed that the epiphytic bacteria from seaweeds, *Ulva lactuca and Dictyota dichotoma* have highest antagonistic effect against *Staphylococcus* sp., *Streptococcus* sp. and *Proteus* sp. The bioactive of the compound has to be tested against other human pathogenic bacteria in order to make it as a wide range of antibiotics. It is obvious that strain UL1 DD1 and DD2 constitutively produces a novel compound which may be the substance responsible for antimicrobial activity. Thus, the epibiotic bacteria associated to this these seaweeds may yield a vast array of new compounds with novel activities that will provide new drugs in the fight against a number of pathogens currently resistant to conventional antibiotic therapies.

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