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Research Article

ANTAGONISTIC EFFECT OF HARD CORAL ASSOCIATED BACTERIA FROM TUTICORIN COASTAL WATERS

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ABSTRACT

The objective of this research to isolate the coral reef associated bacteria against 10 selected pathogens. The isolation and screening of the bacteria strains were done by pour plate method on Zobell Marine Agar (ZMA) medium. The coral associated bacteria were tested for antagonistic effect by double agar overlay method and partial identification of bacteria was used by morphological and gram staining method. Culturable heterotrophic marine bacteria present on the surface of coral *Acropora nobilis* was isolated. Among the isolates a broad spectrum activity was observed by the AN19 and AN161 strains with a zone of inhibition of 6 against E. coli and Bacillus cereus. This study is to assess the production of secondary metabolites with bioactive substances by coral reef associated bacteria was carried out from coral species, *Acropora nobilis*.

Keywords: *Acropora nobilis*, Antagonistic bacteria, Human pathogens, Tuticorin coastal waters.

INTRODUCTION

Marine microorganisms have developed unique metabolic and physiological capabilities that not only ensure survival in extreme habitats but also offer the potential for the production of metabolites which would not be observed from terrestrial organisms[1]. Marine microorganisms are of considerable current interest as a new and promising source of biologically active compounds. They produce a variety of metabolites, some of which can be used for drug development. Interactions between epibiotic marine bacteria and their host organisms are known to play a significant role in marine ecosystems but this association has received little attention. The important microhabitats for marine bacteria are the sediments, biotia and abiotic and internal tissues of invertebrates. Marine animals are well known to have developed symbiotic relationships with numerous microbes. This is particularly true of the bacteria, which are widely distributed on the surfaces and within the tissues of marine animals. The importance of bacterial symbiosis is growing in recognition that bacteria may be the true producers of many compounds isolated from marine organisms[1].

The study of marine bacteria has also led to the realization that microorganism form specific symbiotic relationship with marine organisms which may be responsible for the production of some bioactive compounds[2]. Corals have also long been known to harbor many different species of bacteria in their gastric cavity and on their surface, where their carbohydrates rich mucus is exploited as a medium for microbial growth[3]. Other invertebrates, such as sponges, may harbor photosynthetic bacteria within their tissue, much as the corals harbor algae. It has also been known that many of these bacteria not only provide a substantial nutrient source for the corals, but also provide many antibacterial substances against competing strains of bacteria. Sajad et al (2011)[4] had analyzed pathogenic microorganisms for found them to produce antimicrobial compounds. Thus, corals were found to produce and be endowed with their own microbial defenses, but the strains which inhabited on their surface were found to be producing their own defenses. A few studies have suggested that coral may associate with specific microbes. Santavy (1995)[5] observed that *Porites astreoides* samples collected from throughout the Caribbean harbored bacteria-filled ovoid. It has also been shown that some corals harbor nitrogen-fixing microbes to obtain fixed nitrogen from associated microbes that are fed and protected in an anaerobic environment within the colony[6].

Studies related to the antagonistic activity of epiphytic bacteria attached to the corals against human bacterial pathogens are too meager. So the present work is also aimed to isolate the epiphytic bacteria from the *Acropora nobilis* and to test the inhibitory effect of the isolates against selected human ten bacterial pathogens.

MATERIALS AND METHODS

The epibiotic bacteria were collected by swabbing a tiny area (2-3 cm^2) of the surface of live hard corals, *Acropora nobilis* using a sterile cotton swab from the Tuticorin coastal waters (Lat 8º45 and Long 78º13'E). Then the swabs were placed in sterile bags, brought to the laboratory under aseptic condition for the isolation of epibiotic bacteria. The swab was then placed in 2 ml of sterile seawater and vortexed. From this, serial 10-fold dilutions of each of the samples were prepared and aliquots of 0.1 ml were plated on Zobell Marine Agar 2216 (ZMA) according to the method of Chelossi *et al*, (2004)[7] and Reddy *et al*, (2010)[8] by employing the conventional pour-plate technique in triplicates. Plates were incubated for a week at 24-27ºC. The number of pigmented and non-pigmented and gram positive and gram negative strains was noted. Perceptible different morphotypes were isolated in pure culture on ZMA. Axenic culture were obtained by streaking and re-streaking on ZMA plates and subsequently stored in ZMA slants at 4ºC.

Antagonistic assay against pathogens:

To test the antagonistic effect of the isolated bacterial strains against 10 selected human pathogens such as, *Bacillus cereus* (ATCC 10876), *B. subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *S. epidermidis* (ATCC 12228), *Enterobacter aerogenes* (ATCC 13048), *Streptococcus pneumoniae* (ATCC 6301), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Shigella dysentriae* (ATCC 13313), *Klebsiella pneumoniae* (ATCC 10031) and *Vibrio cholerae* (ATCC 15748). All the human pathogens and were obtained from Christian Medical College (CMC, Vellore, India). Double agar overlay method was used for the assay of antagonistic bacteria against the test pathogens[9]. Colonies of antagonistic bacteria were developed on ZMA plates by spotting 12-18 hour old culture and incubating at 24-27ºC for 40 hours. All the test organisms were cultured in Tryptone Soya Broth (TSB) and the 12-18 h old cultures were used for the experiments. About 10 µL of the culture was suspended in 8 ml of soft Tryptone Soya Agar (TSA) with 0.7% w/w agar was poured immediately over the macro-colonies of the antagonistic marine bacteria on the ZMA plates. The plates were incubated at 24-27ºC for 24 hours. The cleared zone around the macro-colonies of the antagonistic bacteria was measured and the radius of zone of inhibition was noted in mm.

RESULTS

Out of the 324 bacterial strains isolated from the coral surface, 73% (236) were identified as Gram-negative, 87% (283) were non-pigmented and only 12% (39) were producers. 62% (24) of the

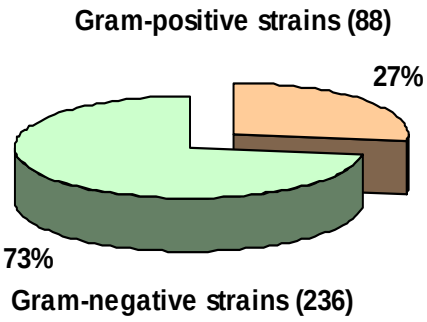
Gram-negative strains and 77% (30) of the pigmented strains showed activity.

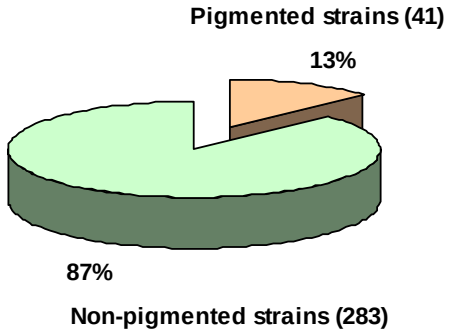
Table I shown the inhibitory activity of epiphytic bacteria isolated from *Acropora nobilis* against selected 10 human pathogens. It was noted that the strains AN19 and AN161 showed inhibition against *Escherichia coli* and *Bacillus subtilis* respectively. The other strains inhibited the test strains moderately with maximum zone sizes of not more than 5.5 mm. It was noted that all of the producers inhibited at least eight of the test bacterial strains (Fig.1).

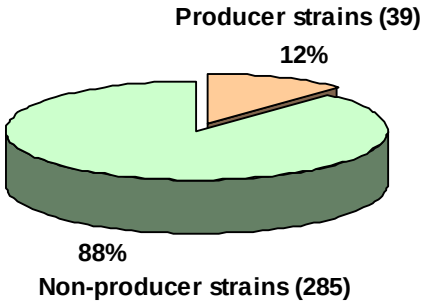
Table I: *Acropora nobilis* associated bacteria exhibiting antagonistic activity against human pathogens

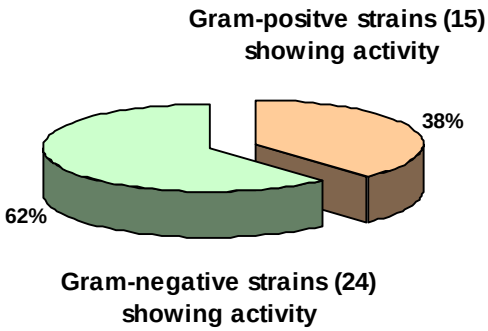
Pathogens	*E. coli*	*Shigella dysentriae*	*Staph. epidermidis*	*Staph. aureus*	*Klebsiella pneumonia*	*Pseud. aerogenosa*	*Vibrio cholerae*	*Strep. pneumonia*	*Strep. faecalis*	*B. cereus*
Isolates	**Radius of the zone of inhibition (mm)**									
AN4	5	3	3.5	3	4	4.5	4.5	3.5	4.5	3.5
AN19	6	3	5	3	3	1.5	4	5	4	2.5
AN47	3	3.5	T	T	T	1.5	2.5	T	T	T
AN71	4	3.5	4	3	2.5	3	T	3	4	3.5
AN87	3.5	2.5	3	2	2.5	3	3	2.5	3	2.5
AN95	3	4	3	4	2	3.5	2	T	3	T
AN130	2.5	2	1.5	T	-	T	3	2.5	1.5	1.5
AN141	3	2	3	3.5	4	3	T	2	2.5	T
AN149	2	1.5	T	2	2.5	3.5	T	1.5	T	4
AN152	3	3.5	4	2.5	3	4.5	4	4.5	4.5	2.5
AN161	3.5	2	2	3	2.5	3.5	3.5	4	5	6
AN175	3.5	2	1.5	3	3.5	4	2.5	3	1.5	3
AN180	4.5	4	3	3.5	2.5	3.5	2.5	3	3.5	3.5
AN189	2	2	1.5	T	2.5	3	3.5	3	2.5	5.5
AN196	3	2	3	3.5	3	2.5	2	2.5	2	3
AN203	2.5	2	3.5	3	2.5	3	2.5	2	3	T
AN205	1.5	T	-	-	1.5	T	-	T	T	3.5
AN221	3.5	3.5	3	4	3.5	3	2.5	3	3.5	4
AN230	2.5	2	2.5	3.5	3	3.5	2.5	3	4	3.5
AN247	2.5	3	3.5	2.5	3	4	3.5	4	3.5	3
AN258	4	4.5	4	3.5	4.5	4	3	5	4.5	5
AB271	4.5	5	4.5	5.5	3.5	4	3.5	3	4.5	5.5
AN280	3.5	3	2.5	3.5	4	3.5	3	2.5	2	2.5
AN321	2	1.5	3	2.5	3	1.5	2	2.5	2	3

T- Trace, - Nil









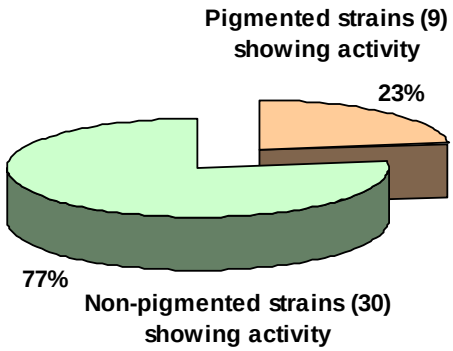


Fig. 1: Bacterial strains isolated from *Acropora nobilis* and antagonistic effect

DISCUSSION

The production of antimicrobial substances by marine bacteria isolated from the different locations in the sea has been known for a long time. Some of these bacteria show significant antibacterial properties[10,11]. Microbial natural products remain one of the most important sources of lead compounds for the pharmaceutical industry. Despite a shift toward alternative sources such as synthetic combinatorial libraries, the pharmaceutical pipeline remains filled with traditional, microbial-derived products or their derivatives[12]. It is generally believed that marine invertebrates are sources of a diverse array of bioactive metabolites with great potential for development as drugs and research tools. However, in many cases, microorganisms are known or suspected to be the biosynthetic source of marine invertebrate natural products. Over the past decade, marine microorganisms have become recognized as an important and untapped resource for novel bioactive compounds. Marine bacteria have been recognized as new sources of therapeutic leads and have shown to be capable of producing a large number of diverse chemicals[13]. The growing interest in this resource is adequately demonstrated by the number of novel metabolites recently reported from marine bacteria and the number of research laboratories throughout the world working in this field[14]

In the present study, out of the 632 bacterial strains were isolated from the coral *Acropora nobilis*, a majority of the strains were identified as Gram-negative (73.5%) and non-pigmented (87.5%). There is already a work by Wahbeh and Mahasneh (1984)[15] to report the heterotrophic bacterial load isolated from the seagrasses, A total of 9 genera were identified and out of these, three were gram negative. Gnanambal *et al*, (2005)[16] had observed that out of the 352 bacterial strains isolated form the gorgonid corals, *Subergorgia suberosa* and *Junceella juncea,* 61% were identified as Gram-negative. A higher number of Gram-negative strains observed in the present study also supported by the work of Fenical (1993)[1] who reported that the bacteria present in seawater are mainly Gram-negative rods. Lower number of pigmented strains obtained in the present study is in line with the findings of Jeyasekaran *et al*, (2002)[17] who have reported that pigmented bacterial population in the marine samples from seawater, sediments, sea plants and bivalves was lower by about 2-3 log counts than the total culturable bacterial population. Also Strahl *et al*, (2002)[18] had revealed that the bacterial strains isolated from various regimens of the marine environ showed that 82.76% were Gram-negative. Another work by Chelossi *et al*, (2004)[7] deals with similar aspects and their findings imply that 58% of the aerobic heterotrophic bacterial strains isolated from the sponge, *Petrosia ficiformis* were identified as Gram-negative.

It was noted that the epiphytic bacterial strains isolated from *A. nobilis* (AN19 and AN161) inhibited the growth of E. coli and *Bacillus subtilis* to 6 mm respectively and the strains AN19 and AN161 inhibited the growth of *Staphylococcus epidermidis and Streptococcus feacalis* to 5 mm respectively. There is a work of Devereux *et al*, (2003)[19] to report that bacterial isolates (11%; 12/111) of the submerged macrophyte, *Vallisneria americana* inhibited the growth of fungus *P. aphanidermatum*. In general, bacterial activities in coral beds are high due to the increased availability of organic matter and nutrients. Inhibition of the bacterial pathogenic strains by the antagonistic bacteria isolated form the marine samples are already reported earlier by Patil *et al*, (2001)[20]. Inhibition zones of up to 5 mm were observed for 3 coral species against human pathogens as reported by Jeyasekaran *et al*, (2002)[16]. A higher degree of inhibition was conferred by 3 of the isolates of the gorgonian corals isolated from *Subergorgia suberosa* and *Junceela juncea* with maximum zones of inhibition against *Escherichia coli* (5.5 mm) as reported by Gnanambal *et al*, (2005)[15].

CONCLUSION

Thus it is generally concluded that numerous natural products from marine invertebrates show striking similarities to known metabolites of microbial origin, suggesting that microorganisms are at least involved in their biosynthesis or are in fact the true sources of these respective metabolites. Thus, in the present investigation, the recovery of strains with antibacterial activity suggests that hard corals represent an ecological niche which harbors a largely uncharacterized microbial diversity and a yet unexploited potential in the search for new secondary metabolites.

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