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Short Communication

Isolation of antagonistic marine bacteria from the surface of the gorgonian corals at Tuticorin, south east coast of India

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Culturable heterotrophic bacteria present on the surface of two gorgonian corals, *Subergorgia suberosa* and *Junceella juncea* were isolated and the number of pigmented and non-pigmented strains was noted. The antagonistic effect of the marine isolates was tested against 8 human pathogens and 7 fish pathogens. It was observed that 82% (289) of the isolated bacterial strains were found to be non-pigmented and 61% (214) were identified as Gram-negative. Only 13% (46) of the isolated bacteria were found to be antagonistic against both human and fish pathogens. 74% (34) of the producer strains were found to be non-pigmented, however, mild antagonistic activity was found to be exhibited also by the pigmented strains. Percentage of Gram-negative strains showing activity was found to be 68% (145). All the indicator strains tested were inhibited by at least 10 of the antagonistic marine bacteria. A higher degree of inhibition was conferred by 3 of the isolates (G_{110}, G_{111} and G_{113}) with maximum zones of inhibition against *Escherichia coli* (5.5mm) by the strain G_{113}. The strains showing higher degree of inhibition against human pathogens (G_{110}, G_{111} and G_{113}) showed potent inhibition against the fish pathogens too with maximum inhibition against *Proteus mirabilis* (5mm) by the strain G_{110}. The symbiotic bacteria present on the surfaces of these gorgonian corals may yield novel metabolites.

[**Key words**: Bacteria, corals, Tuticorin, *Subergorgia suberosa, Junceella juncea*, symbiotic bacteria, antagonistic activity]
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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 plants and animals are well known to have developed symbiotic relationships with numerous microbes. Microbes are ubiquitous in the marine environment and not surprisingly, mucus-covered coral surfaces are often colonized by bacteria and other microorganisms[1,2]. It appears that these microbes are not generally detrimental to their coral hosts[3]. The importance of bacterial symbiosis is growing in recognition that they may be the true producers of many bioactive compounds isolated from corals, sponges, ascidians and other marine invertebrates[4]. In the past 20 years, the pharmaceutical industry has been successful in containing problems due to single resistance determinants. However, the advent of multiple resistance mechanisms has severely limited the effective use of many major classes of drugs. So searches for new drug classes with novel mechanism of action are of relevance. There is a growing concern about the use and particularly the abuse of antimicrobial drugs not only in human medicine but also in aquaculture. Hence, the need of the hour is a search for novel antibacterial compounds with therapeutic potential for which the pathogens may not have developed resisitance[5]. The symbiotic microorganisms from the marine environs are a rich source of new metabolites with a wide variety of biological activities and some of them display significant practical applications[6]. There are studies which have focused on the metabolites of the gorgonian corals *Eunicea fusca*[7], *Lophogorgia* sp.[8], *Pseudopterogorgia elizabethae*[9], etc. But works investigating the symbiotic bacteria on gorgonid corals as potential sources of antimicrobial drugs are too sparse. Gorgonian corals, *Subergorgia suberosa* and *Junceella juncea* are found relatively common in turbid near-shore environs below 5 m depths. The symbiotic bacteria attached to these gorgonian corals are being less studied as sources of potent antibacterial compounds. So a preliminary attempt was undertaken to isolate the antagonistic bacteria associated with the surface of these two gorgonids.

Viable heterotrophic bacteria were obtained by swabbing a small area (1 cm^2 in triplicates) of the external surface of live gorgonian corals, *Subergorgia suberosa* (Octocorallia: Alcyanacea: Subergorgiidae) and *Junceella juncea* (Octocorallia: Alcyanacea: Ellisellidae) from Tuticorin coastal waters, south east coast of India, using a sterile cotton swab, which was then placed in 2 ml of sterile seawater and vortexed. Serial 10-fold dilutions of each solution were prepared and aliquots (0.1 ml) were plated on ZoBell Marine Agar 2216 (ZMA)[10]. Plates were incubated for 7 days at 27ºC. The number of pigmented and non-pigmented strains was noted. Perceptible different morphotypes were isolated in pure culture on ZMA. Bacterial isolates were kept in ZMA slants at 4ºC. Gram staining was performed for all the isolated strains. To test the antagonistic effect of the isolated bacterial strains, 8 human pathogens [*Bacillus cereus* (ATCC 10876), *B. subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *S. aureus* (ATCC 29737) and *Shigella dysentriae* (ATCC 13313)] and 7 fish pathogens [*Proteus mirabilis* (MTCC 1429), *Serratia marcescens* (MTCC 97), *Aeromonas hydrophila* (ATCC 7966),
A. formicans (shrimp isolate), *Micrococcus* sp. (fish isolate), *V. harveyi* (shrimp isolate) and *V. vulnificus* (ATCC 27562)] were used as test strains. All the human pathogens and the fish pathogens, *Proteus mirabilis*, *Serratia marcescens*, *Aeromonas hydrophila*, *V. vulnificus* were obtained from Christian Medical College [CMC, Vellore, India] and the remaining fish pathogens were isolated from the above mentioned sources. Double agar overlay method was used for the assay of antagonistic bacteria against the human pathogens[11]. Colonies of antagonistic bacteria were developed on ZMA plates by spotting 18 h old culture and incubating at 30ºC for 40 h. All the test organisms were cultured in Tryptone Soya Broth (TSB) and the 18-24 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 30ºC for 24 h. The cleared zone around the macro-colonies of the antagonistic bacteria was measured and the radius of zone of inhibition was noted (in mm). Antagonistic activity of the isolated marine bacteria was also tested for the fish pathogens following the method of Gauthier[12]. About 1 ml of 18-24 h old culture of fish pathogens in TSB was mixed with molten TSA (supplemented with 1% NaCl) and was poured immediately on the petriplates and allowed to harden. Marine bacteria were cultured on ZMA plates and a small amount of cell paste was scraped off and deposited onto the surface of TSA plates seeded with the test bacterium. After a diffusion time of 30 min, plates were incubated at 30ºC for 24 h. A clear zone of inhibition around the cells of marine bacteria indicated antibacterial activity and the radius of zone of inhibition was noted (in mm). Some of the producer strains were identified up to the generic level using the biochemical methods outlined in Bergey's manual of systematic bacteriology[13,14].

A total of 352 bacterial strains were isolated from the gorgonids, *Subergorgia suberosa* and *Junceella juncea* and among them 82% (288) of the bacterial strains were found to be non-pigmented of which 61% (214) were identified as Gram-negative. Majority of the pigmented colonies were yellow, red, brown, orange and black in colour. Only 13% (47) of the isolated bacteria were found to be antagonistic against both human and fish pathogens. About 74% (35) of the producer strains were found to be non-pigmented and only 26% (12) were pigmented. However, mild antagonistic activity was exhibited by many pigmented strains also. In the present study, the percentage of Gram-negative strains showing activity (32) was higher (68%) than the Gram-positive strains (32%). Screening of the marine bacterial isolates against human pathogens (Table 1) showed that all the indicator strains tested were inhibited by at least 10 of the antagonistic marine bacteria. A higher degree of inhibition was conferred by 3 of the isolates (G_{110}, G_{111} and G_{113}) with maximum zones of inhibition against *Escherichia coli* (5.5 mm) by the strain G_{113}. However, some of the strains (G_{89} and G_{90}) were able to display mild antagonism. Data for the inhibitory activity of the isolated marine bacteria against the fish pathogens are shown in Table 2. The strains showing higher degree of inhibition against human pathogens (G_{110}, G_{111} and G_{113}) showed potent inhibition against the fish pathogens also with maximum inhibition against *Proteus mirabilis* (5 mm) by the strain G_{110}. However, strains G_{162} and G_{163} showed inhibition against *Serratia marcescens* with zones of 4 mm each and G_{162} and G_{163} against *Aeromonas hydrophila*. Strains G_{189}, G_{289} and G_{320} displayed only mild activities. The present study showed that major antibiotic producing strains belong to the genera *Vibrio* ($G_{110,}$ G_{111}), *Pseudomonas* (G_{113}), *Micrococcus* ($G_{89,}$ G_{90}) and *Bacillus* ($G_{162,}$ G_{163}).

Table 1—Surface bacteria associated with gorgonids antagonistic to human pathogens
Zone of inhibition (values show radius of the zone of inhibition in mm)

Producer Strains	*Bacillus cereus*	*B. subtilis*	*Escherichia coli*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staphylococcus epidermidis*	*S. aureus*	*Shigella dysentriae*
G1	2	2.5	2	2	2	1.5	1.5	2
G5	2	2.5	2	1.5	1.5	1.5	2	2.5
G8	2.5	3	3	3	2.5	3	3	2.5
G9	2	2.5	2.5	2.5	2	2.5	2	2
G10	2	2	2	1.5	2	2	1.5	1.5
G25	--	T	--	--	2	2.5	1.5	1.5
G30	1.5	--	--	--	--	T	--	--
G32	T	--	--	1	--	--	--	--
G41	2.5	2	2	1.5	1	1	--	--
G56	2	--	--	1	T	--	1.5	--
G60	1.5	--	1.5	--	T	--	1.5	--
G67	--	--	--	--	--	--	1.5	--
G89	1	--	1	1.5	--	--	1.5	1.5
G90	1.5	--	--	1.5	--	--	1.5	--
G110	4	4	4.5	4	3.5	4	4	4.5
G111	4	4	5	4	4	4.5	4	4.5
G113	5	5	5.5	5	4.5	5	5	4.5
G162	1.5	1	1	--	--	--	--	--
G189	--	--	T	--	1	1	--	--
G203	--	T	--	1.5	1.5	1.5	1	1.5
G209	1	--	1.5	1.5	1	1.5	--	T
G225	1.5	--	--	--	--	--	--	--
G289	--	--	--	--	T	--	--	--

T= Trace; --=No activity

Table 2—Surface bacteria associated with gorgonids antagonistic to fish pathogens
Zone of inhibition (values show radius of the zone of inhibition in mm)

Pathogens	*Proteus mirabilis*	*Serratia marcescens*	*Aeromonas hydrophila*	*A. formicans*	*Micrococcus* sp.,	*Vibrio harveyii*	*V. vulnificus*
G1	2	2	2.5	--	--	T	1.5
G5	1.5	1	1	1.5	--	1.5	1
G8	--	--	--	1.5	--	T	--
G9	1	--	T	1	--	--	--
G10	T	--	1.5	1	1.5	1	1
G25	T	1.5	1.5	1	1.5	1	1.5
G30	5	--	4	4	4	4.5	4
G32	4	4	4	4	3.5	4	4
G41	4	4	3.5	3.5	4	4	3.5
G56	1	--	1	2	1	T	--
G60	1.5	4	2	2	2	1.5	1.5
G67	1.5	4	2	2	2	1.5	1.5
G89	1	1	1.5	1	1	--	1.5
G90	--	--	--	--	--	--	T
G110	2	2	1.5	1.5	1	2	2
G111	1.5	1	1	--	T	1	1.5
G113	1.5	1	1	--	1.5	1	1
G162	--	--	--	--	T	--	--
G189	2	1.5	2	2	1.5	2	--
G203	1.5	1.5	1.5	1.5	1.5	1.5	1.5
G209	1	1	--	1	T	--	--
G225	--	1.5	--	1.5	1.5	--	T
G289	1	1	--	1	1	1	1

T= Trace; --=No activity

A lower percentage of pigmented strains (18%) were isolated from the surface of the gorgonian corals, *Subergorgia suberosa* and *Junceella juncea*. This observation is similar to the findings of Jeyasekaran *et al*.[15] who reported that pigmented bacterial population was lower by about 2-3 log counts than the total culturable bacterial population observed in the marine samples. In the present study, of the 352 isolates, a total of 214 accounting to about 61% of the bacterial strains were Gram-negative which is in line with an earlier work[4] which reports that the bacteria present in seawater are mainly Gram-negative rods. A higher percentage of antagonistic strains was found to be non-pigmented (74%) which contradicts an earlier report that states that most of the antibiotic-producing marine bacteria grown on marine agar are pigmented[16]. Present finding suggests that 68% of the producer strains isolated was identified to be Gram-negative, which is in line with the view that majority of the antagonistic bacteria isolated from the Tuticorin coast of Tamil Nadu[15] were found to be Gram-negative and only one strain was Gram-positive. However this observation disagrees with the findings of Fenical[4] who reported that the Gram-negative rods isolated from the marine samples have proven to be chemically unproductive. The antagonistic bacteria isolated from the surface of the gorgonids were able to inhibit most of the test strains used for the experiment. Inhibition zones of up to 22 mm were observed for 3 coral species against human pathogens[15]. It has been documented that bacteria associated with the soft coral, *Dendronephthya* sp. are suggested to produce bioactive compounds against the attachment of bacteria onto the surface of these organisms[17]. Presence of antagonistic bacteria on the surface of this soft coral is to control biofouling. It has been hypothesized that this organism may control biofouling on their surfaces by regulating the bacterial species composition. Ducklow & Mitchell[3] found that some species of the marine bacterium *Vibrio* are adapted for living in the mucus layers of living corals. Actinomycetes, recognized as sources of antimicrobial compounds have been isolated from various marine invertebrates such as corals[18].

A better recovery of strains from the surface of these gorgonian corals with antibacterial activity suggests that these organisms represent an ecological niche which harbors a largely uncharacterized microbial variety. Thus, the symbiotic bacteria attached to the surface of these gorgonid corals may yield a vast array of new compounds with novel activities.

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