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Antimicrobial Activity of Bacteria Associated with Seaweeds against Plant Pathogens on Par with Bacteria Found in Seawater and Sediments

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Authors' contributions

This work was carried out in collaboration between both authors. Author KA designed the study, performed the statistical analysis, wrote the protocol and critically edited the manuscript. Author TS managed the analyses of the study, wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: We report antimicrobial activities of bacteria associated with 10 native and one invasive species of seaweeds on par with bacteria found in the seawater and sediment. Bacteria exhibiting antimicrobial activity were phylogenetically analysed using 16S rRNA gene.
Place and Duration of Study: Samples of seaweeds, seawater and sediments collected at 6 localities of south east coast of India between December 2009 and January 2010 during monsoon season.
Methodology: Culturable bacteria in seaweeds (epibiotics and endobiotics), seawater and sediments were isolated through serial dilutions using 1.5% ZoBell marine agar (HiMedia, India). Bacterial isolates producing antibiotics were identified by screening against commercial antibiotics and they were subjected to morphological, Gram's staining and biochemical studies. Chemical property and stability of antimicrobial substances obtained from the promising bacteria active against plant pathogens were studied. Phylogenetic analysis of antibiotics-producing marine bacteria was made using 16S rRNA gene sequencing technique.

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Results: A number of673 isolates obtained through the isolation process were found to be the member of 27 bacterial genera, with species of *Bacillus* recording a maximum of 40.2%. Generally species of bacterial isolates in the association (seaweeds: epibiotics, 39.54% and endobiotics, 40.74%, seawater 8.61% and sediments 11.11%) produced antibiotics and active against plant pathogens (*Xanthomonas axonopodis* pv. *citri*, *X. oryzae*pv. *oryzae* and *Ustilaginoidea virens)* were associated with seaweeds (epibiotics 33.46% and endobiotics 43.11%) and sediments (23.43%). Extracellular components of active bacteria are proteins and retaining bioactivity at pH 7.0, up to 40°C and antifungal property up to 60°C. Extracts obtained from the active bacteria are nonpolar lipophilic substances exhibited only antifungal activity.
Conclusion: Bacterial population were considerably higher in seaweeds as compared to seawater and sediments, and at the same time higher bacterial population was recorded in Gulf samples than the open coast samples. Most of the bacterial isolates associated with seaweeds were found to produce antibiotics.

Keywords: Seaweeds; bacteria; endobiotics; epibiotics; antimicrobial activity; plant pathogens.

1. INTRODUCTION

Isolation of bacteria associated with seaweeds (marine macroalgae) has attracted interest in recent years in a quest for novel bioactive compounds, due to the increasing demand for new therapeutic drugs from natural products in order to combat pathogens having multidrug resistance [1]. Bacteria that reside in (endobiotic) or on the surface (epibiotic) of seaweeds are exposed to a highly competitive environment. In order to survive some bacteria synthesis compounds show an inhibitory effect on the growth and attachment of co-occurring bacterial species competing for the same niche [2,3]. However, few studies have been conducted on endobiotic bacteria of seaweeds [2,4]. Even though studies on isolation of bacteria from marine environment have been conducted in India, they are in connection with soil, sediments and fauna [5,6]; only very few studies are conducted on seaweeds, and they also are restricted to aquatic and animal pathogens [7]. As a tropical country, India has vast coastal waters that experience wide fluctuation of climate, and seaweeds enduring such conditions are expected to contain bacteria with a wide array of chemical compounds.

Bacterial blight of rice caused by *Xanthomonas oryzae* pv. O*ryzae* (Ishiyama) and dye and canker of citrus caused by *X. axonopodis*pv. *Citri* (Hasse) Vauterinare major bacterial diseases in tropical countries [8], which results in severe yield losses of up to 50%, depending on the growth stage, geographical location and seasonal conditions of crops[9]. False smut caused by the fungus *Ustilaginoidea virens* (Cooke) Takah is an emerging epidemic disease in rice in India and other rice-growing countries, with a disease incidence record of 2% to 75% [10]. Biological pest control through identifying, understanding and utilizing various sources of microorganisms or using microbial products (antibiotics, enzymes, siderophores) is considered safe to control plant diseases when compared to pesticides of chemical origin [11,12]. Even though bacteria isolated from various marine sources exhibit antibiotic and enzymatic potential against pathogens causing diseases in plants, human and animals [13], screening of bacteria associated with seaweeds as a source of antibiotics and enzymes against plant pathogens has been very limited [13,14]. In this article, we report associations of bacteria as epibiotics and endobiotics with 10 native and one invasive species of seaweeds collected at selected localities along the coasts of South India and compared the bacterial diversity on seaweeds with that of seawater and

sediments. Isolated bacteria were screened using commercial antibiotics exhibiting antimicrobial activity against plant pathogens were drawn phylogeny using 16S rRNA gene.

2. MATERIALS AND METHODS

Based on the availability during collection along the coasts of South India in the monsoon season, seaweeds collected mentioned in the Fig. 1 and Table 1 were used in the present study.

2.1 Sample Collections

Healthy thallus of each seaweed species collected using sterile forceps was immediately kept in sterile polybag containing seawater and sealed immediately with more than three-fourths of air space inside the bags. Samples of the sediments and seawater associated with the thallus were also collected at the respective seaweed collection site and brought to the laboratory.

2.2 Isolation of Bacteria from Seaweeds, Seawater and Sediments [4,15]

For the isolation of epibiotic bacteria, fresh seaweed thallus weighing 1.0 g was swabbed aseptically with sterile cotton in 10 ml sterile water and left for 30 min. For the isolation of endobiotic bacteria, the sample after swabbing the epibiotic bacteria was homogenized under aseptic conditions using 10 ml of sterile water. Sediment sample weighing 1.0 g was extracted in sterile water using orbital shaker for 30 min and the volume was made up to 10 ml. Different serial dilutions such as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared from the 10 ml of made-up samples (seaweeds and sediments) as well as from the 10 ml of seawater samples. For each dilution, 100 µl swabs were spread on Petri plates containing approximately 15 ml of 1.5% ZoBell marine agar (HiMedia, India). The plates were incubated at 25±2ºC and bacteria colonies with different morphologies appeared were picked up every 6 h up to 4 days and streaked on the fresh plates containing ZoBell marine agar. Pure culture of each isolate was confirmed by subsequent restreaking. Then, bacteria isolates were designated with unique codes and stored in glycerol suspension (glycerol/bacterial broth of 1:1 v/v) in Eppendorf tubes at -80ºC for further investigation.

2.3 Screening of Marine Bacterial Isolates for Antibiotics Production [15]

According to the morphological, Gram's staining and biochemical characteristics described in the *Bergey's manual* [16], 243 strains belonging to 27 bacteria categorized from 673 isolates enumerated from various samples of seaweeds, sediments and seawater were evaluated for antibiotics production using commercial antibiotics (HiMedia, India) of ampicillin (25 mcg/disc), azithromycin (30 mcg/disc), chloramphenicol (10 mcg/disc) and streptomycin (25 mcg/disc) through disc diffusion method [15] impregnated on the Petri plate containing Mueller-Hinton agar (300.00 g L^{-1} Beef, 17.50 g L^{-1} casein hydrolysate, 1.50 g L^{-1} starch, 17.00 g L^{-1} agar, pH 7.3). Bacteria grown in the medium showing resistance against commercial antibiotic by not developing inhibition zone around the discs were considered as antibiotic producers. Thus 36 bacteria from 243 strains found as antibiotic producers and they were taken up for further screening against plant pathogens.

2.4 Screening of Marine Bacteria against Plant Pathogenic Bacteria [3]

Based on the commercial antibiotics screening, 36 bacteria found producing antibiotics were evaluated against plant pathogens which obtained from the culture depository of the Department of Botany, Alagappa Government Arts and Science College, Karaikudi, India. Culture broth of the 36 bacteria grown in 100 ml Zobell marine broth in 250ml of Erlenmeyer flask for 4 days was centrifuged (15,000 x g for 10 min at 30ºC) and 100 µL of culture supernatant was aseptically saturated with sterile 6.0mm diameter Whatman No. 1 filter paper impregnated onto Petri plates containing approximately 15 ml of 1.5% peptone sucrose agar (10.0 g L^{-1} peptone,10.0 g L^{-1} sucrose, 1.0 g L^{-1} sodium glutamate, 20.0 g L^{-1} agar, pH 7.2) spread with 100 µL of 24-hour-old culture of plant pathogenic bacteria and incubated at 25ºC. Antibacterial activity was recorded as diameter zone of inhibition in and around the disc after 48 h of incubation.

2.5 Screening of Marine Bacteria against Plant Pathogenic Fungus [13]

Sterile 15mmdiameter Whatman No.1 filter paper discs were saturated with 500 µL of culture supernatant and allowed to air dry aseptically. Initially Petri plates were seeded approximately with 10 ml of 3% potato dextrose agar (PDA) (200.0 g L^{-1} potato, 20.0 g L^{-1} dextrose, 15.00g L^{-1} agar in distilled water, pH 5.6). A loop full of fungal mycelia mixed evenly with 10 ml of 1.5% PDA was poured over preseeded 3% PDA plates. Then, the plates were impregnated with saturated bacterial culture discs and incubated at 25°C. Antifungal activity was measured as diameter zone of inhibition in and around the disc after 48 h of incubation.

2.6 Extraction of Active Substances from Three Promising Marine Bacteria [17]

Pellets and culture supernatant of *Bacillus cereus* ULT15, *B. megaterium* UT10 and *Lysinibacillus xylanilyticus* GR154 showed promising activities against plant pathogens were separately extracted in ethyl acetate at least thrice till the solvent become colourless using separating funnel. The extracts were pooled and concentrated in Rotary vapour under reduced pressure and stored at 0°C till assay. The crude extracts of 100 µg reconstituted in ethyl acetate were loaded on 6.0mm and 15mm Whatmann No. 1 paper discs for conducting antibacterial and antifungal assays respectively. Antibacterial and antifungal properties of the culture supernatant (100 µL) of three bacterial strains were also conducted. Each experiment was conducted thrice and mean values are expressed.

2.7 Properties ofAntimicrobial Substances [17]

To determine the temperature stability, active culture supernatant and cell extracts obtained from the 4 day old bacterial broth were transferred to 5ml screw capped tubes and kept at different temperatures (30, 40, 50, 60, 70, 80, 90 and 100°C) for 1h in water bath and then antibacterial and antifungal assays were carried out. For determining the pH stability, culture supernatant (3 ml) and cell extracts were mixed with equal volume of 0.1 N phosphate buffer at different pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and incubated for 1h at 30°C and then assay was carried out. To determine the biochemical nature of the active substances, culture supernatant and cell extracts were mixed with proteinase K and lysozyme (1.0 mg ml^{-1}, Medox, India) as 1:1 ratio (v/v) separately and incubated for 1 h. After incubation, enzyme-

treated culture supernatant and extracts were tested for bioactivity. Samples without enzyme treatment served as controls.

2.8 PCR Amplification, 16S rRNA Gene Sequencing and Phylogenetic Analysis of Antibiotics-producing Marine Bacteria

Based on the antimicrobial activity against plant pathogens, identification of 13 promising marine bacteria was confirmed through 16S rRNA gene sequencing and drawn phylogeny [3,17] and the nucleotide sequences were deposited in the DNA Data Bank of Japan (DDBJ)/GenBank.

3. RESULTS AND DISCUSSION

3.1 Enumeration of Seaweeds Associated Bacteria on Par with Seawater and Sediments

As shown in Fig. 1 and Table 1, the seaweed species collected from the one to four localities for whether the bacteria on seaweeds is environmentally acquired or species specific, which was verified by the bacterial population and diversity in seaweeds, seawater and sediments of each locality. It is interesting that, bacterial population in seaweeds far exceeds than in seawater and sediments at the respective sampling sites. This may ascribed to more space and nutrients for bacterial growth in seaweeds than environments (seawater and sediments) [18,19]. In our study, Gulf of Mannar (Rameswaram), one of the marine biodiversity hot spots in India, recorded the maximum number of species of marine organisms [20] and a higher bacterial diversity than that of open coasts. Abundant diversity of other marine organisms and less disturbance by the water current in the Gulf resulted in high detritus organic material content that favours higher bacterial population than in the open coast samples [21]. Similar to Sutha et al.'s [4] findings, seaweeds with large thallus surface recorded high endobiotic bacteria population.

Based on Gram staining and biochemical characteristics, 673 bacteria isolates belonging to 27 genera were collected from the seaweeds, with *Bacillus* recording the maximum contribution of 40.2% (Table 2).

Species of *Bacillus* and *Aeromonas*, which form the majority of the population associated with seaweeds, were proportionally less and main the dominance in the seawater and sediments whereas bacteria recorded as a few specific to seaweed species (*Citrobacter diversus, Enterobacter intermedius, Clostridium* sp. and *Bacillus lacterosporus*) was not found in the seawater and sediments.This indicated that some bacteria are substratum specific suggesting that the association of bacteria with seaweeds is consanguineous [22] with that, it provides specific nutrition required for the growth of bacteria and for yielding corresponding products (such as amino acids, antibiotics and propitious toxins) [19].

3.2 Screening of Marine Bacteria Isolates for Antibiotics Production

Among the total isolates enumerated, 14.8% of 36 isolates that produce antibiotics are associated mainly with seaweeds (epibiotics, 39.54% and endobiotics, 40.74%) rather than with seawater (8.61%) and sediments (11.11%). Among the 36 isolates showing antibiotic activity, 13 exhibiting antimicrobial activity against plant pathogens were associated with

seaweeds (epibiotics, 33.46%; and endobiotics, 43.11%) and sediments (23.43%), but not with seawater (Fig. 2).

Fig. 1. Green seaweeds *Caulerpa scalpelliformis* (a), *Ulva lactuca* (b), *Ulva fasciata* (c) and *Chaetomorpha linum* (d) ;red seaweeds *Gracilaria edulis* (e), *Gracilaria corticata* var. *corticata* (f), *Hypnea valentiae* (g), *Grateloupia filicina* (h) and *Kappaphycus alvarezii* (i) and brown seaweed *Sargassum wightii* (j). All are native species except i which is invasive species

Table 1. Bacterial populations as CFU (Colony Frequency Unit) enumerated from the samples of seaweeds, seawater and sediments collected at different localities along the coasts of South India during December 2009/January 2010

Sl.No.	Sample		Epibiotic(1) and Endobiotic(2)	Locality (CFU ×10^4 dilution)					
				Chennai (Kovalam), Lat: 13.06; Long: 80.24	MR Pattinam Lat:11.47; Long: 78.21	Thondi Lat:9.75; Long:78.9 9	Rameswaram (Gulf) Lat:9.28 ; Long: 79.31	Kanyakumari Lat:8.08 ; Long: 77.53	Thiruvanandapuram Lat: 8.53; Long: 77.10
1	Green	*Caulerpa scalpelliformis*	1				13	8	
		(Brown ex Turner)Agardh	2				89	6	
2		*Ulva lactuca* Linnaeus	1	5		10	96	7	
			2	7		20	123	18	
3		*Ulva fasciata*Delile	1						8
			2						6
4		*Chaetomorpha linum*(Müller)	1						12
		Kützing	2						14
5	Red	*Gracilaria edulis*(Gmelin) Silva	1			8	92	8	
			2			19	53	11	
6		*Gracilaria corticata* var. *corticata*	1				12		
		(Agardh) Agardh	2				9		
7		*Hypnea valentiae* (Turner) Mont.	1		9	5	52	5	
			2		7	19	72	9	
8		*Grateloupia filicina*	1						4
		(Lamouroux) Agardh	2						3
9		*Kappaphycus alvarezii* (Doty)	1			33	74		
		Doty ex Silva	2			10	58		
10	Brown	*Sargassum wightii*Greville-Healthy	1				58	18	
			2				69	8	
11		*Sargassum longifolium*(Turner)	1					9	
		Agardh	2					11	
12	Seawater			1	1	3	8	3	4
13	Sediment			4	2	5	10	6	7

*Values presented at the respective sampling sites where collection made.
Serial number 9 is invasive species and others are native seaweeds

Table 2. Number of isolates and percentage (%) of each bacterium recorded from the samples of seaweeds, seawater and sediments collected at different localities along the coasts of South India during December 2009/January 2010

Sl. No	Bacteria	Seaweeds		Seawater	Sediment	Total isolates	%
		Epibiotic	Endobiotic				
1	*Nesseria* sp.	-	2	-	-	2	0.29
2	*Nesseria vellumolla*	3	3	-	-	6	0.88
3	*Aeromonas* sp.	7	8	5	4	24	3.55
4	*Aeromona shydrophila*	3	5	-	-	8	1.18
5	*Aeromonas salmonella*	-	2	-	-	2	0.29
6	*Aeromonas veronii*	2				2	0.29
7	*Citrobacter diversus*	-	1	-	-	1	0.14
8	*Enterobacter intermedius*	1	-	-	-	1	0.14
9	*Escherichia coli*	2	2	-	-	4	0.59
10	*Klebsiella oxytoca*	1	1	-	-	2	0.29
11	*Clostridium* sp.	-	1	-	-	1	0.14
12	*Bacillus alvei*	17	21	7	6	51	7.54
13	*Bacillus cereus*	11	13	3	3	30	4.44
14	*Bacillus coagulens*	6	2	-	-	8	1.18
15	*Bacillus lacterosporus*		1	-	-	1	0.14
16	*Bacillus licheniforms*	1	3	-	-	4	0.59
17	*Bacillus macquariensis*	6	7	1	4	18	2.66
18	*Bacillus megaterium*	7	3	12	8	30	4.44
19	*Bacillus subtillis*	41	32			73	10.84
20	*Bacillus thuringiensis*	2	2	12	28	44	6.51
21	*Bacillus* sp.	107	113	22	29	271	40.2
22	*Staphylococcus aureus*	7	6	-	-	13	1.92
23	*Lactobacillus delbruecki*		4	-	-	4	0.59
24	*Lactobacillus fermenti*	1	1	-	-	2	0.29
25	*Micrococcus varians*	1	1	-	2	4	0.59
26	*Corynebacterium* sp.	2	1	-	-	3	0.44
27	*Cornebacterium kutsceri*	29	35	-	-	64	9.47
Total isolates		**257**	**270**	**62**	**84**	**673**	**100%**

- Not recorded

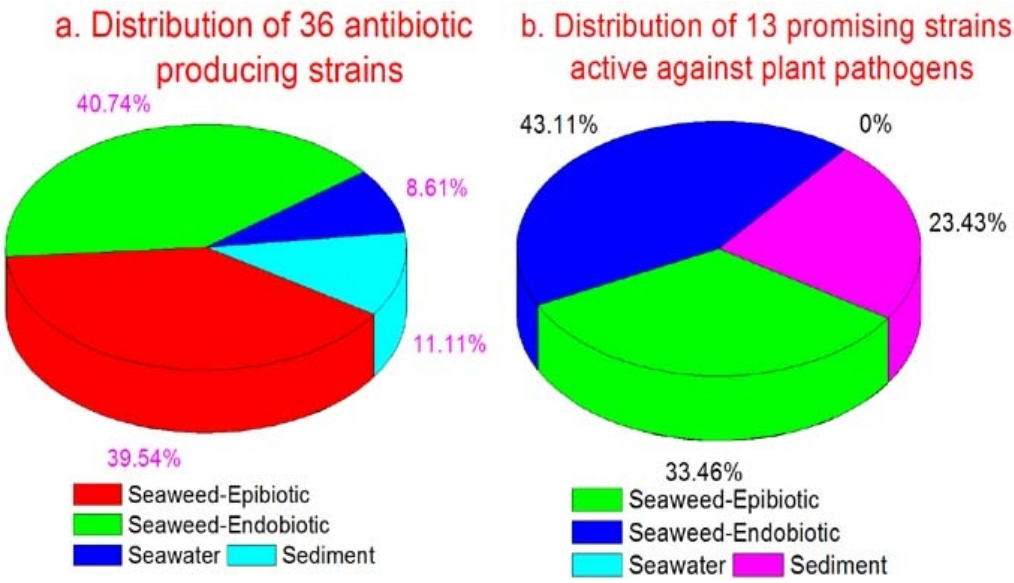

Fig. 2. Active bacteria distributed in seaweeds, sediments and seawater collected at different localities along the coast of south India during December 2009/January 2010

This suggests that bacteria living in a competitive environment (seaweeds) are capable of synthesizing an array of potential antimicrobial substances [19]. According to Zheng et al. [19], the proportion of active bacteria associated with marine invertebrates and seaweeds is higher than that isolated from seawater and sediments.

3.3 Antimicrobial Activity of Active Marine Bacteria and Properties of Antimicrobial Substances

Although cell pellets of 13 isolates exhibited good antifungal activity, the culture supernatant as well as the pellet of *Lysinibacillus xylanilyticus* GT134 showed maximum antifungal activity (Fig. 3A, B, C, D, E). This results show that extracellular components of marine bacteria present in the culture supernatant possess antibacterial and antifungal properties whereas substances extracted from the bacteria cells exhibit only antifungal activity. Crude extracts obtained in ethyl acetate from the culture supernatant as well as bacterial pellet were not anti-bacterially active and ethyl acetate extract obtained from the bacterial pellets showed only antifungal activity. The culture supernatant retains antibacterial activity at pH 7.0 up to 40°C and antifungal property up to 60°C (Fig. 3F). The bioactivity of the culture supernatant was affected as a result of treatment with proteinase K and lysozyme.

Loss of bioactivity in culture supernatant as result of treating proteinase K and lysozyme shows that the extracellular compounds of the bacteria are protein which possesses antibacterial and antifungal properties whereas ethyl acetate extract obtained from the active bacterial cell contains nonpolar lipophilic substances showing only antifungal property [17].

3.4 Phylogeny of Active Bacteria

Phylogenetic analysis of 16S rRNA gene sequencing of the 13 active bacteria associated with brown seaweed *Sargassum wightii*, green *Ulva lactuca* and 3 red seaweeds (*Gracilaria*

edulis, *Grateloupia filicina* and *Hypnea valentiae*) collected at different localities showed high levels of sequence similarity (Figs. 4 and 5) as reported earlier [3,7].

However, very little dissimilarity in the sequences existed between the strains isolated from different seaweeds, between the strains isolated from the same seaweed species found at different localities and between different active bacteria isolated from the specific seaweed collected at the given locale. These results support the claim that seaweeds and associating bacteria maintain consanguineous relationship on nutritional requirement in the given environment [19]. As evidence to this, three bacteria species – *Bacillus cereus* ULT1 (JQ739719), *Lysinibacillus fusiformis* ULT5 (HM573313) and *Bacillus thuringiensis*ULT14 (HM573312) – isolated from *Ulva lactuca* collected from the same locale (Thondi) showed very high similarity. *Lysinibacillus xylanilyticus* SK411 (JQ739715) associated with *Sargassum wightii* collected at Kanyakumari showed little difference with five other strains: GT132 (JQ677989) and GT134 (JQ677988) isolated from *Gracilaria edulis* collected at Thondi as one group; HVT234 (JQ739716) and GSTP512 (JQ677987) isolated from *Hypnea valentiae* and *Grateloupia filicina* collected at Thondi and Thiruvananthapuram, respectively, as another group; and GR154 (JQ677990) associated with *Gracilaria edulis* as a separate identity [5].

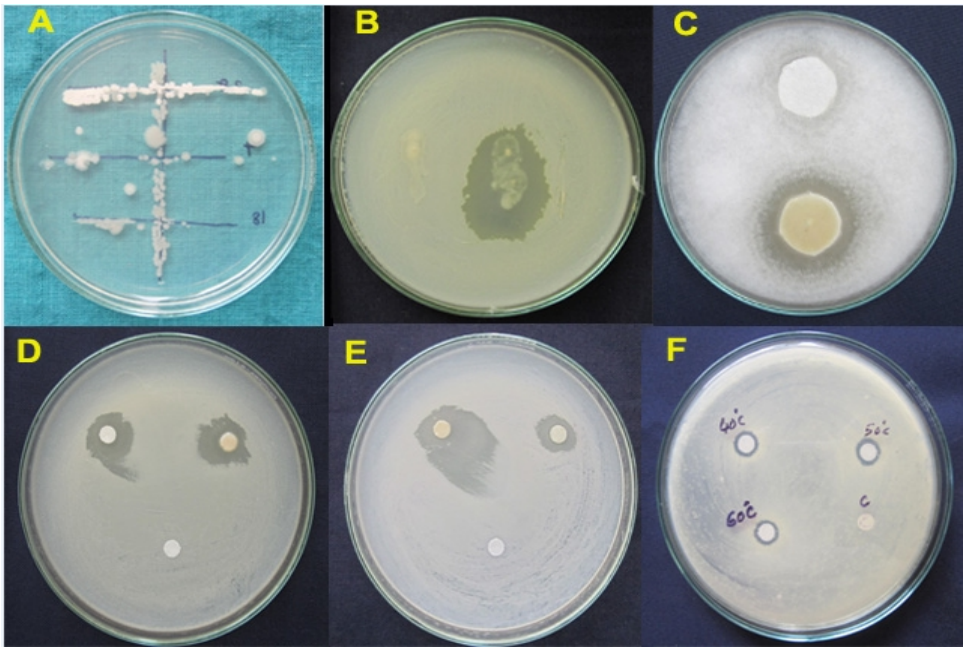

Fig. 3. Growth of plant pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* inhibited by marine active bacterium *Lysinibacillus xylanilyticus* GT134 through cross streaking (A), diffusion of extracellular substances from bacterial cell in the plate assay (B), antifungal (C) and antibacterial (D and E) activities of culture supernatants at different temperature (F)

Table 3 lists the 13 isolates that exhibit antimicrobial activities. Culture supernatant of all active bacteria possesses higher antibacterial activity than pellet samples. By contrast, substances obtained from the pellet of *Bacillus megaterium* UT10 exhibited a higher antibacterial activity than its supernatant.

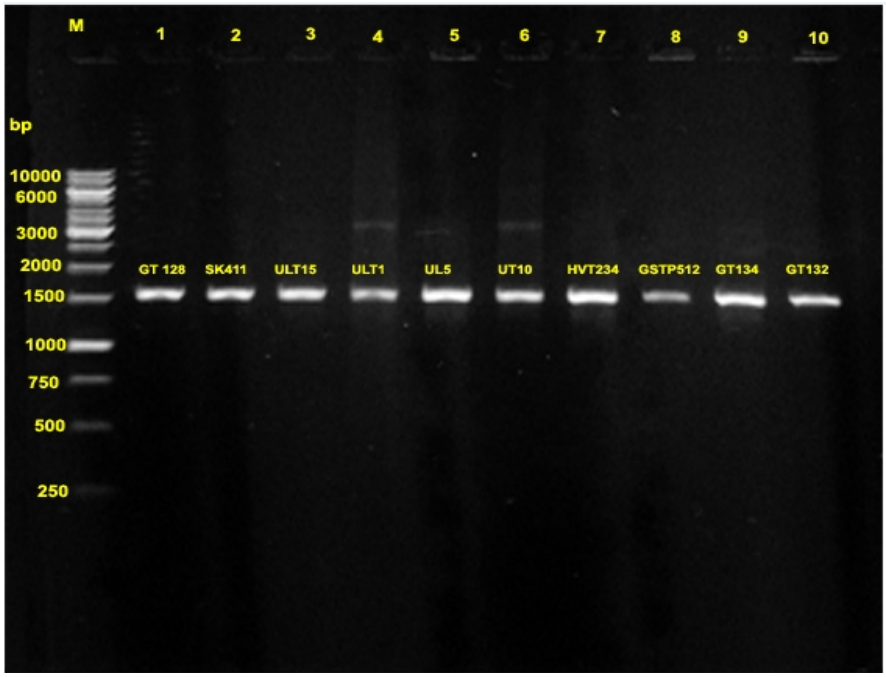

Fig. 4. Isolated 16S rDNAof active marine bacteria separated through agarose gel electrophoresis

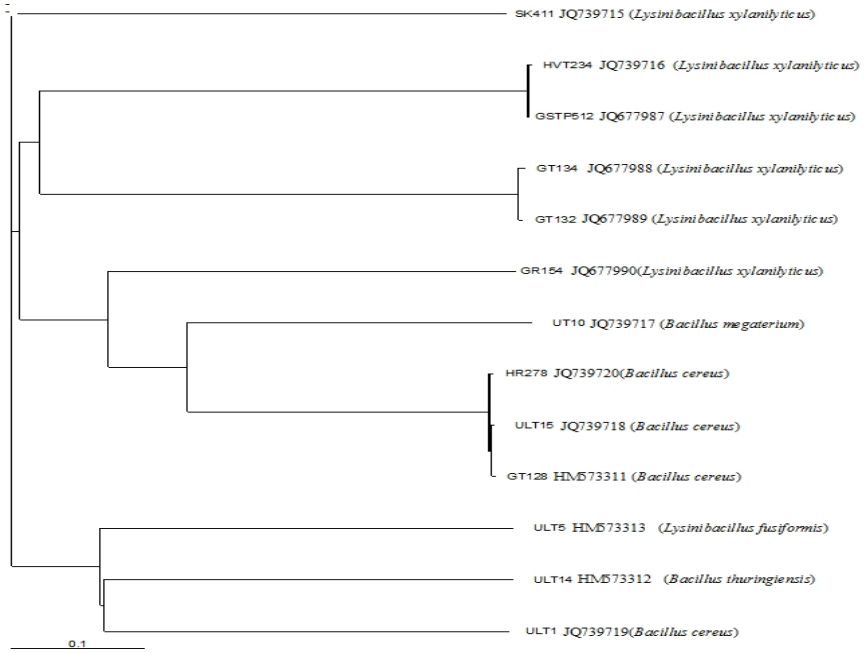

Fig. 5. Neighbor joining phylogenetic tree of 13 active bacteria isolated from the seaweeds and their closest NCBI (Mega BLAST) relatives drawn based on the 16S rRNA gene sequences

Scale bar represents 0.1 substitutions per nucleotide position

Table 3. Antimicrobial activities (zone of inhibition as mm diameter) of culture supernatant (A) and pellet (B) of bacteria isolated from the samples of seaweeds, seawater and sediment collected at different localities along the coast of south India during December 2009/January 2010

Sl.No.	Active bacteria with Genbank Accession Number	Antibacterial activity				Antifungal activity (*Ustilaginoidea virens*AGF01)	
		*X. oryzae*pv. *oryzae* AGB11		*X. axonopodis*pv. *citri* AGB12			
		A	B	A	B	A	B
1	*Bacillus cereus* GT 128,HM573311	10	15	12	11	-	17
2	*Bacillus cereus* SK411,JQ739715	-	6	09	-	16	-
3	*Bacillus cereus* ULT15,JQ739718	6	-	8	-	-	19
4	*Bacillus cereus* ULT1,JQ739719	6	6	13	-	-	-
5	*Lysinibacillus fusiforms* UL5,HM573313	14	7	16	-	-	18
6	*Bacillus megaterium*UT10,JQ739717	-	30	10	50	25	30
7	*Lysinibacillus xylanilyticus* HVT234,JQ739716	16	-	22	9	-	17
8	*Lysinibacillus xylanilyticus* GR154,JQ677990	6	11	15	11	19	36
9	*Lysinibacillus xylanilyticus* GSTP512,JQ677987	30	-	18	-	17	18
10	*Lysinibacillus xylanilyticus* GT134,JQ677988	8	15	-	-	22	36
11	*Lysinibacillus xylanilyticus* GT132,JQ677989	30	15	32	11	-	19
12	*Lysinibacillus xylanilyticus* HR278,JQ739720	-	15	-	-	16	17
13	*Bacillus thuringiensis* UL14,HM573312	-	8	10	15	-	-

- Nil activity

4. CONCLUSION

Bacterial population in seaweeds far exceeded than seawater and sediments and in Gulf samples it is further higher than the open coasts. Species of bacteria dominated in the association with seaweeds (epibiotics-39.54% and endobiotics-40.74%) than seawater (8.61%) and sediments (11.11%) producing antibiotics showed promising antimicrobial activities against two bacteria and a fungi cause diseases in paddy are associated with seaweeds (epibiotics-33.46% and endobiotics-43.11%) and sediments (23.43%). The 16SrRNA gene sequencing of 13 active bacteria supports the bacteria-seaweeds relationship as consanguineous.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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