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Epiphytic Bacterial Communities in Seagrass Meadows of Oligotrophic Waters of Andaman Sea

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Abstract

The epiphytic microbial communities of seagrass (*Cymodocea rotundata* and *Thalassia testudinum*) along with sediments and seawater of the seagrass ecosystem of Andaman Sea were assessed for heterotrophic bacterial communities. Young leaves of *C. nodosa* and *T. testudinum* sediments and water samples were collected from two different locations at Burmanala and Havelock island. Young leaves were swabbed and transferred into Alkaline peptone water along with 1 gm of sediments and 1 ml of seawater for initial microbial enrichments, followed by culture of microbes in Salt Yeast Extract Agar. Thiosulphate Citrate Bile Salt agar was used for seagrass leaves, sediments and water for culturing of *Vibrio* like microbes. The observed colonies were morphologically (colour) identified first and then further identification was done by Gram staining and various biochemical tests. Sediment bacterial count was 10-fold higher than seagrass and water column communities. Morphologically 29% of total bacterial colonies identified were of grey and white colour. *T. testudinum* bacterial epiphytes were 2-fold higher than *C. rotundata* communities. Gram negative bacterial isolates of *Pseudomonas*, *Vibrios and Aeromonas* dominated the bacterial colonies than gram positive bacteria of *Bacillus* and *Micrococcus*. *Vibrios* were only observed on seagrass leaves. Seagrass harboured more gram positive bacterial colonies than gram negative, which were dominated in the sediments of seagrass ecosystem. The bacterial communities of landward site of Burmanala and Havelock were similar whereas 2-fold higher communities were observed at the seaward site of Havelock site on *T. testudinum*. Seagrass ecosystem bacterial epiphytic communities are diverse in oligotrophic waters.

Subject Areas

Marine Biology

Keywords

Intertidal Communities, Microbes, Oligotrophic Waters, Heterotrophic Bacteria

1. Introduction

The Andaman and Nicobar group of Islands are situated in the South-east of Indian subcontinent in the Bay of Bengal, Indian Ocean. The Andaman Sea surrounding these islands comprises of a rich marine diversity of flora and faunal assemblages [1]. The intertidal flora of these islands is dominated by seagrass ecosystem with patches of various seaweeds [2]. Seagrass ecosystems are distributed worldwide [3] [4] and are highly productive and dynamic coastal ecosystems [3] [5]. They provide various ecosystem services, such as nursery and breeding grounds for fishes, carbon storage, nutrient cycling and serve as food for many herbivore fishes, sea turtles and sea mammals [6] [7]. They are highly productive ecosystems particularly in oligotrophic waters [3] [4]. This high productivity in seagrass ecosystem also generates a great amount of organic matter and serves as a good substratum for a rich diversity of microorganisms forming an integral part of the seagrass ecosystem [8]. These microbes (*i.e.* bacteria) play important role in recycling of organic materials [3].

Microorganisms of bacterial origin play a significant role in productivity and degradation of organic matter, *i.e.*, microbial loop [9]. Microbial loops are essential in all types of marine ecosystems but most important in coastal oligotrophic waters where the available nutrients are less and the ecosystem depends on microbes for recycling of organic matter [3] [10]. These microbial communities form a significant part of the epiphytic communities on seagrass ecosystem consisting of bacteria, fungi and protozoa [8] [11]. The distribution and abundance of epiphytes on seagrass leaves depend mainly on the morphology and life span of the portion colonized by epiphytes [8] such as the young leaves or mature leaves. These bacterial communities of seagrass leaves are diverse and include various genus of *Alteromonas*, *Brochothrix*, *Moraxella*, *Marinobacter*, *Pseudomonas* and *Vibrio* [12]. Instead of the entire leaf, bacterial epiphytes prefer young leaf tips and mature leaf bases, which has been observed in *Zostera marina* and *Posidonia oceanica* leaf tips and base [12] [13]. This apico-basal pattern of epiphyte colonization is also observed for other seagrass species like *Cymodocea rotundata* and *T. hemprichii* [8].

These epiphytic communities not only contribute to the net community production of seagrass ecosystem but also support other functions, such as herbivory and transfer of energy to higher trophic levels [14]. Epiphytes also help seagrass in nitrogen fixation [8] [15] and nutrient cycling [16]. [15] reported 4% - 38% of nitrogen needed for primary production by *Thalassia testudinum* is provided by the epiphytic cyanobacteria on their leaves. Similarly, 17% of NH_4^+ removed form water column in *T. testudinum* meadows is due to the presence of bacterial epiphytes on their leaves [16].

Most studies on bacterial epiphytes of seagrass have considered individual seagrass species [17] [18] [19] [20] or a combination of seagrass and sediments from seagrass ecosystem [13] [21] [22]. Individual seagrass species have shown a wide range of bacterial epiphytes associated within the seagrass leaves preferring the apico-base pattern, for example, *Halophila stipulacea* of Red Sea was observed with *Cyanobacteria* and nitrogen fixing bacteria on leaves [17], whereas *Bacillus, Vibrios, Micrococcus* and *Staphylococcus* sp. were observed on *H. ovalis*, *H. uninervis* and *H. stipulacea* of Jordan [23]. Similarly, sulphur-oxidising *Gammapro teobacteria* and sulfur-reducing *Deltaproteobacteria* were observed on *Zostera marina* leaves from Chesapeake Bay [20] and *Z. noltii* from Bassin d' Arcachon [19], whereas *Cyanobacteria sp*. was observed in the leaves of *Thalassia* and *Cymodocea* sp. of Tanzania [5]. The sediment bacterial communities of seagrass ecosystem were more dominated with organicmatter cycling communities such as, *Desulfovibrio* and *Acetobacter* sp. in sediments of *H. wrightii* meadows of Florida coast [18], sulfate-reducing bacteria in *Posidonia oceanica* meadows of Mediterranean Sea [24] and *T. testudinum* and *H. wrightii* of Florida Bay [25]. However, few studies also have been carried on seagrass and sediments together for bacterial epiphytes, such as *T. testudinum* of Bahamas [21], *Posidonia oceanica* of Mediterranean Sea (Novak, 1984) and *T. testudinum* of Florida Bay [22].

Considering the importance of Indian seagrass ecosystem and scarce studies on bacterial epiphytic population of oligotrophic waters of Andaman Sea, we try to assess the heterotrophic bacterial communities that survive on seagrass *C. rotundata* and *T. testudinum* leaves as epiphytes and the associated water and sediments within seagrass meadows of Andaman Sea to have an overview of the microbial diversity of seagrass meadows in oligotrophic waters.

2. Materials and Methods

2.1. Study Area

Two study areas were selected with seagrass meadows of Andaman and Nicobar Islands in Andaman Sea, India. Both stations are situated in low-lying areas with similar hydrodynamic conditions, exposed twice in a day during low tides. At each location two sites were chosen, one close towards the land side and one towards the open ocean. Burmanala (11˚33'22.293''N: 092˚43'43.961''E) is situated on the South-east coast of Andaman island. This low-lying area gets exposed during low tides and the luxuriant growth of seagrass *Cymodocea rotundata* is observed in this intertidal ecosystem. Havelock (11˚58'44.45''N: 92˚56'36.86''E) is situated in north east of Andaman island. This intertidal region is dominated by seagrass *Thalassia testudinum*.

2.2. Sample Collection

Seagrass leaves (*C. rotundata* and *T. testudinum*) were collected from both

Burmanala and Havelock sampling stations (2 sites each) during low tide. Young and slightly mature seagrass leaves were plucked by sterile forceps. Seawater samples (n = 3) were collected in sterile screw cap bottles for all four sites of each station. Sediment samples (n = 3) were collected by scooping out the upper 1 cm of sediment using a spatula from the same area seagrass were collected and stored in sterile glass containers. Water, sediment and seagrass were stored in portable ice box and transferred to laboratory for further analysis. Physical parameters pH, temperature and salinity was measured at both sites of each station using pH meter, thermometer and salinometer respectively.

In the laboratory *C. rotundata* and *T. testudinum* leaves were placed in sterile trays and washed gently three times with a spray of sterile filtered seawater to remove the unattached bacterial population along with any other debris. After washing the leaves, a sterile cotton swab was used to swipe the seagrass leaves. This swab was then transferred to a glass tube containing 30 ml Alkaline Peptone Water (APW, pH-8.5, Hi-media) and incubated for 6 - 8 hours at 37˚C for enrichment of bacterial communities. 100 µL of inoculation was pipetted out from each tube and transferred to sterile petridishes to which 15 - 20 ml of sterilized Salt Yeast Extract Agar (SYEA, Hi-media) was added. After solidification plates were inverted and incubated at 37˚C for 24 hours. Plates were observed for growth (appearance of colonies) after incubation and the plates with less growth were further incubated for 12 hours. Then colonies were counted using a colony counter and the morphology (colour) of the colonies were recorded. All the petriplates containing colonies between 30 - 300 were counted using the Quebec colony meter. Colonies with different morphology were picked and purity of the colonies was determined by streaking it on Nutrient Agar (NA, Hi-media) plates. These colonies were re-streaked onto fresh NA plates with 3-streak method and incubated at 37˚C for 12 hours. Pure isolates from these colonies were confirmed microscopically for purity and then transferred to agar slants or stabs for storage which was used for gram staining and biochemical tests. Routine biochemical test was carried out by following standard method and the isolates were characterized in accordance with the prevalent identification scheme appropriate for marine heterotrophic bacteria [26].

Thiosulphate Citrate Bile Salt (TCBS, Hi-media) agar was used for the culture of Vibrio sp. like organisms from seagrass, sediment and water samples. From pre-inoculated samples of seagrass, sediment and seawater in APW, 1 ml was transferred to sterile glass tubes containing 10ml of sterilized 50% seawater. The tubes were vortexed and immediately filtered onto 0.45 µm GF/F membrane Millipore filter paper and placed on TCBS agar plates. These plates were incubated for 12 to 18 hours and the colonies were counted and the individual colonies were streaked again on TCBS for obtaining pure cultures.

3. Results

The seawater pH was similar and salinity was lower in sediments than water column at both sites of Burmanala and Havelock. Heterotrophic bacterial count was 10-fold higher in sediment than water column and seagrass at both locations (Table 1). In Burmanala the seaward site had higher bacterial count than landward site, whereas the opposite trend was observed at Havelock site (Table 1). *T. testudinum* epiphytic bacterial count was similar with *C. rotundata* at Burmanala landward site and 2-fold higher at the seaward site of Havelock (Table 1).

16 different isolates with five different morphological features were obtained from the seagrass leaves, sediments and water column (Table 2). The white and grey coloured colonies accounted for 59% of total colonies and were dominated in sediment and sweater (Figure 1 and Figure 2). The green and yellow coloured colonies that represent Vibrio cholera like organisms (VCLO) and Vibrio parahaemolyticus like organisms (VPLO) represented 17.5% of the total colonies and were observed only on seagrass leaves. The sediment bacterial communities were dominated by Gram negative whereas the epiphytes of seagrass were dominated by Gram positive bacterial communities (Table 2). Identification of the isolates confirmed that more than 50% represented *Pseudomonas* followed by *Bacillus*, *Micrococcus*, *Aeromonas* and *Vibrios*.

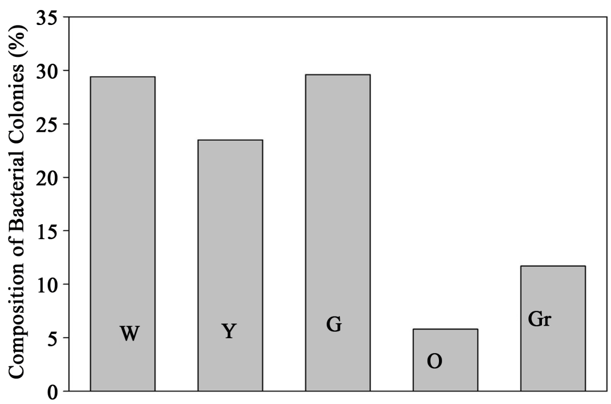

Figure 1. Total composition of different bacterial colonies based on morphology (colour) from Burmanala and Havelock. W (white), Y (yellow), G (grey), O (orange) and Gr (Green).

Table 1. Heterotrophic bacterial counts in water (CFU/ml) and sediment (CFU/mg) and in seagrass (CFU/ml) *C. rotundata* and *T. testudinum* at two sites of Burmanala and Havelock stations of Andaman Island.

Station	Sample	Bacterial Count (CFU/mg or CFU/ml)/Sites	
		A	B
Burmanala	Water	37.16×10^3	59.9×10^3
	Sediment	30.4×10^4	51.5×10^4
	C. rotundata	16.2×10^3	10.9×10^3
Havelock	Water	2.3×10^3	1.5×10^3
	Sediment	16×10^4	15.9×10^4
	T. testudinum	15×10^3	21.4×10^3

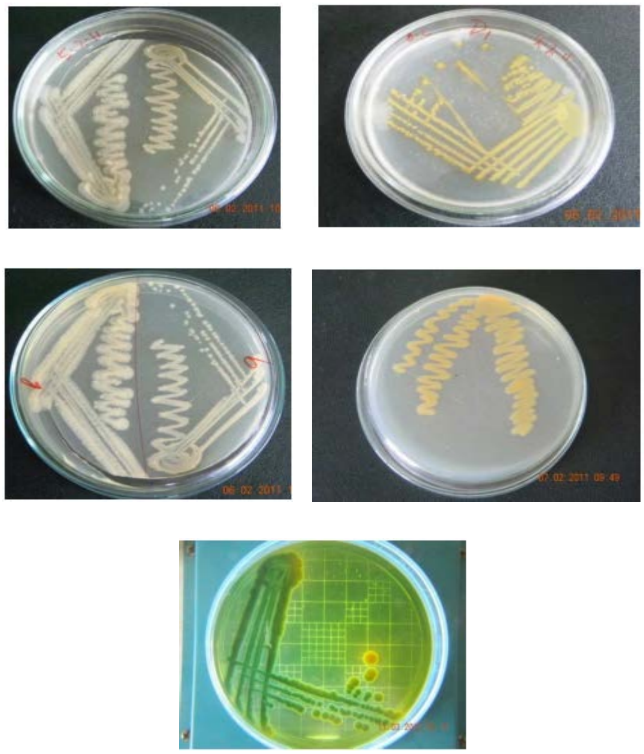

Figure 2. Nutrient agar plate streaks of White, Yellow, Grey, Orange and Green strains isolated from seagrass.

Table 2. Biochemical test of isolated bacterial colonies from Sediment (Sd), Seawater (Sw) and Seagrass (Sg) samples of Burmanala and Havelock stations of Andaman Island.

No	Colony	Sample	Gram	Biochemical Test			
			(+ve, −ve)	TSI	MIO	LIA	Citrate
1	White	Sd	+ve	K/A g^- H^-	$M^+I^-O^{nr}$	K/A (−ve)	+ve
2	Grey	Sd	−ve	K/A g^+ H^-	$M^+I^-O^+$	K/A(−ve)	−ve
3	Grey	Sd	+ve	K/A g^+ H^-	$M^+I^-O^+$	K/A(−ve)	+ve
4	Grey	Sd	−ve	A/A g^- H^-	MIO^{nr}	K/A(−ve)	−ve
5	Yellow	Sd	−ve	A/A g^- H^-	MIO^{nr}	nr	−ve
6	Orange	Sd	−ve	NG	NG	NG	NG
7	White	Sd	−ve	K/A g^+ H^-	$M^+I^-O^+$	K/A(−ve)	−ve
8	Yellow	Sw	−ve	A/A g^- H^-	MIO^{nr}	nr	−ve
9	White	Sw	+ve	A/A g^- H^-	MIO^{nr}	K/A(−ve)	−ve
10	White	Sw	−ve	A/A g^- H^-	MIO^{nr}	nr	−ve
11	White	Sw	+ve	NG	NG	NG	NG
12	Yellow	Sg	−ve	A/A g^- H^-	MIO^{nr}	nr	−ve
13	Grey	Sg	+ve	K/N	ND	ND	ND
14	Green	Sg	+ve	K/N, H^+	MIO^{nr}	nr	−ve
15	Orange	Sg	+ve	A/A g^- H^-	MIO^{nr}	nr	−ve
16	Grey	Sg	−ve	K/A g^+ H^-	M^- I^-O^{nr}	K/A (−ve)	–

A/A (Acid slant/Acid butt), **K/A** (Alkaline slant/Acid butt), $\mathbf{g^-}$ (Gas (−ve)), $\mathbf{H^-}$ (H_2S(−ve)), **K/N** (no reaction), **NG** (no growth), **nr** (no reaction), $\mathbf{M^+}$ (Motility positive), $\mathbf{O^+}$ (Ornithine decarboxylase positive), $\mathbf{I^-}$ (Indole negative), **ND** (not done), **TSI** (Triple sugar Iron agar) **MIO** (Mobility Indole Ornithine), **LIA** (Lysine Iron Agar), **Citrate** (Simmons's citrate).

4. Discussion

Intertidal seagrass leaves, sediments and water column were observed with various heterotrophic bacterial communities of *Pseudomonas*, *Bacillus*, *Micrococcus*, *Vibrios* and *Aeromonas* on seagrass leaves and in sediments and water column of Burmanala and Havelock of coastal Andaman Sea. Similar bacterial isolates were also observed from Andaman waters by [27] and in the intertidal ecosystem [28]. The microbial community observed in sediments were higher than water column and seagrass leaves, similar to the findings for Andaman Sea [27].

Both seagrass *C. rotundata* and *T. testudinum* harboured various heterotrophic bacterial communities, which agree with findings that seagrass leaves host diverse and potentially mutualistic bacterial isolates on their leaf surface [20]. Hosting diverse heterotrophic bacterial isolates was also observed on other seagrass leaves, such as *Vibrios* and *Pseudomonas* on *Zostera marina* [12] [20], *H. stipulacea* [29], *Bacillus*, *Vibrios* and *Micrococcus* on *H. uninervis* [23]. *Bacillus, Vibrios* and *Pseudomonas* as epiphytes on *T. testudinum* and *C. rotundata* of our results were similar to bacterial epiphytes of *T. testudinum* and *C. rotundata* in South China Sea [30]. We report the presence of *Vibrios* in seagrass leaves as epiphytes for the first time in Andaman Sea, however, other reports have observed *Vibrios* in seaweeds in these waters [28]. Heterotrophic bacterial communities in our studies were higher on *T. testudinum* than *C. rotundata*, similar observations have been made on *T. testudinum* than *C. rotundata* in South China Sea [30].

The heterotrophic bacterial diversity increases various microbial metabolic processes on the leaf surface of seagrass, such as sulphide oxidation, methane oxidation, iron reduction, nitrogen fixation and sulfate reduction that are influenced by seagrass leaves and certainly beneficial to the plant in oligotrophic waters [20] [30]. Though these microbes as epiphytes are beneficial to plants, their growth is also restricted on seagrass leaves by antimicrobial agents' seagrasses secrete only allowing certain microbes to be established on leaf surface as epiphytes [31] [32]. The bacterial community on *T. testudinum* was higher than *C. rotundata* in our studies which can be related to the quantity of antimicrobial compounds these seagrasses secrete [32] and their host-microbe interaction in a particular environment [33]. Though, similar types of microbes were observed on both *C. rotundata* and *T. testudinum* in our studies, the interaction between these microbes and seagrass are species-specific and remains same even in different environmental conditions [20]. However, the microbial epiphytes of seagrass leaves and in sediments were similar in our studies, except the *Vibrios* on seagrass leaves, which can be due to the different roles these microbial communities play on leaves and in sediments [20] [33]. Similarly, presence of *Pseudomonas* on the leaf surface is due to the excreted sugars from leaf surface [34] and *Pseudomonas* also helps the seagrass meadows to contribute to carbon bio-oxidation [35].

Bacterial colonies of sediments were dominated by gram negative bacterial colonies of *Pseudomonas* and *Aeromonas,* which agrees with the dominance of gram negative bacteria in marine sediments [36] due to the leaching of easily degradable sugars, organic acids and amino acids from seagrass roots and rhizomes into sediments [20] [37] and reduction of inorganic compounds like sulfate and acetogenic substances [37] [38]. The similarity of bacterial communities in sediment and seagrass leaves suggests the vertical input of organic matter from the seagrass leaves into sediment [39] that later colonise the sediments. Higher bacterial isolates from sediments also suggests that the microbes in sediments play a significant role in oligotrophic waters like Andaman Sea in cycling of carbon and nutrients and contributing to the productivity of these waters [40]. Presence of *Bacillus* and *Pseudomonas* in the sediments favours the seagrass in increasing stability of the seagrass meadows and stimulating mineralization of aerobic and anaerobic matter [41] [42] [43]. Presence of *Pseudomonas* in sediments suggests the presence of excreted sugars from roots and decaying leaves in sediments [34].

Bacterial colonies were higher in sediments followed by seagrass and water column in our studies, similar observations have been made in seagrass meadows elsewhere [30]. Lower bacteria in the water column is due to the low nutrient concentrations in oligotrophic Andaman waters as nutrient concentration plays a major role for growth of bacteria in water column [44].

Our results suggest that in seagrass meadows there is a closed loop of heterotrophic bacterial population as observed in seagrass leaves, sediments and water column. This closed loop represents the similar kind of active bacterial population that are attached on the seagrass leaves, found in the sediments and water column. This loop can be due to the nutrient limitation of these oligotrophic waters and being in a closed loop helps the bacteria in acquiring and cycling nutrients and maintaining productivity of the ecosystem. Both seagrass was associated with diverse heterotrophic bacterial isolates which can be related to the diverse compounds seagrasses release as by-products of primary productivity. This raises the need of understanding the plant-microbe interaction in details in oligotrophic waters of Andaman Sea, which can provide us better understanding of the role heterotrophic bacterial population plays in these intertidal seagrass meadows. However, the presence of pathogens like *Vibrios* in Andaman waters reflects the effects of human impacts on the coastal ecosystems and that can affect the beneficial epiphytic population of seagrass through competition and transfer of pathogens to higher food webs by seagrass depended herbivory.

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Conflict of Interest

The author declares that there is no conflict of interest between any funding agencies or organizations for this research work.

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