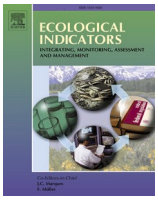
Ecological Indicators 121 (2021) 107067

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Carrageenophyte-attached and planktonic bacterial communities in two distinct bays of Vietnam: Eutrophication indicators and insights on ice-ice disease

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ARTICLE INFO

Keywords:
16S rRNA diversity
Kappaphycus
Euchema
Pollution
Vibrio

ABSTRACT

The composition of the bacterial community of carrageenophyte-attached and planktonic bacteria was studied in two bays of Vietnam with contrasting anthropogenic inputs to assess their role as ecological indicators. Clear differences (~73% dissimilarity) between carrageenophyte-attached bacteria and bacterioplankton were detected in terms of genus composition: mainly *Agaribacter*, *Ruegeria*, *Alteromonas*, the Pir4 lineage and *Vibrio* for the carrageenophytes and *Candidatus Actinomarina*, HIMB 11, NS groups and SAR clades for the bacterioplankton. The copiotrophic nature, potential for complex-polymer degradation, and ability to form and defend biofilms were common features inferred for the carrageenophyte-attached microbiome. Significant differences between the bays were detected in the concentration of most inorganic nutrients. More eutrophic conditions and presumptive wastewater pollution in Cam Ranh (CR) bay were primarily indicated by the dominance of *Rubripirellula*, *Leptobacterium*, *Hypnocyclicus* and *Porphyrobacter* and their correlations with phosphate. In terms of bacterioplankton, the influence of intensive aquaculture in CR bay was suggested by the dominance of the NS5 and NS4 marine groups, the SUP05 cluster, Flavobacteriaceae unclassified and SAR 11 clade III as well as their strong correlations with ammonium and phosphate. The link between silicate and other inorganic nutrients suggests freshwater input in CR bay. Arenicellaceae unclassified and *Formosa* were also potential indicators of eutrophication. Operational taxonomic units (OTUs) of *Marinagarivorans*, *Cobetia*, *Vibrio*, *Alteromonas* and *Pseudoalteromonas* were typical of the carrageenophytes showing ice-ice disease symptoms. *Vibrio* and *Alteromonas* were also common among healthy macroalgae, and differences at the OTU level suggested potential succession of species from the healthy to the diseased state. The probable beneficial roles of some bacteria, such as *Ruegeria*, *Cutibacterium* and unidentified members of the family Rhizobiaceae, were discussed. This study provides pioneering insights into the bacterial community composition of carrageenophytes and highlights their ecological value as strong indicators of the sources of organic matter, anthropogenic impacts and health status of marine systems.

1. Introduction

Coastal systems are endangered by water pollution from diverse sources, which causes decreases in water quality and eutrophication. Moreover, climate change is likely to interact synergistically with the spread of eutrophication and the intensification of anoxia, acidification,

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https://doi.org/10.1016/j.ecolind.2020.107067
Received 25 May 2020; Received in revised form 31 August 2020; Accepted 8 October 2020

harmful algal blooms, benthos degradation and nekton mortality (Rabalais et al., 2009; Lauringson et al., 2012; Kopprio et al., 2015). Intensive aquaculture impacts marine environments, increases nutrient loads and directly or indirectly reduces the abundances of habitat-forming species, such as macroalgae, corals, seagrasses and mangroves. Macroalgae are valuable ecosystem engineers and key organisms for nutrient mitigation in integrated multi-trophic aquaculture (IMTA). Different trophic levels for efficient seafood production within a framework of sustainability and environmental awareness are combined in IMTA. The red algae *Kappaphycus* and *Euchema* have been proposed as excellent candidates for multitrophic-systems because of their efficient nutrient removal and their commercial value (Hayashi et al., 2010, 2017; Tan et al., 2017).

In addition, because of the rising demand for algal-based products, there is increasing interest in improving and expanding macroalgal cultivation and related biotechnologies. Species of *Kappaphycus* and *Euchema,* referred together as eucheumatoid seaweeds or carrageenophytes, provide the main raw material for producing carrageenan. The taxonomy of carrageenophytes is complex because of their morphological plasticity and the high number of commercial varieties. Even new strains of eucheumatoid seaweeds have been created through somatic hybridization and mutagenesis (Tan et al., 2017). Carrageenan is a sulphated polysaccharide with emulsifying, stabilizing and gelling properties, which is important and widely used in the food, cosmetic and pharmaceutical industries. Furthermore, various carrageenans are known to immobilize viruses and bacteria and inhibit tumour growth and have been used in cutting-edge technologies, such as tissue regeneration (Loureiro et al., 2017). However, the culture of carrageenophytes is currently threatened by disease outbreaks (Hayashi et al., 2010).

Some bacteria can invade algal thalli and degrade algal compounds, such as carrageenan or agar. The ice-ice disease has been described in eucheumatoid seaweeds as the bleaching and formation of white spots on the thallus, and presumptive causative agents identified include *Vibrio* sp., *Cytophaga* sp., *Alteromonas* sp. and *Flavobacterium* sp. (Largo et al., 1995; Vairappan et al., 2008; Hayashi et al., 2010). Moreover, some macroalgae are important reservoirs of potential human pathogens, such as like *Vibrio vulnificus* or *Vibrio parahaemolyticus* (Mahmud et al., 2008; Martinez and Padilla, 2016). The tight association between algae and microorganisms is not only detrimental. Several epiphytic bacteria also facilitate macroalgal growth, development and reproduction and are crucial for the exchange of nutrients and secondary metabolites; some provide also resistance to pathogens, grazers and fouling organisms (reviewed by Goecke et al., 2010; Friedrich, 2012; Hollants et al., 2013). Microorganisms associated with algae are also a promising reservoir for the discovery of bioactive compounds. For example, a microorganism associated with *Kappaphycus alvarezii* has been recently described as a source of novel antibiotics (Luyen et al., 2019).

Most research on the algal microbiome has been performed from isolates extracted by culturable techniques. The culturability of bacteria in seawater is considered low and has been estimated to be between 0.1 and 0.001% (Amann et al., 1995). In contrast, next-generation sequencing techniques offer a better picture of the composition of the entire bacterial community and have been used in only few studies to date. Moreover, some bacteria respond quickly to changes in aquatic environments and have been widely used as indicators of anthropogenic impacts. Bacterial community composition based on 16S rRNA diversity can provide a robust indicator of ecological change, biogeochemical processes and water pollution (Becker et al., 2017; Huang et al., 2018; Zeng et al., 2019; Kopprio et al., 2020). Studies of bacterial community composition are rare in species of *Kappaphycus* and *Euchema* and have not been conducted in coastal waters of Vietnam. Here, we aimed 1) to characterize bacterial communities attached to carrageenophytes and surrounding bacterioplankton, which are ecological indicators, in two Vietnamese bays of contrasting anthropogenic impacts, 2) to assess potential bacterial markers of water pollution or higher eutrophic conditions, and 3) to provide insight into putative detrimental and beneficial bacteria for carrageenophytes. We hypothesized that there would be strong differences in the composition of carrageenophyte-attached and bacterioplankton communities under the different anthropogenic impacts of the two bays on the coast of Vietnam.

2. Materials and methods

2.1. Study sites

The coastal areas of Vietnam are characterized by valuable marine resources, and their environmental conditions and geographic features are optimal for aquaculture production. According to Dung and Nhan (2001), *Kappaphycus* and *Euchema* species grow well and are expanding in Cam Ranh (CR) and Van Phong (VP) bays, which are located in the Vietnamese province of Khánh Hòa on the western side of the South China Sea: N 11° 53′ 00″, E 109° 10′ 00″ for CR bay and N 12° 40′ 16″, E109° 16′ 41″ for VP bay. The semi-enclosed CR bay covers 119 km^2, has a mean depth of 10 m and requires 6 days to flush its volume of 1719 km^3. (Barthel et al., 2009). VP bay comprises an area of ~510 km^2, has a mean depth of 15 m and requires 9 days to flush its volume of 1838 km^3. Compared with CR bay, VP bay is strongly stratified (Barthel et al., 2009). The loads of inorganic and organic nutrients from intensive aquaculture in many coastal areas of Vietnam have produced severe eutrophication problems that have resulted in the development of harmful algal blooms and hypoxia (An and Thu, 2007). The water quality of CR bay has been degraded by an excess of nutrients and a loss of seagrasses primarily because of intensive aquaculture development (Chen et al., 2016; Quang et al., 2017).

2.2. Sampling and nutrient analyses

Five sampling stations were selected starting from coastal (CR1 and VP1) to more open waters (CR5 and VP5). Water quality parameters were measured *in situ* with a Manta + multiprobe (Eureka). Water was sampled 30 cm below the surface in CR and VP bays in May of 2017. For bacterioplankton, duplicate samples of ~300 mL were filtered through 0.2 μm GTTP Isopore membranes (Merck Millipore) and stored with RNA*later* (Sigma-Aldrich) at −20 °C. Water for dissolved inorganic nutrients and dissolved organic carbon was filtered with a Syringe filter Minisart PES High Flow (Sartorius) and stored at the same temperature in chemically clean HDPE 50 mL bottles. Eucheumatoid macroalgae or carrageenophytes were collected according to their abundances and accessibility to each sampling point. Two samples of *Kappaphycus alvarezii*, the same portion of the thallus from different individuals, were collected at each station in CR bay. In VP bay, three samples of *Kappaphycus striatus* were collected at VP1, and four samples of *Euchema denticulatum* were collected across the remaining stations. For each species, an individual showing an ice-ice disease was collected during the survey. Ammonium, nitrate, nitrite, phosphate and silicate were determined according to standard methods (Grasshof et al., 1999) with an auto-analyser (Evolution III, Alliance Instrument). Dissolved organic carbon (DOC) was measured by high-temperature catalytic oxidation with a Shimadzu TOC-VCPN analyser. Reference water (Hansell Lab.) and internal standards were used to check the performance of the analyser.

2.3. DNA extraction and 16S rRNA analysis

DNA was extracted following the protocol of Griffiths et al. (2000): first with hexadecyltrimethylammonium bromide (CTAB, Carl Roth) and later with phenol–chloroform-isoamyl alcohol (25:24:1, Sigma-Aldrich). After a centrifugation step at 16,000*g* at 4 °C for 10 min, the DNA was precipitated from the aqueous layer with 2 volumes of 30% polyethylene glycol 6000 (Sigma-Aldrich) – 1.6 M NaCl at 4 °C for 2 h. Afterwards, the samples were centrifuged at 17,000*g* at 4 °C for 90 min,

and the pellets were washed with ice-cold 70% ethanol (Roth) and resuspended in Tris-EDTA buffer (Sigma-Aldrich). The set of primers Bact-341F (sequence 5′ - 3′: CCT ACG GGN GGC WGC AG) and Bact-785R (GAC TAC HVG GGT ATC TAA KCC) were selected according to Klindworth et al. (2012) to amplify the V3-V4 hypervariable region of the 16S rRNA gene. The company LGC genomics sequenced this amplicon with a 2 × 300-bp paired-end run on an Illumina MiSeq sequencer.

Removal of primer sequences and demultiplexing from the raw paired-end reads were conducted by the aforementioned company. Trimming and merging of sequences were performed with Trimmomatic v0.36 (Bolger et al., 2014) and PEAR v0.9.8 (Zhang et al., 2014), respectively. Minimum Entropy Decomposition MED v2.1 (Eren et al., 2015) was used for clustering of Operational Taxonomic Units (OTUs). Representatives of the OTUs were submitted to SilvaNGS (v132, https://ngs.arb-silva.de/silvangs/) using the threshold of sequence similarity of one and the remaining variables as default. Singleton, doubleton, mitochondrial, chloroplast and archaeal sequences were removed from the analysis. Sequence data for this study were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession number PRJEB38710 using the data brokerage service of the German Federation for Biological Data (GFBio, Diepenbroek et al., 2014) in compliance with the Minimal Information about any (x) Sequence (MIxS) standard.

2.4. Statistical analysis

Pooling of taxa, determination of relative sequence abundances and calculation of diversity indexes were performed in R v3.5.1 (R Core Team, 2018) and additional packages, such as vegan (Oksanen et al., 2019) and iNEXT (Hsieh et al., 2019). Statistics and graphics were conducted with R, XLSTAT (Addinsoft, 2018), PRIMER v6 + PERMANOVA and Xact 7.21d. Differences between the type of sample (carrageenophyte-attached or planktonic) and the bays were evaluated using permutational multivariate analysis of variance (PERMANOVA). If there were interactions between the PERMANOVA factors, pair-wise tests were performed. Data were log-transformed, and PEMANOVA was calculated from a Bray Curtis similarity matrix. In the event of significant differences, similarity percentage analysis (SIMPER) was conducted to identify the main taxa contributing to similarities and dissimilarities. Comparisons between the types of samples were performed at the class and genus level as well as between the bays within each type of sample at the genus and OTU level.

Differences in nutrient concentrations between the two bays were evaluated with a Mann-Whitney test. Pearson coefficients (r) assessed the main positive and significant correlations between the relative sequence abundance of genera and nutrient concentrations. Distance-based linear models (DistLM) and distance-based redundancy analysis (dbRDA) were used to evaluate relationships between nutrient concentrations and the distribution of genera in the carrageenophyte-attached and planktonic samples. Akaike's information criterion for small sample sizes (AICc) and best procedure was used for DistLM. All statistical tests were considered significant at $p < 0.05$. A heat map was performed with the most abundant OTUs (greater than 1.6%) to compare healthy algae with those with ice-ice disease.

3. Results

3.1. Carrageenophytes-attached vs. planktonic bacteria

At the class level, PERMANOVA revealed significant differences between sample types (Pseudo-F = 11.4, $p < 0.001$) and bays (Pseudo-F = 4.1, $p = 0.019$); an interaction between these factors was also detected (Pseudo-F = 1.6, $p = 0.140$). According to the pair-wise tests, the types (carrageenophyte-attached or bacterioplankton) differed significantly in VP ($t = 3.5$, $p < 0.001$) and CR bays ($t = 4.7$, $p < 0.001$). Gammaproteobacteria, Alphaproteobacteria and Bacteroidia were the main classes contributing to the similarity within each group: carrageenophyte-attached or planktonic (Table 1). Gammaproteobacteria showed the maximum percentage of similarity within carrageenophyte-attached bacteria, and Alphaproteobacteria showed the maximum percentage of similarity within bacterioplankton. The total dissimilarity between carrageenophyte-attached and planktonic bacteria was ~35%. Acidimicrobiia, Marinimicrobia SAR 406 unclassified, Rhodotermia and Planctomycetacia were the main classes contributing to this value (Table 1). The first three were typical for the planktonic fraction, while the last one was characteristic for carrageenophytes.

At the genus level, PERMANOVA also revealed significant differences between the sample types (Pseudo-F = 30.7, $p < 0.001$) and the bays (Pseudo-F = 3.6, $p = 0.004$); no interaction between the factors was detected (Pseudo-F = 2.2, $p = 0.04$). The mean composition of genera was clearly different between carrageenophyte-attached and planktonic bacteria, but differences were more difficult to detect within the same type of sample (Fig. 1). The total dissimilarity between carrageenophyte-attached and planktonic bacteria was ~73%, and the main bacterioplankton genera contributing to this value were the following: *Candidatus Actinomarina*, HIMB 11, NS marine groups and SAR clades (Table 1). Typical carrageenophytes were *Agaribacter*, *Ruegeria*, *Alteromonas*, Pir4 lineage and *Vibrio*.

3.2. Water quality and correlations of nutrients with bacterial genera

The values of pH (~8) and salinity (~34) were similar in both bays, whereas the mean sea surface temperature was a few degrees higher in VP bay (32.6 °C) compared with CR bay (27.6 °C). Significant differences between the bays were detected in the concentrations of nitrite, ammonium, phosphate and silicate (Table 2) and eutrophic conditions were more prevalent in CR bay. Table 2 describes the main correlations of nutrients with bacterial genera. In the carrageenophyte-attached bacteria, *Rubripirellula*, *Leptobacterium*, *Hypnocyclicus* and Alphaproteobacteria unclassified followed similar correlational patterns with nitrite, phosphate and silicate. The trend with *Porphyrobacter* was comparable but only with phosphate and nitrite. In the bacterioplankton, NS5 and NS4 marine groups, SUP05 cluster, and SAR11 clade III showed a similar pattern with the mentioned inorganic nutrients and ammonium. Most of the correlations of nutrients with bacterial genera in the bacterioplankton had higher correlation coefficients and lower p values.

3.3. Carrageenophyte-attached bacteria: differences between the bays

Based on the DistLM, the dissolved nutrients explained ~34% of the total variation in the set of genera within the carrageenophytes. The first axis of the dbRDA (dbRDA1) ordinated with the samples at each bay (Fig. 2A). On the negative side were samples collected from CR bay, which showed higher concentrations with silicate, phosphate and DOC. Samples collected from VP bay generally showed higher values of nitrate on the positive side. Fig. 2B describes the correlations of nutrients with the two main axes.

At the positive extreme of dbRDA2 was sample 5 of CR bay, which had the maximum value of ammonium (0.4 μM). After SIMPER analysis, the dissimilarity at the genus level was ~54% and the main contributing genera were *Porphyrobacter*, *Vibrio*, *Hypnocyclicus*, *Labilibacter* and Parvularculaceae unclassified, which showed higher abundances in CR bay (Table 3). In contrast, typical genera of VP bay contributing to the dissimilarity were *Urania*-1B-19, *Cobetia*, Alteromonadaceae unclassified, *Agaribacter* and *Pseudoalteromonas*. At the OTU level, PERMANOVA revealed also significant differences between the bays (Pseudo-F = 3.4, $p = 0.004$). In carrageenophyte-attached bacteria, the similarities between genera within CR and VP bay were ~38% and ~31%, respectively. The dissimilarity between the bays was ~77%, and the principal OTUs contributing to this value are detailed in Table 3.

Table 1
Main bacterial classes and genera contributing to the similarity and dissimilarity between the type of sample after SIMPER analysis.

	Carrageenophyte-attached (C)	%	Planktonic (P)	%	C vs. P	%	Ty
Classes	Total similarity	69.4	Total similarity	77.4	Total dissimilarity	35.3	
Main contributors	Gammaproteobacteria^{G}	14.5	Alphaproteobacteria^{Al}	11	Acidimicrobiia^{Aci}	6.6	P
	Alphaproteobacteria^{Al}	13.4	Gammaproteobacteria^{G}	10.7	Marinimicrobia SAR406 unclass	6.6	P
	Bacteroidia^{B}	11.4	Bacteroidia^{B}	10.5	Rhodothermia	6.3	P
	Deltaproteobacteria^{D}	8	Oxyphotobacteria^{O}	8.4	Planctomycetacia^{P}	6.3	C
	Planctomycetacia^{P}	8	Acidimicrobiia^{Aci}	8.4	Mollicutes	5.8	P
	Actinobacteria^{Act}	6	Actinobacteria^{Act}	7.8	Verrucomicrobiae	5.5	P
	Oxyphotobacteria	5.9	Verrucomicrobiae	7.1	Phycisphaerae	5.1	C
	Phycisphaerae	5.4	Deltaproteobacteria	5.9	Campylobacteria	5	C
Genera	Total similarity	49.2	Total similarity	65	Total dissimilarity	73.2	
Main contributors	*Vibrio*^{G}	3.4	HIMB11^{Al}	3.5	*Candidatus Actinomarina*^{Aci}	1.6	P
	Alteromonas^{G}	3.2	SAR86^{G}	3.3	HIMB11^{Al}	1.5	P
	Ruegeria^{Al}	2.9	Flavobacteriaceae unclass^{B}	3.2	NS5 marine group^{B}	1.4	P
	Agaribacter^{G}	2.5	AEGEAN-169 unclass^{Al}	3.2	NS4 marine group^{B}	1.4	P
	Hyphomonadaceae unclass^{Al}	2.1	*Candidatus Actinomarina*^{Aci}	3.2	SAR116 clade unclass^{Al}	1.4	P
	Pir4 lineage^{P}	2.1	NS5 marine group^{B}	2.9	SAR86 clade unclass^{G}	1.4	P
	Rhizobiaceae unclass^{Al}	2	NS4 marine group^{B}	2.9	*Litoricola*^{G}	1.3	P
	Blastopirellula^{P}	1.9	SAR116 clade unclass^{Al}	2.9	SAR 11 clade Ia^{Al}	1.2	P
	Cutibacterium^{Act}	1.8	*Cyanobium* PCC-6307^{O}	2.8	Cryomorphaceae unclass^{B}	1.2	P
	Epibacterium^{Al}	1.7	*Synechococcus* CC9902^{O}	2.8	*Agaribacter*^{G}	1.2	C
	Alteromonadaceae unclass^{G}	1.7	OM60(NOR5) clade^{G}	2.5	*Ruegeria*^{Al}	1.2	C
	Muricauda^{B}	1.7	NS9 marine group unclass^{B}	2.4	SAR 11 clade II unclass^{Al}	1.2	P
	Woeseia^{G}	1.7	*Litoricola*^{G}	2.4	*Alteromonas*^{G}	1.2	C
	Robiginitalea^{B}	1.7	Cryomorphaceae unclass^{B}	2.3	Thiotrichaceae unclass^{G}	1.1	P
	P3OB-42^{D}	1.7	PeM15 unclass^{Act}	2.3	Pir4 lineage^{P}	1.1	C
	Pseudoalteromonas^{G}	1.7	SAR 11 clade II unclass^{Al}	2.1	*Vibrio*^{G}	1.1	C

unclass: unclassified, Caption superscript: indicates correspondence between class and genus (e.g., ^{G} = Gammaproteobacteria), Ty: type of sample (C or P) where the taxon contributing to the dissimilarity dominated

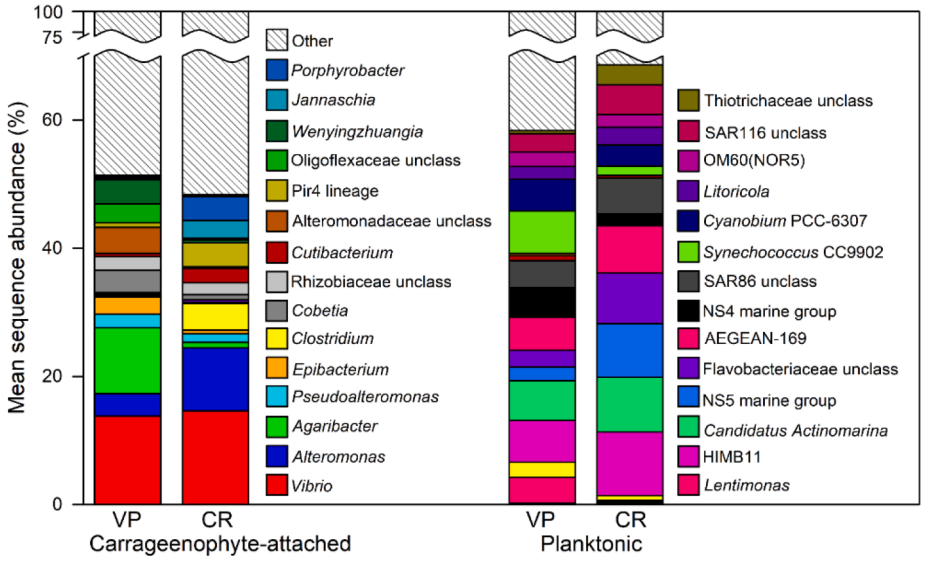

Fig. 1. Mean sequence abundance of the main bacterial genera on the carrageenophytes and surrounding water at Cam Ranh (CR) and Van Phong (VP) bays. unclass: unclassified.

3.4. Bacterioplankton: differences between the bays

After DistLM, dissolved nutrients within the seston fraction explained 50% of the total variation in the distribution of genera. The first axis of the dbRDA does not clearly separate the bays; nevertheless, the samples from CR bay were clustered on the negative sides of both axes (Fig. 3A). Silicate and DOC were negatively correlated with dbRDA1, whereas phosphate and silicate were primarily negatively correlated with dbRDA2 (Fig. 3B).

Sample 5 at VP bay, which had the maximum value of nitrate (1.5 μM), was located at the positive extreme of dbRDA1. After SIMPER analysis, the similarities of bacterioplankton were 77% at CR bay and 60% at VP bay. The total dissimilarity between the bays was ~38%, and the main genera characteristic of VP bay were *Coraliomargarita*, *Lentimonas*, *Phycisphaera* and NS2b marine group (Table 4). In CR bay, the main genera contributing to this value were SUP05 cluster, Thiotrichaceae unclassified, Ilumatobacteraceae unclassified, Microbacteriaceae unclassified and OM43. At the OTU level, PERMANOVA indicated that there were significant differences between the bays (Pseudo-F = 5.8, $p < 0.001$). At this taxonomic level, the similarities between the CR and VP bays were 65% and 41%, respectively. The total dissimilarity was ~60%, and the main OTUs contributing to this value are detailed in Table 4.

Table 2
Nutrient comparison between Van Phong (VP) and Cam Ranh (CR) bays and main Pearson correlations of nutrients with relative sequence abundances of bacterial genera.

	Mean comparison				Main positive correlations					
	VP	CR	Mann-Whitney		Carrageenophyte-attached			Planktonic		
	Mean ± SD	Mean ± SD	U	p	Genus	r	p	Genus	r	p
Nitrate	0.82 ± 0.53	0.71 ± 0.05	101	0.35	*Algicola*	0.74	<0.001	NS2b marine group	0.75	<0.001
					Oligoflexaceae unclass	0.72	0.001	OM182 unclass	0.73	<0.001
					Psychrosphaera	0.66	0.004	*Ruegeria*	0.71	<0.001
					SAR 116 clade unclass	0.6	0.011	*Aliihoeflea*	0.71	<0.001
					Candidatus Actinomarina	0.6	0.011	*Psychrobacter*	0.68	0.001
Nitrite	0.04 ± 0.03	0.23 ± 0.01	<0.01	<0.001	*Rubripirellula*	0.57	0.016	NS5 marine group	0.9	<0.001
					Leptobacterium	0.56	0.019	NS4 marine group	0.9	<0.001<0.001
					Hypnocyclicus	0.55	0.021	SUP05 cluster	0.9	<0.001
					Alphaproteobact unclass	0.53	0.03	SAR 11 clade III	0.8	<0.001
					Porphyrobacter	0.5	0.041	Flavobact unclass	0.79	
Ammonium	n.d.	0.11 ± 0.15	37.5	0.002	*Altererythrobacter*	0.59	0.013	*Formosa*	0.78	<0.001
					Peredibacter	0.58	0.015	NS5 marine group	0.75	<0.001
					Puniceicoccus	0.55	0.021	NS4 marine group	0.75	<0.001
					Hirschia	0.55	0.022	SUP05 cluster	0.72	<0.001
					Proteus	0.54	0.025	SAR 11 clade III	0.67	0.001
Phosphate	0.20 ± 0.02	1.03 ± 0.05	<0.01	<0.001	*Rubripirellula*	0.58	0.015	NS5 marine group	0.92	<0.001
					Leptobacterium	0.56	0.019	NS4 marine group	0.92	<0.001
					Hypnocyclicus	0.56	0.02	SUP05 cluster	0.92	<0.001
					Alphaproteobact unclass	0.53	0.03	Flavobact unclass	0.87	<0.001
					Porphyrobacter	0.5	0.041	SAR 11 clade III	0.78	<0.001
Silicate	3.76 ± 1.26	19.8 ± 0.32	<0.01	<0.001	*Rubripirellula*	0.54	0.026	NS5 marine group	0.93	<0.001
					Leptobacterium	0.53	0.028	NS4 marine group	0.93	<0.001
					Hypnocyclicus	0.53	0.03	SUP05 cluster	0.88	<0.001
					Alphaproteobact unclass	0.5	0.042	Flavobact unclass	0.8	<0.001
								SAR 11 clade III	0.8	<0.001
DOC	151 ± 63	149 ± 13	69	0.5	*Pseudooceanicola*	0.6	0.011	Arenicellaceae unclass	0.78	<0.001
					P3OB-42 unclass	0.54	0.025	*Lutibacter*	0.73	<0.001
					Algisphaera	0.51	0.038	*Marinobacterium*	0.72	<0.001
					Aliihoeflea	0.5	0.04	*Lentisphaera*	0.65	0.002
								Arcobacter	0.63	0.004

All nutrient concentrations in μM. DOC: dissolved organic carbon. Differences according to Mann-Whitney test. SD: standard variation, n.d.: not detected. unclass: unclassified. Alphaproteobact: Alphaproteobacteria. Flavobact: Flavobacteriaceae

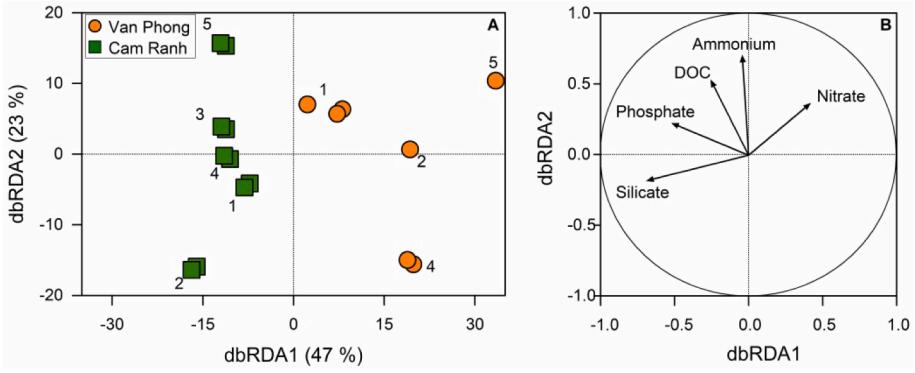

Fig. 2. Distance-based redundancy analysis (dbRDA) assessing relationships between nutrient concentrations and the distribution of genera in carrageenophyte-attached bacteria.

3.5. Insights on ice-ice disease

Elevated Inverse Simpson index values characterized the healthy algae compared with those under the ice-ice disease (Fig. 4, grey captions). The OTUs of the heat map represent more than 54% of the total sequence abundance of the healthy ones, and more than 60% for those experiencing disease. Healthy *Euchema denticulatum* was dominated by OTUs of *Wenyingzhuangia*, *Vibrio*, *Algicola*, *Thalassobius* and *Urania*-1B-19, while those experiencing disease were primarily *Rivularia* PC-7116, *Marinagarivorans* and *Lewinella*. There were differences in the OTU level for Alteromonadaceae unclassified and *Agaribacter*. In the case of *Kappaphycus striatus*, OTUs of Rhizobiaceae and Parvaculaceae unclassified, *Sulfovorum*, P3OB-42, *Agaribacter*, *Pseudooceanicola*, *Kordimonas* and *Cutibacterium* were typically healthy. OTUs of *Vibrio*, *Cobetia*, *Marinomonas* and *Pseudoalteromonas* represented *K. striatus* under the ice-ice disease condition. Under this condition, most of the OTUs of the genus *Vibrio* and some of *Alteromonas* were more abundant in *Kappaphycus alvarezii*. The main healthy OTUs were members of the genera *Hypnocyclicus*, *Porphyrobacter*, Rhizobiaceae and Marinilabiliaceae unclassified, *Bacteroidetes* BD2-2, *Perspicuibacter*, Pir 4 lineage and *Ruegeria*.

4. Discussion

4.1. Putative roles of dominant bacteria attached to carrageenophytes

Epiphytic bacteria were clearly different from those of

Table 3
Main carrageenophyte-attached genera and OTUs contributing to the dissimilarity between the bays after SIMPER analysis.

	VP vs. CR	%	Bay		VP vs. CR	%	Bay
	Total dissimilarity	53.6			Total dissimilarity	76.6	
Main genera	*Porphyrobacter*	1.7	CR	Main OTUs	*Porphyrobacter* 1821	0.4	CR
	Vibrio	1.3	CR		*Urania-1B-19* 0999	0.4	VP
	Urania-1B-19	1.3	VP		*Agaribacter* 0931	0.3	VP
	Hypnocyclicus	1.3	CR		*Alteromonas* 3324	0.3	VP
	Cobetia	1.3	VP		*Epibacterium* 1837	0.3	VP
	Labilibacter	1.3	CR		*Alteromonas* 4320	0.3	VP
	Parvularculaceae unclass	1.2	CR		Pir4 lineage 0221	0.3	CR
	Leptobacterium	1.2	CR		Pir4 lineage 0680	0.3	CR
	Rubripirellula	1.2	CR		*Hypnocyclicus* 0205	0.3	CR
	Alteromonadaceae unclass	1.2	VP		*Agaribacter* 3297	0.3	VP
	Agaribacter	1.2	VP		*Vibrio* 3867	0.3	VP
	Rhizobiaceae unclass	1.2	CR		*Pseudooceanicola* 2931	0.3	VP
	Pir4 lineage	1.1	CR		*Agaribacter* 0923	0.3	VP
	Pseudoalteromonas	1.1	VP		*Vibrio* 4983	0.3	CR
	Epibacterium	1.1	VP		*Vibrio* 3856	0.3	VP
	Arcobacter	1.1	CR		*Phycisphaera* 3439	0.3	VP

unclass: unclassified, bay (VP or CR) where the taxon contributing to the dissimilarity dominated, OTU: operational taxonomic unit or MED node

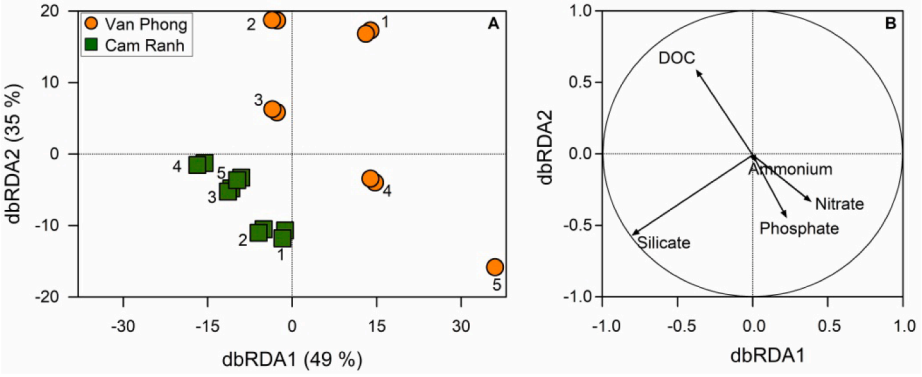

Fig. 3. Distance-based redundancy analysis (dbRDA) assessing relationships between nutrient concentrations and genera distribution in bacterioplankton.

Table 4
Main planktonic genera and OTUs contributing to the dissimilarity between the bays after SIMPER analysis.

	VP vs. CR	%	Bay		VP vs. CR	%	Bay
	Total dissimilarity	37.9			Total dissimilarity	59.9	
Main genera	*Coraliomargarita*	2.6	VP	Main OTUs	SUP05 cluster 2498	0.5	CR
	SUP05 cluster	2.6	CR		*Lentimonas* 1196	0.5	VP
	Thiotrichaceae unclass	2.0	CR		Thiotrichaceae unclass 2942	0.5	CR
	Lentimonas	1.7	VP		SAR86 unclass 3792	0.5	CR
	Ilumatobacteraceae unclass	1.7	CR		NS5 marine group 2654	0.5	CR
	Phycisphaera	1.5	VP		*Cyanobium* PCC-6307 1459	0.4	CR
	Microbacteriaceae unclass	1.4	CR		*Coraliomargarita* 3906	0.4	VP
	OM43	1.4	CR		Flavobacteriaceae unclass 4943	0.4	CR
	Acholeplasma	1.4	CR		NS5 marine group 2650	0.4	CR
	Cryomorphaceae unclass	1.4	CR		Thiotrichaceae unclass 2939	0.4	CR
	Litoricola	1.4	CR		NS5 marine group 2275	0.4	CR
	NS5 marine group	1.4	CR		HIMB11 4075	0.4	CR
	NS4 marine group	1.4	CR		NS4 marine group 2148	0.4	VP
	NS2b marine group	1.4	VP		*Formosa* 3673	0.3	CR
	MB11C04 marine group	1.4	VP		Flavobacteriaceae unclass 2020	0.3	CR
	SAR 11 clade III	1.3	CR		Arenicellaceae unclass 3622	0.3	CR

unclass: unclassified, bay (VP or CR) where the taxon contributing to the dissimilarity dominated, OTU: operational taxonomic unit or MED node

bacterioplankton, even at the class level, confirming one of our initial hypotheses. Several studies support this finding (Burke et al., 2011; Friedrich, 2012; Aires et al., 2013); in our study the dissimilarity was greater than 70% at genus level across the entire data-set. Epiphytic bacteria on macroalgae generally can form biofilms, degrade algal polymers, inhabit organic-rich environments and possess competitive advantages against other microorganisms (e.g., Friedrich, 2012). Gammaproteobacteria, the class with the highest similarity within the carrageenophytes, has these characteristics. For example, *Vibrio* spp. are the most common isolates from red algae, form biofilms on several substrates, present enzymes for the degradation of algal compounds, and are producers of substances with antimicrobial and antifouling properties (Goecke et al., 2010; Hollants et al., 2013; Takemura et al., 2014).

Within the gammaproteobacteria, the family Alteromonadaceae (mainly *Agaribacter*, *Alteromonas* and unidentified members in this study) and *Pseudoalteromonas* possess some of these aforementioned

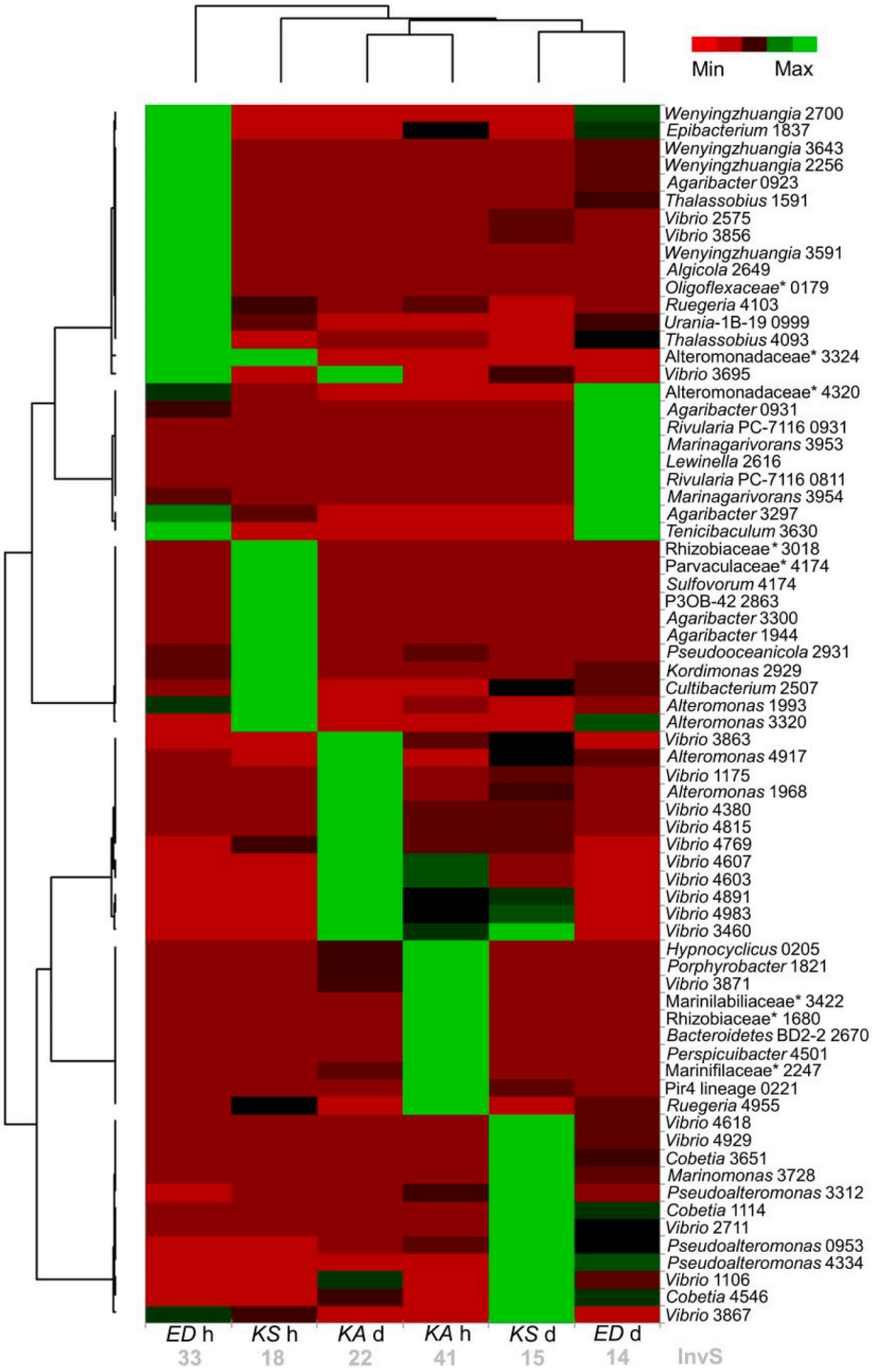

Fig. 4. Heat map comparing the main bacterial operational taxonomic units (OTUs) on healthy and ice-ice diseased species of carrageenophytes. ED: *Euchema denticulatum*, KS: *Kappaphycus striatus*, KA: *Kappaphycus alvarezii*, D: diseased, H: healthy, InS: inverse Simpson diversity.

features for *Vibrio*, which transform them into successful colonizers of macroalgae (Goecke et al., 2010; Hollants et al., 2013). Several species of the family Alteromonadaceae are generally associated with organic-rich environments, are even attached to marine animals and display extensive degradative properties on diverse substrates (López-Pérez and Rodriguez-Valera, 2014). The name of the genus *Agaribacter* was proposed because of its ability to degrade agar (Teramoto and Nishijima, 2014). As reviewed by Goecke et al. (2010), several species of *Alteromonas* and *Pseudoalteromonas* are common epiphytes of macroalgae, are sources of bioactive compounds with antibiotic and antifouling activities, and use agarases, carrageenases, alginases, cellulases and pectinases to catabolize algal polymers.

Some alphaproteobacteria, particularly those of the family Rhodobacteraceae (mainly *Pseudooceanicola*, *Epibacterium* and *Ruegeria* in the carrageenophytes of this study), are characteristic of marine systems rich in organic nutrients. *Pseudooceanicola* had the highest correlation coefficient with DOC in this study. Members of Rhodobacteraceae are ubiquitous primary surface colonizers and produce extracellular polymeric substances (EPS) facilitating the settlement of other bacterial communities (Dang et al., 2008). The genus *Epibacterium* was described

originally as epibiotic on marine algae (Penesyan et al., 2013). Chemical communication through diverse compounds is suspected to be a characteristic of some algal epibionts; for example, *Ruegeria* sp. was able to detect sulphuric compounds released by algae and to release auxins for algal growth (Cirri and Pohnert, 2019). According to Hollants et al. (2013), *Ruegeria* spp. have potential functions in the defence and morphogenesis of macroalgae. The family Rhizobiaceae is widely known for their symbiosis with plants and have some key functions (e.g., nitrogen fixation or nutrient uptake); some can insert bacterial DNA in the genome of higher plants and facilitate their genetic engineering (Carareto Alves et al., 2014). Some members of the family Rhizobiaceae have been used for the genetic modification of microalgae and possess probiotic and growth-enhancing properties (Schwenk et al., 2014).

Two classes of Planctomycetes, Planctomycetacia and Phycisphaerae, were characteristic of carrageenophytes and contributed considerably to the dissimilarity with bacterioplankton. Planctomycetes are copiotrophic microorganisms widely known for their tight association with macroalgae and for their wide enzymatic pool for the degradation of sulphated polysaccharides (Lage and Bondoso, 2014; Bondoso et al., 2017; Faria et al., 2018). Pir 4 lineage and *Blastopirellula* were important genera of the class Planctomycetacia within the carrageenophytes. According to Faria et al. (2018), Planctomycetes attached to algae showed unique proteins related to metal binding systems, phosphate metabolism, chemotaxis, and the stress response. Campylobacteria was the other class contributing to the dissimilarity and the order Campylobacterales within this class was found abundantly in aquatic systems with higher concentrations of organic nutrients (Kopprio et al., 2020). This taxon may be associated with the organic-matter-rich microenvironments of the carrageenophytes.

Similar to Planctomycetes, the family Flavobacteriaceae of the class Bacteroidia (principally *Muricauda*, *Robiginitalea*, *Wenyingzhuangia* and *Leptobacterium* in the carrageenophytes of this study) comprises copiotrophic bacteria with enzymes that degrade polymers and algal particles (Fuhrman et al., 2015; Yilmaz et al., 2016; Bunse and Pinhassi, 2017). Several new species in close association with red algae have been described within this family (e.g., Yoon et al., 2015). Furthermore, the Bacteroidetes are degraders of complex biopolymers and polysaccharides, including carrageenan (Ficko-Blean et al., 2017; Jain et al., 2019). The Actinobacteria are also typical of red algae (Hollants et al., 2013), and this class plays an essential role in the production of antibiotics and degradation of complex polymers (Alvarez et al., 2017). In sum, bacteria of diverse phyla with the ability to degrade complex organic molecules and interact chemically with the algal host were characteristic of the carrageenophytes.

4.2. Potential eutrophication markers attached to carrageenophytes

Rubripirellula, *Leptobacterium*, *Hypnocyclicus* and *Porphyrobacter* were potential markers of eutrophication, as indicated by their correlations with phosphate. Moreover, their similar patterns with silicate (with the exception of *Porphyrobacter*) suggested a freshwater origin. Ammonium was not detected in VP bay, and its concentrations were low in CR bay; these observations affect its accuracy as a pollution marker. The origins of DOC are multiple and range from phytoplankton blooms to anthropogenic sources; by contrast, nitrate is generally linked to more oceanic water masses (e.g., Bachmann et al., 2018). Water nutrients explained only 34% of the variation within carrageenophyte-attached bacteria; the microenvironment on the red algae offers more stable nutrient conditions than the surrounding water. Several other factors such as biogeography, phylogeny and even stochasticity influence the bacterial community composition of macroalgae (Burke et al., 2011; Friedrich, 2012; Aires et al., 2013).

Although significant differences in some nutrient concentrations were detected, the contrast between the bays during our sampling was not as strong as was expected. This phenomenon might be explained by a phytoplankton bloom. As previously mentioned (section 2.1), the water quality in CR bay has been degraded because of intensive aquaculture development; consequently, some bacterial taxa were indicative of polluted water. Although correlation does not necessarily indicate causality, *Rubripirellula*, *Leptobacterium*, *Hypnocyclicus* and *Porphyrobacter* dominated in CR bay and showed elevated percentages of dissimilarities. SIMPER analysis is more sensitive to bacterial abundances and focuses on dominant genera. Therefore, subsequent discussion highlights genera that make greater contributions to the dissimilarities between the bays.

Rubripirellula was a potential pollution marker at CR bay, while Pir 4 lineage, Urania-1B-19 and Phycisphaera were probably specific epibionts of carrageenophytes. Some Planctomycetes are characteristic of polluted environments, while others are specifically associated with macroalgae (Lage and Bondoso, 2014; Bondoso et al., 2017). The Bacteroidetes *Leptobacterium* and *Labilibacter* were likely markers of the copious amounts of organic matter at CR bay. *Hypnocyclicus* dominated the bacterial community composition of the hindgut of a crayfish (Foysal et al., 2019) and may be associated with the impact of intensive aquaculture at CR bay. Elevated abundances of *Hypnocyclicus* and *Arcobacter* in CR bay were likely markers of sewage pollution. These two genera are indicators of high levels of faecal pollution and raw wastewater (Collado et al., 2008; Cui et al., 2020; Kopprio et al., 2020).

Several species of *Porphyrobacter* have been isolated from seawater, and some of them can degrade xenobiotic compounds (e.g., Leys et al., 2005), which is a common feature of members of the family Sphingomonadaceae that facilitates persistence in polluted environments. *Porphyrobacter algicida* shows algicidal activity against phytoplankton (Kristyanto et al., 2017); this species or a close related one might be an epiphyte of *K. alvarezii* and prevent phytoplankton fouling in eutrophic systems. Species of the genus *Vibrio* are commonly detected in red algae, and toxigenic strains of *V. cholerae* have been associated with sewage pollution (Kopprio et al., 2020 and references therein); nevertheless, the resolution of OTU made it impossible to conclude whether *Vibrio* spp. act as markers of anthropogenic impacts. The family Parvularculaceae is characteristic of saline environments and has been recently linked to sludge (Zheng et al., 2019). However, the lack of correlative evidence of the family Parvularculaceae, *Vibrio*, *Arcobacter* and *Labilibacter* reduces the likelihood that they provide robust eutrophication markers.

4.3. Characteristics of dominant bacterioplankton

Several bacterioplankton classes and genera were the main contributors to the dissimilarities with carrageenophyte-attached bacteria and indicated that conditions were generally eutrophic in the bays. The highest value of dissimilarity was observed in Acidimicrobiia (mainly *Candidatus Actinomarina* in this study), which is widely distributed in the photic zone of tropical oceans and, in some cases, mirrors the distribution of *Synechococcus* (Ghai et al., 2013). The cyanobacteria of the class Oxyphotobacteria, primarily *Synechococcus* and *Cyanobium* in this study, were important components of the bacterioplankton in bays. *Synechococcus* generally dominates in coastal and nutrient-rich conditions (Fuhrman et al., 2015; Bunse and Pinhassi, 2017). The South China Sea is dominated by alphaproteobacteria and secondarily by cyanobacteria in surface seawater (Zhang et al., 2018). The dominance of alphaproteobacteria in coastal zones of the South China Sea was also observed in this study.

Within alphaproteobacteria, HIMB11 belongs to the copiotrophic family Rhodobacteraceae and is considered an opportunist. HIMB11 persists on relatively few substrates and uses alternative energy metabolism until conditions are favourable for exponential growth, such as a phytoplankton bloom (Durham et al., 2014). During blooms, Verrucomicrobia populations can become abundant using their diverse complement of glucoside hydrolases to degrade polysaccharides (Bunse and Pinhassi, 2017). Another group that may indicate eutrophic conditions are the Marinimicrobia or SAR 406, which are persistent in oxygen minimum zones and positively correlated with chlorophyll *a* concentrations (Yilmaz et al., 2016). SAR 86 consists of ubiquitous and

abundant gammaproteobacteria with important enzymes for the metabolism of polysaccharides (Hoarfrost et al., 2020), which are favoured under higher concentrations of organic nutrients. Other important copiotrophic gammaproteobacteria of the bacterioplankton and the role of some Bacteroidetes as pollution markers are discussed in the next section. The dominant taxa detected were indicative of eutrophic conditions in the bays.

4.4. Presumptive water pollution indicators in bacterioplankton

NS5 and NS4 marine groups, SUP05 cluster, Flavobacteriaceae unclassified and SAR 11 clade III were strong indicators of eutrophication, as evidenced by their strong correlations with the main inorganic nutrients as well as by their elevated contribution to dissimilarities according to SIMPER analysis. Bacterioplankton are more dependent on the conditions of the water column, as indicated by the higher percentage of variation explained by nutrient concentrations (~50%) and the stronger correlative evidence. NS marine groups and unidentified Flavobacteriaceae dominated the farming effluent of *Litopenaeus vannamei* (Becker et al., 2017). Higher eutrophic conditions in CR bay also indicate lower oxygen concentrations in the water column. SUP05 are sulphur-oxidizing bacteria broadly distributed in oxygen minimum zones and have the potential to consume ammonium to produce nitrite under anoxic conditions (Shah et al., 2017). SAR 11 has been linked to anoxia (Tsementzi et al. 2016), and clade III is considered a freshwater lineage (Herlemann et al., 2014). Furthermore, the correlational patterns of SAR 11 clade III, NS5 and NS4 marine groups, SUP05 cluster, Flavobacteriaceae unclassified with silicate and other inorganic nutrients indicated that nutrient loads had a freshwater origin.

In addition to SUP05, other dominant gammaproteobacteria markers of higher eutrophic conditions at CR bay were Arenicellaceae unclassified, *Formosa*, OM43 and SAR86. DOC and ammonium were strongly correlated with Arenicellaceae unclassified and *Formosa*, respectively. Other potential markers of pollution, but without correlative evidence, were unidentified genera of the families Thiotrichaceae, Ilumatobacteraceae and Cryomorphaceae, *Litoricola* and *Acholeplasma*. The family Thiotrichaceae comprises sulphur-oxidizing bacteria, is valuable for biological phosphate removal and dominates in oxic-settling-anoxic process for the treatment of wastewater (Guo et al., 2019; Karlikanovaite-Balikci et al., 2019). The family Cryomorphaceae was also dominant in aquaculture effluent (Becker et al., 2017), and Ilumatobacteraceae was enriched in the intestine of crabs (Sun et al., 2020). *Litoricola* was a dominant genus in the water and sediments from aquaculture ponds of the shrimp *Litopenaeus vannamei* (Huang et al., 2018). *Acholeplasma* (Mollicutes) is generally associated with estuarine waters and with the microbiome of vertebrates (Yu et al., 2018). In sum, the strongest indicators of higher eutrophic conditions at CR bay were NS5 and NS4 marine groups, SUP05 cluster, Flavobacteriaceae unclassified, SAR 11 clade III, Arenicellaceae unclassified and *Formosa*.

4.5. Insights on ice-ice disease: potential detrimental and beneficial bacteria

The loss of diversity in bacterial communities often reflects the presence of disease. This phenomenon has also been observed in some macroalgae (e.g., Quéré et al., 2019); however, in other cases most of the differences were not significant (Liang et al., 2019). The bacterial community composition at the OTU level changed considerably depending on the carrageenophyte species and the health state. *Marinagarivorans* may not only consume organic molecules on the algal surface but may also degrade the walls and invade the thallus of *E. denticulatum*. A strain of *Cobetia* isolated from rotten seaweeds contained alginate lyases (Gong et al., 2017), and a member of this genus may degrade and invade *K. striatus*. However, species of *Cobetia* have been reported to function in the defence and morphogenesis of macroalgae (Hollants et al., 2013).

OTUs of *Vibrio* were common but not exclusive to the diseased state in *K. striatus* and *K. alvarezii*. *Vibrio* and *Alteromonas* have been reported as aetiological agents of the ice-ice disease (Vairappan et al., 2008; Goecke et al., 2010; Hayashi et al., 2010). OTUs of *Alteromonas* were also characteristic of *K. alvarezii* under this condition, while those of *Pseudoalteromonas* were typical of *K. striatus*. As reviewed by Egan et al. (2014), these three genera comprise common pathogens in marine macroalgae and cause several other syndromes and diseases. In the case of ice-ice disease, changes in salinity and temperature generally trigger the oxidative burst producing the whitening of the thallus, which is followed by the colonization of pathogenic or saprophytic microorganisms. It is often difficult to differentiate pathogens from saprophytes using this amplicon-based approach.

A succession of bacteria at the species or strain level may occur from the healthy state to the diseased state, because OTUs of *Vibrio*, *Alteromonas*, *Agaribacter* and Alteromonadaceae unclassified are generally different between both states. Some OTUs of Vibrio are associated with the healthy state of *E. denticulatum*, and, as mentioned previously, some species are beneficial for macroalgae. Moreover, there is evidence that some species of *Vibrio* affect the survival, morphogenesis and reproduction of macroalgae (Takemura et al., 2014). *Ruegeria* likely contributed to the functions of growth and defence in *E. denticulatum* and *K. alvarezii*. Urania-1B-19 in *E. denticulatum* and Pir 4 lineage in *K. alvarezii* might have some roles related to stress resistance and nutrient exchange. *Wenyingzhuangia* spp. (Flavobacteraceae), are epiphytes of red algae (e.g., Yoon et al., 2015) and may be involved in the degradation of sulphated organic compounds.

Unidentified genera of the family Rhizobiaceae were common in healthy *K. striatus* and *K. alvarezii*; as mentioned previously, they may have probiotic, growth-enhancing and nutritional functions. *Thalassobius* has been described as one of main bacteria causing the lysis of dinoflagellate cultures (Wang et al., 2010), and antifouling properties of the genus are suspected to occur on *E. denticulatum*. *Algicola bacteriolytica* was named because of its unique bacteriolytic activity on diverse bacteria and may defend *E. denticulatum* against potential pathogens. Moreover, the crude extract of *K. alvarezii* has not shown any antibacterial activity (Chuah et al., 2017); thus, this carrageenophyte may depend on epibionts for its defence against pathogens. *Cutibacterium* may produce antibiotics on *K. striatus*, while *Porphyrobacter* may prevent biofouling on *K. alvarezii*.

5. Conclusions and outlook

Bacterial communities in the carrageenophytes and bacterioplankton were strong indicators of the source (algae or water) of organic matter and the higher anthropogenic impacts at CR bay. The combination of correlative evidence with dissimilarity analyses between the bays facilitated the detection of reliable eutrophication markers. Bacterioplankton were deduced to be more dependent on environmental nutrients and were consequently more sensitive to eutrophication or pollution. The ability to degrade algal polymers, such as sulphated polysaccharides, is a common characteristic of most bacterial epiphytes; these molecules are likely used by red algae to sustain commensal and beneficial bacteria. Under certain circumstances or environmental stresses this relationship may change, and some bacteria may use their enzymatic pool to invade the thallus and cause disease or degrade dead tissue. However, there appears to be a succession of *Vibrio* and *Alteromonas* species from healthy to diseased carrageenophytes.

Our approach provides preliminary, correlational (not causal) evidence on the putative beneficial and detrimental bacteria and their potential roles. Some potential functions were inferred also with this 16S rRNA approach, but further metagenomic and metatranscriptomic studies are needed to confirm the presence of functional genes and their expression. Nevertheless, our study is valuable because it offers a pioneering to characterize the entire composition of commercially and ecologically important carrageenophytes. The impacts of intensive

aquaculture on eutrophication can be mitigated integrating carrageenophytes into multitrophic systems. The carrageenophytes are not only a source of carrageenan but are also ecosystem engineers, key players for nutrient mitigation and sources for the discovery of new microorganisms with novel bioactive compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank A. Kunzmann for sampling assistance and A. Fricke for logistical support during quarantine.

Funding

This work was financed by the projects NĐT.11.GER/16 and Aquaweed (031B0121).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107067.

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