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Association of Vibrio Bacterial Diversity with Coral Reefs in Bangka

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ABSTRACT

Coral reef ecosystems include micro and macro organisms that interact with each other to form holobions both in mutualism and pathogenic ways. One part of the holobiont species on coral reefs is the bacterial community belonging to the genus *Vibrio.* Holobiont species present in coral reef ecosystems are found on the surface of mucus, coral tissue, and under the aragonite skeleton. The method used in this research is the morphological species identification method including colony shape, colony color, colony shape, cell shape, spore, gram test, acid fast test, and capsule. Identification was carried out on pure isolates taken from the coral mucus of *Leptoseris scabra*, *Pavona venosa*, and *Acropora tuneis* was taken from the Bangka Sea. The results showed that pure isolates taken from the sampling location in the Bangka Sea obtained 1 isolate identified as *Vibrio cholerae*. So it can be concluded that the member of the genus Vibrio associated with Acropora coral reefs in the Bangka Sea is *V. chloreae*.

1. INTRODUCTION

Indonesia's coastal and marine areas have the highest potential and biodiversity (mega-biodiversity) in the world and are included in the CTC (Coral Triangle Center) area [1]. Bangka Island is one of the islands with very good potential or coral reefs [2]. However, additional research is still needed to determine this. Because Bangka Island is also a center for tin mining, the status of the coral reef ecosystem in Bangka the sea can also be affected. Coral reef ecosystems can be changed by ecological and anthropogenic conditions [3]. It is known that various symbiotic micro- and macro-organisms inhabit coral reef habitats [4]. Holobion is an association between coral reefs and micro- and macro-organisms such as bacteria and microalgae [5]. These intricate symbiotic interactions enhance nutrient uptake and recycling, allowing corals to thrive in oligotrophic settings and build their skeletons [6]. Although some microorganisms can be opportunistic, the resulting interactions are mutualistic symbiosis.

Included in the variety of cnidarian-dinoflagellate symbiosis are stony corals (Anthozoa, Scleractinia: *Acropora humilis*), fire corals (Hydrozoa: *Millepora platyphylla*), blue corals (Anthozoa, Octocorallia: *Heliopora coerulea*), and inverted jellyfish (Scyphozoa: *Cassiopea* sp.) [7]. All of these marine cnidarian holobiont systems are closely involved in a mutualistic partnership with Symbiodiniaceae dinoflagellates. In addition, it is known that the bacterial community associated with coral animals is very diverse and abundant, including the Vibrionaceae family [8]. Bacterial community dynamics take advantage of various places in coral animals, such as mucus produced on the surface of corals, intracellular spaces or niches in coral animal tissues, spaces in coral animal skeletons, and seawater around coral animals [9].

Due to the complexity of the holobiont coral, further study of the interactions between bacteria and Symbiodiniaceae is a challenge. The contribution of the Symbiodiniaceae to the health and function of the holobion in coral reef ecosystems has been studied by studying their physiology, structural diversity, and stress tolerance over time [8]. The population of bacteria associated with coral reefs is twice as diverse as algae [10, 11]. Bacterial groups including Marinobacter (Gammaproteobacteria), Labrenzia, and Roseobacter (Alphaproteobacteria) have been found to be connected to the [6, 10]. Interestingly, it is also known that the same bacterial taxa positively influence the growth of many species of phytoplankton [10].

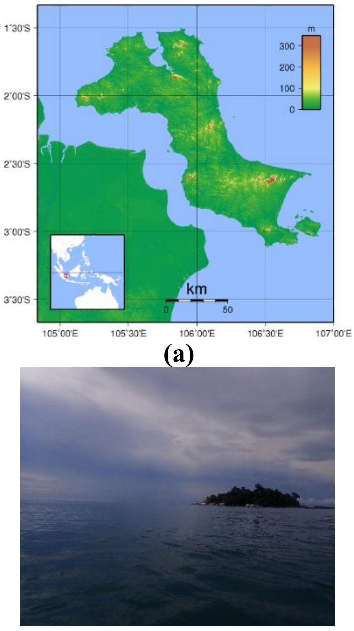
Research on bacteria from the genus Vibrio found that these bacteria grow or are distributed in seawater, offshore sediments, coral reefs or seafood products such as fish, shellfish, shrimp and crabs. Assessing the presence of Vibrio pathogens in tissue necrosis, Rubio-Portillo et al. [12] evaluated the *Vibrio* spp. assemblages associated with healthy and unhealthy colonies of these two shallow coral species. Geographic differences exist between the Vibrio communities associated with *O. patagonica* and *C. caespitosa*, but they become more uniform in diseased specimens of both species. According to Amin et al. [13] the Vibrio community discovered based on pyrH gene phylogeny of 685 isolates from seawater directly linking to Ishigaki coral holobionts consisted of 22 known and 12 possible unknown Vibrionaceae species. *V. hyugaensis, V. owensii,* and *V. harvey* were the most abundant species, followed by *V. maritimus/V. variabillis, V. campbellii, V. coralliilyticus*, and *Photobacterium rosenbergii*. Our research is a preliminary investigation of the bacteria of the genus Vibrio that are related with coral reefs, which obviously requires further investigation. In line with what was done by Amin et al. [13], so that only the morphological identification step.

Although extensive research has been conducted on the diversity of Vibrio species reported to be associated with coral reefs, research conducted in the Bangka Sea, which has different ecological and sociocultural characteristics, is of particular concern. In 2017, offshore Mining Business Permits (MBP) covered 9.35% of total marine area of Bangka Belitung and more than 50% of the marine MBPs were in coastal fishing and tourism areas [14]. Tining activity has the potential to change the abiotic structure of coral reefs. Turbidity, pH, and water temperature can be the cause of extreme environmental changes. Vibrios which were originally harmless bacteria can become pathogenic bacteria as a bacterial response to protect themselves against environmental changes [15]. This study seeks to obtain a morphological picture of the diversity of bacteria associated with coral reefs in the Bangka Sea among members of the genus Vibrio. This description of the diversity associated with coral reefs will be one of the proofs that damage to coral reefs has the potential to change the pathogenic properties of Vibrio bacteria.

2. MATERIALS AND METHODS

2.1 Research locations

This research was conducted in the Bangka Sea in June 2022, with the sampling location between the two sides of the island (Figure 1). The dive site is located at 1°38'41" South and 105° 22'04" East with a temperature of 29°C.



(b)

Figure 1. Map of sampling locations (a) Bangka Island; (b) The Location of Jerangkat Beach

2.2 Sampling

This study was conducted by collecting data at three different observation stations based on varying environmental gradient conditions, namely the in-shore, mid-shore, and offshore parts of Jerangkat Beach. At a depth of 5 meters, data was collected using the Transect method along 15 meters with a wide visibility of 2 meters. The corals that were identified and collected at each location were the prominent corals at that station. All corals found in the transect area (2×2 meters) were photographed with an underwater camera and identified using [16].

2.3 Isolation of the genus vibrio

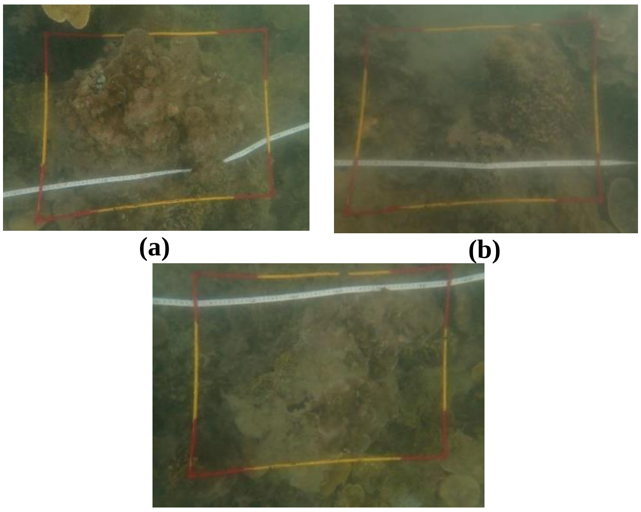
Using the streak plate method, coral samples taken from mucus were inoculated on TCBS media and cultured for 24 hours at 30°C [17]. The temperature of 30°C was chosen because in general bacteria grow optimally at temperatures of 30-37°C, and bacteria from the genus Vibrio grow optimally at temperatures of 25-30°C [18].

2.4 Identification of the genus vibrio

Bergey's Manual of Systematic Bacteriology [19] guides the identification of Vibrio bacteria by examining their morphological and biochemical characteristics; acid resistance test, Gram stain.

3. RESULTS

At station I the in-shore section was identified as Dipsastraea pallida (Figure 2a), while at station II, the mid-shore section was identified as Acropora abrotanoides (Figure 2b), and at station III the off-shore section was identified as Acropora muricata (Figure 2c).

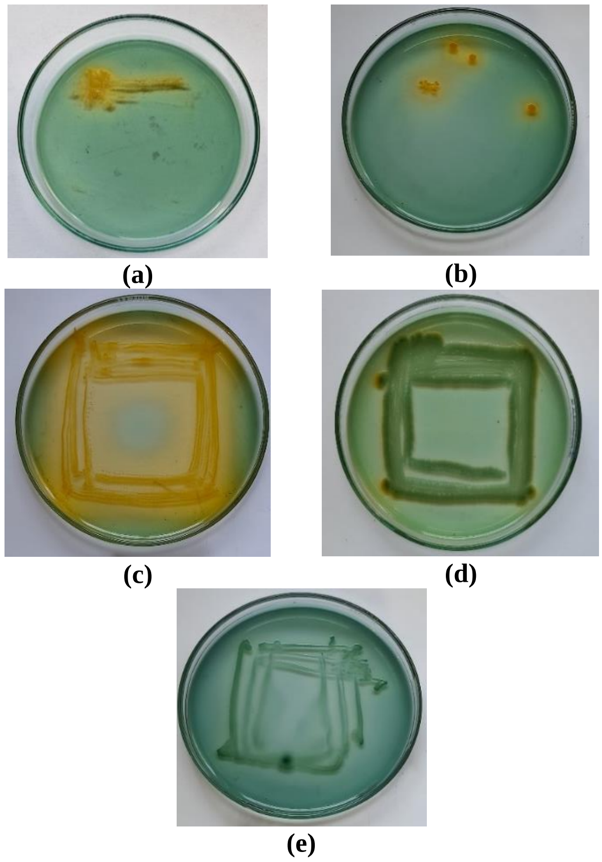


(c)

Figure 2. Dominant coral reefs in each observation at (a): Station I: *Leptoseris scabra*, (b): Station II: *Pavona venosa*, and (c): Station III: *Acropora tuneis*

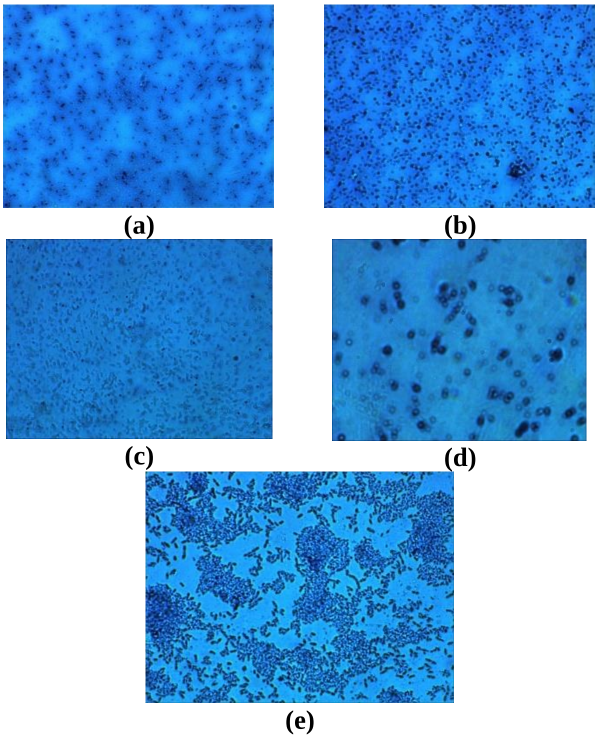
Bacteria belonging to the genus Vibrio were isolated from coral slime samples identified at each station. Bacteria belonging to the genus Vibrio were cultivated on TCBS media at 30°C for 24 hours, then their morphological and biochemical characteristics were determined. Figure 3 illustrates the bacterial colonies growing on TCBS media after 24 hours.

Bacterial cell morphology of various pure culture isolates from various stations (Figures 3 and 4) and biochemical tests (Table 1) were then used to classify the growing bacterial colonies as *Vibrio chloreae*.



(a) (b) (c) (d) (e)

Figure 3. Bacterial colonies associated with Bangka coral reefs, (a) and (b): Bacterial colonies isolated from Mucus *Dipsastraea pallida*; (c) and (d): Bacterial colonies isolated from Mucus *Acropora abrotanoides*; and (e): Bacterial colonies isolated from Mucus *Acropora muricata*



(a) (b) (c) (d) (e)

Figure 4. Morphology of bacterial cells associated with coral reefs, (a,c, and e): Bacil, and (b, and d): Coccus

The shape of the bacilli dominated the morphology of the bacterial cells from the five isolates. In addition to identification by looking at the shape of the cells, further identification tests were carried out by observing the presence or absence of spores, gram test, acid fast test, and capsules. Table 1 displays the results of the identification of bacterial colonies associated with coral reefs as a whole.

4. DISCUSSION

4.1 Holobiont species associated with coral reefs

Because they are not renewed continuously during the diurnal cycle, coral tissue is a more stable medium than SML. It has been shown that the bacterial community associated with coral tissue differs significantly from that of slime [20]. In addition, Rohwer et al. [21, 22] showed that tissue-specific microbial communities were maintained over time and distance, while separate bacterial communities were associated with various coral species. Because of these clear differences between SML and tissue, it is very important to analyze the microbial community of each coral colony layer separately. So far, relatively few microbiological studies [14, 23] have been conducted to determine the relationship between the bacterial population in SML and the tissue layers of coral colonies subjected to thermal stress. Recent studies [23, 24] have not provided information on the simultaneous shifts of bacterial communities in SML and coral holobiont tissues.

It is projected that the frequency and intensity of thermal anomalies will increase due to global climate change [25]. It is anticipated that increasing sea surface temperatures (SST) will place further pressure on corals and associated microbes [23, 26]. Ritchie and Smith [27] found that *Vibrio* spp. comprised 30% of the bacterial community cultivated in *bleached Montastraea annularis*, whereas no *Vibrio -affiliated cultures* were isolated from healthy coral colonies. In addition, the assemblage of coral-associated bacteria shifts to *Vibrio -dominated communities* just before the onset of stress-related visual symptoms during bleaching episodes [23]. On the other hand, *Endozoicomonas* spp. has been found to be a common bacterium in the healthy Porites microbiome [22]. Meyer et al. [28] also found that the presence of lesions on Porites astreoides was associated with a decrease in the relative abundance of *Endozoicomonas* spp.

Numerous studies agree that a comprehensive understanding of healthy and diseased coral microbiota is essential for a deeper understanding of coral disease [29]. Most studies comparing microbial communities in healthy versus bleached corals have been based on single sample time points [30]. These studies may not adequately account for the highly diverse geographic and temporal aspects of this assemblage [31]. Other long-term investigations of the coral–bacterial connection include in situ studies [22, 23, 32], which incorporate extra uncontrolled environmental variables that make it more challenging to interpret the data. Despite the fact that the microbial diversity obtained under controlled experimental conditions may not reflect the actual diversity observed in situ [33], this method will highlight how different environmental elements affect coral-microbial relationships [34]. Currently, no experiments have compared changes in bacterial populations between SML and coral colony tissues in response to increased heat stress.

Table 1. Morphological identification of bacteria associated with coral reefs

Sample code	Colony Form	Bacteria shape	spores	Gram test	Acid Test	Capsule
PJK 002		Basil	Endospore	Negative	Acid Resistant	Negative
PJH 004		coccus	Endospore	Positive	Acid Resistant	Negative
PJH 005		coccus	Endospore	Positive	Acid Resistant	Negative
PJK 006	Irregular	coccus	Negative	Positive	Acid Resistant	Negative
PJK 007		Basil	Negative	Positive	Acid Resistant	Negative
PJK008		coccus	Negative	Positive	Acid Resistant	Negative
PJK009		coccus	Negative	Positive	Acid Resistant	Negative
PJH 010	Round	coccus	Negative	Positive	Acid Resistant	Negative
PJH 011		coccus	Endospore	Positive	Acid Resistant	Negative
PJK 012		coccus	Negative	Positive	Acid Resistant	Negative
PJK 013		coccus	Endospore	Positive	Acid Resistant	Negative
PJH 012		coccus	Endospore	Negative	Acid Resistant	Negative
PJH 014	arch	Basil	Negative	Positive	Acid Resistant	Negative
PJK 015	Round	coccus	Endospore	Negative	Acid Resistant	Negative
PJK 016	arch	Basil	Negative	Positive	Acid Resistant	Negative
PJK 017		coccus	Negative	Negative	Acid Resistant	Negative
PJH 018	Arch	Basil	Negative	Positive	Acid Resistant	Negative
PJK 018		Basil	Negative	Negative	Acid Resistant	Negative

This is the first study to measure bacterial diversity in SML and coral tissue over time under experimentally controlled temperature stress conditions. Simultaneous analysis of communities at both levels can reveal the dynamic movement of commensals and pathogens between slime and tissues of unhealthy coral hosts. Coral-associated bacterial community profiles were analyzed, with particular emphasis on investigating the relative shifts of members of the genera *Endozoicomonas* and *Vibrio* as members of potentially beneficial and pathogenic microbial communities, to demonstrate possible shifts from native microbiota to communities dominated by potential pathogens when host corals experience stress hot.

The coral holobiont is a complex system consisting of bacteria from all three domains (*Eukaryota*, *Bacteria*, and *Archaea*) and several viruses [23, 35]. As one of the microbial groups associated with corals, bacteria have different interactions with their hosts [36] (may be mutualistic or pathogenic). Microalgae are another microbial category associated with corals, long with bacteria [37]. extrinsic (e.g., abiotic and community states composition micro- and macro-organisms in the surrounding environment) and intrinsic variables (e.g., microbial interactions and host physiology) impact the structure of the host-associated microbiome community [35]. Identification of the function of each element in predicting microbiome diversity and community structure is a major goal in microbial ecology, especially in the context of environmental change [38]. Over-exploitation, pollution, climate change, and disease are currently causing damage to coral reefs [26].

Continuous environmental degradation can have an indirect impact on various microorganisms that live around it [22]. This damage can also be exacerbated when algae dominate an ecosystem [39]. According to Bourne et al. [23], the symbiotic relationship between coral animals, endobiotic algae, and many prokaryotic microbes can compromise the integrity of the "coral holobiont". This incident can increase the possibility of bleaching or bleaching. It is known that the bacterial community associated with coral biota has high diversity and abundance [22]. Usually, coral bacterial communities are varied, patchy, and consist of hundreds to thousands of different bacterial species [23]. Recurrent bacterial taxa, such as Marinobacter (Gammaproteobacteria), Labrenzia, and other Roseobacter (Alphaproteobacteria), have been found to be associated with various species of Symbiodiniaceae [11].

4.2 Diversity of *Vibrio* sp. Related to Coral Reefs

Corals have symbiotic relationships with many beneficial bacteria, which can protect against pathogen invasion by secreting antibiotics [40], suppressing pathogen metabolism [40, 41] or digesting pathogens themselves [42]. The coral holobiont displays a core bacterial microbiome (found in 30 to 100 percent of individual coral species) that is associated with increased environmental adaptation and ecological function [43, 44]. The nuclear bacterial microbiome is highly specific [33, 35]; hence, changes in core microbiome abundance provide insight into the adaptation strategies of coral holobions in response to environmental stress in diverse habitats. Understanding the endosymbiotic Symbiodiniaceae and bacterial community structure, and their variations, is critical to understanding the acclimatization of the coral holobion to different climatic regimes and their resilience to climate change.

In addition to affecting the acclimatization and immunological response of host corals [45], coral microbial communities also interact with each other. Fluorescence in situ hybridization (FISH) investigations revealed that Actinobacter and Ralstonia are closely related to Symbiodiniaceae [46]. While some studies have found that Endozoicomonas can protect Symbiodiniaceae from bleaching pathogens, it has not been shown to do so [47, 48]. Altermonas and Cyanobacteria also supply Symbiodiniaceae coral larvae with nitrogen [6]. The microbiome is highly structured and forms complex interconnected microbial networks in which microbes associate with each other either directly or indirectly through processes such as competition, facilitation, and inhibition, according to previous studies [49]. However, it is not known what is the likely driver of Symbiodiniaceae (SBI) bacterial interactions and their relationship to microbial diversity. This information helps evaluate the stability and acclimatization of the coral holobion symbiosis.

The Vibrionaceae family of bacteria showed an unexpected increase in tarballs and crude oil contaminated areas [36]. Vibrio is widely distributed in aquatic ecosystems, and several species are zoonotic or human diseases [50]. Vibrios are of great importance to the seafood sector due to the fact that their accumulation in filter-feeding animals poses a danger of disease transmission to humans [51]. Although Vibrios are native to marine ecosystems, environmental conditions such as temperature and salt control their growth and distribution [52].

5. CONCLUSION

Based on studies conducted at three different stations, it is known that *Dipsastraea pallida* at Station I, Acropora abrotanoides at Station II, and *Acropora muricata* at Station III dominate the coral reefs at Jerangkat Beach, Bangka Regency. Moreover, *Vibrio chloreae* is the morphological name for bacteria associated with coral reefs. Subsequent research is to further explore the association of Vibrio bacteria with other components of the holobiont such as microalgae, macroalgae, vertebrates or invertebrates on the coast of Bangka. This research is complicated by the distance between the research site and the laboratory, which necessitates the isolation of samples in a makeshift room that is susceptible to contamination.

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