Abundance assessment of indicator bacteria for coral health in the Pemuteran Waters, North Bali, Indonesia

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Abstract. The increase of anthropogenic land-based activities in the Pemuteran village, a tourist spot in the northern part of Bali Island, threatened the health of its coral reefs. Therefore, this study aimed to assess the impact of anthropogenic activities in the coral reef by using two indicator bacteria (Enterococci and Vibrio). Study sites were chosen based on human activities whereas coral genus was selected following the three most abundant genera in each site. The mucus of coral fragments and the water column overlying the reefs were sampled. These samples were both tested for the presence of Vibrio in TCBS nutrient at 37°C for 24 hours and Enterococci in Slanetz and Bartley nutrient at 41°C for 24 hours. Results showed that the abundance of Enterococci in the mucus of all coral genera were relatively similar, whereas its abundances in seawater were significantly higher than those in mucus. In contrast to Vibrio, the abundances in mucus in all coral genera were significantly higher than those in seawater. Despite the likely relativeness to the natural characteristics of both indicator bacteria, the extremely high concentration of total organic carbon and nitrate in the water column, particularly nitrate, significantly enhance the abundance of Enterococci. The high level of nutrients was detected in the site which has the highest human activities, milkfish culture and marine recreational spot. The abundant of Vibrio may reveal the cause of the high prevalence of coral diseases in this area, whereas Enterococci indicate that anthropogenic pollution has reached its coral reef ecosystem.
Key Words: coral-reef, anthropogenic pollution, Enterococci, Vibrio, total organic carbon, nitrate.

Introduction. Pemuteran water is one of the tourist destination spots in the northern part of Bali Island. It is well described by the increasing number of hotels, restaurants and dive centers along the coastal zones (Prodjo 2016). However, these tourist service providers may not equip their facilities with the wastewater treatment that protects the underground and surface water from anthropogenic pollution. Studies showed that contaminants from the septic tank transferred to the surrounded coastal zones increased the nutrients concentration as well as the presence of human enterobacteria in the nearby environment (Lapointe et al 1990; Paul et al 1995).

The coral reef ecosystem supports habitat for various marine organisms and plays an important role in tourism such as seen in Bali Island (Warren-Rhodes et al 2003). The massive decrease of coral health was reported in world reefs, particularly those which are close to the tourism zone or dense populated areas (Green & Bruckner 2000). Anthropogenic pollution that contains nutrients and pathogens reach the coral reef ecosystem through land runoff, rivers and boat disposal (Bruno et al 2003; Nobles et al 2000). However, the distribution in the coral reef ecosystem remains unclear. The contribution of these untreated wastewaters to the outbreak of coral disease has shown by the presence of bacteria *Serratia marcescens* in the White Pox disease in coral *Acropora palmata* in Karibia Reef (Sutherland et al 2010). This bacterium is found as a pathogen in the human digestive and respiratory system (Miranda et al 1996; Shi et al 1997).

Enterococci are one of the microbiological indicators of fecal pollution for marine waters, recommended by The United States Environmental Protection Agency (USEPA) (USEPA 2000). Meanwhile, Vibrio is an important coral holobiont in which some of them may build a symbiosis with the hosts (Moreira et al 2014; Rubio-Portillo et al 2014; Munn 2015) while others serve as pathogens (Munn 2015). This bacteria is abundant in the high organic environments such as found in untreated wastewater or aquaculture (Colwell 1996; Reichardt et al 2007), therefore these bacteria may serve as indicator bacteria for coral health. The vast development in tourism industries in Bali Island makes it is necessary to assess the environmental health, in particular its coral reefs which support the economy of the surrounded areas. Furthermore, the presence of these indicator bacteria in the coral reefs harm for the tourist`s health as they may expose to the contaminated seawater (Cheung et al 1990).

Material and Method

Description of the study sites. Study sites were situated in the Selini Beach, Pemuteran Village, Gerogak District, Buleleng Regency, North Bali Island (Figure 1). Reefs were selected based on the anthropogenic land-based activities. Sites 1 and 2 have low population, sites 3 and 4 have high population, whereas site 5 was positioned further from the coastal zones than other sites, and is the spots for scuba diving and snorkeling. Milkfish ponds were located on the coastal areas at site 4 which managed by the traditional fisherman. The average distance between these sites was 1 km. Coral genus was selected following the three most abundant genera in each site. Coral fragments of three colonies were randomly taken by using SCUBA in a 2 x 20 m belt transect. Coral fragments were brushed by using toothbrush and mucus was placed in 50-mL conical bottom tubes. Corals were pictured by using a camera and identified according to Veron (2008) and Suharsono (2008).

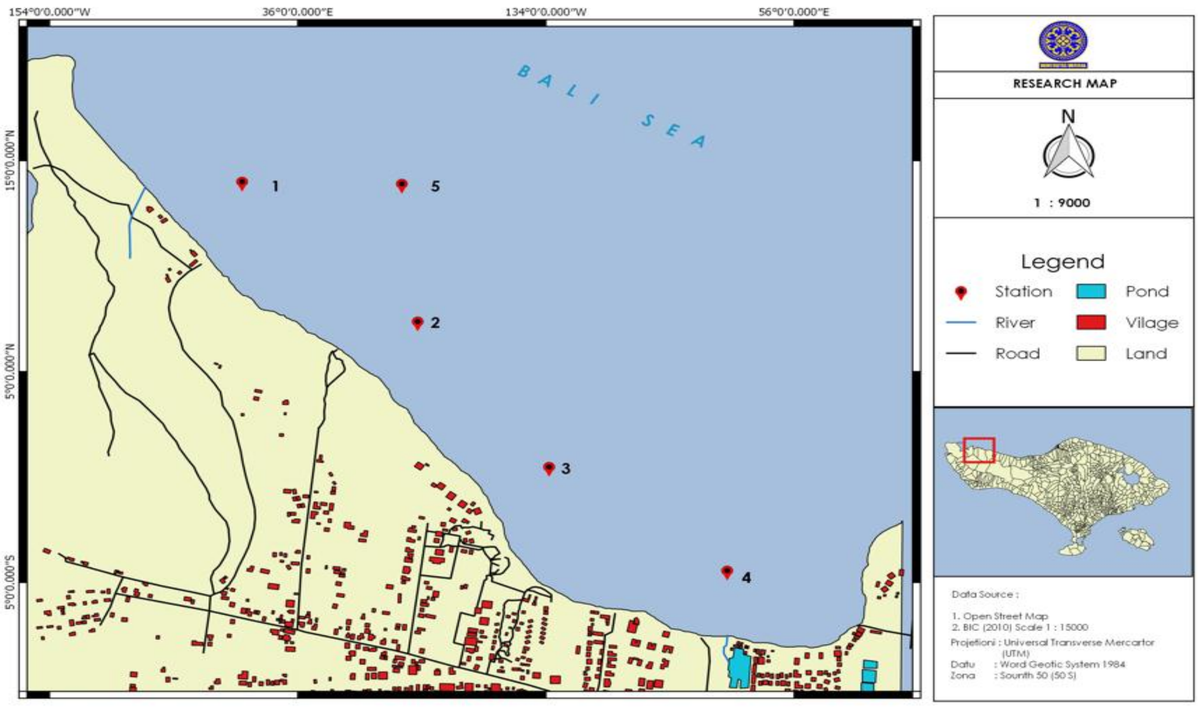

Figure 1. The study sites of selected reefs in Pemuteran waters, Bali, Indonesia.

Microbiological analyzes. Samples were firmly homogenized and filtered onto sterile 47-mm, 0.45-μm-pore-size mixed cellulose ester membranes (up to 100 mL for water and 10 mL for mucus). Filter membranes were placed on Slanetz and Bartley agar media for the assessment of Enterococci and TCBS-agar media for Vibrio. Slanetz and Bartley agar plates were incubated at 41°C for 24 hours, and colonies exhibiting a blue halo were counted as Enterococci. TCBS-agar plates were incubated at 37°C for 24 hours, and red colonies were calculated as Vibrio. Bacterial enumerate was indicated as colony forming units (CFU 100 mL^{-1}).

Measurement of total organic carbon and nitrate. Water was sampled from the overlying water column of the reefs in 1-liter polypropylene bottles to measure the concentration of nitrate and total organic carbon. The samples were kept at low temperatures when transported to the laboratory. Nitrate was analyzed by the Brucine method (Jenkins & Medsker 1964) and total organic carbon was analyzed by the potassium permanganate titrimetry method. The overlying water column`s temperature was measured by using a mercury thermometer.

Statistical analyzes. A Pearson correlation test was used to analyze the statistically significant relationships between the bacteria colony densities, total organic carbon and nitrate concentrations. Statistical analyzes were performed by using SPSS version 16.

Results. The coral density varies among sites whereas the dominant genus were Porites, Pocillopora and Acropora (Figure 2), therefore the coral mucus were only collected from these three coral genera.

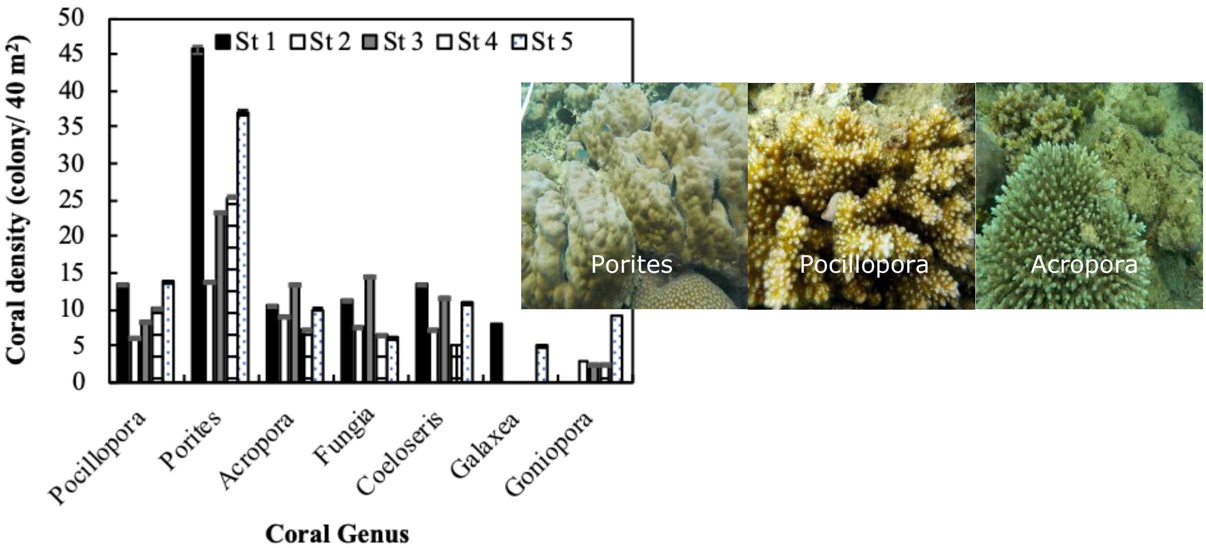

Figure 2. Coral density per genus at study sites.

Correlation between the total organic carbon, nitrate and abundance of indicator bacteria. The level of water temperature and total organic carbon were relatively similar among sites (Table 1). The detection limit of Spectrofotometer used for measurement of nitrate concentrations was 0.01 mg L^{-1}, and it was found that nitrate concentration at sites 1-3 were under this limit. However the level at sites 4 and 5 were extremely high. In contrast to total organic carbon which was significantly high at all sites. These nitrate and total organic carbon levels have exceeded the limit according to Keputusan Menteri KLH No. 51/2004 (Regulation Law No. 51 2004 of the Indonesian Environmental Ministry) which was set to marine organisms (nitrate in seawater 0.008 mg L^{-1} and total organic carbon in seawater 20 mg L^{-1}).

Table 1

The concentration of total organic carbon, nitrate and sea surface temperatures (mean±standard deviation) in the water column overlying reefs (seawater)

Site	*Nitrate (mg L^{-1})*	*Organic carbon (mg L^{-1})*	*Temperature (°C)*
1	<0.01±0.00	54.56±0.36	27.7±0.58
2	<0.01±0.00	49.72±0.36	27.7±0.58
3	<0.01±0.00	60.04±0.63	27.7±0.58
4	0.51±0.01	52.03±0.97	29.0±0.00
5	0.15±0.22	50.98±1.32	30.0±0.00

The abundances varied among indicator bacteria, samples (mucus and seawater) and sites. The abundances of Enterococci in the seawater were extremely higher than those in the coral mucus as the highest were at sites 4 and 5. It is similar to Vibrio that reached the highest density in seawater at site 4 (Figure 3). However, the abundances of Vibrio were significantly lower than Enterococci in the same seawater site samples. There was even no Vibrio found in site 1 (Figure 3). The average densities of Enterococci in coral mucus were rapidly increased to more than 10-fold in sites 4 and 5 (Figure 3). The Vibrio abundances in coral mucus in all coral genera were even too many to count (Figure 3). There were no relatively differences in both indicator bacteria abundances among the coral genus.

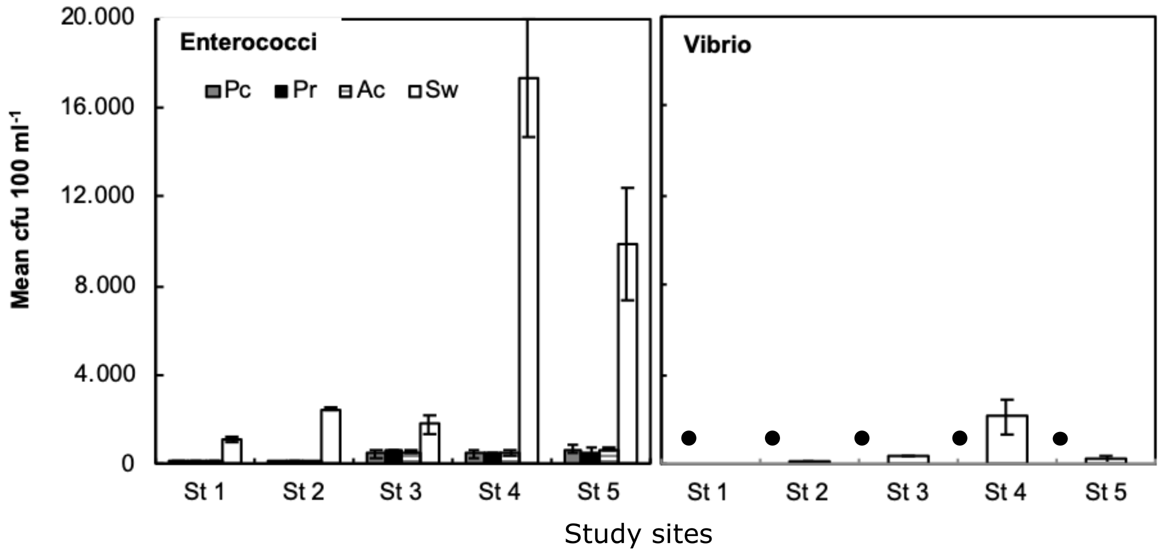

Figure 3. Mean abundances of Enterococci and Vibrio among the coral mucus and seawater at all sites. Pc: Pocillopora, Pr: Porites, Ac: Acropora, Sw: Seawater, "●": Vibrio abundances in the coral mucus were not able to be shown due to its high amount.

Both Enterococci (r = 0.97) and Vibrio (r = 0.95) abundances in the seawater were significantly increased along with increasing nitrate concentration (Table 2). A similar trend was also demonstrated in the abundances of Enterococci in all coral mucus that increased with an increase in nitrate concentration (Table 2). However, these correlations were not as high as Enterococci in the coral mucus ($r_{Pocillopora}$ = 0.47, $r_{Porites}$ = 0.37, $r_{Acropora}$ = 0.47). Moreover, the correlation assessment between *Enterococcus* abundances in all coral mucus and total organic carbon concentration (Table 2) illustrated with the low correlation values whereas the correlation assessment between Enterococci and Vibrio abundances in seawater and concentration of total organic carbon indicated the opposite results with low correlation values (Table 2). The correlation between *Vibrio* abundances in all coral mucus and nitrate and total organic carbon could not be analyzed due to its high abundances, make it impossible to count.

Table 2
The correlation coefficient (*r*) and significant levels (*P*-values) obtained from the correlation analysis between the indicator bacteria abundances, nitrate and total organic carbon

Indicator bacteria	*Sample*	*Nutrients*	*Correlation coefficient (r-value)*	*Significance (P-value)*
Enterococci	Seawater	Nitrate	0.97	0.997
		Organic C	-0.40	0.253
Vibrio		Nitrate	0.95	0.994
		Organic C	-0.11	0.433
Enterococci	Coral (*Pocillopora*)	Nitrate	0.47	0.788
		Organic C	0.13	0.586
Vibrio		Nitrate	a	a
		Organic C	a	a
Enterococci	Coral (*Porites*)	Nitrate	0.37	0.731
		Organic C	0.40	0.745
Vibrio		Nitrate	a	a
		Organic C	a	a
Enterococci	Coral (*Acropora*)	Nitrate	0.47	0.787
		Organic C	0.24	0.654
Vibrio		Nitrate	a	a
		Organic C	a	a

"a": Data could not be analyzed.

Discussion. The highly significant abundances of Enterococci in the seawater compared to in the coral mucus is likely due to this bacteria might have degraded in the seawater by seawater temperature and UV before they inhabited the coral mucus, as these are several stress environmental factors once the enteric bacteria enter the seawater (Morita 1993). Also, as it is not naturally a marine microorganism, therefore it likely needs special adaptation to survive in the coral mucus. In contrast to Enterococci, Vibrio found abundantly in coral mucus as it is symbiont of corals and other marine organisms, some of them contribute nutrients to the host while other become the disease pathogens such as Porites Ulcerative White Spots (Arboleda & Reichardt 2010), and Porites White Patch Syndrome (Séré et al 2013). However our result was not in line with the studies of Lipp et al (2002) and Lipp & Griffin (2004) that found the concentration of Enterococci was higher in seawater relative to the coral mucus. Presumably, our results are related to the hydro-oceanography features of this area that reduced the resident time to Enterococci in the water column. However, both this studies (Lipp & Griffin 2004 and Lipp et al 2002) did not provided any hydro-oceanographic data.

It is suggested that the extremely abundant Vibrio in the coral mucus at all sites might be caused by organic carbon that produced in coral mucus and exacerbated with the input from anthropogenic activities. As found in sites 3 and 4 which had intensely anthropogenic activities in the coastal areas such as restaurants and hotels, while there were anchored boats at site 5 that it might dispose untreated wastewater into this site. There were 26 hotels operated in Pemuteran village according to the BPS Kabupaten Buleleng 2016 (Statistics Indonesia Buleleng Regency) which might dispose of the untreated wastewater which contains pathogens and nutrients to the surrounded coral reefs. Additionally, the presence of traditional milkfish ponds at site 4 might release its residue with high nutrient concentrations. This result is supported by the studies of intensive milkfish farming near coral reef sites (Reichardt et al 2007). Coral mucus consists of 70% dissolved organic carbon and nutrient (phosphate) that induce the growth of bacteria (Nakajima et al 2009). As, coral mucus is inhabited by several microbes (Bentis et al 2000; Kellogg 2004), including bacteria (Bayer et al 2013), thus the enrichment of organic carbon disrupts the balance of its mutual relationship. Investigation of the effect of elevated organic carbon in three different coral species demonstrated that it caused coral tissue loss and bleaching (Kuntz et al 2005; Kline et al

2006). Both nitrate and organic carbon induce the growth of microorganisms. However, the abundances of both Enterococcus and Vibrio likely were intensified by the excessive nitrate concentration as both of indicator bacteria reached the highest abundance at sites 4 and 5. Studies indicated that the enrichment of nitrate caused bleaching and partial mortality (Kuntz et al 2005) and accelerated the progression of Black Bland Disease (Voss & Richardson 2006). In addition, multiple evidences show that elevated nutrient and organic carbon input may decrease the coral`s immune system thus proliferate disease infection (Bruno et al 2003; Kaczmarsky et al 2005; Voss & Richardson 2006). This finding reveals the high disease prevalence of Ulcerative White Spot disease found at all these study sites (Karim 2019) as studies showed the strong correlation between coral diseases and excessive nutrient concentration, in particular N-based nutrients (Patterson et al 2002). According to Arboleda & Reichardt (2010) and Séré et al (2013), Vibrio has been detected in several coral diseases such as Porites Ulcerative White Spots and Porites White Patch Syndrome. Therefore, the enormous abundance of Vibrio in all coral mucus may increase the risk of healthy corals to the diseases. Moreover, the high abundance of Enterococci in the seawater indicates that anthropogenic pollution has reached its coral reef ecosystems. Furthermore, it may harm the health of tourists as they may expose to potential pathogens.

Conclusions. The abundance of Enterococci in the mucus of all coral genera was relatively similar, whereas its abundance in seawater was significantly higher than those in mucus. In contrast to Vibrio, the abundance in mucus in all coral genera was significantly higher than those in seawater. The extremely high concentration of total organic carbon and nitrate in the water column, particularly nitrate, significantly enhance the abundance of Enterococci. The high level of nutrients was detected in the site which has the highest human activities, aquaculture and marine recreational site. The abundance of Vibrio reveals the cause of the high prevalence of coral diseases in this area, whereas Enterococci indicated that anthropogenic pollution has reached its coral reef ecosystems. In turn, the presence of these bacteria implies the health risk of both coral reef ecosystems and the tourists.

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