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Prevalence and Bacteria Associated with White Band Disease on *Acropora sp.* from Gili Labak Island Sumenep District Indonesia

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Prevalence and Bacteria Associated with White Band Disease on *Acropora* sp. from Gili Labak Island Sumenep District Indonesia

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Abstract. Coral reefs have many problems including coral diseases. One of the coral diseases that infect *Acropora* sp. from Gili Labak Island is the White Band Disease (WBD). Their prevalence and bacteria associated with the WBD on *Acropora* sp. is warranted further investigation. This study aims to determine the disease prevalence and bacteria associated with WBD on *Acropora* sp. from the Gili Labak Island. The samples were collected from coral infected. The laboratory analysis includes bacterial isolation, DNA extraction, DNA amplification, electrophoresis, sequencing, and phylogenetic analysis. The results showed that the disease prevalences of WBD on *Acropora* sp. were between 0 to 23%, in which the highest prevalence was found in the site where marine tourism activities and boat mooring occurred. Four bacterial isolates were identified based on morphological features. These four isolates were then analyzed using the Mega 5.2 program and resulted in a complete nucleotide sequence and had similarities to the bacteria present in the Gen Bank through BLAST analysis. The result of BLAST analysis showed that ACWB2A isolate had 99% similarity with *Vibrio alginolyticus* bacteria; ACWB 6 had 99% similarity with *Vibrio owensii*; and isolates 5 and 8 had 99% similarity with *Pseudoalteromonas rubra* bacteria. These are the first record that *Pseudoalteromonas rubra* bacterium is associated with White Band Disease.

Keywords: White Band Disease, *Acropora* sp., bacteria, vibrio, *Pseudoalteromonas*

1. Introduction

Coral reefs are benthic animals that live in the bottom of the waters. The animal is largely colonized in its life composed of calcium carbonate (CaCO_3) as a result of secretion from Zooxanthellae. Coral reefs are an area where various important economic fishes (Lutjanidae, Haemulidae, Lethrinidae, Scaridae, and Siganidae) [1-3], invertebrates (mollusks, crustaceans, and sea cucumbers), and seaweeds spawn and live [4]. Among the world's total fish catches in the 1970s, more than 10% was contributed by reef-related fisheries [5]. Coral reefs also play an important role to protect shoreline and preserve species diversity [6]. Furthermore, coral reefs also give various contributions to the social and cultural aspect of communities such as tourism activities [4] and cultural and religious values [6].

Indonesian coral reefs are in serious threat as more than 50% of reefs are in poor condition. Damage to coral reefs can be caused by human activities such as the use of bombs and cyanide



poison, anchor drainage, walking on the reef, using muroami fishing gear, mining of rocks, and sand mining [7]. In addition, the cause of coral damage can be caused by coral diseases. One of the diseases that occurred on coral reefs in Gili Labak is White Band Disease. Gili Labak Island is a small island in Sumenep District with vibrant marine tourist activities.

White Band Disease (WBD) is a disease that infects *Acropora sp.* coral species from Gili Labak Island Indonesia. It is described by Raymundo *et al.* [8] that this WBD disease can only be observed on *Acropora* corals. Meanwhile, WBD is also found on *Acropora cervicornis* dan *Acropora palmate* in the Caribbean [9], Venezuela and Columbia [10]. The bacteria associated with WBD were varied in different places and coral. WBD prevalence and bacteria associated with WBD that infects *Acropora sp.* coral in Gili Labak Island waters are still unknown, so what bacteria associated with WBD is warranted further investigation. This research will add the information of the WBD disease associated with the bacteria that could be used for further management of coral disease in the area.

2. Methods

The survey and sample collection were conducted on 27-30 April 2017 at Gili Labak Island, Sumenep Regency. The sampling location is at the coordinate point S: 07° 02' 10.11" and E: 114° 02' 42.20" (Figure 1). Bacteria Culture was conducted on 1-13 May 2017 at the Marine Biotechnology Laboratory of Universitas Trunojoyo Madura and on May 18-23, 2017 at the Tropical Marine Biotechnology Laboratory of Diponegoro University, Semarang for PCR analysis.

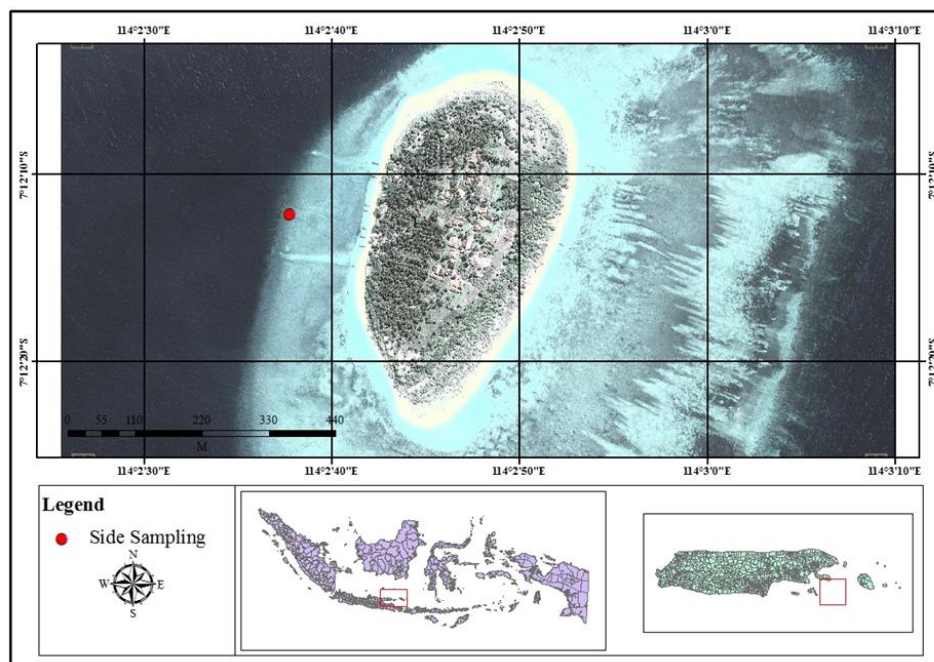


Figure 1. Map showing the location of sampling site in Gili Labak Island Sumenep Indonesia

2.1. Data Collection Procedure

In this research, field data were collected by identifying corals infected with WBD. Identification of corals infected with WBD was performed using the Coral Finder based on Kelley [11] and Veron [12], while the identification of WBD was based on Raymundo *et al.* [8].

Survey for disease prevalence was conducted using underwater visual survey using 2 × 20 m transect. Three transects were used in each site. Four sites were observed to represent all places of the island, namely north, east, south, and west side. The data were then subjected to Equation 1.

$$\text{Coral disease prevalence} = \frac{\text{Number of diseased colonies}}{\text{Total number of colonies}} \times 100\% \quad (1)$$

A sample of the diseased colony was also taken and stored into ziplock and then transported to the laboratory using a cool box with dry ice.

2.2. Isolation and Purification

The sample was then isolated to grow bacterial WBD disease. The isolation stage began with a dilution of the sample up to 10^{-10} dilutions, and the dilutions of 10^{-5} , 10^{-7} , and 10^{-10} dilutions were taken as 100 μ L by micropipette and isolated on Zobell 2216E media then incubated upside down for 2 $\times$ 24 hours. The isolation and purification of bacterial isolates were performed based on morphological appearance by streak method until pure culture obtained [13].

2.3. DNA Amplification Extraction

After obtaining the pure culture then the DNA was extracted to attain the DNA template of the bacteria. The extraction was done by taking bacterial cells from the agar plate and transferred into a tube and added with chelex.

DNA amplification was done by inserting a 27F and 1492R primer into a sterile tube of 2 μ L each and added with 6 mL of ddH₂O. Then it was added with 2.5 μ L of extraction sample and finally combined with 12.5 μ L of Kapa/Promega that made the total volume in tube was 25 μ L. PCR analysis was carried out for 30 cycles, which is the pre-denaturation stage for 3 min with temperature at 95 $^{\circ}$ C, 1 minute denaturation stage at 95 $^{\circ}$ C, 1 minute annealing stage at 55 $^{\circ}$ C, 1 minute pre-extension at 72 $^{\circ}$ C and 7 minutes extension stage at 72 $^{\circ}$ C [14].

2.4. Sequencing and Phylogenetic Analysis

The 16 srDNA sequence results from the sample were analyzed using the Mega 5 program. This program was also used to construct phylogenetic trees. The DNA sequences were compared with Gen Bank data from NCBI website (<http://www.ncbi.nlm.gov>) using BLAST in Mega 5.

3. Results and Discussion

The means of White Band Disease prevalence on *Acropora sp.* in the Gili Labak Island were between 0 and 23.4%, with the highest prevalence found in the north side (Figure 2). The north side is a location used for boat mooring and place where tourist does the activities such as snorkeling and kayaking. Meanwhile, the East and South side of the island does not have a high intensity of human activities. Our finding confirms that tourist activities could increase the prevalence of coral disease. Lamb *et al.* [15] found that coral disease prevalence increased 3-fold at high tourist used area.

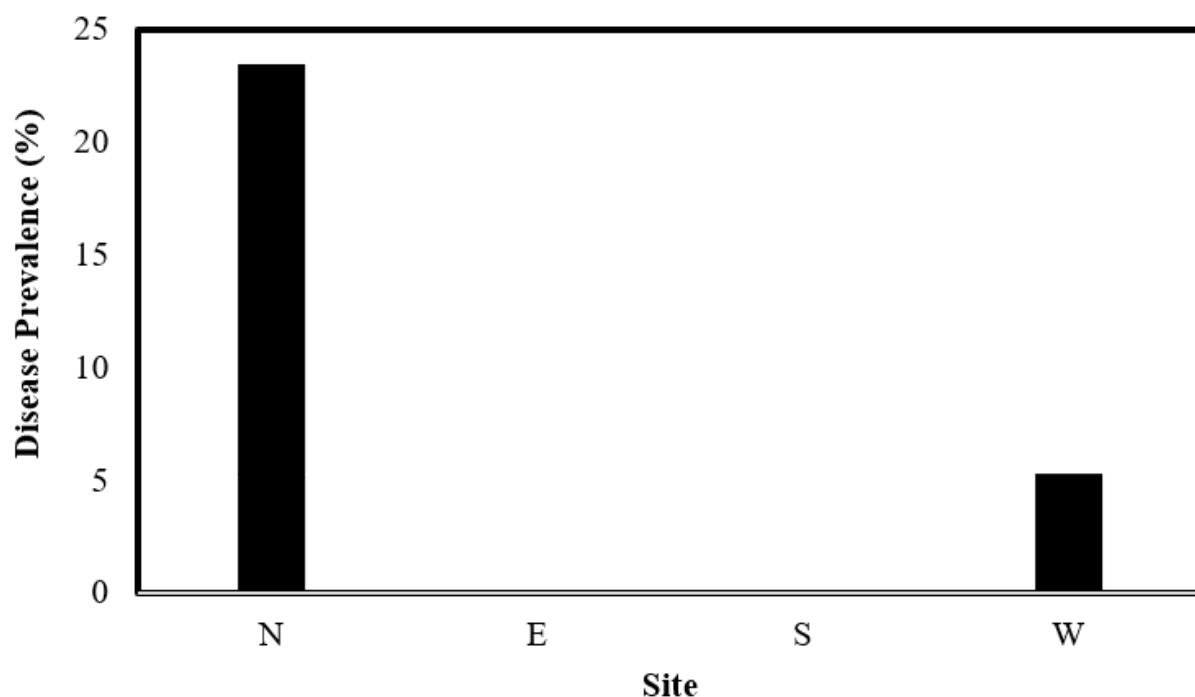


Figure 2. The mean of white band disease prevalence on *Acropora sp.* at Gili Labak Island Indonesia. N: North; E: East; S: South; and W: West site.

Four isolates were found based on the morphological observation of bacterial colonies. These bacterial isolates are identified by morphological appearance such as color, shape, and texture (Figure 3 and Table 1).

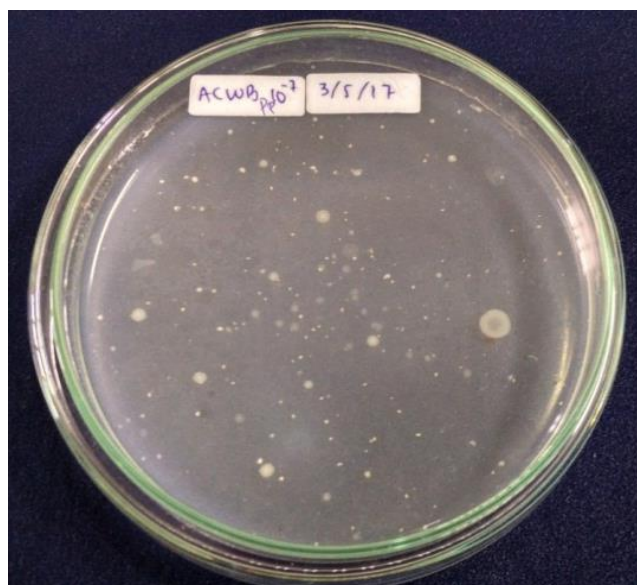


Figure 3. The colonies of bacteria associated with WBD

Table 1. The morphology of the bacterial colonies associated with White Band Disease on *Acropora* sp. corals

No	Isolate Code	Colour	Configuration	Elevation
1.	ACWB 2A	Pure white	Irregular and spreading	Flat
2.	ACWB 5	Milky white	Round	Flat
3.	ACWB 6	White with pure in the middle	Round	Flat
4.	ACWB 8	Yellowish	Round	Umbonate

The amplification process was carried out as much as 30 cycles to increase the amount of bacterial DNA so it could be analyzed for sequencing. Four isolates amplification showed that they had about 1500 bp which was suitable for sequencing (Figure 4).

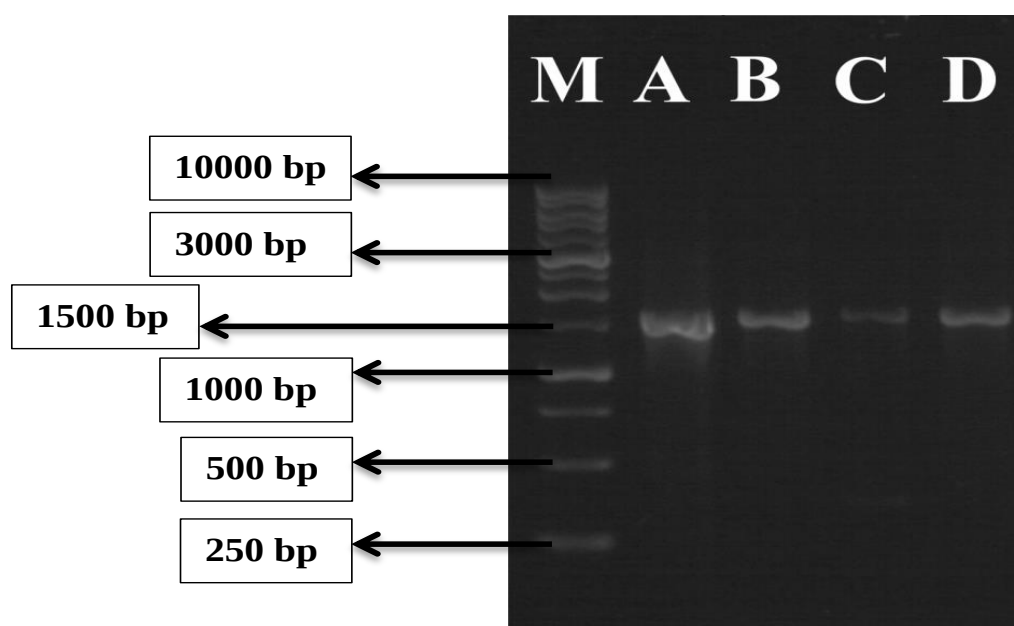


Figure 4. The photos of the amplification of single band DNA (single band), **M.** Marker; **A.** ACWB 2A; **B.** ACWB 5; **C.** ACWB 6; **D.** ACWB 8

3.1. Phylogenetic Analysis

The BLAST analysis showed that ACWB2A isolate had 99% homology with *Vibrio alginolyticus* (ATCC 17749 strain), ACWB 6 isolate had 99% homology with *Vibrio owensii* bacteria (DY05 strain), while ACWB 5 and ACWB 8 isolates had 99% homology with *Pseudoalteromonas rubra* bacteria (ATCC 29570 strain). Hagstrom [16] explains that if the likeness is $\geq 97\%$ then it can be considered as the same species. Phylogenetic analysis is a continuation of the BLAST analysis. This analysis makes it possible to create a phylogenetic tree (a phylogenetic tree) that can be used to see the proximity between the sample DNA and the DNA in the Bank Genes. The phylogenetic analysis confirms that ACWB2A isolate similar to *Vibrio alginolyticus* (ATCC 17749 strain), ACWB 6 isolate similar *Vibrio owensii* bacteria (DY05 strain) (Figure 5).

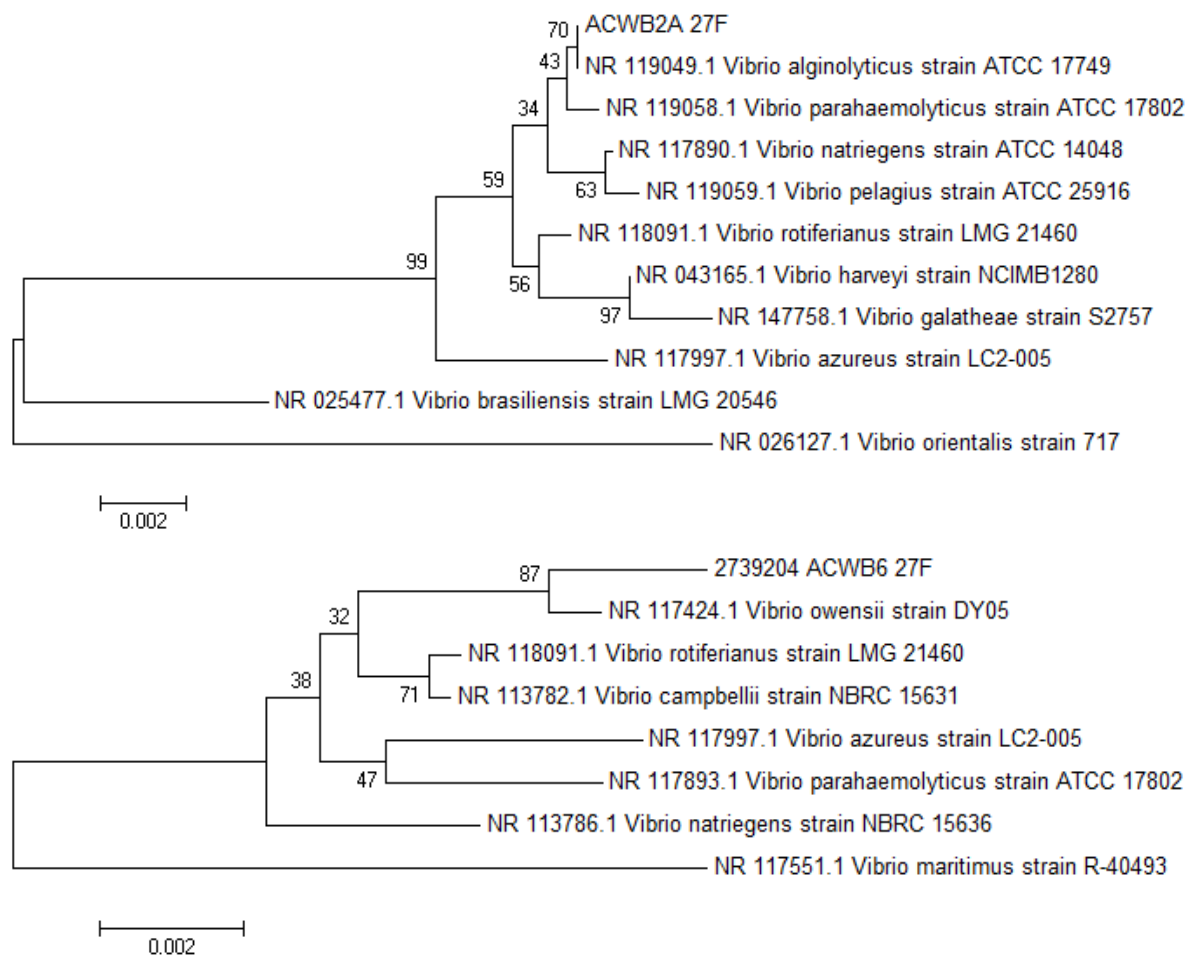


Figure 5. Phylogenetic tree of ACWB 2A and ACWB 6 isolates

ACWB 5 and ACWB 8 bacterial isolates are bacterial isolates that share a close similarity with *Pseudoalteromonas rubra* bacteria (Figure 6).

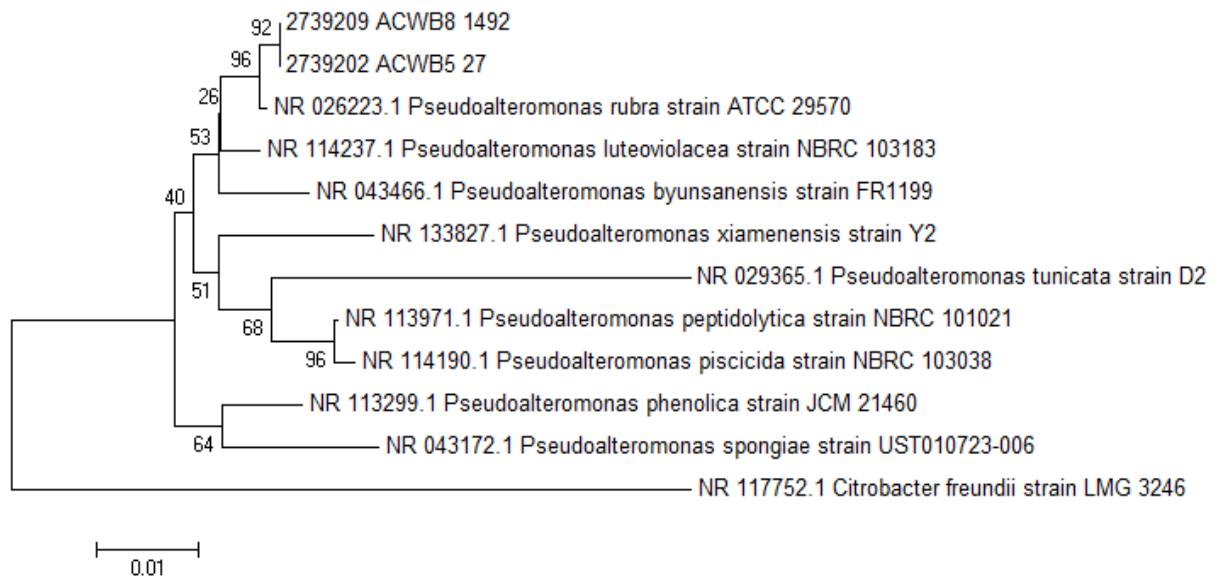


Figure 6. The phylogenetic tree of ACWB 5 and ACWB 8 isolates

Vibrio bacteria are aerobic, but there is also anaerobic facultative. *Vibrio* bacteria including motile bacteria because of its movement using flagella, in addition, are classified as gram-negative bacteria with the shape resembling a curved stem like a comma. Gignoux-Wolfsohn and Vollmer [17] reported that bacteria causing White Band Disease (WBD) might be species from *Vibrio* and *Rickettsia*. Previous studies showed that *Vibrio* bacteria found on coral *Acropora* affected by White Band Disease had bacteria *Vibrio carchariae* [18].

Vibrio alginolyticus bacteria are also found in another coral disease such as Yellow Band Disease (YBD) occurred in *Montastrea* sp. [19]. Cunning *et al.* [20] also found *Vibrio fortis* and *Vibrio harveyi* dominate in the healthy and diseased YBD coral *Montastrea faveolata*. *Vibrio alginolyticus* bacteria are also suspected as a pathogenic bacterium on *Porites andrewsi* infected with White syndrome disease [21]. *Vibrio alginolyticus* bacteria are scattered in the ocean and estuary; these bacteria can survive under high salinity conditions. Several studies have shown that the *Vibrio alginolyticus* bacteria are considered a species that lives freely in water and sediment and can survive in nutrient-poor seawater [22]. Therefore, there is a high possibility that the *Vibrio alginolyticus* bacteria are associated with WBD in the Gili Labak Island. Ritchie and Smith [19] explained that the diseased corals contain a higher proportion of *Vibrio* bacteria than healthy corals. In addition, *Vibrio alginolyticus* bacteria are in high concentrations during summer, when the temperature becomes hotter than usual [22]. Gili Labak Island waters might become a habitat that is very suitable for the growth of *Vibrio alginolyticus* bacteria.

Vibrio owensii bacteria are found on *Acropora hyacinthus* coral affected by White Syndrome [23]. Ushijima *et al.* [24] explained that the *Vibrio owensii* bacteria potentially lead to White Syndrome disease on the coral *Montipora capitata*. *Vibrio owensii* bacteria, including gram-negative bacteria, are facultatively anaerobic, have a slightly curved rod shape with a width of 1.0 μm and a length of 3.1 μm [25]. Tout *et al.* [26] explain that *Vibrio* bacteria are most commonly found on coral reefs.

This research found *Pseudoalteromonas rubra* bacterium was associated with *Acropora* sp. coral infected with White Band Disease. These are the first time that *Pseudoalteromonas rubra* bacterium found is associated with White Band Disease in *Acropora* sp. Tout *et al.* [26] stated that *Pseudoalteromonas* bacteria in the marine environment are most often associated with coral reefs. These bacteria were also found to be symbiotic with soft corals *Sarcophyton* sp. [27-28].

Pseudoalteromonas rubra bacteria are Gram-negative bacteria and can be found in marine waters attached to the surface of rocks, algae, coral reefs, marine animals, sediments, and can be found in coastal waters. *Pseudoalteromonas rubra* bacteria are aerobic, having a straight or slightly curved stem shape of $0.2\text{-}1.5\ \mu\text{m} \times 1.8\text{-}4.0\ \mu\text{m}$ [29]. These findings contribute to the body of knowledge that *Pseudoalteromonas rubra* bacterium was found also to be associated with *Acropora sp.* coral infected with White Band Disease.

4. Conclusion

Based on the research results, it can be concluded that the WBD prevalences on *Acropora sp.* were between 0 and 23.4%, with the highest prevalence at the north side used for boat mooring and marine tourist activities. The bacteria associated with WBD were also found. The similarity between ACWB 2A and *Vibrio alginolyticus* bacteria was 99%, ACWB 6 isolate with *Vibrio owensii* bacteria was 99%, and ACWB 5 and ACWB 8 both had similarity of 99% with *Pseudoalteromonas rubra* bacteria. These three bacteria provide new information about the bacteria associated with White Band Disease on *Acropora sp.* from Gili Labak Island, Madura, Indonesia.

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