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Identification of Antipathogenic Bacterial Coral Symbionts Against *Porites Ulcerative White Spots* Disease

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Abstract. Coral reef ecosystems are ecosystems that are vulnerable and susceptible to damage due to the exploitation of ocean resources. One of the factors that cause coral damage is the disease that attacks the coral. *Porites Ulcerative White Spots* (PUWS) is a coral disease found in Indonesia and attacks the coral genera *Porites* allegedly caused by pathogenic microbial attacks. The purpose of this study was to identify the symbiotic bacteria on healthy coral that have antipathogenic potency against PUWS. The method used in this research was descriptive explorative. Sampling was done in Kemujan Island, Karimunjawa. Bacteria were isolated from healthy coral and coral affected by PUWS disease. Streak method was used to purify coral bacteria, while overlay and agar diffusion were used to test antipathogenic activity. Bacterial identification was carried out based on polyphasic approach. The results of this study showed that coral bacterial symbionts have antipathogenic activity against PUWS disease. The selected bacteria NM 1.2, NM 1.3 and KPSH 5. NM1.2 were closely related to *Pseudoalteromonas piscicida*, *Pseudoalteromonas flavipulchra* and *Bacillus flexus*, respectively.

Keywords: Antipathogens, Symbiotic Bacteria, Coral, Coral Disease, PUWS

1. Introduction

Coral reef ecosystems are ecosystem consisting of animals, plants, fish, shellfish and other biota contained in the tropics that require the intensity of sunlight to live [1]. Coral reef ecosystems are ecosystems that are vulnerable and susceptible to damage due to the exploitation of ocean resources such as exploitation of coral reefs building materials, souvenirs, medicinal materials, cosmetic materials and aquarium ornament. Also, household waste, industrial waste, agricultural waste, transportation



waste and the used of explosives in fishing. Coral disease infection is one of the major factors that worsen the condition of global coral reefs [2].

Karimunjawa islands have coral reefs dominated by coral of *Porites* sp. Coral *Porites* sp. has a widespread throughout Indonesia [3]. This is due to *Porites* sp. is a coral capable of living in various environmental conditions such as in areas that have high and low sedimentation and areas with high and low salinity [4]. While *Porites* sp. able to live in various environmental conditions, the genus *Porites* can also get sick if the environmental around the coral has been infected with the disease.

PUWS is a coral disease found in Indonesia and attacks the coral genera *Porites* allegedly caused by pathogenic microbial attacks. The coral affected by PUWS, the surface of the coral frame will be damaged by the occurrence of white spot regularly [5,6]. In Karimunjawa Islands there are not many studies that study coral diseases especially PUWS. Based on these descriptions it is necessary to have further studies on the presence of PUWS disease in *Porites* sp. in Karimunjawa Island, considering that *Porites* sp is the dominant coral in that area by identifying the symbion bacteria on healthy corals and coral that have antipathogenic potential to corals that are attacked by PUWS.

2. Material and methods

2.1. Sampling and isolation of bacteria symbionts with coral

The coral was collected by scuba diving at depths of 1m to 10m from Kemujan Island, Karimunjawa (S 05° 48' 779" ; E 110° 29' 955"). Several coral colonies were taken using stools and hammers with methods of cutting coral tissue on healthy corals and corals infected disease. Then the sample was labeled into a sterile plastic bag and stored in a cool box [7]. The environmental parameters including salinity and depth at the site around the reef were measured. The tissue samples were scraped off by using a sterile knife. The resultant tissues were then tenfold serially, diluted, spread on the half strong ZoBell 2216E marine agar medium and incubated at room temperature for 48h. On the basis of morphological features, colonies were randomly picked and purified by making streak plates [8].

2.2. Screening of the isolates for anti-pathogenic activity

The bacterial isolates symbionts with healthy coral were tested for anti-pathogenic effect by the agar overlay method (were not presented in this paper) against selected pathogens bacteria. Each of disease bacterial isolated was used for anti-pathogenic effect study. Healthy coral bacteria were taken one ose and planted on ZoBell 2216E marine medium agar on the plates and formed into small spheres. The plates wrapped with plastic wrap and were incubated at room temperature for 48 h. The bacteria causing PUWS disease were grown on liquid ZoBell 2216E for 48 h while shook used a shaker. About 2 mL of the test cultures were suspended in 200 mL of ZoBell 2216E soft medium agar and were poured immediately over the colonies of the healthy coral bacteria on the plates. Then plates were incubated at room temperature for 48 h, antibacterial activity was defined by the formation of inhibition zones around the bacterial colony [8].

All bacterial strains selected from overlay test were rescreened for their activity using agar disk diffusion method. The paper disks diameter 8 mm that previously impregnated with 100 µL of each diseased isolate were placed on the surface of plates. Then, 35 µL of selected isolated were put on each paper disk. The plates were then incubated for 48 h. At the end of incubation period, the zones of inhibition were measured.

2.3. DNA Extraction, PCR amplification and sequencing of 16S rRNA

DNA extraction was conducted by using chelex method (Susilowati *et al.*, 2015). DNA extracts for 16S rRNA gene sequences were amplified by PCR using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492 R (5'-TGGTTACCTTGTTACGAC TT-3') (Weisburg

et al., 1991; Sabdono *et al.*, 2015). DNA sequencing was conducted at PT Genetika Science, Jakarta. The result of DNA sequences were preliminarily aligned with Clustal W Multiple Alignment and the phylogenetic analyses were performed by using MEGA 5.05 [9].

3. Result and Discussion

Isolates of healthy coral bacteria were collected that provided 53 isolates had antimicrobial activity. After diffusion testing, the most active isolate were NM 1.2, NM 1.3 and KPSH 5 (Figure 1).



Figure 1. The inhibition zones formed on agar disk diffusion method

The decrease of anti-pathogenic activity using agar disk diffusion method due to penetration of antibacterial coral bacteria into the medium surrounding the disk was too slow to adequately show their potency to inhibit the growth of pathogenic bacteria.

The homology searched results using the BLAST system were presented in Table 1. The precise taxonomic position of coral bacteria and provided more detail information on their phylogenetic position among their closest relatives were showed in Figure 2.

Table 1. Overview of 16S rRNA gene sequences retrieved from anti-pathogenic coral bacteria

Isolate	Length (bp)	Closest Species	Similarity (%)	(BLAST ncbi)	Accession Number (GenBank)
NM1.2	1249	<i>Pseudoalteromonas piscicida</i>	92 %	NR113971.1	LC101432
NM1.3	1363	<i>Pseudoalteromonas flavipulchra</i>	99 %	NR025126.1	LC101433
KPSH 5	1430	<i>Bacillus flexus</i>	99 %	NR024691.1	LC101435

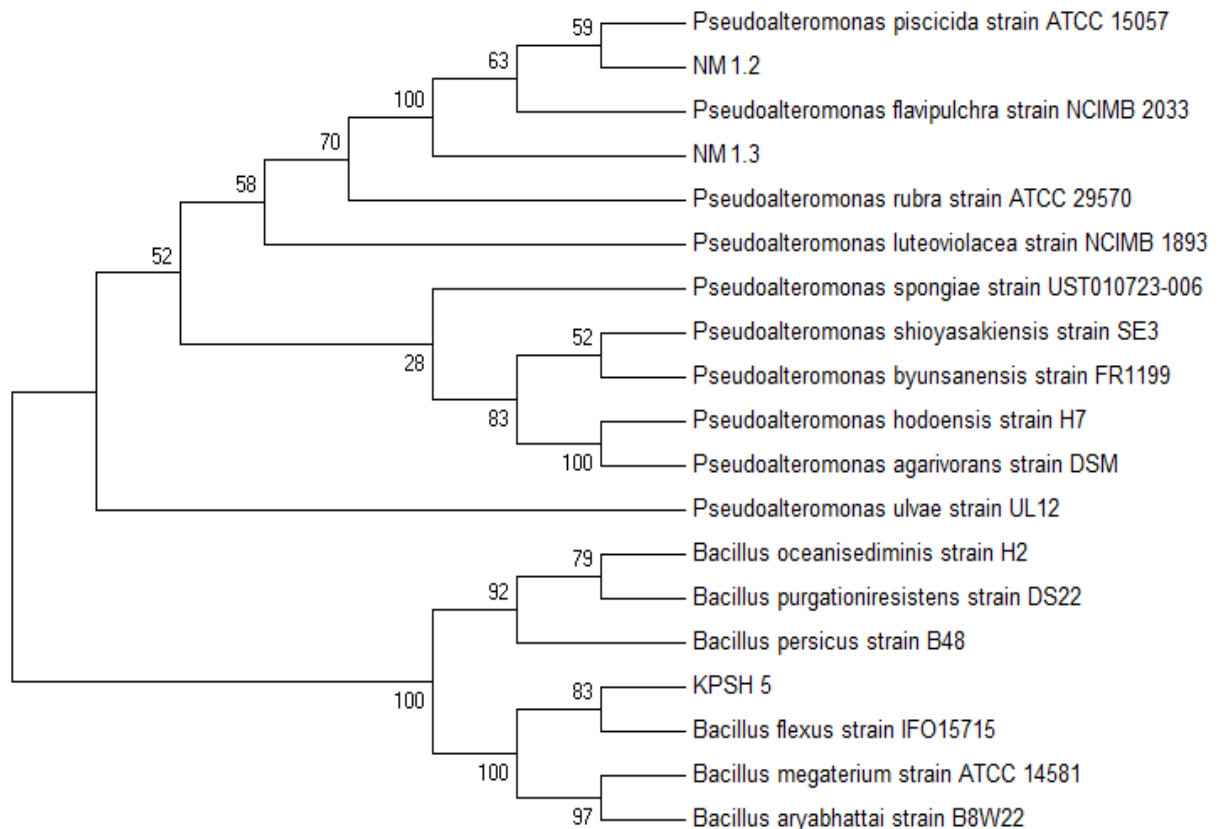


Figure 2. Phylogenetic Tree Isolate NM 1.2, NM 1.3 and KPSH 5 Had Anti-pathogenic Activity

The resulted BLAST homology from NCBI (National Center for Biotechnology Information) showed that the active strain bacteria NM 1.2 92% similarity to *Pseudoalteromonas piscicida* base on 16S rRNA gene sequence. These strain has been submitted to DDBJ (DNA Data Bank of Japan) with the accession number LC101432. Isolate NM 1.3, 99% similarity to *Pseudoalteromonas flavipulchra* with the accession number LC101433. Isolates NM 1.2 and NM 1.3 were isolates similarity to *Pseudoalteromonas* sp. Bacteria *Pseudoalteromonas* sp. was marine bacteria and bacteria that infect most marine organisms. Antibacterial activity of *Pseudoalteromonas luteoviolacea* TAB 4.2 associated with *Acropora* sp. able to inhibit the growth of coral bacteria and pathogens [10].

The resulted BLAST homology showed that the active strain bacteria KPSH, 99% similarity to *Bacillus flexus* and had been submitted to DDBJ (DNA Data Bank of Japan) with the accession number LC101435. In marine environments, *Bacillus* sp. can produce metabolites as antimicrobial, anti-fungal or have high toxicity, therefore has a high potential for the search new antimicrobial compounds [11-13].

4. Conclusion

Three bacterias symbionts with corals were able to inhibit *in vitro* bacterial growth of bacteria isolated from disease corals. The molecular identification by partial 16S rRNA gene sequencing revealed that they were closely related to the genera *Pseudoalteromonas* and *Bacillus*. It is important to continue coral disease research such as research on the prevention of coral disease so damage to corals can be reduced.

5. Acknowledgements

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