Causative agent of White Band Disease From Culkturable Bakterial Community Associated With Healthy and Diseases Coral Acropora humilis and Acropora tortusa from Karimunjawa Islands, Indonesia.

by Sarjito Sarjito

Submission date: 19-Aug-2021 04:51AM (UTC+0700)
Submission ID: 1632970064
File name: is_and_Acropora_tortusa_from_Karimunjawa_Islands,_Indonesia..pdf (703.45K)
Word count: 106
Character count: 380

Ecologia

ISSN 1996-4021

Academic
Journals Inc.

www.academicjournals.com

Ecologia 2 (2): 52-59, 2012
ISSN 1996-4021 / DOI: 10.3923/ecologia.2012.52.59

Causative Agents of White Band Disease From Culturable Bacterial Community Associated with Healthy and Diseased Corals *Acropora humilis* and *Acropora tortuosa* from Karimunjawa Islands, Indonesia

[1,2]Hasbullah Iwan Hakim, [3]Ocky Karna Radjasa, [4]Miftahuddin Majid Khoeri, [5]Gilang Aji Pratama, [5]Dewi Nasima, [3]Ambariyanto, [6]Sarjito and [4]Herawati Sudoyo

[1]Graduate School of Coastal Resource Management, Diponegoro University, Semarang 50275, Indonesia
[2]Fish Quarantee Station, Depati Awir, Pangkal Pinang 33171, Pangkal Pianang, Bangka-Belitung, Indonesia
[3]Department of Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia
[4]Marine Microbiology Unit, Eijkman Institute for Molecular Biology, Jakarta 10430, Indonesia
[5]Center for Marine Research Studies, Semarang 50232, Indonesia
[6]Department of Fishery, Diponegoro University, Semarang 50275, Central Java, Indonesia

Corresponding Author: Ocky Karna Radjasa, Department of Marine Science, Diponegoro University, Semarang 50275, Central Java, Indonesia

ABSTRACT

An effort was carried out to determine the diversity of causative agents from culturable bacterial community associated with healthy and diseased of coral *Acropora* species collected from Tanjung Gelam waters, Karimunjawa islands, North Java sea, Indonesia. Bacterial isolates were successfully grouped based on rep-PCR and 10 representative isolates were further analyzed for antagonistic test resulted in the formation of bacterial consortium. Infection test resulted in the confirmation that bacterial consortium as the causative agent of the white band disease type I in coral *Acropora humilis* and type II in *Acropora turtousa*. A generic composition indicates of the causative agent included them member of genera *Vibrio, Pseudoalteromonas* and *Bacillus*.

Key words: Diversity, causative agents, white band disease, coral, *Acropora*, Karimunjawa islands

INTRODUCTION

Coral reefs are widely known as some of the most productive ecosystems on earth and are certainly the most productive and species-rich environments in the oceans. Coral reefs provide critical protection to coastlines from storm damage, erosion and flooding by reducing wave action as well as the reservoir of marine natural products (Radjasa *et al*., 2011; Puspasari *et al*., 2011).

In addition to threats from human activities (Bryant *et al*., 1998), the emerging infectious diseases have now been regarded as serious threat for coral reefs around the world (Harvell *et al*., 2007). This emergence is believed has been fostered by ecological factors (Daszak *et al*., 2001) such as climate change (Rosenberg *et al*., 2007). The global warming issue in which an average of global temperature increased 0.6±0.2°C in the last century (IPCC, 2007), would definitely threaten coral reef ecosystems as it has been confirmed that certain coral diseases are indeed the result of the expression of temperature-regulated bacterial virulence genes (Rosenberg and Ben-Haim, 2002).

White band disease is among the reported coral diseases that commonly found in branching corals such as *Acropora* spp. (Smith and Thomas, 2008). It has been suggested that, diseases in

corals are the reflection of complex interaction between the causative pathogens, hosts and environment. Bacteria are known to be the main component of coral holobionts with higher diversity and are believed to respond in the disease events (Sunagawa *et al.*, 2009). Thus, profiling the bacterial community associated with coral diseases will play important role for better understanding the emerging coral diseases (Work *et al.*, 2008).

Karimunjawa islands, in the North Java Sea are part of marine national park. Incidences of white band disease among branching corals *Acropora* spp. have been observed, however, less reports have documented on the diversity of microbial community associated with the healthy and diaseased *Acropora* from this area. Here, we report the diversity of the causative agents of white band disease of bacteria associated corals based on culture-dependent approach.

MATERIALS AND METHODS

Sampling site: Diseased coral *Acropora* species were determined by using Manta tow technique. Upon the finding of diseased and healthy colonies at a depth of approximately 3 meters, mucus samples were collected *in situ* from the surfaces of 3 healthy and 3 diseased corals using a 20 mL syringe by scuba diving from Tanjung Gelam waters, Karimunjawa islands, North Java Sea (Fig. 1). Upon collection, syringes were put onto strerile Whirlpak bags. Upon retrieval, all sample bags were put on coolbox until further preparation.

Bacterial isolation: Coral mucus from 3 healthy and 3 diseased corals *Acropora humilis* and *Acropora tortuasa* were serially diluted with sterile seawaters and spread onto each respective surface agar of ZoBell 2216E medium and incubated for 48 h. The appeared colonies were purified and isolated by using streak method.

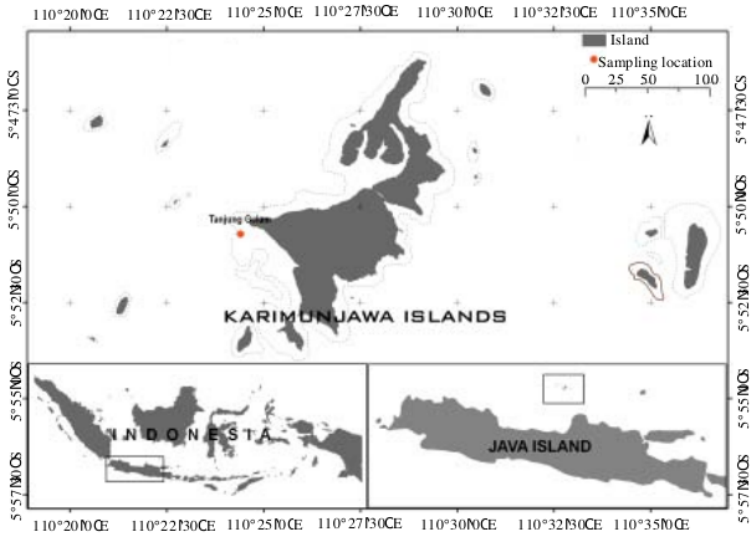

Fig. 1: Sampling site for the collection of coral *Acropora* spp. from Tanjung Gelam waters

DNA extraction, amplification and rapid grouping: Genomic DNA of each bacterial isolate from both healthy and diseased corals were obtained by freeze and thaw (Radjasa *et al.*, 2007a). A total of 19 isolates were grouped based on rep-PCR as previously reported methods (Radjasa *et al.*, 2007b; Sarjito *et al.*, 2009).

Antagonistic test: Ten representative isolates from both healthy and diseased corals based on constructed dendrogram of rep-PCR were tested for antagonistic against each other. One petri dish containing medium ZoBell 2216E was inoculated with a 24 h old of each difffent isolate grown in a ZoBell 2216 broth medium and spread with glass spreader. Nine sterile paper discs were put onto the agar surface which were then poured with a 20 µL of each of the remining isolates. The petries were incubated for 24 h and were checked for the presence of inhibition zones in the surrounding each paper disc.

Infection test: Ten isolates that all passed the antagonistic test were prepared to form a bacterial consortium by inoculating them onto one single flash containing 100 mL ZoBell 2216E broth medium. The flash was incubated for 2×24 h and was checked for density by using a spectrophotometer at 550 nm. Two months old colonies of *Acropora tortousa* and *Acropora humilis* were put onto controlled aquariums and were acclimated for 10 days. Both corals were then inoculated with a 5 mL of bacterial consortium by using a syringe and were observed daily for the occurrence of white band symptons.

DNA sequencing: PCR amplification of partial 16S rRNA genes of bacterial isolates, purification of PCR products and subsequent sequencing analysis were performed according to Radjasa *et al.* (2007a). The determined DNA sequences of strains were then compared for homology to the BLAST database. A phylogenetic tree was constructed using maximum-likelihood analysis and phylogenetic analysis was performed with the PAUP software package (Radjasa and Sabdono, 2008).

DNA sequences: DNA sequences of all bacterial isolates have been deposited in the DNA Database Bank of Japan (DDBJ) in the following accession numbers: AB675037-675045.

RESULTS

A total of 19 bacterial isolates were obtained from both healthy and diseased corals *A. tortuosa* and *A. humilis* (Table 1). All isolates were successfully grouped by using a rep-PCR technique (Fig. 2) followed by dendrogram analysis (Fig. 3) resulted in the occurrence of 10 different group representing bacterial isolates of both healthy and diaseased corals.

Based on the constructed phylogenetic tree, it was shown that 10 representative isolates were closely affiliated with the following bacterial genera of *Vibrio, Pseudoalteromonas* and *Bacillus* (Fig. 4).

The antagonistic test revealed that none of the 10 representative isolates based on rep-PCR analysis showing any antagonistic activity against each other. Therefore, it satified the requirement to form a bacterial consortium needed for infection test.

Following infection test on 2 months old corals *A. humilis* and *A. tortuosa* by a bacterial consortium, it was indeed observed that white band disease symptons occurred on the infected

Table 1: List of isolates collected from healthy and diseased corals

Strain	Coral status	Coral species
GD1.1	Diseased	*A. humilis*
GD1.2	Diseased	*A umilis*
GD1.3	Diseased	*A. humilis*
GD1.4	Diseased	*A. humilis*
GD2.1	Diseased	*A. tortuosa*
GD2.2	Diseased	*A. tortuosa*
GD2.3	Diseased	*A. tortuosa*
GD2.4	Diseased	*A. tortuosa*
G1.1	Healthy	*A. humilis*
G1.2	Healthy	*A. humilis*
G1.3	Healthy	*A. humilis*
G1.4	Healthy	*A. humilis*
G1.5	Healthy	*A. humilis*
G1.6	Healthy	*A. humilis*
G1.7	Healthy	*A. humilis*
G2.1	Healthy	*A. tortuosa*
G2.2	Healthy	*A. tortuosa*
G2.3	Healthy	*A. tortuosa*
G2.4	Healthy	*A. tortuosa*

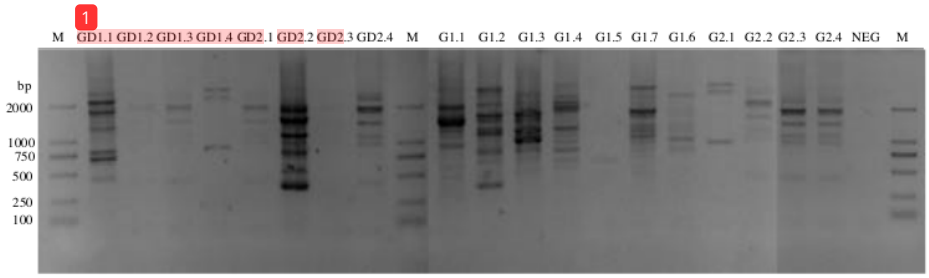

Fig. 2: Gel electrophoresis of rep-PCR of bacterial isolates

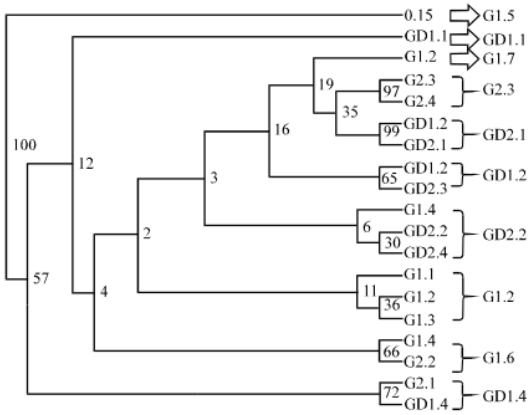

Fig. 3: Constucted dendrogram based on rep-PCR analysis

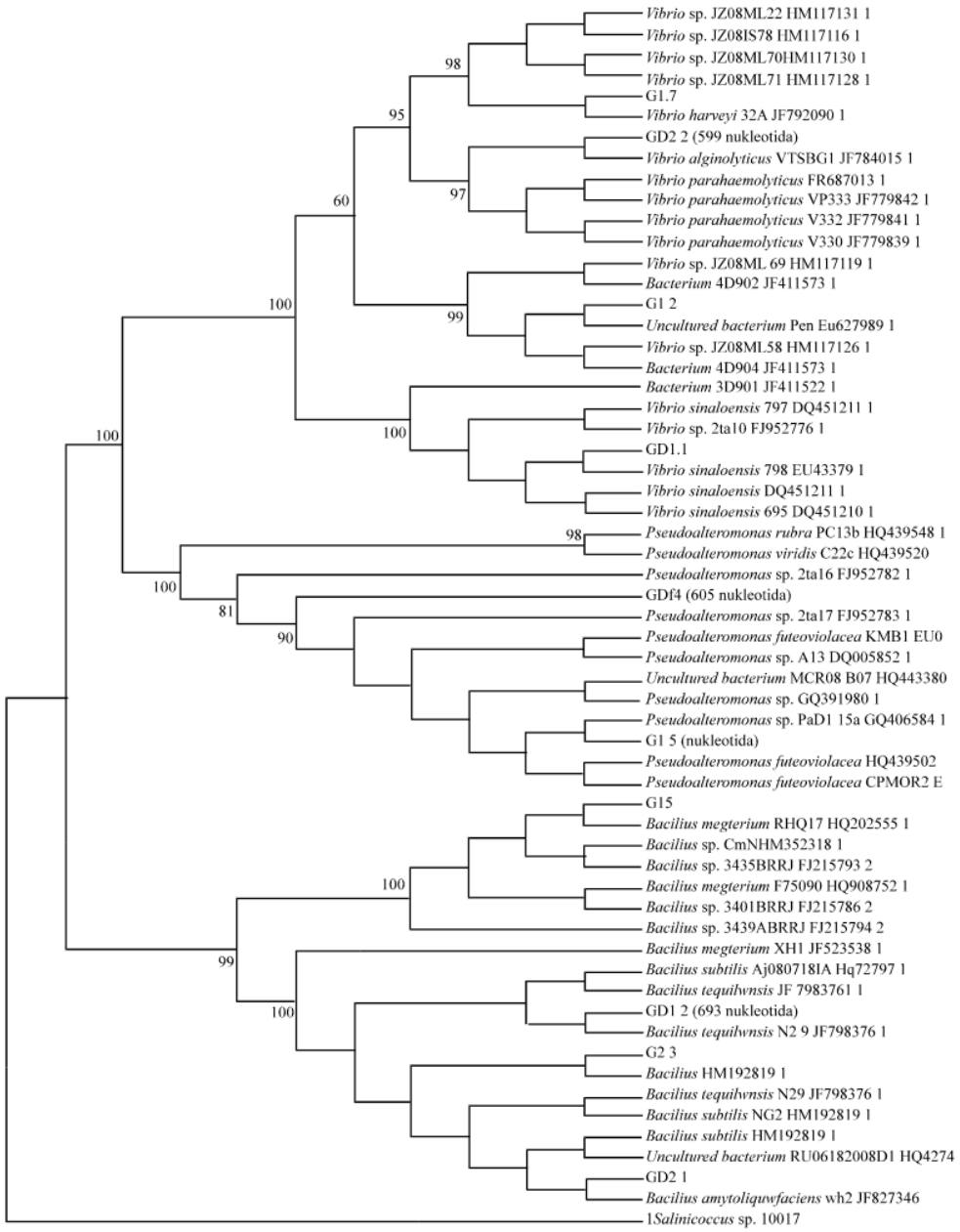

Fig. 4: Phylogenetic tree of the causative agent of white band disease

corals. It interesting to note that bacterial consortium caused a white band disease type I on coral *A. humilis* and white band type II on coral *A. tortuosa* after 4 and 11 days of incubation, respectively (Fig. 5).

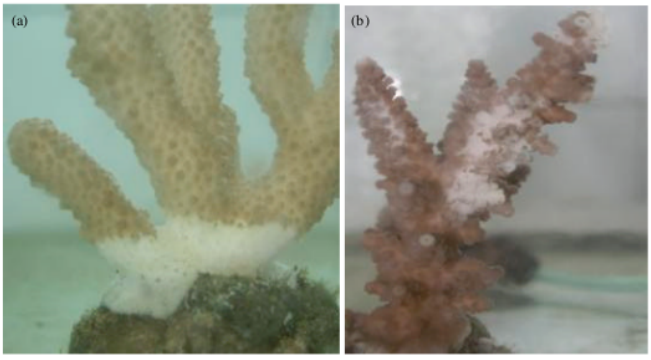

Fig. 5(a-b): Infection test by bacterial consortium on coral *A. humilis* (a) and *A. tortuosa* (b)

DISCUSSION

Water temperature, is among ecological factors that exhibited statistically significant relationships with coral diseases (Rosenberg *et al.*, 2007). The emergence of coral diseases as a result from seawater temperature rise has become a serious threat for the sustainable use of coral reefs. Unfortunately, one of the yet least understood aspects on coral diseases is the diversity of the causative agents of particular disease. In addition, the understanding on the diversity of causative agents agents also plays important role in the management of coral diseases, especially to prevent the spreading of particular disease in particular location, season and ecological parameter.

Present results highlight the diversity of the causative agents of white band disease of culturable bacteria associated with healthy and diseased corals *A. humilis* and *A. tortuosa*. Despite the fact that there is a disparity between culture-dependent and culture-independent approaches (Pantos and Bythell, 2006), in estimating the bacterial community associated with corals, isolation of culturable parts of these associants would help in profiling the causative agents of white band disease in coral *Acropora* spp.

Rapid grouping based on rep-PCR techniques followed by the construction of dendogram allowed the estimation of richness of culturable bacterial community associated with healthy and diseased corals. This study supports the power of rep-PCR in determining the diversity of marine bacteria as previosly reported (Radjasa *et al.*, 2007b; Sarjito *et al.*, 2009).

It is interesting to note that 10 isolates representing the diversity of both healthy and diseased corals showed no antagonistic activity among each other. Thus this fact satified the requirement of forming a bacterial consortium needed for infection test.

The present study indicates that despite the same composition of bacterial consortium infected both corals, the white symptons produced by the consortium observed in two different coral species were indeed different. In the controlled aquariums, colony of coral *A. humilis* showed white band disease type I in which the infection started from the base of coral speading into the tip (Rosenberg *et al.*, 2007). On the other hand, coral *A. tortuosa* showed white band disease type II (Ritchie and Smith, 1995) where, the infection started from the branch and moved forward the tip of branch. In addition, both corals showed different vulnerability toward the infection, where in *A. humilis* the infection was observed on the 4 days and in *A. tortuosa* was on the day 11 days after inoculation.

Molecular identification of the causative agents of white band disease revealed that the generic composition of these causative agents included the member of *Vibrio, Pseudoalteromonas* and *Bacillus*. There is no significant differences on the diversity of the causative agents from culturable bacterial community between healthy and diseased corals. This finding was in accordance with a previous results in which no dramatic differences in the bacterial community between healthy and white band disease based on culture-independent analysis (Casas *et al*., 2004). The present study shows that, the identification of bacteria in healthy corals that were similar to known pathogens or bacteria that were previously isolated from diseased, stressed or injured marine invertebrate (Sunagawa *et al*., 2009), may suggest a role of exogenous opportunistic pathogens of broad host range. Alternatively, colonization by opportunistic pathogens or uncontrolled growth of commensals may have taken advantage of a compromised host immune system caused by a primary agent and/or unfavorable environmental conditions.

The fact the bacterial consortium was the causative agent of white band disease in corals *A. tortuosa* and *A. humilis* in the present study, supports the previous argument (Richardson, 2004) that coral disease is not caused by single pathogen, but rather a pathogen consortium.

ACKNOWLEDGMENTS

This study was partially funded by a grant from The US Department of State's Biosecurity Engagement Program (BEP), USA (grant # IDB1-21003-JA-08) through the US Civilian Research and Development Foundation (CRDF). Hasbullah Iwansyah Hakim was awarded graduate research grants from Agency for Education and Training, Ministry of Marine Affairs and Fisheries, Indonesia (Contract No. Pusdik KKP.B09/SJ.2/KP.510/III/2010) and COREMAP II (Coral Reef Rehabilitation and Management Program), Ministry of Marine Affairs and Fisheries, Indonesia (Contract.No. PMO/NCU COREMAP II. 24/KP3/2010).

REFERENCES

Bryant, D., L. Burke, J. McManus and M. Spalding, 1998. Reefs at Risk: A Map-Based Indicator of Threats to the World's Coral Reefs. World Resources Institute, Washington, DC., USA., Pages: 56.

Casas, V., D.I. Kline, L. Wegley, Y. Yu, M. Breitbart and F. Rohwer, 2004. Widespread association of a *Rickettsiales*-like bacterium with reef-building corals. Environ. Microbiol., 6: 1137-1148.

Daszak, P., A.A. Cunningham and A.D. Hyatt, 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta. Trop., 78: 103-116.

Harvell, D., E. Jordan-Dahlgren, S. Merkel, E. Rosenberg and L. Raymundo *et al*., 2007. Coral disease, environmental drivers and the balance between coral and microbial associates. Oceanography, 20: 172-195.

IPCC, 2007. Synthesis report, 2007. Intergovernmental Panel on Climate Change (IPCC) Geneva, Switzerland.

Pantos, O. and J.C. Bythell, 2006. Bacterial community structure associated with white band disease in the elkhorn coral *Acropora palmata* determined using culture-independent 16S rRNA techniques. Dis. Aquat. Organ., 69: 79-88.

Puspasari, F., Z. Nurachman, A.S. Noer, O.K. Radjasa, M.J.E.C. van der Maarel and D. Natalia, 2011. Characteristics of raw starch degrading α-amylase from *Bacillus aquimaris* MKSC 6.2 associated with soft coral *Sinularia* sp. Starch, 63: 461-467.

Radjasa, O.K. and A. Sabdono, 2008. Ecological role of a softcoral-associated bacterium Arthrobacter sp. on marine biofilm-forming bacteria. Microbiol. Indones, 2: 7-12.

Radjasa, O.K., D. Nasima, A. Sabdono, K. Kita-Tsukamoto and K. Ohwada, 2007a. Characterization of psychrotrophic bacteria from sea waters of makasar strait, Indonesia. J. Boil. Sci., 7: 658-662.

Radjasa, O.K., T. Martens, H.P. Grossart, T. Brinkoff, A. Sabdono and M. Simon, 2007b. Antagonistic activity of a marine bacterium *Pseudoalteromonas luteoviolacea* TAB4.2 associated with coral *Acropora* sp. J. Biol. Sci., 7: 239-246.

Radjasa, O.K., Y.M. Vaske, G. Navarro, H.C. Vervoort, K. Tenney, R. G. Linington and P. Crews, 2011. Highlights of marine invertebrate-derived biosynthetic products: Their biomedical potential and possible production by microbial associants. Bioorg. Med. Chem., 19: 6658-6674.

Richardson, L.L., 2004. Black Band Disease. In: Coral Health and Disease, Rosenberg, E. and Y. Loya (Eds.). Springer, New York, USA., pp: 325-336.

Ritchie, K.B. and W.G. Smith, 1995. Carbon-source utilization patterns of coral-associated marine heterotrophs. J. Mar. Biotechnol., 3: 107-109.

Rosenberg, E. and Y. Ben-Haim, 2002. Microbial diseases of corals and global warming. Environ. Microbiol., 4: 318-326.

Rosenberg, E., C.A. Kellogg and F. Rohwer, 2007. Coral microbiology. Oceanography, 20: 146-154.

Sarjito, O.K. Radjasa, A. Sabdono, S.B. Prayitno and S. Hutabarat, 2009. Phylogenetic diversity of the causative agents of vibriosis associated with groupers fish from Karimunjawa Islands, Indonesia. Curr. Res. Bacteriol., 2: 14-21.

Smith, A.J. and J.D. Thomas, 2008. White band syndromes in *Acropora cervicornis* off Broward county, Florida: Transmissibility and rates of skeletal extension and tissue loss. Proceedings of the 11th International Coral Reef Symposium, July 7-11, 2008, Fort Lauderdale, FL., USA., pp: 180-184.

Sunagawa, S., T.Z. DeSantis, Y.M. Piceno, E.L. Brodie and M.K. DeSalvo *et al.*, 2009. Bacterial diversity and white plague disease-associated community changes in the caribbean coral *Montastraea faveolata*. ISME J., 3: 512-521.

Work, T.M., L.L. Richardson, T.L. Reynolds and B.L. Willis, 2008. Biomedical and veterinary science can increase our understanding of coral disease. J. Exp. Mar. Biol. Ecol., 362: 63-70.

Causative agent of White Band Disease From Culkturable Bakterial Community Associated With Healthy and Diseases Coral Acropora humilis and Acropora tortusa from Karimunjawa Islands, Indonesia.

ORIGINALITY REPORT

10%	%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	C. R. Catacchio, R. Ragone, G. Chiatante, M. Ventura. "Organization and evolution of Gorilla centromeric DNA from old strategies to new approaches", Scientific Reports, 2015 Publication	10%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

Causative agent of White Band Disease From Culkturable Bakterial Community Associated With Healthy and Diseases Coral Acropora humilis and Acropora tortusa from Karimunjawa Islands, Indonesia.

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9