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Original Paper

ANTIBACTERIAL ACTIVITY OF BACTERIAL SYMBIONT OF SOFT CORAL *Lobophytum* sp. AGAINST MDR BACTERIA *Escherichia coli* and *Staphyllococcus aureus*

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ABSTRACT

The improper and uncontrolled uses of antibiotics againts pathogenic bacteria have resulted in the occurrence of Multi Drug Resistant (MDR) strains. There is now an urgency to find alternative antibiotics to combat the MDR strains especially Escherichia coli and Staphyllococcus aureus. Soft coral associated microorganisms are among the most interesting and promising marine natural product sources which produce polyketide and non ribosomal peptide products with various biological activities. In this study, marine bacteria were isolated from soft coral Lobophytum sp. collected from North Java Sea, and were screened for antibacterial activity against MDR strains. One out of 13 bacterial isolates were succesfully screened and were found to be active against both MDR strains, in which isolate LBTGA2 was active against resistant strains E. coli and against resistant S. aureus, respectively. The active isolate also amplified PKS (Polyketide Synthases) gene fragments necessary for the biosynthesis of polyketides. The molecular identification based on partial 16S DNA nukleotide sequences indicates that the active isolate was closely related to Paenibacillus campinasensis.

Keywords: Antibacterial ; Marine bacteria ; Soft coral *Lobophytum* sp. ; MDR

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INTRODUCTION

Nowadays the development of antimicrobial resistance and emerging multiresistant pathogens has raised concern the medical community in the world. MDR bacteria are bacteria that have been resistant to two or more classes of antibiotics (Sastroasmoro, 2005). The improper and uncontrolled uses of antibiotics againts pathogenic bacteria have resulted in the occurrence of antimicrobial resistance, which has become a major health problem world wide (Goldmann and Huskins, 1997; Radjasa *et al*., 2007a).

Invertebrates are the main components of coral reefs which have pronounced pharmacological activities. However, one of the most serious bottlenecks in developing natural products from coral reefs has been the availability of biomass to gain sufficient amounts of substances for preclinical and clinical studies. Exploitation is further complicated by the fact that most of these metabolites possess highly complex structures, making them difficult to be produced economically via chemical synthesis.

There is an environmental consideration by the researchers begining to search for the source material as a new bioactive compounds from marine environment. Marine invertebrates have been known to be prolific producer of secondary metabolites from the coral reef ecosystem and has been the target of bioactive natural product sourcing (Radjasa *et al*., 2007a). Burgess *et al*. (2003) mentioned that bacteria symbiotically

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associated with soft corals can synthesize secondary metabolites similiar to host. This allows the bacterial symbionts to produce antibacterial compounds that may be used to combat MDR bacteria.

It has been considered that the problem of supply has hampered the development of most secondary metabolites from marine organisms and plants, thus, it is important to highlight the possible role of marine bacteria associated with seagrasses in providing an alternative to the commercial metal-based antifouling coatings. Bacteria-seagrass association that occurs on the seagrass surface then could be of great interest to search for potential use as commercial antifoulants.

The use of specific primers in PCR amplification to detect fragments of genes that are important in the biosynthesis of secondary metabolites have been carried out to detect the genetic ability of microorganisms to produce compounds which belong to the Polyketide Synthase (PKS) and Non-Ribosomal Peptide synthetase (NRPS) (Ansari *et al*., 2004).

Here, we report the antibacterial potential of bacteria symbionts of softcoral *Lobophytum* sp. againts MDR bacteria.

MATERIAL AND METHODS

Sampling and isolation of soft coral-symbiont bacteria

Colonies of soft coral were collected from the vicinity of Tanjung Gelam waters, Karimunjawa islands, Jepara, North Java Sea, Indonesia by scuba diving. Upon collection soft coral colonies were put into sterile plastic bags (Whirl-Pak, Nasco, USA). The tissues were then rinsed with sterile seawater and homogenized with blender. The homogenized tissues were serially diluted, spread on ½ strength ZoBell 2216E, incubated at room temperature for 2 days. On the basis morphological features, colonies were randomly picked and purified by making streak plates (Madigan *et al*., 2000; Radjasa *et al*., 2007a).

Antibacterial test

Antibacterial test of soft coral-symbiont bacteria against MDR bacteria was performed by using an overlay method. Multi Drugs Resistant (MDR) bacteria (*Escherichia coli* and *Staphylococcus aureus*.) used in this study were obtained from Laboratory of Clinical Microbiology, Eijkman Institute, Jakarta. Culture of each MDR bacterium in the logarithmic phase was mixed with Zobell soft agar medium (1% v/v), which were then poured on to the respective agar surface previously inoculated with soft coral-symbiont bacteria and incubated for 4 days. The plates were then incubated at room temperature for 2 days. Antibacterial activity was defined by the formation of inhibition zones around the bacterial colonies. (Radjasa *et al.*, 2007a).

PCR Amplification and DNA Sequencing

Amplification was performed according to the method of Radjasa *et al*. (2007b). DNA genomic of secondary metabolite producing-strain for PCR analysis were isolated from cell materials which were taken from an agar plate, suspended in sterile water (Sigma, Germany) and subjected to five cycles of freeze (-80 ^{o}C) and thaw (95 ^{o}C). PCR amplification of partial 16S rRNA gene of bacterial symbiont of *Lobophytum* sp., purification of PCR products and subsequent sequencing analysis were conducted according to the method of Radjasa *et al.,* (2007c). The determined DNA sequence of strains were then compared for homology to the BLAST database.

PCR-based screening of PKS producing bacterial strains

Amplification of Polyketide synthase gene fragments was carried out with the PKS degenerate primers KSDPQQF (5' MGN GAR GCN NWN SMN ATG GAY CCN CAR CAN MG 3') dan KSHGTGR (5' GGR TCN CCN ARN SWN GTN CCN GTN CCR TG 3') (Radjasa, 2005).

DNA engine thermal cycler (MyCycler, Biorad), PCR run comprised 45 cycle with cycle: initial denaturation at 94 ° C for 2 minutes, denaturation (94 ° C for 1 min),

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annealing (55 ° C for 1 minutes), and extension (72 ° C for 2 min) respectively.

DNA Sequencing Analysis

Result of DNA sequencing analysis of the best isolate was compared for homology to the NCBI GenBank databases using Basic Local Alignment Search Tool database (BLAST) and FASTA searches (www.ncbi.nlm.nih.gov) (Altschul *et al.*, 1990; Radjasa *et al.,* 2008). Sequences were aligned using the program ClustalX (Thomson *et al.*, 1997; Radjasa *et al*., 2008). For phylogenetic calculations the Mega 3.1 version software were used (Isnansetyo and Kamei, 2003).

RESULTS AND DISCUSSION

Result

There were 13 isolates from symbiont bacteria soft coral *Lobophytum* sp. tested, however only one isolate was found to inhibit the growth of 2 MDR strains bacteria **(Table 1)**.

Table 1. Antimicrobial activity of soft coral *Lobophytum* sp. bacteria against MDR strains.

No	Isolate	Inhibition zone (mm)	
		E. coli	***S. aureus***
1	LBTGA2	9.700±0.265	10.967±0.635

Further screening for the presence of gene fragments of *Polyketide Synthase* (PKS) of the active isolate showed that the active strain was capable of amplifying PKS gene fragments (**Fig. 1**).

M LBTGA2

Fig. 1. PCR amplification of PKS gene fragments +, LBTGA2 (soft coral *Lobophytum* sp. bacterium), M is DNA markers.

Molecular identification of the active *Lobophytum* sp. isolate based on partial 16S rDNA showed that the active isolate was closely related to *Paenibacillus campinasensis* **(Table 2)**.

Table 2. Molecular identification of active isolate associated with ***Lobophytum*** sp.

No.	Bacterial isolate	Long (bp)	Closest relative	Homology (%)	Accession number
1	LBTGA2	851	*Paenibacillus campinasensis* strain 324	97	NR_024857.1

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Discussion

Problem of pathogenic bacteria that have been resistant to various antibiotics have become widespread in society, therefore the study was conducted and focused to address the problem. The research focused on bacterial symbiont of a particular organism is an effort to answer concerns about the problem of supply of marine natural products from coral reefs, because the concentration of bioactive compounds contained in marine invertebrates is limited, less only reached 10^{-6} % of wet body weight (Radjasa *et al*., 2007a). Perez Matoz *et al*., (2007), reported that bacterial symbiosis with marine invertebrates can synthesize the active compounds that are identical to the host.

Actinomycetes marine agar medium used because actinomycetes are a goup of microbes that most produce antibiotics bioactive compounds (70%), fungi (20%), bacteria (10%) (Atlas, 1998). *Lobophytum* sp. is one of marine invertebrates that produce secondary metabolites. Harper *et al*., (2001) mentioned that chemical compounds resulting from the body of marine invertebrates are useful to prevent and defend against predator attacks, the media competition, preventing bacterial infection, help the process of reproduction, and to prevent ultra violet rays stings.

This study also strongly revealed the ecological rationale for soft coral *Lobophytum* sp. associated microorganism for the maintenance of antimicrobial defenses. Seawaters typically contains 10^7 viruses, 10^6 bacteria, 10^3 fungi, and 10^3 microalgae/mL (Engel *et al*., 2002), including those which have been identified as causative agents in marine infectious diseases (Correa, 1997; Radjasa *et al*., 2007a). It is predicted that production of bioactive secondary metabolites can act as the basic mechanism of antimicrobial defense, because considering that marine invertebrates and their symbionts continuously exposed to a vast array of potentially harmful microorganism.

The present study indicated that marine bacteria associated with soft coral *Lobophytum* sp. showed strong growth inhibition against indicator microorganism (**Table 1**). It is believed that the emergence of MDR bacteria is correlated with improper uses of antimicrobial agents, such as many prescription not taken correctly, antibiotics sold without medical supervision, and spread of resistant microbes due to the lack of hygiene.

DNA sequence of one active isolate were found to have % homology 97% of to previously report sequence (**Table 2**). In this study, isolate LBTGA2 which was closely related to *Paenibacillus campinasensis*, inhibited growth of *E. coli* and *S. aureus*. Very limited information is available on the antibacterial activity of the member of genus *Paenibacillus*. Recently, Yoon *et al.,* (1998) reported that an alkaphilic, endospore-forms bacterium isolated from Brazilia soil was studied and proposed as a new Paenibacillus sp., this bacterium LBTGA2 was succesfully amplified PKS gene fragment. As reported by Radjasa *et al.,* (2008) that polyketides is one of the largest group of natural products many of which are clinically important drugs. Polyketides such as daunorobicin, erythromycin, lovastin, and rapamycin are derived from sequential condensation of short carboxylic acids. In addition, Radjasa *et al.* (2008) reported that polyketide natural products are common metabolites of blue-green algae (cyanobacteria). Cyanobacteria produced a myriad array of secondary metabolites, including alkaloids, polyketides, and non ribosomal peptides, some of which are potent toxins (Neilan *et al*., 1999; Radjasa *et al*., 2008).

CONCLUSION

Soft coral *Lobophytum* sp. associated bacteria provides evidence of antibacterial potential against MDR strains. On the other hand, in minor numbers, soft coral-associated bacteria might be capable of producing polyketides, because of the detection of PKS gene fragment as well as antibacterial activities. Soft coral-associated microbes could overcome the acknowledged supply problem as marine natural product with search secondary metabolites. Further works are needed to clarify the antibacterial compounds responsible for the growth inhibition toward MDR strains.

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