Journal of Coastal Development
Volume 13, Number 2 : Febraury 2010 : 113-118

ISSN : 1410-5217
Accredited : 83/DIKTI/Kep/2009

Original Paper

ANTIBACTERIAL ACTIVITY OF BACTERIAL SYMBIONTS OF SOFTCORAL *Sinularia* sp. AGAINST PATHOGENIC RESISTANT BACTERIA

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Received :December, 28th 2009 ; Accepted :January,26th 2010

ABSTRACT

Infections caused by resistant microbes may cause failure to respond to medical treatments, resulting in prolonged illness and greater risk of death. Treatment failures also lead to longer periods of infection, which increase the numbers of infected people moving into the community and thus expose the general population to the risk of contracting a resistant strain of infection. Soft corals have been known to produce secondary metabolites, some of which may have anticancer, antifouling, antibacterial activity. It has been suggested that natural products from marine invertebrates have striking similarities to metabolites of their association microorganisms. The aim of this study was to isolate and characterize bacterial symbionts of soft coral Sinularia sp. having antibacterial activity against pathogenic Multi Drugs Resistant bacteria (Staphylococcus aureus, Escherichia coli, and Enterobacter). Five were successfully screened for antibacterial against resistant pathogenic bacteria. Two isolates, SNTGZ10 and SNTGZ11 were found to inhibit the growth of MDR Staphylococcus aureus, SC4TGZ3 and SC4TGZ11 inhibited the growth of MDR Escherichia coli and Enterobacter sp. , while isolate SC4TGZ4 inhibit the growth of MDR Enterobacter sp. Molecular identification revealed that: SNTGZ10 and SNTGZ11 were closely related to Virgibacillus; SC4TGZ3 to Pseudovibrio; SC4TGZ4 to Alphaproteobacteria; and SC4TGZ11was closely related to Microbulbife. The bacterial symbionts of softcoral Sinularia sp. offer potential source of antibacterial compounds in particular against MDR strains.

Keywords: Antibacterial activity, softcoral, Sinularia sp., bacterial symbionts

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INTRODUCTION

Resistance in microbes including bacteria, virus, or protozoan to therapeutics is neither surprising nor new. It is, however, an increasing challenge as drug resistance accumulates and accelerates, even as the drugs for combating infections are reduced in power and number. Drug-resistant bacterial pathogens pose a serious and growing menace to all people, regardless of age, gender, or socioeconomic background, a picture that

ISSN : 1410-5217
Accredited : 83/DIKTI/Kep/2009

holds true for developed and developing nations alike. Indeed, microbial resistance threatens to reverse many of the therapeutic miracles of the past half century.

A rapidly expanding list of antimicrobial-resistant organisms is affecting us in a variety of ways. The vast majority of infections that people acquire in hospitals, for example, are caused by bacterial agents, such as *Staphylococcus aureus*, that are resistant to penicillin. In many hospitals in the United States, nearly half of these penicillin-resistant staphylococci are also resistant to second generation, Penicillinase-resistant drugs, such as Methicillin. Compounding matters, the antibiotic Vancomycin, currently one of the few available treatments for Methicillin-resistant Staphyloccocal infections, is now showing increasing signs of losing ground as Vancomycin resistance becomes ever more common among the most frequent infectious agents in hospitals such as staphylococci, streptococci, pneumococci, enterococcus (Lemon, 2003).

Staphylococcus aureus is a gram positive bacteria, pathogen to human that can make food intoxication or infection such as skin infection, pneumonia, meningitis, endocarditis or sepsis with surpuration in many organ (Brooks et al, 2004).

On the other hand, *Escherichia coli* and *Enterobacter* sp. are the member of the family *Enterobacteriaceae. E. coli* is, the type species of the enterics. *E. coli* is such a regular inhabitant of the intestine of humans that it is used by public health authorities as an indicator of fecal pollution of drinking water supplies, swimming beaches, foods, etc. *E. coli* is the most studied of all organisms in biology because of its natural occurrence and the ease and speed of growing the bacterium in the laboratory. A few strains of *E. coli* are pathogenic (one is now notorious, strain 0157:H7, that has been found to contaminate raw hamburger, vegetables, unpasteurized milk and drinking water). *E. coli* causes intestinal tract infections (usually acute and uncomplicated, except in the very young) or uncomplicated urinary tract infections and neonatal meningitis. Enterobacter sp. has been known to cause urinary tract infection and sepsis (Todar, 2008).

Marine organisms including those from coral reef ecosystems have become sources of great interest to natural product chemistry, since they provide a large portion of bioactive metabolites with different biological activities (Faulkner, 2000). It has been suggested that natural products from marine invertebrates have striking similarities to metabolites of their association microorganism (Proksch et al., 2002). Understanding of marine invertebrate microbial association is a fundamental step in studying biologically potential active, possible medicinal compound from associated microorganism. Isolating bioactive compound producing bacteria is obviously offers a much better approach than cultivating and harvest invertebrates, which are in most cases extremely difficult (Radjasa and Sabdono, 2009).

This article describes the characterization of bacterial symbionts of soft coral *Sinularia* sp. having antibacterial activity against medically multi drugs resistant pathogenic bacteria (MDR) (*S. aureus*, *E. coli*, and *Enterobacter),* which were characterized by using 16S rDNA approach.

MATERIALS AND METHODS

Sampling of *Sinularia* sp. and isolation of bacterial symbionts

Sampling was conducted at Tanjung Gelam Islands, Karimunjawa-Jepara, North Java Sea Indonesia. Colonies of softcorals were collected by scuba diving. Upon collection softcorals were put into steril plastic bags

ISSN : 1410-5217
Accredited : 83/DIKTI/Kep/2009

(Whirl-Pak, Nasco USA). The tissue were then rinsed with sterile seawater and homogenized with blender. The homogenized tissues were serially diluted, spread on half strength ZoBell 2216E marine agar medium and incubated at room temperature for 2x24 hours. On the basis of morphological features colonies were randomly picked and purified by making streak plates (Madigan *et al.,* 2000).

Antibacterial test

Antibacterial test of bacterial symbiotns against pathogenic resistant bacteria was performed by using an overlay method. Pathogenic resistant bacteria (*S. aureus, E. coli, Enterobacter* sp.) used in this study were obtained from Clinical Microbiology Laboratory of Kariadi Hospital Semarang). Culture of each bacterium in the logarithmic phase was mixed with TSB soft agar medium (1% v/v), which were poured on to the respective agar surface previously inoculated with bacterial symbionts that had been incubated for 4 days at room temperature. Then the plates were incubated at room temperature 2x24 hours. Antibacterial activity was defined by the formation of inhibition zones around the bacterial colonies.

PCR amplification and DNA sequencing

PCR amplification was carried out according to the method of Radjasa et al (2007a). Universal primers as described by Weisburg et. al. (1991) was used for PCR amplification. Genomic DNA of strains for PCR analysis were obtained from cell materials 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 or partial 16S rRNA gene of the active bacterial symbionts and subsequent sequencing analysis were performed according to method of Radjasa et al (2007b). The determined DNA sequences of strains were compared for homology to the BLAST database.

RESULTS AND DISCUSSION

Results

There were 109 isolates collected from softcoral *Sinularia* sp, and 5 isolates were found to inhibit the growth of pathogenic resistant bacteria (*S. aureus, E. coli, Enterobacter* sp.) as shown in the **Table 1.** Two isolates SNTGZ10 and SNTGZ11 inhibited only the growth of *S. aureus*, and SC4TGZ4 inhibited the growth of *Enterobacter* sp. On the other hand, two other isolates, SC4TGZ3 and SC4TGZ11 were capable of inhibiting the growth of *E. coli* and *Enterobacter* sp.

Table 1. The growth inhibition zone of *S. aureus, E. coli,* and *Enterobacter* sp.

NO	Strain	*S. aureus*	*E. coli*	*Enterobacter* sp.
1	SN TGZ10	1,933 ± 1,01	-	-
2	SN TGZ11	2.867 ± 1,46	-	-
4	SC4 TGZ3 .	-	2,467 ± 0,60	10.28±0.006
5	SC4 TGZ4	-	-	6,900 ± 3,61
6	SC4 TGZ11	-	0,667 ± 0,64	10.207±0.04

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Table 2 . Molecular identification of the active isolates obtained from softcoral *Sinularia* sp. is presented in the

No	Kode Bakteri	Length	Closest Relative	Homology	Access. No
1	SNTGZ10	492	*Virgibacillus salarius*	99 %	AB197851.2
2	SNTGZ11	482	*Virgibacillus marismortui*	98 %	GQ181204.1
3	SC4TGZ4	458	*Alpha proteobacterium*	81 %	DQ097264.1
4.	SC4TGZ3	493	*Pseudovibrio* sp.	99 %	FJ952802.1
5	SC4TGZ11	504	*Microbulbifer variabilis*	99 %	AB266055.1

A phylogenetic tree showing the appropriate of affiliation of the active strain SNTGZ10, SNTGZ11, SC4TGZ3, SC4TGZ11 and SC4TGZ4 is shown in the **fig1.**

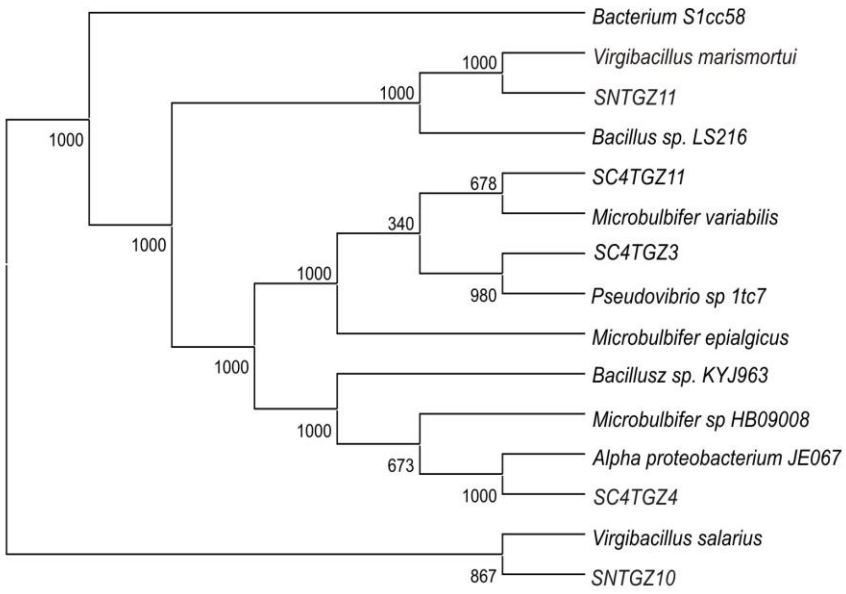

Fig 1. Philogenetic tree of active bacterial symbionts of sofcoral *Sinularia* sp.

Disscussion

Multi drugs resistance has become a global concern. Transferable drug resistance represents a major threat to the treatment of infectious diseases in both humans and animals, including farmed fish. The use of antimicrobial agents in both human and

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Accredited : 83/DIKTI/Kep/2009

veterinary medicine exerts a strong selective pressure inducing resistance to antimicrobial agents among bacteria. Generally, bacteria with the highest level of resistance are isolated from environments contaminated with antimicrobial agents, e.g., hospitals, fish farms, sewage effluents, and wastewater (Kruse and Sorum, 1994).

Marine organisms including those from coral reef ecosystems have become sources of great interest to natural product chemistry, since they provide a large proportion of bioactive metabolites with different biological activities (Faulkner 2000). In particular, marine invertebrates with high species diversity in the tropical coral reefs are often rich in secondary metabolites and are preferential targets in the search for bioactive natural products (Sammarco and Coll, 1992).

Globally, since 1995, there are signals of decreased interest in the search of new metabolites from traditional sources such as macroalgae, molluscs, tunicates and octocorals, and the number of annual reports on marine sponges stabilized. On the contrary, the metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms (Kelecom, 2002).

It has been estimated that less than 2% of microbial flora have been successfully isolated from marine environment as pure cultures. It is expected that still quite a few parts of unexplored culturable invertebrate-associated microorganisms exists in the reef environments (Radjasa *et al.* 2007a, b)

Virgibacillus salarius is a novel spesies of the genus Virgibacillus. *Virgibacillus salarius* is a gram-positive, endospore-forming, rod-shaped and moderately halophilic bacterium. Another *Virgibacillus salarius* was isolated from a salt-crust sample collected from Gharsa salt lake (Chott el Gharsa), Tunisia. It was identified based on polyphasic taxonomy including genotypic, phenotypic and chemotaxonomic characterization and it was closely related to the type strains of *Virgibacillus marismortui* and *Virgibacillus olivae*, with 16S rRNA gene sequence similarities of 99.7 and 99.4 %, respectively. Biochemical analysis resulted in determination of major fatty acids iso-C(15 : 0), anteiso-C(15 : 0) and anteiso-C(17 : 0) (33.3, 29.2 and 9.8 %, respectively); phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine were the main polar lipids and MK-7 was the predominant menaquinone (approximately 100 %) (Hua et al., 2008).

Microbulbifer is a genus from phylum of Proteobacteria found in sea water. Members of this genus can degrade complex carbohydrates such as cellulose, alginate, and chitin. Recently, *Microbulbifer degredans* was renamed *Saccharophagus degredans*. Because of the diverse enzymatic activities found associated with this microorganism. Proteobacteria is the same phylum as Alphaproteobacteria, but different class. Proteobacteria has 2 group classes α (Alpha) proteobacteria and γ (Gamma) proteobacteria. *Microbulbifer variabilis* is from the group of Gammaproteobacteria and *Pseudovibrio* sp. is from the group of Alphaproteobacteria.

CONCLUSIONS

Soft corals *Sinularia* sp. exhibited secondary metabolite producing-marine bacteria with antibacterial activity against pathogenic resistant bacteria (*S. aureus, E. coli,* and *Enterobacter* sp.). This finding offers the opportunity to develop newly potent antibiotics in a sustainable way for handling the occurrence of multi drugs resistant bacteria.

ISSN : 1410-5217
Accredited : 83/DIKTI/Kep/2009

ACKNOWLEDGEMENTS

The work was part of a research grant provided by Directorate General of Higher Education within the *Skala Prioritas Nasional Batch II Programme* (Contract No. 304/SP2H/PP/DP2M/VI/2009) and Public Health Faculty Diponegoro University Semarang (Contract No. 257/J07.1.18/PN/2009). We thank the students of Department of Marine Science Diponegoro University for their supports in field and laboratory.

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ISSN : 1410-5217
Accredited : 83/DIKTI/Kep/2009