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ANTIBACTERIAL ACTIVITY OF A PIGMENT PRODUCING-BACTERIUM ASSOCIATED WITH *Halimeda* sp. FROM LAND-LOCKED MARINE LAKE KAKABAN, INDONESIA

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ABSTRACT

A pigment producing-bacterium associated with green alga Halimeda sp., was successfully isolated from a land-locked marine lake Kakaban, East Borneo, Indonesia and screened for an antibacterial activity against pathogenic Staphylococcus aureus. The bacterium was identified as Pseudoalteromonas piscicida based on its 16S rDNA and was found to produce xanthophyll pigments and to amplify gene fragments of Non-ribosomal peptide synthetase (NRPS).

Keyword: Antibacterial activity, *Halimeda* sp., xanthophylls, Kakaban lake,

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INTRODUCTION

Lake Kakaban globally known as Jellyfish lake and Halimeda lake, is located on the uninhabited island of Kakaban, which is part of the Derawan Islands in the East Borneo, Indonesia. The lake was originally the lagoon of an atoll, formed by corals over a period of two million years. As a result of movements in the earth's crust the coral reef was raised above the sea level, trapping 5 km^2 of seawater within a 50 meter high ridge, effectively creating a landlocked marine lake. The lake is surrounded by a narrow mangrove belt and the entire island is covered with dense tropical vegetation. The coastline of Kakaban Island is encircled by a steeply sloping coral reef. Only one other similar lake ecosystem is known; it is found on Palau in Micronesia, about 1000 km east of the Philippines.

The uniqueness of this lake is that the species of the calcium carbonate-producing green alga, *Halimeda* spp., cover the bottom of the shallow areas of the lake. Thus, the lake is also well known as the Halimeda lake.

In this work, we reported the potential of marine bacterium associated with green alga *Halimeda* sp. for the production of secondary metabolites against pathogenic *Staphylococcus aureus* coupled with PCR based-screening for the presence of non-ribosomal polypeptide synthetases. Additional aim is to detect the presence of pigment. To the best of our knowledge, this

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is the first report on the bioprospecting study of bacteria associated with green algae from Kakaban Lake.

MATERIALS AND METHODS

Collection of samples and bacterial isolation

Colonies of green alga *Halimeda tuna* were collected from Lake Kakaban by hands from a depth of approximately 1 meter. Upon collection colonies were put into sterile plastic bags (Whirl-Pak, Nasco, USA) and put into cool-box. The tissues were then rinsed with sterile seawater and cut with a sterile knife. The resultant tissues were serially diluted, spread on ½ strength ZoBell 2216E marine agar medium and incubated at room temperature for 48 hours. On the basis of morphological features, colonies were randomly picked and purified by making streak plates (Madigan *et al*., 2000).

Inhibitory interaction test

Inhibitory interaction tests of coral-associated bacteria against test bacteria were performed by using an overlay method as described (Radjasa *et al.,* 2007). The following bacteria *Staphylococcus aureus* (DSM 6672) obtained from the German Culture Collection (DSMZ, Braunschweig, Germany) was used as a tested strain.

PCR amplification

Non-ribosomal peptide synthetases (NRPS) primers were prepared and amplification of peptide synthetase gene fragments was carried out with the NRPS degenerated primers as described (Radjasa *et al.,* 2007). PCR amplification of partial 16S rRNA gene of marine bacterium H1.7, purification of PCR products and subsequent sequencing analysis were performed according to the methods of Thiel and Imhoff (2003), respectively. The determined DNA sequences of strains were then compared for homology to the BLAST database.

Extraction of pigments

Pigment was extracted from the cells of *Pseudoalteromonas piscicida* with cool methanol, and purified by column chromatography and then by high-performance liquid chromatography, as described by Limantara *et al.,* (1994).

RESULTS AND DISCUSSION

Screening among 7 marine bacteria associated with algae *Halimeda* sp. by using test organism revealed that only one isolate, H1.7 capable of inhibiting the growth of *Staphylococcus aureus,* while the rest of isolates showed no activity. The measurement of inhibition zone as indicator of the antibacterial potential of isolate H1.7 against pathogenic bacterium is presented in Table 1. As shown in the figure 1, the isolate was also capable of amplifying the gene fragments of Non-ribosomal peptide synthetases (NRPS) indicated by the presence of single band having similar height to the positive control of *Pseudomonas fluorescens* DSM 50117.

Table 1. Antibacterial activity of isolate H1.7 against pathogenic *Staphylococcus aureus*

Isolate	Closest relative	Homology (%)	Tested pathogen	Inhibition Zone (cm)
H1.7	*Pseudoalteromonas piscicida*	99	*Staphylococcus aureus*	2,93 ± 0,49

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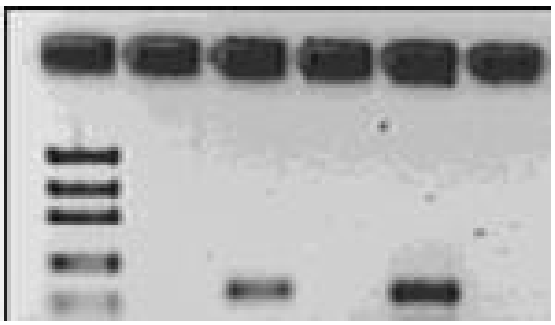

Fig.1. PCR amplification of NRPS gene fragments; + control *Pseudomonas fluorescens* DSM No. 50117; M is DNA markers

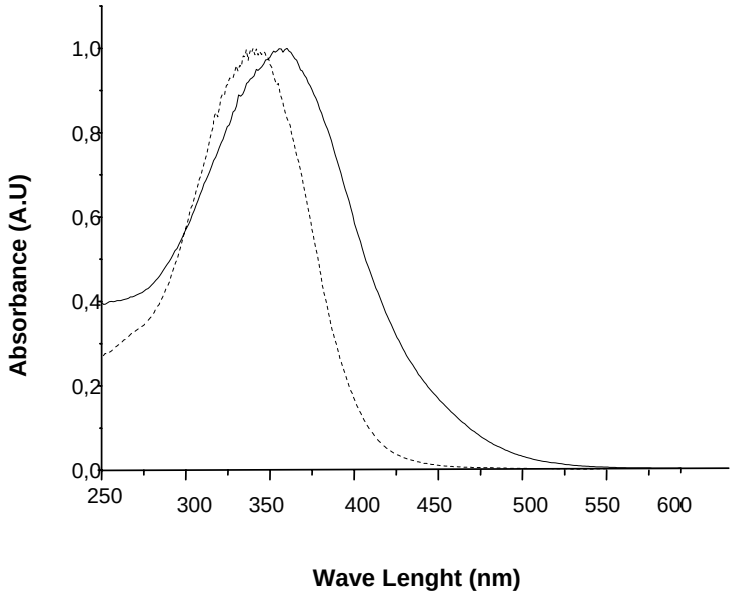

Fig 2. Spectral pattern of Trans and Cis-xanthophyll in acetone solution. — : spectrum before passing HCl gas; …. : spectrum after passing HCl gas

Table 2. Composition of pigments produced by algae bacterium H1.7

Peak no	Pigment composition	Percentage of pigment (%)
1	Xanthophyll *trans*	83.46
2	Xanthopyll *cis*	16.53

An attempt was carried out to estimate the potential of marine bacteria associated with algae *Halimeda* sp. from Lake Kakaban as the source of antibacterial compounds in particular against the pathogenic *Staphylococcus aureus.*

It is interesting to note that algae bacterium *Pseudoalteromonas* sp. H1.7 showed strong growth inhibition against *Staphylococcus aureus*. This raises the

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possibility the use of algae bacteria as the source of antibacterial compounds for controlling the pathogenic bacterium *Staphylococcus aureus*

Our results highlight algae-associated bacterium (H1.7) carrying the NRPS gene. Growth inhibition of pathogenic bacterium by NRPS strain H1.7 demonstrates the so far uncharacterized secondary metabolites of strain H1.7 lead to antagonistic activity. This bacterium is 99% identical to *Pseudoalteromonas piscicida* based on its 16S rRNA gene sequence. Alteromonadales and Vibrionales of the Proteobacteria were among the dominant producers of antibiotics on marine snow from the Southern California Bight (Long and Azam 2001) and the German Waddensea (Grossart *et al.,* 2004). Species of *Pseudoalteromonas* have also been isolated from tunicates (Holmstrom, 1998) and sponges (Ivanova *et al.,* 2002).

The members of genus Pseudoalteromonas are well known to produce various peptide natural products including antibacterials. (Radjasa *et al.,* 2007) reported that a marine bacterium *Pseudoalteromonas luteoviolacea* TAB4.2 isolated from a coral *Acropora* sp. showed antibacterial activity against both coral bacteria and pathogenic bacteria.

Bowman (2007) mentioned that the genus *Pseudoalteromonas* is a marine group of bacteria belonging to the class *Gammaproteobacteria* that has come to attention in the natural product and microbial ecology science fields in the last decade. Pigmented species of the genus have been shown to produce an array of low and high molecular weight compounds with antimicrobial, anti-fouling, algicidal and various pharmaceutically-relevant activities.

The present work highlights the production of natural pigments by a presumably symbiotic algae bacterium (H1.7) carrying the NRPS gene. The produced pigments were found to belong to the group of carotenoid, especially the member of xanthophylls, Xanthophyll *trans (*83.46%)and , Xanthopyll *cis* (16.53%), respectively

Interestingly, the organism closest related to H1.7, *Pseudoalteromonas piscicida,* produces yellow pigment as isolate H1.7 (Bowman, 2007). The algicidal strain Y, also closely related to *P. piscicida*, forms broad spectrum antimicrobial yellow brominated substances that LC-MS analysis revealed to comprise 12 different but likely structurally similar compounds (Skerratt, 2001).

CONCLUSION

In conclusion, green algae *Halimeda* sp. obtained from a land-locked marine lake Kakaban exhibited pigment producing-marine bacteria with antibacterial potential against tested strain. The present study highlighted the potential use of green algae associated bacteria as the sustainable source of marine pigments as well as marine antimicrobial compounds.

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Absorbance

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