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Research Article

Endophyte microbial characteristic of soft corals Lobophytum sp and Sinularia sp collected from Maspari Island waters, South Sumatera

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Abstract

Soft corals have bioactive compounds to potential as marine natural products, but the over exploitive to destroy of that ecosystem. Therefor endophyte microbial isolation can be effort to prevent that matters. This research aimed to isolate and characteristic on the entophyte microbial of soft coral Lobophytum sp and Sinularia sp that collated from Maspari island waters. Methodology of research was establishing growth of microbial samples (bacterial and fungal), isolation and characterization. Total of bacteria colony of Lobophytum sp were obtained about five isolates, and Sinularia sp were about four isolates. The macroscopic characteristic showed that whole bacteria had white colors. Those colonies had undulate, entire and curl (the edge of colony) and circular and irregular (for colony shape). For fungal of Lobophytum sp were obtained about three isolates, while Sinularia sp had only one isolate. The fungal colonies macroscopic characteristic showed yellow, green and white color, while shaped and edges colonies were thickened. Spread, thin, round, dark, and the whole of isolates had filamentous hyphae.

Keywords: entophyte microbial, Lobophytum sp., Sinularia sp., soft coral.

1. INTRODUCTION

Soft corals have bioactive compounds that can be used antimicrobial [1-3]. Over exploitative of soft corals can destroy of ecosystem due to their slow growth [4]. To utilize bioactive compounds on soft corals without damaging their habitat can use on endophyte microbes

Endophyte microbes are microbes that live inside their host and symbiotic with each other. They can produce the same bioactive compounds as their host. Several studies have shown that endophyte microbes associated with soft corals have potential as antimicrobials [5, 6].

One of locations that can be found soft coral Lobophytum sp and Sinularia sp species is Maspari island waters, south Sumatera with position at Bangka Strait. Some soft corals found on this island have potential as described earlier. Therefore, a study of endophyte microbes those are symbiotic with coral soft Lobophytum sp and Sinularia sp species.

2. EXPERIMENTAL SECTION

The research was conducted on September to October 2017. Soft coral samples were used Lobophytum sp and Sinularia sp that collected from Maspari island waters, South Sumatera with coordinate position 3o 15' 57" S and 106o 12' 59" E. For soft coral identified refer to [7-10].

2.1. Establish culture of endophyte microbial

Soft coral sample (10 g, fresh weight) washed with sterile sea waters about 2 to 3 times and coped to smalls. For endophyte bacteria is grown in liquid Zobell medium, and fungal in Potato Dextrose Broth (PDB) (9:1 v/w). Then It is incubated and shaker refer to [6].

2.2 Isolation of endophyte microbial

Isolation and characterization macros copies of endophyte bacterial were dilution, enrichment, plantation and observation under microscopies. A medium used autoclaved Zobell solid medium about 20 ml in petri dish, respectively. The marine biota (coral, seaweed and mangrove) were grown medium, then it diluted of gradually (10-1, 10-2, 10-3, 10-4, 10-5 and 10-6). The last three dilutions were planted with pout plate technique. This method used refer to [6, 11, 12].

Endophyte fungal isolation was dilution and plantation in medium Potato Dextrose Agar (PDA) that autoclaved about 15 ml in petri dish, respectively. This method used refer to [12, 13].

2.3 Purification of endophyte microbial

The isolate bacteria grouped used macroscopic observed for purification and cultures with autoclaved Zobell solid medium about 20 ml in petri dish, respectively. The isolate of endophyte bacteria

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Table 1 Compositions of Zobell medium for endophyte bacterial isolate

Materials	Total
Agar*	15.0 g
Peptone	2.5 g
Yeast Extract	0.5 g
Sea water	1.0 L

* added for solid medium

inoculated 1 ose needle, and incubated at 280C for three days. The purification methods of bacteria used refer to [14]. For isolate fungal used autoclaved PDA medium about 20 ml in petri dish, respectively. Isolate of endophyte fungal must be a single colony, and then characterized refer to [15].

3. RESULTS AND DISCUSSION

3.1. Endophyte bacteria of soft coral

The result grown of endophytes bacteria showed produced of color, smell and foam that indicated bacteria grown in medium. Day 1, media showed still not changed of color, smell and foam, while medium changed were day 4th to yellow of Lobophytum sp species and dark brown of Sinularia sp species as seen in Figure 1.

This was done bacteria began to grow can be seen from the change of color, smell and foam from the metabolic process. A color changes also occurred in [6] on samples of Sinularia sp with in same medium that showed dark brown color.

The result of isolation in petri dish was characterized by the growth of bacterial colonies. Macroscopics characterization was done visually including elevation, edges, size and color of colony as seen in Table 2.

Based on Table 2 showed that bacteria colonies not grown in dilutions of 10-5 and 10-6. Isolates grown on Lobophytum sp samples were five pure isolates, while Sinularia sp were four pure isolates as seen in Figure 2. The bacteria isolated from the both samples had white colored, small sized and flat elevated colonies. For bacteria colonies from Lobophytum sp showed edges had undulate and entire, shaped were circular and irregular. while bacteria of Sinularia sp had colonies edges were undulate, entire and curl. Isolates obtained [6] that had same shape of colony, but different characteristics other due to different samples [6]. The association bacteria of soft coral Lobophytum sp were found 158 isolates [5]. Bacterial isolates were obtained so large due to different sample treatments, which that isolates were carried out of endophyte and epiphytic.

3.2 Endophyte fungal of soft coral

Physical characteristics that changed in liquid media such as color and smell showed indicated that endophyte fungus had occurred microbial growth process. For Day 1 were not change of color and smell. The colors and smells changed dark yellow and stink (of Lobophytum sp), and dark brown (of Sinularia sp) had occurred day 4th as seen in Figure 2.

The result of fungal isolates was obtained three types with yellow, green and white color features of Lobophytum sp sample, while only one fungus isolate type with white color of Sinularia sp. There were obtained 15 isolates of fungal from Sinularia sp and two of them had potential as antifungal, whereas had color of colony were white and black. It had different of this result due

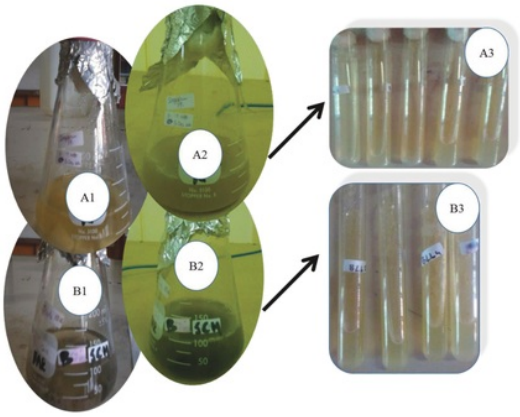

Figure 1. Culture of Endophyte bacteria in liquid medium, A1) Lobophytum sp of 1st day; A2) Lobophytum sp of 4th day; A3) isolates of Lobophytum sp; B1) Sinularia sp of 1st day; B2) Sinularia sp of 4th day; and B3) isolates of Sinularia sp

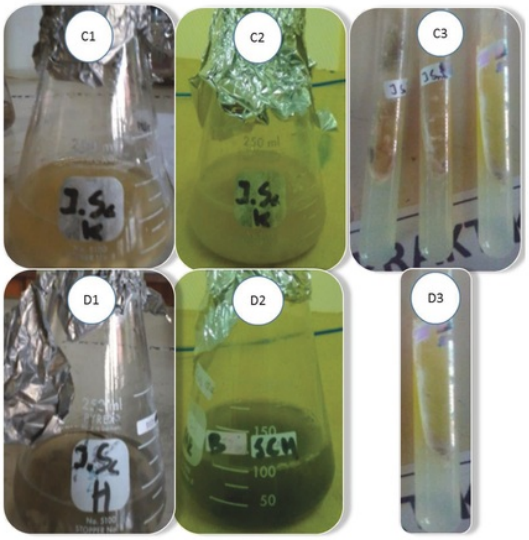

Figure 2. Culture of endophyte fungal in liquid medium, C1) Lobophytum sp of 1st day; C2) Lobophytum sp of 4th day; C3) isolates of Lobophytum sp; D1) Sinularia sp of 1st day; D2) Sinularia sp of 4th day; and D3) isolates of Sinularia sp

to treatment of sample, whereas she isolated epiphyte and endophyte on the fungal associates.

Based on Table 3, fungal isolates were named code Lb1, Lb2 and Lb3 on Lobophytum sp sample, while named code Sn1 only on Sinularia sp. The Lb1 isolate had colonies of yellow color, thickened and spread. Lb2 had green color, thickened, mycelium regularly and black of colony edges. Lb3 had white color, thin, round and dark of colony edges. While Sn1 isolate had white color, thickened and spread, the whole of isolates had fibrous hyphae. There were obtained 15 fungus isolates from Sinularia sp and two of them had potential as antifungal [16].

CONCLUSION

Soft coral Lobophytum sp and Sinuaria sp species had endophyte

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Table 2 Macroscopic of endophyte bacteria colonies

Soft coral	Dilutions	Colony color	Colony size	Elevation	Boundary	Shape
Lobophytum sp.		*white (1)*	small	*flat*	*undulate*	*circular*
	10^{-4}	*white (2)*	small	*flat*	*undulate*	*circular*
		white (3)	small	*flat*	*entire*	*irregular*
		white (4)	moderate	*flat*	*undulate*	*irregular*
	10^{-5}	*na*	na	*na*	*na*	*na*
	10^{-6}	*na*	na	*na*	*na*	*na*
Sinularia sp		*white (1)*		*flat*	*undulate*	*circular*
	10^{-4}	*white (2)*	small	*flat*	*entire*	*circular*
		white (3)	small	*flat*	*entire*	*irregular*
		white (4)	small	*flat*	*curl*	*irregular*
		white (5)	small	*flat*	*curl*	*circular*
	10^{-5}	*na*	na	*na*	*na*	*na*
	10^{-6}	*na*	na	*na*	*na*	*na*

na: unavailable

Table 3 Macroscopic of endophyte fungal colonies

Soft coral	Isolate code	color	shape	Hyphae
Lobophytum sp	Lb1	yellow	thickened and spread	filamentous
	Lb2	green	thickened, mycelium regularly and black of colony edge	filamentous
	Lb3	white	thin, round and dark of colony edge	filamentous
Sinularia sp	Sn1	white	thickened and spread	filamentous

microbial, where obtained endophyte bacterial and fungal. The bacterial colonies were found more in Lobophytum sp compared to in Sinularia sp, but the fungal colonies obtained in Sinularia sp were more variable. The both soft coral samples had macroscopics characteristic of endophyte microbial were obtained white colors, small sized and flat elevated colonies for bacteria isolates, while for fungal isolates had yellow, green and white color features. The shapes of colonies were thickened. spread, thin, round, dark edge, and the whole of isolates had filamentous hyphae.

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