Screening of Antibacterial MDR derived from Sponge Associated Fungus of Riung Water, Nusa Tenggara Timur

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Abstract

Marine sponge-associated fungi are the sources of bioactive compounds with various pharmacologicals potency. This study aimed to isolate the sponge-associated fungi as the producer of the MDR anti-bacterial compounds. The associated fungi were isolated from the sponges collected from Riung water, Nusa Tenggara Timur. Five of the best isolates were cultured on MEA to obtain the methanolic extract for further studies. The antagonistic test was conducted using overlay method towards the MDR Staphylococcus aureus and Escherichia coli. A total of 33 fungi were isolated from 19 sponge specimens. The antagonistic test showed that 19 isolates were active against both S. aureus and E. coli, and 13 of them were merely active against one of the bacteria. However, only five isolates have strong activity against one or both of the bacteria. The KN-15-3 had the strongest activity against S. aureus (18.75±0.777mm) and E. coli (15.10±0.141mm) at the concentration of 400 µg.disc^{-1} so it can be developed further as a source of drug candicate.

Keywords: *Fungi symbiont, Sponges, MDR Antibacterial, Staphylococcus aureus, Escherichia coli.*

Introduction

The sponge is a rich resource of metabolite compounds with various bioactivity and structural classes (Mayer *et al*. 2010). Some compounds have a pharmacological potential such as an anticancer (Trianto *et al*., 2014), antibacterial (Qaralleh *et al*., 2010), antifungal (Sik *et al*. 2006), antiinflammatory (Yang and Andersen, 2002), antimalaria (Fattorusso *et al*. 2011), and antioxidant (Trianto *et al*., 2011). However, some researchers proved that many secondary metabolites are originally produced by microorganism whether as a associated or as a food (Gandimathi *et al*., 2008). Naturaly, marine fungi live in the sea water (Kawaroe *et al*., 2015), sediments (Wei *et al*., 2004), and associated with marine organisms.

Some researches showed that many microorganisms live associated with the marine invertebrates, such as sponge (Friedrich *et al*., 2001; Mohamed *et al*., 2008). Marine sponge-associated fungi are potential producers of bioactive compounds, which are useful as medicinal materials (Lee *et al*., 2013; Zheng *et al*., 2013). Some of those compound promising to be developed as a basic material for medicine (Wiese *et al*., 2011).

Infectious diseases caused by microorganisms still become health problem, which caused the morbidity and mortality levels to be higher. For example, infection by *E. coli* cause diarrhea and meningitis. The emerge of resistant pathogens toward the recent medicines, is one of the main problems in the infectious diseases treatment. The multidrug resistance pathogen become the urgent focus of the research for a new antibiotic production (Rajasekar *et al*., 2012). In this paper we will discuss about the isolation and screening of associated fungi from the sponges collected from Nusa Tenggara Timur, Indonesia.

Materials and Methods

The Isolation of Sponge Associated Fungi

A sampling of the sponges was conducted in the depth of 2-5 meters using *SCUBA diving* in the

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Riung waters, Nusa Tenggara Timur. The specimens were kept in a cool box. Then, the sponges were cut in about 2x2cm^2 after cleaned with sterile sea water. The sponges samples were put in a prepared media Malt Extract Agar (MEA), and incubated for 24-36 hours in room temperature. Every growing fungi colony was observed, then separated based on its morphology (colony color, size, and shape). The isolation process was repeated until pure isolates obtained.

Screening of the Isolates

The antibacterial activity test was conducted using *overlay* methods (Terkina *et al*., 2006). The fungal isolates were inoculated in the solid medium marine ZoBell 2216E and incubated for 1-2 days. The *S. aureus* and *E. coli,* the collection of Microbiology Laboratory of Faculty of Medicine of Diponegoro University, were prepared in a ZoBell 2216E medium for 1x24 hours as the antagonism. The suspension of the agonist bacteria was taken 1 mL and dissolved into 100 mL *soft agar* ZoBell 2216E (1% from the *soft agar* total volume). The *Soft agar* poured to the solid medium contained the sponge-associated fungi. The activity of the isolates were indiacated by an inhibition zone around the colony.

Activity Test of the Crude Extract

Five active isolates were grown in the *Malt Extract Broth (MEB)* to provide the crude extract. The extraction was conducted according to the Rajasekar *et al*. (2012) methods with a small modification.

Table 1. The fungal isolates of the sponges collected from Riung Water

No	Isolate code	Color and characteristic
1.	KN-1-1	Yellow, no hypha, convex
2.	KN-2-1	Milky white, with hypha, flat
3.	KN-2-2	Dark green, with hypha, convex
4.	KN-2-3	Yellow, irregular
5.	KN-3-1	Light brown, round, irregular
6.	KN-4-1	Yellow, irregular, disperse
7.	KN-5-1	Milky white, no hypha
8.	KN-5-2	Grey, round, no hypha, convex
9.	KN-6-1	Milky white, no hypha
10.	KN-6-2	Yellow, irregular
11.	KN-7-1	Yellow, irregular, spreads, convex
12.	KN-9-1	Cream, irregular, disperse, flat
13.	KN-10-1	White, no hypha, convex
14.	KN-10-2	White, no hypha, disperse, convex
15.	KN-10-3	Milky white, no hypha, irregular, convex
16.	KN-11-1	Milky white, no hypha, convex
17.	KN-11-2	Milky white, no hypha, round, concave in the center
18.	KN-12-1	White, no hypha, round, convex
19.	KN-12-2	Yellow, disperse, convex
20.	KN-13-1	White, no hypha, disperse, convex
21.	KN-13-2	Blackish green, spores, disperse, convex
22.	KN-13-3	White, spores, convex
23.	KN-14-1	Cream, irregular, convex
24.	KN-15-1	Dark brown, spores, flat
25.	KN-15-2	White, no hypha, convex
26.	KN-15-3	Blackish white, no hypha, concave center
27.	KN-16-1	White bone, disperse, irregular, flat
28.	KN-17-1	White, no hypha, irregular, convex
29.	KN-17-2	White, no hypha, round, convex
30.	KN-18-1	Cream, irregular, spreads, convex
31.	KN-19-1	White, no hypha, irregular, convex
32.	KN-19-2	Yellow, irregular, convex
33.	KN-19-3	Yellowish white, spores, disperse, flat

Fungal *mycelium* was taken in sterile and soaked in methanol for 24 hours with one repetition. The extracts were tested for *S. aureus* and *E. coli* at a concentration of 400 µg.disc^{-1} using the disk diffusion agar method (Radjasa *et al*., 2007).

Result and Discussion

The Isolation of Sponge Associated

A total 19 sponges were collected from the Riung Water. All of the sponges were belong to Demospongia class. Purification of the associated fungi from the sponges provided 33 isolates based on their morphological characteristics. The brief description of the 33 isolates is presented in Table 1.

Sponges are known as the host for the marine microorganism including fungi (Li *et al.,* 2009). Sponges filter the water for their food and oxygen, where the microorganism and the organic particles entering the sponges' body in the process. Sponges have choanocytes surrounded by cilia which actively create a current water in the sponge. The sponge is able to pump approximately 1,200 times of its own volume of water per day (Hooper, 2000). Some microorganism including bacteria and fungi live symbiotically with the sponges. The prior study showed that most of the fungi isolated from the sponges are included in *Ascomycota, Zygomycota* and mitospores fungi (Li *et al*., 2009). In some cases sponge's symbionts often contribute considerably to the total sponge biomass.

Screening of the Isolates

The overlay test of the fungal isolates against the *S. Aureus* and *E. coli* showed there were 19 of associated fungi were active towards both of the tester bacteria *(broad spectrum),* ten isolates were active to oneof the agonist bacteria, and four isolates were not active. Illustration of the overlay method conducted in this research is demonstrated in Figure 1. Among the active isolates, totally six of them showed remarkably strong activity either against two or one pathogen. The detailed results of the *overlay* test were presented in Table 2.

The associated microorganisms have been proven as genuine producer of some bioactive compounds isolated from sponges either the intact or the precursor molecule. The correlation host-symbionts molecules has been describes by (Kobayashi and Ishibachi, 1993). Three diketopiperazines isolated from the sponge *Tedania ignis* were able to produce from cultured bacteria *Micrococcus* sp. Polybrominated diphenyl ether that well known to be produced by the sponge Lamelodysidea herbacea can also be produced from a culture of the associated bacteria *Vibrio* sp.

Activity Test of the crude Extract Associated Fungi Isolate Antibacterial

The best five of the fungi isolates were cultured in a medium scale to obtain methanolic extract. The extracts were tested against the *S. aureus* and *E. coli* as presented in Table 3.

Based on the antibacterial activity test of the crude extracts, the biggest resistance zone towards *S. aureus* (18.75±0.777 mm) and *E. coli* (15.10± 0.141 mm) at a concentration 400 µg.disc^{-1} by the fungal isolate KN-15-3. The marine fungi associated with the marine invertebrate are the producer of the new bioactive compounds, promised to produce antibiotic compounds, anticancer and anti-inflammatory agent (Belofsky *et al*., 1999; Li *et al*., 2009; Swathi *et al*., 2013). Some of the marine sponge-associated fungi produce unique secondary metabolic which is different and more specific than the fungi isolated from the terrestrial (Suryanarayanan, 2012).

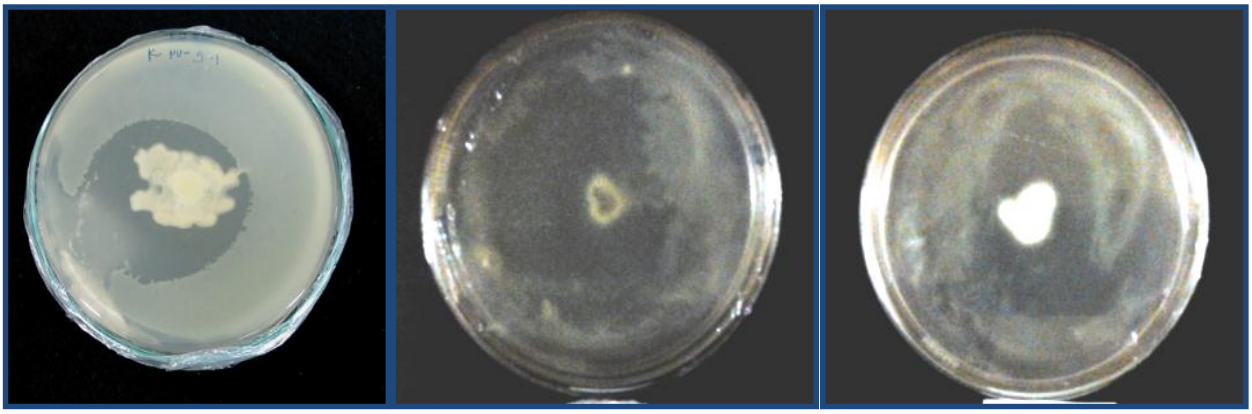

Figure 1. The overlay tests result of associated sponge fungi towards *S. aureus* and *E. Coli.*

Table 2. The *overlay* test result of associated sponge fungi isolates towards *S. aureus* and *E. Coli*

No.	Fungi code	*E. coli*	*S. aureus*
1	KN-1-1	-	+
2	KN-2-1	+	+
3	KN-2-2	+	+
4	KN-2-3	+	+
5	KN-3-1	++	++
6	KN-4-1	-	+
7	KN-5-1	+	-
8	KN-5-2	++	+
9	KN-6-1	+	-
10	KN-6-2	+	+
11	KN-7-1	-	-
12	KN-9-1	+	+
13	KN-10-1	+	+
14	KN-10-2	-	+
15	KN-10-3	-	+
16	KN-11-1	+	+
17	KN-11-2	-	-
18	KN-12-1	+	+
19	KN-12-2	+	+
20	KN-13-1	+	-
21	KN-13-2	+	+
22	KN-13-3	-	-
23	KN-14-1	++	++
24	KN-15-1	+	-
25	KN-15-2	++	++
26	KN-15-3	+	+
27	KN-16-1	+	+
28	KN-17-1	+	+
29	KN-17-2	+	-
30	KN-18-1	+	++
31	KN-19-1	+	++
32	KN-19-2	-	+
33	KN-19-3	-	-

Table explanation: "+" mark = active, "-" mark = non-active, "++" mark = highly active (the inhibition zone ≥ 6 cm)

Table 3. The fungal biomass, extract content and the inhibition zone towards *S. aureus* and *E. coli* (mm) of fungal isolates cultured in MEB.

No	Associated Fungi	Fungi Biomass (gr)	Extract Weight (gr)	% extract	Bioactivity	
					S. aureus (400 µg.disc^{-1})	*E. coli* (400 µg.disc^{-1})
1.	KN-5-2	14.91	0.5623	3.77	10.3±0.141	7.63±0.188
2.	KN-17-1	0.96	0.5988	62.37	7.2± 0.282	6.48±0.447
3.	KN-14-1	35.6	1.3773	3.86	10.35±0.353	10.0±0.283
4.	KN-19-1	9.01	0.6602	7.32	17.36±0.188	7.35 ± 0.070
5.	KN-15-3	32.86	0.3252	0.98	18.75±0.777	15.10±0.141

However, the production of bioactive compounds was highly affected by nutrition, age, and the environmental factors (Li *et al*., 2009; Swathi *et al*., 2013). The ability of the isolates produced the bioactive compound indicated that the isolates has a potent to be developed as a drug source.

Conclusion

Some of associated fungi isolated from the sponges collected from the Riung waters NTT have the antibacterial MDR *S. aureus* and *E. coli* activity. The KN-15-3 isolate has the strongest activity towards both of the pathogenic bacteria, so it deserved to be developed as a drug material source.

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