Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 16, No. (3) : 2014 : 475-478

ISSN-0972-3005

ISOLATION AND SCREENING FUNGAL SYMBIONT IN GREEN ALGA *ULVA RETICULATA* AS CANDIDATE OF ANTIBIOTIC PRODUCER

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(Received 15 Feburary, 2014; accepted 16 March, 2014)

Key words : *Fungal symbiont, Ulva reticulata, Antibiotic producer*

Abstract - The isolation of fungal symbiont from *Ulva reticulata* by direct planting method on Potato Dextrose Agar plate was performed. Preliminary screening of the fungal symbionts as a candidate of antibiotic producer involved a fermentation process in Potato Dextrose Broth supplemented with 0.5 mg % yeast extract using shaker at 120 rpm for 7 days. Antimicrobial activity of fermentation supernatants was examined using disc agar diffusion method against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Candida albicans* and *Malasezzia furfur*. We found that three isolates, identified by macroscopic and microscopic characteristics, were molds genus *Aspergillus, Penicillium* and *Cladosporium* and one of them was unidentified fungi. Supernatant of *Aspergillus, Penicillium*, *Cladosporium* and the unidentified fungi demonstrated a high antifungal activity against *C. albicans*, verified by the diameter zone of inhibitions of 28.04 mm, 24.98 mm, 25.38 mm, and 27.73 mm, respectively. Only supernatant of the unidentified fungi had antifungal activity against *M. furfur*. A high antibacterial activity against *S.aureus* was shown by the supernatant of *Penicillium* and *Chladosporium* with diameter zone of inhibitions of 19.15 mm and 16.56 mm, respectively. All isolates had low antibacterial activity against *E.coli* and *S. thyposa*. Fungal symbionts in *Ulva reticulata* from Takalar, South Sulawesi are potential as a candidate of antibiotic producer, especially antibacterial activity against *S.aureus* and *C.albicans*.

INTRODUCTION

Many essential compounds act as antibacterial and antifungal derived from seaweed or marine algae. Marine algae are classified as Rhodophyta (red algae), Phaeo-phyta (brown algae) and Chlorophyta (green algae) (Chanda *et al.*, 2010). Some of green alga was more active than other groups of alga screened for their antibacterial activity and they are potential sources of bioactive compounds and should be investigated for natural antibiotics (Kandhasamy and Arunachalam, 2008; Osman *et al.*, 2010). For example, Green alga Ulva reti-culata and *Ulva lactuca* extracts showed promising antimicrobial activity against bacterial and fungal human pathogens (Kolanjinathan and Stella, 2011). *Ulva fasciata* contains sterol alkaloid, phenoloic, flavonoid, terpenoid, glicoside had width spectrum antibacterial activity (Premalatha *et al.*, 2011; Selvin and Lipton, 2004)

Marine algae live in symbiosis with certain microorganisms such as fungi and bacteria. Epi-phytic and endophytic fungi live on the surface and in the inner tissues or even in the cell of their hosts, respectively. It is believed that algae and their associated microbial symbionts should represent a good source of biologically active secondary metabolites (Schulz *et al.* 2008; Suryanarayanan *et al.* 2010). For example, Ascochyta salicorniae, an endophytic and obligate marine fungus was isolated from the green alga *Ulva* sp. (Claudia *et al.*, 2000). New marine derived antibiotics, 43 epi- and endophytic fungal strains were also isolated from the surface or the inner tissue of different marine plants and invertebrates. Through preliminary and secondary screening, 10 of them were found to be

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able to produce broad-spectrum antimicrobial metabolites (Zhang *et al.*, 2009).

The purpose of this study was to search for antibiotic-producing fungal symbiont in green alga *Ulva reticulata* from Takalar South Sulawesi, Indonesia. In this paper, we report the isolation, identified the fungi by macroscopic and microscopic characteristic and preliminary screening of fungal symbiont from *Ulva reticulata* as antibiotic producer and by diffusion agar assay using several microbial test.

MATERIALS AND METHODS

Collection and Preparation Green Alga

The green alga *Ulva reticulata* were collected from Punaga Coast Takalar Regency, South Sula-wesi. Immediately after collection, they were washed in fresh seawater to remove the sand and other extraneous matter. The alga were transported to the laboratory in cool box.

Isolation of Fungal Symbiont

The alga were divided two groups: first group was not subjected to surface sterilization, while second group was subjected to surface sterilization according to Flewelling method with modification (2011). The algae was rinsed with sterile sea water, followed by immersion in 1 % sodium hypochlorite for 1 minutes and rinsed again with sterile sea water. After surface sterilization, fragments of approx. 1 cm x 1 cm from sterile algae were inoculated on Petri dishes containing PDA supplemented with chloramphenicol 50 mg/L to suppress bacterial growth. All the plates were incubated at room temperature for up to 12 days. The fungi were transferred to fresh PDA plates, incubated for 1 week and periodically checked for purity. Identification were done by macroscopic and microscopic characteristic.

Production of bioactive compound by submerged fermentation

Production of antimicrobial compound was conducted according to Radu method with modification (2003). The endophytic fungi were fermented with a submerged fermentation with shaking 120 rpm for 7 days using PD-Y medium (2.4 % Potato Dextrose Broth, 0.5 % Yeast extract, 1 % NaCl). After incubation, the media were sonicated for 5 minutes and centrifuged at 3000 rpm for 15 minutes. The supernatant was used as sample test.

Antimicrobial assay by agar diffusion methods

Antibacterial activity was evaluated by agar diffusion method. Supernatant was pipetted 20 µL onto paper disc (diameter 6 mm), each disc was placed on Muller Hinton Agar (pH 7 + 0.2) which contained 1 mL bacteria suspension (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*) and incubated for 24 hours at 37°C. Inhibition results are expressed as diameter of the clear halo surrounding each disc on cultivated agar plates. Tetracycline antibiotic 30 ppm was used as positive control. Antifungal activity was tested using similar method using Potato Dextrose Agar and fungal fungal suspension (*Candida albicans* and *Malessezia furfur).* Incubation time was 24 -48 h at room temperature.

RESULTS AND DISCUSSION

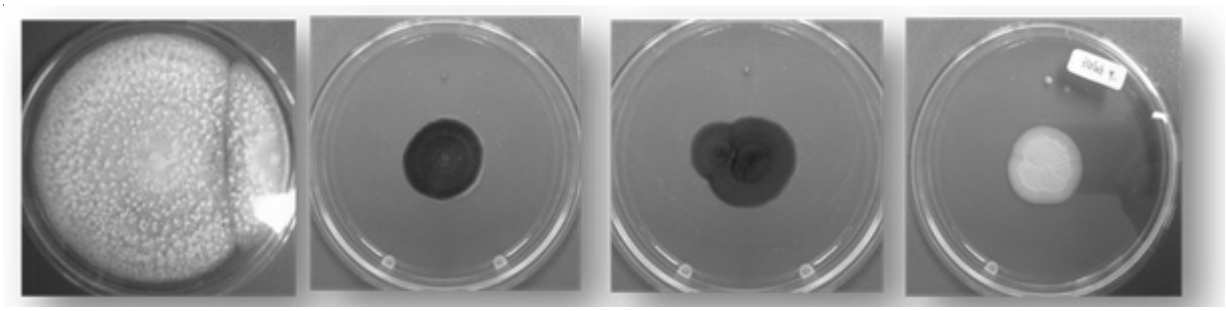
Isolation of Fungal Symbiont

A total of 4 fungal symbiont were obtained from Punaga Coast Takalar Regency, South Sulawesi. The macroscopic and microscopic characteristics are shown in Figure 1.

Strain FSUr-1, FSUr-2, FSUr-3 were identified as *Genus Aspergillus, Penicillium, Chlados-porium*, respectively. FSUr-4 was unidentified. Several studies found that genus *Aspergillus* and *Penicillium* presented in fungal symbiont in marine alga. The genus *Aspergillus* has been known to be a major contributor to the secondary metabolites of marine fungal origin, for example, genus *Aspergillus* has been isolated by Zhang *et al*. (2007) from marine brown alga *Colpomenia sinuosa* similarly, Gamal-Elden *et al.* (2009) has isolated fungal endophyte from green alga Ulva sp, which was identified as *Penicillium* sp.. Zhu *et al.* (2009) has also isolated *Penicillium*, from green alga *Blidingia minima*. Gao *et al.* (2011) found that *Penicillium chrysogenum* QEN-24S, an endo-phytic fungus isolated from an unidentified marine red algal species of the genus Laurencia, displayed inhibitory activity against the growth of pathogen *Alternaria brassicae* in dual culture test.

Antimicrobial activity of the isolates

Antimicrobial activity obtained from fermentation supernatant of 4 fungal symbionts was examined by disc agar diffusion method against *Staphylococcus*



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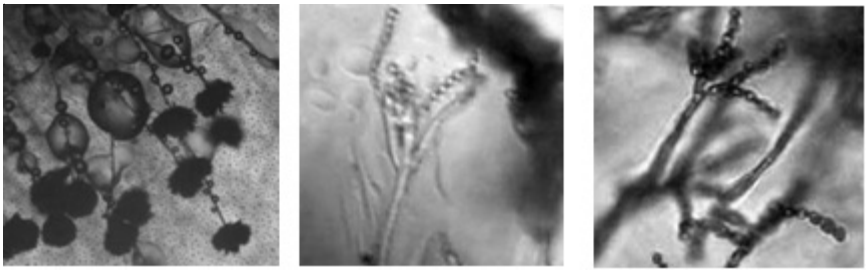

B

Fig. 1 Macroscopic and microscopic characteristic of fungal symbiont isolated from *Ulva reticulata*
A. Colony of Culture FSUr-1, FSUr-2, FSUr-3, FSUr-4 grown on PDA for seven days, respec-tively
B. Microscopic characteristic (conidia and conidiaphores) of FSUr-1, FSUr-2, FSUr-3, respectively

Table 1. Antimicrobial activity of supernatant of Symbiont fungi from green Alga *Ulva reticulata*, in the agar diffusion assay

Sample /(mm) Supernatant of isolates	Diameter zone of inhibition					
	Bacteria				Fungi	
	B.subtilis	*S.aureus*	*E.coli*	*S.thypi*	*C.albicans*	*M.furfur*
FSUr-1	11.53+0.6	9.70+ 0.33	10.08+ 0.86	8.72+ 0.42	28.04+ 1.26	7.47+ 0.52
FSUr-2	22.03+ 0.83	19.15+ 1.22	15.08+ 0.91	13.54+ 0.9	24.98+ 1.15	10.56+ 1.24
FSUr-3	19.95+ 0.81	16.56+ 1.37	13.74+ 0.78	13.34+ 0.15	25.38+ 1.59	15.16+ 1.01
FSUr-4	12.2+ 0.07	10.97+ 0.53	12.74+ 0.75	11.71+ 0.05	27.73+ 1.72	17.49+ 0.94
Chloramphenicol			18.36+ 0.77	9.59+ 0.09		
Tetracycline	22.01 + 1.18	11.89 + 1.47				
Nystatin					8.95 + 1.30	7.91 + 0.23

Inhibition zone in mm including disc, expressed as Mean ± SD (n = 3);

aureus, Escherichia coli, Bacillus subtilis, Candida albicans and *Malasezzia furfur* The result shows in Table 1 and Fig. 2.

Supernatant of *Aspergillus, Penicillium, Cladosporium* and the unidentified mold against *C. albicans*, demonstrated a high antifungal activity, verified by the diameter zone of inhibitions of 28.04 mm, 24.98 mm, 25.38 mm, and 27.73 mm, respectively. A high antibacterial activity against *S.aureus* was showed by the supernatant of *Penicillium* and *Chladosporium* with diameter zone of inhibition were 19.15 mm and 16.56 mm, respectively. All isolates had low antibacterial activity against *E. coli* and *S. thyposa*. Only supernatant of the unidentified fungi had antifungal activity against *M. furfur*. According to Ibbitassam *et al.* (2009), the antibacterial activity was classified from less active which was (10 mm<diameter of inhibition<16 mm), to moderately active (16 mm<diameter of inhibition <20 mm), to highly active (diameter of

inhibition > 20 mm) and they found *Ulva* sp had antibacterial activity against *S. aureus* s higher than *E. coli*.

CONCLUSION

We found four fungal symbiont from green alga *Ulva reticulata* from Punaga Coast Takalar Re-gency, South Sulawesi. Three of them (FSUr-1, FSUr-2, FSUr-3) are potential as candidates for of antibiotic producer.

ACKNOWLEDGEMENT

This article has been orally presented at 5th International Seminar of Indonesian Society for Microbiology Indonesian Society for Microbiology, Manado, 2012.

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