Antibacterial Activity of Cultivable Endophytic Bacteria from Mangrove Tree *Rhizopora mucronata*

Erny Qurotul Ainy[1] & Maya Anggraini Sembiring[1]
[1]Program Studi Biologi UIN Sunan Kalijaga
Jl. Marsda Adisucipto No.1, Telp. (0274) 519739, Fax. (0274) 540971 Yogyakarta 55281
E-mail:erny.ainy@uin-suka.ac.id.

ABSTRACT

Rhizopora mucronata has been considered as a promising source of endophytes with high bioactivities. The objective of this study were examining the potency of endophytic bacteria isolated from mangrove tree (*R. mucronata*) for their antibacterial activity against two bacterial species, *Eschericia coli* and *Staphylococcus aureus* and screening the antibacterial compounds from endophytes extracts. Stems tree were collected from Wanatirta mangrove forest in Kulonprogo, Yogyakarta. Seven endophytic bacterial isolated from stem were evaluated based on morphological characteristics. Antibacterial activity of the isolates was tested by paper disc assay method and data was recorded. Antibacterial compounds from the crude extract endophytes were determined. The results demonstrated that IBT4 and IBM1 isolates that identified as members of Bacillus were found exhibiting antibacterial activity and producing alcaloid and saponin.

Keywords: Antibacterial activity, Endophytic bacteria, Isolation, Mangrove, *Rhizopora mucronata*

INTRODUCTION

Mangroves are trees that located mainly in a transition zone between land and sea in the (sub) tropics regions. The zone is characterized by different ecological factors, such as temperature, tidal currents and sedimentation. The aerial of mangroves stabilize this environment and provide a habitat for many species of organisms (Nagelkerkeren et al., 2008). As its abundance of nutrients and shelter, mangrove forest represents a rich of biodiversity. Therefore, mangrove forest are well-known with its diversity of microorganisms. Microbial communities of mangrove can adapt to fluctuating environmental condition and tend to represents as a source of biotechnological compounds that could be exploited. Mangrove microorganisms can produce various enzymes, antibiotics and salt tolerant genes. However, less than 5% of the species present have been described (Thatoi, Behera, Mishra, & Dutta, 2013). Among mangrove microorganisms, bacterial populations are greater than fungi (K. Kathiresan, 2005).

Endophytic bacteria refer to bacteria living in inside surface of plants or extracted from inside plants and having no damaged effects on the plants (Mano & Morisaki, 2008). It is estimated that all plant species become host for one or more endophyte microorganisms (Etminani & Harighi, 2018; Kapoor, Jamwal, & Gandhi, 2019).

Endophytic bacteria has been applied as a source for the production of bioactive compounds. However, reports on the mangrove as the source of microbial endophytes are still limited (C.Jose & Christy, 2013).

Rhizophora mucronata belongs to family Rhizophoraceae. It has been successfully used for treating a variety of diseases over hundreds of years such as angina, dysentery, hematuria, constipation, nausea, and diabetes. The certain medicinal properties of *Rhizophora mucronata* depend on the part like (Bibi et al., 2019; Gurudeeban, Ramanathan, & Satyavani, 2015). In Japan and China this mangrove has been used as an antidiarrheal (Tarman, Safitri, & Setyaningsih, 2013). However, excessive deforestation and mass exploitation of some important mangrove plants lead to many of them becoming endangered. Apart from being an eco-hazard, extracting bioactive componds from plants is also ver laborious, costly, and time consuming. Thus, exploring mangrove endophytic bacteria as ecofriendly sources of high-value phytochemicals is highly desirable (Jamwal & Gandhi, 2019).

Endophytes are manufacturer of bioactive compounds inside plants and they are also responsible for the hosts' medicinal properties and it can be used by plants for defense against pathogens and some of the compounds also can be applied as pharmaceutical drugs (Rahman, Shinwari, Iqrar, Rahman, & Tanveer, 2017). Medicinal properties of different parts of mangrove plant may be fully or partially dependent on the endophytes (C.Jose & Christy, 2013).

Infectious disease still becomes a global issue because of the development and spreading of drug resistant pathogens. However, isolation of mangrove endophytic bacteria for antimicrobial activity in Indonesia is limited. Whereas, natural resources are the potential source of novel bioactive compounds and can be used in treatment for various infection disease.

It is predicted that the use of endophytic bacteria as a source of raw material to be more efficient economically than using medicinal plants (Tarman et al., 2013). The aim of this study is obtaining endophytic bacteria from mangrove tree *Rhizopora mucronata*, characterizing of the endophytic bacterial isolates, and determining the antibacterial activity of the bacterial extracts against *Eschericia coli* and *Staphylococcus aureus*, and evaluating its antibacterial constituents.

MATERIAL AND METHOD

Isolation and purification of endophytic bacteria froms stem of *R.mucronata*

R. mucronata mangrove stem samples were collected from the banks of the Wanatirta Mangrove Forest in Bogowonto Kulon Progo Yogyakarta. The stem was cut into pieces about 5 cm in length and then washed by running water. The surface stem was sterilized with ethanol solution 70% for 1 minute followed by washing it with NaOCl 0,3% for 5 minutes, and rinsed three to five times with sterile distilled water. Stem pieces were blotted on the blotting paper and placed on Nutrient Agar (NA(media) which had been added by ketoconazole (0.3 gram/100 ml) for the isolation of endophytic bacteria (Rahman et al., 2017). Petri dishes containing plant samples were then incubated at room temperature for 2-5

days. The isolation process repeated till monocultures were obtained for further experimentations. Bacterial colonies growing were differentiated on the basis of morphological colony characters. Bacterial isolates were picked from plates and purified on TSA by streaking techniques and incubated at 27-29ºC for 48 hours .

Screening of bacterial endophyte producing antibacterial of *R.mucronata*

Testing the selection of endophytic bacteria that have the potential to be antibacterial is carried out using the Paper Disc Diffusion method. The tested bacteria used were *Staphylococcus aureus* and *Eschericia coli*. Each bacterial suspension of the test on NB (Nutrient Broth) media that was in the mid log phase was taken 0.1 mL and pipetted into the NA media and spread evenly using a Dirgalsky stem. 10 µL of endophytic bacterial suspension was absorbed on a sterile 5 mm diameter disc and dried. The disc then was placed on the NA media which had been inoculated with the tested bacteria, then incubated for 24 hours at 35ºC and observed whether there is a clear zone formed. The tree disks with different concentration of extract were placed gently on solidified seeded test with the help of sterile forceps. Then, the plates were inverted and kept in a refrigerator at 4°C for about 24 hours. This period offtime was sufficient for the material to diffuse into the medium over a substantial area. Then, all plates were incubated at 35ºC for 18 h and diameters of the zones of microbes growth inhibition was measured (Gurudeeban et al., 2015).

Production of bacterial secondary metabolites

Pure endophytic bacterial isolates were grown in NB medium with incubation period of 24 hours at 30ºC taken 1 ml and transferred to 5 ml of MHB (Mueller-Hinton Broth) medium and incubated for 24 hours at 35ºC (Elita, 2013). The fermentation process of endophytic bacteria uses MHB medium. The bacterial colony suspension was then taken as much as 1 ml and transferred into an Eppendorf tube that had contained 9 ml of MHB medium. The Eppendorf tube which contained bacterial suspension was then incubated in the incubator shaker for 48 hours at 30^0 °C. After incubation, the medium which contained bacterial suspension then centrifuged at 5,000 rpm for 20 minutes. The supernatant is taken using a sterile pipette and put in a sterile test tube. The supernatant will be used at the secondary metabolite test stage.

RESULT

Using the plant tissues cultured in Petri dishes, endophytic bacteria were successfully isolated (Table1). There were 7 isolated bacterial endophytes from stem *R. mucronata.* The presence of endophytic bacterial species and their population density is highly variable depending on the bacterial species, host, end environmental condition (Chebotar et al., 2015).

Table 1. Endophytic bacteria from stem *R. mucronata*

No	Isolates	Characteristics of						
		Colony					Cell	
		Texture	Colour	Form	Elevation	Margin	Form	Gram type
1	IBT 1	Non-mucous	Cream	Circular	Convex	Undulate	Coccus	+
2	IBT 2	Mucous	Yellow	Circular	Convex	Entire	Coccus	+
3	IBT 3	Non-mucous	Yellow	Circular	Convex	Entire	Bacil	+
4	IBT 4	Mucous	Transparant	Circular	Raised	Entire	Bacil	+
5	IBT 5	Non-mucous	Cream	Circular	Flate	Entire	Bacil	+
6	IBM 1	Non-mucous	Cream	Circular	Convex	Entire	Bacil	+
7	IBM 2	Mucous	Cream	Circular	Raised	Entire	Coccus	-

Two isolated bacteria, IBT 4 and IBM 1, displayed anti-bacterial activities against the selected bacteria by producing inhibition zones. Table 2 summarizes data on the antibacterial activity of endophytic bacteria of *R. mucronata*. Both IBT 4 and IBM1 could inhibit the growth of *E.coli* more strongly than *S.aureus.*

Table 2. Antibacterial activity of extract endophytic bacteria from *R. Mucronata* against *E. coli* and *S. aureus*

No	Isolates	Diameter of halo zone (mm)	
		Escherichia coli	*Staphylococcus aureus*
1	IBT 1	-	-
2	IBT 2	-	-
3	IBT 3	-	-
4	IBT 4	10,5	5
5	IBT 5	-	-
6	IBM 1	5	4
7	IBM 2	-	-

The presence of phytochemicals such as alkaloids, tannins, flavonoids in mangrove plants responsible for the antimicrobial effect. Table 3 shows the constituents of secondary metabolites that produced by mangrove endophytic bacterial.

Table 3. Secondary metabolit produced by endophytic bacteria from *R. mucronata*

No	Metabolites	Endophytic bacteria	
		IBT 4	IBM 1
1	Alkaloid	+	+
2	Flavonoid	-	-
3	Saponin	+	+
4	Tanin	-	-
5	Terpenoid	-	-
6	Steroid	-	-

Table 4. Identification of endophytic bacteria of *R.mucronata* at genus level (*Generic Assignment*) with antibacterial potency

No	Key characters	Endophytic bacteria		
		IBT 4	IBM 1	Bacillus
1.	Cell arrangement	Pair	Single	Single/Pair
2.	Cell form	Bacil	Bacil	Bacil
3.	Gram type	+	+	+
4.	Catalase production	+	+	NA
5.	H_2S production	-	-	+/-
6.	Citrate hydrolysis	-	-	+/-
7.	Starch hydrolysis	-	+	NA
8.	Fermentation of carbohydrates:			
	Glucose	+	+	+
	Lactose	+	+	+
	Sucrose	+	+	+
	Maltose	+	+	+

NA (*Not Applicable*)

DISCUSSION

Endophytes associated with medicinal plants are rich sources of secondary metabolites with antimicrobial activity. They spend their whole life cycle within plant tissues without causing any infections or signs of disease (Bacon and White, 2000; Saikkonen et al., 2004). In addition, these endophytes may produce the same metabolites in vitro and within host plant tissue (Kusari et al., 2013; Dos Santos et al., 2016). In the present study, the antibacterial activity of endophytic bacteria of *R.mucronata* were analyzed.

R.mucronata displayed a broader spectrum of biological activities such as antidiabetic (in vitro and in vivo), antioxidant, antiinflamatory, analgenic, anti-HIV, antimicrobial (Bibi et al., 2019). However, new discoveries in this plant research lead to the development and opening of many areas to explore. The plant is now gaining importance to develop some more new search for the future development. Therefore, considering its versatile medicinal uses, there is an ample scope for future research on *R.mucronata* and hence further pharmacological investigations are warranted.

The present study conducted to find out the the presence of bacterial endophytes in stem tissue of this plant. By using nutrient agar, seven endophytes were isolated from stem tissue of the plant.

The result of antibacterial activity showed that the diameters of inhibition zone indicating antibacterial activity produced by endophytic bacteria was diverse. It is due to the difference of each bacterium's sensitivity, where they posseses different biological capabilities in response to bacterial agents. The structural differences between cell wall of Gram negative and Gram positive bacteria is one of the most influential factors to the antibacterial activity. The other factors are concentration or intensity of the antimicrobial agents, the number of microorganism tested, species of microorganisms, temperature, the presence of organic matter, acidity, and alkalinity (Le et al., 2019).

Bioactive components of bacterial extracts can be determined using phytochemical test. It is a qualitative test applied to detect the contents of secondary metabolites present in the samples (Copriyadi et al., 2005). The test results showed that alkaloid and saponin compounds were detected in the both IBT4 and IBM1 isolates of endophytic. These results clarified the reason the both endophytes' antibacterial activity.

Based on the morphological and biochemical characterization of the endophytes they are identified as *Bacillus*. Further research work is needed to explore more about the role of these bacterial endophytes in the metabolism of the plant.

CONCLUSION

Seven bacterial endophytes with different morphology were isolated from *Rhizophora mucronata* stem. The selected bacteria, IBT4 and IBM1, showed antibacterial activity against *Eschericia coli* and *Staphylococcus aureus* and produced alkaloid and saponin. Based on their macroscopic and microscopic characteristics, both IBT4 and IBM1 were suspected as *Bacillus*.

REFERENCES

Bibi, S. N., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., Rengasamy Kannan, R. R., … Pandian, S. K. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves-a comprehensive review. *Marine Drugs*, Vol. 17. https://doi.org/10.3390/md17040231

C.Jose, A., & Christy, P. H. (2013). Assessment of antimicrobial potential of endophytic bacteria isolated from Rhizophora mucronata. *International Journal of Current Microbiology and Applied Sciences*, *2*(10), 188–194. Retrieved from https://www.ijcmas.com/vol-2-10/Anu C.Jose and Priscilla Helen Christy.pdf

Chebotar, V. K., Malfanova, N. V., Shcherbakov, A. V., Ahtemova, G. A., Borisov, A. Y., Lugtenberg, B., & Tikhonovich, I. A. (2015). Endophytic bacteria in microbial preparations that improve plant development (review). *Applied Biochemistry and Microbiology*, *51*(3), 271–277. https://doi.org/10.1134/S0003683815030059

Etminani, F., & Harighi, B. (2018). *Isolation and Identification of Endophytic Bacteria with Plant Growth Promoting Activity and Biocontrol Potential from Wild Pistachio Trees*. *34*(3), 208–217. https://doi.org/athol. https://doi.org/10.5423/PPJ.OA.07.2017.0158

Gurudeeban, S., Ramanathan, T., & Satyavani, K. (2015). Antimicrobial and Radical Scavenging Effects of Alkaloid Extracts from Rhizophora Mucronata. *Pharmaceutical Chemistry Journal*, *49*(1), 34–37. https://doi.org/10.1007/s11094-015-1218-8

Jamwal, V. L., & Gandhi, S. G. (2019). Endophytes as a Source of High-Value Phytochemicals: Present Scenario and Future Outlook. In S. Jha (Ed.), *Endophytes and Secondary Metabolites* (pp. 571–590). https://doi.org/10.1007/978-3-319-90484-9_14

K. Kathiresan, S. Z. (Syed Z. Q. (2005). *Biodiversity of mangrove ecosystems*. Hindustan

Pub. Corp.

Kapoor, N., Jamwal, V. L., & Gandhi, S. G. (2019). Endophytes as a Source of High-Value, Bioactive Metabolites. In S. Jha (Ed.), *Endophytes and Secondary Metabolites* (pp. 1–32). https://doi.org/10.1007/978-3-319-76900-4_9-1

Le, N. T. T., Bach, L. G., Nguyen, D. C., Le, T. H. X., Pham, K. H., Nguyen, D. H., & Thi, T. T. H. (2019). Evaluation of factors affecting antimicrobial activity of bacteriocin from lactobacillus plantarum microencapsulated in alginate-gelatin capsules and its application on pork meat as a bio-preservative. *International Journal of Environmental Research and Public Health*, *16*(6). https://doi.org/10.3390/ijerph16061017

Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., … Cheng, G. (2017). Antimicrobial activity and resistance: Influencing factors. *Frontiers in Pharmacology*, *8*(JUN), 1–11. https://doi.org/10.3389/fphar.2017.00364

Mano, H., & Morisaki, H. (2008). Endophytic bacteria in the rice plant. *Microbes and Environments*, *23*(2), 109–117. https://doi.org/10.1264/jsme2.23.109

Nagelkerken, I., Blaber, S. J. M., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., … Somerfield, P. J. (2008). The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquatic Botany*, *89*(2), 155–185. https://doi.org/10.1016/j.aquabot.2007.12.007

Rahman, L., Shinwari, Z. K., Iqrar, I., Rahman, L., & Tanveer, F. (2017). An assessment on the role of endophytic microbes in the therapeutic potential of Fagonia indica. *Annals of Clinical Microbiology and Antimicrobials*, *16*(1), 1–12. https://doi.org/10.1186/s12941-017-0228-7

Tarman, K., Safitri, D., & Setyaningsih, I. (2013). Endophytic Fungi Isolated from Rhizophora mucronata and Their Antibacterial Activity. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, *8*(2), 69. https://doi.org/10.15578/squalen.v8i2.88

Thatoi, H., Behera, B. C., Mishra, R. R., & Dutta, S. K. (2013). Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: A review. *Annals of Microbiology*, *63*(1), 1–19. https://doi.org/10.1007/s13213-012-0442-7

Certificate
of Recognition

No. 16283/UN22/PM.03/2019

Awarded to

Erny Qurotul Ainy

as

Oral Presenter

During the KOBI 2nd International Conference 2019
"MANAGEMENT OF TROPICAL BIODIVERSITY FOR HUMAN WELFARE: FROM ECOSYSTEM TO MOLECULAR"

September 6-8, 2019
Conference Building, Universitas Tanjungpura Pontianak
Indonesia

Rector of Universitas Tanjungpura

Prof. Dr. Garuda Wiko, S. H., M. Si
NIP. 19650128 199002 1 001

Chairman of Indonesia Biology Consortium (KOBI)

Prof. Dr. Budi Setiadi Daryono, M.Arg.Sc
NIP. 19700326 199512 1 001