Potential Antibacterial Activity of Endophytic Fungi

Penicillium sp. and Trichoderma sp. Derived From Mangrove Ceriops Tagal (Perr.) C.B.Robb and Bruguiera sp.

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Abstract. This study aims to isolate endophytic fungi from the roots of mangrove from Riau Province’s Mangrove Forest and to screen some isolates with ability to inhibit the growth of pathogenic bacteria. A total of 28 endophytic fungi have been isolated from mangrove samples identified as Bruguiera sp. and Ceriops tagal (Perr.) C.B.Robb. Out of 28 isolates, 17 isolates (60.71%) were isolated from direct plating of sterile segment of root samples, while 11 isolates (39.28%) were isolated from suspension of grinded sterile segment in aliquot sterile sodium chloride. The potency of all isolated endophytic fungi to produce antibacterial agents were identified by doing antagonistic study against Escherichia coli, Staphylococcus aereus and Vibrio alginolyticus. The results showed that the highest antibacterial activity was produced by the isolates of C7B, B1C, C4E, and C4D with clear zones diameter of 17.91 ± 0.84 mm; 17.78 ± 0.83 mm; 17.66 ± 0.83 mm; 16.72 ± 1.15 mm, and 13.65 ± 0.27 mm, respectively. Based on macroscopic and microscopic analysis, those fungal isolates were identified as Penicillium sp. (C7B) and Trichoderma sp. (B1C, C4E, C4D).

1. Introduction

Antibiotics have been a valuable resource to humans since the discovery of penicillin in 1928, but the spread of antibiotic resistance in bacterial pathogens has become a major public health problem. Canica et al. [1] reported that antibiotic resistant bacteria already cause an excess of 700,000 deaths each year worldwide. Furthermore, the Centre for Disease Control and Prevention has recently stated that the world is on the verge of entering the “post-antibiotic era”, one where more people will die from bacterial infections than from cancer. It indicates that the need for new antibiotic classes is urgent.

Mangrove forests, that constitute a dynamic transition zone between terrestrial and marine habitats, have adapted to a unique habitat with muddy saline waters, extensive salinity, high temperature and moisture, high microbial and faunal competition, and brackish tidal activities [2, 3]. Around 50–70 species of mangrove plants are distributed in tropical and subtropical climates in the world while a total of 41.4% of global mangroves are found in South and Southeast Asia [4, 5]. Rhizophoraceae is
the most abundant mangrove tree family and comprises four genera: *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora* [6]. According to Arobaya and Wanma [7], Indonesia has 27% of the world's total mangrove forest, equivalent to 4.25 million ha, and 143 billion ha are in Riau Province.

Leaves, stems, bark, roots and fruits of mangrove plants are a valuable resource for folk medicine. It has been reported that mangrove plants host a great variety of endophytes comprising a consortium of soil, marine and freshwater fungi; it is well known that mangrove-derived endophytic fungi are promising sources of natural products and drug with an amazing array of bioactivities [8, 9; 10]. Although several mangrove plants worldwide have been studied for their endophytic fungal association, there is no report on the endophytic fungi of mangrove from Riau Province's Mangrove forest. The objective of this study was to explore Riau mangroves derived endophytic fungi as a source of new bioactive compounds against bacterial pathogens.

2. Methodology

2.1. Sampling site and endophytes isolation

Mangrove samples were taken randomly from Sei Pakning (Siak Regency) and Tenggayun Beach (Bengkalis Municipality). Mangrove species of all samples were identified at University herbarium. The roots were brought to the laboratory in separate sterile polyethylene bags and were then used immediately for fungal endophytes isolation.

Endophytic fungi were isolated from all roots samples following our previously published method [11]. Hyphae tips that grew out from the isolation process were sub-cultured onto the surface of PDA without antibiotic. Pure cultures were then maintained at 4°C until being used for further analysis.

2.2. Antagonistic test

Pure fungi isolates were then subjected onto antagonist study against Gram negative and Gram positive pathogenic bacteria i.e., *Escherichia coli*, *Staphylococcus aureus* and *Vibrio alginolyticus*. A plug of 5-day-old culture of each fungal endophyte was inoculated on the centre of Mueller Hinton Agar containing each pathogenic bacteria (OD 0.1) and incubated for 24-48 h. All treatments were performed in triplicate. Inhibition zones produced around each endophytes was measured and all those inhibition data were then analyzed statistically with ANOVA and *Duncan's New Multiple Range Test* at p ≤ 5%. Endophytic fungus with the highest diameter of inhibition zone was then subjected into macroscopic and microscopic analysis.

3. Results and Discussion

Antibiotic resistant, which is defined as the reduction of effectiveness of an antibiotic during treatment of infectious diseases, have become increasing widespread worldwide. Multiple drug resistance genes from different organisms can be acquired by the same microbe and it causes the emergence of multi drug resistant microorganisms called “superbugs”. According to Wang et al. [12], super resistance gene (NDM-1) - that causes enzymatic degradation of β-lactam antibiotics – makes the bacteria resistant against broad range antibiotics. Similarly, other researchers reported that million cases of multi drug resistant (MDR) *Mycobacterium tuberculosis* are now resistant to current antibiotics [13]. The increasing number of MDR strains has contributed to an urgent need to discover novel antibacterials to combat those resistant bacteria. Instead of exploration of new antimicrobial agents from natural products, there is a renewed focus on the search of lead candidates from endophytic sources.

The term ‘endophyte’ was introduced in 1866 by De Bary and was initially applied to any organism found within a plant [14]. Now, endophytes are defined as microorganisms that live within the plant tissues without causing any damage to the host and are classified as fungi, bacteria or algae [15]. There are five categories for the rationale behind choosing the plants for endophytic study: plants in ethnopharmacology, plants that grow in special habitats such as mangrove or swamps, healthy plants that are surrounded by diseased plants, plants that occupied certain land mass, and plants that grow in high biodiversity areas. In this study, endophytes were isolated from Mangrove plants due to its unique...
environment. Endophytes from mangrove are well known act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties [8, 16].

A total of 28 fungal endophytes have been isolated from root samples of Mangroves collected from Bengkalis and Siak area in Riau Province, Indonesia (Figure 1). Those mangrove samples were identified as Ceriops tagal (Perr.) C.B.Rob and Bruguiera sp. Out of 28 isolates, antagonist test results showed that 17 isolates were able to inhibit the growth of E. coli and produced inhibition zone ranging from 9.80 ± 0.71 mm to 17.78 ± 0.83 mm; only 6 isolates produced inhibition zone around V. alginolyticus (diameter were 9.95 ± 2.65 mm to 17.91 ± 0.84 mm); while 8 isolates were active against gram positive bacteria, S. aureus with inhibition zones of 9.80 ± 0.71 to 13.65 ± 0.27 mm (Table 1). Comparison of overall endophytes and from each species of mangrove against pathogenic bacteria tested was given in Figure 2.

| No. | Isolates code | Sampling area | Mangrove Species | Diameter of inhibition zone (mm) |
|-----|---------------|---------------|------------------|----------------------------------|
|     |               |               |                  | E. coli                          |
| 1   | B1C           | I             | Bruguiera sp.    | 17.78 ± 0.83°C                  |
| 2   | B1A           | I             | Bruguiera sp.    | 10.06 ± 0.72°C                  |
| 3   | B5C           | V             | Bruguiera sp.    | -                               |
| 4   | B5D           | V             | Bruguiera sp.    | -                               |
| 5   | B5E           | V             | Bruguiera sp.    | -                               |
| 6   | B5A           | V             | Bruguiera sp.    | 12.10 ± 0.48°C                  |
| 7   | B1D           | I             | Bruguiera sp.    | -                               |
| 8   | C4A           | IV            | Ceriops tagal    | 15.15 ± 0.56°C                  |
| 9   | C6C           | VI            | Ceriops tagal    | 9.80 ± 0.71°C                   |
| 10  | C7A           | VII           | Ceriops tagal    | 14.72 ± 2.24°C                  |
| 11  | C4B           | IV            | Ceriops tagal    | 15.43 ± 1.10°C                  |
| 12  | C6B           | VI            | Ceriops tagal    | 11.44 ± 0.22°C                  |
| 13  | C6A           | VI            | Ceriops tagal    | 10.84 ± 0.53°C                  |
| 14  | C2            | II            | Ceriops tagal    | 11.15 ± 0.36°C                  |
| 15  | C4C           | IV            | Ceriops tagal    | 16.72 ± 1.15°C                  |
| 16  | C6D           | VI            | Ceriops tagal    | -                               |
| 17  | C7D           | VII           | Ceriops tagal    | -                               |
| 18  | B1B           | I             | Bruguiera sp.    | 11.63 ± 0.44°C                  |
| 19  | B5B           | V             | Bruguiera sp.    | 12.78 ± 1.50°C                  |
| 20  | B1E           | I             | Bruguiera sp.    | -                               |
| 21  | B1F           | I             | Bruguiera sp.    | -                               |
| 22  | B1G           | I             | Bruguiera sp.    | -                               |
| 23  | C4E           | IV            | Ceriops tagal    | 12.20 ± 0.21°C                  |
| 24  | C4F           | IV            | Ceriops tagal    | -                               |
| 25  | C7B           | VII           | Ceriops tagal    | 15.77 ± 2.22°C                  |
| 26  | C4D           | IV            | Ceriops tagal    | 16.56 ± 0.53°C                  |
| 27  | C7C           | VII           | Ceriops tagal    | 12.98 ± 1.64°C                  |
| 28  | C7E           | VII           | Ceriops tagal    | -                               |

- : Not detected

Table 1. Fungal endophytes, sources, and their antibacterial activities
Interestingly, four isolates (C4E, C7B, C6C, and C4A) were active in inhibiting the growth of all pathogenic bacteria used in this study, and all of them were isolated from *Ceriops tagal* (Perr.) C.B.Rob; while there are 8 isolates are potential sources of broad spectrum antibiotics since they could inhibit the growth of both gram negative and gram positive bacteria. The most active endophyte, indicating by the highest diameter zones, against *E. coli*, *V. alginolyticus*, and *S. aureus* was isolate with code of B1C, C7B, and C6B, respectively. The isolates coded as C4E, C7B, C4D, and B1C were then characterized macroscopically and microscopically to identify their species and our finding showed that they were *Penicillium* sp. (C7B) and *Trichoderma* sp. (B1C, C4E, C4D) (Figure 3).

It is well known that *Penicillium* sp. is penicillin producing fungi, and many researchers have reported potential antimicrobial activity of *Trichoderma* sp. [17, 18]. Several reports highlighted isolation of different classes of chemicl constituents from endophytic fungi as part of the secondary metabolites products including steroids, triterpenoids, tetrahydroisosorbicillinol, bisorributenolide, agistatine B, parvisporicin, dihydrospororothiolide, pseudocitreoindole, stilbene, kojic acid, acetyl-kojic acid, p-hydroxybenzoic acid ,enodine, chloroemodine, and ergosterol-peroxide, hydroxy-phenylethyl alcohol, nicotinic acid, galactitol, naphthoquinone, phenylacetamide and many more [19, 20,21].
4. Conclusions
Endophytic fungi associated with Mangrove plants in Riau Province are potential sources of antimicrobial agents, especially *Ceriops tagal* derived endophytes. A number of 60.7% of isolated fungi were able to inhibit pathogenic bacteria tested, and four of them were active against both gram negative and gram positive bacteria.

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References
[1] Caniça M, Manageiro V, Abriouel H, Moran-Gilad J and Franz C M A P 2018 *Trends in Food Science & Technology* **84** 41-44
[2] Yao H, Sun X, He C, Maitra P, Li X and Guo L 2019 *Microbiome* **7**(57) 1–15.
[3] Rajamani T, Suryanarayanan T S, Murali T S and Thirunavukkarasu N 2018 *Fungal Ecology* **36** 109–116
[4] Narendran R and Kathiresan K 2016 *Biocatalysis and Agricultural Biotechnology* **6** 189–194
[5] Singh D K, Sharma V K, Kumar J, Mishra A, Verma S K, Sieber T N and Kharwar R N 2017 *Scientific Reports* **7**(1) 1–14
[6] Kumar V, Cheewangkoon R, Gentekaki E, Maharachchikumbura S S N, Brahmanage R S and Hyde K D 2019 *Phytotaxa* **393**(3) 251–262.
[7] Arobaya A dan Wanma 2006 *Warta Konservasi Lahan Basah* **14**(4) 4-5.
[8] Yan Z, Wen S, Ding M, Guo H, Huang C, Zhu X, Huang J, She Z and Long Y 2019 *Marine Drugs* **17** 1–12.
[9] Hyde K D, Xu J, Rapior S. et al 2019 *Fungal Diversity* **97** 1-81
[10] Ananda K and Sridhar K R 2002 *Canadian Journal of Microbiology* **878** 871–878.
[11] Lorenita M, Haryani Y, Puspita F and Trihartomo D 2013 *Journal of Agricultural Technology* **9** 565–570
[12] Wang L, Hu C and Shao L 2017 *International Journal of Nanomedicine* **12** 1227–1249
[13] Prestinati F, Pezzotti P and Pantosti A 2015 *Pathogens and Global Health* **109**(7) 309–318
[14] Wilson W R, Karchmer A W, Dajani A S, Taubert K A, Bayer A, Kaye D, Bisno AL, Ferriero P, Shulman S T and Durack D T 1995 *American Heart Association* **6**(274) 1706-13
[15] Schulz B and Boyle C 2006 *Microbial root endophytes* **9** 367
[16] Gouda S, Das G, Sen S K and Shin H 2016 *Frontiers in Microbiology* **7** 1–8.
[17] Handayani D, Rivai H, Mulyana R, Suharti N, Rasyid R and Hertiani T 2018 *Journal of Applied Pharmaceutical Science* **8**(2) 049–053
[18] Amatuzzi R F, Cardoso N, Poltronieri A S, Poitevin C G, Dalzoto P, Zawadeneak M A and Pimentel I C 2018 *Brazilian Journal of Biology* **78**(3) 429-435
[19] Li G H, Wang X B, Liu F F, Dang L Z, Li L, Yang Z S, Xin X and Zhang K Q 2010 Chemistry and Biodiversity 7 1790-5.

[20] Tao M H, Li D L, Zhang W M, Tan J W and Wei X Y 2011 Journal of Chinese Medicinal Materials 34 221-3.

[21] Zhao J, Mou Y, Shan T, Li Y, Zhou L, Wang M and Wang J 2010 Molecule 15 7961-7970.