Isolation And Antimicrobial Test Of Endophytic Bacteria From Pearl Grass (Hedyotis Corymbosa (L.) Lamk)

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Abstract: Endophytic bacteria are bacteria that live in healthy plants without causing disease and as alternative of antimicrobial compound producers. The endophytic bacteria improve the health and productivity of plants by suppressing pathogen and promote plant growth. Pearl grass (Hedyotis corymbosa (L.) Lamk) is a medicinal plant that has been used for years and has many benefits. The purposes of this study are to isolating and screening of endophytic bacteria from pearl grass against five pathogenic bacteria (Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Shigella disentriae and Pseudomonas aeruginosa). The macroscopic morphology observation was done to observed the morphology of endophyte bacteria colonies. The result obtained that total of endophyte bacteria isolate on pearl grass plant was 15 isolates, that were four isolates from leaves, five isolates from stems and 1 isolate from flower. Based on the screening results, nine isolates of endophytic bacteria have potential activity (characterized by the formation of inhibition zone) against antimicrobca. The inhibition zone may indicate that those isolates produce compounds that have antibacterial effects. Those isolates are A3, A7,A12,A13,B3.2, B7,B11, D9, and BU4. Four isolates (A7,A12,A13, and B7) of endophytic bacteria have potential activity again E.coli. Two isolates (A3 and D9) of endophytic bacteria have potential activity against St. aureus. Five (A12, A13, B7, B11, BU4) of endophytic bacteria have potential activity against Sa. typhimurium. Three (B3.2, B7 and B11) of endophytic bacteria have potential activity against Sh. disentriae. Two (A3, and B7) of endophytic bacteria have potential activity again P. aeruginosa. The most inhibition zone showed by B7, so it can be concluded that B7 is the most potential isolate as a novel source of antibacterial compound.

Index Terms: Antibacterial, Endophytic Bacteria, Pearl Grass.

1. INTRODUCTION

Endophytic bacteria are beneficial bacteria that grow in plants and can increase plant growth under normal and challenging conditions. They can benefit the host plant indirectly by improving plant health by targeting pests and pathogens with antibiotics, hydrolytic enzymes, nutritional restrictions, and by protecting plants. To provide this benefit, bacteria must colonize the plant’s endosphere after colonizing the rhizosphere. Colonization was achieved using battery properties which involved motility, attachment, degradation of plant polymers, and avoidance of plant defenses [1]. Endophytic bacteria colonize inner host tissues, sometimes in high numbers, without damaging the host or eliciting strong defense responses. Unlike endosymbionts they are not residing in living plant cells or surrounded by a membrane compartment [2]. In many cases, endophytic bacteria behave in a reciprocal relationship. The habit is the intercellular space of plants, large areas of interconnected space that contain high levels of carbohydrates, amino acids, and inorganic nutrients, which serve the purpose of supporting the growth of intercellular bacteria [3]. The diversity of endophytic invaders depends on several specific factors of bacteria, plants and the environment. Some endophytic bacteria can have a broad host range and can be used as bioinoculants in developing safe and sustainable farming systems, biotechnology and the health field [1]. Bacteria reported as endophytes include a significant range of both Gram-positive and -negative bacteria belonging to genera of Alpha-, Beta-, and Gammaproteobacteria, as well as Actinobacteria and Firmicutes [3]. Each plant has a secondary metabolite as an antibacterial which is produced by endophytic bacteria in a colony. Of the 30,000 plant species on earth have one or more endophytic bacteria and fungi that are useful as antibacterial and antifungal [4].

One example of medicinal plants is pearl grass (Hedyotis corymbosa (L.)). Hedyotis corymbosa (L.) Lam. (Rubiaceae) is a weedy annual herb, found mainly during the rainy season in fields throughout tropical East Asia, Indonesia, India, Sri Lanka, and the Philippines [5]. Identify the components of secondary metabolites pearl grass was conducted using Gas Chromatograph Mass Spectrometri (GCMS). GCMS results showed that 20 compounds derived from the group of flavonols, monoterpenes, triterpenes, cycloterpenes, sesquiterpenes, phenolics, organic acids, flavones. Compounds that identified were: catechol, camphene, limonene, myrcene, pinene, camphor, cineole, geraniol, citronellol, gallic acid, ascorbic acid, β Caryophyllene, β elemene, β farnesene, β-selinene, apigenin, kaempferol, luteolin, catechin, betulinic acid. Some of the important functions of compounds were antioxidant, antibacterial, antinflammatory, anticancer, antitumor, antileukemic, hepatoprotector, antiallergic, expectorant, hypoglycemia, hipocholesterolemic, antitussive, analgesic, chemoprotective agent [6]. The plants pearl grass oil extracts has shown good antibacterial and antifungal activity. The essential oil of Hedyotis corymbosa showed a zone of inhibition to about 19.3±1.1 mm for Escherichia coli for Staphylococcus aureus 18.1±1.5 mm for Pseudomonas aeruginosa as 19.2±1.3 mm for Streptococcus pneumonia as 17.7±1.7 mm for Candida albicans as 13.8±1.8 mm for Candida parapsilosis as 14.3±2.5 mm and for Candida tropicalis as 12.5±1.3 mm [7]. At present many new antibiotics are obtained from herbal extracts as antimicrobials because the use of chemical antibiotics is very...
expensive and can damage human health. So a lot of research is done about antibiotics from herbal plant extracts, however, the use of extracts from herbal plants is ineffective because it requires a large amount of plant parts to be extracted. Therefore, for productivity in producing new antibiotics, isolation and selection of endophytic microbes as antimicrobials from pearl grass plants is carried out. This study aims to obtain endophytic bacterial isolates from pearl grass and test the antimicrobial activity against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Shigella dysenteriae, Pseudomonas aeruginosa and Candida albicans.

2 MATERIAL AND METHOD

2.1 Sample Collection
Plant samples, including leaves, flowers, roots and steams, were collected from Tulang Bawang University (Latitude : 5°25'01.2"; Longitude : 105°16'04.0”). For bacterial isolation, each part of the plants was placed into a sterile plastic bag and transferred to the biology laboratory of Tulang Bawang University.

2.2 Isolation of Endophytic Bacteria
Endophytic fungi were isolated from healthy pearl grass taken from young leaves, roots, stems and flowers because they contain lots of organic acids and phenol compounds. These compounds prevent the development of pathogens. For isolation of endophytic bacteria washed leaves, roots, stems and flowers samples were washed with running tap water for 5 minutes. This sample was surface sterilized by immersing them sequentially in 70% ethanol for 1 minute, 1% sodium hypochloride (NaOCl) for 5 minute and 70% ethanol for 30 seconds and rinsed thoroughly with sterile distilled water for five times. The excess water was dried under laminar airflow chamber. The surface sterilized samples were then cut under aseptic conditions. Surface sterility test was performed for each sample to ensure elimination of surface microorganisms. Cut samples was then placed on isolation medium (15 gram of pearl grass powder, 15 gram of agar powder, 1000 mL aquades). The plates were incubated in incubator at 37 °C overnight. After that, it was observed and measured the inhibition zone formed using the calipers.

2.3 Test Microorganisms
Pathogenic strain, Gram-positive bacteria Staphylococcus aureus and Gram-negative Escherichia coli, Shigella dysenteriae, Salmonella typhimurium and Pseudomonas aeruginosa clinical isolates were used as test microorganism in this study. All pathogenic strains were ob-tained from Balai Penyidikan dan Pengujian Veteriner Regional III.

2.4 Assays of Antimicrobial Activity
Pure endophytic bacterial isolates were grown in Nutrient Broth (NB) medium and antimicrobial activity was determined against eight pathogenic bacteria (Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Shigella dysenteriae and Pseudomonas aeruginosa). One use of pathogenic bacteria were regenerated inside 5 mL of NB (Nutrient Broth) media and endophytic bacteria originating from stock slant media were inoculated to new NA media, then incubated at 37 °C for 24 hours. A total of 0.4 mL of pathogenic bacterial culture was poured into a petri dish and then added to 20 mL of liquid NA at ± 40 °C. Petri dishes were shaken so that pathogenic microbial suspensions were evenly homogenous. Endophytic bacterial isolates were spotted on media that already contain pathogens. The plates were incubated at 37 °C overnight. After that, it was observed and measured the inhibition zone formed using the calipers.

3 RESULT AND DISCUSSION
Based on the morphology characteristics of colony, we obtained fifteen isolates A3, A7, A12, A13, A14, B3.1, B7, B11, D8, D9, D10, D12, dan BU4 endophytes bacteria of pearl grass. Each pure isolate was tested further for the antimicrobial activities. The samples used in this study were roots, stems, leaves, and flowers of pearl grass. Endophytic bacteria that had been successfully grown and isolated were 15 isolates. The fifteen isolates, five of them were isolated from pearl grass roots, five other isolates from pearl grass stems, 4 isolates were isolated from pearl grass leaves and 1 isolate from pearl grass flowers. The number of endophytic bacterial populations that can be isolated in the living tissue parts of the leaves, stems and pearl grass roots are spread evenly where each tissue section contains 3-4 bacterial isolates. The results of this study indicate that endophytic bacteria can live in any tissue (leaves, stems, roots, flower) of pearl grass. Each isolate observed its colony shape. The results of observing the shape of the colony can be seen in Table 1. Figure 1 Figure 2 below. The results of the observation showed that the shape of bacterial isolate colonies had almost the same characteristics, namely: the shape of the colony in a round shape, the surface (elevation) was convex, the margins were flat, the color of the colony was white, had a negative gram and the shape of the isolate cell was shaped the bacil. Isolates A7, A12 and D9 have irregular shaped colony while other isolates are circular. B11 and D8 isolate forms are coccus, D12 isolate cells form staphylococcus, Bu4 forms streptobacilli. Other isolates which have different characteristics were isolates B3.1 and A13. The B13 isolate colony's color is pink while the A13 isolate is yellow. However, the colors of the other isolate colonies were almost all white, from shiny clear white to creamy dull white.

Table 1.

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate Code</th>
<th>Colony Size</th>
<th>Colony colour</th>
<th>ColonyShape</th>
<th>ColonyElevation</th>
<th>ColonyMargin</th>
<th>Gram</th>
<th>Cell shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A3</td>
<td>large</td>
<td>White</td>
<td>circular</td>
<td>Raised</td>
<td>entire</td>
<td>negatif</td>
<td>bacilli</td>
</tr>
<tr>
<td>2</td>
<td>A7</td>
<td>large</td>
<td>White</td>
<td>irregular</td>
<td>Flate, raised margin</td>
<td>irregular</td>
<td>negatif</td>
<td>bacilli</td>
</tr>
<tr>
<td>3</td>
<td>A12</td>
<td>large</td>
<td>White</td>
<td>irregular</td>
<td>Flate, raised margin</td>
<td>irregular</td>
<td>negatif</td>
<td>bacilli</td>
</tr>
<tr>
<td>4</td>
<td>A13</td>
<td>moderate</td>
<td>Yellow</td>
<td>circular</td>
<td>raised</td>
<td>entire</td>
<td>negatif</td>
<td>bacilli</td>
</tr>
<tr>
<td>5</td>
<td>A14</td>
<td>moderate</td>
<td>White</td>
<td>circular</td>
<td>convex</td>
<td>entire</td>
<td>negatif</td>
<td>bacilli</td>
</tr>
</tbody>
</table>
Endophytic bacteria can enter the plant tissue generally through roots, but parts of plants exposed to direct air such as flowers, stems, leaves (through stomata) and cotyledons, can also be a pathway for endophytic bacteria. Endophytic bacteria that have entered the plant can grow only at a certain point or spread throughout the plant. These microorganisms can live in vascular vessels or in the intercellular space [8], roots, stems, leaves and fruit [3,9]. The interaction of endophytic bacteria and plants is a form of symbiosis. Symbiosis between plants and endophytic bacteria is neutral, mutualism or commensalism [3]. The symbiosis of mutualism between endophytic bacteria and plants, in this case endophytic bacteria get nutrients from plant metabolism and protect plants against pathogens, while plants get derivatives of nutrients and active compounds needed during their lives [9]. The results of this study showed that endophytic bacteria were found in the roots, stems, leaves and flowers of pearl grass, although in the flower section only 1 isolate was found. States that the number of endophytic bacteria in plants cannot be determined with certainty, but these bacteria can be detected by isolating the agar media [3]. The agar media used to isolate endophytic bacteria in this study was endophytic bacterial isolation media. This media is a media that contains pearl grass powder. Endophytic bacteria can live on these isolation media because of the complex nature of the media required by endophytic bacteria and have a composition similar to conditions in plants. Endophytic bacteria from pearl grass begin to show growth after pieces of plant parts are inoculated in this medium for ± 48 hours (2 days). States that the selection time for incubation for at least 2 days aims to ensure that the growing bacteria are endophytic bacteria, not a bacterial contaminant. In addition, the addition of nystatin (antifungal) to the media also aims to optimize the results of isolation [8, 9, 10, 11]. Endophytic bacterial colonies that were successfully isolated from pearl grass showed diversity, both in terms of color, shape and speed of growth. This is in accordance, endophytic bacteria in one host plant generally consists of several genera and species. The diversity of endophytic bacteria in a plant is also influenced by plant growth conditions, especially soil conditions. In some cases, plants of the same type or species have endophytic bacteria which are not always the same. In some plants there are specific and specific endophytic bacteria inhabiting these plants [12]. The results of screening of endophytic bacterial isolates from pearl grass plants against pathogenic bacteria are shown by the formation of inhibitory zones in the form of clear areas around endophytic bacterial colonies (Table 2). A total of 15 endophytic bacterial isolates were tested against 5 pathogenic bacteria, namely Staphylococcus aureus, Escherichia coli, Shigella dysentriae, Pseudomonas aeruginosa and Salmonella typhymurium as well as 1 yeast pathogen Candida albicans. A total of 12 endophytic bacterial isolates, namely A3, A7, A12, A13, B3.2, B7, B11, D8, D9, D10, D12 and BU4 showed the formation of inhibitory zones against one or several pathogenic bacteria. However, it does not show the formation of inhibitory zones against C. albicans. While isolates A14, B3.1 and B8 showed no inhibition and did not form clear areas like other isolates.
Based on the test results, 12 endophytic bacterial isolates from pearl grass showed inhibition of pathogenic bacteria. The isolates were from roots (4 isolates), stems (3 isolates), leaves (4 isolates), and flowers (1 isolate). The formation of clear areas around the colonies of endophytic bacterial isolates indicates the possibility of antibacterial compounds capable of killing or at least inhibiting the growth of pathogenic bacteria. Conducted similar tests with endophytic bacteria from Indonesian medicinal plants. The endophytic bacteria isolated showed inhibitory activity against pathogenic bacteria marked by the formation of clear areas around the endophytic bacterial colony [9]. Host plants are habitat for endophytic bacteria because of symbiotic mutualism when endophytic bacteria obtain nutrients which are vitamins, polysaccharides and fatty acids from their host plants. On the other hand, bacteria produce several products such as amino acids, antibiotics, and toxins that are beneficial for growth, metabolism, and increase the chemical resistance of host plants [13]. Infections due to pathogenic bacteria make chemical resistance that has self-protection for the host plant becomes necessary. Thus, antibacterial bioactive compounds produced by endophytic bacteria will be influenced by the condition of the host plant. Antibacteria produced by endophytic bacterial isolates in pearl grass in this study are thought to have different mechanism of action against resistant bacteria such as Gram-positive and Gram-negative bacteria. However, the mechanism involved in the activity against bacteria as a synergy of several bioactive compounds or one of the compounds has not been determined because the extraction of the bioactive compound has not been carried out to find out the bioactive compounds contained in the antibacterial produced by the bacteria. Discovered a new antibiotic called teixobactin from uncultured bacteria using in situ methods, and can be used to treat MRSA and Mycobacterium tuberculosis infections without creating resistance. Based on the 16S rRNA gene, the microorganism is Eleftheria terrae which belongs to the β-proteobacteria class. The mechanism of teixobactin regarding activity against resistant bacteria is by inhibiting cell wall synthesis by binding to lipids II (peptidoglycan precursors) and Lipid III (cell walls of precursor teichoic acid) from bacteria [14].

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### REFERENCES


Fig. 1. Magnetization as a function of applied field. Note that “Fig.” is abbreviated. There is a period after the figure number, followed by one space. It is good practice to briefly explain the significance of the figure in the caption.