Analysis of 16S rRNA Sequence of Endophytic Bacteria Isolate BS1 from *Piper betle* [L.] Stem

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***Abstract*—** *Piper betle* [L.] is a medicinal plant that has long been used by Indonesian people as an anti vaginal or oral candidiasis. Utilization of bacterial endophytes from medicinal plants is a new way to get the antibacterial compounds without having to directly extract from the medicinal plants. Endophytic bacteria (BS1 isolate) of the plant *Piper betle* [L.] has been isolated and demonstrated antibacterial ability against several pathogenic bacteria, such as : *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*, with the greatest inhibition against *Staphylococcus aureus*. The result of 16S rRNA analysis using BLAST showed that BS1 isolate was related to *Pseudomonas* sp. with 98% identity.

***Keywords*—**Endophytic bacteria, *Piper betle* [L.], *Pseudomonas* sp., 16S rRNA

I. Introduction

PIPER betle [L.] (Green Betel) is a medicinal plant that has been used in Indonesia for many years. The use of *Piper betle* [L.] leaves has long been known to prevent and cure cough, reduce bad breath and prevent infections caused by fungi and bacteria [1]. The study of *Piper betle* [L.] in the health sector have been carried out. *In vitro* assay of *Piper betle* [L.] leaves that extracted with sterile distilled water showed inhibition activity against *Streptococcus mutans* [2]. Methanol extracts of *Piper betle* [L.] leaves also has antimalarial activity against *Plasmodium berghei*. According to the study, those effects are associated with a high antioxidant content in *Piper betle* [L.] leaves extract [3]. *Piper betle* [L.] also has inhibition activity against xanthine oxidase [4] and also antifungal against Candida and Aspergillus (*in vitro* assays) [5].

According to those studies, *Piper betle* [L.] has been proven as multi-benefits medicinal plant. However, to obtain the benefits, it requires big amount of the plant and the extraction process is also take longer time. Based on this reason, research of *Piper betle* [L.] is directed to endophytic bacteria. Endophytic bacteria are bacteria that live inside the host plant tissues without causing disease symptoms [6]. Endophytic bacteria that have entered into the plants can grow only at one particular point or spread throughout the plant. These microorganisms can live in the vascular vessels or in the intercellular spaces [7], roots, stems, leaves and fruit [8,9].

Some endophytic bacteria have ability to produce potential products: *Streptomyces* sp. NRRL 30566 from *Grevillea pteridifolia* produce kakadumycin, an antibiotic compound that solved in methylene chloride [10]; *Pseudomonas viridiflava*, the plant-associated bacteria, produce unique antimycotics named ecomycins [11]; endophytic bacteria isolated from *Artemisia annua*, *Bacillus polymixa*, can produce antimalarial artemisinin in the synthetic liquid medium [12].

Endophytic bacteria isolate BS1 from *Piper betle* [L.] is expected to produce similar compounds as the host plant or at least produce an active antibiotic compound. The aim of this study is to analyze endophytic bacteria isolate BS1 from *Piper betle* [L.] that able to inhibit pathogenic bacteria (based on previous study) using 16S rRNA marker.

II. Procedure For Analysis Endophytic Bacteria Bs1 Isolate

A. DNA Template Preparation of BS1 Isolate

Colonies of BS1 isolate suspended in 0.5 mL of sterilizes saline then centrifuged at 10000 rpm for 10 min. The pellet is resuspended in 0.5 mL of InstaGene Matrix (Bio-Rad, USA). Incubated 56°C for 30 min and then heated 100°C for 10 min. After heating, supernatant can be use for Polymerase Chain Reaction (PCR).

B. PCR and Sequencing for 16S rRNA Gene

The primers used in this study are 27F and 1492R. DNA template volume added as much as 1 mL in 20 mL of PCR total reaction. The PCR reaction perform at 94°C for 45 second, 55°C for 60 second, and 72°C for 60 second with 35 cycles. Unincorporated PCR primers and dNTPs removed from PCR products by using Montage PCR Clean up kit (Millipore). The PCR result then sequenced and analyze using

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BLAST. The sequence data compared with 16S rRNA in GenBank (NCBI).

III. Result And Discussion

The endophytic bacteria isolate BS1 from *Piper betle* [L.] stem used in this study obtained from previous research. Isolate BS1 has showed inhibition zone against *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*, with the biggest inhibition zone showed toward *Staphylococcus aureus* [13]. Based on the analysis of 16S rRNA sequences using the BLAST program, BS1 isolate was related to *Pseudomonas* sp. with 98% identity. The result indicates that this bacteria is likely to be a potential novel species.

Analysis of 16S rRNA genes was used because this gene have composed of variable and conserved regions. Variable regions used to detect distant relationship, because this area is different in all bacteria. While the conserved regions used to detect relationship that are close to this area because of relatively similar to the bacteria in same genus. Universal primers for 16S rRNA gene in bacteria can be used to analyze the relationship between all kinds of bacteria. In general, comparison of the sequence of bases in 16S rRNA gene can distinguish organisms, especially bacteria, to the genus level [14].

Pseudomonas is a type of bacteria that widely spread and some are pathogenic, especially in animals. *Pseudomonas* has been known to interact with plants or also called plant-associated bacteria. One study about endophytic bacteria is *Pseudomonas viridiflava*. This endophytic bacteria isolated from several grasses and capable of producing ecomycin, an antimycotic (antifungal) compound [11].

Pseudomonas isolated from other place, such as from marine or contaminated soil can produce specific compound such as biodegradation compound or anticancer agent [15,16]. *Pseudomonas* isolated from seaweed also can produce a specific peptide [17]. Thus, isolate BS1 which defined as *Pseudomonas* sp. has a possibility to produce specific compounds, in this case expected to produce antibacterial compounds. However, further research is needed to determine the type of compound and its mechanism in inhibiting the growth of bacteria.

IV. Conclusion

Based on 16S rRNA analysis using BLAST, BS1 isolate from *Piper betle* L. was related to *Pseudomonas* sp. with 98% identity.

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