

# Analysis of 16S rRNA Sequence of Endophytic Bacteria Isolate DM6 from *Coleus scutellariodes* [L.] Benth. Leaves

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**Abstract**—*Coleus scutellariodes* [L.] Benth. is a medicinal plant from Indonesia. It's leaves are commonly used to treat diarrhea, asthma, bronchitis, cough, launched the menstrual cycle, neutralize toxins, appetite enhancer, accelerates the maturation absces, and can kill intestinal worms. Utilization of bioactive compounds from plant's extract is not efficient because we need a large biomass. Alternative that can be done is using endophytic bacteria which several genera are known to produce antibiotics, anticancer compounds, antifungal, antiviral, and could act as an insecticidal agent. Based on previous research, endophytic bacteria isolate DM6 from *C. scutellariodes* [L.] Benth. leaves had the ability to inhibit the growth of several pathogenic bacteria, such as: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Salmonella enteritidis* which the greater inhibitory activity to Gram-positive bacteria. The result of 16S rRNA sequence analysis using BLAST showed DM6 isolate was related to *Brevibacillus parabrevis* strain HDYM-15 with 96% identity.

**Keywords**—*Brevibacillus parabrevis*, *Coleus scutellariodes* [L.] Benth., endophytiv bacteria, 16S rRNA

## I. INTRODUCTION

ENDOPHYTIC bacteria is a symbiotic microorganism that live inside plant tissues and not show negative effects on their host plant [1]. Endophytic bacteria can colonize in a certain section or scattered in all parts of the plant and endophytes can live in the vascular vessels or in the intercellular space [2]. Endophytic bacteria come from the environment and enter to the plant via stomata, lentikula, or root [3]. Endophytic bacteria are ubiquitous in most plant species [4] and can be isolated by surface-disinfected or extracted plant tissues [5].

*Coleus scutellariodes* [L.] Benth. is one of herbal plant in Indonesia which traditionally used to treat various illnesses e.g. diarrhea because its contain antidiarrhea and antibacterial compounds [6]. Methanol extract of the leaves

miana able to inhibit the growth of *Salmonella enteritidis* and *Staphylococcus aureus* [7]. Ethanol extract of *C. scutellariodes* [L.] Benth. leaves at concentrations of 10% and 20% has antibacterial power against *S. aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella paratyphosa* [8].

Bioactive compounds which contained in herbal plants are believe to be influenced by the coexistence between plant and microbes. The microbes have reported can produce secondary metabolites that are beneficial as antitumor, antifungal, antibacterial, and can produce compounds to regulate plant growth [9]. Several genera of endophytic bacteria are known to produce natural products such as antibiotics, anticancer compounds, antifungal, antiviral, and could act as insecticidal agents, for example: *Streptomyces griseus* which isolated from *Kandelia candel* were able to produce  $\rho$ -Aminoacetophenonic acids as antimicrobial [10] and *Pseudomonas viridiflava* from grass which able to produce ecomycins b and c as antimicrobial compounds [11].

Molecular characterization is an important step to identify endophytic bacteria isolate. One of the ways to identify certain types of bacteria is using 16S rRNA gene. 16S rRNA is a small subunit ribosomal in all prokaryotic organisms that can be used to identify bacteria [12]. This study is to identify DM6 isolate from *C. scutellariodes* [L.] Benth. by using 16S rRNA sequence analysis.

## II. PROCEDURE FOR 16S rRNA SEQUENCE ANALYSIS OF ENDOPHYTIC BACTERIA ISOLATE DM6

### A. Preparation of Template DNA

Colonies are picked up with a sterilized toothpick, and suspended in 0.5 mL of sterilizes saline in a 1.5 mL centrifuge tube, then centrifuged at 10,000 rpm for 10 min. After removal of supernatant, 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 PCR

### B. DNA Amplification

Previously isolated DNA amplification performed using the PCR machine. Primers used were 27F (5'-AGA GTT TGA

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TCM TGG CTC AG -3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T -3'). 1  $\mu$ L of template DNA was added in 20  $\mu$ L of PCR reaction solution. Amplification was performed with 35 cycles. Denaturation conditions 94 °C for 45 sec, annealing 55 °C for 60 seconds, elongation of 72 °C for 60 seconds.

#### C. Purification of PCR Product

PCR primers and dNTPs from PCR products which unincorporated are removed by using Montage PCR Clean up kit (Millipore).

#### D. Sequencing results of DNA Amplification

PCR products were subsequently sequencing and the results are analyzed using BLAST to identify the species of endophytic bacteria.

### III. RESULT AND DISCUSSION

Identification of bacteria isolates was performed using molecular approaches based on 16S rRNA sequence. The use of 16S rRNA sequence analysis because its represent all phylogenetic information and more practical [13]. 16S rRNA gene found in all prokaryotic organisms, and consist of conservative and varied sequences that can be used to identify bacteria through differences and variations of the base pair [13]. Identification of the species of an organism determined from the percentage of sequence similarity with the 16S rRNA sequences which available in GenBank by  $\geq 99\%$  while for genus identification is  $\geq 97\%$  and to identify new genus determined by the percentage of similarity is  $< 97\%$  [14].

In previous study, endophytic bacteria isolate DM6 from *C. scutellariodes* [L.] Benth. were able to inhibit pathogenic bacteria, such as: *Bacillus cereus*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Escherichia coli* [15,16]. 16S rRNA sequence analysis using BLAST showed DM6 isolate was related to *Brevibacillus parabrevis* strain HDYM-15 with 96% identity. The result indicates that this bacterium is likely to be a potential novel species.

*Bacillus* is bacterial genera which ubiquitous in soil and several species can produce an antibiotics with different chemical structure [17]. Antibiotics compounds which produced by *Bacillus* mainly have a peptidic nature and the majority of them are produced by *Bacillus subtilis* and *Bacillus brevis* [18]. *Brevibacillus parabrevis* (*Bacillus brevis*) is a Gram-positive bacteria which able to produce a variety of oligo-peptide antibiotics and lipopeptide. *B. parabrevis* known to synthesize two kinds of antibiotic peptides, cyclopeptide tyrocidine and linear gramicidin [19].

These antibiotics which produced by *Bacillus* are mainly effective against Gram-positive bacteria [17]. This is related with the result of our preliminary screening that inhibitory activity of DM6 isolate is greater to Gram-positive bacteria than Gram-negative bacteria [15, 16]. This result give a positive signal that DM6 isolate or *Brevibacillus parabrevis* strain HDYM-15 can produce an antibiotics compounds that still need to be further investigation.

### IV. CONCLUSION

16S rRNA sequence analysis using BLAST showed DM6 isolate from *C. scutellariodes* [L.] Benth. was related to *Brevibacillus parabrevis* strain HDYM-15 with 96% identity.

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