

Antibacterial Activity of Endophytic ER-2 Bacterial Isolates from Plant *Cotylelobium melanoxylo*

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Abstract. Endophytic microbes are microorganisms that colonize the parts of plant organs and interact with host plant without causing any symptoms or diseases in the host plant. Endophytic microbial colonization is found in various types of plant organs, namely: roots, stems, leaves and seeds. This study is focused on endophytic bacteria mostly conducted intensively on wild plants or forests in the tropical ecosystems. Various trees or tropical forest plants have potentials as traditional medicine and in cooperation with the bacteria. The *C. melanoxylo* has been utilized by local community as antidiarrheal medicine because of the content of active antibacterial compounds. The presence of endophytic bacteria in tissues of the stem of *C. melanoxylo* was observed the bacteria were directly isolated from the tissues by planting pour plate on Tryptic Soya Agar (TSA) medium. The antibacterial activity of endophytic bacteria isolates was examined using a dual cultural assay against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923 as a model bacterial. Morphological, physiological, and biochemical characters were identified based on the ability of the isolates to grow. The endophytic bacteria isolate of strain ER-2 was inhibitat the growth of *E. coli* ATCC 35218 and *S. aureus* ATCC 25923.

Keywords: *Cotylelobium melanoxylo*, bacteria, endophytes, antibacterial

1 Introduction

The search for secondary metabolites from endophytes in various plants is very important to obtain antimicrobial compounds (Tenguria et al., 2011). During this time, natural secondary metabolites use more plants, especially medicinal plants to extract their active compounds. Most secondary metabolites are taken from parent plants (Radji, 2005). To get the compounds that are expected to require a lot of plant biomass. Plants used sometimes grow in tropical rainforest ecosystems. If this is done continuously it will damage the forest ecosystem without any effort to preserve it. Besides that, the plants used are sometimes very difficult to grow by vegetative propagation and require decades to be harvested. The discovery of secondary metabolites that have the potential to produce antimicrobials from endophytic fungi can reduce dependence on plants. The use of endophytic bacteria as a producer of secondary metabolites which have the potential as raw materials for drugs can be produced in large quantities with a short period of time.

Endophytic microbes (bacteria or fungi) can produce various active compounds which are useful as antimicrobial materials (Verma et al., 2009). Secondary metabolite compounds produced by endophytic microbes include alkaloids, flavonoids, phenols, peptides, quinones,

steroids, terpenoids, poliketone, benzopyranon, xanthon, tetralon and others (Tan & Zou, 2001; Tenguria et al., 2011). These compounds can play a role in producing anticancer, antimicrobial, antidiabetic, antimalarial, antiviral and antioxidant compounds (Radji, 2005; Pimentel et al., 2010; and Tenguria et al., 2011). The existence of various problems in the health sector such as types of cancer, the presence of bacterial resistance to drugs, the spread of diseases caused by protozoa (Strobel, 2003).

The search for sources of active compounds from natural ingredients needs to be continued to reduce dependence on raw materials for medicines from chemicals. Samples from various sources, including bacteria from different places, are tested for their potential ability to produce antimicrobials. The discovery of endophytic fungi that can produce antimicrobials can be used for natural medicine raw materials. Antimicrobials produced from endophytic fungi are obtained through a series of screening processes.

2 Materials and methods

2.1 Endofit endophytic bacterial activity test

Testing the activity of endophytic bacteria is carried out with disc agar technique. *E. coli* pure culture aged 18-24 was taken 1 ml then put into a petri dish (Idramsa et al. 2015). Then 10 ml of Nutrient Agar (NA) was poured into a petri dish with pour plate technique and then left to cool. Then the endophytic bacteria were taken by cutting the medium with a diameter of 6 mm containing 48 hours of endophytic bacteria colonies inserted into a petri dish that had been cultured with *E. coli* bacteria and incubated for 3 days at room temperature. Furthermore, it was observed that the inhibition zone was formed by measuring the diameter of the inhibitory zone using calipers.

2.2 Characterization of endophytic bacteria

Morphological observations of endophytic bacterial colonies aim to recognize the forms of growth, colonies and characteristics of bacterial colonies in various forms of media. The observation of the morphological characters of endophytic bacteria was first cultured in a pure manner which was inoculated aseptically on a medium to be tilted, to stand upright, a liquid nutrient medium and a medium in the cup. The medium is incubated at room temperature for 24 - 48 hours. Pure cultures of endophytic bacteria are tested biochemically based on their ability to grow and their activity on media containing carbohydrates. The characters observed follow characterization according to Holt et al. (1994).

3 Results and discussion

3.1 Endophytic bacterial antagonist test

Based on the results of the selection, ER-2 isolates was selected which showed activity against Gram-positive and Gram-negative. Some endophytic bacteria can inhibit or reduce the growth of other microbes in a medium. The effect of this inhibition is due to the presence of compounds produced in the form of toxins, antimicrobial components, bacteriocins, antibiotics

and so on. Antibacterial activity test meant that between one bacterium and another bacterium the competition was marked by the formation of a clear zone of images (2). Endophytic bacteria ER-2 strains are able to inhibit the growth of gram-negative E.coli pathogenic bacteria (ATCC 35218) and gram-positive S. aureus bacteria (ATCC 25923).

The difference in diameter of the inhibitory zone is influenced by the difference in the ability of the diffusion of the active compound into the agar and the difference in bacterial sensitivity. Antimicrobials that are effective against a small number of microorganisms or single taxonomic groups have narrow spectrum activity (Black & Black, 2008). The same statement was also stated by Pal & Paul (2013) isolates that showed antibacterial activity against the growth of Gram-positive and Gram-negative test bacteria were said to have a wide spectrum.



Fig 1. Endophytic bacterial antagonist test (a) bacteria patogen E.coli dan (b) S. aureus (B)

3.2 Characterization of endophytic bacteria

Endophytic bacteria colonies grow 4-7 days on TSA media, gram-positive round shape (0.5-3 mm), colorless, non motile, and rough surface. Morphological observations on the colonies, at the beginning of the growth of a single round white colony, then the colony after 7 days of incubation of the colony diameter widened. Colony in the form of a filament, height increases, edges are filamentous. The surface of the colony is not shiny and translucent, non-pigmented and harsh surface texture. The observation results of morphological, physiological and biochemical characters of ER-2 endophytic bacteria isolate in Table 1.

Characterization physiology include acid production (fructose, lactose and sucrose) negative (+), grew at 25-3 temperature range 0°C and optimum growth at a temperature of 25 °C, is growing at a pH range of 5-10, were able to grow on NaCl salinity levels (%) 2.5-15% and optimum growth at salinity levels of 2.5-10%. According to Willey et al. (2008) stated that each bacterium has a pH range to grow and optimum pH growth. In general, the optimum pH of growth of most bacteria is in the range between pH 6.5 and 7.5 (Pelcaar et al ., 2009). Based on the test results showed that isolates of endophytic bacteria ER-2 strain can grow tolerant to 15% NaCl levels. The optimum growth was at 2.5-10.0 (w/v).

Table 1. Phenetic characters of bacteria endophytic ER-2

Characteristics	Results ER-2
Colony	
Form	filamentous
Elevation	raised
Margin	filamentous
Appear	dull
Optical proverty	opaque
Pigmentation	white

Texture	rough
Growth at temperature	
4 °C	-
10 °C	-
25 °C	++
30 °C	+
35 °C	-
40 °C	-
55 °C	-
Growth at pH	
5	++
6	+++
7	++
8	++
9	++
10	++
Growth at NaCl (%)	
2.5	+++
5.0	+++
7.5	+++
10.0	+++
12.5	++
15.0	++
20.0	-
22.0	-

4 Conclusions

Based on the results of the study it can be concluded that the isolates of ER-2 endophytic bacteria from plant stems of *C. melanoxydon* had antibacterial activity against *E. coli* and *S. aureus* bacteria with medium resistance categories.

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