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Biochemical identification of endophytic bacteria isolates from medicinal plant kayu jawa *lannea coromandelica* (houtt.) merr

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ABSTRACT

Medicinal plants and their endophytes are a source of valuable bioactive compounds and their secondary metabolites which contribute to more than 80% of natural medicines available in the market. A plant that has long been used as a traditional medicinal herb for healing is the Kayu Jawa (*Lannea coromandelica* (Houtt) Merr). This study aimed to characterization and identification of endophytic bacterial isolates isolated from medicinal plants Kayu Jawa (L. *coromandelica* (Houtt.) Merr). This research is a laboratory experimental research. In total, 8 isolates were selected as representatives of the bacterial community living in the tissues of Kayu Jawa (L. *coromandelica* (Houtt.) Merr). The microscopic observation was performed by Gram staining. Biochemical identification was conducted using Vitek 2. The results showed that *Bacillus* was the dominant genus in this study followed by *Staphylococcus* and *Pantoea*. Based on the research that has been done, it can be concluded that the endophytic bacteria isolated from Kayu Jawa *Lannea coromandelica* (Houtt) Merr, six isolates belonging to *Bacillus* and another two belonging to *Staphylococcus* and *Pantoea* genera. However, for the exploration of this prospective application, it is very important to do a systematic study of the biological and distribution of endophytic bacteria in the host.

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INTRODUCTION

Infectious and parasitic diseases account for about half of all deaths worldwide (Menpara & Chanda, 2013). Medicinal plants and their endophytes are a source of valuable bioactive compounds and their secondary metabolites which contribute to more than 80% of natural medicines available in the market (Singh & Dubey, 2015). Endophytic microorganisms are

storehouses of new secondary metabolites that can serve as excellent sources of drugs for antiarthritis, antimicrobial, anticancer, antidiabetic, anti-insect, and immunosuppressant. currently, only a few plants have been investigated for their diversity and potential endophytes to produce bioactive secondary metabolites. The discovery of new antimicrobial secondary metabolites and bioactive compounds from various types of endophytic microorganisms is an important alternative to overcome the increasing levels of drug resistance to various pathogenic microorganisms (Gouda, Das, Sen, Shin, & Patra, 2016).

Endophytes are reported to produce a number of bioactive metabolites in one plant or microbes which act as excellent medicinal sources for the treatment of various diseases and have potential applications in agriculture, medicine, food and the cosmetic industry (Gouda et al., 2016). These secondary metabolites are categorized into various functional groups, alkaloids, benzopyran, chinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones, xanthones, and many more. A number of bioactive compounds, such as camptothecin, diosgenin, hypericin, paclitaxel, podophyllotoxin, and vinblastin, which have been produced commercially, are produced from various endophytic fungi, each of which has been cultivated for the pharmaceutical industry (J. Zhao, Shan, Mou, & Zhou, 2011).

A plant that has long been used as a traditional medicinal herb for healing is the Kayu Jawa (*Lannea coromandelica* (Houtt) Merr). This plant is easily found in Indonesia and is often used as a wound medicine by pounding the bark and then attaching it to the wound. This plant can also be used as cough medicine, ulcer medicine and appetite enhancer by drinking boiled water from the leaves. In addition, this plant has several properties and benefits, including helping to heal sprains, bruises, heart disease, dysentery and mouth sores (Rao, Einstein, & Das, 2014). The results of the study by Majumder (2013) stated that the methanol extract of L. *coromandelica* (Houtt.) Merr stem bark also has antibacterial activity. Babu & Kumar (2013), reported that the results of phytochemical screening of the chloroform extract of L. *coromandelica* (Houtt.) Merr contained alkaloid, tannin and steroid chemical compounds. Ethanol extract and water-alcohol combination of L. *coromandelica* (Houtt.) Merr bark are also reported to show hepatoprotective potential due to the presence of dihydroflavonol compounds in the bark. The ethanol extract of L. *coromandelica* (Houtt.) Merr bark has antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*(Rahmadani, 2015).

However, very few studies that utilize these symbiotic organisms and groups of bioactive metabolites. For the past few decades, it has been proved that the discovery of new bioactive compounds decreased. While plants are widely explored for the discovery of new chemical compounds for various therapeutic purposes, endophytic microorganisms play an important role as sources of bioactive compounds, with potential uses in the health sector and in drug discovery. Studies of this kind can improve understanding of endophytes and address the need for new and beneficial compounds needed to combat various pathogens associated with human health.Previous research has obtained endophytic bacterial isolates from the roots, stems and leaves of the Kayu Jawa *Lannea coromandelica* (Houtt.) Merr and the initial identification of endophytes bacterial isolates has been completed and obtained 12 endophytes bacterial isolates that have the potential to inhibit the growth of pathogenic bacteria (Astuty, Syam, & Sari, 2019) and will be continued with biochemical identification of endophytes bacterial isolates that were successfully isolated which is the aim of this study.

RESEARCH METHODS

Research Design

This research is a laboratory experimental research. Scientific literature studies, in this case a review of publications of previous research results, were carried out to strengthen the basic principles, concept formulations and technologies to be used in this research.

Population and Samples

Endophyte bacterial isolates in this study were obtained from previous studies(Astuty et al., 2019). In total, 8 isolates were selected as representatives of the bacterial community living in the tissues of Kayu Jawa (L. *coromandelica* (Houtt.) Merr).

Procedures

Endohyte isolates preparation

Eight endophyte isolates selected were subcultured on Mannitol Salt Agar (MSA) medium and incubate accordingly. Mannitol salt agar (MSA) has been used as a selective medium and was found to be valuable for use with specific specimens.

Figure 1. Endophyte isolates on Mannitol Salt Agar (MSA)

Mannitol salt agar (MSA) media was used to identify the isolates growing on high salt concentrations, and a positive test consisted of a color change from red to yellow, indicating a pH change to acidic. All isolates showed positive result.

Biochemical identification

Aseptically transfer 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0)into a clear plastic (polystyrene) test tube (12 mm x 75 mm).Use a sterile stick or swab to transfer a sufficient number of morphologically similar coloniesto the saline tube prepared in step 2. Prepare a homogenous organism suspension with adensity equivalent to a McFarland No. 0.50 to 0.63 using a calibrated VITEK®2DensiCHEK™ Plus (age of suspension must not exceed 30 minutes before inoculating card). Place the suspension tube and GP card in the cassette.The cassette containing the bacterial suspension and card is transferred to the vacuum chamber station automatically. The bacterial suspension is transferred to the wells by the device. The transfer tube is cut automatically. The card is transferred to the incubator chamber after 15 minutes, after incubation it will be analyzed automatically

Data Analysis

Data analysis was carried out descriptively by microscopic observation and identification of the isolates. The collected data were tagged and presented in tabular form...

RESULTS

In this study,8 isolates were selected as representatives. Although it is known that the isolates come from different parts of the plant, based on the results of Gram staining, the 8 endophyte isolates are Gram-positive bacteria. (Table 1)

Table 1. Results of Gram staining of endophyte isolates

Isolate code	Gram staining
KJD1	Positive
KJD3	Positive
KJB2	Positive
KJB3	Positive
KJB4	Positive
KJA1	Positive
KJA2	Positive
KJA3	Positive

Based on biochemical observations made using Vitek 2-compact BIOMERIEUX, results showed that *Bacillus* was the dominant genus followed by *Staphylococcus* and *Pantoea*.

Table 2. Biochemical identification of endophyte isolates using Vitek 2

Isolate code	Identification result	Probability	Level of confidence
KJD1	*Staphylococcus lentus*	95%	Very good identification
KJD3	*Bacillus pumilus*	93%	Very good identification
KJB2	*Bacillus pumilus*	93%	Very good identification
KJB3	*Bacillus licheniformis*	86%	Acceptable identification
KJB4	*Bacillus cereus*	91%	Good identification
KJA1	*Bacillus pumilus*	93%	Very good identification
KJA2	*Bacillus cereus*	91%	Good identification
KJA3	*Pantoea* sp	98%	Excellent identification

DISCUSSION

Endophyte associated with plants in various forms, including bacteria (actinomycetes or mycoplasma) or fungi that colonize in the plant tissue. More than 200 genera from 16 phyla of bacterial species have been reported to be endophytic and most of the species belong to the phylum *Actinobacteria*, *Proteobacteria*, and *Firmicutes*(Golinska et al., 2015). The diversity of endophytic bacteria ranges from gram-positive bacteria and gram-negative bacteria, such as *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Bacillus*, *Brevibacterium*, *Microbacterium*, *Pseudomonas*, *Xanthomonas*, and others (Sun, He, Xiao, Ye, & Tian, 2013). The endophytic bacteria varied in nature and are known to produce various metabolites.

In this study, the identification of these isolates by the molecular method, the VITEK® 2 method. The molecular method is accurate and it brings many benefits and improvements in the case of bacteria that are difficult to grow. Previous studies have shown that the VITEK® 2 system could give reliable identification and susceptibility results with pure bacterial cultures in medicine and clinical practice (Moreno-Vázquez, Larrañaga, Uberhuaga, Braga, & Pérez-Ruíz, 2014) and detect antimicrobial activity in plant extracts or essential oils (Bubulica et al., 2012; Sakkas et al., 2016). Based on biochemical observation was conducted using Vitek 2-compact BIOMERIEUX, our results indicated that *Bacillus* was the dominant genus in this study followed by *Staphylococcus* and *Pantoea*(Table 1). *Bacillus* and *Acitenobacter* spp. are part of the 16 genera that have been identified as endophytes (Gouda et al., 2016). These species have been isolated from roots, stem and leaves of L. *japonica*(L. Zhao et al., 2015). Although *Staphylococcus* spp. is a member of a human microflora, several species such as *Staphylococcus epidermidis* were previously reported as endophytic plants (Vendan, Yu, Lee, & Rhee, 2010). Chaudhry and Patil(2016)have

indicated that *Staphylococcus* species is adapted in various hosts and has demonstrated the characteristics of protection and development for its plants. This is the endophyte characteristics in their plants host.Rustamova et al., (2020)were isolated and identified*Pantoeaananatis* from medicinal plant tissue of V. *anthelmintica*. Janardhan and Vijayan (2012) also observed *Pantoeaananatis* from the medicinal plant *Lantana camara* from Malaysia.

Endophytes are often tissue and season specific. Geographical location influences the distribution patterns of endophytes because they occupy unique biological niches as they grow in many different environments. Geographical location and season greatly influence the distribution pattern of endophytes. Several reports indicate that seasonal changes affect the growth and presence of endophytes in medicinal plants. Therefore, different types of bacterial endophytes tend to inhabit the plants in different seasons. Soil type is the main factor determining the diversity of endophytic bacteria in plants (Nair & Padmavathy, 2014).

In addition, the genus *Bacillus* is found in diverse environments, is Gram-positive, aerobic, rod-shaped and forms endospores. Bacillus is capable of producing compounds such as amylase, protease, antibiotics, and surfactants. Due to its sporulation ability, variations in morphological characteristics, and production of antimicrobial substances, the Bacillus strain was able to survive a wide range of conditions. High production levels and direct release of peptides into the extracellular space makes strains of Bacillus organisms suitable for the production of antimicrobial compounds (Dehghanifar, Keyhanfar, & Emtiazi, 2019). Because endophytes are known to produce similar secondary metabolites as their host plant, these can be isolated, identified and further investigations performed on their produced secondary metabolites for drug development.

CONCLUSION

Based on the research that has been done, it can be concluded that the endophytic bacteria isolated from Kayu Jawa *Lannea coromandelica* (Houtt) Merr, six isolates belonging to *Bacillus* and another two belonging to *Staphylococcus* and *Pantoea* genera. Endophytic bacteria have the potential to produce bioactive metabolites that can be explored to control disease and improve human, animal and plant health through their development as materials for new drugs, antibiotics, biofertilizers, biopesticides, biofertilizers, etc. However, for the exploration of this prospective application, it is very important to do a systematic study of the biological and distribution of endophytic bacteria in the host plant.

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