Jurnal Pertanian Tropik **ISSN NO: 2356- 4725/p- ISSN : 2655-7576**
Vol.9.No.1. 2022 (05) 032-044 **DOI: 10.32734/jpt.v9i1, April.8656**

Potential of Endophytic Bacterial Isolates as Antagonist Agents Against Xanthomonas sp. Causes of Leaf Blight on Acacia Seeds (Acacia crassicarpa)

Potensi Isolat Bakteri Endofit Sebagai Agens Antagonis Terhadap Bakteri *Xanthomonas* sp. Penyebab Penyakit Hawar Daun Pada Bibit Akasia (*Acacia crassicarpa)*

Fifi Puspita[1], Muhammad Ali[1], Romayanju Siagian[1*], Bayo Alhusaeri[2]

[1] Jurusan Agroteknologi, Fakultas Pertanian, Universitas Riau, Jl. BinaWidya Km 12,5, Riau, 28293, Indonesia
[2] PT. Arara Abadi

**Corresponding Author*: siagianromayanju@gmail.com

ABSTRACT

Acacia is a type of plant that recommended for development of industrial forest plantations (HTI) especially pulp and paper. According to Central Statistics Agency (2020), area of acacia HTI in Riau Province 2019 was 2,335,450 ha with a log output of 31,509,228m^3 and total pulp export of 2,952.4 million US dollars. The need for paper pulp has increased every year. Problems in acacia nursery area is presence of disease caused by Xanthomonas sp. Bacterial leaf blight causes significant losses in procurement of acacia seedlings, so we need an alternative control with environmentally friendly and sustainable by utilizing potential of biological agents in controlling leaf blight. This aimed to study potential of endophytic bacterial isolates against bacterial pathogen Xanthomonas sp. by adopting a Completely Randomized Design (CRD). The results showed that there were 9 isolates of endophytic bacteria that had inhibitory power against Xanthomonas sp., bacterial isolates in hypersensitivity test did not show symptoms of necrosis in tobacco leaves, results of physiological and biochemical tests on levan test and VP test showed differences in reaction results on tested endophytic bacteria isolates and it was found isolate of endophytic bacteria B8 was effective as an antagonist agent in controlling disease.

Keywords: *acacia; characterization; endophytic bacteria; in vitro; in vivo; Xanthomonas sp.*

ABSTRAK

Akasia merupakan salah satu jenis tanaman yang direkomendasikan untuk pembangunan hutan tanaman industri (HTI) khususnya *pulp* (bubur kertas) dan kertas. Menurut Badan Pusat Statistik (2020), luas areal HTI akasia di Provinsi Riau pada tahun 2019 ialah 2.335.450 ha dengan produksi kayu bulatnya sebesar 31.509.228m^3 dan total ekspor bubur kertas sebesar 2.952,4 juta dolar AS. Kebutuhan bubur kertas tiap tahunnya mengalami peningkatan. Permasalahan di areal pembibitan akasia adanya gangguan penyakit yang disebakan oleh *Xanthomonas* sp. Penyakit hawar daun bakteri menyebabkan kerugian yang cukup signifikan dalam pengadaan bibit akasia, sehingga diperlukan alternatif pengendalian yang bersifat ramah lingkungan dan berkelanjutan dengan memanfaatkan potensi agens hayati dalam mengendalikan penyakit hawar daun. Penelitian ini bertujuan untuk mempelajari potensi isolat bakteri endofit terhadap patogen bakteri *Xanthomonas* sp. dengan mengadopsi Rancangan Acak Lengkap (RAL). Hasil penelitian menunjukkan bahwa terdapat 9 isolat bakteri endofit yang memiliki daya hambat terhadap *Xanthomonas* sp., isolat bakteri pada uji hipersensitif tidak menunjukkan gejala nekrosis pada daun tembakau, hasil uji fisiologis dan biokimia pada uji levan dan uji VP terdapat perbedaan hasil reaksi pada isolat bakteri endofit yang diuji dan diperoleh isolat bakteri endofit B8 efektif sebagai agens antagonis dalam mengendalikan penyakit.

Kata Kunci: akasia; karakterisasi; bakteri endofit; *in vitro*; *in vivo*; *Xanthomonas* sp.

Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.9.No.1. 2022 (05) 032-044 DOI: 10.32734/jpt.v9i1, April.8656

INTRODUCTION

Acacia is a type of plant that is recommended for the development of industrial forest plantations (HTI) which plays a role in supporting the development of the wood industry, especially *pulp* and paper. According to the Central Statistics Agency (2020), the area of acacia HTI in Riau Province in 2019 was 2,335,450 ha with a log production of 31,509,228 m3^{with} a total pulp export of 2,952.4 million US dollars and an increase of 0 ,19 percent from 2018. The need for paper pulp has increased every year, both domestic and export needs, so it is necessary to provide sufficient raw materials. The supply of raw material for pulp is closely related to the availability of seeds in the field. The availability of quality seeds will affect plant growth and the final production of the commodity will increase. Quality seeds can be obtained with proper handling. This is done to avoid problems that occur in the acacia nursery area, one of which is disease disorders.

Disease disorders that occur in acacia nurseries are bacterial leaf blight. Bacterial leaf blight causes significant losses in the procurement of acacia seeds. The control efforts that have been carried out have not shown optimal results, so there is a need for other control alternatives that are environmentally friendly and sustainable, namely the use of biological agents such as endophytic bacteria. The group of endophytic bacteria that has been widely used comes from the genera Bacillus, Pseudomonas and Burkholderia. Research on endophytic bacteria that has been carried out, among others, isolates of endophytic bacteria (BD4.2E1) is able to suppress bacterial leaf blight disease and increase yield on shallots with an effective suppression of 60.06% disease percentage compared to controls (Resti *et al*., 2013). Rajendran *et al.* (2006) added that endophytic bacteria were able to control bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacerum* on cotton, controlling blood disease in banana plants and endophytic bacteria *Bacillus* spp. *Indigenos* from tomato roots were able to suppress bacterial wilt disease by *Ralstonia solanacearum* (Marwan *et al*., 2011). This study aims to obtain biochemical physiological characters and to test endophytic bacteria that have antagonistic activity against *Xanthomonas* sp.

RESEARCH METHODS

The materials used in this study were isolates of endophytic bacteria from oil palm plants from Fifi Puspita, isolates of endophytic bacteria from acacia and eucalyptus, isolates of pathogenic *Xanthomonas* sp. originating from PT. Arara Abadi, *Nutrient Agar* (NA) medium, *Yeast Dextrose Calcium Carbonate Agar* (YDCA) medium, *Luria Bertani* (LB), 0.5% peptone, 3% H_2O_2 solution , *N,N,N,N-Tetramethyl-P-Phenylenediamine* 1%, *Oxidase-Fermentative* (OF) medium, paraffin oil, starch medium, *Lugol's iodine solution,* 5% sucrose, liquid glucose, alpha napthol solution, 40% KOH, object glass, 70% alcohol, filter paper , *plastic wrap* and sterile distilled water.

Tools used in this study were petri dishes, test tubes, Erlenmayer, 500 ml measuring cup, Ose needle, Bunsen lamp, micro pipettes of various sizes, *automatic mixer*, incubator, oven, *laminar air flow*, shaker, refrigerator, analytical balance, *microwave*, handsprayer.

Rejuvenation of Endophytic Bacteria and Pathogens

Rejuvenation of endophytic bacterial isolates and *Xanthomonas* sp.was done by taking one culture tube of endophytic bacteria and *Xanthomonas* using a sterile needle, transferred to NA media for endophytic bacterial isolates and YDCA for *Xanthomonas* sp. by scratching on the surface of the NA and YDCA media in a petri dish and stored in the incubator for 48 hours.

Endophytic Bacteria Antagonist Test Against *Xanthomonas* sp.

The test was carried out using the disc paper method. Antagonistic test of endophytic bacteria isolates against *Xanthomonas* sp. carried out by using

cultured isolates of endophytic bacteria and *Xanthomonas* sp. aged 48. Each culture was inoculated as much as one ose into 10 ml of *Luria Bertani* (LB) media in a sterile test tube, then homogenized using a *rotary shaker incubator* for ± 13 hours at a speed of 100 rpm.

Each endophytic bacterial isolate that had been*shaker* was transferred to *a laminar air flow cabinet* and then 10 l of each endophytic bacterial isolate was taken to be transferred to 0.5 cm filter paper using a micro pipette and allowed to dry. *Xanthomonas* sp. The *shaker* was transferred using *cotton buds* to be spread evenly on the surface of the NA medium in a sterile petri dish and allowed to dry. The surface of the dry NA media was then placed as many as 3 pieces (as a replication) of dry filter paper which had been dripped with each endophytic bacterial isolate and incubated at room temperature.

Endophytic bacterial hypersensitivity test on tobacco leaves

Hypersensitivity test to isolates of endophytic bacteria was carried out using a 2-month-old tobacco plant (*Nicotiana tabacum* L.) as an indicator plant. Hypersensitivity reactions were tested by making a suspension with a dilution factor^{108}from each isolate of endophytic bacteria aged 48 hours. The suspension obtained was then put into a *syringe* (without a needle) and infiltrated at the corners between the leaves of the tobacco plants to wet the intercellular spaces without penetrating to the top layer of the leaves.

Physiological and Biochemical Characterization of Endophytic Bacteria

Physiological and biochemical characterization of endophytic bacterial isolates with high antagonistic activity included catalase test, oxidase test, OF test, starch hydrolysis test, levan test, *Voges Proskaur* (VP) test. The test results are presented in the form of descriptive data.

Preparation of Acacia

Seeds The acacia seedlings used were varieties of *Acacia crasicarpa* which were approximately one month old with healthy plant conditions. The seeds used have a uniform height, diameter and number of leaves. Seedlings that have attack growth are placed in the area of acacia seedlings that have symptoms of blight caused by *Xanthomonas* sp.

Preparation of Endophytic Bacteria Suspension The antagonist test *in vitro*.

Endophytic bacteria were incubated for 48 hours to make a suspension of endophytic bacteria. The endophytic bacterial isolates aged 48 hours were ground using a ose needle and dissolved in 10 ml of distilled water. A total of 1 ml of the parent culture was taken and placed into 9 ml of distilled water for dilution. The dilution of each endophytic bacteria was carried out until it reached a dilution factor of 10^8.

Endophytic bacteria with a dilution factor of 10^8 1 ml of each was taken and transferred into 100 ml of sterile NB (*Nutrient Broth*) media, the suspension of each endophytic bacterial isolate was shaken*again* overnight at 100 rpm. Suspension that has been *shaken* overnight can be diluted again according to the needs in the field. Endophytic bacterial suspension was sprayed onto each *Acacia crassicarpa* according to the treatment and repeated until the entire leaf surface was moistened.

Application of Endophytic Bacteria on Acacia Seeds

Application of endophytic bacteria to acacia leaves is carried out by spraying the suspension of each endophytic bacteria that has been prepared on the entire surface of the plant leaves according to the treatment and replication using *a hand sprayer* by spraying each acacia seed to wet all the acacia seedlings. Spraying is done once a week for 1 month on acacia seedlings.

Parameter Observation

Antagonist Power of Endophytic Bacteria Against *Xanthomonas* sp. In *Vitro*

Observation of inhibition zone of endophytic bacteria isolates against *Xanthomonas* sp. counted from the second to the seventh day. The calculation of the inhibition is done by measuring the clear zone

formed and expressed in millimeters using a *caliper*.

Hypersensitivity Reaction (HR)

Macroscopic observations of endophytic bacterial colonies were carried out after 48 hours of inoculation to determine the color change of tobacco leaves after endophytic bacterial isolates were injected. Bacteria are classified as pathogenic if there are symptoms of necrosis on the injected tobacco leaf and vice versa if there are no symptoms of necrosis on the injected tobacco leaf, then the bacteria are not classified as pathogens.

Physiological Characteristics of Endophytic Bacterial Physiological Biochemical Isolates

Catalase reaction

Observation of the results of the catalase reaction was seen after being left for 5 minutes with visible air bubbles on the glass slide. Air bubbles indicate that these bacteria can reduce H_2O_2 (Lelliot and Stead, 1987).

Oxidase Reaction

Observation of the reaction results of the oxidase reaction was marked when the color of the colony changed to purple (magenta). The color change of the bacterial colonies on the glass slides indicated a positive reaction.

Oxygen demand reaction (Oxidation-Fermentation = OF)

Changes in the color of the medium to yellow in the non-paraffinized tube and no color change occurred in the paraffin-treated tube indicating *oxidative* of glucose. Changes in the color of the medium to yellow occurred in both tubes indicating fermentative metabolism. If there is gas production, it will be seen in the paraffin-treated tube.

Starch Shydrolysis reaction

Observation of the results of the starch hydrolysis reaction when the starch hydrolysis medium was blue indicated that the starch was not hydrolyzed. Bacteria that hydrolyze starch are characterized by the formation of bright or clear areas around the bacterial colonies (Lelliot and Stead, 1987).

The reaction for levan formation

Bacteria classified as Levan are positive if the colonies that develop on 5% *Sucrose Nutrient Agar* are convex/convex in shape. The levan test was negative, if the surface of the bacterial colony was not convex and the growth of the colony was underdeveloped.

Voges Proskaur (VP) reaction

VP observations were made by observing the color change of the endophytic bacterial solution. A positive reaction is indicated by a pink solution (Stolp and Gadkari, 1983).

Early symptoms of the disease

Observation of the initial symptoms of the disease is carried out when the symptoms appear on the leaves for the first time. Initial symptoms appear at the tip of the leaf, the center of the leaf, the base of the leaf in the form of a small red line measuring approximately 1-3 mm. Plant parts infected with *Xanthomonas* sp. on the leaves will slowly experience tissue death around the point of attack. Blight on acacia leaves will increase if the environmental conditions are favorable with the appearance of symptoms of spots and small changes in leaf color.

Disease incidence (%)

Percentage of disease incidence is obtained by using the formula:

$$KP = \frac{n}{N} \times 100\%$$

Information:
KP : Percentage of disease incidence
n : Number of plants affected
N : Number of test plants

Number of surviving seedlings

Observations were made by calculating the percentage of surviving plants with the formula:

$$S = \frac{JTB}{JTU} \times 100\%$$

Information:
S : Percentage of surviving plants
JTB : Number of surviving plants
JTU : Total number of test plants

Seedling height (cm)

Measurement of plant height was measured from the base of the stem with a limit of 2 cm from the base of the stem to the tip of the highest point using a ruler.

Number of leaves (strands)

Observations were made by counting the total number of leaves formed from the sample plants.

Acacia seedling stem diameter (cm)

The stem diameter was measured above 2 cm from the base of the stem using a *caliper*.

RESULTS AND DISCUSSION

Antagonistic Power of Endophytic Bacterial Isolates Against *Xanthomonas* sp. In *Vitro*

Isolate B9 has a higher inhibitory power against *Xanthomonas* sp. compared with all treatments and not significantly different from isolates B4, B1, B15, B6, B8, B3. This was presumably because the endophytic bacterial isolate B9 had a larger inhibition zone. The inhibition zone is the activity of secondary metabolites of endophytic bacteria in inhibiting the growth of pathogens by interfering with the metabolism of pathogens, inhibiting the synthesis of cell walls of pathogenic bacteria, interfering with the permeability of the cell membranes of pathogenic bacteria, inhibiting protein synthesis of pathogenic bacteria and, damaging the synthesis of nucleic acids of pathogenic bacteria (Brooks *et al.*., 2005). Antagonistic power of endophytic bacteria isolates against *Xanthomonas* sp. can be seen in Table 1 below.

Table 1. Antagonistic power of endophytic bacteria isolates against *Xanthomonas* sp.

Isolate	Resistence (mm)
B0	0,000d
B1	6,178a
B2	0,000d
B3	4,083abc
B4	6,267a
B5	0,000d
B6	5,369ab
B7	0,000d
B8	4,333abc
B9	6,321a
B10	0,000d
B11	0,000d
B12	0,000d
B13	0,000d
B14	0,000d
B15	6,070a
B16	3,440bc
B17	0,000d
B18	0,000d
B19	0,000d
B20	2,439c

The numbers followed by unequal lowercase letters are significantly different after data transformation $\sqrt{y}+0.5$ according to DNMRT test results) at 5% level .

Antibiotics are classified as secondary metabolites produced by endophytic bacteria in metabolic pathways by enzymes that are not needed by plants. Antibiotics are agents produced by living microorganisms which in low concentrations can inhibit or kill other organisms (Hasim, 2003).

Factors that influence the formation of the inhibition zone are the rate of diffusion of antibacterial compounds into the media and their interaction with pathogenic bacteria. The growth rate of endophytic bacteria and the sensitivity of pathogenic bacteria to antibacterial compounds in endophytic bacteria can also affect the resulting inhibition zone (Cappuccino and Sherman, 1999 *cit* Setyati *et al*., 2016).

The poor role of secondary metabolites in endophytic bacteria resulted in the inability to produce antibacterial compounds needed to suppress pathogenic bacteria. Endophytic bacteria that are not able to grow and develop properly fill the space as a result, they are unable to compete for nutrients in the petri dish.

According to Schulz *et al*. (2006) *cit* Fithriyah (2015), competition is one of the factors that influence the formation of inhibition zones. This is supported by the opinion of Yulianti (2013) which states that microorganisms are unable to become biological agents due to the inability of endophytic bacteria to adapt to the

environment and unable to compete with pathogens.

Hypersensitivity reaction (HR)

Hypersensitivity reaction is a plant response to rapid and localized cell death. Based on the results of the hypersensitivity reaction test on tobacco plants, all isolates of endophytic bacteria that have been tested showed negative results, which were non-pathogenic.

Hypersensitivity reactions appear in pathogen-infected plants in the form of necrosis and cell death to limit the movement of pathogens (Wahyudi *et al*., 2011). Hypersensitivity reactions are characterized by the absence of symptoms of necrosis that appear on tobacco leaves after application in the form of injection of a suspension of endophytic bacteria on tobacco leaves.

Catalase reaction

The presence of bubbles in the catalase test indicates that endophytic bacteria have the enzyme catalase. Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide is toxic to cells because this material will inactivate enzymes in cells. Hydrogen peroxide is formed during aerobic metabolism, so that microbes that grow in an aerobic environment must decompose the toxic material Lay (1994) *cit* Alfian (2008).

Oxidase Reaction The oxidase

Test is used to determine the presence of one of the four cytochrome groups, namely cytochrome c (Johnson and Case, 2010). Isolates of endophytic bacteria on glass slides showed positive results, which was indicated by the appearance of a purple color on the slides indicating that endophytic bacteria had cytochrome oxidase enzymes that play a role in the oxidation and reduction of electrons.

Jawetz *et al*. (2010) stated that some organisms produce oxidase enzymes that play a role in catalyzing the oxidation and reduction of electrons. Cytochrome oxidase enzymes in endophytic bacteria play an important role in electron transport during aerobic respiration.

OF reaction (Oxidation-Fermeentation)

OF reaction on endophytic bacterial isolates was fermentative which was characterized by a change in the color of the medium from blue to yellow in both the paraffin-treated and non-paraffinized media. The change in the color of the media to yellow was caused by a decrease in the pH of the media due to organic acids produced by endophytic bacteria. This acidity is caused by small amounts of endophytic bacterial fermentation products such as acetic acid, lactic acid and CO2 gas (Swings and De Ley, 1977).

Evita (2005) also added, if a yellow color is formed on a medium covered with paraffin and not covered in paraffin, it means that bacteria metabolism uses carbohydrates by fermentation and if a yellow color is formed on a medium that is not covered with paraffin, it means that the metabolism of bacteria in using carbohydrates is organically. oxidation.

Starch Hydrolysis

Reaction Starch hydrolysis reaction in endophytic bacteria was characterized by the formation of a clear zone around the growth area of endophytic bacteria after being given a few drops of Lugol's iodine solution. Asadullah (2014) stated that the clear zone formed around the isolate indicated amylolytic isolate activity, namely the ability of the isolate to hydrolyze selective media found in growth media by producing amylase.

Pricilia *et al*. (2018) also stated that the large clear zone formed around the media containing endophytic isolates indicated the amount of glucose produced from the hydrolysis reaction of starch by amylase. The -amylase enzyme produced by endophytic bacteria can hydrolyze starch into simpler saccharides such as maltose and glucose. In starch hydrolysis, -amylase plays a role in breaking bonds with the configuration of starch. The hydrolysis of starch by the -amylase enzyme is divided into two pathways, namely hydrolysis of amylose and hydrolysis of amylopectin.

Levan

Reaction A positive levan reaction was indicated by the formation of a single colony of endophytic bacteria on Levan media. Colonies formed on 5% NA medium were shiny, mucoid, convex like a dome shape. Levan or polyfructose is an extracellular capsule produced through the activity of the enzyme levan sucrose. The enzyme sucraseis an enzyme that breaks down sucrose and uses the energy released to combine sugar units to form polymer chains of sugars (poly sugars). Most groups of bacteria use sucrose as a carbon source to produce the enzyme levan sucrose (Fahy and Hayward, 1983 *cit* Asrul, 2005).

The results of the levan test on endophytic bacterial isolates B20 did not show colonies and were not convex, which indicated that the endophytic bacteria did not have the activity of the enzyme levan sucrase that produces polyfructose. On the other hand, other endophytic bacteria have dome-shaped colonies which indicate the activity of the enzyme levan sucrase. The enzymes responsible for the synthesis of glucose polymers are known as fructosyltransferase, glucosyltransferases.

Ningtyas (2016) reported that bacteria have fructosyltransferase and glucosyltransferase enzymes that can hydrolyze sucrose into fructose (levan) and glucose (dextran). Fructose serves to help the adhesion and aggregation of bacteria, while glucose is the main food source for bacteria.

VP (Voges Proskauer) reaction

The Voges Proskaeur (VP) test was used to determine the ability of bacteria to produce the final product acetyl-methyl carbinol (acetoin) from the fermentation of 2,3 butanediol. The endophytic bacterial isolates showed a positive reaction which was indicated by a change in the color of the medium to pink or red, while the negative reaction was indicated by the absence of a color change in the medium. This indicated that the isolated endophytic bacteria were unable to completely oxidize glucose to acid.

According to Sunatmo (2007), the VP test aims to determine the ability of a bacterium to oxidize glucose by producing acid as the final product and with a high concentration such as acetylmethyl carbonyl from organic acids as a result of glucose metabolism. In addition, Sari (2014) also stated that with the addition of KOH (potassium hydroxide), the presence of acetoin was indicated by a change in the color of the medium to pink.

Early symptoms of disease

Isolation of endophytic bacteria B8 showed early symptoms of disease which tended to be slower, namely 32,667 days after application compared to other treatments and not significantly different from isolates of endophytic bacteria B4, B6, B16. The slow onset of disease in the B8 endophytic isolates was thought to be due to the role of endophytic bacteria in the acacia seedling tissue. The treatment of endophytic bacteria on symptoms that appear early in the disease can be seen in Table 2.

Table 2. Early symptoms of disease in acacia seedlings

Treatment of Endophytic Bacteria	Early symptoms of attack (days after application)
B0	7,000c
B1	14,000bc
B3	14,000bc
B4	18,667ab
B6	18,667ab
B8	32,667a
B9	14,000bc
B15	14,000bc
B16	18,667ab
B20	14,000bc

Figures followed by unequal lowercase letters are significantly different after data transformation $\sqrt{y}$ according to DNMRT test results) at the 5% level.

The slow onset of disease symptoms in B8 endophytic bacterial isolates is thought to be due to the role of endophytic bacteria in the acacia seedling tissue. Endophytic bacteria spend part or all of their life cycle by colonizing inter or intracellular tissues without causing disease, triggering the host plant to produce phytoalexins due to pathogen attack and as growth stimulants (Strobel and Daisy, 2003).

The role of endophytic bacteria in acacia seedling tissue is related to secondary metabolite compounds contained in endophytic bacterial isolates that are able to work effectively in suppressing the growth of pathogens. The working system of metabolites produced by bacteria by destroying cell walls, changing cell permeability, damaging the nuclear membrane so that it will result in inhibition of cell growth or cell death, inhibits enzyme work (Pelczar and Chan *cit* Sariyanto, 2006).

Disease incidence (%)

Treatment of endophytic bacteria on the incidence of acacia seedling disease can be seen in Table 3.

Table 3. Disease incidence in acacia seedlings

Treatment of Endophytic Bacteria	Number of live plants (%)
B0	20,454abc
B1	12,086cd
B3	19,408abc
B4	21,646ab
B6	12,892bcd
B8	8,270d
B9	8,610d
B15	20,746abc
B16	27,763a
B20	17,890abc

The numbers followed by unequal lowercase letters are significantly different after transforming the data arcin +0,25 according to the results of the DNMRT test) at the 5% level

Table 3 The treatment of B8 endophytic bacteria isolates tended to be lower in disease incidence parameters to other endophytic bacterial isolates and was not significantly different from endophytic bacterial isolates B8, B1 and B6. This is presumably due to the role of endophytic bacterial isolates in acacia seedling tissue that produces secondary metabolites.

Munif *et* al. (2012) stated that the mechanism of endophytic bacteria in inducing resistance in plants is done by colonizing the tissue in plants so as to stimulate plants to increase the production of metabolite compounds. Secondary metabolite compounds produced in the form of antibiotic compounds and antimicrobial compounds play a very important role in inducing plant resistance to pathogen attack.

The role of endophytic bacteria in acacia seedling tissue that produces secondary metabolites in the form of peroxidase enzymes, increased chitinase activity, -1,3 glucanase, and *pathogenesis related* proteins, phytoalexins. Peroxidase enzymes are needed by plants to produce plant defense compounds such as lignin, chitin, and several compounds that make up cell walls (Harni and Ibrahim, 2011). The results of research by Resti *et al*. (2013), obtained 6 isolates of endophytic bacteria isolated from shallot roots and had effectiveness in suppressing bacterial leaf blight 28.32 – 64.30 %, and the increase in shallot yields reached 50.65 – 214.85%.

Number of live seedlings

Treatment of endophytic bacteria on the number of live seedlings of acacia seedlings can be seen in Table 4.

Table 4. Number of live seeds on acacia seedlings

Treatment of Endophytic Bacteria	Number of live plants (%)
B0	71,667a
B1	71,667a
B3	46,667b
B4	61,667ab
B6	61,667ab
B8	78,333a
B9	71,667a
B15	48,333b
B16	46,667b
B20	60,000ab

The numbers followed by unequal lowercase letters are significantly different after transformation of arcin data $\sqrt{y}$ according to DNMRT test results) at the 5% level.

Table 4. shows that the treatment of B8 endophytic bacteria isolates tended to have more number of viable seeds compared to other endophytic bacterial isolates. This is

presumably due to the good interaction between endophytic bacteria in colonizing the acacia seedling tissue to produce secondary metabolites. The number of live seedlings in the treatment of B8 endophytic bacterial isolates can be related to the initial parameters of the emergence of disease symptoms and the percentage of disease incidence. Parameters of early symptoms of endophytic bacterial isolates B8 were able to inhibit the appearance of symptoms of bacterial leaf blight caused by *Xanthomonas* sp. by 32,667 days after application, while the parameter of disease incidence was able to suppress the incidence of disease by 8.270%. So that the parameter of observing the number of live plants on B8 endophytic bacteria isolates can produce a larger percentage of the number of live seeds.

The less than optimal role of endophytic bacteria in forming secondary metabolites in plant tissues resulted in the inhibition of the function of secondary metabolites produced by bacteria to suppress the development of pathogens. In addition, the factors that cause the low number of plants that live in the field are caused by the large incidence of disease that occurs and the onset of disease symptoms. The early emergence of the disease can spur the development of pathogenic bacteria to attack acacia seedlings, so that the role of endophytic bacteria found in acacia seedling tissue is unable to perform its role effectively.

Seedling height (cm)

Table 5. Shows that the treatment of endophytic bacterial isolates B1 tends to be higher than the treatment of other endophytic bacteria. This is due to the role of endophytic bacteria in producing secondary metabolites and the genetic characteristics of acacia seedlings which tend to be the same so that the growth process of seedlings is relatively the same. Treatment of endophytic bacteria on acacia seedling height can be seen in Table 5.

Table 5. Plant height on acacia seedlings

Treatment	plant height (cm)
B0	13,715c
B1	17,185a
B3	16,490ab
B4	15,643abc
B6	16,614a
B8	16,248ab
B9	14,199bc
B15	15,367abc
B16	13,725c
B20	15,775abc

The numbers followed by unequal lowercase letters are significantly different according to the results of the Duncan New Multiple Range Test (DNMRT) at the 5% level.

The increase in the height of acacia seedlings is influenced by phytohormones produced by endophytic bacteria . Ryan *et al*. (2008) reported that endophytic bacteria can stimulate growth directly through the synthesis of compounds that help absorb nutrients from the environment including the synthesis of indole acetate and gibberellins. One of the mechanisms is to produce growth hormones such as indole-3-*acetic acid* (IAA) and auxin compounds, one of which functions as a plant growth promoter (Hallmann *et al*. 2001; Eliza 2004).

Number of Leaves (sheets)

Table 6. shows that the treatment of endophytic bacterial isolates B20 tends to have a higher number of leaves produced compared to other endophytic bacterial isolates. This is thought to be due to the role of endophytic bacteria in acacia seedling tissue. Surette *et al.* (2003) *cit* Surya Murthi, R (2015) stated that endophytic bacteria are able to increase plant growth, either directly or indirectly. Endophytic bacteria directly produce nutrients for plants, such as nitrogen, phosphate and other minerals and produce growth hormones such as ethylene, auxin or IAA (*Indole Acetic Acid)* and cytokinins. Treatment of endophytic bacteria on the number of leaves of acacia seedlings can be seen in Table 6.

Table 6. Number of leaves on acacia seedlings

Treatment of Endophytic Bacteria	Number of leaves (strands)
B0	5,989c

B1	6,857abc
B3	7,639ab
B4	7,200ab
B6	7,788a
B8	6,503bc
B9	7,433ab
B15	6,915abc
B16	7,342ab
B20	7,790a

The numbers followed by unequal lowercase letters are significantly different after data transformation $\sqrt{y}$ +0.5 according to DNMRT test results) at the 5% level

Endophytic ability to increase growth hormones such as auxin and cytokinins that function as a stimulant for shoot formation and cell elongation, cell growth, and stimulate the growth of shoots and shoots of plants (SU *et al*., 2011). Agustiansyah *et al*. (2013) added that biological agents produce IAA which functions to stimulate growth. IAA produced by bacteria will be utilized by plants to undergo metabolic processes in the plant body so that it helps the process of increasing the number of acacia seedling leaves.

Acacia Seedling Stem Diameter (cm)

Treatment of endophytic bacteria on stem diameter of acacia seedlings can be seen in Table 7.

Table 7. Stem diameter of acacia seedlings

Perlakuan Bakteri Endofit	Diameter Batang (cm)
B0	1,871ab
B1	1,9764a
B3	2,098a
B4	2,097a
B6	2,071a
B8	1,906ab
B9	1,8575ab
B15	2,112a
B16	1,684b
B20	1,996a

The numbers followed by unequal lowercase letters are significantly different according to the results of the Duncan New Multiple Range Test (DNMRT) at the 5% level.

Table 7. shows that the treatment of endophytic bacteria B15 tends to have higher stem diameter compared to the treatment of other endophytic bacteria isolates. It is suspected that the role of endophytic bacteria in acacia seedling tissue is to produce secondary metabolites produced by endophytic bacteria in the form of phytohormones. Phytohormones are found in acacia seedling tissue due to endophytic bacteria that can help acacia seedlings in stem diameter growth. Plant hormones regulate several aspects of plant growth and development, such as the formation and maintenance of meristems (SU *et al.*, 2011). In addition, endophytic bacteria are used as biocontrol agents and growth promoters so that they can increase the availability of nutrients and produce growth-promoting hormones such as IAA, GA3 and Cytokinins (Gusmaini *et al*., 2013).

CONCLUSION

The results of the endophytic bacterial antagonist test in vitro, in 9 isolates of endophytic bacteria as antagonistic agents against Xanthomonas sp. (B1, B3, B4, B6, B8, B9, B15, B16, B20). The results of physiological and biochemical tests that endophytic bacteria have different reactions. Endophytic bacteria isolate B8 was effective as an antagonist agent against the pathogen *Xanthomonas* sp.

ACKNOWLEDGMENTS

The authors would like to express their deepest gratitude to those who have assisted in the research

REFERENCES

Agustiansyah, S. Ilyas, Sudarsono, M. Machmud. 2013a. Characterization of rhizobacteria that have the potential to control *Xanthomonas oryzae* pv. *oryzae* and increase the growth of rice plants. *J.* Tropical HPT.13:42-51.

Alfian, AYDP 2008. Isolation and Identification of Endophytic Bacteria in the Stem of *Artmesia Annua*,L. Tested

for Antibacterial Potential against *Escherecia coli* and *Staphylococcus aureus*. Thesis.(Unpublished). Sanata Dharma University. Yogyakarta.

Asadullah M (2014) Isolation of Amylolytic Bacteria from Rice bran and Ability Test for Production of Coarse Amylase Enzyme in Various Types of Production Media. Essay. Faculty of Science and Technology UIN. Poor.

Asrul, Arwiyanto, T., Maryudani. 2004. Effect of treatment of tomato seeds with *Pseudomonas putida* pf-20 on bacterial wilt disease (*Ralstonia solanacearum). Agroscience* 17(3): 419-430

Central Bureau of Statistics. 2020. Statistics of Indonesia's Foreign Trade According to ISIC Code 2018-2019. BPS. Jakarta.

De Ley and Swings J., (1976), "Phenotype description, numerical analysis and a proposal for an improved taxonomy and nomenclature of the genus Zymomonas Kluvyer and Van Niel", International Sys. Bacteriology, no. 26, p. 146-157.

Eliza., Munif A., Djatnika I., and Widodo. 2007. Physiological Characters and Role of Antibiosis of Graminae Root Bacteria on *Fusarium* and Banana Plant Growth Stimulator. Journal*of Horticulture* 17(2):150-160.

Evita, CT2005. Organic Waste Degrading Bacteria at the Padang City Cold Water Final Disposal Site. ESSAY. Andalas University. field

Fahy PC and Hayward AC. 1983. Media and methods for isolation and diagnostic tests. In: Fahy PC & Persley GJ (Eds.) Plant Bacterial Diseases. A Diagnostic Guide. pp. 337–378. Academic Press.

Fithriyah, NL (2015). Isolation And Identification Of Endophytic Bacteria From Kebar Grass (*Biophytum* sp.) As Producing Antibacterial Compounds Against *E.coli* And *S.aureus*. Essay. Faculty of Science and Technology. Maulana Malik Ibrahim State Islamic University. Poor.

Gusmaini, D. Sopandie, SA Aziz, A. Munif, and N. Bermawie. 2013. The Potential of Endophytic Bacteria in Increasing Growth, Production and Levels of Androgrfolids in Sambalito Plants. *Journal of Littri*, 19(4):167-177.

Halaman, J. 2001. Plant interaction with endophytic bacteria. Biotic interactions in plant-pathogen associations. CAB International. pp 87-119.

Harni.R., and MSDIbrahim. 2011. The potential for endophytic bacteria to induce resistance of pepper plants to *Meloidogyne incognita*. Research Institute for Spices and Various Industrial Plants. *Journal of Littri* 17 : 118 – 123.

Hasim. 2003. Planting Grass, Harvesting Antibiotics. Jakarta : Kompas No. 127. The 39th year

Jewetz, E., Melnick, Joseph L., Adelberg, EA 2010. Medical Microbiology. New York: McGraw Hill Companies.

Johnson, TR, and Case, CL 2010. Laboratory Experiments in Microbiology 9 th edition. Pearson Benjamin Cummings, San Francisco. Page. 25.

Lay, B. 1994. Microbial Analysis in the Laboratory. 79-101, 129-132. Management of PT. Grafindo Persada. Jakarta.

Lelliott, R.A. and DE. Stead. 1987. Methods in Plant Pathology. Vol. II. Method for the Diagnosis of Bacterial Diseases of Plants,. Blackwell Scientific Publications. Oxford. London. Edinburgh.

Marwan, Husda, SS Meity, Giyanto and NAAbdjad. 2011. Isolation and selection of endophytic bacteria to

control blood disease in banana plants. *J. Tropical HPT*. Vol. 1, No. 2:13-121.

Munif A, Wiyono S, Suwarno. 2012. Isolation of endophytic bacteria from upland rice plants and their potential as biocontrol agents and plant growth promoters. *J Fitopatol Indonesia* 8 (3):57-64.

Ningtyas, AIL, 2016, Differences in Concentration and Antibacterial Activity Test of Kluthuk Banana Stem Ethanolic Extract (*Musa balbisiana* Colla.) Against Staphylococcus aureus and *Pseudomonas aeruginosa*, FMIPA Sebelas Maret University, Surakarta

Pelczar, Michael J and Chan, ECS 2007. Fundamentals of Microbiology Volume I. Jakarta: UI Press.

Pricilia S, Astuti W, Marliana E (2018) Screening of Amylase, Lipase and Protease Producing Endophytic Bacteria from Macaranga hullettii King leaves ex Hook.f. *Atomic Journal* 3(2) : 102-105.

Rajendran, L., D. Saravana Kumar, T. Ragunchander, R. Samiyappan. 2006. Endophytic Bacteria Induction Of Defense Enzymes Against Bacterial Blight Of Cotton. Tamil Nadu (IN): Tamil Nadu.

Resti, Z., T. Habazar, DP. Son and Nasrun. 2013. Screening and identification of endophytic bacterial isolates to control bacterial leaf blight on shallots. *J HPT Tropical*. 13(2):167–178.

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett. 278:1-9.

Sariyanto.N., 2006. Exploration of Antagonist Agents Potentially Suppressing Fusarium Wilt Disease in Bananas. (Essay). Pest and Plant Disease Study Program, Faculty of Agriculture, Bogor Agricultural University

Stolp, H and D. Gadgari. 1983. Nonpathogenic Members of the Genus Pseudomonas. P. 719-741 in The Prokaryotes A Handbook on Habitat edited by Star, MP, HG Truper, A. Balows, and HG Schlegel. Springer-Verlag New York.

Strobel G and B. Daisy. 2003. Bioprospecting for microbial endophytes and their natural products. *Journal of Microbiology* and Molecular Biology Review. 67(4).

SU, YH, YB LIU, and XS ZANG. 2011. Auxin-cytokinin interaction regulates meristem development. *mole. Plants*. 28:1-10.

Sunatmo IT. 2007. Microbiological Experiments in the Laboratory. Jakarta: Ardy Agency.

Surette MA, Stunz AV, Lara RR & Nowak J. 2003. Bacterial endophytes in processing carrots (*Ducus carota* L. Var. Satvus): their localization, population density, biodiversity and their effects on plant growth. P. soil. 253:381-390.

Surya Murthi, R. Lisnawita, Syahrial Oemry. 2015. The Potential of Endophytic Bacteria to Increase Growth of Tobacco Plants Infected with Root Claw Nematodes (*Meloidogyne* spp.). *Journal of Agroecotechnology* . E-ISSN No. 2337- 6597 Vol.4. No.1, December 2015. (583) :1881-1889

Wahyudi TA, Meliah S, Nawangsih AA. 2011. *Xanthomonas* oryzae pv. Oryzae bacteria causing leaf blight in rice: Isolation, Characteristics, and Study of Mutagenesis with Transposon. Makara Sains, 15(1): 89-96.

Yulianti, Titiek. 2013. Utilization of endophytes as biological control agents of plant pests and diseases. Bulletin of

tobacco, fiber and industrial oil crops. 5(1): 40-49.