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Natural Ingredients of Beans as Growth Medium and Propagation of Endophytic Bacteria from Lowland Eggplant

Arika Purnawati*, Safira Rizka Lestari, Iis Purnamawati

Agrotechnology Department, Agriculture Faculty, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia

Corresponding author:
E-mail:
arika_p@upnjatim.ac.id

ABSTRACT

Endophytic bacteria are defined as a group of microbes that live in plant tissues that do not cause disease and are mutually related to the host plant, can be isolated from all parts of the plant were roots, stems, leaves, and seeds. Endophytic bacteria have the potential to produce natural products for agriculture, increase plant growth, and support the sustainable development of agriculture. The growth of endophytic bacteria *in vitro* generally uses a medium that contains nutrients including macronutrients, namely elements (C, H, O, and N), micronutrients, namely non-metallic elements (S and P), metallic elements (Ca, Zn, Na, K, Cu). , Mn, Mg, and Fe), vitamins, water, and energy. To meet these growth requirements, currently the growth and propagation of endophytic bacteria generally still use synthetic media which are expensive and in the field, mainly by farmers, are not easy to obtain. The study aimed to obtain an alternative medium made from beans for the growth and propagation of endophytic bacteria from lowland eggplants. The types of beans were soybeans, green beans, red beans, cowpeas, peanuts, and Nutrient Agar (NA) as control. Inoculation into the medium was done using the spread plate technique. This technique is preceded by a dilution and at dilution of 10^{-7} was taken 0.1 ml then to be spread on the growing medium. Observations were growing and colony counting was done 24 hours after inoculation. The results were: (1) endophytic bacteria from lowland eggplant can grow well on all mediums with the best growth on NA medium, (2) all media can be used as the propagation of endophytic bacteria from lowland eggplant plants, (3) the assumed of the growth curve is logarithmic with 4 phases were lag phase, exponential phase, stationer phase, death phase.

Keywords: Beans as ingredients of the medium, growth, propagation, endophytic bacteria, lowland eggplant

Introduction

Endophytic bacteria are defined as a group of endophytic microbes that live in plant tissues without causing negative impacts, including not causing plant disease and mutualism between host plants and endophytic microbes, can be isolated from all parts of the plant, namely roots, stems, and leaves, so have potential to produce a natural product for agriculture, live in the internal tissues of plants and without causing direct negative effects. does not hurt plant tissues and indicates the possibility of a mutualistic symbiotic relationship between endophytic bacteria and their hosts (Malvanova, 2013). Endophytic bacteria can be isolated from all parts of the plant, namely roots, stems, leaves, seeds, and also have potential in agriculture, including producing secondary metabolites that have the potential as an elicitor of plant resistance to pathogens with the Induced Systemic Resistance (ISR) mechanism, capable of increasing plant growth called Plant Growth Promoting Rhizobacteria (PGPR) because it can increase the availability of nutrients and produce growth hormone (Gao et al., 2010).

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The growth of endophytic bacteria is influenced by growth media and nutrition factors to meet energy needs, build cells, and synthesize protoplasm and other cell parts so that the growing media needs to contain elements of C, H, O, N, P as the main elements and non-metallic elements such as sulfur and phosphorus, metallic elements such as Ca, Zn, Na, K, Cu, Mn, Mg, and Fe, vitamins, water (Purnawati et al., 2016), so it is necessary to grow medium containing these elements such as Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA). The reality in the field for the growth and propagation of endophytic bacteria requires a growing medium that is cheap, easy to obtain, and easily made by farmers so that it is easy to apply to control plant pest organisms (OPT) (Purnawati et al., 2016). Based on this, an alternative medium made from natural ingredients is needed such as sorghum flour, soybean flour, corn flour, snail flour, earthworm flour, and golden snail flour, which are natural ingredients for the growth and multiplication of endophytic bacteria that increase the population of endophytic bacteria in solid and liquid consistency (Purnawati et al., 2016).

The purpose of the study was to obtain a growth medium and the propagation of endophytic bacteria from lowland eggplant made from natural ingredients as an alternative medium.

Material and Methods

Isolation and purification of endophytic bacteria from eggplant tissue

The samples used were leaves, stems, and roots in healthy and fresh conditions from lowland plants, namely eggplant, which were taken near plants that were attacked by bacterial wilt disease in the Jombang area. The plant samples were then washed with running water and cut 1-3 cm long. The sample pieces were surface disinfected by soaking in ethanol 70% (30 sec), sodium hypochlorite solution 5% (5 min), soaking in ethanol 70% (30 sec), and rinsed using sterile equates (3 min). Two pieces of each plant organ sample were planted in a petri dish containing Nutrient Agar (NA) medium for bacterial growth. Each sample was placed in a prone position. While the incubation conditions for samples grown on NA media were 37^0C for 24 hours. The bacteria that grew were then purified on a new medium (Purnawati, et al., 2014).

Preparation of Medium

Preparation of natural medium :

(1) Soybean flour medium: 1 kg of soybeans washed with clean running water and removed all dirt attached to the soybeans until clean. Soybean soaked in clean water for 4 hours while kneaded to clean the epidermis. Then the soybeans were washed again using water and drained for 15 minutes. Soybeans are dried in the sun for 2 days, roasted for 10 min, and then ground into fine flour (Agro Inovasi, 2009).
(2) Green beans fluor medium: mung bean soaked in hot water for 4 hours until the epidermis is broken and loose. Then roasted until dry and ground into flour (Agro Inovasi, 2009).
(3) Peanut flour medium: peanuts are soaked in boiling water for,r 3 min while stirring, dried at 130-150°C for 3 hours so that the water content is less than 6%, and cooled. The dried peanuts are rubbed with hands so that the husks are removed and then winnowed so that the separated epidermis can be removed and the seeds without the husks are obtained, then wrapped in a strong thick cotton cloth, and pressed so that most of the oil comes out. The results of pressing are cake and peanut oil. Peanut meal was ground with a grinding machine until smooth (60 mesh). Finely ground cake is called peanut flour (Intan, 2018).
(4) Cowpea flour medium: cowpeas are washed and soaked in water for 14 hours, then steamed for 10 minutes, dried and the husks removed. The seeds are then finely ground and sifted until they become flour (Larasati, 2015).
(5) Red bean fluor medium: red beans are washed and soaked in water then steamed for 10 minutes, dried and the husks removed. The seeds are then finely ground and sifted until they become flour (Larasati, 2015).

Preparation of NA :
Weighing 39 g of NA and dissolved in 1 l of distilled water.

Natural medium and NA medium test

Before carrying out the media test, each of the ingredients that had become flour was weighed 39 g/l plus agar for a solid medium. Natural medium and NA were sterilized using an autoclave at 121^0C, 1.5 atm for 15 min then 10 ml was poured into a Petri dish. The test was done using inoculation of endophytic bacteria into each medium. Cultures were incubated in an incubator at 26^0C for 24 hours. Observations were incubation period, shape, and color of the colonies based on the Taxonomic Outline of the Prokaryotes Bergey's Manual of Systematic Bacteriology Second Edition (Garrity et al., 2004), population and colony size of endophytic bacteria. was calculated using the dilution technique and at 10^{-8} was calculated.

Results and Discussion

The results of alternative medium from natural ingredients for the growth and propagation of endophytic bacteria from lowland eggplant plants are:

1. Shape and Color of Colonies
   Shape dan color of colonies (Table 1).

Table 1. Shape and color of colonies

		Shape and color colonies	
		Shape	Color
NA medium	B1	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white
NA medium	B2	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white
NA medium	B3	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white
NA medium	B4	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white

To be continued…

NA medium	B5	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white
NA medium	B6	round	white
soy fluor medium		round	white
green bean fluor medium		round	white
peanut fluor medium		round	white
cowpea fluor medium		round	cloudy white
red bean fluor medium		round	cloudy white

2. Effect of Medium to Number of Colonies
   A number of colonies in each natural medium (Fig 1):

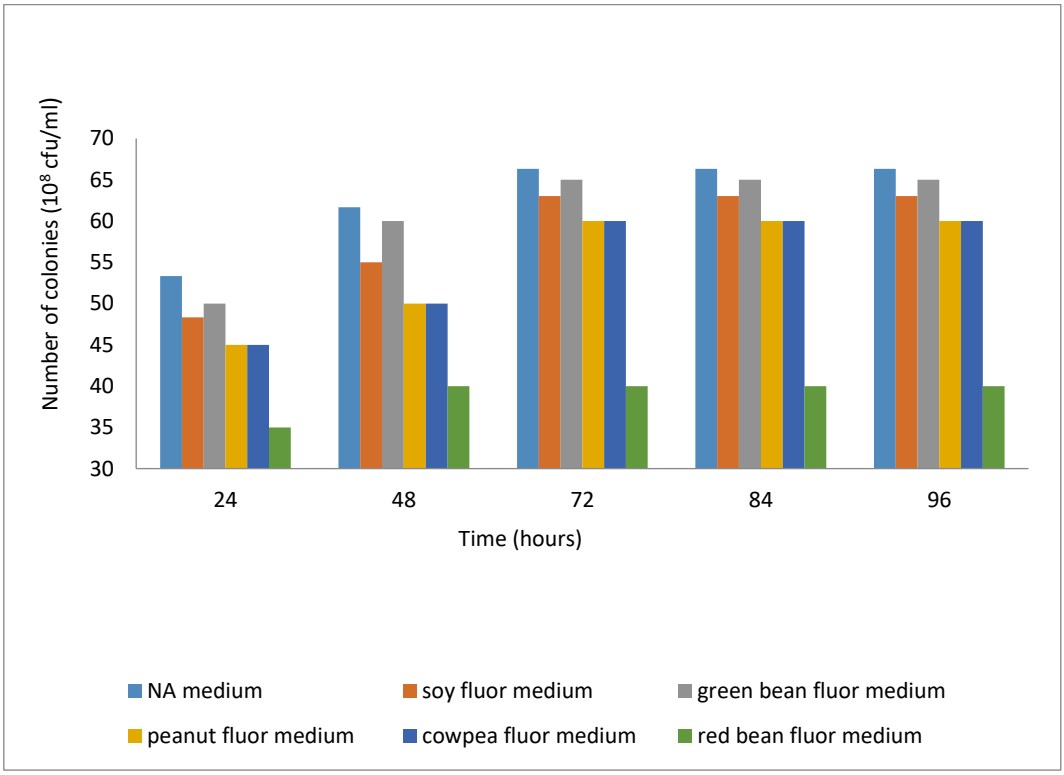

Figure 1. Effect of medium to number of colonies

3. Effect of Medium-to-Diameter Colonies
   Diameter colonies in each medium (Fig 2).

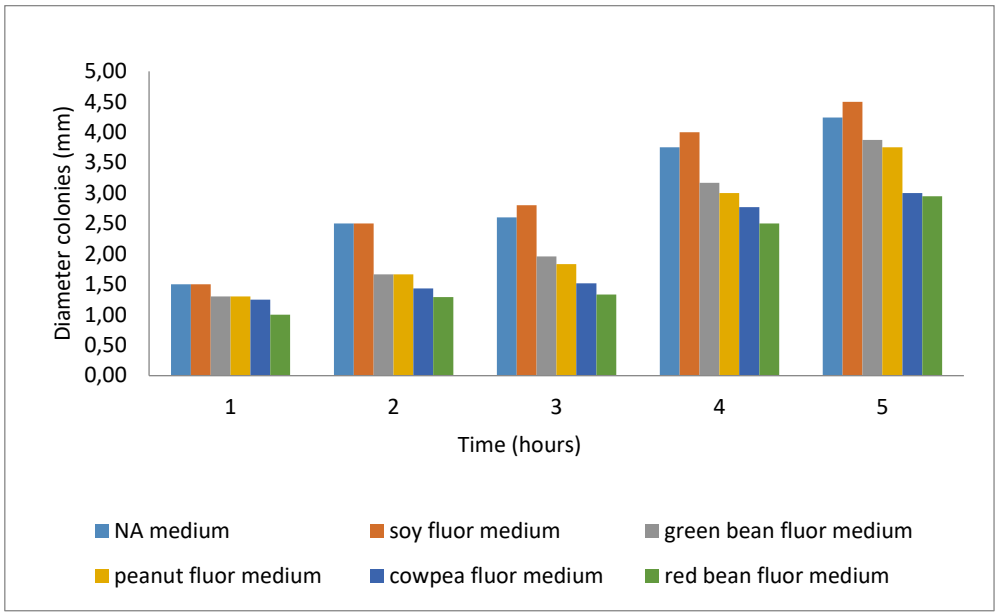

Figure 2. Effect of medium to diameter colonies

The results of the study in Table 1, Figures 1 and 2 were obtained with the reason that endophytic bacterial colonies from eggplant plants were generally influenced by nutrients in the growing medium, macro and microelements that play a role in the structure and function of cells. Each flour that became the growing medium in this study contained nutritional components needed for the growth of bacterial colonies, namely carbohydrates, and proteins. Also contains C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. Waspadji (2003) states that soybean, green bean, peanut, cowpea, and red bean contains chemical compositions and substances such as carbohydrates, proteins, fiber, P, Ca, Fe, Na, K, and many other components like a vitamin.

In addition, colonies, number of colonies, and diameter of colonies are also influenced by physical and environmental factors such as O_2 concentration, hydrogen ion concentration (pH), and temperature. Todar (2015) states that bacterial growth needs nutrition. which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo.

Conclusion

1. Endophytic bacteria from lowland eggplant grow well in a natural ingredients medium.
2. Natural ingredients medium can use for the propagation of endophytic bacteria.
3. Soybean and green bean are good natural ingredients medium for the growth and propagation of endophytic bacteria from lowland eggplant.

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