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The Utilization of Coconut Water and Molasses as Fluorescent Pseudomonad Propagation Medium

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Abstract — Fluorescent pseudomonad are groups of bacteria that can be used in inducing plant resistance to pathogens. To be easily applied and stored, fluorescent pseudomonad must be reproduced in a particular medium. The aim of this research is to identify the ability of coconut water and molasses as the propagation medium of fluorescent pseudomonad. This research used a complete randomized design with 5 treatments and 3 replications. The treatment is the incubation period of fluorescent pseudomonad in coconut water and molasses medium, which is: A = incubation period of 0 weeks (control); B = incubation period of 2 weeks; C = incubation period of 4 weeks; D = incubation period of 6 weeks; and E = incubation period of 8 weeks. The results showed the highest number of bacteria in the coconut water propagation medium is at 6 weeks after incubation (wai) of $5.7.10^{15}$ cfu/mL and the least is at 8 wai of $1,3,10^{8}$ cfu/mL. While in the propagation medium molasses, the largest number of bacteria is at 2 wai is $2,6,10^{11}$ cfu/mL, and no more bacteria found in sugar drops medium at 6 wai.

Keywords — Fluorescent Pseudomonad, Coconut Water, Molasses.

I. INTRODUCTION

Fluorescent pseudomonad are groups of bacteria that are grouped into Plant Growth Promoting Rhizobacteria (PGPR). Rhizobacteria is a bacteria that colonizes plant roots and usually lives as endosymbiosis. Various species of rizhobacteria can colonize the plant rhizosphere and can also be found within the roots [1]. Fluorescent pseudomonad live freely in the soil, rhizosphere, and phyllosphere. The use of fluorescent pseudomonad for the control of plant diseases has been widely reported. The success of fluorescent pseudomonad as a biocontrol agent due to several characters that it has, such as: having a high ability of living in rhizosphere, broad-spectrum, easy to reproduce, environmental saving, compatible with other rhizobacteria, drought, heat, and UV radiation tolerant [2].

Several species of fluorescent pseudomonad produce siderophores, HCN antimicrobials, IAA, and phosphate solvent compounds, and show growth-promoting activity [3]. Introduction of olive plant root with several strains of fluorescent pseudomonad is very influential in reducing incidence of Verticillium wilt disease [4]. Black pepper plants introduced by some Pseudomonas fluorescens strains have the potential to suppress root rot disease caused by Phytophthora capsici in greenhouses [5]. Five P. fluorescens strains that is being selected in vitro had antimicrobial activity against several types of fungi such as Alternaria cajani, Curvularia lunata, Fusarium sp., Bipolaris sp. and Helminthosporium sp. [6].

Fluorescent pseudomonad from the root zone of Mimosa invisa able to induce the resistance of banana plants to the Blood Disease Bacteria (BDB) and Fusarium wilt by Fusarium oxysporum f.sp cubence [7]. Fluorescent pseudomonad strains Pj1, Pj2, and Pj3 can suppress BDB attacks on Barangan banana seedlings through increased enzyme activity of phenylalanine ammonia liase and peroxidase [8]. Furthermore, found 10 strains characterizing the fluorescent pseudomonad bacteria, and the physiological character of each strain showed differences in the production of fluorescence pigments. The best Mi.1 strain to control BDB in vitro [9].

To grow and multiply the fluorescent pseudomonad required a medium. Mass production of these bacteria can use

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xanthan gum as a growing medium. A growing medium consisting of 20% xanthan gum can keep bacteria for two months at 40 oC [10]. The population fluorescent pseudomonad able to live for five weeks in 1,5% methyl cellulose media, and storage at 5 °C [11].

The research that developed to date still uses solid media in petri dishes that have been formulated in such a way by certain factories that the price is quite expensive. For trading, it is necessary to grow bacteria in a medium that has the same culture effectiveness as the solid medium. The research has been done utilizing coconut water and molasses as a fluorescent pseudomonad propagation medium. This availability of this medium is quite a lot because coconut water is a waste of copra industry, while molasses from sugar factory. Thus, the utilization of these two wastes becomes a promising option in the effort of developing propagation media in order to mass-produce fluorescent pseudomonad.

Coconut water contained 91,23% water, 0,29% protein, 0,15% fat, 7,27% carbohydrate, and ash 1,06%. Besides, there are also nutrients in the form of sucrose, dextrose, fructose, and vitamin B complex [12]. Molasses is the final product of sugar making which contains no sugar which can be crystallized in the conventional way, and still contains 50 - 60% sugars, amino acids and minerals. The high sugar content of molasses resulted in frequent use an additional source of carbohydrates in the growth medium of microorganisms [13].

II. Research Methods

A. *Study Design*

Fluorescent pseudomonad used is the Pj1 strain. Coconut water and molasses are used as bacterial propagation medium. The study design was Completely Randomized (RAL) with 5 treatments and 3 replications. The treatment is the incubation period of fluorescent Pseudomonad in coconut water medium and molasses, which is: A = incubation period of 0 weeks (control); B = incubation period of 2 weeks; C = incubation period of 4 weeks; D = incubation period of 6 weeks; and E = incubation period of 8 weeks.

B. *Propagation of fluorescent pseudomonad in coconut water/molasses medium.*

Coconut water / molasses of 49 mL were put into bottles and sterilized in an autoclave temperature of 121°C for 15 minutes. After sterile and cold, 1 mL of fluorescent pseudomonad is added with density of $3,10^8$ cells/mL and incubated according to treatment at room temperature.

C. *Observation.*

The number of fluorescent pseudomonad bacteria in each medium was observed 0, 2, 4, 6, and 8 weeks after incubation using formula as follows [14] :

JB = A x B
Description: JB = number of bacteria per mL
A = number of bacterial colonies
B = dilution factor

III. Results And Discussion

The result of the variance analysis of the number of fluorescent pseudomonad bacteria in each type of propagation medium with different incubation periods shows a significant difference. Table 1. shows the differences in the number of bacteria on coconut water growing media at different incubation period. The number of fluorescent pseudomonad bacteria on the coconut water medium 2 weeks after incubation (wai) at room temperature was $2,1.10^{13}$ cfu/mL, continued to rise for up to 6 weeks ($5,7.10^{15}$ cfu /mL), but after 8 weeks there was a decrease in the number of bacteria becoming $1,3.10^8$ cfu /mL. The growing medium of molasses showed an increase in the number of bacteria from 2 wai which is $2,6.10^{11}$ cfu /mL, but after 4 weeks there was a decrease to $6,3.10^8$ cfu /mL. No more bacteria are found in the medium of growing sugar cane drops at 6 wai.

Table 1. The number of Pseudomonad fluorescent in coconut water propagation medium at different incubation periods.

Incubation period (wai)	Number of bacteria (cfu/mL)
A (control)	$1,0.10^7$ a
B (incubation period of 2 weeks)	$2,1.10^{13}$ b
C (incubation period of 4 weeks)	$2,3.10^{14}$ c
D (incubation period of 6 weeks)	$5,7.10^{15}$ d
E (incubation period of 8 weeks)	$1,3.10^8$ e

Different letters on the numerical values indicate significant different among incubation period at p<0,05 to DNMRT

The propagation medium and incubation period can determine the number of fluorescent pseudomonad. Coconut water as a propagation medium, has the highest bacterial count at 6 wai, and at 8 wai the amount of bacteria is decreasing. Coconut water contains nutrients in the form of 7,27% carbohydrates, 0,29% protein, and 0,15% fat. Carbohydrates can be a source of carbon for fluorescent pseudomonad, and proteins as a source of nitrogen. The results of carbohydrate oxidation can be a source of energy for fluorescent pseudomonad. However, this activity can produce organic acids in coconut water [12]. Organic acids in a carrier can lower the pH. These organic acids include acetic acid and butyric acid. *Fusarium oxysporum* f. sp. *lycopersici* and *Ralstonia solanacearum* are depressed due to the presence of both organic acids in the soil [15].

Molasses can also be used as a propagation media for fluorescent pseudomonad bacterial. Table 2. shows an increase in the number of bacteria from 2 wai which $2,6.10^{11}$ cfu/mL, but after 4 weeks there was a decrease to $6,3.10^8$ cfu/mL. At 6 wai, no more bacteria are found in the molasses growing media.

Table 2. The number of fluorescent pseudomonad in molasses propagation medium at different incubation periods

Incubation period (wai)	Number of bacteria (cfu/mL)
A (control)	$1{,}0.10^{7}$ a
B (incubation period of 2 weeks)	$2{,}6.10^{11}$ b
C (incubation period of 4 weeks)	$6{,}3.10^{8}$ c
D (incubation period of 6 weeks)	0,00 d
E (incubation period of 8 weeks)	0,00 d

Different letters on the numerical values indicate significant different among incubation period at p<0,05 to DNMRT

Propagation of fluorescent pseudomonad using a growing medium of molasses shows that the bacteria live up to 4 wai with bacterial numbers of $6{,}3.10^{8}$ cfu/mL. After 6 and 8 wai there is no longer fluorescent pseudomonad visible living on the medium of molasses. This is different from the coconut water propagation medium which still shows the presence of fluorescent pseudomonad bacteria up to 8 wai. In this case it can be stated that the nutrients present in molasses are only available 4 wai for the life of the fluorescent pseudomonad. After 4 wai fluorescent pseudomonad run out of nutrients and fluorescent pseudomonad able to live in a molasses medium is no longer found. The residue of the fluorescent pseudomonad metabolism in molasses can be toxic to bacteria.

Molasses is an excellent basic medium for biocontrol agents. A combination of molasses and yeast extract (1: 1) can increase the number of fluorescent pseudomonad bacteria P-6 after 20 hours storage time at 27 °C, which is $3{,}7.10^{9}$ cfu / mL. Furthermore, the number of bacteria dropped to $2{,}3.10^{9}$ cfu/mL at the end of the observation. High concentrations of molasses (40 g/L) had no significant effect on increasing bacterial growth, because high concentrations of carbon sources could be toxic to cells [16]. Bacterial growth will be stopped in a medium due to the depletion of nutrients and the build-up of metabolic waste that is toxic [17]. Secondary metabolic production in the medium may cause a decrease in pH, and may inhibit the growth of fluorescent pseudomonad [18].

IV. Conclusion

Propagation of fluorescent pseudomonad can use coconut water and molasses medium. The number of fluorescent pseudomonad bacteria in the most coconut water propagation medium at 6 weeks after incubation (wai) was $5{,}7.10^{15}$ cfu/mL, and decreased to $1{,}3.10^{8}$ cfu/mL at 8 wai. The largest number of fluorescent pseudomonad bacteria in molasses medium was seen at 2 wai of $2{,}6.10^{11}$ cfu/mL, and at 6 wai no more fluorescent pseudomonad was found.

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