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Utilization of Semi Natural Media With Hydrolysis Method Using Pepaya and Pineapple for Growth of *Pseudomonad Fluorescent* Isolate Pf-122

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Abstract

Pseudomonad fluorescent isolate Pf 122 is a biological agent bacteria that is often used to control soil borne pathogens. The aim of the study was to determine the growth of bacteria in a variety of semi-natural media that have been hydrolyzed by using papaya or pineapple and those that are not hydrolyzed. The media is expected to be used as a substitute for Kings' B, which is relatively expensive, for the growth of *Pseudomonad fluorescent*. This study was compiled using a Completely Randomized Design (CRD) with 13 treatments and repeated 4 times on a laboratory scale. The research treatment consisted of several media including Kings' B, crab, pineapple crab, papaya crab, snail, pineapple snail, papaya snail, lamtoro, pineapple lamtoro, papaya lamtoro, tolo, pineapple tolo and papaya tolo. The results showed the highest population of bacteria in the medium of crabs and snails. The average population of bacteria in semi-natural media is more than the population of bacteria in the Kings 'B media. The growth of bacteria is the second best, namely bacterial growth in pineapple crab media, papaya crab, anyway, Kings'B. Media that is not good for bacteria growth are pineapple snail, papaya snail, pineapple toasted, pineapple lamtoro and papaya lamtoro because it doesn't show the growth of bacteria. Compared to crab, snail and pineapple crab media, growth of *Pseudomonad fluorescent* isolate Pf 122 bacteria shows significant differences with Kings 'B. Thus the medium of crabs, snails, pineapple crabs and tolo can be used as a substitute for Kings' B.

Keywords: Hydrolysis, media, *Pseudomonad fluorescent*, semi natural

INTRODUCTION

The existence of biological agents in nature is very diverse. One type of biological agent that is often used to control plant pathogens is pseudomonads fluorescent. *Pseudomonad fluorescent* are a group of bacteria that produce fluorescent pigments, are saprophytic and can grow in organic media that have high protein content. This is the nature that causes *Pseudomonads fluorescent* to live in liquid organic waste, such as coconut water waste which has a protein content (Syukur et al., 2006). *Pseudomonads fluorescent* have very diverse types with various specific characteristics. One of the pseudomonad fluorescent isolates that has been proven to be able to control various plant pathogens is pseudomonad fluorescent isolate Pf-122 bacteria (hereinafter referred to as Pf-122 bacteria).

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Pseudomonad fluorescent isolate Pf-122 bacteria is one of 130 pseudomonad fluorescent isolates, which can inhibit *Ralstonia solanacearum* growth *in vitro* with more inhibition zones from 15 mm (Wuryandari *et al.*, 2005).

In previous studies, semi-natural media have been used as growth media of Pf-122 bacteria. The media is in the form of snails, paddy crabs and lamtoro, but the results of the growth of Pf-122 bacteria are not maximal. This is because the process of making media by boiling uses only distilled water. This method causes the protein not to be fully hydrolyzed, so that the high protein content cannot be utilized optimally by the Pf-122 bacteria. Enzymatic protein hydrolysis using papaya and pineapple is the process of breaking protein polymers into their constituent monomers with the help of papain and bromelin contained by papaya and pineapple. The content of each of these enzymes serves to hydrolyze proteins in natural media such as lamtoro, tolo nuts, snails, and rice crabs.

Based on this, this study was conducted to optimize the use of protein from natural ingredients snails, leucaena, rice crabs and tolo nuts as medium for Pf-122 bacteria. The growth of Pf-122 bacteria is expected to be optimal by the using cheap and easily available natural media.

METHODS

This research was conducted from January to March 2018. The research was conducted at the Plant Health Laboratory of the Faculty of Agriculture, Universitas Pembangunan Nasional Veteran Jawa Timur. The materials used in this study included snails, rice crabs, lamtoro, tolo beans, aquades, 70% alcohol, young pineapple, young papaya, Kings B media, and Pf-122 bacteria collection Dr. Ir. Yenny Wuryandari MP.

Research stages

Making Hydrolysis Media

Each natural material in the form of lamtoro, tolo beans, snails, and rice crabs was roughly crushed using mortar for crabs, tolo and lamtoro, while for snails cut using a knife. Natural ingredients that have been crushed plus pineapple or papaya juice with a ratio of 1: 3 and left overnight. Natural ingredients that have been hydrolyzed are taken as much as 1 liter then added 20g of sugar and 15 g of agar. Then put into erlenmeyer sterile and sterilized using autoclave for 20 minutes.

Making Media without Hydrolysis

Semi-natural media without the hydrolysis process is made by roughly destroying each natural material such as tolo, lamtoro, crabs and snails then boiling them using distilled water. The decoction of natural ingredients is taken as much as 1 liter then added 20 g of sugar and 15 g of agar. Then put into sterile erlenmeyer and sterilized using autoclave for 20 minutes.

Pf-122 Bacterial Propagation Test

Bacteria are grown in semi-natural media in solid form, using the pour plate method. Before it is grown on semi-natural media, a dilution series of bacterial suspensions is carried out. The dilution results poured into the petri dish are the results of a 10^{-3},10^{-4},10^{-5} and 10^{-6} dilutions of 0.5 ml using a micropipette, then pouring solid semi-natural media which has previously been liquefied by 10 ml.

Observation Parameters

1. The incubation period of Pf-122 bacteria in each medium is semi-natural.
2. The population of Pf-122 bacteria in each semi-natural medium, on day 1 to day 3
3. The amount of dissolved protein in each semi-natural medium.

RESULT AND DISCUSSION

The Incubation Period of Bacterial Growth in Each Semi Natural Media

The incubation period for bacterial growth is the initial time for bacterial growth to be inoculated in semi-natural media. In this study, observation of the incubation period was carried out every day for three days. The growth velocity of Pf 122 bacteria in each semi-natural medium showed different diversity (Table 1).

Table 1. Incubation time of bacterial in semi natural media

No.	Media	Incubation time (days to-)		
		1	2	3
1.	KB	√	√	√
2.	K	√	√	√
3.	KN	-	√	√
4.	KP	-	√	√
5.	B	√	√	√
6.	BN	-	-	-
7.	BP	-	-	-
8.	T	√	√	√
9.	TN	-	√	√
10.	TP	-	-	-
11.	L	-	√	√
12.	LN	-	-	-
13.	LP	-	-	-

Description: √ = there is growth; - = there is no growth

The growth of Pf-122 bacteria on the best natural media is in the medium of rice crabs, snails, and tolo which are not hydrolyzed because they grow on the first day. Pf-122 bacteria did not grow on papaya snail media, pineapple snail, tolo papaya, pineapple lamtoro, and papaya lamtoro, it showed no growth until the third day. The speed of growth of Pf-122 bacteria in the media of rice crabs, snails and tolo without hydrolysis was caused by the complete and non essential essential protein and amino acid content of each ingredient. According to Andi *et al.*, (2009) the essential amino acid content in paddy crabs is leucine 8.36%, arginine 8.38% and lysine 6.96%, while for non-essential amino acids, among others aspargin reaches 12.87%, acid glutamate 11.53% and tyrosine 1.91%. According to Wakhid (2010), snails are one source of animal protein which has a high crude protein content of 53.121%. According to Masauna et al. (2013), the tolo beans are ripe every 100 grams containing 10 g of water, 22 g of protein, 1.4 g of fat, 51 g carbohydrates, 3.7 g of vitamins; 3.7 g of carbon, 104 mg of calcium and other nutrients and the energy produced is around 1420 kj / 100g. Young seeds every 100 g contain 88.3 g of water, 3 g of protein, 0.2 g of fat, carbohydrates, 1.6 g of vitamin, 0.6 g of carbon and the energy produced is around 155 kj / 100 g.

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Number of Pf-122 bacteria in semi-natural media

Based on the results of analysis of variance, the growth of Pf-122 bacteria in each hydrolyzed natural medium showed significantly different results. When viewed from bacterial growth on rice crab media starting the first day of incubation until the third day shows the best growth and increases quite sharply. Bacterial growth on lamtoro media showed good growth on the third day. Bacterial growth in papaya paddy crab media increased quite sharply on the third day (Figure 1). Thus the best semi-natural media that can be used as a substitute media for Kings'B is the medium of rice crab without hydrolysis. The results of the variance analysis on rice crab media showed that the average number of the largest population was 1.93 x 10^8 CFU/ml, followed by growth in snail media with an average bacterial population of 6.91 x 10^7 CFU / ml.

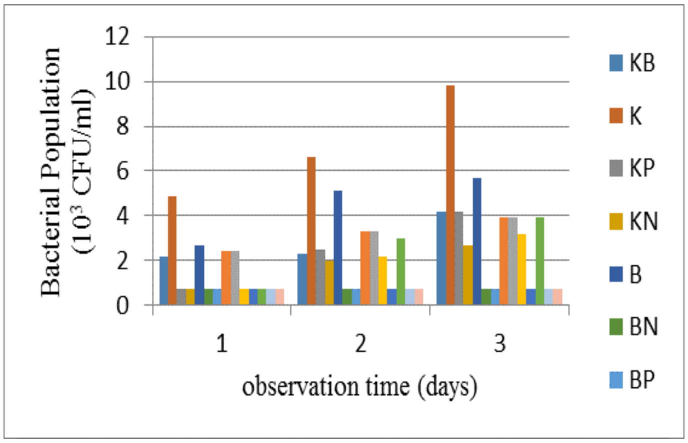

Figure 1. Population of pf-122 bacteria in various natural media for three days. (KB: Bacterial growth on Kings'B media), (K: Bacterial growth in rice crab media), (Bacterial growth on snail media).

The growth of Pf-122 bacteria between crab and snail media is not significantly different from Kings' B media, so that the media can be recommended as a substitute media for Kings'B. The medium of rice crab which has been hydrolyzed with papaya also shows good bacterial growth with an average of 2.91 x 10^7 CFU / ml. The growth of Pf 122 bacteria in papaya paddy crab media, the population is the same as bacterial growth on Kings'B media. Whereas in snail, toil and lamtoro media hydrolyzed using papaya and pineapple, the growth of Pf-122 bacteria is not good. The population of Pf-122 bacteria in these media was very small and some did not grow. The population of Pf-122 bacteria in pineapple toloof media on the third day was only 4 x 10^5CFU / ml, whereas in tolo papaya media, pineapple snail, papaya snail, pineapple lamtoro, papaya lamtoro did not show Pf-122 bacteria (Table 2).

Table 2. Total population of Pf-122 bacteria (CFU/mL)

Treatment	Observation Day (*incubation time)		
	1	2	3
KB	(8,7x 10^6) (2,2 x 10^3)bc	(2,2x 10^7) (3,7 x 10^3)bc	(2,8x 10^7) (4,2 x 10^3)ab
K	(3,5x 10^7) (4,9 x $10^{3)}$ c	(7,3x 10^7) (6,6 x 10^3)c	(1,9x 10^8) (9,8 x 10^3)b
KP	0 a	(8,6x 10^6) (2,5 x 10^3)ab	(2,9x 10^7) (4,2 x 10^3)ab
KN	0 a	(5,9x 10^6) (2 x 10^3)ab	(9,9 x10^6) (2,7 x 10^3)ab
B	(1,2x 10^7) (2,7 x 10^3)bc	(5,6x 10^7) (5,1 x 10^3)bc	(6,9x 10^7) (5,7 x 10^3)b
BN	0 a	0 a	0 a
BP	0 a	0 a	0 a
T	(8x 10^6) (2,4 x 10^3)bc	(1,5x 10^7) (3,3 x10^3)b	(2,3x 10^7) (3,9 x 10^3)a
TN	0 a	(2x 10^5) (2,2 x 10^2)a	(4x 10^5) (3,1 x 10^2)a
TP	0 a	0 a	0 a
L	0 a	(1,3x 10^7) (3 x 10^3)ab	(2,6x 10^7) (3,9 x 10^3)ab
LN	0 a	0 a	0 a
LP	0 a	0 a	0 a

Information:

Numbers accompanied by the same letters in the same column are not significantly different from the BNJ 5% test; right data is a transformation data; left data is the original data; KB : Kings' media as a control.

The growth and isolation of Pf-122 bacteria was caused by the different content and type of protein in each medium. The population of bacteria in rice crab media and snails with pineapple rice crabs and papaya rice crabs and other alternative media is likely due to the formation of other compounds which actually kill the Pf-122 bacteria when grown in these natural media. Snails contain mucus which is antiseptic and can kill bacteria. No growth of Pf-122 bacteria on snail hydrolysis media of pineapple and papaya, allegedly due to the hydrolysis process which makes the whole mucus in the snail more and more released, which affects the growth of Pf-122 bacteria. This is supported by Berniyati et al., (2007), that achasin slime snail protein is a protein that can work by attacking or inhibiting the formation of common parts of bacterial strains such as the cytoplasmic membrane. The aim of achasin in *Staphylococcus aureus*, is its cytoplasm, by attacking the cytoplasmic membrane and causing the cell wall to peel and sink into the cytoplasm (Berniyati et al., 2007).

Whereas in terms of the hydrolysis enzyme, it also has several additional chemicals. Pineapple has an enzyme, one of which is the bromelin enzyme. Bromelin has been shown to show a variety of fibrinolytic, antiedematous, antithrombotic and anti-inflammatory activities both in vitro and in vivo. Bromelin also has anti-lesion properties that can prevent bacteria. Therefore, bromelin is possible to prevent the sticking of bacteria, thus exerting antibacterial action (Nc. Praveen *et al.,* 2014).

Temperature is also one of the important aspects affecting the growing media used for bacterial growth. According to (Nielsen, 1997) in (Anggraini, 2015), the temperature treatment for 50 °C hydrolysis for 2 hours has the highest dissolved protein content, while the treatment of hydrolysis temperature of 60 °C for 6 hours has the lowest protein content. Hydrolysis will reduce protein molecular weight and increase the number of polar clusters. This can cause protein content in each of the natural media to decrease so that Pf-122 bacteria cannot be utilized.

Analysis of Dissolved Protein Levels in Semi Natural Media

The results of the analysis of dissolved protein levels in semi-natural media are found in table 3. When looking at the results of protein analysis, it can be concluded that hydrolysis activities on natural ingredients actually make dissolved protein levels drop. Dissolved protein content greatly influenced the growth of Pf-122 bacteria.

Table- 3. Results of dissolved protein level analysis

No.	Media	Dissolved protein levels (%)
1.	Kings'B	14,58
2.	Bekicot	8,22
3.	Bekicot Nanas	4,18
4.	Bekicot Pepaya	4,01
5.	Kepiting	7,90
6.	Kepiting Nanas	3,22
7.	Kepiting Pepaya	3,15
8.	Lamtoro	6,90
9.	Lamtoro Nanas	4,18
10.	Lamtoro Pepaya	5,91
11.	Kacang Tolo	5,56
12.	Tolo Nanas	4,13
13.	Tolo Pepaya	3,61

Source: Results of analysis of dissolved protein levels from semi natural media research samples at the research institute and industrial consultants (BPKI).

Low levels of dissolved protein cause the number of Pf-122 bacteria population to decrease and even those bacteria that do not grow on the media. The results of analysis of dissolved protein from paddy crabs hydrolyzed with pineapple or papaya and tolo pineapple hydrolysis were low, causing Pf-122 bacteria to grow less than those without hydrolysis. For media snail, lamtoro, and tolo which are hydrolyzed with papaya or pineapple, produce low dissolved protein compared to not hydrolyzed. This has the effect of not growing the Pf 122 bacteria. High dissolved proteins in the medium of rice crabs, snails, lamtoro and tolo cause a high population of Pf-122 bacteria.From the results of the analysis it can be concluded, the higher the percentage of dissolved protein content, the higher the population of Pf-122 bacteria, while the low percentage of dissolved protein causes low bacterial growth (Table 3). The causes of the low levels of dissolved protein are caused by several factors, namely the improper selection of the age of hydrolysis materials, the compound content of pineapple and papaya, and compounds contained in natural ingredients of snails, lamtoro, tolo and rice crabs, and temperature.

Bromelin as a hydrolysis enzyme can be obtained from pineapple plants from stalks, skins, leaves, fruits and stems. The content of the enzyme is more in the flesh of the fruit, this is indicated by a higher activity compared to the activity in the stem (Supartono 2004). The temperature treatment in making hydrolysis media also causes low levels of dissolved protein. The temperature used in this study is 70o-75oC. According to the results of the study (Anggraini 2015), the temperature treatment of the edamame hydrolysis process 50 ° C for 2 hours had the highest dissolved protein content, while the hydrolysis treatment temperature of 60 ° C for 6 hours had lowest protein content. As a result of the temperature treatment the dissolved protein content decreases. Hydrolysis will reduce protein molecular weight and increase the number of polar clusters. Protein hydrolysis can cause proteins that initially do not dissolve into dissolved proteins, which are then hydrolyzed by the enzyme papain into amino acids.

Several factors that influence the activity of protease enzymes are pH, temperature, concentration and incubation or ripening time (Zarei *et al.*, 2014). Mcbroom and Oliver-hoyo (2007), explained that there was an inverse curve pattern between the incubation time and enzyme activity in hydrolyzing proteins. This pattern is caused by the kinetic behavior of the enzyme on the substrate, so that at certain times there will be anticlimactic activity. The most papain content is found in young papaya fruit. Papaya sap contains quite a lot of proteolytic enzymes (protein decomposers) (Warisno, 2003).

Lamtoro (*Leucena leucocephala*) is one of the tree legumes containing high protein and carotenoids. Legume leaves contain high concentrations of phenolic compounds, especially tannins and mimosins as well as lamtoro leaves (Jayanegara & Sofyan, 2008). Mimosin is a class of aromatic amino acids. Mimosin is found in the seeds and leaves of the Leucaena species (D'Mello, 2000). Mimosin is a non-protein amino acid that has a structure similar to tyrosine, and is present in several species of mimosa in the genus Leucaena. Lamtoro leaves mimosine content ranges from 2% -6% and varies depending on the level of maturity (Aung *et al.,* 2006). Mimosin (β-N- (3-hydroxy-4-pyridone) contains high polyphenol compounds including tannin which binds to proteins, so that proteins become not "available" for Pf 122 fluorescent pseudomonad bacteria and cause negative effects on growth (Wang *et al* ., 2000).

Bacteria that do not grow on tolo beans that have been hydrolyzed using papaya are thought to be due to tolo beans containing polyphenol compounds. It is like Laurena *et al.* (1984) that polyphenols / tannins are compounds that determine the color of cowpea seed skin, the darker the color the higher the content of polyphenol compounds. The role of polyphenols is as a protein binder so that it becomes unavailable. Protein can be high after hydrolysis, but it cannot be used for bacterial growth, because protein is not in accordance with the desired bacteria. Polyphenols can form complexes with proteins and carbohydrates and can reduce the bioavability of minerals.

CONCLUSION

1. *Pseudomonad fluorescent* isolate Pf 122 bacteria grow well because it has a fairly short incubation period of one day which is grown in natural media of rice crab.

2. The highest population of *Pseudomonad fluorescent* isolate Pf 122 bacteria in natural media of paddy crab was 1.93 x 10^8 CFU / ml.
3. There is a correlation between the percentage of dissolved protein and the population of *Pseudomonad fluorescent* isolate Pf 122 bacteria, which is the high population of high protein content.

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