B -08

PSEUDOMONAD FLUORESCENT PRESERVATION USING TAPIOCA AND RICE FLOUR CARRIER AND THE ADDITION OF GLYCEROL STABILIZER

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Abstract

Pseudomonad fluorescent is a biological agent produces signal compounds for plantation to produce antimicrobial compounds. *Pseudomonad fluorescent* preservation is needed to enable storing and ease application. The objective of research is to explore the best carrier for *Pseudomonad fluorescent* preservation. The research used Complete Randomized Design with 6 treatments and 3 replications. The treatments were tapioca (addition 0.03, 0.04, and 0.05 mL of glycerol); and rice flour (addition 0.03, 0.04, and 0.05 mL of glycerol). Observations done against the number of *Pseudomonad fluorescent* on 10, 20, 30, 40, 50, and 60 days of storage. Data analysis used ANOVA significant level of 5% and further testing DNMRT significant level of 5%.The result showed that the number of bacteria on every storage period (10, 20, and 30 days) were not significantly different. The significant difference was shown on storage period of 40, 50, and 60 days.The highest number of bacteria on 40 days of storage was treatment C (tapioca+ glycerol 0.05mL) which is 5.05 (logN), the lowest was treatment E (rice flour+ glycerol 0.04mL) which is 3.84 (logN). The highest number of bacteria on 50 days of storage was treatment C (tapioca+ glycerol 0.05mL) which is 4.88 (logN), the lowest was treatment E (rice flour+ glycerol 0.04 mL) which is 3.40 (logN). The highest number of bacteria on 60 days of storage was treatment A (tapioca+ glycerol 0.03mL) which is 5.04 (logN), the lowest was treatment D and E (rice flour+ glycerol 0.03mL and rice flour+ glycerol 0.04mL) which is 4.01 (logN).

Keywords: *Pseudomonad fluorescent*, preservation, carrier

INTRODUCTION

Pseudomonad fluorescent is a group of bacteria that colonize the plant root zone (Rhizobacteria). Utilization of *Pseudomonad fluorescent* is one of the potential alternatives to be developed as biological control agents of plant diseases. According to Saravanan et al., (2004) *Pseudomonad fluorescent* can be isolated from the survace area of plant roots and effectively reduce soil borne diseases. In addition, Bashan and de-Bashan (2002) stated that suggested *Pseudomonad fluorescent* can increase the availability of iron for plants, and produce growth hormones such as auxin, gibberellin, cytokinin, and ethylene.

Pseudomonad fluorescent have been used as a biological agent for several fungi and other plant pathogens. The ability of *Pseudomonad fluorescent* suppress the pathogen

populations are associated with its ability to protect the roots from soil pathogen infection by colonizing the surface of roots and producing chemical compounds such as antifungal and antibiotics as well as the competition on the absorption of Fe (Supriadi, 2000). Several species belonging to the group of *Pseudomonad fluorescent* including *Pseudomonas fluorescens, P. putida, P. aeruginosa* and *P. aureofaciens* (Elat and Chet 1987, cit Karthikeyan et al., 2006)."

P. *fluorescensfrom* the rhizosphere of banana can reduce the discoloration of the vascular tissue of banana due to Fusarium wilt plant disease, and induces accumulation of enzyme resistance at the root. Suppression of pathogens can occur by various mechanisms such as competition for nutrients, root colonization, antibiosis through the production of antibiotics. In addition, *P. fluorescens* can also produce plant growth regulators such as auxin and gibberellins which promote the growt hand yield of banana (Saravanan et al., 2004).

The results of the study of Pauland Sarma (2006) reported some strains of *P. fluorescenscan* dissolve the complex compounds of P in the soil so that is available to plants. N and P were also found more into the black pepper plant tissue after being introduced by *P. fluorescens*. All these factors not only stimulate the roots to the high absorption of nutrients and minerals, but also the health of the roots look much better.

Pseudomonad fluorescent isolates PfPj1 derived from bananas roots can inhibit growth of Blood Disease Bacteria (BDB) which cause blood disease of banana plants and also increase the growth of banana plants (Advinda, 2004). Advinda (2010) alsoreported that the*Pseudomonad fluorescent*isolates PfPj2, PfPj3, and Cas.3 are the best in controlling BDB. Armaleni (2013) stated *Pseudomonad fluorescent* isolates Cas.3 can also inhibit the growth of *Ralstoniasolana cearum* bacterium disease causing wilt tomato plants.

Until now the use of *Pseudomonad fluorescent* generally in the form of suspension of bacterial cells must be multiplicated first in a petri dish in a laboratory. *Pseudomonad fluorescent* which were still in a petri dish do not have a long shelf life. Therefore it is necessary to find a method and proper storage techniques to be easily applied, stored, and used in the field. Storage collection (preservation) *Pseudomonad fluorescent* bacteria aims to keep microbial culture alive, and its characteristics are also expected to remain stable and unchanged.

The effective and simple preservation of microbial can use a variety of carrier that are organic or inorganic. Chotiah (2008) stated that the preservation methods used should minimize loss of viability during the preservation process, so that after preservation, the culture will live for a long time. Suprapti (2005) suggested a carrier in the form of tapioca can be used for preservation of bacteria, because it has rich energy, and has the nutrients needed by the bacteria including carbohydrates, proteins and fats. Sulistiani (2009) reported that rice flour can also be used as a preservation medium for microorganisms. High starch content in rice flour can provide a carbon source or adequate nutrition in bacteria.

The carrier xanthan gum of 20% can keep bacteria *Pseudomonad fluorescent* for two months at a temperature of 40 ° C (Kloeper and Schroth 1981, cit Cook and Baker, 1983). While Sabaratnam and Traquair (2002) using inorganic materials talc as a carrier Streptomyces. These bacteria are able to live 100% in the talc to 14 weeks of storage (temperature 4 °C).

Besides the right technology for the successful preservation of bacteria, the addition of stabilizer need to be used in this technology in order to extend storage period. It is based on research reported by Özaktan and Bora (2004) that the addition of glycerol stabilizer before bacteria *Pantoea agglomerans* strain Eh 24 added talc carrier can maintain viability for four months.

In order to find the right technology for *Pseudomonad fluorescent* preservation, preservation technology has been applied to a carrier using a material that is both organic rice flour and tapioca. Besides the added stabilizer glycerol with different concentrations of *Pseudomonad fluorescent* before mixed with the carrier.

RESEARCH METHOD

Pseudomonad fluorescent isolates used were Cas.3 (collection Advinda 2007). The research used Complete Randomized Design with 6 treatments and 3 replications. The treatments were tapioca (addition 0.03, 0.04, and 0.05 mL of glycerol); and rice flour (addition 0.03, 0.04, and 0.05 mL of glycerol). Observations done against the number of *Pseudomonad fluorescent* on 10, 20, 30, 40, 50, and 60 days of storage. Data analysis used ANOVA significant level of 5% and further testing DNMRT significant level of 5%.

Rejuvenation and Propagationof Pseudomonad fluorescent

Pseudomonad fluorescent Cas.3 homogenized by vortex and rejuvenated in a petri dish on King's B solid medium with streak plate method, and incubated for 48 hours. Inoculum propagation is done by take an ose pure culture in a petri, then cultured in the medium of 25 mL of King's B liquid in 100 mL erlemeyer and shake for 24 hours (preculture). Then 1 mL preculture was then taken and put into 50 ml of King's B liquid medium and shake for 2 x 24 hours (main culture).

Suspension Provision of Pseudomonad fluorescent

*Pseudomonad fluorescent*Cas.3 (main culture) is taken as 1 mL, and then inserted into a test tube containing 9 mL of sterile distilled water. Dilution of the suspension is done up with a population density of 3×10^8 cells/mL (scale 1 Mc. Farland's).

Provision Suspension Pseudomonad fluorescent

Suspension *Pseudomonad fluorescent*Cas.3by 10 mL (population $3x10^8$ cells /mL) was put into a test tube and centrifuged at 3000 rpm for 10 minutes. Supernatant was discarded to obtain pellets. Wet cells (pellet) from *Pseudomonad fluorescent* Cas.3 which is in the test tube was added with glycerol according to treatment that is 0,03 mL, 0,04 mL, and 0,05 mL, then homogenized with a vortex. The pellet was mixed evenly into 1 gram of sterile carrier on heat-resistant plastic, and then incubated at room temperature according to treatment.

Observation

Data were collected for *Pseudomonad fluorescent* bacterial counts at 10, 20, 30, 40, 50, and 60 days of storage

RESULT AND DISCUSSION

Results

The data in Table 1.shows the number of *Pseudomonad fluorescent* bacteria preserved in a carrier tapioca and rice flour and addition of stabilizer glycerol. *Pseudomonad fluorescent* storage for 10, 20, and 30 days of treatment have not shown any significant difference statistically. Statistically significant differences seen in the storage 30, 40, and 60 days.

Table 1. The number of *Pseudomonad fluorescent* on the carrier as well as tapioca and rice flourwith the addition of glycerol (storage of 10 to 60 days)

Treatment	The number of bacteria (log N)					
	10 days	20 days	30 days	40 days	50 days	60 days
A(tapioca+glycerol 0,03 mL)	5,22	5,41	4,87	4,87 ab	4,48 ab	5,04 b
E(tep. beras+glycerol 0,04 mL)	5.32	4,64	3,93	3,84 a	3,40 a	4,01 a
B(tapioka+glycerol 0,04 mL)	5,46	5,48	4,58	4,67 ab	4,63 ab	5,02 b
C(tapioka+glycerol 0,05 mL)	5,47	5,46	4,85	5,05b	4,88 b	4,58 ab
D(tep. beras+glycerol 0,03 mL)	5,56	5,45	4,45	4,44 ab	3,64 ab	4,01 a

F(tep. beras+glycerol 0,05 mL)	5,61	5,06	4,30	4,17 ab	4,00 ab	4,14 a

Discussion

Pseudomonad fluorescent preservation using rice flour and as much as 0.05 mL of glycerol stabilizer generates the highest number of bacteria on storage for 10 days, and the lowest in the preservation using tapioca and the addition of 0.03 mL of glycerol. At 20 days of storage the highest number of bacteria are visible on preservation using tapioca and the addition of 0.04 mL of glycerol, and the lowest in the preservation using rice flour and the addition of 0.04 mL of glycerol. Preservation using tapioca and the addition of 0.03 mL of glycerol showed the highest bacterial counts after 30 days of storage, and the lowest using rice flour and the addition of 0.04 mL of glycerol.

After 40 and 50 days of storage, treatment given showing the significant difference. The highest number of bacteria present in preservation using tapioca and the addition of 0.05 mL of glycerol. This treatment is significantly different compare to preservation treatments using rice and the addition of 0.04 mL of glycerol. After 60 days of storage the highest visible number of bacteria present in preservation using tapioca and the addition of 0.03 mL of glycerol. However, these findings did not show significant differences with treatment using tapioca preservation and addition of 0.04 mL of glycerol and glycerol 0.05 mL.

Vidhyasekaran and Muthamilan (1995) research showed that a variety of carrier have different effects and to maintain populations of bacteria during storage. They found the carrier talc and peat bacteria able to survive up to 240 days, and decreased 30 days later at room temperature storage. Sallam *et al*., (2013) reported the use of talc as a carrier of *P. fluorescens* bacteria is able to maintain the number of bacteria to four months at the storage temperature of 4^0C. In the five months a decline in the number of bacteria occurs, and *P. fluorescens* is no longer found in the sixth month in a carrier.

From the results of this study shows that the addition of tapioca with glycerol is the best stabilizer in maintaining the ability of *Pseudomonad fluorescent* live up to 60 days of storage. Although there was a slight decrease in the number of bacteria up to 50 days of storage, but there was an increase on 60 days of storage.

CONCLUSION AND SUGGESTION

Conclusion

The conclusion of this study is:

a. The number of bacteria at each storage period (10, 20, and 30 days) were not significantly different
b. Significant difference between treatments shown in the storage period of 40, 50, and 60 days.
c. Tapioca with the addition of glycerol is best in maintaining the ability of *Pseudomonad fluorescent* live up to 60 days of storage.

Suggestion

From the research, it is advisable to apply the *Pseudomonad fluorescent* in the carrier tapioca with stabilizer glycerol into the field.

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