The effectiveness of various Rhizobacteria carriers to improve the shelf life and the stability of Rhizobacteria as Bioherbicide

T C Rakian¹, L Karimuna¹, M Taufik², G A K Sutariati¹, Muhidin¹ and U Fermin¹

¹Department of Agrotechnology, Faculty of Agriculture, Universitas Halu Oleo, Kendari, Southeast Sulawesi 93212, Indonesia
²Department of Plant Protection, Faculty of Agriculture, Universitas Halu Oleo, Kendari, Southeast Sulawesi 93212 Indonesia
Email : tresjia_junus@yahoo.com

Abstract. Deleterius Rhizobacteria (DRB) has a potential to control of weed and act as a bioherbicide. Developing a method to weed control that environmentally sound friendly has been increasingly studied. Rhizobacteria can form colonies on weed rooting and synthesize the secondary metabolite compounds. The effectiveness of rhizobacteria as bioherbicide is determined by its survival to be stored for a long time. The objective of this study is to obtain the type of carrier which effectively maintains the life and stability of DRB. Therefore it is necessary to do in vivo and in-vitro research. This study consists of two stages of testing the effectiveness of the carrier in increasing the shelf life of rhizobacteria and testing the effectiveness stability as a bioherbicide on *Ageratum conyzoides* weed after storage for 20 weeks. Research was conducted in Agronomy Lab, Agriculture Faculty, Halu Oleo University Kendari, Since August to December 2016. Research found that the talc powder and chaff charcoal powder were effective as a carrier of rhizobacteria and able to maintain the viability of rhizobacteria *Bacillus lentus* A05 and *Pseudomonas aeruginosa* A08 for five months and also able to maintain the stability of rhizobacteria as bioherbicide.

1. Introduction

Weed control is generally done conventionally using synthetic chemicals that is herbicide. The use of herbicides is considered as the most appropriate and fast method, because it is very effective to suppress the growth of weeds. However, continuous use of synthetic herbicides will have a negative effect on the environment, human health, and can cause weeds to become resistant to herbicides [1-3]. To reduce the negative effects of using synthetic herbicides in weed control is by developing biological controls using microorganisms or biological agents to regulate weed population density [4].

Microorganisms are used as bioherbicides because they do not have a negative impact on the environment, are effective during plant lifetimes and some agents can produce compounds that function as growth hormones thereby providing multiple benefits to the plants [5-6]. Rhizobacteria that have the ability to inhibit the growth of weeds are called deleterious rhizobacteria (DRB), which is a non parasitic bacteria, capable of forming colonies on weed roots and synthesizing secondary metabolite compounds [7].

The effectiveness of rhizobacterial applications as bioherbicides in the field is determined by the survival of rhizobacteria both in storage and in the field. Application of field rhizobacteria in the form of suspension will be ineffective due to unsupportive environmental factors, such as very high solar radiation, rainfall, and nutrients for rhizobacteria for survival in both storages and field.
Therefore, rhizobacteria applications should be packed in a carrier material that does not contain toxins and does not affect the ability of these rhizobacteria as the bioherbicide. The formulations of *P. putida* strain 30 and 180 in the talc carrier can still live in observation for six months with a density of colonies greater than 1 x 10⁸ cfu g⁻¹ [8]. In addition to *P. putida*, research suggests that *B. subtilis* in a talk formulation can still live with a density of 1.0 x 10⁷ cfu g⁻¹ colonies on observation 45 days after application [9].

2. Materials and Methods
The study consisted of two stages of testing the effectiveness of the carrier in increasing the shelf life of rhizobacteria as the first stage. Furthermore, in the second stage of rhizobacteria originating from storage for 20 weeks, it was taken and tested its stability as a bioherbicide on *A. conyzoides* weed. Both stages of this study were conducted on a laboratory scale. Research was conducted in Lab of Agronomy Faculty of Agriculture Halu Oleo University Kendari, from August to December 2016.

2.1. Effectiveness of carrier in the increasing shelf life of rhizobacteria
The test was performed in a formulation, prepared by adding a carrier material (talc, bentonite, kaolin and ground burned rice husk) of 50 g each into 50 ml of a bacterial suspension with a density of 10⁷ ml⁻¹, then mixed evenly for 10 minutes. After that, it is inserted into a bottle of formulation and stored at room temperature (22°C- 24°C). Determination of life bacteria in the formulation material is done every 14 days with spread method. The bacterial formulation of 1 g was diluted with sterile aquades until 10⁻¹⁰ dilution. Furthermore, bacterial cultures of each formulation dilution of the formulation material are spread on TSA media. At the first 14 days observation, the dilution was carried out to 10⁻¹⁰ then dispersed at dilution levels of 10⁻⁹ and 10⁻¹⁰. It aims to see if bacteria still grow on the dilution. Each further observation remains dilution until level 10⁻¹⁰, except in the dilution there is no growing colony, the next observation is dilution at one level below (10⁻⁹, 10⁻⁸, and so on). The observation of rhizobacterial life ability in each formulation was done until the age of 5 months in storage.

2.2. Stability of rhizobacteria in carrier as bioherbicide
Rizobacterial isolates that have been stored in various carrier materials for 20 weeks are taken and dispersed in a petri dish. After 24 hours, rhizobacteria were grown using ose needle, then rizobacteria was reproduced by growing in TSA medium and incubated for 48 hours, then made in a liquid suspension with a cell density of 10⁶ml⁻¹. Test of the ability of rizobacterial isolates as bioherbicides was performed by seed biopriming treatment on *A. conyzoides* seeds, disinfected seeds were inserted into rizobacterial isolate suspension and incubated for 24 hours using shaker. Weed seeds *A. conyzoides* which have been incubated in suspension for 24 hours Is added in a plastic plate which is dampened with a dampened sterile CD in an oven with 121°C for 24 hours, each cup planted with 25 weed seeds then placed in a germinator.

3. Results and discussion
3.1. Effectiveness of carrier in the increasing shelf life of rhizobacteria
The growth patterns of rhizobacterial isolates of *B. lentus* A05 and *P. aeruginosa* A08 on various carrier types for 20 WAA (weeks after application) showed that the growth of DRB isolate up to the shelf life of 4 WAA experienced a logarithmic phase of a rapidly increasing population of DRB isolates. Furthermore, in the shelf life of 6 WAA, the growth of DRB isolates tended to have decreased the number of bacterial colonies up to the shelf life of 20 WAA. Observations on the shelf life of 2 to 20 WAA indicate that the isolate of *B. lentus* A05 can live on various carriers such as talc, bentonite, kaolin and ground burned rice husk (figure 1). As for the isolate rhizobacteria *P. aeruginosa* A08, able to live for 20 MSA in talc carrier material, bentonite and ground burned rice husk, but on kaolin carrier with a shelf life of 10 WAA have no live bacteria.
Figure 1. The growth model of *Bacillus lentus* A05 and *Pseudomonas aeruginosa* A08 in various rhizobacterial carriers.

The talc carrier material is called magnesium silicate (Mg₃Si₄O₁₀(OH)₂) and is available in powder form [10]. Chemical composition of Mg₃Si₄O₁₀(OH)₂ is magnesium 26.28%, silicon 10.10%, oxygen 63.36% and 0.36% hydrogen so that talc is a carrier that can provide nutrients for rhizobacterial viability, especially the fulfillment of nutrients O and Mg. Rhizobacteria need nutrients of C, O, N, H, P, and S, for element O required about 20%. Besides it also needed micro elements such as Mg [11]. The talc carrier is an appropriate material compared to tapioca starch, chitosan and corn flour to *Bacillus* sp [12].

The best carriers for *B. lentus* A05 and *P. aeruginosa* are also shown by ground burned rice husk. Analyzed of ground burned rice husk showed that the moisture content contained in it was 7.40%, C-organic was 7.51%, N total was 0.49%, P₂O₅ was 0.07%, K₂O 0.08% with CEC 88.08 cmol kg⁻¹ and the pH of H²O was 6.73 [13]. The content contained in ground burned rice husk is very suitable with the nutritional needs of rhizobacteria both *B. lentus* A05 and *P. aeruginosa*. Microbes need a source of key nutrients such as carbon, protein, water, vitamins, and minerals to support its growth [11].

3.2. Stability of rhizobacteria in carrier as bioherbicide

The carrier of rhizobacteria (talc, bentonite, and ground burned rice husk) in addition to improving the survival of rhizobacteria in storage for five months can also maintain the stability of rhizobacteria as bioherbicides. The results showed that *P. aeruginosa* A08 and *B. lentus* A05 derived from the carrier were still capable of suppressing the weed of *A. conyzoides* when compared with the control. However, the best treatment was demonstrated by the isolate *P. aeruginosa* A08 using talc and ground burned rice husk powder as the carrier. *P. aeruginosa* derived from the carrier of talc and ground burned rice husk, can inhibit germination of *A. conyzoides*, 77.92% (figure 2), inhibit root length of 79.53% and 81.89% respectively (figure 3). The talc and ground burned rice husk can maintain the stability of *P. aeruginosa* as bioherbicide.
Figure 2. Effect of rhizobacterial isolate *Bacillus lentus* A05 and *Pseudomonas aeruginosa* A08 on vigor of *Ageratum conyzoides*.

*P. aeruginosa* A08 isolate has a biochemical characteristic that produces HCN (*hydrogen cyanide*) with 5.26 ppm concentration [14-15]. Another research showed that *P. putida* produces HCN compounds that have capable to inhibit the growth of weeds *Abutilon theophrasti* [16]. *P. fluorescens* also can...
inhibit the growth of downy brome weed (Bromus tectorum L. Brote) to 86%, because _P. fluorescens_ rhizobacteria produce hydrogen cyanide and indole-3-acetic acid [17]. Cyanide is a growth inhibitor that has been shown to play a role in plant respiration, carbohydrate metabolism, CO₂ and nitrate assimilation. HCN produced by DRB isolates in weed seed rhizosphere can cause phytotoxicity similar to that of herbicide. The isolate _B. lentus_ A05 can also inhibit the germination and length root of _A. conyzoides_ and produce high amounts of IAA compounds [14-15]. The mechanism inhibition of downy brome weeds by _P. fluorescens_, in addition to producing HCN is also due to the production of IAA (indole-3-acetic acid) with high concentrations [12].

4. Conclusions

The best carriers for rhizobacterial isolates potentially as bioherbicides were talc and ground burned rice husk; this is because the carrier can retain the shelf life of _B. lentus_ A05 and _P. aeruginosa_ A08 to 20 WAA. In addition to maintaining shelf life, the carrier can maintain the stability of rhizobacteria as bioherbicide, especially in _A. conyzoides_.

References

[1] Kughur P G 2012 The effects of herbicides on crop production and environment in makurdi local government area of Benue State Nigeria _Journal of Sustainable Development in Africa_ 14(4) 206-216
[2] Mada D, Duniya N and Adam I G 2013 Effect of continuous application of herbicide on soil and with crop protection machinery in Southern Adamawa State. _International Refereed Journal of Engineering and Science_ 2(6) 44-49
[3] Khan M S, Chaudhry P, Wani P A and Zaidi A 2006 Biotic effects of the herbicides on growth, seed yield, and grain protein of green gram. _J. Appl. Sci. Environ._ Mgt. 10(3) 40-46
[4] Sembel D T 2010. Pengendalian Hayati Hama-hama Serangga Tropis dan Gulma. Penerbit Andi Yogyaarka. p282.
[5] Silva H S A, Romeiro R S R, Macagnan D, Vieira B A H, Pereira M C B and Mounteer A 2004 Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities _Biol Control_ 29 288-295
[6] Yan Z, Ryu C M, McIntoy J, Reddy M S, Woods F, Wilson M and Kloepper J W 2002. Induction of systemic resistance against tomat late blight by PGPR _Phytopathology_ 92(12) 1329-33
[7] Kremer R J and Kennedy A C 1996 Rhizobacteria as biocontrol agent of weed _Weed Technol._ USA 10 601-609
[8] Bora T, Ozakhtan H, Gore E and Aslan E 2004 Biological control of _Fusarium oxysporum_ sp. Melonis by wettable powder formulations of the two strains of _Pseudomonas putida_. _J. Phytopathology_ 1529(7) 471-75
[9] Amer G A and and Utkhode R S 2000 Development of formulations of biological agents for management of root rot of lettuce and cucumber. _Can. J. Microbiol_ 46(1) 809-16
[10] Nakkkeeeran S, Fernando W G D and Siddiqui Z A 2005 _PGPR: Biocontrol and Biofertilization_. Chapter 10. Plant Growth Promoting Rhizobacteria Formulations and Its Scope in Commercialization for the Management of Pests and Diseases. (Netherlands) pp: 257-296.
[11] Rahayu W P and Nurwirita C C 2012. _Mikrobiologi Pangan_. (Bogor: Penerbit IPB Press) 132p
[12] Devi H S, Munir E and Priyani N 2015 _Viabilitas Bacillus sp. BK17 pada Berbagai Bahan Pemabawa_ (Medan: Fakultas MIPA Universitas Sumatera Utara Medan)
[13] Nurbaity A, Setiawan A and Mulyani O 2011 Efektivitas arang sekam sebagai bahan pembawa pupuk hayati mikoriza arbuskula pada produsik sorgum. _Agrinimal_ 1(1) 1-6
[14] Rakian T C, Karimuna L, Taufik M, Sutariati G A K, Muhidin, Pasolon Y B 2015 The effectiveness of indigenous rhizobacteria as bioherbicide to control of weed _Australian Journal of Basic and Applied Sciences_ 9(31) 707-11
[15] Rakian T C 2015 _The Development of Deleterius Rhizobacteria as A Biological Control of_
Agent of Weed (Bioherbicide) on Soybean Plant (Glycine max L Merril) (Kendari : Dissertation on Halu Oleo University Kendari)

[16] Biswas M M, Zdor R and Miller C 2009 Using Gene Fusions to Study Cyanogenesis in a Weed Deleterious Rhizobacterium Proceedings IXth International Bioherbicide Group Workshop p20-22

[17] Kennedy C and Johnson B N 2001. Host range of a deleterious rhizobacterium for biological control of downy brome Weed Science 49 792–97