The 2nd International Conference On Indigenous Knowledge For Sustainable Agriculture 2023
ISBN : 978-623-161-062-1

ACTIVITY OF PHOSPHATE SOLUBILIZING RHIZOBACTERIA ISOLATES WITH BIOCHAR CARRIER IN DIFFERENT STORAGE PERIOD

Eko Hary Pudjiwati[1], Nunuk Purwati[1]

[1]Agriculture Faculty, Tarakan Borneo University, Indonesia

*email : eko.pudjiwati@borneo.ac.id

ABSTRACT

Phosphate Solubilizing Bacteria is one way to increase the availability of P element in the soil. The application of phosphate solubilizing bacteria requires a carrier material that can maintain the viability of the inoculum and its metabolic activity within a certain period of time. This study aims to determine the activity of native phosphate solubilizing rhizobacterial isolates on biochar carriers and different storage times. The experimental design used was a completely randomized design with two factors and two replications. The first factor is the type of biochar, namely M1=coconut shell biochar and M2= palm shell biochar. The second factor consisted of R0 = without phosphate solubilizing bacteria isolates, R1 = BP1 isolates, R2 = BP2 isolates, and R3 = BP1 + BP2 isolates. The results showed that biochar can be used as a carrier for phosphate-dissolving rhizobacteria without losing its activity in dissolving phosphate. Coconut shell and palm shell biochar did not give a significant difference in the phosphate solubility activity of isolates BP1 and BP2, as well as the BP1+BP2 consortium until the shelf life was 90 days. Phosphate Dissolving Index values range from 2.25 to 2.88 in the medium category

Keywords:

biochar, carrier material, phosphate solubilizing bacteria, phosphate solubilization index,

INTRODUCTION

Phosphorus (P) fixation in the soil causes the availability of P to be low, while this element is a macronutrient that is needed by plants in large quantities. P fertilization becomes inefficient because most of the P elements given to the soil are fixed. To increase the availability of soil P, phosphate solubilizing bacteria (BPF) can be used, namely a group of bacteria capable of converting insoluble phosphate in the soil into a soluble form. The mechanism of dissolving phosphate compounds by phosphate solubilizing bacteria takes place chemically and biologically for both organic and inorganic phosphate forms. Phosphate solubilizing bacteria produce a number of organic acids such as formic, acetate, propionate, lactate, fumarate, oxalate, tartaric,

ISBN : 978-623-161-062-1
Managed By: Faculty Of Agriculture, University Of Borneo Tarakan

glutamic and succinic [1]. These organic acids chelate Al, Fe, and Ca which makes phosphate released from the AlPO4.2H2O, FePO4.2H2O, and Ca3(PO4)2 bonds, so that the level of phosphate (dissolved) in the soil increases [2]. Biological dissolution of phosphate occurs because these microorganisms produce several enzymes, namely phosphatase enzymes [3] and phytinase enzymes [4]. Phosphatase enzymes can decompose phosphate bound by organic compounds into available forms.

The application of phosphate solubilizing bacteria requires a carrier material, namely a material that can be used as a habitat or place for inoculum life before being applied with the aim of maintaining the viability of the inoculum for a certain period of time. The carrier material used must have the ability to activate microbes so that they can grow and develop when used. The carrier material can be solid or liquid. A good carrier material must be non-toxic, have a high water holding capacity, be available in large quantities, be easy to produce, and be able to maintain the growth and survival of bacteria [5].

Biochar can be used as a carrier for phosphate solubilizing bacteria. As a carrier material, biochar can provide a good habitat and increase the activity of microorganisms [6]. Biochar can be obtained from various sources of materials, such as palm shells and coconut shells. The results of the study [7], oil palm shell biochar as a carrier material is suitable as a habitat for bacteria and can maintain bacterial populations during a shelf life of 3-9 months. Coconut shell biochar was able to maintain phosphate-solubilizing microbial populations in a 3-month shelf life compared to corn cob and rice husk biochar [8].

The activity of phosphate solubilizing bacteria is influenced by the type of carrier and shelf life. Testing of phosphate dissolving activity on a laboratory scale is indicated by the formation of a clear zone (holozone). The larger the holozone formed, the stronger the ability of the bacteria to dissolve bound phosphate (P) [9]. This study aims to determine the activity of indigenous phosphate solvent rhizobacteria isolates on biochar carrier materials and different storage times. Phosphate solubilizing rhizobacterial isolates used were the results of previous studies, namely BP1 and BP2 isolates.

METHOD

The research was conducted at the Laboratory of Plant Protection, Faculty of Agriculture, University of Borneo Tarakan. The main ingredients used in this study were phosphate-solubling rhizobacterial isolates, namely BP1 and BP2, palm shell and coconut shell biochar, NB and Pikovskaya media, physiological solution (0.85% NaCl), distilled water and 70% alcohol. The main tools in this research are Laminair Air Flow, autoclave, spectrophotometer, petridish. The experimental design used was a two-factor completely randomized design with 2 replications. The first factor is the type of biochar, namely M1=coconut shell biochar and M2=oil palm shell biochar. The second factor consisted of R0 = control, R1 = BP1 isolate, R2 = BP2 isolate, and R3 = BP1 + BP2 isolate. In order to obtain 8 treatment combinations.

The research implementation began with the preparation of biochar as a carrier, preparation of inoculums (BP1 and BP2 isolates), inoculation, parameter observation and data analysis. Coconut shell biochar and palm shell biochar are processed by

ISBN : 978-623-161-062-1
Managed By: Faculty Of Agriculture, University Of Borneo Tarakan

pyrolysis method at 400°C. The biochar is mashed and filtered using a 100-200 mesh sieve, then put into heat-resistant plastic, each weighing 35 grams. Biochar was sterilized using an autoclave at 121ºC and 1 atm pressure for 15 minutes.

Preparation of inoculum and inoculation of carrier material. First inoculate each bacterial isolate from NA medium aseptically as much as 1 ose into a 250 mL Erlenmeyer flask containing 200 mL of sterile Nutrient Broth (NB) medium, then incubate in a shaker at 150 rpm, temperature 28°C, for 2 x 24 hours. Carrier inoculation was carried out by injecting 15 ml of phosphate-dissolving rhizobacterial isolate according to treatment. Biochar that had been inoculated with phosphate solubilizing rhizobacterial isolates was stored at 28℃ with a shelf life of 7, 30, 60, and 90 days.

Observations were made on all treatment combinations for the Phosphate Dissolving Index parameter as an indicator of the activity of phosphate solubilizing rhizobacteria at storage periods of 0, 7, 30, 60 and 90 days. Testing the solubility index of Phosphate (P) by bacteria was carried out by placing one ose of bacterial colonies on Pikovskaya Agar media. Observations were made after incubation for 72 hours at room temperature. The ability of the isolates to dissolve Phosphate (P) was tested qualitatively based on the size of the clear zone formed around the colony.

The following is the calculation of the Phosphate Dissolving Index (PDI):

$$\textbf{PDI} = \frac{\textbf{Holozone Diameter+Colony Diameter}}{\textbf{Colony diameter}} \textbf{ X 100}$$

Data obtained in the Data Normality Test, Analysis of Variance (ANOVA) and continued with the Duncan Multiple Range Test (DMRT) at the 5% level of confidence. Data analysis using the SPSS 26.0 program.

RESULT AND DISCUSSION

The results showed that phosphate solubilizing rhizobacterial isolates BP1, BP2 and BP1+BP2 were able to qualitatively dissolve phosphate in solid pikovskaya media, as indicated by the presence of a clear zone. Whereas in the treatment without phosphate solvent rhizobacteria isolate, there was no clear zone. The holozone (clear zone) occurs when organic acids are excreted by bacteria which then bind to Ca ions from Ca3(PO4)2 sources in Pikovskaya media and liberate phosphate ions (H2PO4-, HPO42-, and PO43-) to form a clear colored area [10].

The activity of phosphate solubilizing bacteria is influenced by the type of bacteria, carrier material and storage time. At all shelf lives, the carrier material (type of biochar) did not provide a significant difference in the ability to dissolve phosphate for all isolates of phosphate-solubling rhizobacteria except for the shelf life of 30 days (Table 1). The ability of bacteria to dissolve phosphate is indicated by the Phosphate Dissolving Index (PDI) value. Statistically, there was no significant difference in the PDI values of the isolates BP1, BP2 and the BP1+BP2 consortium with coconut shell and oil palm shell biochar carriers for almost all shelf lives. PDI values ranged from 2.25 to 2.88. The difference in PDI values was due differences in the ability of phosphate solubilizing rhizobacterial isolates to produce types and amounts of organic acids. Organic acids produced by phosphate solubilizing

ISBN : 978-623-161-062-1
Managed By: Faculty Of Agriculture, University Of Borneo Tarakan

bacteria include citric, glutamic, succinic, lactic, oxalic, malic, fumaric and tartaric acids [11].

Table 1. Value of Phosphate Dissolving Index (PDI) Combination of Biochar Type and Phosphate Solubilizing Rizobakteri Isolate Type

Treatment	PDI 0 days	PDI 7 days	PDI 30 days	PDI 60 days	PDI 90 days
M1R0	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
M1R1	2,43 b	2,52 b	2,47 bc	2,45 b	2,25 b
M1R2	2,44 b	2,61 b	2,59 c	2,42 b	2,44 b
M1R3	2,54 b	2,43 b	2,88 d	2,32 b	2,37 b
M2R0	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
M2R1	2,41 b	2,44 b	2,85 d	2,33 b	2,34 b
M2R2	2,63 b	2,54 b	2,33 bc	2,48 b	2,48 b
M2R3	2,36 b	2,59 b	2,31 b	2,54 b	2,25 b

Note: numbers followed by the same letters are not significantly different based on the 5% DMRT Test.

There was an interaction on the Phosphate Dissolving Index value at 30 days old, indicating that the type of biochar will affect the ability of rhizobacteria isolates in phosphate dissolving. At the shelf life of 30 days the M1R3 and M2R1 treatments showed the highest PDI value compared to the other treatment combinations. But if you look at the PDI value of each treatment combination at the ages of 0, 7, 30, 60 and 90 days (Figure 1), it shows that there was no significant change in value. The PDI value of this study is in the medium category. This is in accordance with what was stated [12], the ability to dissolve phosphate in the high category if it has a phosphate solubility index value of >4, moderate 2-4, and low <2.

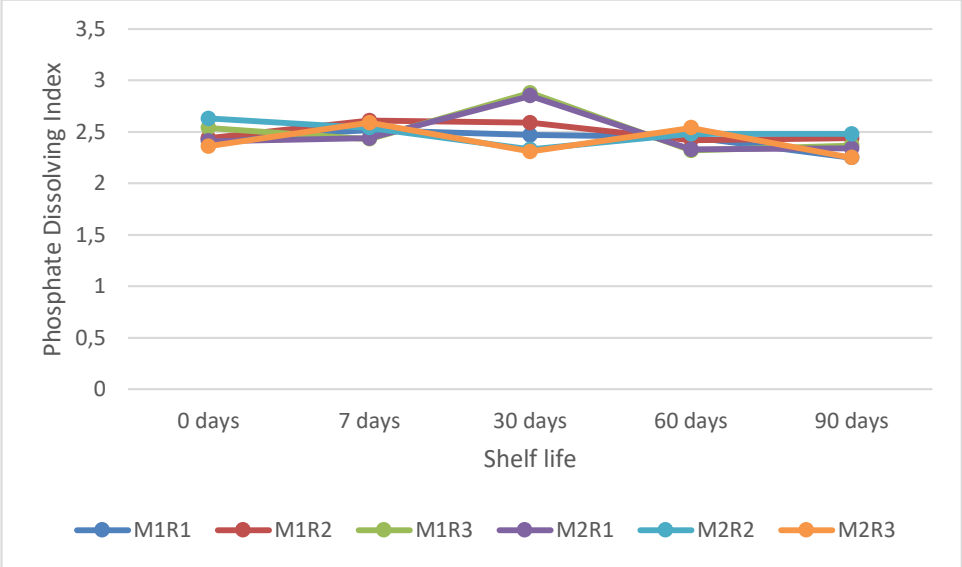

Figure 1. Changes in the PDI Value for 90 days

The insignificant change in PDI value during the shelf life of up to 90 days means that coconut shell biochar and palm shell biochar can be good carriers for phosphate solubilizing rhizobacteria. The characteristics of biochar with its fine particles have greater availability of nutrients [13], the porous

ISBN : 978-623-161-062-1
Managed By: Faculty Of Agriculture, University Of Borneo Tarakan

structure of biochar and high porosity provide a suitable habitat for bacteria, so that the bacteria remain alive and metabolically active for a long time [14].

CONCLUSION

Biochar can be a carrier for phosphate-dissolving rhizobacteria without losing its activity in dissolving phosphate. Coconut shell and palm shell biochar did not give a significant difference in the solubility activity of phosphate isolates BP1 and BP2, as well as the BP1+BP2 consortium until the shelf life was 90 days. Phosphate Dissolving Index values range from 2.25 to 2.88 in the medium category.

ACKNOWLEDGMENTS

The author would like to thank the Dean of the Faculty of Agriculture, University of Borneo Tarakan who has provided funds for the publication of this scientific paper, as well as to those who assisted in conducting the research.

REFERENCES

[1] Istina, IN, Nurhayati and Jakoni. 2019. Indigenous Phosphate Solubilizing Microbes on Soil Productivity Enhance at Riau Province. Journal of Agricultural Dynamics Special Edition Number 3: 27–34.

[2] Widhianingrum, W. 2008. Activity of Phosphate Solubilizing Bacteria in Carrot (Daucus Carota L.) Planting with Various Balances of Organic Fertilizers and Inorganic Fertilizers in Tawangmangu Andisols. Thesis. Faculty of Agriculture, Eleven March University, Surakarta.

[3] Lynch, J.M. 1983. Soil Biotechnology: Blackwell Sci. Pubs. Co. London. 1-199.

[4] Alexander, M. 1977. Introduction to Soil Microbiology. 2nd Ed., John Willey and Son, New York. 467.

[5] Bachtiar, T., Anas, I., Sutandi, A., and Ishak. 2019. Quality Improvement of Rhizobium Carrier Materials and Phosphate Solubilizing Fungi through Gamma Ray Sterilization Co-60 and Effect on the Growth and Production of Soybean (Glycine max L.). Ganendra Nuclear Science and Technology Journal, 22(1):11-23.

[6] Arifin, Z and Suyani, IS. 2022. Use of biochar and nitrogen fertilizers to increase the growth and yield of mustard pakchoy plants on sub-soil (sub-soil). Agrovigor: Journal of Agroecotechnology, 15(1):54–62.

[7] Santi, LP and Goenadi, DH. 2010. Utilization of Bio-char as a microbial carrier for Ultisol Soil Aggregates from Taman Bogo-Lampung. Plantation Tower. 78(2): 52-60.

[8] Husna, M., Sugiyanta., and Pratiwi, E. 2019. The Ability of the Bacillus Consortium on Biofertilizers to Fix N2, Dissolve Phosphate and Synthesize Indole 3-Acetic-Acid Phytohormones. Land and Climate. 43(2): 117-125.

[9] Widawati, S., Suliasih, and Muharam, A. 2010. The Effect of Compost Enriched with Nitrogen Fixing Bacteria and Phosphate Solvents on Pea Plant Growth and Activity Phosphatase Enzyme in Soil. J. Hort. 20(3):207-215.

ISBN : 978-623-161-062-1
Managed By: Faculty Of Agriculture, University Of Borneo Tarakan

[10] Oksana., Irfan, M., Fianiray, A.R. and Zam, S.I. 2020. Isolation and identification of phosphate solubilizing bacteria in the soil of Rumbai District, Pekanbaru. Agrotechnology Research 4(1):22-25. doi:10.20961/agrotechresj.v4i1.36063

[11] Allapragada, P dan Seshachala, U. 2012. Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India. Turk J Biol 36: 25-35. doi:10.3906/biy-1012-160.

[12] Marra., L.M., de Oliveira, S.M., Soares, C.R.F.S. and de Souza Moreira, F.M. 2011. Solubilisation of inorganic phosphate by inoculant strains from tropical legumes. Science Agriculture 68(5):603-609, doi:10.1590/S0103-90162011000500015.

[13] Chen, J., Li, S., Liang, C., Xu, Q., Li, Y.,Qin, H., & Fuhrmann, J. J. 2017. Response of Microbial Community Structure and Function to Short-Term Biochar Amendment in an Intensively Managed Bamboo (Phyllostachys praecox) Plantation Soil: Effect of Particle Size and Addition Rate. Science of the Total Environment, 574: 24-33.

[14] Glodowska, M. 2014. Biochar as A Potential Inoculant Carrier for Plant Beneficial Bacteria. Thesis. Macdonald Campus of Mc Gill University.