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Screening of Fungal Rot Isolates from Cocoa as Phosphate-Dissolving and Their Growth Ability on Three Types of Media

Iradhatullah Rahim [1], Tutik Kuswinanti [2*], Laode Asrul [3], Burhanuddin Rasyid [4]

[1] Faculty of Agriculture, Animal Husbandry, Fisheries, Muhammadiyah Parepare University, South Sulawesi
[2] Department of Plant Pests and Diseases, Fakulty of Agriculture, Hasanuddin University, Makassar
[3] The Environmental Study Center, Hasanuddin University, Makassar, South Sulawesi
[4] Departement of Soil Science, Faculty of Agriculture, Hasanuddin University, Makassar,

ABSTRACT

This paper discuss about the potential of fungal rot isolates as phosphate-dissolving fungi and their vegetative growth ability on three solid media. All fungal rot isolates were collected from decayed cocoa plants in Bila Village, Pitu Riase, Sidrap District, South Sulawesi. The potential to dissolve phosphate was examined on Pikovskaya broth media and measured using spectrophotometer. The Vegetative ability to grow on solid media was tested on Potato Dextrose Agar (PDA), Malt Pepton Agar (MPA), and Malt Extract Agar (MEA). The results showed that the highest quantitative ability to dissolve phosphate was observed on fungal rot isolate BPB, followed by JT, BPG, and BPE1 isolates. MPA medium supported the best mycelial growth compared with others media.

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Key words: Phosphate dissolving fungi, fungal rot isolates, growth media.

INTRODUCTION

Efforts on the agricultural sector activities produces abundant agricultural waste as a by-product. Waste can be straw, srover, stem, leaf, coat of berry, bran, husks, and remaining of pruning.

* Corresponding author
E-mail address: koeswinanti@yahoo.co.id

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These abundant agricultural waste that still not used. It can be serve as a source of organic matter and nutrients that are beneficial to the plant. Organic waste materials can be absorbed perfectly when it degraded completely. Factors that play an important role in the decomposition of litter is the climate, environmental conditions, and the presence of microorganisms. Climate factors include rainfall, humidity, the sunlight intensity, and temperature. The environmental conditions factor are the temperature of the water, the pH of the water, the salinity of the water and the others. In the decomposition process, all these factors interacting with each others. There are associations between physical factors and biological factors. Biological factors have a greater role than physical factors. Biological factors are affected by a host of fungi and bacteria. However, fungi have a greater ability than bacteria to break down the remains of plants (cellulose, hemicellulose, and lignin), could soon make soil organic matter decompose to a simple organic compound. It serves as the primary ion exchanger and release nutrients around plant [2,3,4].

The relationship between organic matter and plant growth can be directly or indirectly. Organic material of natural substrates for microorganisms saprophytic and indirectly provide nutrients for the plants through the activity of microorganisms. During the process of decomposition of organic material, the resulting organic acids such as fulvic and humic acid. Humic acid and fulvic acid is a part that has a large role in chemical reactions as a part of the organic material. Phosphorus deficiency may occur in plants growing on cultivated lands containing phosphate in sufficient amounts. This happens because the plants can only absorb of phosphorus in a form that is available. The form of phosphate in soil can available through the secretion of organic acids by microbes. Microbes may also release inorganic phosphate that can dissolve into the soil through decomposition event. In addition an increase in uptake of micro elements by plants with organic humus that grant. Some types of rot fungi are able to absorb phosphate [5]. However his ability varies greatly depending on the type, power and capability, adaptations to life in different environments. Microbial phosphate from the specific soil if inoculated on other land not necessarily maintain the ability of phosphate dissolving [6,9].

MATERIALS AND METHODS

Isolation of Fungal Rot

Isolates of rot fungi was obtained from decayed stems cacao in central of cocoa cropping the Bila village of Pitu Riase, Sidrap district, South Sulawesi. The fruiting body of 10 kinds of rot fungi were stored in the paper bag until it isolated in the laboratory. Pieces of fungal fruit bodies (1 cm x 1 cm in size) were surface sterilized with 70% alcohol, rinsed 2 times with sterile water and placed on to sterile filter paper. Each piece (ø 7 mm) cultivated aseptically on PDA medium and incubated at room

temperature. After subculturing and purification, isolates are then coded according to the name of place of origin [7].

Testing of Isolates as Phosphate-Dissolving Fungi

The isolates were tested for their quantitative ability in solubilizing phosphate by using Pikovskaya broth medium with $Ca_3(PO_4)_2$ as the phosphate source. Materials of Pikovskaya broth medium are glucose 10 g; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2 SO_4$ 0,5 g; $MgSO.2H_2O$ 0,1 g;MnSO4 25 mg; $FeSO_4$25 mg; KCl 0,2 g, yeast extract 0,5 g; and agar 15 g, dissolved in sterile water until volume of 1 l [8,9]. Pipette 30 ml of the suspension and put in the Erlenmeyer, contain Pikovskaya broth medium, and incubated in rotary shaker at 150 rpm for 7 days. Filter the 20 ml culture with filter paper Whatman No. 42. Filtrate was centrifugated at 1000 rpm for 15 minutes, 5.0 ml of supernatan then poured into test tubes, added with 0.5 ml of concentrated reagents P (12 g ammonium molibdat, 0.277 g kalium antimoltartat) and Reagent dye concentrated (0.53 g ascorbic acid), shaked for a few minutes, and let it standed for 30 minutes. The absorbance of solution was measured with the spectrophotometer at a wavelength of 693 nm. In the same way was done in the Erlenmeyer flask containing Pikovskaya broth medium uninoculated fungi as a control [10].

Growth Ability on Three Different Media

Observation on growth rate of isolates was revealed on three different media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), dan Malt Peptone Agar (MPA), on petridish (Ø 9 cm). The 7 mm mycelium disk of each isolates was cultured on PDA (30 g PDA/L distilled water), MEA (15 g of malt extract, 16 g agar/ L destilled water), and MPA. (15 g of malt extract, 20 g glucose, 5 g of Peptone and 16 g of agar/ L of destilled water) [5]. Isolates were incubated at room temperature. Growth rate of each isolates was measured based on the colony diameter daily until 7 days.

RESULTS AND DISCUSSION

Isolation of Rot Fungi

Nine isolates were successfully cultivated from the fruit body of the rot fungi, namely BSA, BPB, BPC, BPD, BPE1, BSF, BPG, BPH, and JT. Color and morphological characters of isolates are different each other. Variation of color among isolates are pure white, whitish, yellowish, orange and grayish. These color differentiation may be caused by genetic factors or due to environmental and medium conditions. The isolates can be due to variations in addition to genetic factors as well as by environmental conditions in a sampling area and on carbon sources including growth medium, temperature, and pH. The Carbon sources can be obtained in the form of organic and inorganic.

Organic shapes include amino acids, organic acids, polyalcohol and others organic shapes. While the form of inorganic carbon and gases including CO [11,12].

Phosphate-Dissolving Test

Quantitative test results of phosphate dissolving ability of various rot fungi isolates with $Ca_3(PO_4)_2$ as a source of phosphate showed varies between isolates (Table 1). The highest dissolving phosphate was observed on BPB and the lowest one was on BSF isolate. However, all isolate can dissolve phosphates from the source of the phosphate.

Table 1. Solubizing phosphate of $Ca_3(PO_4)_2$ by varies of rot fungi isolates from cocoa

Isolates	pH value	Phosphate-dissolving level (mg/l)
BSA	5.86	2.474
BPB	5.69	3.198
BPC	5.43	2.490
BPD	5.35	2.385
BPE1	5.55	2.927
BSF	5.76	2.036
BPG	6.04	3.073
BPH	5.89	2.510
JT	5.27	3.094

Table 1. showed that pH of culture filtrate varies between 5.27- 6.04. These tend to be acidic and allows for growth of the rot fungi. The optimal pH range for the growth of the *Pleurotus spp.* mycelium, is 5.5% - 6.5 [13]. Often mold spores grow in the substrate with a optimum pH of 4.5-7.0 [14]. The difference of the optimum pH medium value as the pH for the growth of mushrooms varies between strains or among species [15]. Decrease of pH in Pikovskaya broth media caused by organic acids produced by fungus. Organic acids are very useful mainly to increase solubility of phosphate. The higher organic acid is produced, the higher the phosphate dissolved [16,17,11]. Oyster mushroom which is one of the rot fungus, its growth was strongly influenced by pH. If the pH is too low or too high then the growth Oyster mushrooms will be hampered, even will grow another fungus that interferes with the growth of the oyster mushroom itself. The pH is also strongly affect the absorption of phosphate [18].

In a neutral or alkaline soil that has a high content of phosphate, calcium phosphate precipitation occurs. Microorganisms are able to dissolve phosphate and make it available for plants. In contrast, soils acidic commonly calcium ion-poor, hence phosphate precipitated in the form of iron compounds. One way to correct the deficiency of phosphorus is inoculated seed or the soil by

microorganisms solubizing-phosphate. The research by Sethi and Rao 1968, showed that fungi constituting agent better in dissolving phosphate than bacteria [5].

Growth rate of rot fungi isolates

The analysis of variance (ANOVA) of 3 different mediums, types of isolates, and their interaction booth indicates a highly significant effect on the diameter of the colony of rot fungi. Least Significant Difference (LSD) test showed, that isolates BPB significance different with other isolates. BSA isolate does not significance different with BPC. Similarly, the BPD isolate not significant different with BPG isolate, while BPE1 isolates did not significance different with BSF and BPH.

Table 2. Mean of colony diameter (cm) of rot fungi isolates in three different mediums, 7 days after incubation.

Isolates	Media			Mean
	PDA	MPA	MEA	
	Colony Diameter (cm)			
BSA	5.73 c	6.43 def	6.07 cd	6.08 b
BPB	3.40 a	9.00 j	3.40 a	5.27 a
BPC	6.13 de	6.13 de	6.13 de	6.13 b
BPD	6.20 def	7.23 g	6.20 def	6.54 c
BPE1	9.00 j	9.00 j	9.00 j	9.00 e
BSF	9.00 j	9.00 j	9.00 j	9.00 e
BPG	3.93 b	8.92 ij	7.02 fg	6.62 c
BPH	9.00 j	9.00 j	9.00 j	9.00 e
JT	9.00 j	7.80 h	8.68 i	8.49 d
Mean	6.82 a	8.06 c	7.17 b	

Description: The numbers followed a similar letter not significance of the difference using the LSDTest at $\alpha = 1\%$.

Figure 1 shows that on the media PDA, BSF isolate has highest diameter colony and the lowest was BPB isolate. There are 4 isolates that grow maximum fulfill petridish after 7 days of incubation, they are BSF, BPH, BPE1, and JT isolates. On MEA and MPA medium, BPE1 isolate showed highest colony diameter on third day. Isolates that fills the petridish in 7 dpi on MEA medium are BPE1, BPH, BSF, and JT isolates. On MPA media best growth were observed by BPE1, BPH, BSF, and BPB isolates.The best growth of the isolates in each medium varies as each isolates selectively with the content of nutrients. Not all of media types are suitable as a growth medium of fungi. Some of the nutrients needed by all fungi, only some of the elements needed by a particular species, and some of the elements required by particular species that will grow in the media that contain specific amounts of

nutrients [13]. Malt Extract Agar (MEA) proved to be the best solid medium and Malt Broth (MB) as best liquid medium for vegetative growth of *L. cladopus*. [19]. Table 2 showed there are difference between 3 kind of solid medium. The highest mean of colony diameter was on MPA media, 8.06 cm.

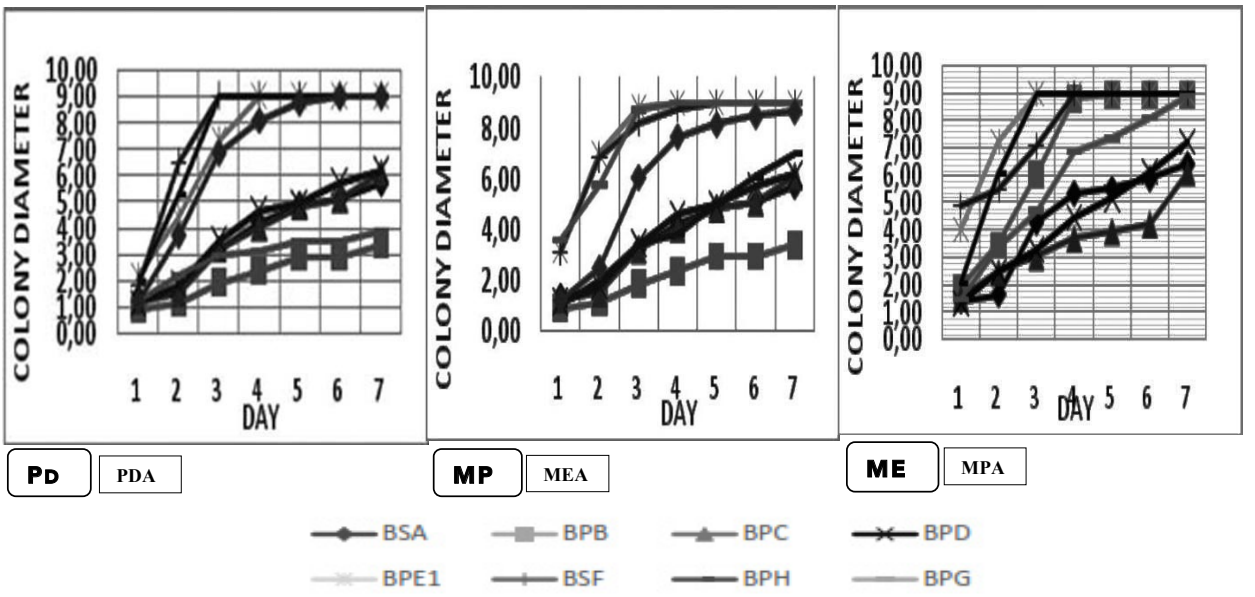

Fig.1. Mean of colony diameter of rot fungi on solid media PDA, MEA and MPA, 7 dpi (days post incubation).

The three tested media were rich in essential nutrients needed for growth and development of fungal rot . PDA medium have a carbohydrate content of nutrients, water, and protein derived from potato, glucose, substrates and in order. MEA medium has a composition of nitrogen, carbohydrates, sodium chloride, and so on. While the media MPA has nutritional nitrogen, carbohydrates, sodium chloride, agar, and pepton. Carbon compounds have two functions, the first for the metabolism of other heterotrophic organisms as mushrooms.

Carbon compound in form of C element needs to provide the process for synthesis of compounds that are used for the creation of living cells such as proteins, nucleic acids, cell wall material, and food. The second function is the main energy source as coming from the process of oxidation of the carbon compounds [20,21].

Conclusions

Present study showed that BPB, JT, BPG, and BPE1 isolates had the highest quantitative ability in solubilizing phosphate.The best media for optimum growth of rot fungi is MPA medium. .

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