THE POTENTIAL OF ENDO PHYTIC FUNGI AS BIOCONTROL AND PHOSPHATE SOLUBILIZATION AGENT IN Capsicum annum

Indah Soﬁani1, Dwi N. Susilowati2, Ivan Permana Putra3

Address(es): 1Department of Biology, Faculty of Mathematics and Natural Sciences, Jakarta State University, Campus A, Jl. Rawamangun Muka, Jakarta Timur 13220, Indonesia.
2Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A Bogor 16111, Indonesia.
3Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Jl. Agatis, Dramaga Campus, Bogor 16680, Indonesia.

*Corresponding author: d_nengsus@ yahoo.com

ABSTRACT

The productivity of chili (Capsicum annum) in Indonesia is currently very low. Some factors that inﬂuenced it included the presence of pathogenic microorganisms which lead to the low availability of phosphate in the soil. This condition became a limiting factor for plant growth and production. Endophytic fungi can be used as antagonistic agents in inhibiting pathogenic fungi and to increase the efﬁciency of phosphate solubilization known as phospholytic fungi. This study aimed to ﬁnd antagonistic agents from endophytic fungi that can suppress the growth of pathogenic fungi and test the ability of endophytic fungi to dissolve phosphate. Fungi isolates used were BB-Biogen collection isolates, consisting of 42 endophytic fungi isolates, and 3 pathogenic fungi isolates (Fusarium sp., Colletotrichum acutatum, Phytophthora capsici) on chili plants (C. annum). The antagonism test was carried out using the dual culture method in the Potato Dextrose Agar (PDA) medium for 5 days incubation at temperature (± 28 °C). The parameters measured were based on the formation of inhibition zones and the calculation of the percentage of growth inhibition of fungi isolates. The test results obtained 7 representative fungi isolates (RIVA4, RIVA5, MIVD2, Aspergillus niger, Cladosporium sp., Colletotrichum acutatum, Phytophthora capsici), and Chaetomium globosum). Based on the calculation of the percentage of growth inhibition, fungi isolates with RIVA5 code have a higher potential in inhibiting the growth of all three pathogenic fungi. Calculation of the percentage of endophytic fungi inhibition of RIVA5 were 70.3% (Fusarium sp.), 63.3% (C. acutatum), and 60% (P. capsici). Phosphate test was carried out by the cork borer method in a Petri dish (diameter 9 cm) with a distance of 3 cm. Incubation was carried out at room temperature (± 28 °C) for 5 days. Percentage inhibition of endophytic fungi against pathogen was calculated using the formula:

\[ H = \frac{J1 - J2}{J1} \times 100\% \]

Where:

\[ H = \text{Percentage of inhibition} \]

\[ J1 = \text{the radius of the pathogenic fungi colony towards the edge of the Petri dish} \]

\[ J2 = \text{the radius of the pathogenic fungi colony that is headed toward the fungus} \]

Keywords: Capsicum annum, endophytic fungi, pathogenic fungi, phospholytic fungi

INTRODUCTION

Chili plants (Capsicum annum) is one of the most popular vegetable commodities in Indonesia and has a high economic value (Marryono et al., 2015). Chili is one of the main commodities for Indonesian farmers because it can be planted in various fields and known to have high adaptability (Ali, 2006). Chili productivity in Indonesia is still very low, due to many factors such as pathogenic microorganisms which can reduce the quality and quantity of production (Than et al., 2008; Kim et al., 1999).

Indonesian farmers generally still use a lot of synthetic pesticides because of the ease of obtaining and effectiveness. Even though many research results show that the excessive use of synthetic pesticides results in environment problems and endanger to human health (Nantawanit et al., 2010). A fairly safe and environmentally friendly control is needed as an alternative control, one of which as the result, fungi isolates with RIVA5 code have a higher potential in inhibiting the growth of all three pathogenic fungi. Based on the calculation of the phospholytic index, A. niger isolates have a high phosphate solubility index value of 5.

MATERIAL AND METHODS

Sample Collection

Endophytic fungi isolates and pathogenic fungi were obtained from Biogen Culture Collection. About 18 isolates of endophytic fungi were obtained from Rhodomyrtus tomentosa, and 15 isolates from Melastoma malabathricum, 9 isolates from the Alpinia malaccensis. While as many as 3 isolates of pathogenic fungi (Fusarium sp., Colletotrichum acutatum, Phytophthora capsici) were taken from Capsicum annum.

Morphology Observation of Fungal Endophyte

The fungi were ﬁrstly grouped on the bases of their colony shape, size and texture, mycelia type, color, and diameter. Fungal colonies with similar characteristics were grouped into the same morphotypes.

Antagonism test of fungi

Antagonism test of fungi was carried out by the dual culture method based on Naik et al., (2009). Pathogenic and endophytic fungi were inoculated on PDA medium in a Petri dish (diameter 9 cm) with a distance of 3 cm. Incubation was carried out at room temperature (± 28 °C) for 5 days. Percentage inhibition of endophytic fungi against pathogen was calculated using the formula:

\[ H = \frac{J1 - J2}{J1} \times 100\% \]

Where:

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\[ J2 = \text{the radius of the pathogenic fungi colony that is headed toward the fungus} \]
Phosphat solubilization activity test

Endophytic fungal isolates were inoculated using sterile straws and transferred into Pikovskaya medium in a petri dish. The media was incubated at room temperature (± 28 °C) for 7 days. Indications of phosphate dissolution by fungi can be characterized by the formation of clear zones around colonies. The activity of fungi in phosphate degradation is expressed by the value of the Phospholytic Index measured using the following formula:

$$\text{Phospholytic Index} = \frac{\text{diameter of clear zone (cm)}}{\text{diameter of fungi colony (cm)}}$$

RESULTS AND DISCUSSION

A total of 41 endophytic fungi were characterized based on their morphology on PDA medium. Each endophytic colony shows a unique and varied appearance of colonies. Most of endophytic fungi have circullar shape, flat elevation, hypha cottony texture, aerial mycelium, and average colony size after 11 weeks by 6.8 cm (Fig. 1; Table 1).

| Table 1 Culture characteristics of the Endophytic Fungi on PDA |
|---------------------------------------------------------------|
| **Isolate code** | **Shape** | **Colour** | **Elevation** | **Texture** | **Mycelium** | **Edge** | **Size (cm) of colony after 11 days** |
|------------------|-----------|------------|---------------|-------------|--------------|---------|-------------------------------------|
| RIVA1            | circular  | brown      | raised        | Cottony     | aerial       | entire  | 7.2                                 |
| RIVA2            | circular  | dark green | raised        | Cottony     | aerial       | entire  | 7.2                                 |
| RIVA3            | irregular| white      | raised        | Cottony     | aerial       | dentate | 8                                   |
| RIVA4            | circular  | white      | flat          | cottony     | aerial       | filamentous | 8.5                           |
| RIVA5            | circular  | white      | flat          | Cottony     | aerial       | filamentous | 9.5                           |
| RIVD1            | irregular| white, black| convex        | Cottony     | aerial       | dentate | 9                                   |
| RIVD2            | circular  | white      | raised        | Cottony     | aerial       | entire  | 3                                   |
| RIVD3            | irregular| black, white| convex        | Cottony     | aerial       | dentate | 7.4                                 |
| RIVD4            | irregular| black, white| convex        | Cottony     | aerial       | dentate | 2.5                                 |
| RIVD5            | irregular| black, white| convex        | Cottony     | aerial       | dentate | 2.8                                 |
| RIVD7            | irregular| black, white| convex        | Cottony     | aerial       | dentate | 2.8                                 |
| RIVD8            | circular  | white      | raised        | Cottony     | aerial       | entire  | 2.7                                 |
| RIVD9            | irregular| black, white| convex        | Cottony     | aerial       | dentate | 9                                   |
| RIVD10           | irregular| black, white| convex        | Cottony     | aerial       | dentate | 2.6                                 |
| RIVD11           | irregular| black, white| convex        | Cottony     | aerial       | dentate | 3.2                                 |
| RIVD14           | circular  | white      | raised        | Cottony     | aerial       | entire  | 1.5                                 |
| RIVD15           | circular  | white      | raised        | Cottony     | aerial       | entire  | 8.4                                 |
| RIVD16           | irregular| black, white| convex        | Cottony     | aerial       | dentate | 9                                   |
| MIVF3            | irregular| grey       | flat          | fluffly      | aerial       | undulate | 2                                   |
| MIVF4            | circular  | white      | raised        | Cottony     | aerial       | undulate | 8.3                                 |
| MIVF5            | circular  | white      | convex        | Cottony     | aerial       | filamentous | 8.3                           |
| MIVF6            | circular  | white      | convex        | Cottony     | aerial       | dentate | 8.2                                 |
| MIVF7            | circular  | white      | raised        | Cottony     | aerial       | dentate | 8.5                                 |
| MIVA1            | circular  | white      | raised        | Cottony     | aerial       | filamentous | 2.5                           |
| MIVA2            | irregular| white      | Flat          | Cottony     | aerial       | entire  | 9                                   |
| MIVA3            | circular  | white      | raised        | Cottony     | aerial       | entire  | 9                                   |
| MIVA4            | circular  | white, cream| cream to yellow| Cottony     | aerial       | entire  | 9                                   |
| MIVB1            | irregular| white      | raised        | Cottony     | aerial       | dentate | 6.5                                 |
| MIVB2            | circular  | grey       | Flat          | Cottony     | aerial       | filamentous | 7                                   |
| MIVD1            | irregular| white      | Flat          | Cottony     | aerial       | entire  | 7                                   |
| MIVD2            | circular  | white      | Flat          | Cottony     | aerial       | entire  | 9                                   |
| MIVF1            | circular  | white      | Flat          | Cottony     | aerial       | filamentous | 9                                   |
| MIVF2            | irregular| white      | Flat          | Cottony     | aerial       | entire  | 9                                   |
| Colletotrichum boninense | circular | black, white| cream to yellow| Cottony     | aerial       | dentate | 7.7                                 |
| Aspergillus sydowi | circular | black      | Yellow       | Cottony     | aerial       | entire  | 7.5                                 |
| Cladosporium oxysporum | circular | black      | Yellow       | Cottony     | aerial       | entire  | 8.5                                 |
| Aspergillus niger | circular  | black      | Yellow       | Cottony     | aerial       | entire  | 8.8                                 |
| Cladosporium sp. | circular  | black      | Yellow       | Cottony     | aerial       | entire  | 8.3                                 |
| Guignardia mangiferae | circular | black      | Yellow       | Cottony     | aerial       | entire  | 8.7                                 |
| Chaetomiun globosum | circular | grey, cream| cream to yellow| Cottony     | aerial       | entire  | 8.7                                 |
| Diaporthe anacardii | circular | white      | cream to yellow| Cottony     | aerial       | entire  | 7.6                                 |
Antagonistic testing was carried out by the dual culture method, in which endophytic fungi isolates and pathogenic fungi isolates were grown together in a petri dish (Naik et al., 2009). It aims to create a mechanism of competition that occurs between the two. When pathogenic fungi isolated are inoculated in a medium that already contains endophytic fungi, the growth, and development of endophytic fungi are inhibited due to reduced space and nutrients. The results confirmed that 7 isolates have a higher antagonistic ability than others in inhibiting the growth of pathogenic fungi. The parameters observed in antagonistic testing are the presence of inhibitory zones between pathogenic and endophytic fungi, and the reduction of pathogenic fungi mycelium. Based on the data obtained, it is known that the most potential endophytic fungi isolates in inhibiting the 3 pathogenic molds isolates are RIVA5 isolates with inhibition values of 70.3% (Fusarium), 63.3% (Colletotrichum acutatum), 60% (Phytophthora capsici) (Table 2).

Table 2 Value of growth inhibition of pathogenic fungi by potential endophytic fungi

| No | Isolate code/ name     | Growth inhibition (%) of pathogenic fungi isolates |
|----|------------------------|--------------------------------------------------|
|    |                        | Fusarium sp. | Colletotrichum acutatum | Phytophthora capsici |
| 1  | RIVA4                  | 16           | 61                      | -6                    |
| 2  | RIVA5                  | 70.3         | 63.3                    | 60                    |
| 3  | MIVD2                  | 7.4          | 45.4                    | 37                    |
| 4  | Aspergillus niger      | 40           | 20                      | 0                     |
| 5  | Cladosporium sp.       | 40           | 50                      | 0                     |
| 6  | Cladosporium Oxysporum | 66           | 31.52                   | 0                     |
| 7  | Chaetomium globosum    | 52           | 56                      | 0                     |

Control fungi isolates without antagonistic treatment and fungus isolates with dual culture antagonist treatment had differences in colony growth (Fig. 2). Treatment fungi isolates showed reduced mycelium, non-sporulating, and had smaller diameters while control isolates without antagonistic treatment showed normal my growth of mycelium, which was not reduced, and sporulated. That is because there is an antagonistic interaction between endophytic fungi isolates and pathogenic fungi isolates in antagonist testing.

Endophytic fungi isolates in dual culture treatment have antagonistic ability to inhibit the growth of pathogenic fungi colonies. This is in line with Talaprat et al. (2017) which stated that the inhibition of the growth of mycelium colonies of pathogenic fungi is due to the antagonistic nature of endophytic fungi. Antagonistic interaction is a form of defense that includes self-defense, territory, and nutrition. The reduction of hyphal or mycelium width that occurs in pathogenic fungi is suspected due to the antagonistic nature of endophytic fungi. Pathogenic fungi lack nutrients to grow when they are grown with endophytic fungi in the same medium so that the mycelium that is formed becomes less and there is no sporulation. The inhibition zone formed is due to the antagonism characteristic of endophytic fungi isolates. The inhibition zone is a clear zone that indicates inhibition of fungi growth due to the secretion of metabolite compounds by endophytic fungi isolates. The presence of antagonistic mechanisms in endophytic fungi against pathogenic fungi is a form of antibiosis. Secondary metabolites are metabolite compounds that function as inhibitors of growth of pathogenic fungi. These compounds are not essential for growth and are produced at certain times. Secondary metabolites are a form of self-defense from adverse environmental conditions. Secondary metabolites are in the form of pelysis enzymes, volatile compounds, sidospores, or other toxic compounds. Compared to bacteria and plants, fungi are among the most productive producers of secondary metabolites (Keller et al., 2005). Differences in the ability of antagonism between fungus can be caused by many things, including the speed of spore formation, the number of antibiotic compounds produced and the differences in specific enzymes produced. Some antagonistic mechanisms are space and nutritional competition, production of antifungal compounds, and lytic enzymes (glucanase, chitinase, and protease) (Chet and Chernin, 2002). The mechanism of space and nutrient competition occurs when endophytic fungi try to obtain limited space and nutrients when grow together with pathogens so that the growth activity of pathogenic fungi colonies is disrupted due to lack of nutrients and space to grow (Janisieiwicz & Korsen, 2002; Sharma et al., 2009). Lytic enzymes cause degradation of protein components making up the fungal cell walls, resulting in inhibition of cell wall growth in mold mycelium (Chet and Chernin, 2002; Nunes 2012).

Regarding to the phosphate test, it was found that endophytic fungi isolates which can dissolve phosphate, including 4 fungi isolates, namely MIVA4, MIVF7, Aspergillus sydowii, and A. niger. A. niger has the highest phospholytic ability among other fungi isolates, with a phospholytic index value of 5.0 (Table 3). The media used for phosphate test is psychovaya media, which turbid into white because it contains insoluble P such as calcium phosphate. After 48-72 hours of incubation, the potential for microorganisms to grow on tricalcium phosphate agar will indicate the presence of a clear zone (Fig. 3), while other microorganisms do not exhibit this characteristic.

The mechanism of biological phosphate dissolution occurs because these microorganisms produce enzymes such as the enzyme phosphatase and phytase enzyme. The activity of the enzyme phosphatase produced by these fungi is known through the phospholytic index.
The observations showed a high Phosphate dissolving index and had a fast-growing ability obtained from Aspergillus niger isolates. Aspergillus are known to be everywhere and grow on almost all substrates. Aspergillus is a dominant group of phosphate solvating fungi found in acid soils in Indonesia. This Aspergillus genus has high potential in dissolving Phosphate bound to become Phosphate available in the soil.

The difference in the value of the phospholytic index in each isolate shows the difference in the activity of the enzyme phosphatase in hydrolyzing phosphate. Isolates with high index values indicate high extracellular phosphatase activity, and vice versa in isolates with low index values, phosphatase activity is also low extracellular.

**CONCLUSION**

Based on the results RIVA5 has the highest potential in inhibiting the growth of all three pathogenic fungi. Calculation of the percentage of hyphal or mycelium width inhibition of RIVA5 were 70.3% (Fusarium sp.), 63.3% (C. acutatum), and 60% (P. capsici). In the phosphate test, it is known that A. niger endophytic fungi isolates have the highest ability among other fungi isolates in hydrolyzing phosphate, with phosphate solubility index value of 5.

**REFERENCES**

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