DIVERSIFICATION OF ENDOFIT MUSHROOM IN SOYBEAN PLANTS AND ANTAGONISM ABILITY TO PATHOGEN Fusarium, sp.

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Abstract

Endophytes are microorganisms (bacteria, fungi, or aktinomisetes) that live and colonize within the host tissues without causing negative effects, and even give many advantages to its host. One of the advantages is as a biological control agent both for insect pests and plant-causing pathogens. As a biological agent, endophytes can reduce crop damage by insects, nematodes, or disease-causing pathogens through plant resistance induction. In addition, endophytes can also function as biological agents through antagonistic interaction and competition. The Long Term Objective of this study is to obtain potential endophytic fungi as biological agents of insect pests and pathogens; the ongoing mechanism; as well as endophytic applications in agriculture, especially plantation crops. Specific Objectives test the potential of enndofite fungi that have the potential to control the pathogen carried by Fusarium sp. on soybean crops.

Keywords: *endofit, fusarium*

1. INTRODUCTION

Soybean Plants [Glycine max (L.) Merr.] Is one of the most strategic commodities and plays an important role for the life of Indonesian people both as food and feed. Demand for soybeans continues to increase as the food and feed industry grows and the improvement in education leads to increased public awareness of health and food quality. This will eventually increase consumption of quality foodstuffs,includingsoybeans (Sudaryanto, 1996). Fusarium genus is a pathogenic fungus that often attacks soybeans from the nursery to cause wilting symptoms in soybean crops. This causes the crops to decrease resulting in a decrease in soybean production. According to Sudantha (2010), one of the obstacles in the development of soybean crop is Fusariumoxysporum f. sp. glycine that causes the disease to fall apart and wilt. Li et al. (2008) adds that F. solani can also cause soybean sprouts. Biological control of seed-borne pathogens is a method of control currently being developed by using endophytic bacteria. Endophytic bacteria can stimulate growth regulators, fix nitrogen, and increase resistance induction to plant pathogens (Dalal and Kulkarni 2013a). According to Nandhini et al. (2012), endophytic bacteria are capable of producing compounds that can be used as chemical resistance against pathogenic microbes that infect plants. Utilization of endophytic bacteria from soybean plant can be used as control agent of pathogenic fungus carried by soybean seed. According to research Shu- Mei et al. (2008), bacterial endofit Bacillus amyloquafaciens from soybean plant can inhibit growth of F. oxysporum

pathogen fungi 80.2-96.7% in vitro. Seed treatment may be administered by immersion using a filtrate culture of endophytic bacteria. B. endoradicis is also found to be in the roots of soybean crops (Zhang et al., 2012).

The potential for endophytic fungus is large enough to be developed as a biological control agent, since it lives in plant tissue, thus contributing directly to inhibiting the development of plant pathogens (Niere 2002), and enhancing plant growth. The endophytic fungi that have been isolated and act as biological control agents are the coniothyriumminitans that can suppress the pathogen germination of Sclerotiniasclerotiorum (Huang 2001). In addition, Barnet et al. (2003) found that C. minitans were able to survive in highly sterilized soil. This defensive ability is a good aspect for controlling Sclerotiniasclerotiorum soil pathogens and as a control agent for other pathogens.

The endophytic fungi protect the plant from pathogen attack through mechanism of competition, induction of resistance, antagonism, and mycoparasite (CABI 2004). The fungus can also induce host metabolism response, thus becoming resistant to plant degradation so that the product is increased (RedlineCarris1996).

2. METHODS

The plant part used is the root (lower, middle, top), stem (tip, middle, bottom), leaf (root, bottom, shoot), root rhizomes and rodoma stems (for binahong). Plant samples in fresh condition cleaned with water flowing then cut into pieces along 2-5 cm and separated according to the part of the plant. The sample piece is then surface sterilized by soaking it in technical alcohol for 1 minute, Chlorox 5.25% solution for 5 minutes, and technical alcohol for 2 minutes. The sterilized sample pieces were cut and chopped and then planted in a NA containing nystatin medium. The media containing the sample was incubated at room temperature in the dark and observed daily until there was colony growth. Endophytic bacteria grown are purified one by one and preserved in order to tilt.

Isolation of pathogenic fungi carried by soybean seed was done by blotter test method. Soybean seeds sterilized the surface using 1% NaOCl for 1 minute and washed with sterile water 3 times then dried. A total of 10 seeds were planted in petri dishes containing sterile suction paper with replicates 40 times. The seeds were incubated at room temperature for 24 hours with light-hour 12-hour N-UV irradiation and 12 hours of darkness. Incubation is continued by placing the seeds in a room with a temperature of -20 ° C for 24 hours. The seeds were incubated back at room temperature by 12 hours of light and 12 hours of darkness until day 8 and 14. The emerging pathogenic fungus was observed and the rate of infection was calculated using the formula:

$$\text{Fungal infections carried by seed} = \frac{\Sigma \text{ infected seed}}{\Sigma \text{ seeds are planted}} \times 100\%$$

The most high-grade fungi were purified on potato dextrose agar medium (ADK) and incubated for 5-7 days at room temperature, then characterized and identified macroscopically and microscopically to obtain Fusariumoxysporum.

Pengujian antagonis antara jamur endofit dengan Jamur Patogen menggunakan metode oposisi langsung, yaitu dengan cara menumbuhkan isolat jamur endofit dengan patogen secara berhadapan dengan jarak 3 cm pada cawan Petri berdiameter 9 cm dengan media PDA. Inokulasi antara jamur endofit dengan Jamur Patogen dilakukan pada waktu bersamaan. Biakan uji diinkubasi pada suhu kamar (28-30℃) sampai dengan patogen tumbuh memenuhi cawan Petri. Daya hambat jamur antagonis diketahui dengan menghitung pertumbuhan koloni

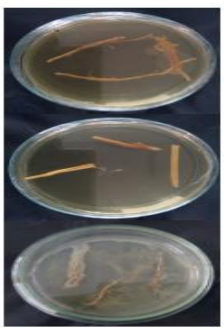
$$\text{Daya hambat} = \frac{(Y-X)}{Y} \times 100\%$$

Y, diameter koloni cendawan patogen normal (cm); dan X, diameter koloni cendawan patogen yang terhambat pertumbuhannya (cm).

3. RESULT AND DISCUSSION

Bacterial Isolation and Endophytic Fungi from Soybean Plants

From the isolation and identification of endophytic fungi from soybean plants, two fungal isolates have been found in root tissue and one fungal isolate on stem tissue.



The diversity of the genus of fungi and endophytic bacteria of soybean plants is presented in Table 1.

Plant Tissue	Genus	Number of Species
Root	Sp. 1 Sp. 2	1 1
Stem	Sp 3	1
TOTAL		**3**

Table 1. Genetic Variety of Endophytic

Fungi in Soybean Plants

Endophytic fungus is a fungus contained in plant tissue systems that do not cause symptoms of disease in host plants. Endophytic fungi spend partly or even the entire life cycle of colonies inside and outside the living tissue cells of their host plants. We can explore endophytic fungi in plant tissue systems such as leaves, fruits, twigs / stems and roots. In some types of endophytic fungi are known to stimulate plant growth and increase host resistance to pathogen attack. Masyarah (2009) in Kurnia et al (2014) states that plant species are scattered on the face of the earth, each plant contains one or more endophytic microorganisms comprising bacteria and fungi capable of producing biological or metabolite compounds that can function as an antiser, growth regulators and the production of hydrolytic enzymes such as amylase, cellulase, xylanase, ligninase, chitinase. This is caused by the endophytic fungus seizing the nutrients of the pathogen (nutritional competition) so that there is a change

in the pathogenic hyphae that will cause the growth of pathogen inhibited. Endophytes generally come from fungi or bacteria class. About 300,000 plant species are known to be endophytic hosts (Strobel et al., 2004) with various forms of mutualistic, commensalistic, and parasitic symbiosis (Aly et al, 2011).

2.Test of Fungal Antagonists of Pathogens and Endophytic Fungi as Soybean Biodiversity Control Agent

Antagonistic testing between pathogenic fungi and endophytic fungi using direct opposition method, ie by growing endophytic fungal isolates with pathogens in the presence of a distance of 3 cm on a Petri dish with a diameter of 9 cm with PDA media.Inoculation between endophytic fungi and pathogenic fungi.done at the same time. The test culture was incubated at room temperature (28o-30oC) until the pathogen grew to fill the Petri dish. Inhibitory power of antagonistic fungus is known by calculating colony growth by using the formula:

$$I = \frac{R1 - R2}{R1} x100\%$$

Information :
I = Percentage of Inhibition
R1 = Finger Colonies Pathogens whose growth direction away from colonies of fungi antagonist nut.
R2 = Finger Colonies Pathogens whose growth direction approaches the colony of fungi antagonist nut.
Based on the results of antagonistic test of fungi and bacteria endophytes and pathogenic fungi obtained data as follows:

Isolate	Fungi	The percentage of inhibitory power of day-					
		2	3	4	5	6	7
Isolat 1	*Fusarium* sp	4.77	15.82	29	42.12	48.8	51.25
Isolat 2	*Fusarium* sp	0.77	8.57	25.40	38.20	46.0	51.19
Isolat 3	*Fusarium* sp	0.52	7.79	8.56	9.12	10.7	10.71

Table 2.Percentage of inhibitory power and Fusarium sp.

Based on Table 2 it is known that the treatment of endophytic and pathogenic fungi have a significant effect on colony growth area. Based on the result of the difference test, it was found that the growth area on the 1st day until the seventh day of treatment in isolate 1 (51.25%) and isolate 2 (51.19%) was significantly different than isolate 3 (10.71%). This indicates that isolate 1 and isolate 2 fungi can be used as biological agents to inhibit the growth of Fuasariumsp due to its rapid growth. Rapid Endofit Fungus Growth leads to competition of nutrition and living space with Fusarium sp. This is in accordance with Soesanto (2008) which states that competition for nutrition plays a major role in almost all biological control agents. In addition to competition for nutrition as well as the competition of living space. From these data it can be seen that the growth of antagonistic agents derived from rhizosphere and plant tissue has rapid growth and can be used as a biological agent. This is in accordance with Carrol (1998) which states that in general the type of biological agents developed are natural microbes, both microbes that live in soil, water, organic matter, and plant tissues.

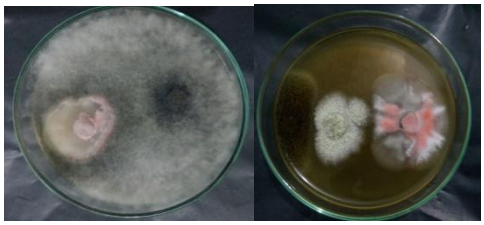


Figure 4.Fusariumsp Fungus Antagonism Test and Endophytic Fungus

The role of endophytes as biological agents began a lot of research since the existence of phenomena about the ability of plants in the face of biotic and abiotic stress associated with the presence of endophytes in the network. Endophytes prevent disease progression by producing siderophores (Kloepper et al., 1980), producing toxic metabolites for pathogenic fungi (Schnider-Keel et al. 2000), or the occurrence of space and nutrient competition (Kloepper et al., 1999). M'Piga et al. 1997 suggests that endophytes also have the ability to reduce the production of toxins produced by pathogens that are not pathogenic to plants or induce plant resistance against pathogens. (Yulianti, 2012).

Endophytic mechanisms in protecting plants against pathogen attack include:

1. Inhibition of growth of pathogens directly through antibiotic compounds and lytic enzymes produced;
2. Indirect inhibition through endophytic stimulation of plants in the formation of secondary metabolites such as salicylic acid, jasmonic acid, and ethylene which function in increasing plant resistance against pathogens or acting as antimicrobials such as phytoalexin;
3. Stimulation of plant growth so as to be more resistant to pathogen attack;
4. Colonization of plant tissue so that pathogens are difficult to penetrate; and
5. hyperparasit (Gao et al. 2010 in Yulianti, 2012).

4. CONCLUSION

Based on the research results obtained conclusion that is:

1. The results of the invitro test showed that 2 endofite fungal isolates derived from the roots and 1 isolate of endophytic fungi from the stem.
2. Antagonis test results based on the percentage of inhibitory power indicates isolate fungus sp 1 has inhibitory power with percentage 51.25% and sp 2 has inhibitory power 51.19% while sp3 has 10.71% inhibitory power in inhibiting fascial pathogen seed Fusarium sp.

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