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Glucanase and Chitinase from Some Isolates of Endophytic Fungus *Trichoderma spp.*

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ABSTRACT. Endophytic fungi are those fungi that are able to grow in plant tissue without causing symptoms of disease. It is thought that these fungi may confer on the host plants degree of resistance to parasitic invasion. Endophytic fungi have been isolated from stem tissue and these fungi are known to be antagonistic to pathogenic fungi. These endophytes produce chitinase and β-1,3-glucanase enzymes. Based on the fact that chitin and β-1,3-glucan are the main skeletal polysaccharides of the cell walls of fungal patogen. The aim of this research is to do potential test on some of isolates of Trichoderma's endophytic (L-1,L-2, Is-1, Is-2 and Is-7) in the chitinase and β-1,3-glucanase activity in effort to determine endophytic which be chossen to be gene resource for the next research. The gene will be transformed to citrus plant japanese citroen in effort to make citrus plant transgenic resistance to phytopatogenic invasion. The result of this research is endofit namely L-1 is the most potential endophytic fungi with chitinase activities is 4,8 10^{-2} Unit and glucanase 24,2. 10^{12} Unit. The addition of chitin and cell wall of phytophtora causes chitinase activity significantly increase, and also addition of laminarin and cell wall of phytophtora makes glucanase activity increase.

1. Introduction

Endophytic fungi are fungi that can grow in plant tissues of both stems, roots and leaves of plants [1] without causing symptoms of the disease even increasing the degree of resistance of host plants to parasitic attacks [2]. The relationship between endophytic microbes and their host plants is a form of symbiotic mutualism and ensured endophytic microbes will increase the degree of resistance of host plants to parasitic attacks [3]. Currently, these endophytes receive great attention because they can produce bioactive compounds such as antibiotics, enzymes because of their great activity in killing pathogenic microbes. Besides being able to produce antimicrobial compounds, endophytic microbes are also capable of producing potentially anticancer, antimalarial, anti HIV, antioxidant and so on. Liu et al [4] has successfully isolated 39 endophytes contained in *Artemisia annua*. Lu et al [1], from his research found that bioactive compounds isolated from endolitic microbes *Colletotrichum sp*. In *Artemisia annua* has the ability to inhibit the growth of the pathogenic fungi *Gaeumannomyces graminis* var. Tritici, *Rhizoctonia cerealis*, *Helminthosporium sativum* and *Phytophthora capisici.*

Trichoderma spp is one of the endophytes of fungi that grow on the root surface of various plants and soil. Trichoderma can inhibit the growth of pathogenic fungi by producing enzymes capable of degrading cell walls such as chitinase, β-1,3-glucanase, proteases, mannanases and other hydrolysed enzymes [7-9]. It has long been known that in addition to producing antibiotics Trichoderma also acts as a parasite for other fungi [10]. Trichoderma has the ability to compete with other microorganisms in terms of obtaining nutrients. It is also said that Trichoderma is able to degrade pectinases and other enzymes that have an important role for the life of pathogenic fungi [10]. β -1,3-glucan are the main framework of polysaccharides of the cell wall of pathogens. It is possible that chitinase and β-1,3-glucanase are enzymes involved in breaking the cell wall during the antagonistic action of the endophytes. A set of chitinases and gene glucanases have been isolated from *T. virens* and structural

features and their expression has been deactivated [2]. Endophytic fungi have been successfully isolated from the citrus trunk tissue and these fungi are known to be antagonistic to pathogenic fungi. These endophytes produce chitinase and glucanase enzymes. Based on the fact that chitin and *Trichoderma harzianum* is a fungus that can produce chitinase [11, 12] so it can function as a controlling plant diseases. Chitinase is an extraeluler enzyme produced by fungi and bacteria [12] and plays an important role in the breakdown of chitin.

A number of endophytic fungi have been successfully isolated from citrus trunk tissues and rice stalks that are tested as antagonists against citrus plant pathogenic fungi. These endophytes are known to produce chitinase enzymes and 1,3-β glucanase enzymes. It is key that the enzyme is involved in the cell wall termination of phytopathogenic pathogens during the antagonist action of the endophytes. Such enzymes may act directly by degrading the cell wall of pathogens, and indirectly by releasing fragments of cell walls that may act as elicitors of a defense reaction such as the occurrence of phytoalexin accumulation [13].

2. MATERIALS AND METHODS

2.1 Materials

Endophytic isolates were obtained from a collection of endophytic isolates labeled L-1, L-2, Is-1, Is-2 and Is-3 from the biotechnology HPT laboratory of the Faculty of Agriculture Universitas Brawijaya, Malang. L-1 and L-2 are derived from orange stems while Is1, Is2 and Is 7 are derived from rice stem tissue. Chitin as the enzyme substrate khitinase and laminarin as glucanase substrate. In order to obtain the enzyme chitinase and glucanase is done by inoculating Trichoderma isolate on PDA medium (Potato Dextrose Agar). Then incubated for 5 days at 28 ^{o}C. After 5 days, the PDA media was centrifuged at 5000 rpm, 4 ^{o}C for 10 minutes. The supernatant (enzyme solution) obtained was then used for the enzyme activity of khitinase and glucanase. The chitinase enzyme activity test was conducted by mixing a 0.25 mL enzyme solution with 0.25 1% colloidal chitin solution in 50 mM phosphate buffer pH 6.7, incubated in a water bath temperature of 45 ^{o}C for 6 hours, after which the solution plus the Somogyi - Nelson and is read with a 550 nm. Glucanase enzyme activity test was done by mixing 0.25 ml of enzyme solution with 0.25 mL 1% laminarin substrate in Tris -HCl pH 8 buffer solution, incubated at 37 ^{o}C for 6 hours, plus Somogyi-Nelson reagent and quantized with spectrophotometer at 550 nm. One unit of activity is defined as the amount of enzyme liberated by 1 µmol GlcNAc/min under certain conditions.

3. RESULTS AND DISCUSSION

3.1 Activity test of chitinase enzyme from Trichoderma spp.

The results of chitinase enzyme activity test of several species of *Trichoderma spp* presented in Figure 1. Endophytes *Trichoderma spp*. L-1 has higher enzyme activity than Trichoderma spp at 4.8 x 10^{-2} units. On the other hand, the endophytes of Trichoderma Is-7 have the lowest enzyme activity, at 3.2 x 10^{-2} unit.

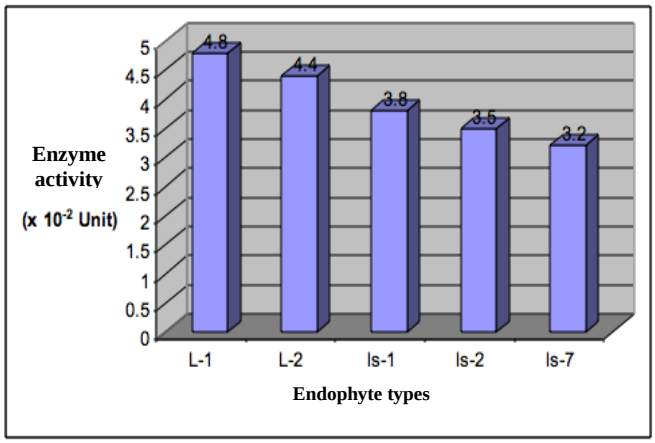

Figure 1. The relationship between endophytic type and chitinase enzyme activity

Trichoderma spp. is a fungus that can produce chitinase. Khitinase is an extracellular enzyme produced by fungi and bacteria [12]. In this study *Trichoderma spp.* grown with chitosan substrate. The difference of enzyme activity on the five types of *Trichoderma spp*. have been determined because of the different species of *Trichoderma spp*. According to Nugroho [13], the ability of *Trichoderma spp*. to produce enzymes varies greatly between strains, which may be due to differences in the gene that codes it. From the previous test it was found that the highest activity of several isolates tested, Trichoderma L-1 endophytic fungus had the highest enzyme activity of chitinase. In the next stage will be tested how the activity of enzyme chitinase when Trichoderma L-1 grown on the media with the addition of chitin. For this purpose, the concentration of chitin added to the medium is as follows: 0; 0.2; 0.4; 0.6; 0.8 and 1%, the results are presented in Figure 2.

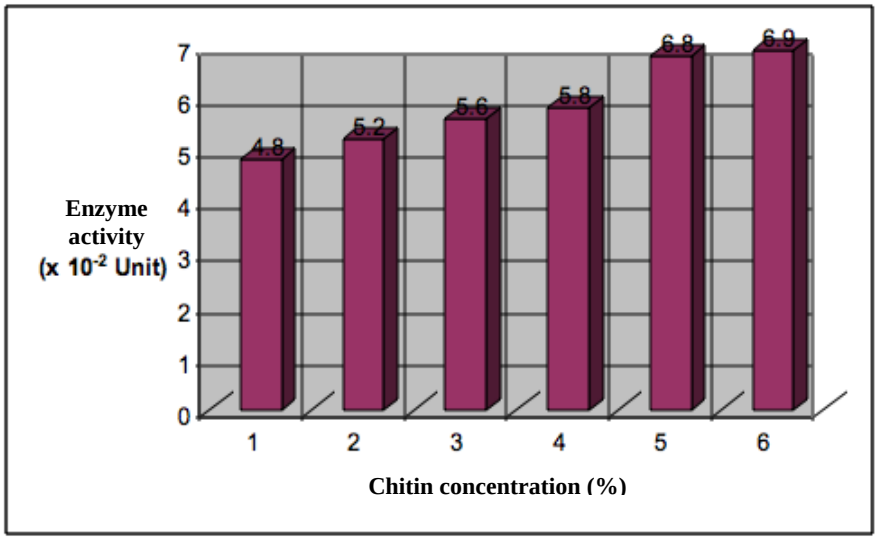

Figure 2. The relationship between the concentration of chitin on the activity of chitinase

From the experiment it was found that higher concentration of chitin added to the medium would increase the activity of chitinase. Chitin is a polymer composed of N-acetyl glucosamine. Chitin is a constituent of fungal cell walls and bacteria [14, 15]. The activity of chitinase will increase when the bean and pea plants are induced by pathogenic fungi.

3.2 Test of chitinase activity exposed to Phytophthora cell wall

The results of chitinase activity test on Phytophthora cell wall are presented in Figure 3. There is a significant relationship between cell wall concentration and chitinase activity. The higher concentration of Phytophthora cell wall added to the media, will increase the activity of chitinase. At 1% concentration, chitinase activity increased 3.2 x 10^{-2} unit higher than control.

Phytophthora spp. is the cause of fruit rot disease, stem cancer, leaf blight, seed blight, and wilting of water shoots. Chitin is a constituent of fungal cell walls and bacteria [14]. Boller et al. [3] states that the activity of khitinase enzymes will increase when the bean and pea plants are induced by pathogenic fungi. In this study, the increase in enzyme activity is suspected as a natural reaction mechanism performed by the enzyme chitinase because of the fungus pathogen, in this case is the cell wall *Phytophthora spp.* added to the media.

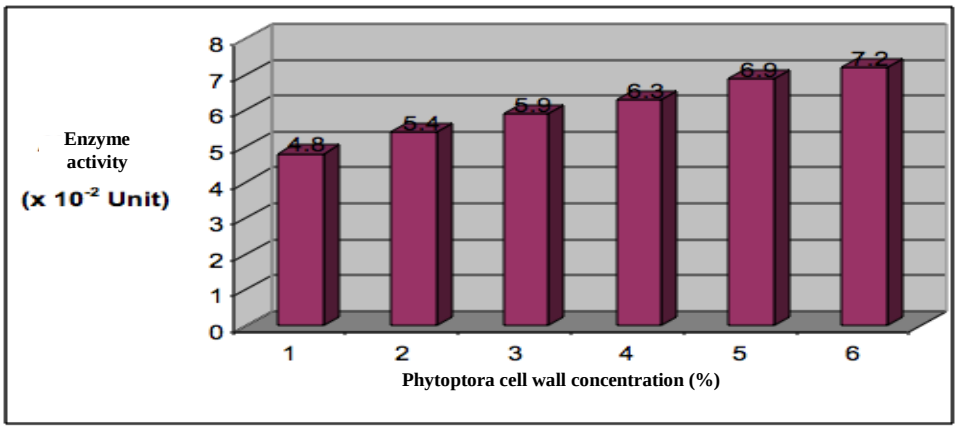

Figure 3. The relationship between Phytophthora cell wall concentrations and chitinase activity

3.3 Activity test of glucanase enzyme from Trichoderma fungus

The results of the glucanase activity showed that Trichoderma L-1 had the highest enzyme activity compared with other types of Trichoderma. (Figure 4). The glucanase activity on L-1, L-2, Is-1, Is-2 and Is-7 was 24.2 x 10^{-2} units, 22.4 x 10^{-2} units, 18.7 x 10^{-2} units, 18.9 x 10^{-2} Units and 18.7 x 10^{-2} units, respectively.

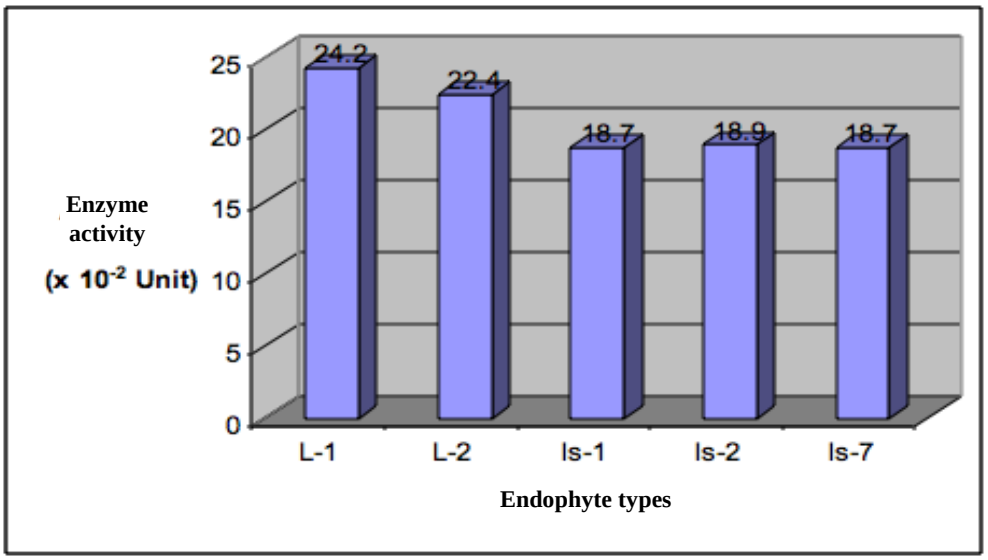

Figure 4. Relationship between types *Trichoderma spp* with their activity

In addition to produce chitinase, *Trichoderma spp*. is a fungus that can produce glucanase. Glucanase is an enzyme that hydrolyzes β-1,3-glucan, which is a constituent of fungal cell and bacterial cells [14]. The difference of enzyme activity on the five types of *Trichoderma spp*. tested because of the different species of *Trichoderma spp*.

3.4. The results of glucanase activity test with laminarin which is added to the PDA medium
To test the activity of glucanase used laminarin substrate added to PDA media. From the experiment it was found that the higher concentration of laminarin added to the medium would increase the activity of the glucanase (Figure 5).

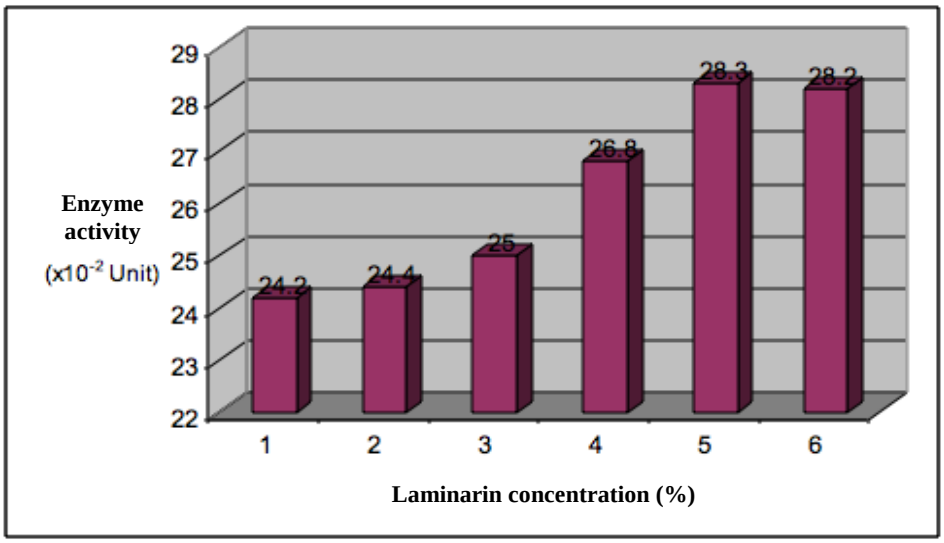

Figure 5. Relationship between laminarin concentration on glucanse activity

In addition to chitin, the fungus cell wall is also composed of β-glucans as a component of the cell skeleton and α-glucans as components that strengthen cell. From the results of his research Thrane *et al.* [15] found that the activity of β-1-3-glucanase increased when he was grown together with the presence of the antagonistic fungus Pitheum ultimum, which is the antagonistic fungus of *T. harzianum*.

3.5 Test of glucanase enzyme activity exposed to Phytophthora cell wall
The results of the test of glucanase activity on Phytophthora cell wall are presented in Figure 6. There is a significant relationship between cell wall concentration and glucanase activity. The higher concentration of Phytophtora cell wall added to the media, will increase the activity of glucanase. At the concentration of 1% cause glucanase activity increased 7 x 10^{-2} Unit higher than the control.

In this study an increase in enzyme activity is thought to be a natural reaction mechanism performed by the enzyme glucanase due to the presence of pathogenic fungi, in this case is the cell wall of *Phytophthora spp*. added to the media. Suzuki, et al. (1995) found that tobacco cell culture given chitinase and β-1,3-glucanase was able to suppress the growth of *P. infestans*. Noronho and Ulhoa [16] from the research they get their activity β-1,3-glucanase in culture containing the cell wall of *Rhizoctonia solani, Sclerotium rolfsii* and *Pythium spp*. Sivan and Chet in [5] found that the production of laminarinase (β-1,3-glucanase) enzyme by *T. harzianum* was induced by the cell wall of the target fungus added in the medium.

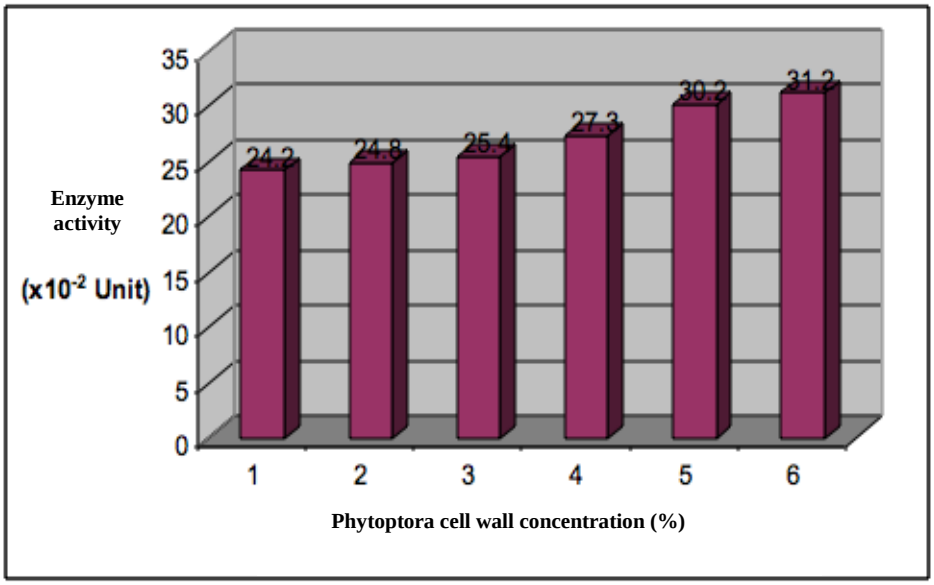

Figure 6. Relationship addition of Phytophthora cell wall concentration with glucanase activity

4. CONCLUSION:

From the research results can be concluded that Trichoderma fungus L-1 is the most potential endophytes with the highest chitinase and glucanase activity. The addition of chitin, laminarin or cell wall Phytophthora spp. causing an increase in enzyme activity of chitinase and glucanase. The addition of chitin, laminarin or cell wall causes increased activity of chitinase and glucanase.

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