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Potential Of White Turmeric (Curcuma Zedoaria) Endophyte Bacteria As A Producer Of Protease Enzymes

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Abstract— **Enzyme technology is an alternative for experts and environmentalists to replace various chemical processes in producing enzymes in industry. Protease enzymes represent one of the three largest groups of industrial enzymes. Known sources of protease enzymes come from animals, microbes and plants. Plants are the largest source of protease enzymes (43.85%) and followed by bacteria (18.09%). *Curcuma zedoaria* is generally used as medicine and as an ingredient in cooking. This research aims to determine the potential of white turmeric (*Curcuma zedoaria*) endophyte bacteria as a producer of protease enzymes. The research method used in this study was experimental and descriptive. Protease activity was determined using Skim Milk Agar medium. The results of the study showed that endophytic bacteria in white turmeric rhizomes (*Curcuma zedoaria*) have quite high potential in producing protease enzymes with a proteolytic index of 1.98.**

Keywords— Endophytic bacteria; White turmeric; Protease; Enzyme

I. INTRODUCTION

The development of the enzyme industry has been rapid and occupies an important position in the industrial sector. Increasing public awareness of environmental problems and pressure from experts and environmentalists have made enzyme technology an alternative to replace various chemical processes in the industrial sector (Soeka et al., 2011). Protease enzymes represent one of the three largest groups of industrial enzymes and find applications. The known sources of protease enzymes come from animals, microbes and plants. Plants are the largest source of protease enzymes (43.85%) followed by bacteria (18.09%), fungi (15.08%), animals (11.15%), algae (7.42%) and viruses (4, 41%) (Mahajan and Shumat, 2010).

Proteases are enzymes that catalyze the breaking of peptide bonds within proteins and polypeptides, producing oligopeptides or free amino acids as a result. Proteinases, which catalyze the degradation of protein molecules into more manageable fragments, and peptidases, which hydrolyze polypeptide fragments into amino acids, are two types of protease enzymes. Every live cell produces proteases for intracellular use or to be released into the environment for nutritional and defensive purposes. Aside from being physically necessary, they have also found various crucial functions to play in the health, food, textile, and medicine industries over the years (Correa et al., 2014).

Protease enzymes occupy around 60% of total enzyme sales in the world (Yuniati et al., 2015). The protease enzyme is one of the commercial enzymes that has high economic value, reaching US$ 200 million per year. Protease enzymes are a support for increasing the value of human life along with the development of biotechnology which is able to improve protease enzymes and their applications (Jisha et al., 2013). Protease enzymes are widely used in the detergent, food, leather, pharmaceutical and other chemical industries (Li et al.,

Curcuma zedoaria or what is known as turmeric/white ginger is one of the Curcuma genus which is widely used as medicine and as an ingredient for cooking. In Indonesia, Curcuma zedoaria leaves are used as an additional spice to improve the taste of fish dishes and other foods (Srigusa et al., 2007). In medicine, Curcuma zedoaria has long been used by various ethnicities in Indonesia, Malaysia and India (Malek et al., 2004).

Bacteria that grow and spend at least one life cycle in the host plant tissue without causing negative effects on the plant are called endophytes (Pimentel et al, 2011). Plants can become hosts for microorganisms to produce metabolites to increase nutrient absorption, stimulate growth and increase plant biomass, as well as induce plant resistance to pathogens (Xia et al. 2015). These bacteria live in symbiotic mutualism with host plants (Wani et al., 2015). Endophytic bacteria and their host plants have a mutualistic symbiotic relationship, allowing the bacteria to produce the same bioactive compounds as those contained in their host plants (Nursanty and Suhartono, 2012).

Microbes are a source of enzymes that are very often used in plants and animals. One of the enzymes produced by endophytic microbes is proteolytic. Protease enzymes, apart from being useful in metabolic processes, are also used in various food factories (Rizaldi et al., 2018). Based on the potential of these endophytic bacteria, research was carried out to utilize white turmeric rhizomes to find isolates of endophytic bacteria that have the potential to produce protease enzymes.

II. METHOD OF RESEARCH

2.1. Isolation of Endophytic Bacteria

The rhizome samples were washed with running water for 10 minutes, then cut into several pieces 2-3 cm long. The sample was then sterilized by immersing it in a 75% alcohol solution for 1 minute. The rhizome pieces were then soaked in 5.3% sodium hypochlorite solution for 5 minutes and alcohol for 0.5 minutes, then dried using sterile tissue paper. Sample pieces were cut with a sterile knife and placed on the surface of Nutrient Agar (NA) media (Tomita, 2003) in a petri dish, then incubated at 28oC for 2–15 days. The bacterial isolate that grows is then purified until a single isolate is obtained.

2.2. Characterization of Bacteria Isolates

Morphological characterization of endophytic bacteria was carried out using macroscopic and microscopic observations. Macroscopic observations include shape, size, color, edge shape and elevation of the colony (Wulandari and Purwaningsih 2019). Microscopic observations were carried out using Gram staining (Lay, 1994) to determine the shape and arrangement of cells. The Gram staining stage begins with making bacterial preparations and then fixing them over fire. The preparations were treated with crystal violet solution for 1 minute and then washed with water. Next, the preparations were treated with Lugol's solution for 1 minute, washed with running water and dried. 96% ethanol was then added to the preparation, washed with running water and dried in the air. The next step is to add the safranin solution to the preparation for 15 seconds, wash it with running water and let it dry. Observations were made using a microscope.

2.3. Protease Enzyme Activity Testing

Protease Enzyme Activity Testing Protease enzyme activity testing using Skim Milk Agar (SMA) media. SMA media consists of 1 g of 0.1% peptone; 5 g NaCl 0.5%; 20 g gelatin, and 500 ml distilled water. 100 g of skim milk was dissolved in 500 ml of distilled water and sterilized at 100^{o}C for 10 minutes. Next, the milk medium is mixed with sterile agar medium and poured into a petri dish. Bacterial isolates were grown in petri dishes and incubated at 28^{o}C for 48-72 hours. The clear zone formed around the isolate indicates that the isolate can degrade proteases (Hamdani et al., 2019) and the index is calculated using the formula:

$$\text{Proteolytic Index} = \frac{\text{colony diameter-clear zone diameter}}{\text{colony diameter}}$$

III. RESULT AND DISCUSSION

3.1. Isolation and Characterization of Bacterial Isolates

The isolation results obtained 1 isolate of endophytic bacteria from the rhizome of the white turmeric plant. The macroscopic morphological characteristics of endophytic bacteria are circular in shape, small in size, smooth edges, raised elevations with a milky white color. Meanwhile, microscopic morphological characteristics showed that the endophytic bacterial isolates were rod-shaped/bacilli and were gram-negative bacteria. Sulistiyani (2014) stated that the endophytic bacteria of white turmeric are dominated by Gram negative bacteria. The results of the isolation and characterization of white turmeric rhizome endophytic bacteria can be seen in Figure 1.

A) B)

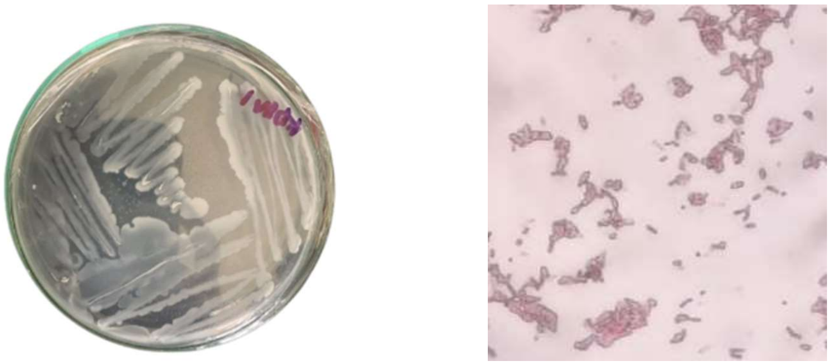

Figure 1. Endophytic Bacteria Isolate (A) and after gram staining, Gram Negative Bacteria (B)

Endophytic bacteria are bacteria that live and colonize plant tissue through mutualistic symbiosis. Plants can become hosts for microorganisms to produce metabolites to increase nutrient absorption, stimulate growth and increase plant biomass, as well as induce plant resistance to pathogens (Xia et al., 2015). The endophyte community provides benefits, one of which is as an enzyme producer. Enzymes from microorganisms are more profitable and their production is faster than enzymes from plants and animals. It is known that bacteria are one of the superior microorganisms in enzyme production (Pricilia et al., 2018).

Isolation of endophytic bacteria from white turmeric rhizomes showed that there were colonies of endophytic microorganisms on the rhizomes. This is in line with Sulistiyani et al. (2014) which stated that the population of endophytic bacteria from the white turmeric plant was mostly found in the rhizomes. The population of endophytic microbes from the roots or rhizomes is the highest compared to other plant parts because the roots are the place where microbes enter plant tissue (Dalal and Kulkarni 2013). Endophytic bacteria can be found in almost all parts of the host plant including roots, stems, leaves, fruit and tubers. Populations and types of bacteria are not only influenced by species, variety, age, health and plant development phase, but also by environmental factors such as soil, climate and season (Elmagzob et al., 2019)

3.2. Potential Protease Enzyme Activity

The results of the protease enzyme activity test showed that the white turmeric rhizome endophytic bacteria isolate had the potential to produce protease enzymes. This can be seen from the clear zone that forms around the paper disc, which shows that the endophytic bacterial isolate is capable of producing enzymes. The results of the enzyme activity test for bacterial isolates can be seen in Figure 2.

Figure 2. Activity Test of Bacterial Isolates in Producing Protease Enzymes

Based on the results of the protease enzyme activity test, the proteolytic index ratio of white turmeric rhizome endophytic bacterial isolates was 1.98. The proteolytic index ratio of the clear zone formed around the isolate on skim milk media indicates that the bacteria are able to hydrolyze the substrate. Soeka and Sulistiani (2017) stated that the formation of a clear zone produced by the test bacteria means that the bacteria are able to produce extracellular protease enzymes. This is in line with research by Widowati et al., (2020) which revealed that there was protease enzymatic activity by bacteria isolated from white turmeric rhizomes.

The proteolytic index of endophytic bacteria in white turmeric is still relatively low. According to Ahmad et al., (2013), the proteolytic index category is classified as low if it is less than 2.1. Meanwhile, the moderate category is if the proteolytic index reaches 2.1-3.1 and the rest is categorized as high proteolytic. Therefore, it is necessary to optimize supporting factors for protease production in bacteria to increase the ability of endophytic bacteria to produce protease.

The clear zone formed is protein that is degraded by proteolytic bacteria into amino acids (Shelegel, 1984), then degraded again into CO_2H_20 and ammonia (NH_3) which are released into the environment. The high proteolytic index can be caused because this isolate has the ability to quickly synthesize and degrade amino acids (Sumardi and Lengkana, 2009). According to Nadeak (2005), the number of enzymes in cells is very dependent on the speed of synthesizing and degrading amino acids, because basically in all forms of life, enzymes are synthesized from amino acids and degraded into amino acids.

IV. CONCLUSION

Based on the research result, it can be conclude that:

1. There are endophytic bacteria in Curcuma zedoaria that live in plant tissue without having a negative effect on the host
2. The endophytic bacteria Curcuma zedoaria has the potential to produce protease enzymes, with a proteolytic index value of 1.98

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