ISOLATION AND CHARACTERIZATION OF INULINASE ENZYME-PRODUCING MICROBES FROM BANANA FRUIT (Musa paradisiaca)

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ABSTRACT

Inulinase (EC 3.2.1.80) is an enzyme that can hydrolyze inulin into fructose or fructo-oligosaccharides. Using the inulinase enzyme as a catalyst in making inulinase-based fructose can obtain a fructose percentage of 90-95 percent. Inulin is a linear polysaccharide (β-2,1-linked d-fructose residue terminated by a glucose residue) that accumulates as a storage carbohydrate in plants. One natural source that can produce inulin is bananas. Bananas are a source of inulin which contains around 0.58-1.09 percent. This research used three types of bananas, including Musa X paradisiaca, Musa paradisiaca formatypica, Musa paradisiaca var sapientum. Apart from that, you can also find out the characteristics of the microbes and enzyme activity produced from the three types of bananas. The results of this research show that the three isolated types of banana are capable of producing the inulinase enzyme which is indicated by the growth of these microbes in ISM media. ISM media (Inulinase Selective Media) is an inulinase mixing media where only inulin-producing microbes can grow on the media. The microbes isolated from the three types of bananas that had the highest inulinase enzyme activity value were Raja Nangka bananas with an enzyme activity value of 0.405 IU/mL. Meanwhile, the lowest inulinase enzyme activity value was in Kepok bananas with an enzyme activity value of 0.088 IU/mL.

Keywords: banana fruit, benefits of inulinase enzyme, Inulinase enzyme, ISM media
INTRODUCTION

Inulinase (EC 3.2.1.80) is an enzyme that can hydrolyze inulin into fructose or fructooligosaccharides. Inulin can be hydrolyzed into monosaccharides and used as a carbon and energy source in the fermentation process (Carrado et al. 2021). The benefits of inulinase include being able to stimulate the immune system, reducing the risk of colon cancer and can be used as a probiotic (Utomo et al. 2022). Inulin is found in many vegetables and fruits, including onions, leeks, garlic, bananas, rye and barley (Mensink et al. 2015).

One plant that can produce inulinase is banana. Bananas (Musa paradisiaca) contain good probiotics and are able to grow in the intestines so they are beneficial for health. The fiber content in bananas is also high, one of the soluble fibers is inulin. The inulin content in bananas is less than in tubers, but bananas have a fairly high inulin content compared to other fruits. The average inulin content in bananas is ±1g/100g, while the inulin content in banana extracts is 2.10 percent (Hidayati & Syauqy, 2015).

Banana is a fruit that contains inulin in the form of fructan and resistant starch. Bananas contain 43-49g of dietary fiber, 1 g of inulin, 10-20 g of gelatin and 6 g of fructooligosaccharides in 100 g of dry matter (Waseem et al. 2022). This research used three types of bananas, namely kepok bananas (Musa paradisiaca formatypica), Ambon bananas (Musa paradisiaca var. sapientum) and Raja Nangka bananas (Musa X paradisiaca).

Kepok banana (Musa paradisiaca formatypica) is a banana that contains high nutritional value, has a good fruit texture, and tastes much better (Ernawati et al. 2021). Apart from that, Kepok bananas also contain fiber such as resistant starch and inulin which have a positive effect on blood glucose. Analysis of nutrients in Kepok bananas shows that the water content reaches 65.5 percent, carbohydrate content is 31.89 percent, antioxidant content is 12.3 percent, protein is 1.75 percent, crude fiber is 1.14 percent, fat is 0.95 percent, ash 0.72 percent, and inulin 0.1265 percent (Ruhdina & Sari, 2023). Inulin fiber plays a role in increasing the production of Short Chain Fatty Acid (SCFA) in the intestine which affects insulin metabolism in the body so that hypoglycemia can occur (Suhaeman et al., 2019).

Ambon banana (Musa paradisiaca var. sapientum) is a type of fruit that has high nutritional value. In previous research, 100 grams of Ambon banana peel contained the minerals potassium 882.38 mg, phosphorus 117mg, calcium 715 mg and iron 1.6 mg. Meanwhile, jackfruit plantain (Musa 1 gram of plantain peel contains the minerals potassium 78.1mg, calcium 19.2 mg, sodium 24.3 mg and iron 0.61 mg (Margetha et al. 2023). These two types of bananas are bananas that can be eaten immediately after they are ripe or table fruit bananas. Inulin levels will be higher when the banana has a sweeter taste. Inulin levels are also influenced by the level of ripeness, namely the higher the level of ripeness, the more bananas contain various types of sugar including glucose, fructose and maltose which are degraded by carbohydrate hydrolase enzymes (Ratnaningrum et al. 2023).

The inulinase enzyme can be obtained by isolating the microbes in the fruit. Microbial isolation can be carried out using several methods including the pour plate method, streak plate
method and spread plate method (Arini, 2016). This research aims to isolate microbes that produce the enzyme inulinase from kepok bananas, Ambon bananas and Raja Nangka bananas. As well as knowing the characteristics of the microbes and enzyme activity produced from the three types of bananas.

**MATERIALS AND METHODS**

Research on “Isolation and characterization of microbes producing the inulinase enzyme contained in bananas (Musa paradisiaca)” was carried out at the Biotechnology Laboratory, Department of Biology, Diponegoro University.

**Preparation of Potato Dextrose Agar (PDA)**

PDA was made by dissolving 8 g of PDA (Potato Dextrose Agar) in 200 mL of distilled water. The medium was sterilized by autoclaving at 121°C at 2 atm pressure for 15 minutes.

**Preparation of Inulinase Selecting Medium (ISM)** (Dion et al., 2022)

Selective media was made by dissolving 2 g of pure inulin (Sigma-Aldrich), 2 g of yeast extract, and 4 g of agar in 200 mL of distilled water. The medium was sterilized by autoclaving 121°C at 2 atm pressure for 15 minutes.

**Isolation of Microbes from Banana Fruit**

The tip of a banana that is rotten (too biologically ripe) is peeled and the flesh is removed using a sterile hose. Inoculate into PDA media using the streak method. Incubate for 24-48 hours at room temperature.

**Microscopic Observation**

The glass slide was dripped with methylene blue. Isolates that grew on Potato Dextrose Agar were taken using a sterile tube and placed on glass slides.

**Inulolytic Activity Screening** (Wijanarka dkk., 2018)

Microbes that grow on PDA are inoculated into ISM (Inulinase Selecting Medium) media using the streak method. Incubate for 24-48 hours at room temperature.

**Preparation of Inulinase Enzyme Production Medium** (Dion et al., 2022)

12g of dahlia tuber flour was dissolved in 400 mL of distilled water, heated for 25 minutes, filtered and added with 0.92g of NH4NO3; 0.92g (NH4)2.HPO4; 0.4g K2HPO4; 0.2g MgSO4.7H2O, and 0.6g yeast extract at pH 5. The production medium was autoclaved at a temperature of 121°C for 15 minutes and a pressure of 2 atm.

**Starter Making** (Wijanarka dkk., 2018)

The starter is made by inoculating one cycle of microbes that grow in ISM media and inoculating them into 5 ml of sterul production medium with pH 5 in a test tube. Microbes were incubated using a Rotary Shaker at a speed of 120 rpm at room temperature for approximately 20 hours. The results of the growing microbial culture are characterized by the production medium becoming cloudy.

**Cell Growth Measurement** (Wijanarka dkk., 2018)

The 20 hour old starter was inoculated into the inulinase production medium. The starter was incubated using a Rotary Shaker at a speed of 120 rpm at room temperature for approximately 24 hours. 3 mL of culture is taken every 6 hours,
to measure cell growth. Cell growth is determined by measuring the Optimal Density (OD) value using a spectrophotometer at 4520 nm.

**Enzyme Production** (Wijanarka *et al.*, 2018)

1 mL of culture fluid was put into an Eppendorf tube. Centrifuged at 3000 rpm for 10 minutes. The supernatant obtained is a crude enzyme and is used to test enzyme activity.

**Measurement of Inulinase Enzyme Activity** (Wijanarka *et al.*, 2018)

The supernatant resulting from centrifugation is a crude enzyme used to determine inulinase and invertase activity. Inulinase activity was measured using the DNS method and was determined based on the amount of 1 mol of reducing sugar released per minute under certain conditions. The composition of ingredients in measuring inulinase enzyme activity is shown in Table 1.

The three test tubes ES (Enzyme Substrate), S (Substrate), E (Enzyme), were incubated at 50 °C for 30 minutes. The enzymatic reaction was stopped by placing the test tube containing the sample in boiling water for 5 minutes. DNS reagent was added as much as 1 mL in cold conditions and heated again for 10 minutes. 5 mL of distilled water was added to all test tubes and measured using a spectrophotometer with a wavelength of 1570. The results of spectrophotometer measurements are 1 mol of reducing sugar released per minute under certain conditions.

**Calculation of Inulinase Enzyme Activity** (Wijanarka *et al.*, 2018)

Measurement of inulinase enzyme activity is based on the reducing sugars formed. Reducing sugar is measured by calculating the absorbance of the enzyme substrate (ES) minus the absorbance of the substrate (S) and enzyme (E) to obtain the following formula:

\[
\text{Enzyme Activity (IU/mL)} = \frac{\text{Abs. ES} - \text{Abs. E - Abs. S} \times \text{fruktosa} \times P \times 1000}{\text{BM fruktosa} \times T}
\]

Information:
Abs. ES: Enzyme and substrate absorbance
Abs. E: Enzyme absorbance
Abs. S: Substrate absorbance
BM Fructose: Molecular mass of fructose (180.1 g/mol)
P: Dilution factor (70x)
T : Incubation time (30 minutes)

**RESULTS AND DISCUSSION**

**Isolation of Inulolytic Microbes from Banana Fruit**

One natural source that can produce inulin is bananas. According to Taufira *et al.* (2019) reported that bananas are a source of inulin which contains around 0.58-1.09 percent. In

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this research, microbes were isolated from kepok bananas (*Musa paradisiaca formatypica*), Ambon bananas (*Musa paradisiaca var sapientum*), Raja Nangka bananas (*Musa X paradisiaca*) and banana liquid. Of the three types of bananas, fruit was taken that was ripe or rotten due to physiological factors and was leaking fluid. This is in accordance with the opinion of Ratnaningrum *et al.* (2023) stated that inulin levels will be higher when the banana has a sweeter taste. Inulin levels are also influenced by the level of ripeness, namely the higher the level of ripeness, the more bananas contain various types of sugar including glucose, fructose and maltose which are degraded by carbohydrate hydrolase enzymes.

Isolation of inulinase from bananas was carried out using the streak plate method on ISM media. This method is used to obtain colonies that are completely separate from other colonies. This is in accordance with the opinion of Arini (2016) that the streak plate method is a method used to obtain colonies that are completely separate from other colonies, thus making the isolation process easier. The streak plate method is carried out by streaking the media by dividing it into 3-4 quadrants in a petri dish.

Based on the isolation results, 6 microbial isolates were obtained from kepok bananas, Ambon bananas, Raja Nangka bananas and banana liquid which were able to grow on media containing inulin. Microbes that grow on

![Microbial isolates](image)

*Figure 1* Microbial isolates that grew on ISM media within a 48 hour incubation period (a) P.A1 (Ambon 1 banana) (b) P.A2 (Ambon 2 banana) (c) P.K1 (Kepok 1 banana) (d) P.K2 (Kepok 2 banana) (e) P.R1 (Raja 1 banana) (f) P.C2 (Banana Liquid 2).
ISM media can be seen in Figure 1. There is isolate P.R1 (Raja 1 banana) which has a convex and shiny colony surface. Isolate P.C2 (Banana Liquid 2) has a flat and slightly shiny colony surface. Isolate P.K1 (Kepok 1 banana) has a colony surface that is round, convex and slightly shiny. Isolate P.K2 (Kepok 2 banana) has convex and shiny colonies. Isolate P.A1 (Ambon 1 banana) has a convex and dull colony surface. Isolate P.A2 (Ambon 2 banana) has a convex and slightly shiny colony surface.

Isolation of inulinolytic microbes was carried out using ISM (Inulinase selecting medium) media. ISM media is a selective media that contains inulin as the only carbon source so that only inulinase-producing microbes are able to grow on this media. This is in accordance with the opinion of Dion et al. (2019) that ISM media is a selective medium with a single carbon source of inulin and tends to only obtain microbial isolates that have inulinase enzyme activity. According to Rizky et al. (2019) the carbon source in the media acts as a booster for enzyme production and as a biocatalyst, thereby speeding up the regeneration process.

The growth of microbes in ISM media can also be caused by the presence of macronutrients and micronutrients in the media. According to Abadianti et al. (2017), microorganisms are able to grow and produce optimal enzymes in media with optimal nutrient composition. Macronutrients such as carbon and nitrogen sources are one of the nutrients that must be available in the enzyme production medium. Apart from macronutrients, microbes also need micronutrients in the form of metal ions which are used in growth and cell metabolic function.

The three types of banana isolated indicate that the three types of banana are capable of producing inulinase because the microbes produced are able to grow in selective inulinase media, namely ISM media with optimal nutrient composition. According to research by Rizky et al. (2019) *R. Solani* which is capable of producing the inulinase enzyme is carrying out a screening procedure by growing *R. solani* in selective media with the only carbon source, namely inulin. The results of the research show that *R. solani* is the best producer of inulin because it is able to grow well on modified media containing inulin.

The morphological characteristics of isolated microbes were carried out using two types of observations, namely macroscopic observations and microscopic observations. Macroscopic observation is direct observation regarding the shape of the colony, color of the colony and surface of the colony. Meanwhile, microscopic observation is the observation of isolated microbial cells using tools, namely a microscope. In microscopic observations, microbial staining was carried out using methyl blue. According to Anggrayeni et al. (2019) *Methylene blue* staining was carried out to determine the differences between yeast and bacterial cells. Dead yeast cells do not have selectively permeable properties in their cell membranes, so methyl blue can enter the cells and do not have the ability to reduce which causes dead yeast cells to remain blue. Meanwhile, living yeast cells can retain methyl blue and have the ability to reduce which causes live yeast cells to be transparent or clear in color. Microbial cells were observed using a microscope at 400x magnification.
The morphology of inulolytic microbial isolates can be observed in Figure 2. The isolates produced were P.A1 (Ambon 1 banana), P.A2 (Ambon 2 banana), P.K1 (Kepok 1 banana), P.K2 (Kepok 2 banana), P.R1 (Raja 1 banana) after being observed using a microscope at 400x magnification, the cell morphology is round. If observed macroscopically, you will see the colonies are round in shape, with white to cream colored colonies, the edges of the colonies are flat with a smooth colony texture. This is in accordance with the opinion of Fadli et al. (2019) based on microscopic observations of the cell shape of yeast isolates, namely round.

Figure 2. Morphology of banana inulolytic microbial isolates (400x magnification) (a) P.A1 (Ambon 1 Banana) (b) P.A2 (Ambon 2 Banana) (c) P.K1 (Kepok 1 Banana) (d) P.K2 (Kepok 2 Banana) (e) P.R1 (Raja 1 Banana) (f) P.C2 (Banana Liquid 2).
Meanwhile, macroscopic morphology, namely the shape of yeast colonies, can be round, irregular, filamentous and rhizoid. Yeast colonies can be yellow, orange, red, cream and white. Each yeast has different colony characteristics so that one type of yeast can be differentiated from another.

The colonies formed on isolate P.C2 (Banana Liquid 2) were not considered yeast because after being observed using a microscope with 400x magnification, the cell morphology looked smaller and longer compared to other isolates. Macroscopic observation shows that the colonies are round and smoother or shiny compared to yeast colonies. This is in accordance with research by Yarza et al. (2021) that the morphological characteristics of bacteria are round cells, small colonies and shiny white colonies. Based on gram staining, they are divided into two, namely gram-positive bacteria and gram-negative bacteria. According to Hamida et al. (2019) gram positive bacteria after gram staining will produce a purple color because their cell walls are composed of thicker peptidoglycan so they are able to maintain the purple color of crystal violet. According to Mu’rofah & Yulian (2023), gram negative bacteria after gram staining will produce a red color with rod-shaped colonies. According to Wulantadi & Purwaningsih, (2019), gram negative bacteria cannot maintain the main paint color because their cell walls contain a lipoprotein layer which will dissolve when washed with ethanol. In this study, gram staining was not carried out because it did not reach the stage of identifying the type of bacteria in the microbe.

Growth of Microbes producing the Inulinase Enzyme

Microbial growth in isolates P.R1 (Raja 1 Banana), P.K2 (Pisang Kepok 2 Banana), P.A1 (Ambon 1 Banana), P.A2 (Ambon 2 Banana), P.K1 (Kepok 1 Banana), and P.C2 (Banana Liquid) can be seen in Figure 3. which shows that at an incubation time of 0 hours it has a low growth OD. This research did not show a lag phase due to using a starter that was 20 hours old as much as 5 percent. This is in accordance with research by Rizky et al. (2019) stated that the lag phase did not occur in the growth of the two types of yeast, which was probably due to the use of a 5 percent microbial culture starter in the starter medium. The starter is growing exponentially and is transferred to another medium with the same growth conditions such as aeration and temperature, then the microbes will not experience a lag/adaptation phase and will enter the logarithmic phase.

The microbial growth test was carried out using a spectrophotometer at 1520 nm. Based on Figure 3, it can be observed that microbes experience a log/exponential phase, namely at incubation time 0 to incubation time 12 hours. The growth rate of microbes has increased drastically, indicating that the microbes are undergoing a growth phase. According to Wahyuningsih & Zulaika (2018), cells that have adapted to the medium or environment will enter the log or exponential phase. According to Wahyuningsih & Zulaika (2018), cells that have adapted to the medium or environment will enter the log or exponential phase. The results showed that P.C2 had the highest average OD at the 6th incubation time, namely 1.892. At the 12th hour incubation time isolates P.A1, P.A2, P.K1, and P.K2 experienced an increase
in growth OD. However, the P.C2 isolate has decreased, indicating that some cells have died. Microbes experience a stationary phase at an incubation time of 12 to 24 hours where the number of microbial cell growth is equal to the number of cell deaths. The growth curve for isolates P.A2, P.K2, and P.K1 decreased, indicating that the microbes were experiencing a stationary phase. According to Abidiani et al. (2017) after an incubation time of 12 hours, the yeast culture began to enter the stationary phase. At this stage, yeast cell growth begins to slow because death begins to occur in the culture. The growth curve in this study did not show a death phase because the incubation time for microbial growth was only 24 hours.

**Inulinase Activity Test**

Enzyme activity is the amount of enzyme that releases 1 µmol of reducing sugar in the form of fructose from the inulin substrate per minute. Inulase enzyme activity was carried out to determine the levels of inulinase produced in the growth phase. Enzyme activity was measured using a spectrophotometer at a wavelength of 4570, carried out for 24 hours and taken every 6 hours. According to Wijanarka & Sarsa (2019), the inulinase enzyme activity test is carried out to determine the amount of inulinase produced. Inulinase is an extracellular enzyme that is secreted out of cells, so it needs to be separated through a centrifugation process between the supernatant (crude enzyme) and biomass. According to Mangunwidjaja et al. (2014), inulinase is divided into two types, namely endoinulinase and exoinulinase. Exoinulinase is a type of inulinase that cuts b-2,1 bonds sequentially to produce fructose, while endoinulinase cuts randomly and hydrolyzes the internal bonds in inulin, thereby producing fructooligosaccharides.

The enzyme activity produced within 24 hours of incubation was the highest activity value, namely in plantain with an enzyme activity value of 0.405 IU/mL. Meanwhile, the lowest activity value was in the Kepok banana, with an enzyme activity value of 0.088 IU/mL in a 12 hour period. This is because within a 24 hour incubation period the enzyme activity value in Raja Nangka bananas is higher than in Ambon bananas. Meanwhile, within a 2 hour incubation period, the enzyme activity value in Ambon bananas was higher than in Kepok bananas. This is in accordance with the opinion of
Wijanarka & Sarsa (2019), the longer the incubation time, the greater the amount of reducing sugar as a result of inulin hydrolysis. This reducing sugar is a catabolite that inhibits inulinase activity if it is in large quantities. According to Abdella et al. (2023), inulinase production varies greatly because its biosynthesis depends on the carbon source used. The high production of inulinase is caused by the induction of inulinase enzyme production by inulin as a specific substrate.

The difference in the enzyme activity test results for each banana is influenced by several factors, one of which is the sweetness level of the banana itself. Of the three types of banana isolated, of course they have different levels of sweetness so that the enzyme activity values produced are also different. According to Ratnaningrum et al. (2023) the sweetness level of bananas will have an effect because the levels of inulin produced will be high when the banana has a sweeter taste. Based on research that has been carried out, the types of bananas that have the highest levels of inulin are king bananas, Ambon bananas, and Kepok bananas, respectively. These results are in accordance with research by Ratnaningrum et al. (2023) that the highest average inulin levels were found in cotton bananas (20.1% ± 0.2), mali bananas (19.8% ± 0.04), bulu Nangka bananas (9.37% ± 0.00), Ambon banana (6.34% ± 0.02), Kepok banana (5.74% ± 0.00), Cere banana (4.63% ± 0.00) and Horn banana (0.46% ± 0.2). According to Ma’ariffattullah et al. (2019) the activity of the inulinase enzyme can be influenced by several factors including pH, temperature, and substrate concentration. The activity of the inulinase enzyme produced is closely related to the amount of reducing sugar produced. The greater the amount of reducing sugar produced, the higher the inulinase activity and vice versa. Determination of inulinase activity can be done with Dinitrosalicylic acid (DNS) reagent.

Samples P.K1 and P.C2 in this study did not show any enzyme activity, because the enzyme activity calculation results showed a minus value. Samples P.R1, P.K2, P.A1 and P.A2 over a certain period of time also showed no enzyme activity, as shown in Figure 4.
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activity. This can happen because after carrying out the growth test, this research did not immediately carry out an enzyme activity test, thereby allowing the enzyme contained in the sample to be damaged. Apart from that, it can also be caused by environmental factors, both in the process of testing enzyme activity and in the irradiation process, where there are several errors that cause contamination of the sample. The occurrence of contamination in the sample will greatly affect the value of the inulinase enzyme activity produced.

CONCLUSION

Based on the research that has been carried out, it can be concluded that there is an inulinase enzyme in Ambon bananas, Kepok bananas and king bananas. The highest enzyme activity value is in plantain with an enzyme activity value of 0.405 IU/mL. The lowest enzyme activity value was in Kepok banana, with an enzyme activity value of 0.088 IU/mL. Within a 24 hour incubation period, the enzyme activity value for Raja Nangka bananas was higher than for Ambon bananas. Meanwhile, within a 12 hour incubation period, the enzyme activity value of Ambon bananas was higher than that of Kepok bananas.

SUGGESTION

The results showed that there were several values of 0 in the inulinase enzyme activity within a certain incubation period. This can happen because the samples were put in the refrigerator first for 3 days due to limited equipment during the research process. Therefore, researchers recommend that further research be carried out directly to test the enzyme activity directly and not put it in the refrigerator first to avoid damage to the enzyme or contamination by certain microbes that cause the enzyme activity to be unreadable.

REFERENCES


Arini, Liss Dyah D. 2016. Mitigasi Escherichia coli dalam Berbagai Makanan di Pusat Jajanan Surakarta (Galabo)


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