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FIN FRUIT ENDOPHYTE BACTERIA PRODUCES THE ENZYMES AMYLASE, LIPASE, PROOTEASE, CELLULASE.

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ABSTRACTCT

The use of enzymes in Indonesia increases every year,So action is needed to align production and enzyme needs in Indonesia. Enzymes can be produced from various sources such as plants, animals and microorganisms. The advantage of using microorganisms in enzyme production is that they can be produced in large quantities in a shorter time and can be produced sustainably. One source of filtering bacteria to produce enzymes is figs. This research aims to examine the extracellular enzyme activity of fig endophytic bacteria and identify endophytic bacteria that are capable of producing extracellular enzymes such as protease, amylase, cellulase and lipase. Isolation and purification of bacteria using NA media for 24 hours. Test extracellular enzyme activity using NA media enriched with starch, Tween 80, skim milk, CMC. Biochemical tests were carried out to identify fig endophytic bacteria that produce extracellular enzymes. The results of the research showed that there were 21 isolates of fig endophytic bacteria, 14 of which were able to produce extracellular enzymes. The results of the identification of fig endophytic bacteria producing extracellular enzymes showed that 8 isolates were thought to be from the genus Bacillus, 3 isolates were thought to be from the genus Micrococcus, 1 isolate was thought to be from the genus Cellulomonas, 2 isolates were thought to be from the genus Acetobacter.
Keywords:*Figs, endophytic bacteria, enzymes, amylase, lipase, protease, cellulase*

Introduction

The use of enzymes in the industrial sector has been applied since 1960 and will be widely used in the future because they are more environmentally friendly and energy efficient (Ompusunggu et al., 2014). Enzymes that are used to produce products in the industrial sector are extracellular enzymes. Extracellular enzymes are able to work outside cells, such as protease, amylase, lipase and cellulase (Remijawa et al., 2020). The enzymes that are widely used and produced to meet industrial needs

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are dominated by lipase, amylase, cellulase and protease enzymes which are included in the hydrolytic enzyme group (Rahmiati et al., 2016). Protease enzymes in the food industry are used to tenderize meat for making bread and cheese (Tondais et al., 2020). The use of cellulase enzymes in the industrial sector as color protection for fabrics and for softening paper pulp (Nababan et al., 2019). The use of lipase enzymes is applied in the manufacture of textiles, cosmetics, pharmaceuticals, pulp and paper (Mosjov et al., 2020). The amylase enzyme is widely used in the food industry in the manufacture of starch syrup, fruit juice and cake production (Phieter et al, 2020).

Fig (Ficus carica) is a flowering plant belonging to the ficus genus (Suherman, 2019). Tin is included in the tribe. Moraceae has 40 genera and 1400 species that are able to grow in tropical and subtropical areas with woody trees, shrubs and epiphytes (Essid et al., 2015). Figs in Indonesia are also known as figs (Akhfiya et al., 2018). Figs contain serine, resin, albumin, rheolytic enzymes, lipase enzymes, peroxidation enzymes, and malic acid (Rahimah & Pujiastuti, 2016). Secondary metabolites and enzymes contained in figs are the result of the activity of endophytic bacteria.

Research on fig endophytic bacteria and their use is currently still rarely carried out. This research is intended to identify endophytic bacteria that are capable of producing extracellular enzymes such as protease, amylase, cellulase and lipase.

Materials and Methods

Fig Fruit Sampling

Figs are taken from the fig orchard located on Jalan Budi Luhur Gang Keluarga Baru No. 99, Dwikora Village, Medan Helvetia District, Medan City. The fig samples used were fresh and ripe Iraqi fig varieties. The fresh figs referred to in this study are figs that are 1-2 days old after being picked from the tree. The use of ripe figs is because endophytic bacteria are able to live in healthy tissue to survive and reproduce, so the use of ripe figs has the potential to produce endophytic bacteria (Tangapo, 2020).

Sterilization of Tools and Materials

The equipment that will be used for bacterial isolation must first be sterilized. The media to be used must also be sterile without other microorganisms growing to avoid contamination. The media and tools were sterilized using an autoclave at 121oC for 15 minutes, then the media was cooled to 50oC (Nababan et al., 2019).

Isolation and Purification of Fig Endophytic Bacteria

23 grams of NA media was dissolved in 1 liter of distilled water then heated, then stirred until homogeneous. The media solution was sterilized using an autoclave with a pressure of 1.5 atm at a temperature of 121oC for 15 minutes (Hudaya et al., 2014). Isolation and purification of fig endophytic bacteria using NA media. The NA media solution was chosen for the isolation of endophytic bacteria because its composition is able to suppress the growth of endophytic fungi. Fresh figs are sterilized for one minute using 2 ml of 70% alcohol, then removed and drained. The figs are then soaked for five minutes in 5.25% Naocl, then drained again and placed in 70% alcohol for 30 seconds. The sample was rinsed using distilled water for one minute with two repetitions (Aryani et al., 2020). The figs are then cut into 1x1 cm pieces. Bacteria were isolated using the direct plating method, which is a method of growing bacteria using 4-5 plant cuttings on NA media, then incubating at 37oC for 48 hours (Ginting et al., 2020). Bacterial isolation was carried out by repeating three times to see the diversity of bacteria.

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Gram Staining of Fig Fruit Endophyte Bacterial Isolates

Gram staining is done by cleaning the glass object using 95% alcohol, then fixing it with a Bunsen flame, then waiting until it cools. The bacterial isolate is taken aseptically as one vial needle and then spread thinly on a glass slide. The specimen was fixed over a busen flame by passing it over the flame three times. The object glass was dripped with crystal violet until the preparation was covered and left for 30-60 seconds at room temperature. Wash the preparation using distilled water for 5 seconds. The object glass was dripped with Lugol's solution and left for 1-2 minutes at room temperature, then rinsed with distilled water for 5 seconds. Then decolarization was carried out by dripping 95% alcohol on the object glass, then rinsing using distilled water for 5 seconds. Next, safranin was dropped onto the object glass and left for 1 minute, then rinsed with distilled water for 5 seconds and aired to dry. The preparation was observed under a microscope. Purple bacteria indicate gram-positive bacteria, while red bacteria indicate gram-negative bacteria (Rahmatullah et al., 2021).

Motility Test

The motility test of fig endophytic bacteria followed the method of Sardiani et al., (2015), namely that the endophytic bacterial isolates were taken 1 dose each and then inoculated by pricking on SIM (Sulphide Indole Motility) media and incubated at 37oC for 2x24 hours. Bacteria that are motile (move) can be seen in the presence of propagation marks around the media used by the needle puncture.

Citrate Test

The citrate test for fig endophytic bacteria followed the method of Hasiolan et al., (2022), namely that the endophytic bacterial isolates were taken 1 dose each and then inoculated by pricking them on Simmon citrate media so that they were slanted and incubated at a temperature of 37oC for 24 hours. Positive tests are marked by the formation of a blue color, while negative tests are marked by a green color (Sardiani et al, 2015).

Catalase Test

The catalase test for fig endophytic bacteria follows the method of Sardiani et al., (2015), namely that the endophytic bacterial isolates are taken 1 dose each and then inoculated on an object glass, 2-3 drops of H2O2 solution are added. The formation of gas bubbles indicates a positive result, while gas bubbles not forming indicates a negative result.

Amylase Enzyme Activity Test

NA media enriched with 1% starch was used as a medium for testing amylase enzyme activity. 2.3 grams of NA media was mixed with 100 mL of distilled water. After that, it is heated and stirred until homogeneous. 1 gram of starch is weighed then mixed into the medium that is being heated and then stirred until homogeneous. The homogenized media was sterilized using an autoclave for 1 hour at a temperature of 121oC to avoid contamination from other microorganisms. The media was cooled to a temperature of ± 40oC. Then the media is poured into a petri dish aseptically and then wait until it solidifies. Endophytic bacterial isolates were taken 1 dose each, then inoculated on starch agar media using the streak method with spot scratches and grown at a temperature of 37oC for ± 48 hours. The activity of the amylase enzyme is shown by the presence of a clear zone around the colony using a dark blue background (Remijawa et al., 2020). The clear zone is formed because the test medium containing starch and dripping with iodine is hydrolyzed by endophytic bacteria to determine the ability of the bacteria to use starch (Gultom et al, 2021).

Lipase Enzyme Activity Test

NA medium enriched with Twen 80 1% was used as a medium for testing lipase enzyme activity. 2.3 grams of NA media was mixed with 100 mL of distilled water and added with 1 mL of Tween 80. The

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media was heated and stirred until homogeneous. The media was sterilized using an autoclave for 1 hour at 121oC to avoid contamination from other microorganisms. The media is cooled to a temperature of ± 40oC, then poured into a petri dish aseptically and then waited until it solidifies. Endophytic bacterial isolates were taken 1 dose each, then inoculated on agar media enriched with Tween 80 using the streak method with spot streaks and grown at a temperature of 37oC for ± 48 hours. Lipase activity can be seen by the formation of a cloudy white zone around the bacterial isolate which is a fatty acid precipitate (Adelita et al., 2019).

Protease Enzyme Activity Test

NA medium enriched with 1% skim milk was used as a medium for testing protease enzyme activity. 1 gram of skim milk and 2.3 grams of NA media mixed with 100 ml of distilled water. The media was heated and stirred until homogeneous, then sterilized using an autoclave for 1 hour at a temperature of 121oC. The media is poured into a petri dish aseptically then wait until it solidifies. Endophytic bacterial isolates were taken 1 dose each and then inoculated on agar media enriched with skim milk using the streak method with spot streaks and grown at a temperature of 37oC for ± 48 hours. Protease activity is indicated by the formation of a clear zone around the bacterial colony (Pricilia et al., 2018).

Data analysis

The research data is presented in the form of images, namely documentation and measurement of enzyme activity indices as well as identification of the characteristics of endophytic bacteria which are arranged in a table. The morphology of bacterial colonies that produce enzymes is best described in narrative form. The enzyme activity index is carried out by measuring the ratio between the diameter of the clear zone and the diameter of the bacterial colony (Murtiyaningsih & Hazmil, 2017). The formula for calculating the enzyme activity index:

$$\text{Indeks Aktivitas Enzim} = \frac{\text{Diameter zona bening-Diameter koloni}}{\text{Diameter koloni}}$$

Extracellular enzyme activity is classified into 4 groups based on the ratio of the diameter of the clear zone to the diameter of the bacterial colony, namely high enzyme activity if the extracellular enzyme index is greater than 2, medium enzyme activity if the extracellular enzyme index is between values 1 to 2, low enzyme activity if the extracellular enzyme index smaller than 1, and there is no activity (Sutari, 2020).

Results and Discussion

The results of the isolation of fig endophytic bacteria (Ficus carica L.) in this study were 21 isolates identified macroscopically and microscopically. Macroscopic identification includes colony edges, colony color, elevation and shape of the colony, and microscopic identification using gram staining (Table 1).

Table 1. Characteristics of Fig Fruit Endophyte Bacterial Isolates (*Ficus carica L.)*

Code Isolate	Form Colony	Color Colony	Edge Colony	Elevation	Cell shape	Gram color
BE1	Circular	yellow	entire	convex	coccus	positive
BE2	Circular	white	entire	flat	coccus	positive
BE3	Irregular	white	undulate	flat	basil	positive
BE4	Irregular	broken white	undulate	flat	basil	positive
BE5	Irregular	white	undulate	convex	basil	positive
BE6	Circular	white	entire	flat	basil	positive
BE7	Irregular	white	entire	flat	coccus	positive
BE8	Filamentus	white	filiform	flat	basil	positive

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BE9	Circular	white	entire	flat	basil	positive
BE10	Circular	white	entire	convex	basil	negative
BE11	Irregular	white	undulate	flat	coccus	positive
BE12	Filamentus	white	filiform	flat	basil	positive
BE13	Irregular	white	lobate	flat	basil	positive
BE14	Irregular	white	undulate	rosed	basil	negative
BE15	Irregular	white	undulate	umbonale	basil	positive
BE16	Irregular	yellow white	undulate	flat	basil	positive
BE17	Irregular	white	undulate	rosed	coccus	positive
BE18	Irregular	white	undulate	flat	coccus	positive
BE19	Circular	pink white	entire	convex	basil	negative
BE20	Irregular	white	undulate	flat	basil	negative
BE21	Circular	white	entire	umbonale	coccus	negative

Macroscopic and microscopic characteristics can be seen in Table 4.1. Macroscopic characteristics of fig endophytic bacteria showed that bacterial colonies consisted of 7 circular isolates, 12 irregular isolates, and 2 filamentous isolates. The color of the colony was dominated by white for 17 isolates, yellow for 1 isolate, white for 1 isolate and pinkish white for 1 isolate. There were 8 isolates of entire colony edges, 10 isolates of undulate, 2 isolates of filiform and 1 isolate of lobate. There were 13 isolates of flat colony elevation, 4 convex isolates, 2 rising isolates and 2 umbonale isolates. Based on the results of microscopic observations (Table 4.1), 14 isolates were bacillus-shaped, 7 coccus-shaped isolates, 5 gram-negative isolates and 16 bacterial isolates. gram positive. Previous research conducted by Gultom et al, (2023), obtained results from 27 isolates of fig endophytic bacteria with irregular, circular, rhizoid, filamentous, complex colonies and colonies tending to be white. Cells tend to be bacillary and gram negative. The results of research by Venita & Gultom, (2023), showed that 6 isolates of fig endophytic bacteria were obtained which had macroscopic characteristics dominated by irregular colony shapes and were white in color. Microscopic observations were dominated by gram-positive coccus forms. Colony colors are different because there are intracellular pigments produced by bacteria (Sudewi et al., 2020). The BE19 isolate has the same color as figs, namely pinkish white, Breed et al. (1957) stated that the white circle-shaped colonies with pink centers were bacterial colonies isolated from figs.

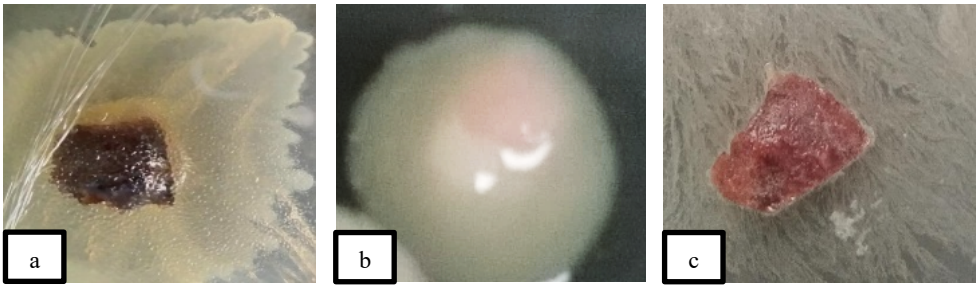

Figure 1.Macroscopic characteristics of fig endophytic bacterial isolates (a. isolate

Identification of endophytic bacteria is carried out using biochemical tests to determine the genus of endophytic bacteria that produce extracellular enzymes. The biochemical tests used are the motility test, citrate test and catalase test. The results of the biochemical test for endophytic bacteria can be seen in table 2.

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Table 2. Results of Identification of Fig Fruit Endophyte Bacterial Isolates (Ficus carica L.) Biochemical Tests

Isolate Code	Motility	Citric	Catalase	Genus
BE1	Negative	Negative	Positive	*Micrococcus*
BE3	Negative	Negative	Positive	*Bacillus*
BE4	Negative	Positive	Positive	*Cellulomonas*
BE5	Negative	Negative	Positive	*Bacillus*
BE6	Negative	Negative	Positive	*Bacillus*
BE7	Negative	Negative	Positive	*Micrococcus*
BE8	Negative	Negative	Positive	*Bacillus*
BE9	Negative	Negative	Positive	*Bacillus*
BE13	Negative	Negative	Positive	*Bacillus*
BE14	Negative	Positive	Positive	*Acetobacter*
BE15	Negative	Negative	Positive	*Bacillus*
BE16	Negative	Negative	Positive	*Bacillus*
BE17	Negative	Negative	Positive	*Micrococcus*
BE19	Negative	Positive	Positive	*Acetobacter* (*Acetobacter roseus*)

Based on the results of a comparison of the characteristics of fig endophytic bacterial isolates (Ficus carica L.) that produce extracellular enzymes using the book Bergey's Mannual of Determinative Bacteriology, it was found that the fig endophytic bacteria isolate BE1 was thought to be from the genus Micrococcus, the fig endophytic bacterial isolate BE3 was thought to be from the genus Bacillus, fig endophyte bacteria isolate BE4 is thought to be from the genus Celullomonas, fig endophyte bacteria isolate BE5 is thought to be from the genus Bacillus, fig endophyte bacteria isolate BE6 is thought to be from the genus Bacillus, fig endophyte bacteria isolate BE7 is thought to be from the genus Micrococcus, fig endophyte bacteria isolate BE8 is thought to be from the genus Bacillus, fig endophyte bacteria isolate BE9 is thought to be from the genus Bacillus, fig endophyte bacteria isolate BE13 is thought to be from the genus Bacillus, fig endophyte bacteria isolate BE14 is thought to be from the genus Acetobacter, bacterial isolate The BE15 fig endophyte is thought to come from the genus Bacillus, the BE16 fig endophyte bacteria isolate is thought to be from the Bacillus genus, the BE17 fig endophyte bacteria isolate is thought to be from the Micrococcus genus, the BE14 fig endophyte bacteria isolate is thought to be from the Acetobacter genus.

Fig endophytic bacteria (Ficus carica L.) are capable of producing the enzymes amylase, lipase, protease and cellulase. A total of 5 bacterial isolates produced the amylase enzyme, 10 bacterial isolates produced the lipase enzyme, 4 bacterial isolates produced the protease enzyme and 7 bacterial isolates produced the cellulase enzyme, presented in Figure 2.

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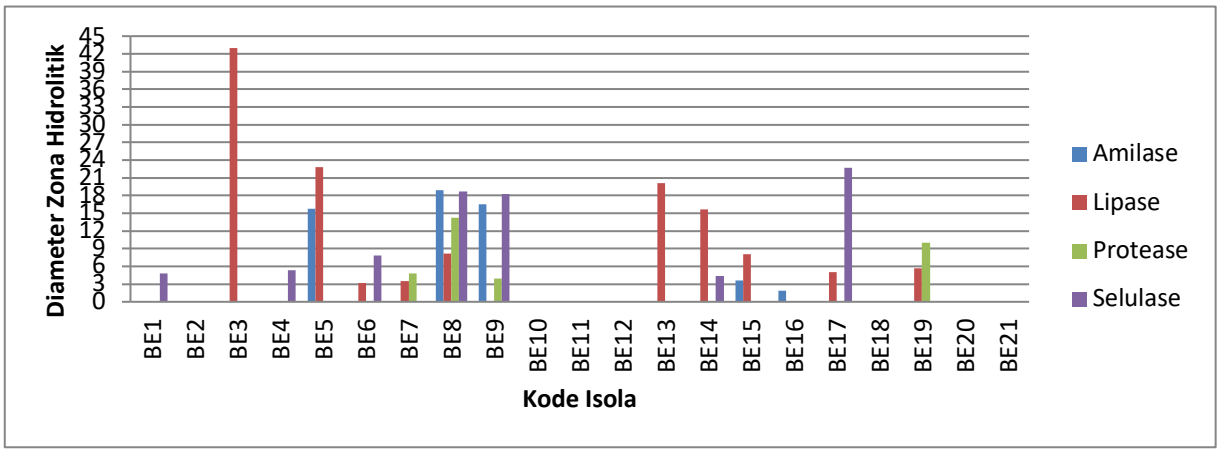

Figure 2.Extracellular Enzyme Activity of Fig Fruit Endophyte Bacteria (Ficus carica L.)

The fig endophytic bacteria that is capable of producing the four extracellular enzymes is isolate BE8. The fig isolate BE9 endophytic bacteria is capable of producing amylase, protease and cellulase enzymes. There are two isolates of fig endophytic bacteria that produce lipase and amylase enzymes, namely isolates BE5 and BE15. There are three isolates of fig endophytic bacteria that produce lipase and cellulase enzymes, namely isolates BE6, BE14, and BE17. Fig endophytic bacteria isolates BE7 and BE19 are able to produce lipase and protease enzymes. Isolate BE16 is only able to produce the amylase enzyme. The fig endophytic bacteria isolates BE3 and BE13 were only able to produce lipase enzymes. Fig endophytic bacteria isolates BE1 and BE4 were only able to produce cellulase enzymes.

Based on Figure 3, three isolates of fig endophytic bacteria produced the amylase enzyme with a high category enzyme activity index and two isolates with a low category enzyme activity. There were five isolates of fig endophytic bacteria producing lipase enzymes with high category enzyme activity, three isolates of fig endophytic bacteria producing lipase enzymes with medium category enzyme activity and two isolates of fig endophytic bacteria producing lipase enzymes with low category enzyme activity.

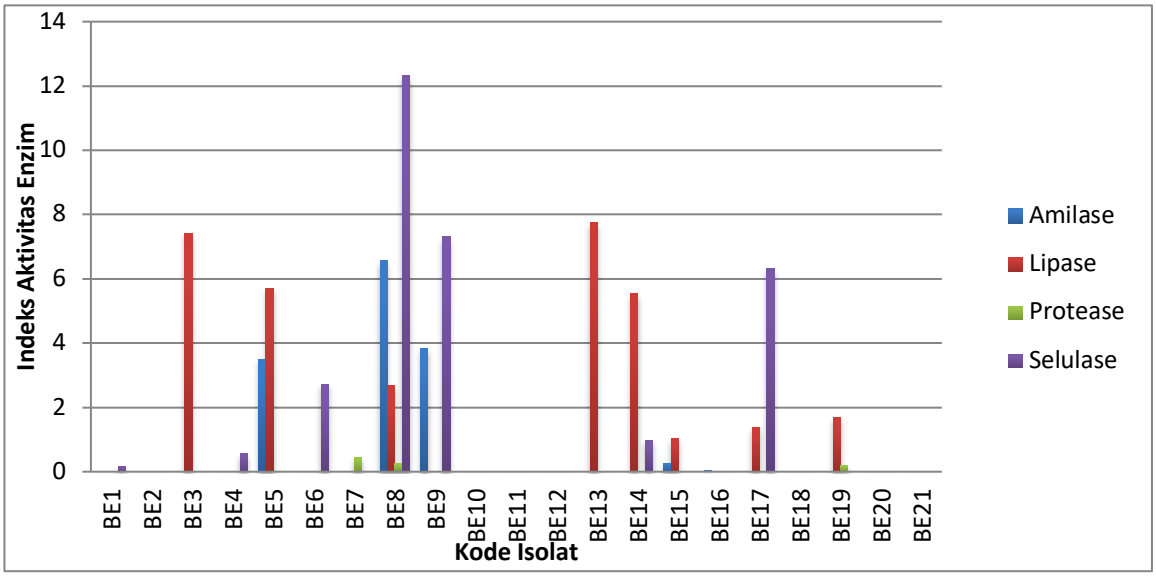

Figure 3.Extracellular Enzyme Activity Index of Fig Fruit Endophytic Bacteria (Ficus carica L.)

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The amylase enzyme activity was produced by four isolates of fig endophytic bacteria, which was characterized by the formation of a clear zone around the bacterial colony. Amylase enzyme activity was obtained by dripping iodine on a test medium containing starch. Iodine reacts with starch to form a dark blue-black color on the media and forms a clear zone around the colonies of fig endophytic bacteria which is due to the ability of fig endophytic bacteria to hydrolyze starch so that the interaction between iodine and starch is reduced (Fazal et al, 2022). The amylase enzyme is able to hydrolyze starch into simple sugars such as glucose, dextrin and maltose (Ginting et al, 2018). The amylase enzyme randomly breaks the glycoside bonds in starch molecules, then hydrolyzes them and produces simple sugars such as oligsaccharides and dextrins (Subagiyo et al, 2017). Starch is hydrolyzed through three stages, namely gelatinization, liquefaction and saccharification (Nangin & Aji, 2015). The research results of Krishnan et al. (2012), papaya fruit endophytic bacteria are able to produce the enzyme amylase.

Lipase enzyme activity was produced by fig endophytic bacteria as many as ten bacterial isolates. The presence of lipase enzyme activity is characterized by the formation of a cloudy white zone around the bacterial colony which is a precipitate of fatty acids (Adelita et al., 2019). Lipase enzyme activity was obtained from tween 80 in the test medium which acts as a substrate and is hydrolyzed by endophytic bacteria into monooleic acid which binds to calcium (Ervina et al., 2020). The lipase enzyme is able to hydrolyze triglyceride, diglyceride and monoglyceride fats into glycerol and free fatty acids (Sarjono et al, 2022). The source of fat or lipids used in the lipase enzyme activity test is Tween 80. Tween 80's ability to increase permeability in cell walls makes it easier to release enzymes from cell walls (Moentamaria et al, 2016). Tween 80 is an oleic acid monoester from polyoxyethylene sorbitan. The ester content in Tween 80 will be hydrolyzed by bacteria into mono-oleic acid, then the mono-oleic acid binds with calcium (from CaCl2.2H2O) which produces a turbid color around the bacterial colony (Ervina et al, 2020). The results of research by Dogan & Taskin (2021), endophytic bacteria from Poaceae plants are able to produce lipase enzymes.

Protease enzyme activity was produced by fig endophytic bacteria in four bacterial isolates. Protease enzyme activity is characterized by the formation of a clear zone around the colony as a result of hydrolysis of skim milk media as a substrate by endophytic bacteria (Rori et al, 2020). The research results of Bhutani et al. (2021), obtained 9 isolates of green bean endophytic bacteria capable of producing protease enzymes. Protease enzymes are able to hydrolyze peptide bonds in protein molecules to produce amino acids or peptides (Susilowati et al, 2020). The protein source used in the protease enzyme activity test is skim milk. Skim milk contains casein which is good for isolating endophytic bacteria that produce protease enzymes (Asril & Leksikowati, 2019). Microorganisms utilize skim milk as a substrate for biomass growth, producing metabolite products, maintenance and cell division (Setyati et al, 2015). The function of the protease enzyme is to break the peptide bonds contained in casein in milk so that a clear zone is formed around the bacterial colony (Zahidah & Shovitri, 2013).

Cellulase enzyme activity was produced by fig endophytic bacteria as many as seven bacterial isolates. Cellulase enzyme activity is characterized by the formation of a yellowish clear zone around the colony. The cellulase enzyme works to convert the cellulose substrate into glucose (Purkan et al, 2015). The source of cellulose substrate in the cellulase enzyme activity test is CMC (Carboxy methyl cellulose). CMC (Carboxy methyl cellulose) is a

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derivative of water-soluble cellulose which is capable of detecting the production of endol 1,4-β glucanase and is rapidly degraded by microorganisms.Cellulase enzyme activity was obtained by dripping Congo red on test media containing cellulose. Congo red interacts with (1,4)- β-d-glucan and (1,3)- β-d-glucan during cellulose hydrolysis to form a yellowish clear zone around the bacterial colony.(Yousef et al, 2019).The research results of Vijayalakshmi et al. (2016), mango endophytic bacteria are able to produce cellulase enzymes.

Conclusions

Fig endophytic bacteriaable to produce the enzymes amylase, lipase, protease, and cellulase. A total of 5 bacterial isolates produced amylase enzymes, 10 bacterial isolates produced lipase enzymes, 4 bacterial isolates produced protease enzymes and 7 bacterial isolates.

The results of the identification of fig endophytic bacteria producing extracellular enzymes showed that 8 isolates were thought to be from the genus Bacillus, 3 isolates were thought to be from the genus Micrococcus, 1 isolate was thought to be from the genus Cellulomonas, 2 isolates were thought to be from the genus Acetobacter.

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