pISSN 2302-1616, eISSN 2580-2909
Vol 12, No. 1, June 2024, pp. 34-46
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i1.41668

Endophytic bacteria isolated from stems and roots of *Acrostichum aureum* Linn. potential for hydrolytic enzyme and α-amylase inhibitor

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ABSTRACT. Endophytic bacteria live symbiotically in plant tissues but do not hurt plants. Endophytic bacteria are widely used in the industrial sector as enzyme producers. This study aims to examine the potential of endophytic bacteria from the stems and roots of sea fern (*Acrostichum aureum* L.) as producers of hydrolytic enzymes and to determine their potential as α-amylase inhibitors. Macroscopic and biochemical tests characterized endophytic bacterial isolates. Hydrolytic enzyme activity test consisted of cellulase, lipase, and laccase enzyme. Isolates that were able to hydrolyze were tested for antidiabetic potential by α-amylase inhibitor test. A total of 24 bacterial isolates were selected for their ability to produce cellulase, lipase, and laccase. The results obtained 24 isolates of endophytic bacteria showed that as much as 33% of stem isolates and 52% of root isolates were able to produce hydrolytic enzymes. The α-amylase inhibition test results of the three endophytic bacteria tested were isolates A.T 2 (17%) and A.A 3 (8%) on 1% starch substrate, and A.T 2 (36%) on 2% starch substrate with a spectrophotometer at a wavelength of 540 nm. Endophytic bacteria isolated from the stems and roots of sea ferns can be developed as an alternative base material for herbal medicines for antidiabetics.

Keywords: *Acrostichum aureum* L.; α-amylase inhibitor; endophytic bacteria; hydrolytic enzyme; sea fern

Article History: Received 27 October 2023; Received in revised form 14 May 2024; Accepted 26 May 2024; Available online 30 June 2024.
How to Cite This Article: Linda TM, Defani SA, Berliansyah A, Febriarti BL, Zul D. 2024. Endophytic bacteria isolated from stems and roots of *Acrostichum aureum* Linn. potential for hydrolytic enzyme and α-amylase inhibitor. *Biogenesis: Jurnal Ilmiah Biologi*. vol 12(1): 34–46. doi: https://doi.org/10.24252/bio.v12i1.41668.

INTRODUCTION

Peatlands are layers of soil rich in organic matter and store much higher carbon than mineral soils (Klingenfuß *et al*., 2014; Anda *et al*., 2021). Based on the organic matter content, peatlands have a fairly high biodiversity, which dominated by trees, shrubs, and ferns (Korasidis *et al*., 2017; Akbar, 2022), including the sea fern (*Acrostichum aureum* L.). Exploration of ferns in the Riau province by Sofiyanti *et al*. (2019) reported 20 species of ferns in Bengkalis and 21 species in Meranti. In these two peatlands, sea ferns were found coexisting with mangroves. Ferns play an important role in ecosystem balance, regulating water management, as traditional medicine (Badhsheeba & Vadivel, 2020), antimicrobial (Rakkimuthu *et al*., 2018; Santhosh *et al*., 2022), anti-inflammatory (Abiola & Adetutu, 2022), antioxidant, and antidote to snake venom (Ultari *et al*., 2021). Previous studies reported that sea fern extract contain phytochemical compounds such as saponins, proteins, steroids, terpenoids (Badhsheeba & Vadivel, 2020), phenolics, flavonoids (Hanin & Pratiwi, 2017), saponins, and alkaloids (Linda *et al*., 2023).

Plants produce phytochemical compounds in response to environmental stress. The largest group of phenolic compounds are flavonoids, which have antioxidant activity and can increase self-defense from diseases induced by free radicals (Tungmunnithum *et al*., 2018). Flavonoids, a class of plant secondary metabolites, have been recognized for their antioxidant properties. These properties are hypothesized to contribute to the prevention of fat accumulation, potentially mitigating obesity, a known risk factor for developing diabetes mellitus (Al-Ishaq *et al*., 2019; Rufino *et al*., 2021). Sea fern leaves, rich in various bioactive compounds including alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids, warrant investigation for their potential antihyperglycemic effects (Nurhasnawati *et al*., 2019; Hanif *et al*., 2022).

Long-standing symbiosis with their plant hosts empowers endophytic bacteria to potentially synthesize diverse bioactive compounds derived from host secondary metabolites (Chigurupati *et al*., 2019; Pratiwi, 2019). Linda *et al*. (2022) reported the phytochemicals of the crude extract of secondary metabolites of six endophytic bacterial isolates from the leaves of sea fern from Bengkalis Island containing alkaloid and saponin compounds. Various studies on the bacterial community associated with plants as endophytes have developed quite rapidly, considering the important contribution of the bacterial community. One of the contributions of endophytic bacteria is as producers of enzymes and therapeutic agents such as antioxidants and antihyperglycemic. Rori *et al*. (2020) obtained seven isolates of endophytic bacteria producing amylase, protease, and cellulase enzymes from *Api-Api Putih* (*Avicennia marina*) leaves.

Information on the diversity of endophytic bacteria from the stems and roots of marine ferns and their potential to produce hydrolytic enzymes and α-amylase inhibitors is limited. This study aims to assess endophytic bacteria' potential from sea ferns (*Acrostichum aureum* L.) stems and roots as hydrolytic enzyme producers and determine their potential as α-amylase inhibitors. This study offers *A. aureum* L. as a promising source for discovering novel α-amylase inhibitors with potential applications in diabetes management, independent of seasonal or geographic limitations.

MATERIALS AND METHODS

Study area. The media and materials used in this study included 13 endophytic bacteria isolated from stem organs and 11 endophytic bacteria isolated from root organs of the sea fern from the Meranti Islands (Fig. 1).

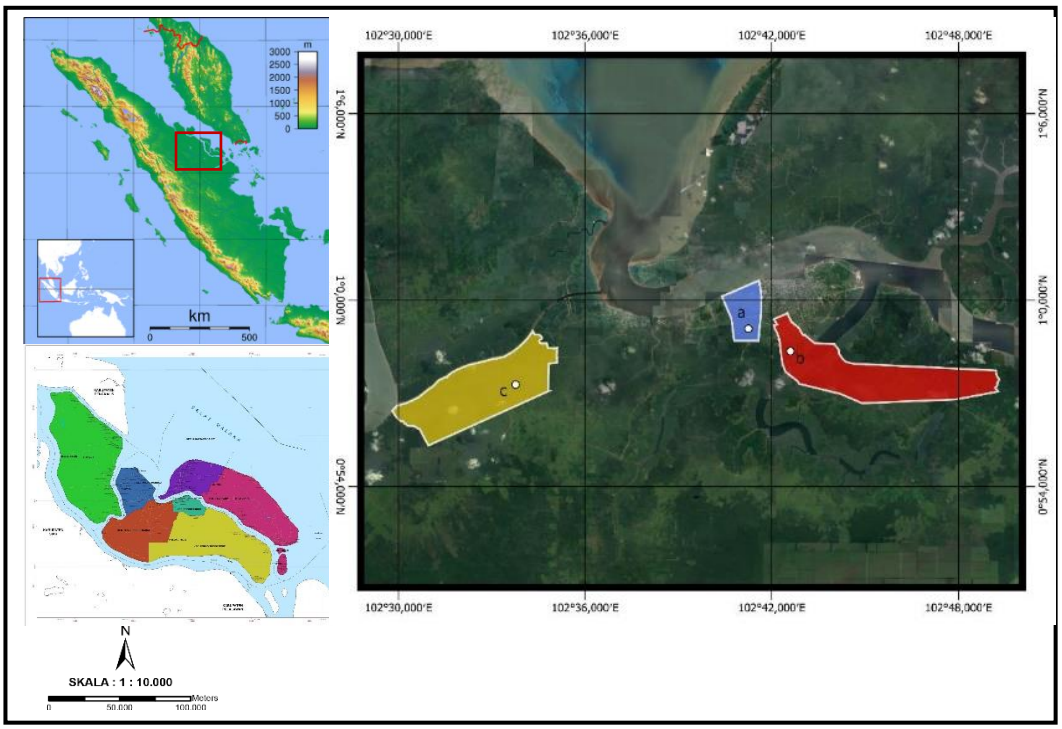

Fig. 1. Location of peatland location area at Meranti, Riau Province, Indonesia: a. Alah air village; b. Banglas Barat village; c. Kundur village

Preparation of bacteria. Eleven isolates of endophytic bacteria isolated from roots: A.T 1, A.T 2, A.T 3, A.T 4, A.B 1, A.B 2, A.A 1, A.A 2, A.A 3, A.A 4, and A.A 5 and 13 bacterial isolates endophytes come from stems: B.T 1, B.T 2, B.T 3, B.T 4, B.T 5, B.B 1, B.B 2, B.B 3, B.B 4, B. A 1, B.A 2, B.A 3, and B. A 4. Sea fern was taken from the peat soil area of Meranti Island, Riau

Province. The isolate subculture was rejuvenated by streak quadrant on NA medium, then incubated at room temperature for 24 h.

Morphological and biochemical characterization endophytes bacteria. Macroscopic and biochemical tests characterized endophytic bacterial isolates. Macroscopic characterization includes colony morphology in the form of colony color, elevation, shape, size, and edges. Biochemical characterization was carried out with gram staining and a 3% KOH test.

Hydrolytic enzyme activity. The endophytic bacteria were spotted on medium with a certain composition (Table 1).

Table 1. Media composition for hydrolytic activity tests

Hydrolytic activity tests	Media composition	Reference
Lipase	Tripton 0.5 g/L, beef extract 0.3 g/L, olive oil 0.5 mL/L, agar 1.5 g/L, dan phenol red 0.01 g/L.	Vertygo (2021)
Cellulase	Starch 10 g/L, KNO_3 2 g/L, NaCl 2 g/L, K_2HPO_4 2 g/L, $MgSO_4.7H_2O$ 0.05 g/L, $CaCO_3$ 0.02 g/L, $FeSO_4.7H_2O$ 0.01 g/L, CMC 1%, agar 18 g/L.	Pesrita *et al.* (2017)
Laccase	Nutrient agar (NA) 28 g/L dan guaiacol 1 mL/L.	Wins *et al*. (2019)

The medium was incubated for three days for cellulase and lipase enzyme tests and four days for laccase enzyme test. At the end of incubation, the medium was added to 1% iodine solution to form a clear zone. The positive test of hydrolytic activity of lipase enzyme is the formation of a yellow zone and a positive test of laccase enzyme with the color change of isolate to brownish. The enzymatic index was measured by following formula (Cornish-Bowden *et al*., 2014):

$$\text{Enzymatic Index} = \frac{\text{Diameter of clear zone}}{\text{Diameter of colony}}$$

Screening of endophytic bacteria. Endophytic bacterial isolates were screened for the ability to grow on selective deMan Rogose Sharpe Agar (MRSA) media. A positive test is characterized by growth after 24 h incubation. Furthermore, positive tests on MRSA media were further tested on MRSA + $CaCO_3$ media with three various concentrations: 0.5%, 0.25%, and 0.1%. Endophytic bacterial isolates were bottled on MRSA + $CaCO_3$ selective medium with a positive test marked by a clear zone after 48 h of incubation. Isolates that were able to hydrolyze were then tested for antidiabetic.

α-amylase inhibitor assay. Preparation of the medium for the production of crude bacterial extracts followed the method (Linda *et al*., 2023). One milli of endophytic bacterial culture (10^8 CFU/mL) was grown in 90 mL NB. Samples were incubated at incubator for 72 h at 30°C agitated at 150 rpm. Centrifugation was performed to separate the bacterial cells at 3,500 rpm for 15 min. The supernatant (crude extract) was tested for their inhibitory activity against the α-amylase enzyme. A sample of 0.5 mL of crude extract of bacterial secondary metabolites was taken, to which 0.5 mL of α-amylase enzyme (0.5 units/mL) was added and incubated for 10 min at 25°C. One mL of starch solution, each on 1% and 2% substrate, was added to the test tube and then incubated for 10 min at 25°C. At the end of the incubation, 2 mL of dinitro salicylic acid (DNS) reagent was added and heated for 5 min at 100°C to stop the reaction. The same treatment was also carried out for the negative control, where 0.5 mL of the sample was replaced with aqua DM. Acarbose positive control with a concentration of 10 ppm was also given the same treatment as the sample. Each treatment was made with three repetitions. The absorbance of each sample solution was measured with a spectrophotometer at λ 540 nm. The formula for the percentage of inhibitor as follows (Langmuir, 1916):

$$\text{I\%} = \frac{\text{Absorben control} - \text{Absorben Sampel}}{\text{Absorben control}}\ x\ 100\%$$

Data analysis. Data from macroscopic, biochemical characterization, enzyme assay and α-amylase inhibitor measurement of each endophytic bacteria were analyzed descriptively presented in tables and figures.

RESULTS AND DISCUSSION

Characterization of endophytic bacteria. Endophytic bacteria from sea fern were obtained as many as 24 isolates that were characterized based on their morphology and biochemical tests. According to Sousa *et al*. (2013), characterization of colony morphology is very important as an indicator of bacterial phenotypic variation. The characteristics of bacterial colonies observed in this study are colony color, elevation, shape, and colony edges. endophytic bacterial colonies showed variations in color, elevation, and shape of colony edges (Fig. 2).

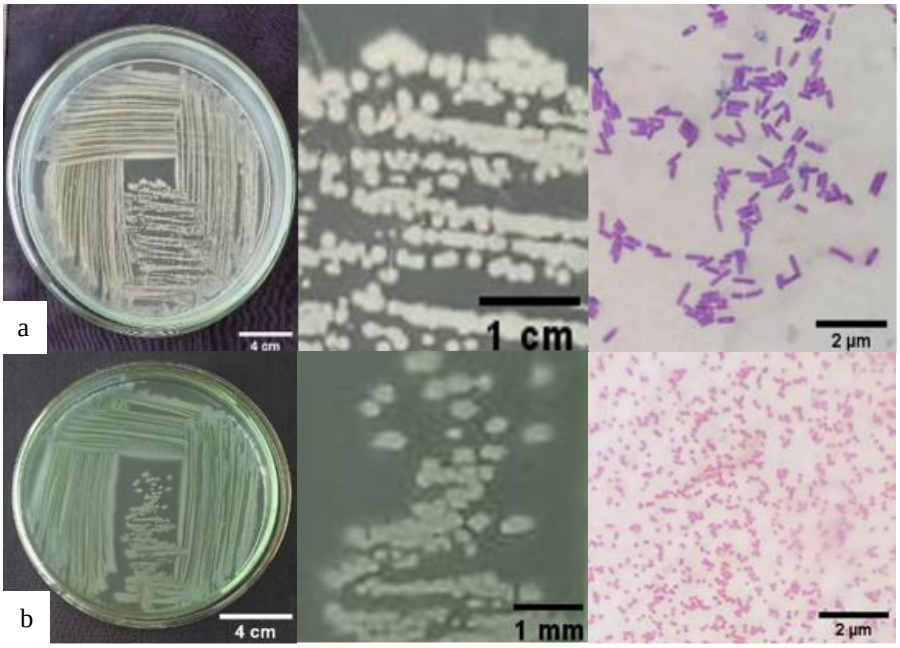

Fig. 2. Morphology of colony and Gram staining of endophytic bacterial: a. isolate B.B3; b. isolate A.T2 (Light microscope, 1000 × magnification)

Various studies have reported endophytic bacteria with milky white and yellow colony colors (Linda *et al*., 2022) from sterile leaves of sea fern, white and yellow from white fire (*A. marina*) (Ramadhanty *et al*., 2021), then yellowish white and white from white fire (Prihanto *et al*., 2018a). Colony elevations observed in this study were flat and convex. Various studies have reported the elevation of endophytic bacteria, namely raised from the roots, stems and leaves of *Api-Api Putih* (Ramadhanty *et al*., 2021), and convex from the respiratory roots of *Api-Api Putih* (Yanti *et al*., 2021). The colony shape of 24 bacteria was circular and irregular. Various studies have reported the form of circular endophytic bacterial colonies from the roots, stems and leaves of *Api-Api Putih* (Ramadhanty *et al*., 2021), and the irregular shapes of the *Pidada Putih* (*Sonnetaria alba*) (Prihanto *et al*., 2018b).

The microscopic characteristics observed in this study were short bacilli. Biochemical characterization is also an important aspect to determine the physiological properties of bacteria. Therefore, cell shape observations, Gram staining tests and 3% KOH tests were carried out on 24 endophytic bacteria. The results of microscopic characterization and biochemical tests are presented in Table 2. A total of 13 isolates were Gram positive and nine isolates were Gram negative. Prihanto *et al*. (2018a) reported the results of the characterization of endophytic bacteria isolated from the roots, stems and leaves of *Api-Api Putih* with the form of bacilli and Gram-positive cells. Pujianto *et al*. (2015) and Pujianto *et al*. (2018) reported the results of the characterization of endophytic bacteria isolated from bitter melon and soursop plants in the root, stem and leaf organs, obtaining results for Gram positive and negative bacteria. Hidayati *et al*. (2014) reported the results of the characterization

of endophytic bacteria isolated from the leaves, bark and roots of rubber plants, Gram-positive and negative endophytic bacteria. Our previous study (Linda *et al*., 2022) reported isolates of endophytic bacteria from sterile leaves of sea ferns with staining results belonging to the Gram-negative category. This shows that there is a great variety of endophytic bacteria found in various types of plants.

Table 2. Microscopic and biochemical characterization of stem and root endophytic bacterial isolates of sea fern

Code of isolates	Biochemical test Gram staining	KOH 3%	Cell Shape
B.T 1	Negative	-	Bacilli- short
B.T 2	Negative	-	Bacilli- short
B.T 3	Negative	-	Bacilli- short
B.T 4	Negative	-	Coccus
B.T 5	Positive	+	Coccus
B.B 1	Negative	-	Bacilli- short
B.B 2	Positive	+	Bacilli- short
B.B 3	Positive	+	Bacilli
B.B 4	Negative	-	Bacilli
B.A 1	Negative	-	Bacilli- short
B.A 2	Positive	+	Bacilli- short
B.A 3	Negative	-	Bacilli- short
B.A 4	Negative	-	Bacilli
A.T 1	Positive	+	Bacilli
A.T 2	Negative	-	Bacilli short
A.T 3	Positive	+	Bacilli
A.T 4	Negative	-	Coccus
A.B 1	Negative	-	Bacilli short
A.B 2	Negative	-	Bacilli
A.A 1	Positive	+	Bacilli
A.A 2	Negative	-	Coccus
A.A 3	Negative	-	Bacilli
A.A 4	Positive	+	Bacilli
A.A 5	Positive	+	Coccus

Note: A= root; B= stems; (+) = Positive KOH test; (-) = Negative KOH test; T= Kundur village; B=Banglas Barat village; A= Alah air village

Hydrolytic enzyme activity. The results of the hydrolysis enzyme activity test showed that of the 13 stem isolates, nine isolates (69%) were positive for the potential to produce cellulase, three isolates (46%) produced laccase, and one isolate (8%) produced lipase. Three isolates (27%), namely B.B 2, B.A 2 and B.A 3 produced two enzyme activities, namely cellulase and laccase. Endophytic bacterial isolates from root organs (11 isolates) obtained 10 isolates (91%) have cellulase, three isolates (27%) produce laccase, and four isolates (36%) produce lipase. One isolate of endophytic bacteria i.e., A.A2 has all three enzyme activities: cellulase, lipase, and laccase. Two isolates (18%), including isolates A.T 4 and A. A 3 has cellulase and lipase activities, and two isolates (18%), namely isolates A.T 1 and A.B 1, have cellulase and laccase activities (Fig. 3). Endophytic bacterial isolates from roots have more activity in hydrolysis enzymes 52%, and stem isolates only 33%.

The cellulase enzyme activity test from 24 endophytic bacteria showed 19 isolates, showed positive results marked by a clear zone around the colonies on the cellulolytic selective medium (Table 3). One of the activities of endophytic bacterial isolates producing cellulase enzyme as shown in Fig. 4. The diameter of the clear zone in the cellulase enzyme activity test ranged from 17.08 to 38.98 mm, with a cellulolytic index range between 1.36 to 2.73 (Table 3). The difference in the cellulolytic index value of the clear zone shows variations in the ability to hydrolyze the substrate of each endophytic bacterial isolate.

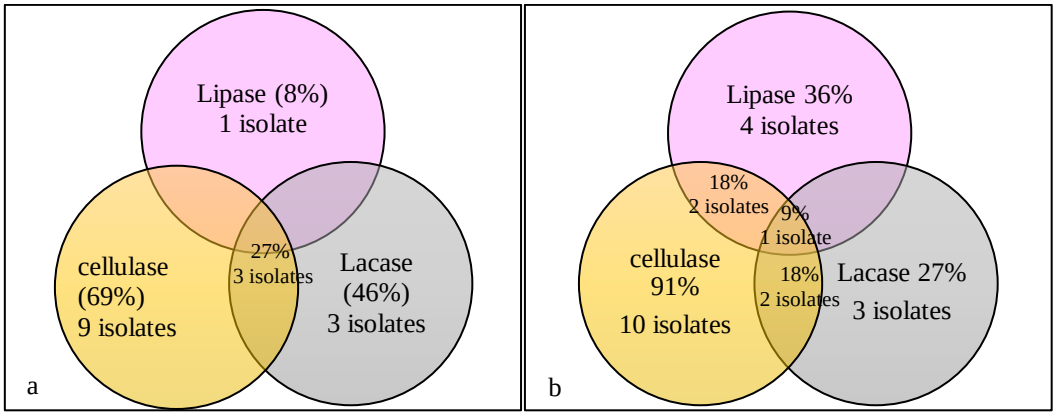

Fig. 3. Venn diagram and comparison of hydrolysis enzyme activity of sea fern endophytic bacteria: a. Venn diagram of stem isolates; b. Venn diagram of root isolates

The cellulase enzyme, a complex consisting of exoglucanase, endoglucanase, and β-glucosidase, exhibits activity towards carboxymethyl cellulose (CMC) primarily due to its endo-1,4-β-glucanase component. CMC, being pure amorphous cellulose, serves as a substrate to assess this specific endo-acting cellulase activity (McCleary *et al*., 2014; Taubner *et al*., 2015). Endophytic bacteria preferentially utilize glucose, a product of cellulase activity, as their primary carbon source for survival (Yang *et al*., 2017; Namwongsa *et al*., 2019). Detection of cellulase-producing endophytes can be achieved through the addition of iodine solution to culture plates. This technique exploits the selective binding of iodine to polysaccharides containing β-D-glucan bonds, allowing for the visualization of cellulase activity zones within the agar medium (Calegari *et al*., 2019; Sharma *et al*., 2020).

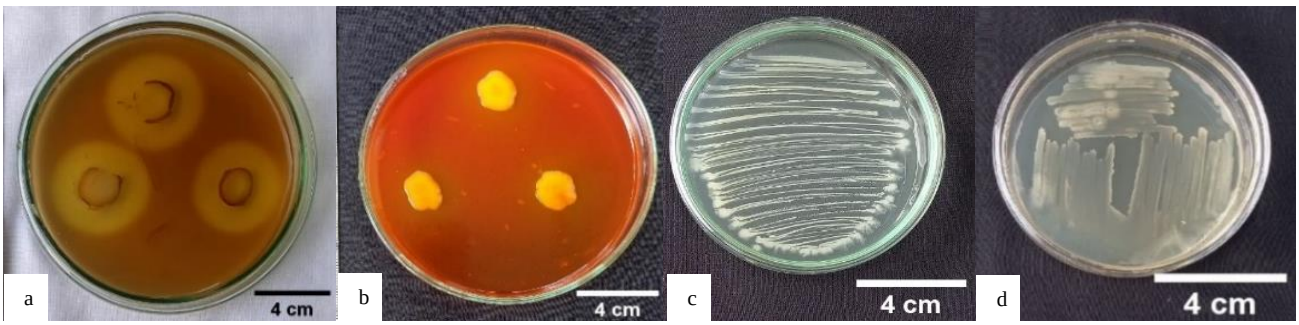

Fig. 4. The ability of endophytic bacterial isolates: a. The clear zone formed in B.A 3 in producing cellulase enzyme with 1% CMC substrate for three days of incubation; b. bacteria endophyte hydrolysis olive oil as substrate with change color from red medium to yellow on around B.A 1; c. Color change of endophytic bacterial isolates to detect laccase for isolate B.B2 on NA medium; d. Isolate B.B2 on NA medium containing 0.1% guaiacol

The highest cellulase enzyme activity was isolate A.A 4 with a cellulolytic index value of 2.73. The difference in cellulase enzyme activity can be caused because each isolate has a different potential in decomposing the substrate in the media. The clear zone formed around the isolate is caused by the isolate's ability to produce cellulase enzymes, which hydrolyze cellulose into glucose. The endophytic bacteria isolated from *Api-Api Putih* leaves produced cellulase enzyme by spotting on 1% CMC medium with a cellulolytic index of 1.92 (Rori *et al*., 2020). The endophytic bacteria from sterile sea fern leaves isolates positively produced cellulase enzyme on 1% CMC medium (Linda *et al*., 2023). Ntabo *et al*. (2018) reported that 19 out of 22 endophytic bacterial isolates from the roots and leaves of *Api-Api Putih* produced cellulase, with the highest cellulolytic index (5.00) on 1% CMC medium.

Table 3. Test results for the ability of endophytic bacterial isolates to produce cellulase enzymes

Code isolate	Diameters colony (mm)	Diameter clear zona (mm)	Index cellulolytic
B.T 1	9.68	22.88	2.36 ± 0.34
B.T 2	9.12	-	-
B.T 3	13.70	31.08	2.27 ± 0.19
B.T 4	12.65	-	-
B.T 5	13.63	-	-
B.B 1	13.967	33.50	2.40 ± 0.11
B.B 2	11.95	23.78	1.99 ± 0.09
B.B 3	17.50	29.75	1.70 ± 0.07
B.B 4	17.35	35.12	2.02 ± 0.08
B.A 1	8.08	-	-
B.A 2	11.41	17.08	1.50 ± 0.05
B.A 3	13.95	35	2.51 ± 0.04
B.A 4	13.51	35.85	2.65 ± 0.08
A.T 1	14.21	19.28	1.36 ± 0.59
A.T 2	13.09	25.89	1.98 ± 0.15
A.T 3	14.41	33.83	2.35 ± 0.32
A.T 4	13.64	28.04	2.06 ± 0.14
A.B 1	15.07	36.84	2.44 ± 0.86
A.B 2	13.48	-	-
A.A 1	18.96	38.98	2.04 ± 0.09
A.A 2	13.87	22.84	1.65 ± 0.19
A.A 3	13.60	30.87	2.27 ± 0.23
A.A 4	11.54	31.54	2.73 ± 0.20
A.A 5	14.87	35.04	2.36 ± 0.05

The lipase enzyme activity test of 24 endophytic bacteria isolates obtained five endophytic bacteria isolates, which showed positive results (Table 4), indicated by a yellow zone around the isolate in a selective medium containing olive oil and phenol red. The yellow zone around the isolate indicates that the bacteria can hydrolyze the substrate (Fig. 4). According to Ramnath *et al*. (2017), the color change from red to orange or yellowish is caused by an increase in acidity caused by the release of fatty acids resulting from lipid degradation by the resulting lipase enzyme. The use of olive oil in the manufacture of selective lipolytic media as a source of lipid carbon will turn yellow when there is lipase enzyme activity. The five isolates were positive of the lipase enzyme, differences in lipolytic index indicated differences in the ability to produce lipase enzymes.

Our previous study reported endophytic bacteria isolated from sterile leaves of sea fern (*Acrostichum aureum* L.) isolate D.SB 51 positive for lipase enzyme with carbon sources of olive oil (Linda *et al*., 2023), and Tween 80. Khan *et al*. (2022) also reported four isolates of endophytic bacteria from the roots of *Arthrocnemum macrostachyum* isolate TKR4 produced the highest lipase enzyme activity of 17.3 IU mL^{-1} with olive oil carbon source. Pramiadi *et al*. (2014) reported a lipolytic index of bacterial isolate 213 of 2.26 and bacterial isolate 361 of 2.57 with 10% olive oil and 0.1% Rhodamine-B.

The results of laccase enzyme activity from endophytic bacteria showed six isolates with positive test. Three isolates derived from the stem (B.B 2, B.A 2, and B.A 3) and three isolates derived from the roots (A.T 1, A.B 1, and A.A 2) of sea fern (*Acrosticum aureum* L.) (Table 5). The laccase assay used selective media with 0.1% guaiacol substrate and was incubated for four days. Positive endophytic bacterial isolates producing laccase enzymes were characterized by a change in the color of bacterial colonies to brownish on the selective medium. One of the results of laccase activity detection is in Fig. 4.

Table 4. The ability of endophytic bacterial isolates to produce lipase enzymes

Code isolates	Diameter colony (mm)	Diameter clear zone (mm)	Index lipolytic
B.T 1	8.95	-	-
B.T 2	11.98	-	-
B.T 3	11.86	-	-
B.T 4	11.23	-	-
B.T 5	8.7	-	-
B.B 1	12.05	-	-
B.B 2	16.24	-	-
B.B 3	8.14	-	-
B.B 4	15.45	-	-
B.A 1	15.21	28.91	1.90 ± 0.05
B.A 2	8.44	-	-
B.A 3	8.10	-	-
B.A 4	7.64	-	-
A.T 1	8.16	-	-
A.T 2	8.90	-	-
A.T 3	11.85	-	-
A.T 4	11.457	44.17	1.96 ± 0.29
A.B 1	10.6	-	-
A.B 2	15.14	24.53	1.62 ± 0.12
A.A 1	11.58	-	-
A.A 2	9.88	35.79	3.62 ± 0.27
A.A 3	19.61	26.26	1.34 ± 0.03
A.A 4	7.51	-	-
A.A 5	8.11	-	-

Laccase enzyme is an enzyme that works on the main substrate of phenolic compounds, one of which is guaiacol. Guaiacol is an aromatic phenolic compound that can be oxidized by the enzyme laccase, which acts as a source of synthetic lignin (Verma *et al*., 2017). Laccase enzyme-producing bacteria as biocatalysts are widely used because of their high capacity to oxidize phenolics and other aromatic compounds. Wins *et al*. (2019) reported five isolates of laccase-producing endophytic bacteria isolated from the roots of *Musa acuminate* (banana) and *Hevea brasiliensis* (rubber) plants with a concentration of 0.1% guaiacol as a substrate characterized by the formation of reddish brown circles around the colonies.

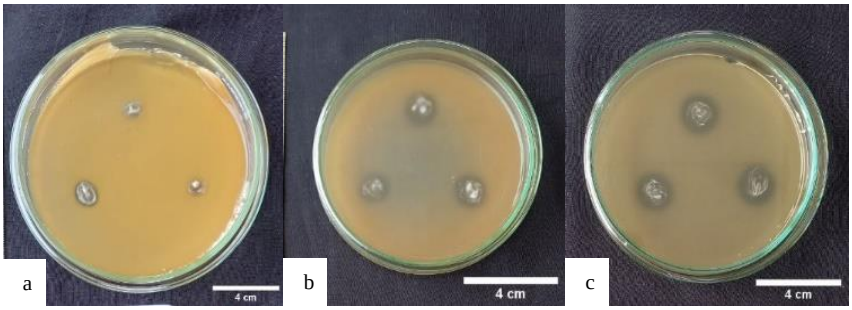

Fig. 5. Variation of clear zone on MRSA media with the addition of $CaCO_3$ isolate A.T 2: a. concentration of 0.5%; b. 0.25%; c. 0.1%

Screening of endophyte bacteria. The results of endophytic bacteria selection on MRSA and MRSA + $CaCO_3$ selective media showed that of the 24 endophytic bacterial isolates, only five could grow on selective media (Table 6). MRSA is a selective medium for lactic acid bacteria (LAB) consisting of dextrose, meat extract, yeast extract, ammonium citrate, magnesium sulfate, peptone, sodium acetate, dicalcium phosphate, and Tween 80, which can support LAB growth (Vitko & Richardson, 2013; Wirawati & Widodo, 2021). In the further test of lactic acid bacteria (LAB) selection, $CaCO_3$ was added with various concentrations of 0.5%, 0.25%, and 0.1%. Adding calcium

carbonate ($CaCO_3$), which is alkaline, can reduce the alkaline pH of the medium with the production of acid produced by LAB so that a clear zone is formed around the LAB colonies (Fig. 5).

α-amylase inhibitor assay. Endophytic bacteria in various plant tissues are partially unexplored but are known as a source of new natural materials in the industrial and pharmaceutical fields. Three endophytic bacterial isolates that were able to grow on MRSA media were continued to measure α-amylase inhibitor activity in vitro, namely isolates A.T 2, A. A 3, and B.A 1. According to Khadayat *et al*. (2020), α-amylase inhibitors play an important role as catalytic agents in starch hydrolysis, ultimately affecting glucose production in the human body. Therefore, controlling the catalytic activity of this enzyme is essential in reducing glucose production. The α-amylase inhibition test results of the three endophytic bacteria tested were isolates A.T 2 and A.A 3 on 1% starch substrate, and A.T 2 on 2% starch substrate. One other isolate, B. A 1 gave negative α-amylase inhibitor activity (Table 7). Akshatha *et al*. (2013) reported negative α-amylase inhibitor results from *Streptomyces* sp. isolated from *Rauwolfia densiflora* plants.

Table 5. The ability of endophytic bacterial isolates to produce laccase enzymes with guaiacol as substrate

Code of isolates	Response of brown bacterial colonies
B.T 1	-
B.T 2	-
B.T 3	-
B.T 4	-
B.T 5	-
B.B 1	-
B.B 2	+
B.B 3	-
B.B 4	-
B.A 1	-
B.A 2	+
B.A 3	+
B.A 4	-
A.T 1	+
A.T 2	-
A.T 3	-
A.T 4	-
A.B 1	+
A.B 2	-
A.A 1	-
A.A 2	+
A.A 3	-
A.A 4	-
A.A 5	-

Note: (+) = Positive laccase enzymes test; (-) = Negative laccase enzymes test

Table 6. Selection of endophytic bacteria from the stems and roots of sea fern on MRSA media and MRSA + $CaCO_3$

Code of isolates	MRSA	MRSA + $CaCO_3$ 0.5%	0.25%	0.1%
B.T 1	-	-	-	-
B.T 2	-	-	-	-
B.T 3	-	-	-	-
B.T 4	-	-	-	-
B.T 5	+	-	-	-
B.B 1	-	-	-	-
B.B 2	-	-	-	-
B.B 3	-	-	-	-
B.B 4	-	-	-	-
B.A 1	+	-	-	-
B.A 2	-	-	-	-
B.A 3	-	-	-	-
B.A 4	-	-	-	-
A.T 1	-	-	-	-
A.T 2	+	+	+	+
A.T 3	-	-	-	-
A.T 4	-	-	-	-
A.B 1	-	-	-	-
A.B 2	-	-	-	-
A.A 1	+	-	-	-
A.A 2	-	-	-	-
A.A 3	+	-	-	-
A.A 4	-	-	-	-
A.A 5	-	-	-	-

Note: (+) = Positive test; (-) = Negative test

The results showed that isolate A.T. 2 at 2% starch substrate concentration has higher inhibitor activity against α-amylase than the control (acarbose), which is 36% (Table 7). Acarbose is a synthetic inhibitor for α-amylase so the test solution containing acarbose produces a positive value of α-amylase inhibitor of 34% on 1% starch substrate and 21% on 2% starch substrate, which means that acarbose can inhibit the work of α-amylase enzyme by closing the active side of the enzyme that will bind to the substrate. Acarbose is a type 2 antidiabetic drug that inhibits the activity of the α-amylase enzyme to delay carbohydrate digestion and reduce glucose absorption to prevent postprandial plasma glucose rise (Ibrahim *et al*. 2017). Linda *et al*. (2023) reported the percentage of α-amylase inhibitor (18.13%) for endophytic bacterial isolate D.SB 5.2 from sterile leaf organs of sea ferns from Bengkalis Regency with Nutrient Broth (NB) fermentation medium incubated for 3 days at 30^{o}C with a concentration of

0.5% starch solution and 0.5 U/mL α-amylase. The results showed that different methods, isolate sources, fermentation medium, temperature, pH, and incubation duration would produce different α-amylase inhibitor values.

Table 7. α-amylase inhibitor activity of endophytic bacteria from stem and root organs of sea ferns in production medium after 72 hours incubation

Substrate (starch)	Inhibitor α-amylase Isolates			Acarbose (Control positives)
	A.T 2	A.A 3	B.A 1	
1%	17%	8%	-	34%
2%	36%	-	-	21%

Notes: (-) = negative inhibitor α-amilase

The α-amylase inhibitor assay remains a cornerstone approach in diabetes management due to its focus on a key enzyme responsible for carbohydrate breakdown. α-Amylase hydrolyzes complex carbohydrates into readily absorbable monosaccharides, like glucose, leading to postprandial hyperglycemia and elevated blood sugar levels. By inhibiting this enzymatic activity, α-amylase inhibitors can potentially prevent the excessive breakdown of starch into glucose, thereby contributing to glycemic control in diabetic patients (Mahmood, 2016; Takahama & Hirota, 2018; Chigurupati *et al*., 2021). This approach is particularly relevant as uncontrolled glucose production, the primary energy source for cellular processes, can lead to detrimental hyperglycemia in diabetic individuals.

CONCLUSION

A total of 24 isolates of endophytic bacteria showed that as much as 33% of stem isolates and 52% of root isolates were able to produce hydrolytic enzymes. The α-amylase inhibition test results of the three endophytic bacteria tested were isolates A.T 2 (17%) and A.A 3 (8%) on 1% starch substrate, and A.T 2 (36%) on 2% starch substrate. Endophytic bacteria isolated from the stems and roots of sea ferns can be developed as an alternative base material for herbal medicines for antidiabetics.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Ministry of Education, Culture, Research, and Technology of Indonesia for the funding through DIKTI Research Program (No. 15496/UN19.5.1.3/AL.04.00 /2023)

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