

Isolation and characterization of endophytic bacteria from sterile leaf of *Acrostichum aureum* from Bengkalis Island (Riau, Indonesia) and its potency for antidiabetic

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Abstract. Linda TM, Febriarti BL, Zul D, Sofyanti N, Berlyansah A, Delfira N, Devi S. 2023. Isolation and characterization of endophytic bacteria from sterile leaf of *Acrostichum aureum* from Bengkalis Island (Riau, Indonesia) and its potency for antidiabetic. *Biodiversitas* 24: 1580-1588. Endophytic bacteria are found in symbiotic plant tissues. These bacteria pose potential sources for the natural product due to their health benefit, e.g., for antidiabetic activity. The present study aimed to isolate and characterize endophytic bacteria isolates from the sterile leaf of sea fern (*Acrostichum aureum* Linn.) from Bengkalis Island (Riau Province), Indonesia. Furthermore, to investigate their potency as an antidiabetic agent. The characteristics of isolates bacteria were described based on colony morphology and biochemical tests. A total of six endophytic bacteria were isolated from the sterile leaf of *Acrostichum aureum*. Their characteristics vary in shape, color, and size. The phytochemical screening indicated the presence of alkaloids and saponin in all the isolates. The results exhibited that the extract of the sterile leaf of *A. aureum* also gave a similar phytochemical compound of isolate endophytic bacteria. The dendrogram constructed from morphological and biochemical characteristics showed the separation of D.SB 4.2 from other six bacteria strains due to the positive antibacterial activity for *Escherichia coli* ATCC35218, *Staphylococcus aureus* ATCC29213, *Salmonella typhi* ATCC14028, and *Bacillus subtilis* ATCC11774. Out of six isolates, only isolate D.SB 5.2 was selected for the antidiabetic test. The isolates showed positive results on the activity as α -amylase inhibitor (18.12%). This study provides important information on endophytic bacteria isolates on *A. aureum* and their potency for antidiabetic agents.

Keywords: α -amylase, *Acrostichum aureum*, antidiabetic, endophytic, phytochemical

INTRODUCTION

Acrostichum aureum Linn. belongs to the Pteridaceae family, known as mangrove, swamp, or sea fern. This species is widely distributed throughout America, Africa, and Southeast Asia (Wu et al. 2018), including Indonesia. This sea fern in Indonesia was recorded from the West Kutai district, East Kalimantan (Nurhasnawati et al. 2019); Kulonprogo, Jogjakarta (Hannin and Pratiwi 2017); Riau Province, i.e., Rangsang Island (Sofiyanti et al. 2019), and Bengkalis Island (Sofiyanti et al. 2020). In addition, *A. aureum* is locally known as *paku laut*, *piai raya*, or *kerakas* (Arbiastutie et al. 2021). Traditionally, *A. aureum* has been broadly utilized for treating many diseases, including peptic ulcers, rheumatism (Wu et al. 2018), stypic, worm remedy (Kale 2015), and wounds (Ramya et al. 2021). Modern researchers also confirm that it has antimicrobial (Ramya et al. 2021), anti-inflammatory (Abiola and Adetutu 2022), antioxidative analgesics, and antidote activities (for snake venom) (Ultari et al. 2021). Usually, the medicinal properties of a plant species are due to the

content of phytochemical constituents (Ishnava and Motisariya 2018). According to Wu et al. (2018), endophytic microbes in plants play an important role in regulating secondary metabolite synthesis in host plants.

Endophytic microbes are microorganisms found in symbiotic plant tissues without causing negative effects on the host (Anjum et al. 2019). Because of the long-term host-parasite relationship, endophytes can produce secondary metabolites similar to their plants' host. The activities of endophytic microbes have been reported for anticancer (Cardoso-Filho 2018; Sulistiyani and Kusumawati 2019), antidiabetic and antioxidant (Chigurupati et al. 2019); therapeutic agent (Ranganathan and Mahalingam 2018); antifungal (Siala et al. 2016); antibacterial properties (Chigurupati et al. 2018; Zulfarina et al. 2022); α -glucosidase inhibitors (Fatin et al. 2018); and α -amylase inhibitors (Chigurupati et al. 2019). Endophytic microbes also produce an enzyme like chitinase (Linda et al. 2018; Bhutani et al. 2021). According to Pujianto et al. (2018) and Sarjono et al. (2020) they are potentially antidiabetic agents due to their ability of endophytic microbes as

inhibitors of α -amylase and α -glucosidase enzymes.

Diabetes is a chronic metabolic disease (Galicia-Garcia et al. 2020) due to the inadequate production of insulin or improper response of the body's cells to insulin (Kousar 2019). In the human body, insulin is vital for glucose homeostasis; therefore, the lack of insulin may cause high blood sugar levels (hyperglycemia) (Cheisson et al. 2018). In Indonesia, diabetes causes the highest mortality of patients. Therefore, diabetic drugs are always in high demand in Indonesia. However, the long-term uses of synthetic drugs have side effects, such as edema with indigestion and hyponatremia (Krentz and Bailey 2005). Therefore, developing safe and natural new drugs for this disease is necessary. Chigurupati et al. (2021) recorded the incredible capacity of endophytic microbes to produce bioactive compounds as an antioxidant and antidiabetic agents from *Durio zibethinus*. Moreover, the endophytic bacteria studies on pteridophyte (ferns and its allies) species has been reported on many taxa, e.g., *Dryopteris* (Das et al. 2017), *Ophioglossum* (Mukherjee et al. 2017), *Azolla* (Yang et al. 2022), and *Asplenium* (Masocha et al. 2022).

In the preliminary study on sea fern, six endophytic bacteria were isolated from the sterile leaf of *A. aureum*. The phytochemical screening from these bacteria showed the presence of alkaloids and saponins compounds (Linda et al. 2022). Alkaloid in the plant is the largest group of secondary metabolites. This compound encompasses neuroactive molecules and is pivotal in plant defense (Matsuura and Fett-Netto 2015). The alkaloid role in diabetes mellitus management has been reported by Adhikari (2021). In the case of endocrine disorders (such as diabetes), this compound can be used as a preventive and curative agent (Ajebli et al. 2021). Therefore, this study aimed to isolate and characterize the endophytic bacteria from the *A. aureum* sterile leaf and examine their potency for antidiabetics.

MATERIALS AND METHODS

Study area

This study was conducted from May to Agustus 2021. The *Acrostichum aureum* was collected from the coastal area of Selatbaru beach at Bengkalis Island, Riau Province, Indonesia N: 1°33'41.425" E: 102°15'43.722, N:1°33'41.364" E: 102°15'42.022 and N:1°33'41.375 (Figure 1). In this study, a sterile leaf of *A. aureum* (Figure 2) was cut using a sterile scalpel and placed in a sterile polyethylene bag. The leaves were directly brought to the laboratory to isolate bacterial endophytes.

Isolation of endophytic bacteria

Colonies of bacteria were isolated from the sterile leaf margin of *A. aureum* and purified with NA media. Endophytic bacteria were isolated using a modified method developed by Hallman et al. (1997). The leaves of *A. aureum* were cleaned under running water and then dried using tissue paper. Samples were cut using a sterile scalpel (4 x 4 cm). The leaves' surface was sterilized by flushing with sterile water two times, then placed the samples in 70% alcohol for 1 min, followed by NaOCl (5.25%) for 5 min. The samples were washed three times using sterile water. Samples (2x2 cm) were then inoculated on the Nutrient Agar (NA) media and incubated at room temperature for 48 h. The colonies of bacteria growing at the leaf margin were then purified in NA media. Endophytic bacterial isolates were further investigated for their morphology, biochemical characterization, and phytochemical compounds.

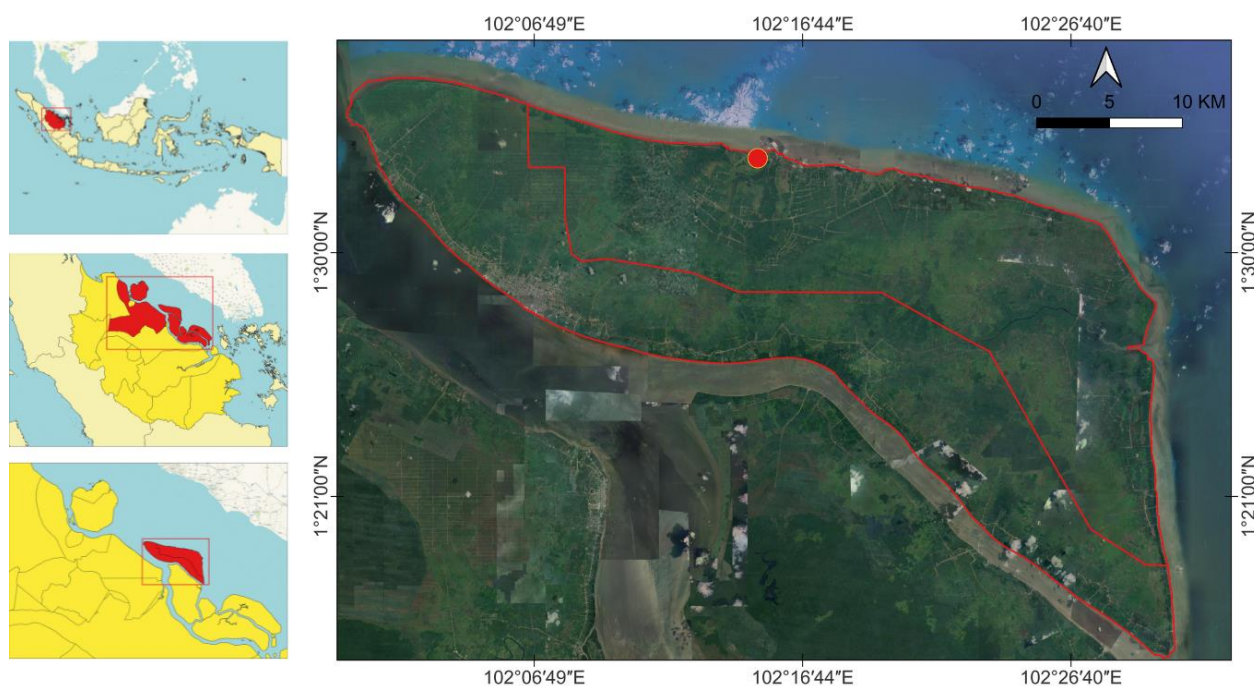


Figure 1. Location of coastal area Selatbaru beach at Bengkalis island, Bengkalis District, Riau Province, Indonesia



Figure 2. Morphology of *Acrostichum aureum*, A. Habit, B. Sterile leaves

Morphology and biochemical characterization of endophytic bacteria

The morphological and biochemical characteristics of the isolates were evaluated to determine their phenotypic properties. Morphological characteristics were based on the colony and cell morphology. The biochemical characteristics examined in this study were catalase production ability. It also identifies Gram-negative bacteria (KOH 3%) and isolates differentiation (Tween 80 hydrolysis test). The Man Rogosa Sharpe Agar (MRSA) medium was used as selective media for the physiology test. Therefore, its ability to detect hydrolytic enzymes included protease tests, CMCase, and lipase. *Escherichia coli* ATCC35218, *Staphylococcus aureus* ATCC29213, *Salmonella typhi* ATCC14028, and *Bacillus subtilis* ATCC11774 were used for the antibacterial test.

Phytochemical screening of endophytic bacteria

Isolates of endophytic bacteria were examined for secondary metabolite production according to Aristina et al. (2019) with modifications. The isolate of endophytic bacteria as much as 10% (v/v) with a population of 10^8 cfu/mL was introduced into 90 mL of Nutrient Broth (NB) and incubated at 150 rpm incubator shaker for 72 hours at 30°C. The secondary metabolites of each bacterial isolate were produced by separation using filtration by Whatman no.42 filter paper. This study tested three phytochemicals, i.e., alkaloid, steroid, and saponin, in the endophytic bacterial extract.

Alkaloid test

A 5 mL crude extract of endophytic bacteria was mixed with 2 mL chloroform and 2 mL of ammonia and filtered. Then 3-5 drops of H_2SO_4 were added to the filtrate and shaken until two layers formed. Next, the top layer was transferred to a test tube, and 4-5 drops of Mayer reagent were added. The positive test result of the alkaloid was characterized by the presence of a white color (Aristina et al. 2019).

Steroid test

Ten drops of AAG(CH_3COOH) and two drops of H_2SO_4 were added to a 5 mL crude extract of endophytic bacteria. The tube was then gently shaken and left for a few minutes. The presence of a green-blue color indicated a positive steroid test (Mondong et al. 2015).

Saponin test

A 5 mL crude extract of endophytic bacteria was taken into a test tube, and then 10 mL of aquadest was added and shaken for 1 minute. A positive saponin test was characterized by the presence of foam on the upper surface (Mondong et al. 2015).

In vitro antidiabetic activity

Endophytic bacteria (D.SB 5.2) used in vitro antidiabetic activity. A total of 10 mL (10^8 CFU/mL) of endophytic bacterial culture was inoculated in 90 mL Nutrient Broth (NB) and then incubated for 72 hours at 30°C. Crude extracts of endophytic bacterial metabolites were centrifuged at 3,500 rpm for 15 minutes. The supernatant obtained was tested for its inhibitory activity against the α -amylase enzyme. In addition, the positive control was acarbose 100 ppm.

A reported technique assayed the α -amylase repressing activity with slight modification. 0.5 mL sample was mixed with 0.5 U/mL of α -amylase (0.5 mL) enzyme, then dissolved into phosphate buffer (pH 6.9) and incubated at 25°C for 10 min. A total of 1 mL of starch solution (0.5%) was added and incubated for 10 min at 25°C. After the second incubation, 2 mL of dinitro salicylic acid reagent was added and heated. The spectrophotometric readings were noted at 540 nm. The percentage of Inhibition (I%) was obtained by applying the following formula (Pujianto et al. 2018).

$$I\% = \frac{Abs_{control} - Abs_{sampel}}{Abs_{control}} \times 100$$

Fourier Transform Infra-Red (FTIR) spectroscopy

The crude extract of isolates D.SB 5.2 was taken, and the spectra were scanned with the wave number range from 4000 to 500 cm^{-1} using Fourier Transform Infra-Red (FTIR) spectroscopy, and then spectra were recorded using IR software.

Data analysis

The data of colony morphology, cell morphology, biochemical test, enzyme test, and antibacterial test were characterized, tabulated, and presented in the figures and tables. The data were then scored and analyzed using NTSyst construct dendrogram.

RESULTS AND DISCUSSION

Morphological characterization of endophytic bacteria

The characterization of endophytic bacteria has been carried out based on the colony morphology and biochemical test. A total of 6 endophytic bacteria were successfully isolated from the sterile leaf of *A. aureum*,

namely D.SB 1.2, D.SB 1.7, D.SB 4.2, D.SB 5.1, D.SB 5.2, and D.SB 6.2. Colony morphology is a visual culture characteristic of bacteria in the agar plate. According to Sousa et al. (2013), colony morphology is pivotal as an indicator of the phenotypic variation of bacteria. The characteristic of bacterial colonies observed in this study was colony color, elevation, shape, size, and edge. Therefore, the colony morphology of endophytic bacteria showed variation in color, elevation, size, and shape of the colony edge (Figure 3).

One of the characteristics used in identifying bacteria was the colony color of bacteria. In the present study, the colony color observed was dull white (4 isolates, i.e., D.SB 1.2; D.SB 1.7; D.SB 4.2 and D.SB 5.1), yellow (1 isolate, i.e., D.SB 5.2), and light yellow (1 isolate, i.e., D.SB. 6.2) (Figure 3A, E, I, M, R, V). Other various colony colors of bacteria that have been recorded are red (Arifiyanto et al. 2021), green to bluish green (Hikmawati et al. 2019), purple (Kis et al. 2015), orange (Usman et al. 2018). According to Ahmad et al. (2012), the colony color depends on the pigmentation bacteria produce. Usually, the pigment that affects the yellow, golden yellow, creamy, and brown coloration of bacteria colonies is zeaxanthin (Thawornwiriyanun et al. 2012). This pigment has been reported in *Flavobacterium* species, *Paracoccus zeaxanthinifaciens*, *Staphylococcus aureus*, and *Xanthomonas oryzae* (Usman et al. 2018). Other pigments of bacteria colonies recorded in the previous study are astaxanthin (pinkish-red) (Choi et al. 2021), canthaxanthin (dark red) (Duy et al. 2021), staphyloxanthin (golden yellow) (Elmesseri et al. 2022); indigoidine (blue), prodigiosin (red), pyocyanin (blue-green) and violacein (purple) (Usman et al. 2018). The previous studies on bacteria pigment recorded the capability of some bacteria to produce varieties of pigment, such as the genus *Agrobacterium* (Rao et al. 2017), *Bacillus*, *Chromobacterium* (Usman et al. 2018), *Flavobacterium*, *Staphylococcus* (Nurjahan et al. 2020) and *Serratia* (Venil et al. 2021).

The colony elevation observed in the present study was flat (3 isolates) and raised (3 isolates). The flat elevation was observed in D.SB 1.2, D.SB 4.2, and D.SB 6.2 isolates (Figure 3B, J and V), while isolates D.SB 1.7, D.SB 5.1 and D.SB 5.23 showed raised elevation (Figure 3F, N, and R). Various elevation of bacteria colony has been recorded in the previous studies, i.e., flat (Sousa et al. 2013), raised (Sulmiyati et al. 2019), convex, umbonate (Kurahman et al. 2020), pulvinate, and crateriform (Tasaki et al. 2017).

The colony forms of six isolated bacteria were similar. All the bacterial colonies were found to be circular. The size of the colony was small and moderate. The diameter of the small-size colony was less than 1 mm, as observed in D.SB 1.2, D.SB 4.2, and D.SB 5.2. On the other hand, a moderate-size colony was about 1 mm in diameter (D.SB 1.7, D.SB 5.1, and D.SB 6.2). The colony margin of all isolated bacteria was entire. In the present study, a total of three cell shapes, i.e., streptococcus (1 isolate), bacilli (3 isolates), and coccus (2 isolates) was observed (Figure 3 D, H, L, P, T, and X). The shape of streptococcus was only found in D.SB 1.2, bacilli in D.SB 1.7, D.SB 4.2, and D.SB 5, while coccus shape was found in D.SB 5.2 and D.SB 6.2 isolates. The other colony form is irregular (Sousa et al. 2013).

Biochemical characterization of endophytic bacteria

Biochemical characterization is also an important aspect of bacteria profiling. Therefore, selected bacterial isolates were tested for gram staining, tween hydrolysis test, hydrolytic enzymes detection test, and antibacterial test. The results of bacterial characterization are presented in Table 1. D.SB 4.2 isolates showed the highest positive result among six isolated bacteria. This isolate showed a positive result on the gram staining test, KOH 3%, and antibacterial test on *E. coli*, *S. aureus*, *S. typhi*, and *B. subtilis*.

The phenetic study was carried out on six strains of endophytic bacterial isolates. The characteristics of each strain were scored and analyzed using NTSyst to obtain a similarity index using Simple Matching. The similarity index ranges from 0.24 to 0.98. The dendrogram was constructed based on the similarity index (Figure 4).

The dendrogram (Figure 4) was divided into two main clusters (I and II) at a 0.25 similarity index. The first cluster (I) consisted of 5 isolates (D.SB1.2, D.SB6.2, D.SB1.7, D.SB 5.1, and D.SB 5.2), and the second cluster (II) only consisted of one isolate (D.SB4.2). The characteristic of D.SB4.2 in Cluster II that differed from other isolates in cluster I was the positive antibacterial activity for *E. coli*, *S. aureus*, *S. typhi*, and *B. subtilis*. Otherwise, isolates in cluster I gave negative results. Cluster I was divided into two sub-clusters (IA and IB). Sub-cluster IA consisted of 2 strains, and sub-cluster IB consisted of 3. This sub-cluster can be distinguished based on the elevation of colonies. In sub-cluster IA, the elevations were flat or convex, while in sub-cluster IB, the elevation of colonies was mostly raised. The highest similarity index (85%) was found in D.SB1.7 and strain D.SB 5.1 strains; both were different in hydrolyzing lipids. D.SB5.1 strain shows a positive result on the physiology of hydrolyzing lipids from olive oil and Tween 80 sources. Based on the phenetic analysis, these two isolates have a very close kinship relationship compared to other isolates. Salaki et al. (2010) state that the numeric-phenetic classification method classifies each microbial strain into a homogeneous taxon group. Those taxon species are based on many phenotypic data, namely macro morphology, colony morphology, use of carbon sources, enzyme reducers, the ability to degrade macromolecules, and other physiological properties.

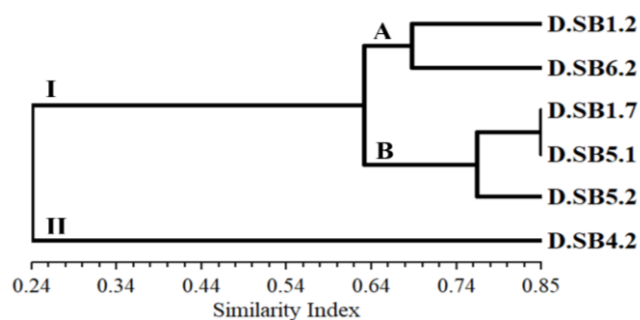


Figure 4. Dendrogram of six endophytic bacterial isolates from *Acrostichum aureum* sterile leaves

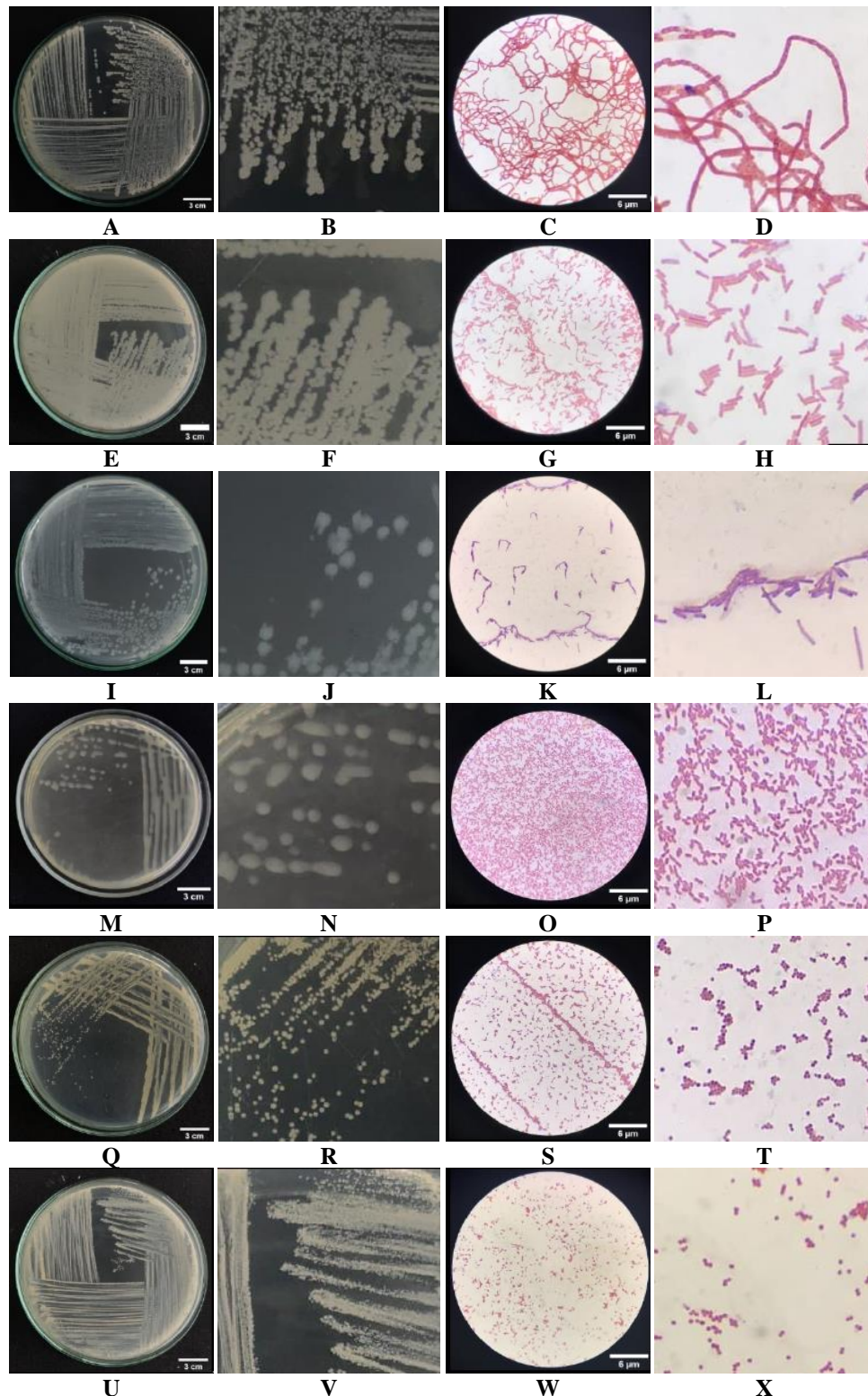


Figure 3. Characteristics colony of endophytic bacteria isolated from *Acrostichum aureum* sterile leaf. A - D. D.SB 1.2 (A. Colony morphology, B. Detail of colony morphology showing elevation, C. Morphology of bacteria cells after gram-negative staining, D. Shape of bacteria cells), E - H. D.SB 1.7, I - L. D.SB 4.2, M - P. D.SB 5.1, R - U. D.SB 5.2, V - Y. D.SB 6.2. 2. (Note: A, E, I, M, Q, U. Colony morphology; B, F, J, N, R, V. Detail of colony morphology showing elevation; C, G, K, O, S W. Morphology of bacteria cells after gram-negative staining; D, H, L, P, T, X. Shape of bacteria cells)

Phytochemical test

Results of the phytochemical test are presented in Figure 5 and Table 2. In this study, three phytochemical compounds, namely alkaloid, steroid, and saponin, were

tested from the extract of endophytic bacteria and sterile leaf extract of the *A. aureum*. All the isolates showed the presence of alkaloids (Figure 5A1- 5A7). The presence of alkaloids in bacteria has also been reported in previous

studies by Purwestri et al. (2016), Munakata et al. (2022), and Ptak et al. (2022). Alkaloid is a nitrogen base compound that poses numerous health benefits. Therefore, this compound exhibited antiproliferation, antimetastatic on cancer (Qiu et al. 2014), analgesic, antimalarial, antihypertensive (Kueté 2014), and antidiabetic activities (Ajebli et al. 2021).

All six isolates showed a negative result for steroids (Figure 5B1-5B7). However, all the isolates exhibited positive tests for saponin (Figure 5C1-5C7). The sterile leaf of *A. aureum* extracts showed the presence of saponin, which was indicated by the presence of foam on the top of the mixture. The result of this study is similar to Badhsheeba and Vadivel's study (2020), which recorded the presence of alkaloids and saponin in ethanol and methanol extract of *A. aureum*. In endophytic bacteria, alkaloids and saponin were also reported by Variani et al. (2021) on the *Serratia marcescens* strain.

In vitro antidiabetic activity

Endophytic bacteria in various plant tissues are largely unexplored, but they are known to be a source of novel natural products for industry and pharmacy. However, for in vitro antidiabetic activity, only one isolate, i.e., D.SB 5.2, was selected for the α -amylase inhibitor test. According to Khadayat et al. (2020), alpha-amylase inhibitory plays an important role as a catalytic agent in starch hydrolysis and ultimately affects glucose production in the human body. Therefore, controlling this enzyme catalytic activity is pivotal in reducing glucose production.

Endophytic bacterial (D.SB 5.2) isolate was examined for their antidiabetic activity using in vitro method inhibited α -amylase. The result showed that isolate D.SB 5.2 had higher inhibitory activity against α -amylase than the control (acarbose), which was 18.13% (Table 3). Acarbose, a type of antidiabetic drug, is especially used for type 2 diabetes to inhibit the work of the α -amylase enzyme to delay the digestion of carbohydrates and reduce glucose absorption to prevent the rise of postprandial plasma glucose (Li et al. 2022). Pujianto et al. (2018) reported a higher percentage of α -amylase inhibitory (72.22%) on endophytic bacteria isolates from *Anona muricata*. In the human body, α -amylase enzymes are essential for carbohydrate digestion and controlling blood glucose levels. According to Chigurupati et al. (2019) and Khan et al. (2019), this enzyme is important in treating type 2 diabetes. Glucose is the main metabolic substrate in producing tissue energy (Guemes et al. 2015). However, uncontrolled sugar production may cause hyperglycemia, usually in diabetic patients.

The results of Fourier Transform Infra-Red spectroscopy (FTIR) revealed that isolates D.SB 5.2 had an O-H (alcohol) function group in the wavelength range of 3054.16-3659.53 cm^{-1} , the C-H function group at wavelengths 2128.11-2146.38 cm^{-1} ; C-O (ester) function group in the wavelength range 1179.36-1197.14 cm^{-1} and C-H (aromatic) functional group at wavelength 596.74-

841.36 cm^{-1} . Based on the results of phytochemical and FT-IR tests, compounds in isolate D.SB 5.2 belonged to a similar group of alkaloids and saponin compounds (Figure 6). The ability to isolate as an antidiabetic is due to the phytochemical compounds of alkaloids and saponins. *Euclea undulata* with alkaloid content inhibited α -amylase activity (Ajayi et al. 2012). Moreover, *Cryptolepis sanguinolenta* stem extract might also be responsible for alkaloids-containing hypoglycemic activity (Deutschlander et al. 2010). *Gymnema sylvestre* can increase animal models' average blood glucose levels and stimulate insulin secretion with the detected metabolite content of saponin derivatives (Yadav et al. 2019).

Table 1. Biochemical characterization of endophytic bacteria

Biochemical Tests	D.SB	D.SB	D.SB	D.SB	D.SB	D.SB
	1.2	1.7	4.2	5.1	5.2	6.2
Gram staining	-	-	+	-	-	-
Endospora	-	-	-	-	-	-
KOH 3%	-	-	+	-	-	-
Catalase	+	+	+	-	+	+
Tween 80	-	-	+	-	-	-
MRSA	-	+	+	+	+	+
<i>Hydrolytic enzyme</i>						
Protease	+	+	+	+	+	+
Cellulase	-	-	+	-	-	+
Lipase	-	-	-	+	-	-
Amylase	+	+	+	+	-	+
<i>Antibacterial test</i>						
<i>E. coli</i> ATCC35218	-	-	+	-	-	-
<i>S. aureus</i> ATCC29213	-	-	+	-	-	-
<i>S. typhi</i> ATCC14028	-	-	+	-	-	-
<i>B. subtilis</i> ATCC11774	-	-	+	-	-	-

Table 2. Phytochemical constituents in endophytic bacteria and the sterile leaf of *Acrostichum aureum*

Isolates	Phytochemical contents		
	Alkaloid	Steroid	Saponins
D.SB 1.2	+	-	+
D.SB 1.7	+	-	+
D.SB 4.2	+	-	+
D.SB 5.1	+	-	+
D.SB 5.2	+	-	+
D.SB 6.2	+	-	+
Sterile leaf (<i>A. aureum</i>)	+	-	+

Note: +: Present, -: Absent

Table 3. In vitro, α -amylase enzyme inhibitors extract from endophytic bacteria isolate.

Sample	Inhibition Value (%)
D.SB 5.2	18.13
Acarbose (Control)	9.61

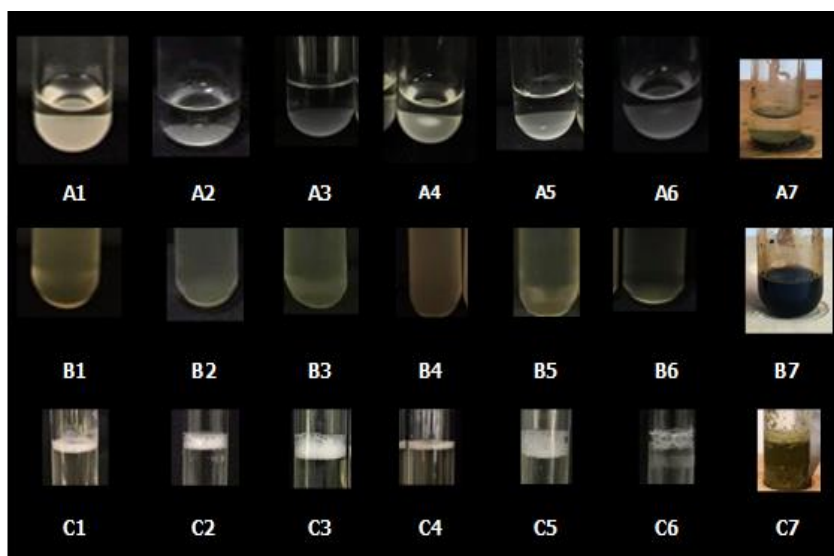


Figure 5. Phytochemical test: A. Alkaloid, B. Steroid, C. Saponin. (Isolate 1: D.SB1.2; 2: D.SB1.7; 3: D.SB4.2; 4: D.SB 5.1; 5: D.SB 5.2; 6: D.SB 6.2; 7: sterile leaf of *Acrostichum aureum*)

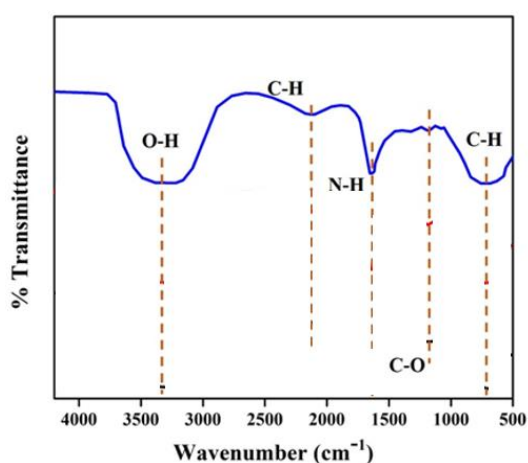


Figure 6. Fourier Transform Infra-Red spectroscopy (FTIR) analysis of crude extract isolate D.SB 5.2 of endophytic bacteria

In conclusion, the present study showed that bacteria isolated from the sterile leaf of *A. aureum* were endophytic. The colony's morphology and biochemical test provided important data on six endophytic bacteria. Three out of one isolate used in antidiabetic activity exhibit the inhibitor activity of α -amylase enzyme (D.SB 5.2) with 18.12% inhibition. This study provides the first information on endophytic bacteria from sterile leaf *A. aureum* inhibitor on α -amylase and their potency as an antidiabetic agent.

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REFERENCES

- Abiola AA, Adetutu OA. 2022. Counteract inflammatory properties of the purified ethanolic extract of *Acrostichum aureum* (Linn.) leaf. *J Appl Health Sci Med* 1 (2): 43-47.
- Adhikari B. 2021. Roles of alkaloids from medicinal plants in the management of diabetes mellitus. *J Chem* 2021: 2691525. DOI: 10.1155/2021/2691525
- Ahmad WA, Ahmad WY, Zakaria ZA, Yusof NZ. 2012. Application of bacterial pigments as colorant. In: *Springer Brief in Molecular Science*. Springer. DOI: 10.1007/978-3-642-24520-6_4.
- Ajayi AF, Akhigbe RE, Adewumi OM, Okeleji LO, Mujaidu KB, Olaleye SB. 2012. Effect of ethanolic extract of *Cryptolepis sanguinolenta* stem on in vivo and in vitro glucose absorption and transport: mechanism of its antidiabetic activity. *Indian J Endocrinol Metab* 16: S91-6. DOI: 10.4103/2230-8210.94265.
- Ajebli M, Khan H, Eddouks M. 2021. Natural alkaloids and diabetes mellitus: A review. *Endocr Metab Immune Disord Drug Targets* 21 (1): 111-130. DOI: 10.2174/1871530320666200821124817.
- Anjum R, Afzal M, Baber R, Khan MAJ, Kanwal W, Sajid W, Raheel A. 2019. Endophytes: as potential biocontrol agent review and future prospects. *J Agric Sci* 11 (4): 113-125. DOI:10.5539/jas.v11n4p113.
- Arbiastutie Y, Diba F, Masriani. 2021. Short communication: ethnobotanical and ecological studies of medicinal plants in a mangrove forest in Mempawah District, West Kalimantan, Indonesia. *Biodiversitas* 22 (6): 3164-3170. DOI: 10.13057/biodiv/d220619.
- Arifiyanto A, Afriani H, Putri MH, Damayanti B, Riyanto CLR. 2021. The biological prospective of red-pigmented bacteria cultured from contaminated agar media. *Biodiversitas* 22 (3): 1152-1159. DOI: 10.13057/biodiv/d220310.
- Aristina RF, Astuti W, Pratiwi DR. 2019. Skrining dan uji fitokimia ekstrak kasar bakteri endofit dari batang pacing (*Costus* sp.). *J Atom* 4 (1): 21-24. [Indonesian]
- Badhsheeba MA, Vadivel V. 2020. Physicochemical and phytochemical contents of the leaves of *Acrostichum aureum* L. *J Global Biosci* 9 (4): 7003-7018.
- Bhutani N, Maheshwari R, Kumar P, Dahiya R, Suneja P. 2021. Bioprospecting for extracellular enzymes from endophytic bacteria isolated from *Vigna radiata* and *Cajanus cajan*. *J Appl Biol Biotechnol* 9 (3): 26-34. DOI: 10.7324/JABB.2021.9304.
- Cardoso-Filho JA. 2018. Endophytic microbes as a novel source for producing anticancer compounds as multidrug resistance modulators. In: Akhtar M, Swamy M (eds.). *Anticancer Plants: Natural Products and Biotechnological Implements*. Springer, Singapore. DOI: 10.1007/978-981-10-8064-7_15.

- Chesson G, Jacqueminet S, Cosson E, Ichai C, Leguerrier A, Nicolescu-Catargi B, Ouattara A, Tauveron I, Valensi P, Benhamou D. 2018. Review of hyperglycaemia: definitions and pathophysiology. *Anaesth Crit Care Pain Med* 37: S5-S8. DOI: 10.1016/j.accpm.2018.02.019.
- Chigurupati S, Marri MR, Kumar A, Nemala AR, Nanda SS, Vijayabalan S, Selvarajan KK. 2018. Bacterial endo-symbiont inhabiting *Durio zibethinus* leaves and their antibacterial potential. *Intl J Pharm Tech Res* 11 (3): 198-205. DOI:10.20902/IJPTR.2018.11301.
- Chigurupati S, Vijayabalan S, Karunanidhi A, Selvarajan KK, Nanda SS, Satpathy R. 2019. Antidiabetic, antioxidant and in silico studies of bacterial endosymbiont inhabiting *Nephelepis lappaceum*. *Ovidius Univ Ann Chem* 30 (2): 95-100. DOI: 10.2478/auoc-2019-0017.
- Chigurupati S, Vijayabalan S, Palanimuthu VR, Das S, dan Bhatia S. 2021. Bacterial endophyte inhabiting *Durio zibethinus* and its radical scavenging and antidiabetic potential. *Indian J Pharm Educ Res* 55 (3): 857-862. DOI: 10.5530/ijper.55.3.159.
- Choi SS, Seo YB, Nam S, Kim G. 2021. Enhanced production of astaxanthin by co-culture of *Paracoccus haendaensis* and lactic acid bacteria. *Front Mar Sci* 7: 597553. DOI: 10.3389/fmars.2020.597553.
- Das G, Park S, Baek K. 2017. Diversity of endophytic bacteria in a fern species *Dryopteris unififormis* (Makino) and evaluation of their antibacterial potential against five foodborne pathogenic bacteria. *Foodborne Pathog Dis* 14 (5): 260-268. DOI: 10.1089/fpd.2016.2243.
- Deutschlander MS. 2010. Isolation and identification of a novel antidiabetic compound from *Euclea undulata* Thunb. Hatfield, Pretoria: University of Pretoria. [Doctoral dissertation]. University of Pretoria, South Africa.
- Duy L, Tran QT, Dang A, Phi-Hung N, Trinh H, Pham L, Nguyen D, Do L, Dung P, Nguyen N, Do M. 2021. Optimization of canthaxanthin extraction from fermented biomass of *Paracoccus carotinifaciens* VTP20181 bacteria strain isolated in Vietnam. *Foods Raw Mater* 9 (1): 117-125. DOI: 10.21603/2308-4057-2021-1-117-125.
- Elmesseri RA, Saleh SE, Elsherif HM, Yahia IS, Aboshanab KM. 2022. Staphyloxanthin as a potential novel target for deciphering promising anti-*Staphylococcus aureus* agents. *Antibiotics* 11 (3): 298-303. DOI: 10.3390/antibiotics11030298.
- Fatin N, Pujiyanto S, Raharjo B. 2018. Uji aktivitas inhibisi α -glukosidase isolat bakteri endofit tanaman duwet (*Syzygium cumini* L. Skeels) sebagai sumber alternatif antidiabetes. *Bioma* 20 (2): 165-169. DOI: 10.14710/bioma.20.2.165-169. [Indonesian]
- Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martin C. 2020. Pathophysiology of Type 2 diabetes mellitus. *Intl J Mol Sci* 21 (17): 6275. DOI: 10.3390/ijms21176275.
- Güemes M, Rahman SA, Hussain K. 2015. What is a normal blood glucose? *Arch Dis Child* 101 (6): 308336. DOI: 10.1136/archdischild-2015-308336.
- Hallman J, Quadt-Hallmann WF, Mahaffee WF, Klopper JW. 1997. Endophytic bacteria in agricultural crops. *Can J Microbiol* 43: 895-914. DOI:10.1139/m97-131.
- Hannin NNF, Pratiwi R. 2017. Kandungan fenolik, flavonoid dan aktivitas antioksidan ekstrak daun paku laut (*Acrostichum aureum* L.) fertil dan steril. *J Trop Biodivers Biotechnol* 2 (2): 51-56. DOI: 10.22146/jtb.29819. [Indonesian]
- Hikmawati F, Susilowati F, Setyaningsih R. 2019. Colony morphology and molecular identification of *Vibrio* spp. on green mussels (*Perna viridis*) in Yogyakarta, Indonesia tourism beach areas. *Biodiversitas* 20 (10): 2891-2899. DOI: 10.13057/biodiv/d201015.
- Ishnava KB, Mottisariya DM. 2018. In-vitro study on α -amylase inhibitory activity of selected ethnobotanical plant extracts and its herbal formulations. *Intl J Pharmacogn Chinese Med* 2 (3): 000136. DOI: 10.23880/IPC-16000136.
- Kale M. 2015. Ethno-Medico-Botanical study on *Acrostichum aureum* L. of South Western Ghats. *Matter: Intl J Sci Technol* 3: 107-108.
- Khadayat K, Marasini BP, Gautam H. 2020. Evaluation of the α -amylase inhibitory activity of *Nepalese* medicinal plants used in the treatment of diabetes mellitus. *Clin Phytosci* 6 (34): 1-8. DOI: 10.1186/s40816-020-00179-8.
- Khan R, Naqvi STQ, Fatima N, Muhammad SA. 2019. Study of antidiabetic activities of endophytic fungi isolated from plants. *Pure Appl Biol* 8 (2): 1287-1295. DOI: 10.19045/bspab.2019.80071.
- Kis M, Gábor S, Emese A, Zsolt R, Péter M, Maróti. 2015. Purple non-sulfur photosynthetic bacteria monitor environmental stresses. *J Photochem Photobiol B, Biol* 151: 110-117. DOI: 10.1016/j.jphotobiol.2015.07.017.
- Kousar S. 2019. Type 1 Diabetes: Causes, symptoms and treatments, review with personal experience. *Curr Res Diabetes Obes J* 11 (4): 555817. DOI: 10.19080/CRDOJ.2019.11.555817.
- Krentz AJ, Bailey CJ. 2005. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. *Drugs* 65 (3): 385-411. DOI: 10.2165/00003495-200565030-00005.
- Kuete V. 2014. Health effects of alkaloids from African medicinal plants. *Tox Survey of African Medic Plants, Elsevier*. DOI: 10.1016/B978-0-12-800018-2.00021-2.
- Kurahman OT, Yulawati A, Haerunnisa L, Supriyatna A, Cahyanto, Suryani, Supriadin A, Hidayat C, Masri M. 2020. The isolation and identification bacteria on Jallalah animal (study on the feeding Tilapia (*Oreochromis niloticus*) with chicken manure as foods). *Elkawnie: J Islam Sci Technol* 6 (2): 222-236. DOI: 10.22373/ekw.v6i2.7770.
- Li J, Wei Q, McCowen KC, Xiong W, Liu J, Jiang W, Thomas RL, Hepokoski M, He M, Shyy JYJ, Malhotra A, Xiong N, Li WX. 2022. Inpatient use of metformin and acarbose is associated with reduced mortality of COVID-19 patients with type 2 diabetes mellitus. *Endocrinol Diabet Metab* 5: 287308. DOI: 10.1002/edm2.301.
- Linda TM, Berlyansah A, Fibrianti BL, Sofiyanti N, Devi S. 2022. Isolation and analysis of bioactive compounds endophytic bacteria of sea fern (*Acrostichum aureum* L.) from Bengkalis Island, Riau. *J Biol Tropis* 22 (1): 46-54. DOI: 10.29303/jbt.v22i1.3104.
- Linda TM, Siregar S, Fitri WD, Martina A, Lestari W, Roslim DI, Hapsoh. 2018. Isolation and screening of culturable endophytic bacteria from leaf of rubber plant that produces of chitinase. *J Phys: Conf Ser* 1116 (5): 052038. DOI: 10.1088/1742-6596/1116/5/052038.
- Masocha VF, Liu H, Zhan P, Wang K, Zeng A, Shen S, Schneider H. 2022. Bacterial microbiome in the phyllo-endosphere of highly specialized rock spleenwort. *Front Plant Sci* 13: 1-15. DOI: 10.3389/fpls.2022.891155.
- Matsuura HN, Fett-Neto AG. 2015. Plant alkaloids: main features, toxicity, and mechanisms of action. In: Gopalakrishnakone P, Carlini C, Ligabue-Braun R (eds.). *Plant Toxins*, Springer, Dordrecht. DOI: 10.1007/978-94-007-6728-7_2-1.
- Mondong FR, Meiske S, Sangi, Kumaunang. 2015. Skrining fitokimia dan uji aktivitas antioksidan ekstrak etanol daun Patikan Emas (*Euphorbia prunifolia* Jacq.) dan Bawang Laut (*Proiphys amboinensis* (L.) Herb). *J Mipa UNSRAT* 4 (1): 81-87. DOI: 10.35799/jm.4.1.2015.6910. [Indonesian]
- Mukherjee A, Bhattacharjee P, Das R, Pal A, Paul AK. 2017. Endophytic bacteria with plant growth promoting abilities from *Ophioglossum reticulatum* L. *AIMS Microbiol* 3 (3): 596-612. DOI: 10.3934/microbiol.2017.3.596.
- Munakata Y, Spina R, Slezack-Deschaumes S, Genestier J, Hehn A, Laurain-Mattar D. 2022. Screening of endophytic bacteria of *Leucojum aestivum* 'gravity giant' as a potential source of alkaloids and as antagonist to some plant fungal pathogens. *Microorganisms* 10 (10): 2089. DOI: 10.3390/microorganisms10102089.
- Nurhasnawati H, Sundu R, Sapri, Supriningrum R, Kuspradini H, Arung ET. 2019. Antioxidant activity, total phenolic and flavonoid content of several indigenous species of ferns in East Kalimantan, Indonesia. *Biodiversitas* 20 (2): 576-580. DOI: 10.13057/biodiv/d200238.
- Nurjahan MT, Saha S, Ninan A, Das S. 2020. Enhanced *Staphylococcus aureus* pigment formation under the influence of gum acacia. *World J Pharm Pharm Sci* 8 (4): 1490-1498. DOI: 10.20959/Wjpps20194-13531.
- Ptak A, Morańska E, Warchoł M, Gurgul A, Skrzypek E, Dziurka M, Laurain-Mattar D, Spina R, Jaglarz A, Simlat M. 2022. Endophytic bacteria from in vitro culture of *Leucojum aestivum* L. a new source of galanthamine and elicitor of alkaloid biosynthesis. *Sci Rep* 12: 13700. DOI: 10.1038/s41598-022-17992-5.
- Pujianto S, Resdiani M, Raharja B, Ferniah RS. 2018. α -Amylase inhibitor activity of endophytic bacteria isolated from *Annona muricata* L. *J Phys: Conf Ser* 1025: 012085. DOI: 10.1088/1742-6596/1025/1/012085.
- Purwestri YA, Kartikasari N, Putri SG, Wilson W, Sembiring L. 2016. Metabolic profiling of endophytic bacteria from *Purwocheng (Pimpinella pruatjan* Molkend) root and antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *AIP Conf Proc* 1744: 020063. DOI: 10.1063/1.4953537.
- Qiu S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y, Wang XJ. 2014. Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chin J Nat Med* 12 (6): 401-406. DOI: 10.1016/S1875-5364(14)60063-7.

- Ramya R, Othman R, Hatta FAM, Sulaiman WSH, Latif NHM. 2021. Characterization of phenolic content and antimicrobial activities of fern species used by Malays in traditional medicine. *Asian J Environ Hist Heritage* 5 (2): 77-91.
- Ranganathan N, Mahalingam G. 2018. Secondary metabolite as therapeutic agent from endophytic fungi *Alternaria longipes* strain VITN14G of mangrove plant *Avicennia officinalis*. *J Cell Biochem* 120 (3): 4021-4031. DOI: 10.1002/jcb.27686.
- Rao MPN, Xiao M, Li WJ. 2017. Fungal and bacterial pigments: secondary metabolites with wide applications. *Front Microbiol* 22 (8): 1113. DOI: 10.3389/fmicb.2017.01113.
- Salaki CL, Situmorang J, Sembiring L, Handayani N. 2010. Analisis keanekaragaman isolat *Bacillus thuringiensis* yang patogenik terhadap serangga hama kubis (*Crociodolomia binotalis*) dengan pendekatan sistematika numerik. *Biota* 15 (3): 469-476. DOI: 10.24002/biota.v15i3.2605. [Indonesian]
- Sarjono PR, Mahardika HDR, Mulyani NS, Ngawidyana, Prasetyawibowo NBA, Ismiyanto. 2020. Aktivitas antidiabetes metabolit sekunder bakteri endofit asal kulit kayu manis. *J Penelitian Sainstek* 25 (2): 143-156. DOI: 10.21831/jps.v25i2.32892. [Indonesian]
- Siala R, Chobba IB, Vallaeys T, Triki MA, Jrad M, Cheffi M, Ayedi I, Elleuch A, Nemi A, Cerqueira F, Gdoura R, Drira N, Gharsallah N. 2016. Analysis of the cultivable endophytic bacterial diversity in the date palm (*Phoenix dactylifera* L.) and evaluation of its antagonistic potential against pathogenic *Fusarium* species that cause date palm bayound disease. *J Appl Environ Microbiol* 4 (5): 93-104. DOI: 10.12691/jaem-4-5-2.
- Sofiyanti N, Isda MN, Juliantari E, Suriatno R, Pranata S. 2019. The inventory and spore morphology of ferns from Bengkalis Island, Riau Province, Indonesia. *Biodiversitas* 20 (11): 3223-3236. DOI: 10.13057/biodiv/d201115.
- Sofiyanti N, Marpaung AA, Suriatno R, Pranata S. 2020. Jenis-jenis tumbuhan paku di Pulau Rangsang, Kepulauan Meranti, Riau dan karakteristik morfologi-palinologi. *J Biol Trop* 20 (1): 102-110. DOI: 10.29303/jbt.v20i1.1711. [Indonesian]
- Sousa AM, Machado I, Nicolau A, Pereira MO. 2013. Improvements on colony morphology identification towards bacterial profiling. *J Microbiol Methods* 95 (3): 327-335. DOI: 10.1016/j.mimet.2013.09.020.
- Sulistiyani TR, Kusumawati DI. 2019. Keragaman bakteri endofit penghasil L-asparaginase bebas L-glutaminase. *J Kefarmasian Indonesia* 9 (1): 28-39. DOI: 10.22435/jki.v9i1.1302. [Indonesian]
- Sulmiyati, Said NS, Fahrodi DU, Malaka R, Maruddin F. 2019. The characteristics yeast isolated from commercial kefir grain. *Hasanuddin J Anim Sci* 1 (1): 26-36. DOI: 10.20956/hajas.v1i1.6519.
- Tasaki S, Nakayama M, Shoji W. 2017. Self-organization of bacterial communities against environmental pH variation: Controlled chemotactic motility arranges cell population structures in biofilms. *PLoS One* 12 (3): e0173195. DOI: 10.1371/journal.pone.0173195.
- Thawornwiriyanun P, Tanasupawat S, Dechsakulwatana C, Techkarjanaruk S, Suntornsuk W. 2012. Identification of newly zeaxanthin-producing bacteria isolated from sponges in the gulf of Thailand and their zeaxanthin production. *Appl Biochem Biotechnol* 167 (8): 2357-2368. DOI: 10.1007/s12010-012-9760-2.
- Ultari A, Handayani D, Eriadi A. 2021. A review: Study of chemical content, bioactivity of mangrove fern plants (*Acrostichum aureum* L.). *EAS J Pharm Pharmacol* 3 (1): 7-12. DOI: 10.36349/easjpp.
- Usman HM, Faouq AA, Baki AS, Abdulkadir N, Mustapha G. 2018. Production and characterization of orange pigment produced by Halophilic bacterium *Salinococcus roseus* isolated from Abattoir soil. *J Microbiol Exp* 6 (6): 238-243. DOI: 10.15406/jmen.2018.06.00222.
- Variani YA, Setyaningrum E, Handayani K, Nukmal N, Arifiyanto A. 2021. Analisis senyawa bioaktif ekstrak metabolit sekunder *Serratia marcescens* strain MBC1. *Indones J Chem Anal* 3 (2): 64-71. DOI: 10.20885/ijca.vol4.iss2.art3. [Indonesian]
- Venil CK, Dufosse L, Velmurugan P, Malathi M, Lakshmanaperumalsamy P. 2021. Extraction and application of pigment from *Serratia marcescens* SB08, an insect enteric gut bacterium, for textile dyeing. *Textiles* 1 (1): 21-36. DOI: 10.3390/textiles1010003.
- Wu X, Huang Q, Xu N, Cai J, Luo D, Zhang Q, Su Z, Gao C, Liu Y. 2018. Antioxidative and anti-inflammatory effects of water extract of *Acrostichum aureum* Linn. against ethanol-induced gastric ulcer in rats. *Evid Based Complement Altern Med* 2018: 3585394. DOI: 10.1155/2018/3585394.
- Yadav D, Kwak M, Jin JO. 2019. Clinical applications of *Gymnema sylvestre* against type 2 diabetes mellitus and its associated abnormalities. *Prog Nutr* 21 (2): 258-269. DOI: 10.23751/pn.v21i2.7780.
- Yang Y, Deng S, Yang Y, Ying Z. 2022. Comparative analysis of the endophytic bacteria inhabiting the phyllosphere of aquatic fern *Azolla* species by high-throughput sequencing. *BMC Microbiol* 22: 246. DOI: 10.1186/s12866-022-02639-2.
- Zulfarina, Rosiana Y, Ayudia D, Darmawati. 2022. Isolasi bakteri endofit dari tanaman Laban (*Vitex Pubescens Vahl*) sebagai antibakteri. *J Sains Teknol* 11 (1): 85-92. DOI: 10.23887/jstundiksha.v11i1.42781. [Indonesian]