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Original Research Article

The Antibacterial and Antioxidant Activities of Endophytic Bacteria from Cassava Leaves (*Manihot esculenta* Crantz)

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ABSTRACT

Endophytic bacteria are bacteria that live in healthy plant tissue without causing harm. Several studies reported that endophytic bacteria produce active compounds that are similar to those released by their hosts and also have medicinal values. Cassava leaves (*Manihot esculenta* Crantz) are recorded to produce antibacterial and antioxidant activity from their compounds. Therefore, Endophytic bacteria from cassava leaves may have great potential to also have antibacterial and antioxidant activity. This study aims to determine the number of isolates, characterize them, and test the antibacterial and antioxidant activity of endophytic bacteria from cassava leaves. Antibacterial activity was tested using the agar diffusion method (Kirby-Bauer) and antioxidants were tested using the DPPH method (*1,1-diphenyl-2-picrylhydrazyl*). The isolation results obtained five isolates of endophytic bacteria that were successfully purified. Based on the results of morphological observations, Gram staining and biochemical tests, we estimated that isolates BEDS1 and BEDS4 belong to *Pseudomonas* while BEDS3, BEDS6 and BEDS7 belong to *Bacillus*. BEDS1 and BEDS2 had antibacterial activity against *Staphylococcus aureus*, while the BEDS7 isolate had antibacterial activity against all tested bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus mutans*. BEDS3 is the isolate that had the highest antioxidant activity with IC_{50} value of 14.94 ppm (very active). Based on the phylogenetic tree from 16S rRNA gene analysis, BEDS3 originated from the genus Bacillus because they form a sister group with *Bacillus* sp. strain nsu-3 with a bootstrap value of 100%.

***Keywords*:** Endophytic bacteria, antibacterial, *Manihot esculenta* Crantz, antioxidant, 16S rRNA, cassava leaves.

Introduction

The cassava plant is a tropical plant that originated from the Amazonia and spread from North Argentina to the rest of South America.[1] In regions like Indonesia, cassava leaves are consumed as a green vegetable and used as an ingredient in traditional medicine. There have been many research reports about the cassava leaves' ability to treat disease, including as an antibacterial and antioxidant. Antibacterials are substances that can kill or inhibit the growth of other bacteria, while antioxidants are substances that can counteract free radicals. Cassava leaf extract has antibacterial activity against *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.[2,3,4] In addition, cassava leaves are also known to have a role as antioxidants. According to Linn et al. Cassava leaves have significant antioxidant activity when tested individually in vitro.[5] This ability of the leaves is due to the presence of secondary metabolites produced.

Secondary metabolites are compounds produced by plants as a form of adaptation to their environment.[6] The results of phytochemical analysis prove that cassava leaves contain alkaloids, saponins, flavonoids and tannins that act as antibacterials as well as phenolics and flavonoids that act as antioxidants.[4,5]

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Secondary metabolite compounds from a plant that are believed to have the potential to be used as antibacterials and antioxidants can be isolated by making extracts from parts of the plant.[7] However, if this extraction becomes widespread, then the availability of plants in the environment will decrease. Therefore, an alternative is needed that can obtain similar bioactive compounds without reducing plant availability, namely by isolating endophytic bacteria from the desired plants.

Endophytic bacteria are bacteria that naturally live in healthy plant tissue and provide several benefits to their hosts through the secondary metabolites they release.[8] Metabolites produced by endophytic bacteria are known to have similarities to the bioactive compounds released by their hosts.[9] For example, endophytic bacteria from lime peel produce secondary metabolites, namely alkaloids, flavonoids and polyphenols that are also in the lime peel.[10] The properties of the host are potentially also in its unique endophytic bacteria. According to Dwi et al. endophytic bacteria isolated from guava leaves are known to have antioxidant activity like their host.[11] Therefore, isolating endophytic bacteria from a plant may be a more efficient way to obtain the desired bioactive compounds. This research aims to isolate and characterize endophytic bacteria from cassava leaves and test their antibacterial and antioxidant activities. Based on the existing literature, research on the antibacterial and antioxidant activity of endophytic bacteria from cassava leaves has yet to be done.

Materials and Methods

Isolation endophytic bacteria

The cassava leaves samples were obtained in 2023 from the Experimental Garden of the Department of Biology, Faculty of Mathematics and Natural Sciences, of Syiah Kuala University in Banda Aceh (5°34'03.9"N 95°22'32.2"E). A piece of cassava leaf was picked

and taken to the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Syiah Kuala University to be washed and sterilized. The cassava leaves taken were light green in colour with no redness and were on stalks 5-6 from the top of the leaf. Cassava leaves that were taken from the collection location were immediately washed using clean running water. The leaves were cut into small pieces and weighed to 1 gram. The surface was then sterilized by immersing the sample in 70% alcohol for 45 seconds, 5.25% sodium hypochlorite for 45 seconds, and 70% alcohol again for 30 seconds. After that, the samples were rinsed with sterile distilled water. The rinsed water during surface sterilization was incubated as a control. Then, the leaf samples were ground using a mortar and 0.1 ml of liquid was taken using a micropipette to be placed on a Petri dish containing sterile NA (Nutrient Agar) media. Next, all of the Petri dishes were incubated at room temperature until the bacteria were growing. Endophytic bacterial isolates grown on NA media were purified using the four-way streak technique to obtain a single isolate that was separate from other colonies. Growing isolates were inoculated into fresh NA media plates. Finally, this Petri dish was incubated in an incubator at 37˚C for 24 hours.

Microscopic and macroscopic characterization of endophytic bacteria
Macroscopic characterization was carried out by visually observing the endophytic bacterial isolates in the dish. Objects were observed and categorized according to shape, edge, colour and elevation of the colony. Microscopic characterization was carried out by Gram staining where 1 drop of NaCl was dropped on the glass slide. Then a small sample of the isolate that grew on NA media was taken using an Ose needle and spread slowly on drops of distilled water on the glass slide. Then, the slides were fixed over a Bunsen flame. Next, the preparation was dripped with drops of crystal violet and left for 1 minute to dripped with distilled water. The preparations were dripped with iodine and left for 1 minute and then rinsed again. The preparations were dripped with 96% alcohol for 15 seconds and rinsed with distilled water. Finally, the preparations were dripped with safranin for 45 seconds and then rinsed again with distilled water. Next, the preparations were dripped with immersion oil and observed under a microscope with 1000x zooming.

Biochemical test
Motility test
Endophytic bacteria were taken using a straight-ended loop. Then, the loop was stabbed perpendicularly into the SIM media and incubated at 37˚C for 24 hours. Positive results (motile) are indicated by encroachment around the loop puncture mark on the media.[12]

Catalase test
1 drop of hydrogen peroxide (H_2O_2) dripped onto the slide. Then the endophytic bacteria were taken using a loop and placed on top of the droplet. If oxygen bubbles form around the droplet, the catalase reaction is positive.[12]

Triple Sugar Iron Agar (TSIA) test
Endophytic bacterial isolates were inoculated into TSIA media by pricking perpendicularly on the butt side and zig-zag streaking on the slant side. Isolates were incubated at 37˚C for 24 hours. The colour change in slant and butt media were observed.[13]

Indole test
Endophytic bacterial isolates were inoculated on Sulfide Indole Motility (SIM) and incubated at 37˚C for 24 hours. After that, 10-12 drops of Kovac's reagent were dripped into bacterial culture. A positive test is indicated by the formation of a red ring layer at the top of the media.[13]

Methyl Red-Vosges Proskauer (MR-VP) test
Endophytic bacteria were inoculated into MR-VP media and incubated at 37˚C for 24 hours. After that, 3-4 drops of methyl red indicator dripped for the MR test. Meanwhile for the VP test, 3 drops of KOH and 3 drops of alpha-naphthol dripped into the bacterial culture. A positive test is indicated by the colour of the media changing to red.[13]

Antibacterial activity test
The test isolate used were clinical isolate collection from Laboratorium of Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. Each 24-hour-old test bacteria was taken using an Ose needle and suspended in a tube containing 5 ml of physiological NaCl. Then, the turbidity obtained was equalized with 0.5% McFarland solution ($1.5x10^8$ CFU/mL). Furthermore, the test bacteria were spread using cotton buds on Mueller Hinton Agar (MHA) media and allowed to harden.[14] The antibacterial test was carried out using the agar plate diffusion method using a sterile straw (5 mm). Isolates of endophytic bacteria that were 24 hours old in the NA medium were taken and placed on the surface of the test bacterial media. The same was done with the positive control, antibiotic chloramphenicol and the negative control (sterile NA media). Each isolate was repeated 4 times. Then, the plates were incubated at 37°C for 24-48 hours. The clear/inhibitory zone formed was observed and measured using a caliper. The formula used to measure the diameter of the inhibition zone was:[15]

$$\frac{(VD - CD) + (HD - CD)}{2}$$

Information :
VD : Vertical Diameter
HD : Horizontal Diameter
CD : Colony Diameter

Production of endophytic bacteria supernatant
First, the endophytic bacterial isolates were purified again on the Nutrient Agar (NA) and incubated for 24 hours at 37°C. The isolate that grew was then taken using an Ose needle and put into the Nutrient Broth (NB). Next, the media was incubated using an incubator shaker at a speed of 170 rpm at a temperature of 37°C for 72 hours (3 days).[16] After that, the bacterial suspension was separated from its biomass using a centrifuge at 4000 rpm for 15 minutes at a temperature of 4°C to obtain the supernatant.[17] This supernatant was used for antioxidant activity.

Antioxidant activity test
The antioxidant activity of endophytic bacteria was measured using the *1,1-diphenyl, 2-picrylhyodrazil* (DPPH) method.[18] Initially, the DPPH solution was made to a concentration of 50 ppm by dissolving 0.0025 g DPPH and 50 mL of methanol pro analysis. Then, a stock solution was made from 200 ppm of endophytic bacterial supernatant (supernatant considered as 1000 ppm). This mother liquor was then diluted into several concentrations, namely 10, 25, 50, 100, and 150 ppm. Ascorbic acid as a positive control was also diluted to the same concentrations. 2 ml of each test solution concentration was taken with 2 ml of DPPH solution. Next, the treatment tubes were incubated at 37°C in the dark for 30 minutes. The absorbance value was measured using spectrophotometry at a wavelength of 517 nm. The inhibition percentage was calculated using the formula:

$$\%Inhibition: \frac{(control\ absorbance - sample\ absorbance)}{control\ absorbance} \times 100$$

The percentage inhibition value obtained from each concentration was used to determine the linear regression equation y=a+bx via Probit analysis, where x was the Log concentration (ppm) and y was the probit value of the percentage inhibition (%).

16S rRNA analysis
Pure isolates of the endophytic bacteria that were considered to have the most potential were sent to PT Genetika Science Indonesia to isolate the DNA and process it into a PCR product. DNA was extracted using the Quick-DNA fungal/bacterial Miniprep Kit (Zymo Research, D6005). Next, DNA was amplified using (2x) Taq Master Mix (K9021; Thermo) and primer 27F(5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R(5′-GGTTACCTTGTTACGACTT-3′).[19] The PCR product was then tested for DNA purity qualitatively using electrophoresis and quantitatively using *nanodrop*. The PCR product was then sent for sequencing to *First Base* (Selangor, Malaysia). The resulting sequences were edited using

Bioedit software. Next, a phylogenetic tree was constructed using the MEGA X program. The method used was *neighbour joining* with 1000 repetitions bootstrap analysis.[20]

Results and Discussion

Isolation of endophytic bacteria

Based on the results of the isolation and purification that had been carried out in this study, the total number of endophytic bacteria obtained was five isolates. The five isolates are believed to be true endophytic bacteria. This is due to the precise surface sterilization process achieved by the absence of bacteria or fungi growing on the control dish containing the last rinse of sterile distilled water. While in the treatment dish, bacterial growth was found. Neem leaves (*Azadirachta indica*) were also successfully isolated with as many as twenty five isolates of endophytic bacteria.[21] This shows that there are may be significant differences in the number of endophytic bacteria produced by the leaves of different plants. Factors that can affect the diversity of endophytic bacteria in a plant include plant types, plant genotypes, geographical conditions of the environment, and tissue types.[22]

All of the endophytic bacteria have different characteristics (Table 1, Figure 1, Table 2). Via Gram stain, it was shown that there were three isolates of endophytic bacteria belonging to Gram-positive bacteria and two isolates belonging to Gram-negative bacteria. This shows that Gram-positive bacteria dominated the Gram-negative bacteria. Singh et al. obtained twenty five isolates of endophytic bacteria from neem leaves with twenty two isolates belonging to the Gram-positive and three isolates belonging to the Gram-negative.[21]

BEDS1 and BEDS4 are thought to come from *Pseudomonas* genus. BEDS1 was positive for catalase, motile, negative for indole test, able to ferment lactose, glucose, and sucrose, positive for citrate test, positive for MR test, and negative for VP test. BEDS4 was positive for catalase, motile, negative for indole test, able to only ferment glucose, positive for citrate test, negative for MR test, and negative for VP test (Table 2). The characteristics of these two isolates are thought to have similarities with those of *Pseudomonas*. Based on Bergey's Manual of Systematic, *Pseudomonas* is a group of rod-shaped Gram-negative bacteria. *Pseudomonas* has features including catalase-positive, motile, rod-shaped, and Gram-negative.[23]

BEDS3, BEDS6 and BEDS7 are thought to belong to the genus *Bacillus*. BEDS3 is positive for catalase, motile, negative for indole test, able to only ferment glucose, negative for citrate test, positive for MR test, and negative for VP test. BEDS6 is motile, able to produce catalase enzyme, indole test negative, able to only ferment glucose, citrate test positive, MR test negative, and VP test negative. The characteristics of the two isolates are similar to *Bacillus*. Based on Bergey's Manual of Systematic, BEDS7 is positive for catalase, motile, negative indole, able to only ferment glucose, negative for citrate test, positive for (MR) test, and negative for VP test. *Bacillus* has rod shapes in single, pair, or chain arrangements, generally producing catalase, motile or non-motile, and coming from the Gram-positive group.[24]

Antibacterial activity of endophytic bacteria

The results of the antibacterial activity test showed that from five isolates, there was only one isolate that was able to inhibit all the tested bacteria and two isolates that were only able to inhibit the *S aureus*. BEDS7 isolate was able to inhibit *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. mutans.* Meanwhile, BEDS1 and BEDS2 were shown to only inhibit *S aureus* (Table 3). The average diameter of the inhibition produced by the three isolates was much smaller when compared to the diameter produced by the positive chloramphenicol control. All of inhibition zones produced by endophytic bacteria in this study were categorized as weak. This is because the diameter of the resulting inhibition zone was less than 5 mm.[25] Four endophytic bacterial isolates obtained from *Imperata* leaves also had antibacterial activity and all of them were in the weak category (inhibition zone diameter ≤5).[14]

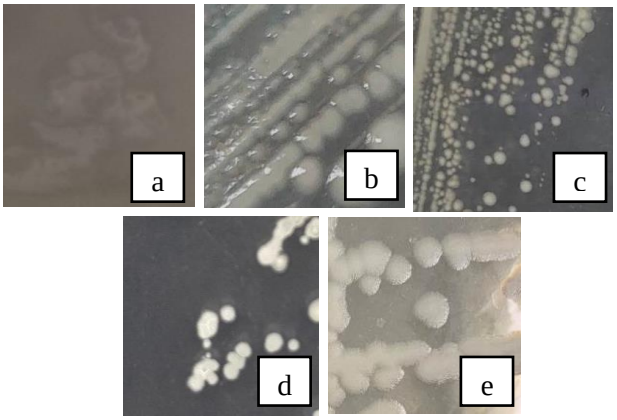

Figure 1: Colony morphology of endophytic bacteria; (a) BEDS1;(b) BEDS3 ;(c) BEDS4; (d) BEDS6;(e) BEDS7

Table 1: Macroscopic and microscopic characteristics of endophytic bacteria isolates

Isolat code	Colony Characteristics				Cell characteristics	
	Colour	Edge	Form	Elevation	Shape	Gram
BEDS1	White	Jagged	Irregular	Flat	Bacilli	Negative
BEDS3	White	Smooth	Round with raised edges	Flat	Bacilli	Positive
BEDS4	Cream	Smooth	Round	Flat	Bacilli	Negative
BEDS6	White	Smooth	Round	Flat	Bacilli	Positive
BEDS7	White	Smooth	round	Flat	Bacilli	Positive

Table 2: Result of biochemical test of endophyte

Isolate Code	Biochemical test						
	Catalase	Motility	Indole	TSIA	Citrate	MR	VP
BEDS1	+	Motile	-	y/y[a]	+	+	-
BEDS3	+	Motile	-	y/y[a]	-	+	-
BEDS4	+	Motile	-	y/y[a]	+	-	-
BEDS6	+	Motile	-	y/y[a]	+	-	-
BEDS7	+	Motile	-	y/y[a]	-	+	-

[a]y/y : yellow slant/ yellow butt
[b]r/y : red slant/ yellow butt

Based on the research results obtained, BEDS7 isolate was able to inhibit all the test bacteria in both the Gram-positive and Gram-negative bacteria groups. However, when seen at the average value of the resulting inhibitory diameter, BEDS7 produced a larger diameter in the Gram-positive bacteria group, namely *S. aureus* and *S. mutans* compared with *E. coli* and *P. aeruginosa* which included Gram negative bacteria. It was also shown that BEDS1 can only inhibit *S. aureus* of all the test bacteria. This shows that Gram-negative bacteria have a better defense against endophytic bacterial isolates obtained in this study. Differences in the cell wall components of the Gram-positive and Gram-negative bacteria are also thought to influence this. The structure of Gram negative bacteria (multilayer) is thought to be more difficult for antibacterial compounds to penetrate. According to Breijyeh et al. Gram-negative bacteria are more resistant than Gram-positive bacteria, and cause significant morbidity and mortality worldwide.[26]

Antioxidant activity of endophytic bacteria

The results showed that there were differences in antioxidant activity produced by the five supernatants of endophytic bacterial isolates tested. The antioxidant ability can be seen quantitatively from the absorbance value produced after 30 minutes of incubation. The absorbance value determines the percentage of inhibition of each concentration, which will then determine the IC_{50} value. The results of the IC_{50} values produced from each isolate can be seen in Table 4.

Table 3: Antibacterial inhibition zone measurement results

Isolat code	Average inhibition zone (mm)				Category[a]
	E. coli	*S. aureus*	*P. aeruginosa*	*S. mutans*	
BEDS1	0	0.21 ± 0.19	0	0	Weak
BEDS3	0	0	0	0	-
BEDS4	0	0	0	0	-
BEDS6	0	0	0	0	-
BEDS7	0.95 ± 0.12	1.47 ± 0.47	0.41 ± 0.18	1.51 ± 0.26	Weak
K(+)	16.42 ± 0.37	27.83 ± 0.48	19.91 ± 0.96	20.25 ± 0.69	Strong
K(-)	0	0	0	0	-

[a]according to Mao et al.[25]

Table 4: Antioxidant result of endophytic bacteria

Isolate code	Concentration (ppm)	Absorbance mean	Inhibition (%)	IC_{50} (ppm)
BEDS1	10	0.434 ± 0.003	40.04	
	25	0.420 ± 0.001	41.99	
	50	0.403 ± 0.005	44.41	130.06
	100	0.365 ± 0.003	49.62	
	150	0.347 ± 0.001	52.07	
BEDS3	10	0.475 ± 0.072	34.4	
	25	0.249 ± 0.010	65.65	
	50	0.164 ± 0.006	77.37	14.94
	100	0.191 ± 0.005	73.63	
	150	0.196 ± 0.017	72.84	
BEDS4	10	0.506 ± 0.075	30.14	
	25	0.288 ± 0.007	60.21	
	50	0.173 ± 0.015	76.04	20.19
	100	0.204 ± 0.005	71.83	
	150	0.242 ± 0.009	66.54	
BEDS6	10	0.698 ± 0.027	3.47	
	25	0.658 ± 0.003	9.164	
	50	0.653 ± 0.034	9.9	3181.60
	100	0.629 ± 0.008	13.118	

	150	0.597 ± 0.005	17.639	
BEDS7	10	0.500 ± 0.016	30.97	
	25	0.500 ± 0.020	31	
	50	0.486 ± 0.001	32.9	3951
	100	0.452 ± 0.003	37.59	
	150	0.439 ± 0.018	39.38	
	10	0.179 ± 0.03	75.3	
	25	0.129 ± 0.002	82.1	
Ascorbic acid	50	0.061 ± 0.005	91.4	1.14
	100	0.053 ± 0.0003	92.6	
	150	0.044 ± 0.001	93.9	

Mean of control absorbance : 0.725

BEDS3 produces the lowest IC_{50} values compared to the other isolates, with 14.94 ppm. The IC_{50} value indicates the concentration required to inhibit 50% of free radicals. This means that BEDS3 only requires a concentration of 14.94 ppm to be able to reduce 50% of the free radicals around it. The IC_{50} value produced by this BEDS3 isolate is classified as very active (Table 5). According to Anggreni *et al*. the antioxidant strength category of a compound is said to be very active when the IC_{50} value < 50 ppm, active when the IC_{50} value between 50-100 ppm, medium when the IC_{50} value ranging from 101-150 ppm, weak when the IC_{50} value ranging from 250-500 ppm, and inactive when IC_{50} more than 500 ppm.[27] Through the graph (Figure 2.), the linear regression equation obtained from the BEDS3 antioxidant test results is y= 0.8047x + 4.0505. IC_{50} value obtained by entering the value y=5 (the probit value of 50 is 5) and finding the x value. The R^2 value (R square) in the graph is 0.67, indicating that the supernatant concentration has an effect of 67% on the inhibition percentage, and 33% of the influence likely comes from external influences. This effect is thought to originate from a decrease in the percentage of DPPH inhibition at concentrations of 100 ppm and 150 ppm (Table 4). Foti explained that high concentrations of cinnamic acid as an antioxidant inhibit free radical scavenging due to COOH ionization which causes changes in molecular structure so that the formation of phenolic anions as electron donors is inhibited.[28] Therefore, the same thing is thought to happen to the compounds contained in the BEDS3 and BEDS4 supernatants, where it is thought that at high concentrations above 50 ppm, ionization or changes in molecular structure occur due to certain chemical reactions, causing electron donors to decrease or their formation to be inhibited.
Apart from quantitative observations, antioxidant activity was also seen qualitatively through changes in the colour of the DPPH solution and samples after incubation, from deep purple to light purple to yellowish. BEDS3 experiences colour changes little by little at each concentration, initially from purple at a concentration of 10 ppm then reddish purple at a concentration of 25 ppm until it starts to turn yellow at a concentration of 50 to 150 ppm. Yuniarti et al. stated that the colour change occurred due to the transfer of electrons from the sample to DPPH, thereby neutralizing DPPH free radicals.[29] Fighting free radicals causes free electrons to pair up, which then causes a loss of colour proportional to the number of electrons taken. The reduction in colour intensity of the DPPH solution is produced by the reaction of the DPPH radical molecule with one hydrogen atom released by the compound in the sample to form a yellow compound. BEDS3 isolate is an endophytic bacterial isolate originating from cassava leaves. The antioxidant activity it produces is not much different from the antioxidant activity produced by its host, cassava leaves. Linn et al. have tested the antioxidant activity of cassava leaf ethanol extract and obtained an IC_{50} value of 17.69 ppm which is classified as very active.[5] This suggests BEDS3 has better antioxidant activity than its host.

Table 5: Antioxidant category of endophytic bacteria supernatant

Sample	IC_{50} (ppm)	Category[a]
BEDS1	130.06	Medium
BEDS3	14.94	very active
BEDS4	20.19	very active
BEDS6	3181.60	Inactive
BEDS7	3951	Inactive
Ascorbic Acid	1.14	Very active

[a]according to Anggreni et al.[27]

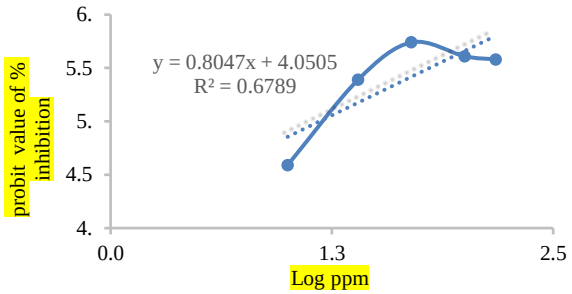

Figure 2: Regression linear graphic of inhibition percentage BEDS3 to DPPH

Molecular identification using 16S rRNA
Molecular identification was only carried out on isolates that were considered to have the most potential, namely BEDS3, which was the isolate producing the highest antioxidant activity. The DNA size of the PCR results shows the alignment with the 1500bp marker (Figure 3).

Table 6: BLAST result from NCBI

16S rRNA identification from BLAST	Similarity percentage
Bacillus sp. *strain* nsu-3	99.93%
Bacillus sp. *strain* 190Cu-As	99.93%
Bacillus cereus strain SH06	99.93%
Bacillus sp. *strain* B4	99.93%
Bacillus sp. *strain* P014	99.93%

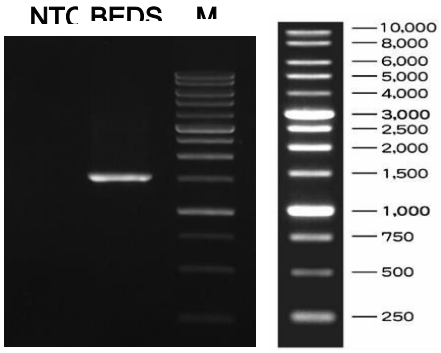

Figure 3: Visualization of BEDS3 Amplicon DNA

This shows that the use of primers 27F and 1492R is appropriate. The sequencing results of the PCR product succeeded in obtaining 1432 base pairs from the BEDS3 isolate. This size is very close to the size of a typical 16S rRNA sequence. The 16S rRNA sequence is around 1500bp in size.[30] Therefore, the sequences obtained can be used to align with other sequence data contained in *GenBank* via the BLAST-N program (*Basic Alignment Search Tool Nucleotide*).

The identification results show that all species have 99.93% of percentage identity (Table 6). This shows that the BEDS3 isolate is likely the same species as one of the BLAST results. If the similarity percentage value resulting from identification of 16S rRNA in microbes is in the range of 97-100% the similarity is at the species level.[31] Based on the phylogenetic tree topology (Figure 4.) BEDS3 forms a *sister group* with *Bacillus* sp. strain nsu-3 with 100%. Bootstrap value. This is a very good value which shows that in 1000 repetitions, BEDS3 and *Bacillus* sp. *strain* nsu-3 adjoining in 1000 instances. This means that BEDS3 is closely related to *Bacillus* sp. *strain* nsu-3. This is also supported by the BLAST result of 99.93%. In addition, the results of morphological and physiological identification showed that the BEDS3 isolate was thought to originate from the genus *Bacillus.* Therefore, through this research it can be confirmed that the BEDS3 isolate comes from the genus *Bacillus*. Several *Bacillus* species have been noted to have antioxidant activity such as *Bacillus pumigatum*, *Bacillus megaterium* and *Bacillus amyloliquefaciens GSBa-1*. [32,33]

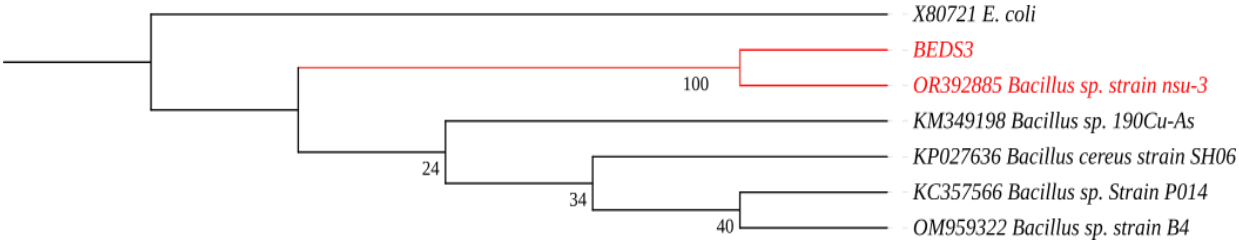

Figure 4: Phylogenetic tree of BEDS3

Conclusion

Five isolates of endophytic bacteria were successfully isolated from cassava leaves with different characteristics. This research has proved that the potential abilities possessed by the plants are also possessed by their endophytic bacteria. Endophytic bacterial isolates from cassava leaves produce antibacterial and antioxidant activity. Based on morphological and biochemical characterization, BEDS1 and BEDS4 are thought to be from *Pseudomonas* genus. BEDS3, BEDS6 and BEDS7 are thought to be from the *Bacillus* genus. Isolates BEDS1 have antibacterial activity against *Staphylococcus aureus* bacteria, while isolate BEDS7 has antibacterial activity against all tested bacteria, namely *S. aureus*, *E. coli*, *P. aeruginosa* and *S. mutans*. BEDS3 produces the highest antioxidant activity in the very active category with 14.94 ppm IC50 value. From the results of analysis using the 16S rRNA gene, this isolate was confirmed to be derived from *Bacillus*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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