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Isolation, Characterization, and Identification of Endophytic Bacteria by 16S rRNA Partial Sequencing Technique From Leaves of *carica papaya* and Its Potential as an Antioxidant

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ABSTRACT. A study about the *Carica papaya* plant has been reported producing antioxidants. The secondary metabolites can be isolated from plants and endophytic bacteria that are symbiotic with papaya plants. The endophytic mycobacteria have many advantages to produce bioactive compounds. This study aims to isolate the endophytic microbes which are symbiotic with papaya, obtaining data of antioxidant activity with the DPPH method and obtaining phytochemical screening qualitative data of secondary metabolites production of the endophytic bacteria. The results from this study are isolated endophytic bacteria which are symbiotic with papaya leave, named Q1. Obtained bacterial isolate has staphylococcus shape and is a type of gram-negative bacteria. The antioxidant activity of secondary metabolites of endophytic bacteria isolates using the DPPH method has IC50 of 22.472 ppm. Phytochemical screening shows that the production of secondary metabolites of endophytic bacteria which were isolated from papaya leave contains alkaloids, flavonoids, tannins, and saponins. Genotypic identification is done by isolation and purification chromosomal DNA using the helix saponin approach, then amplification fragments of 16S rRNA gene with PCR, electrophoresis of 16S rRNA gene, sequencing with Sanger method and phylogeny construction with a distance-based method. The study shows Q1 has the closest correlation with *Pseudomonas deceptionensis*, *Pseudomonas endophytic*, *Pseudomonas psychrophile, Pseudomonas fragi* ATCC 4973, *Pseudomonas fragi* NBRC 3458 from *Pseudomonas* genus.

INTRODUCTION

Endophytic bacteria live in plant tissue in all plant species, usually in living plant tissue [1]. Endophytic bacteria live between plant cells and have symbiotic mutualism with their host plants [2]. The symbiotic relationship of mutualism Endophytic bacteria are bacteria that can produce secondary metabolites almost the same as their host plants [3]. Papaya (*Carica papaya*) leaves contain secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and steroids. These secondary metabolites can act as antioxidant. The secondary metabolites can be obtained not only from leaf extracts but also from endophytic bacteria that are symbiotic in papaya leaves. Plants containing bioactive compounds usually have endophytic bacteria that have similar abilities to those of their hosts in producing metabolites.

It has been reported that the endophytic bacteria isolate EC3-B able to inhibit free radicals by 68.5% with a total phenol of 69 mr greg gallic acid/gram samples. In addition, it performed the highest antibacterial ability at the incubation time of 38 hours with the MIC values obtained of 1.4 mg/mL against *Escherichia coli, Salmonella typhi* and 1.3 mg/mL against *Staphyllococcus aureus* [4].

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This article study isolate of the endophytic microbes that are symbiotic with papaya, data of antioxidant activity with the DPPH method and phytochemical screening qualitative data of secondary metabolites production of the endophytic bacteria.

EXPERIMENTAL

Instruments and Materials

The instruments used were a set of laboratory glassware, a set of laminar air flow, a set of microscope, analytical balance, Shimadzu UV-1280 UV-*Visible* (Ultra Violet-Visible) spectrophotometer. Materials used were yeast (Merck), nutrient agar (Merck), nutrient broth (Merck), methanol (Merck), DPPH (Sigma Aldrich), quercetin (Sigma Aldrich), and ascorbic acid (Merck).

Methods

Isolation of Endophytic Bacteria from C. papaya Leaves Tissue

The sample used in this study was papaya leaf (*C. papaya*) aged 5 months obtained from Tembalang area, Semarang, Indonesia. The isolation phase refers to the method that has been carried out by Sun et al. [5] with some modifications. Papaya leaf samples were sterilized by immersion in 5.25% calcium hypochlorite and 70% alcohol. Afterwards, the samples were rinsed 3 times with sterile distilled water. One gram of sterile sample was then crushed using a mortar. The extract was separated from the residue and it was diluted to obtain 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilution. A total of 20 µL papaya leaf extracts of each concentration and negative control were spread on *Zobell* media in a petri dish using a *spreader* and was then incubated at room temperature for 6 days. Growing bacterial colonies were separated according to their morphological appearance and were *refresh* on *Zobell* agar media to obtain a single colony isolate.

Gram Staining Tests

Gram staining tests were carried out by following standard staining procedures and techniques. The microscopic examination was performed with the oil immersion objective of the bright field microscope.

Secondary Metabolite Production

Endophytic bacterial isolates were rejuvenated on Zobell agar media at room temperature for one day, then were inoculated in 90 mL Zobell liquid media and incubated with a shaker at room temperature at a speed of 75 rpm for 24 hours as a starter [6]. The starter was planted in 210 mL of Zobell liquid media for 24 hours. Subsequently, it was replanted in 700 mL of Zobell liquid media during the production time determined from the bacterial growth curve. The culture obtained was then centrifuged at a speed of 6000 rpm for 10 minutes. The supernatant from the culture was then concentrated using a freeze dryer for 5 days to obtain the secondary metabolite extract of endophytic bacterial isolates.

Antioxidant Activity Assay with DPPH Reagent

Metabolite samples were dissolved in methanol with various concentrations. As much as 1 mL of metabolite samples from each concentration variation were added into 3 mL of 0.1 mM DPPH and then the mixture was left for 30 minutes in the dark. The mixture were then measured their absorbance at a maximum wavelength of 517 nm [7]. In the ascorbic acid sample, the similar protocol was carried out as a comparative antioxidant. Control absorbance and test samples were used to calculate %inhibition using the following formula:

$$\%inhibition = \frac{A_{control} - A_{test\ sample}}{A_{control}} \times 100\% \quad (1)$$

The IC_{50} value can be calculated by creating a linear curve between the concentration of the test solution (x-axis) and the %inhibition value (y-axis). The IC_{50} value is a number that showed about concentration of the test sample that provides reduce in the oxidation process by 50% [8]. Half-maximal inhibitory concentration (IC_{50}) is the most

widely used and informative measure of a sample efficacy. It indicates how much sample is needed to inhibit a DPPH free radical by half, thus providing a measure of potency of an antioxidant

Phytochemistry screening

Phytochemical screening was carried out based on [9]. The phytochemical screening included testing of flavonoid, alkaloids, saponins, triterpenoids and steroids.

Genotypic Identification

DNA Extraction and Purification

The DNA extraction was carried out using the *Chelex saponin* method. 2-3 ose bacterial cultures were put into the *microtube* and 500 mL ddH_2O and 1 mL 5% saponin were added to the Phosphate Buffer Saline (PBS) and kept cold for 24 hours. *The microtube* stored for 24 hours was then centrifuged at 12000 rpm for 10 minutes and the supernatant from the centrifugation was removed. *Pellet* cells result in centrifugation was added of 1 mL of DDH_2O and re-centrifuge. Afterwards, 100 µL of ddH_2O and 50 µL Chelex 20% were added. The mixture was heated to boiling for 10 minutes and was homogeneous using vortex for every 5 minutes and recentrifugation was done. The centrifugation supernatant (DNA solution) was then transferred to a new *microtube* and stored in a *freezer*. The results of DNA extracts stored in *freezers* were then tested for concentration and purity using nanodrop.

Amplification of 16S rRNA gene with PCR

DNA samples obtained were amplified in vitro by the PCR method. The 16S rRNA gene fragment was amplified using a set of 16SF primers, namely at positions 8 to 27 (5' AGA GTT TGA TC(AC) TGG CTC AG 3') – FORWARD and 16SR at positions 1492 to 1510 (5 'TAC GG (CT) TAC CTT GTT ACG A 3 ') - REVERSE. The printed DNA sample was added with 1 µL of 16SF primer, 1 µL of 16SR primer and Taq Polymerase. The sample mixture was then put into a PCR machine. Amplification was done in 30 cycles.

Electrophoresis

Electrophoresis was carried out using 1% agarose gel. A total of 4µl of amplified 16S rRNA gene was inserted into agarose gel wells. Agarose gel was added to the electrophoresis and added 5x TBE until submerged. Electrophoresis was carried out for 60 minutes with a current of 400 mA and a voltage of 100 Volts. Electrophoresis was stopped when the movement of the sample has reached the tip of the gel. The gel was then observed under ultraviolet light using a UV Transilluminator lamp to determine the pattern of DNA bands. The results obtained in the form of DNA bands show chromosomal DNA fragments amplified at the PCR stage, then the nucleotide sequence was read by the sequencing method.

Sequencing

PCR results that have been shown to contain 16S rRNA gene fragments were sequenced. The results of sequencing analysis were DNA forward and reserve sequences. To combine DNA forward and reserve, sequences were carried out using DNA Baser with results in the form of DNA sequence output or DNA sequences. It was then compared with Genbank data to construct phylogeny trees.

Phylogeny Study

The sequencing results were compared with Gen Bank data using the BLASTN (Basic Local Alignment Search Tool Nucleotide) program found in the NCBI (National Center of Biotechnological Information). The results in the form of DNA nucleotide data of the closest bacteria were then carried out phylogenic studies by aligning/aligning using the MEGA 6. The BLASTN results were entered into the Mega 6 program to align the nucleotide sequence of the 16S rRNA gene with other comparative bacteria from the BLASTN results. The process was carried out using the Alignment by Clustal W menu to generate output data. Phylogenetic Analysis menu was used to construct phylogenic trees against bacterial isolates. Results alignment Alignment by Clustal W were used as input data on Phylogenetic analysis. The next step was to choose the construct/Test Neighbor-Joining Tree with the Phylogeny Test using Bootstrap methods for 5000 to obtain a phylogeny tree image. The phylogeny will show the kinship between the sample bacteria with the bacteria that has the closest kinship.

RESULTS AND DISCUSSION

Isolation of Endophytic Bacteria

Table 1 showed that isolate Q1 is a coccus Gram (-) bacteria. Colonies of bacteria grow after 6 hours with a round shape and yellow. Then bacteria transferred to *Zobell* agar media so it is tilted to obtain a single isolate.

TABLE 1. Characteristics of papaya leaf endophytic bacterial isolates

Isolate	colour	Colony shape	Elevation shape	Cell shape	Gram Staining	Appereance	Physical Characteristics
Q1	yellow	Circle	Convex	*coccus*	Negative	shiny	aerob

Bacterial Growth Curve

Observation of a bacterial growth curve aims to determine the phases of bacterial growth. In this study, observations were made to determine the time of production of secondary metabolites from Q1 endophytic bacterial isolates. Bacterial cell growth usually follows a certain growth pattern in the form of a sigmoid growth curve. The pattern in the bacterial growth curve shows the phase of bacterial growth. These phases reflect the state of bacteria in the culture at a particular time. Fig. 1 showed that growth rate of endophytic Q1 bacteria indicates the beginning of the cell death phase at the 84th hour. The time of production of secondary metabolites was done at the 84th hour. When bacteria undergo certain phases, bacteria produce secondary metabolites. There is competition between bacteria to get the nutrients because bacterial nutrition starts to run out. Bacteria carry out metabolic processes to produce secondary metabolites that are used to defend themselves, in the competition for nutrients[10]. The next research phase is the production of secondary metabolites of Q1 endophytic bacteria from papaya leaves.

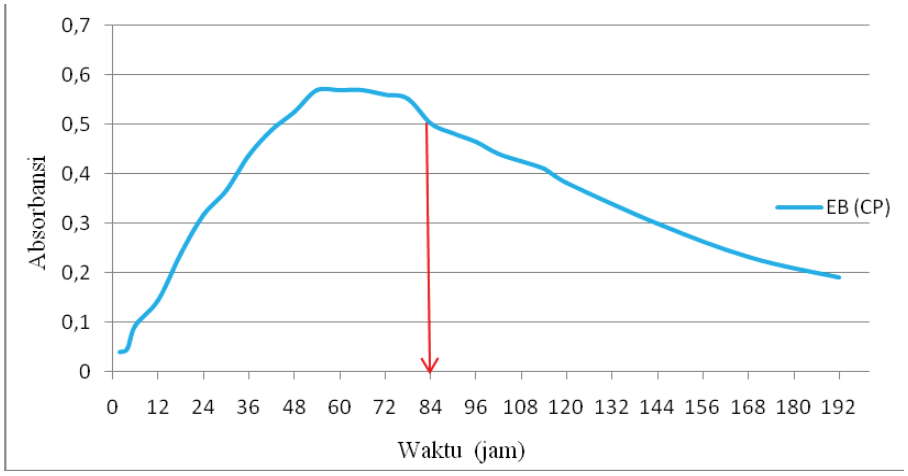

FIGURE 1. Growth curve of Q1 endophytic bacterial

Secondary Metabolite Production

Based on the growth curve of endophytic Q1 bacteria that have been obtained, the secondary metabolites of these bacteria can be produced at the 84th hour, i.e. in the initial phase of death. In this phase, the bacteria have produced the most amounts of secondary metabolites because nutrients have started to run out, so the bacteria secrete secondary metabolites to defend themselves. The secondary metabolites obtained were dried using the freeze-drying method. The metabolites obtained in form of thick brown paste extracts and its mass can be calculated. The weight of the sequential metabolite extract was 1.48 g from 1 L of culture media. The next stage was phytochemical screening including qualitative tests on the presence of alkaloids, flavonoids, saponins, tannins, quinones, steroids and terpenoids. Table 2 shows the results of secondary metabolite phytochemical screening.

TABLE 2. Phytochemical screening of secondary metabolite Q1

Parameter	Results	
	Secondary Metabolite of Q1	Methanol extract of papaya leaves
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tanins	+	+
Steroids	-	-
Triterpenoids	-	-

Based on Table 2, it is known that both ethanol extracts of papaya leaves and extracts of secondary metabolites of endophytic Q1 bacteria isolates from papaya leaves contain alkaloids, flavonoids, saponins, and tannins. According to Strobel and Daisy [10], endophytic bacteria that plant on plants that produce bioactive compounds have the potency to produce bioactive compounds similar to their host plants. This is probably due to genetic transfers from host plants to endophytic microbes [11].

Antioxidant Activity Test using the DPPH Method

Metabolite extract from freeze drying results was used as a sample to test antioxidant activity using DPPH method. The results obtained are shown in Fig. 2. The results obtained by secondary metabolite of Q1 have antioxidant properties which can be shown by the change in color of the solution from purple to yellow. It can be seen that the inhibition percentages of samples experienced a sharp increase from the concentration of 10 to 15 ppm. The graph showed that percent inhibition at 10, 15, 20, 25, and 30 ppm respectively were 28.61%, 37.06%, 46.60%, 55.86%, 61.31%. The percentage of inhibition was used to calculate IC_{50} as the main parameter in determining antioxidant activity. The results of IC_{50} obtained are shown in Table 3.

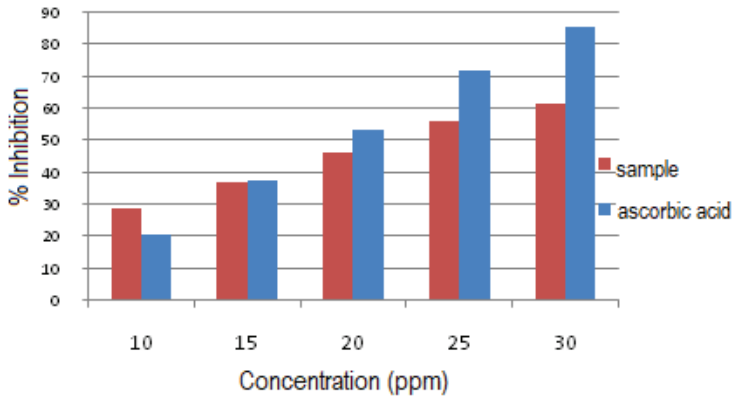

FIGURE 2. Graph antioxidant activity of secondary metabolites Q1 isolate

TABLE 3. Result of the IC_{50} test solution

Larutan Uji	**IC_{50} (ppm)**
Ascorbic Acid	18.88
Q1	22.47

Absorbance is used to find the %inhibition value which can be used to calculate IC_{50} as a parameter in determining the capacity of antioxidant activity. The IC_{50} value indicates the need for antioxidant concentration to reduce 50% of DPPH free radical concentration. As IC_{50} gets smaller, the greater the antioxidant capacity to extract its metabolites [8]. Based on the Table 3, the highest antioxidant capacity was ascorbic acid. The sample extract was 22.47 ppm which means that with a concentration of 22.47 ppm the sample can inhibit 50% of DPPH free radical action. According to Madigan et al. [12], the stationary phase to death is the best phase in producing secondary

metabolites. This is due to the limited amount of nutrients in the phase that causes bacteria to release genes for the synthesis of secondary metabolites that can be used to defend themselves.

Based on Irondi et al. [13], the antioxidant activity of papaya leaf methanol extract by the DPPH method resulted in an IC 50 value of 980 ppm. Papaya leaf extract results have weak antioxidant activity (> 200ppm), while extracts from endophytic bacteria have a very strong activity (<50 ppm). This showed that extracts from endophytic bacteria have ability to inhibit DPPH free radical more effectively than leaves papaya methanol extract.

Genotypic Identification

DNA Extraction and Purification

The identification of molecular biology is based on the nucleotide sequence of the 16S rRNA genes, so that to obtain genes can be done by amplification of the 16S rRNA gene on the chromosome DNA. Chromosomal DNA is obtained by isolation carried out by cell lysis. The purity test results from DNA isolation of endophytic Q1 bacteria can be seen in table 4. Table 4 showed that results of the purity test using nanodrop by Duplo obtained nucleic acid concentrations with an average of 18.7. A good purity score is 1.8 - 2.0. The absorbance rate is based on the ratio of 260/280. The nucleotides, DNA, and RNA will absorb at a wavelength of 260 nm, while proteins and other contaminants absorb at a wavelength of 280 nm.

TABLE 4. Results of nanodrop DNA isolation of endophytic Q1

No.	Sample	Concentration of Nucleic Acid (ng/µl)	Absorbance 260/280
1.	Q1	18.8	1.97
2.	Q1	18.6	1.94

The success of DNA isolation is an important stage as a template for the PCR process. The quality of isolated DNA can be seen from the purity value and DNA concentration, where DNA to be amplified must have high purity. DNA purity was measured based on the ratio of absorbance ratio (260 nm/280 nm). The results of DNA isolation that have been carried out were tested for the purity and concentration of DNA using nanodrop.

Isolation using helix is insulation made with the addition of Chelex which consists of a styrene-divinylbenzene copolymer containing ions pair iminodiacetate by heating to trigger cell lysis. Chelex itself consists of styrene-divinylbenzene copolymer which contains a pair of iminodiacetate ions that act as chelating groups that can bind to polyvalent metal ions such as Mg^{2+} and Ca^{2+}. With the binding of magnesium ions, enzymes that damage DNA, as well as a nuclease, will be inactivated, so that DNA molecules can be protected and not damaged.

The heating factor is a trigger for cell lysis, because when an increase in temperature the phospholipids in the cell membrane will melt which allows more movement to affect cell permeability, so molecules that should not be able to enter the cell can enter the cell and cause cell lysis. The addition of ddH_2O which is pure water is done to reduce the confounding factors to reduce the presence of inhibitors that can inhibit.

Amplification of 16 S rRNA genes

The sequencing stage requires DNA in sufficient quantities, so it is necessary to do amplification or DNA propagation techniques. Amplification of the 16S rRNA gene was done by the PCR method which is a replication technique or multiplying DNA in a cycle using polymerase enzymes and using chromosomal DNA as a template. Amplified gene fragment of the 16S rRNA isolates Q1 seen in Fig. 3. The Fig. 3 showed that DNA amplification using 16S rRNA PCR showed positive results marked by the presence of Q1 bacteria. Amplicons are seen as glowing ribbons. The luminescence of the band is seen in agarose gels due to the addition of EtBr (Ethidium bromide) which gives fluorescent color after reacting with DNA base pairs [14]. Amplitude 16S rRNA gene fragment Q1 isolates by PCR method was obtained measuring around 1500 bp. This proves that the primer is right in recognizing the target 16S rRNA gene fragment in the order of 1492 to 1510 pb.

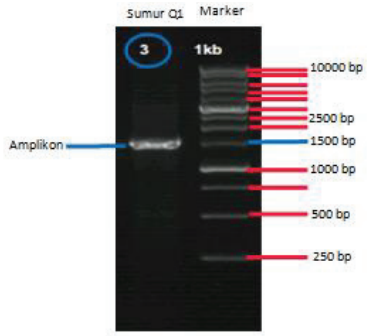

FIGURE 3. Electrophoresis results of 16S rRNA gene fragments of Q1

Q1 Amplicon Sequencing Results

Genotypic identification can be done by knowing the sequence of nucleotide fragments and 16S rRNA gene isolates of Q1 bacteria, so it is necessary to do the Dideoxy-Sanger sequencing method. In this method, the extension of the 16S rRNA gene fragment chain begins and ends using a primer that is complementary to the specific region of the printed DNA nucleotide sequence.

Primary sequencing 1492R and 27F as the start of the sequencing process. The primer was extended using Taq polymerase, an enzyme that replicates DNA. The sequencing process also includes four types of deoxynucleotide bases (DNA-forming units) and chain breakers or nucleotides (chain terminators or ddNTP) in low concentrations. The results of the 16S rRNA gene fragment isolation of Q1 isolates can be seen in the following Fig. 4. The results of the nucleotide base sequence results from the reading of the electrogram starting in specific sites by primers 27F and 1492R are connected with the DNA BASER program. From the results of joining the nucleotide base sequence, the nucleotide sequence a 16S rRNA gene isolate Q1 isolates are intact (Fig. 4). Amplification of the 16S rRNA gene to the 1510 base sequence, but the nucleotide sequence for the 16S rRNA gene fragment of Q1 isolates obtained from sequencing only reached sequence 1460. This difference due to the area where the primary attachment (sequence area 8-27 and 1492-1510) did not produce a specific peak. In the area of attachment, the primer reads like the letter N so the nucleotide sequence of the area cannot be determined.

```
1    TGCAGTCGAGCGGTAGAGAG GTGCTTGCTT CTCTTGAGAG CGGCGGACGG
51   GTGAGTAATA CCTAGGAATC TGCCTGGTAG TGGGGGATAA CGTTCGGAAA
101  CGGACGCTAA TACCGCATAC GTCCTACGGG AGAAAGCAGG GGACCTTCGG
151  GCCTTGCGCT ATCAGATGAG CCTAGGTCGG ATTAGCTAGT TGGTGAGGTA
201  ATGGCTCACC AAGGCTACGA TCCGTAACTG GTCTGAGAGG ATGATCAGTC
251  ACACTGGAAC TGAGACACGG TCCAGACTCC TACGGGAGGC AGCAGTGGGG
301  AATATTGGAC AATGGGCGAA AGCCTGATCC AGCCATGCCG CGTGTGTGAA
351  GAAGGTCTTC GGATTGTAAA GCACTTTAAG TTGGGAGGAA GGGCTGTAGC
401  TTAATACTCT AGTATTTTGA CGTTACCGAC AGAATAAGCA CCGGCTAACT
451  CTGTGCCAGC AGCCGCGGTA ATACAGAGGG TGCAAGCGTT AATCGGAATT
501  ACTGGGCGTA AAGCGCGCGT AGGTGGTTTG TTAAGTTGGA TGTGAAATCC
551  CCGGGCTCAA CCTGGGAACT GCATTCAAAA CTGACTGACT AGAGTATGGT
601  AGAGGGTGGT GGAATTTCCT GTGTAGCGGT GAAATGCGTA GATATAGGAA
651  GGAACACCAG TGGCGAAGGC GACCACCTGG ACTGATACTG ACACTGAGGT
701  GCGAAAGCGT GGGGAGCAAA CAGGATTAGA TACCCTGGTA GTCCACGCCG
751  TAAACGATGT CAACTAGCCG TTGGAAGTCT TGAACTTTTA GTGGCGCAGC
801  TAACGCATTA AGTTGACCGC CTGGGGAGTA CGGCCGCAAG GTTAAAACTC
851  AAATGAATTG ACGGGGGCCC GCACAAGCGG TGGAGCATGT GGTTTAATTC
901  GAAGCAACGC GAAGAACCTT ACCAGGCCTT GACATCCAAT GAACTTTCTA
951  GAGATAGATT GGTGCCTTCG GGAACATTGA GACAGGTGCT GCATGGCTGT
1001 CGTCAGCTCG TGTCGTGAGA TGTTGGGTTA AGTCCCGTAA CGAGCGCAAC
1051 CCTTGTCCTT AGTTACCAGC ACGTAATGGT GGGCACTCTA AGGAGACTGC
1101 GGTGACAAAC CGGAGGAAGG TGGGGATGAC GTCAAGTCAT CATGGCCCTT
1151 ACGGCCTGGG CTACACACGT GCTACAATGG TCGGTACAGA GGGTTGCCAA
1201 GCCGCGAGGT GGAGCTAATC CCATAAAACC GATCGTAGTC CGGATCGCAG
1251 TCTGCAACTC GACTGCGTGA AGTCGGAATC GCTAGTAATC GTGAATCAGA
1301 ATGTCGCGGT GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACACC
1351 ATGGGAGTGG GTTGCACCAG AAGTAGCTAG TCTAACCTTC GGGAGGACGG
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FIGURE 4. Electrophoregram partially sequenced

Phylogeny Trees of Q1

Genotypic characterization can show the kinship of an organism. Nucleotide sequence of a fragment of the 16S rRNA gene of isolates Q1 results from Sequencing used for genotypic characterization. The kinship relationship of Q1 isolates with other bacteria was done by comparing the nucleotide sequence of the GENBank database using the BLAST-N program. The results of the processing are obtained DNA nucleotide data of the closest bacteria. Using Alignment by Clustal W, a phylogeny study was carried out by aligning the nucleotide sequence of the 16S rRNA

gene with other comparative bacteria from the BLASTN results so that the phylogeny tree construction can be carried out. The phylogeny tree construction is done by using the Distance-based method that depends on genetic distance, so it requires multiple sequence alignment (MSA). Based on comparison results, 100 bacterial comparators from the Pseudomonadaceae family were obtained, the majority of the genus Pseudomonas with a 99% similarity level.

Figure 4 is a phylogenic tree of Q1 isolate with 53 bacterial comparators, showing Q1 isolate having the closest kinship with *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* ATCC 4973, *P. fragi* NBRC 3458 of the type of bacterial *Pseudomonas* comparison. The closest can be seen in the following image at Fig. 5. *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* ATCC 4973*, P. fragi* NBRC 3458 equally have the same level of the nucleotide sequence of 99% against Isolate Q1.

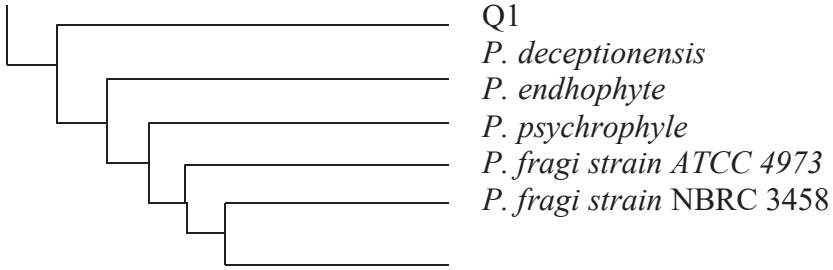

FIGURE 5. Phylogeny Tree of Q1

TABLE 5. Comparison of bacterial characters

Bacteria	Bacteria Characteristics				
	Cell Shape	**O Need $_2$**	**Gram type**	**Growth Temperature**	**Pigment**
P.deceptionensis	*The stem*	Aerobic	Negative	Mesophyll	Green
P. endophyte	*The stem*	Aerobic	Negative	Mesophyll	Yellow Green
P. psychrophile	*The stem*	Aerobic	Negative	Mesophyll	Yellow Green
P. fragi strain ATCC 4973	*The stem*	Aerobic	Negative	Mesophyll	Yellow Green
P. fragi strain NBRC 3458	*The stem*	Aerobic	Negative	Mesophyll	Yellow Green
Q1 Isolate	*The stem*	Aerobic	Negative	Mesophyll	Yellowish Green

Table 5 showed that the shape of Isolate Q1 compare with *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* strain ATCC 4973*,* and *P. fragi* strain NBRC 3458 have similarities of cell form. Alignment of the nucleotide sequence a 16S rRNA gene fragment of Q1 isolates and *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* ATCC 4973, *P. fragi* NBRC 3458 can be seen in Fig. 6. The phenotypic characters of Q1 isolates from nearby bacteria were almost the same but had striking differences in genotypic characters. The difference in genotypic characters between species is seen in the alignment of the 16S rRNA gene nucleotide sequence in Figure 6 which is marked in white. Differences in nucleotide sequences can generally be seen from upstream to downstream.

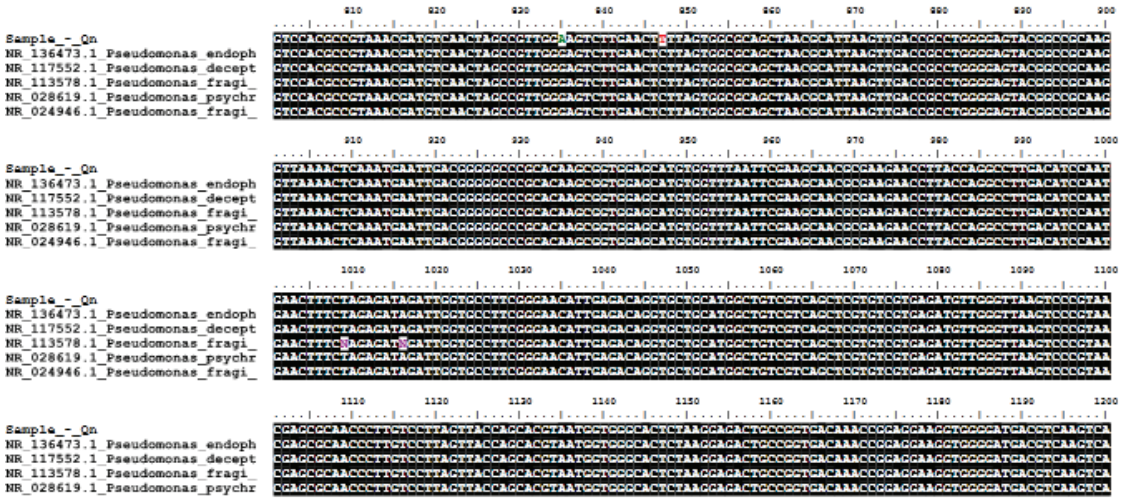

FIGURE 6. Alignment of the Nucleotide Sequence of 16S rRNA Q1 gene fragments with nearby bacteria

P. deceptionensis a mesophyll bacteria (-4-34 ºC), aerobic, gram-negative bacteria, the form of *rod-shaped* or rod, produces pigment *fluorescents* green and is derived from the Antarctic marine sediments. *P. endophyte* is a mesophyll bacteria (35 ºC), aerobes, gram-negative bacteria, rod-shaped, producing green *fluorescents* pigments and derived from the parent tissue of the leaf *Solanum tuberosum* (a type of potato) [15] *P. endophyte is* also resistant to biotic & abiotic organisms [16]. *P. psychrophile* is a mesophile bacteria (-1-40 ºC), aerobic, gram-negative, rod-shaped, and produces acid from D-glucose in aerobic conditions [17]. Based on research that has been done, Q1 isolates have been genotypically identified as having the closest kinship to the bacterium *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* ATCC 4973*, P. fragi* NBRC 3458, which is generally a bacterium belonging to the bacterial group *P. psychrophil*e since are equally capable of producing pigment *fluorescent.*

CONCLUSION

An isolate endophytic bacteria, namely Q1 isolate, that are symbiotic with papaya leaves has a coccus shape and gram-negative bacteria. Phytochemical screening shows that the production of secondary metabolites of endophytic bacteria contains alkaloids, flavonoids, tannins, and saponins. The antioxidant activity of secondary metabolites of endophytic bacteria isolates using the DPPH method has IC_{50} of 22,472 ppm. Q1 isolate has the closest correlation with *P. deceptionensis, P. endophyte, P. psychrophile, P. fragi* ATCC 4973*, P. fragi* NBRC 3458 from *Pseudomonas* genus.

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