Trop J Nat Prod Res, July 2023; 7(7):3342-3350 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Exploration of Endophytic Bacteria in FIGS (*Ficus carica* L.) with Antibacterial Agent Potential

Endang S. Gultom[1]*, Hasruddin Hasruddin[2], Nila Z. Wasni[2]

[1]*Departement of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan, Indonesia*
[2]*Departement of Biology Education, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan, Indonesia*.

ARTICLE INFO

Article history:
Received 11 April2023
Revised 01 June 2023
Accepted 21 July 2023
Published online 01 August 2023

ABSTRACT

Tin or Ara plants are one of the plants that can be used as a source of endophytic bacteria. Figs contain phenols, benzaldehyde, terpenoids, flavonoids, alkaloids, fiber, and polyphenols. This fruit is used by the society to treat asthma, bronchitis, dry cough, diabetes, cancer, coronary heart disease, gout, and osteoporosis. In this research, endophytic bacteria will be used as a source of bioactive compounds that have the potential to be antibacterial. The isolation of endophytic bacteria was carried out using the scatter plate method. The isolated endophytic bacteria were identified macroscopically, microscopically, and molecularly. There are 3 potential isolates that produce antibacterial compounds: TH9, TH10, and TH11. The results of bacterial species barcoding of full length of 16S rRNA bidirectional PCR sequencing of the three isolates were adjusted to the data available at the NCBI Genbank through the BLAST program. TH9 isolate had 99.86% homology with *Bacillus velezensis* strain NM374, TH10 isolate had 99.93% homology with *Stutzerimonas stutzeri* CCUG 11256 strain and TH11 isolate had 99.93% homology with *Staphylococcus wareri* strain CG10.

Keywords: Endophytic bacteria, Antibacterial, Molecular identification, 16S rRNA Gene

Introduction

Infectious diseases are still one of the leading causes of morbidity and mortality in the world. Infection occurs due to the invasion of pathogenic microorganisms. Infectious diseases are usually treated using antibiotics, but irrational implementation and consumption of antibiotics will be dangerous. Bacteria will be resistant to these antibiotics[1], and the effectiveness of antibiotics also decreases when dealing with multidrug-resistant organisms (MDRO). The MDRO groups experiencing resistance include Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Extended spectrum beta-lactamase (ESBL), and so on[2]. Other alternatives to be antibiotics can be obtained from various natural resources such as endophytic bacteria.

Endophytic bacteria are bacterial colonies that live in plant tissues without causing various disease symptoms for their host plants[3]. Endophytic bacteria can be isolated from tissues in plant organs such as roots, stems, leaves, and fruit[4]. Endophytic bacteria can produce the same bioactive compounds as their host plants due to coevolution or genetic transfer from the host plant to the endophytic bacteria[5]. Aristina *et al*.[6] reported that the results of the phytochemical test of the crude extract of 17 isolates of the endophytic bacteria of Pacing stem (*Costus* sp.) were positive from containing alkaloids, steroids, triterpenoids, and saponins[6].

Based on the report of Aryani *et al*.,[7] four isolates of endophytic bacteria from Imperata leaves were able to produce total phenols, flavonoids, alkaloids, saponins, and tannins.

*Corresponding author. E mail: endanggultom@unimed.ac.id
Tel: +626613365

Citation: Gultom ES, Hasruddin H, Wasni NZ. Exploration of Endophytic Bacteria in FIGS (*Ficus carica* L.) with Antibacterial Agent Potential. Trop J Nat Prod Res. 2023; 7(7):3342-3350 http://www.doi.org/10.26538/tjnpr/v7i7.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The potential of endophytic bacteria to produce bioactive compounds with shorter life cycles can be used as an alternative of medicinal plant extracts, which required large amounts of raw materials. As a result, the availability of these plants would be more maintained.

Tin or Ara plants are one of the plants that can be used as a source of endophytic bacteria. Figs contain phenols, benzaldehyde, terpenoids, flavonoids, alkaloids, fiber, and polyphenols[8]. Ramadhan *et al*., (2020) stated that fig fruit extract has the potential to inhibit the growth *Staphylococcus aureus* bacteria at a concentration of 100% using distilled water as a solvent.[8-9] The methanol extract of figs was also able to inhibit the growth of *Streptococcus pneumoniae* colonies at a concentration of 100% which was characterized by the absence of colonies growing on the media. The use of endophytic bacteria was chosen to reduce the exploitation of nutritious plants such as figs. The numerous benefits of figs enable the need for these plants to increase, and its expensive price makes this fruit scarce in Indonesia. This scarcity is also caused by *Cerotelium fici* disease, mosaic virus, and microorganisms.[1].

The potential of these endophytic bacteria is a great opportunity to explore various types of endophytic bacteria in plant tissues. Research related to the discovery and utilization of potential endophytic bacteria has not received much attention. Therefore, it is necessary to explore the endophytic bacteria present in plants, especially figs, and to identify these bacteria to determine their nature and type. Today, the technique to identify microorganisms is growing more and more. The identification of bacteria can be done by molecularly analyzing the bacteria using the 16S rRNA gene.[11] Molecular analysis is considered more precise and accurate than other common microbiological procedures.

The studies related to the identification of endophytic bacteria in fig plants have been reported[12], succeeded in isolating three endophytic bacteria from fig leaves (*Ficus carica* var. Brown Turkey): *Klebsiellaoxycota*, *Pseudomonas* sp., and *Pantoea* sp. Linelejan *et al*.,[13] stated that there were two endophytic bacteria in Sulawesi's unique fig leaves (*Ficus minahasse*), which are *Branchibacterium Muris* and *Pseudacidovorax intermedius.*[13]

In the present study, the antibacterial activity of *Stutzerimonas stutzeri* strain CCUG 1256 and *Bacillus velezensis* strain NM 374 isolated from

FIGS (*Ficus carica* L), and evaluated against *multi drug resistant* organism of *Staphlyloccus lugdunensis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Materials and Methods

Collection and Sterilization of Figs

Samples of fig (*Ficus carica* L.) were taken in Medan Helvetia District, Medan City (the coordinate: 3.595698895136841, 98.63614390004443). Plant sample was submitted to the Herbarium Bandungense, Institute Technology Bandung of Indonesia, Bandung for its identification by Arifin Surya Dwipa, PhD., and the voucher specimen was 3750/II.CO2.2/PL/2022. The samples were then taken to the Microbiology Laboratory of the State University of Medan for sterilization. Sterilization At first the sample was immersed in 96% ethanol for 1 minute, then put into 5.25% sodium hypochlorite solution for 5 minutes, and finally rinsed again using 96% ethanol for three repetitions[14].

Isolation and Purification of Endophytic Bacteria

The sterilized sample was then cut into several pieces and planted on Nutrient Agar (NA) isolation media in a petri dish. The remaining parts of the fruit were crushed, and its juice was filtered and then planted using the methods of pour, streak, and spread plate. Then, it was incubated for 24–48 hours at 37°C. During this period, the growth rate of bacterial colonies was observed. If colony growth appeared, the bacterial colonies could be purified by transferring 1 oz of bacterial colonies into fresh NA medium. The pure breed that was successfully obtained was then re-inoculated into NA slanted agar.[14-15]

Characterization of Endophytic Bacteria

Macroscopic observation of bacterial colonies was carried out by looking at the inoculum morphology of endophytic bacterial isolates. The morphological features observed included shape, color, edges, and elevation of the bacterial isolates, which could be observed from the top of the petri dish.[15-17] Microscopic observation was carried out by Gram staining. The bacteria were spread thinly and fixed on a clean glass slide with a light flame. Specimens were treated with 0.5% aqueous crystal violet for 30 seconds and then washed with water for one minute. Then, drop lugol, leave for 1 minute, and rinse with water. Remove the color with 95% ethanol, rinse the specimen again with water, and stain with safranin for approximately 10 seconds. Finally, wash the specimen with water and observe it under a microscope at a magnification of 100[17-18].

Antibacterial activity Test

The testing of the antibacterial activity of the endophytic bacteria was carried out on the Multi Drug Resistant Organisms (MDRO) test bacteria by inoculating 100 L of the MDRO test bacterial culture using a sterile cotton bud into a petri dish containing MHA media. The petri dish containing the MDRO test bacteria was then divided into several test areas. The positive control used 20 L of ciprofloxacin solution, and the negative control used 20 L of sterile distilled water. Oxoid paper discs were immersed in 20 L of endophytic bacterial culture and placed in the middle of the test area. The petri dish was incubated for 24 hours at 37°C (modified by Nugraheni et al., 2021). When the incubation period ended, the clear zone appearing was observed, and the diameter was measured. The samples that have the potential to produce antibacterial compounds are marked by the creation of clear zones.[15,19]

Molecular identification

Molecular identification of bacteria was based on the PT. Indonesian Science Genetics. Genomic DNA extraction from bacteria was performed using the Quick-DNA Bacterial Miniprep Kit (Zymo Research, D6005). Then, it was amplified using primers 27F (5' – AGAGTTTGATCMTGGCTCAG– 3'), dan 1492R (5' – GGTTACCTTGTTACGACTT– 3'). A total of 1 L of DNA sample was mixed with 9.5 L of dd H2O (double distillated water), 12.5 L of My Taq HS Red Mix, and forward and reverse primers.[15]

The PCR reaction was carried out on a thermocycler (predenaturation: 95 °C for 3 minutes, denaturation: 95 °C for 15 seconds, annealing: 52 °C for 30 seconds, extension: 72 °C for 45 seconds, final extension: 72 °C for 3 minutes). The amplification process was carried out for 35 cycles. The PCR results were checked by the gel electrophoresis technique, followed by the sequencing process. The sequence results were analyzed using the Basic Local Alignment Search Tool (BLAST) on the website http://www.ncbi.nlm.nih.gov, and phylogenetic analysis was performed using the Molecular Evolutionary Genetic Analysis (MEGA) X program.[14,15]

Results and Discussion

Macroscopic and Microscopic Characteristics of Endophytic Bacterial Isolates

Based on the results of isolation of endophytic bacteria in figs, 27 bacterial isolates were obtained. Bacteria were identified macroscopically such as colony shape, elevation, edges and color. The identification results were on Table 1, and the results of the visualization are on Figure 1.

This study resulted in thirteen white bacterial isolates, five cream isolates and three brown isolates. Three yellow isolate and one light yellow isolate were TH25. One orange isolate was TH23, and one transparent or clear isolate was TH22. Based on the colony shape, ten isolates had an irregular colony shape, ten isolates had a circular colony shape, four isolates had a rhizoid colony shape, two isolates had a filamentous colony shape and one isolate had a complex colony shape. Based on the shape of the elevation, twenty isolates were flat and seven isolates had a raised elevation shape (seen in Figure 1). Many studies have been reported on the isolation and characterization of endophytic bacteria carried out on various species of agricultural crops such as coffee, bananas, rice, or others. Endophytic bacteria were isolated from fig (*Ficus carica* L.) through surface sterilization. Hallman et al. (1997) defined endophytic bacteria as bacteria that live in plant tissues and can be isolated through sterilization of the tissue surface[20]. Purwanto *et al*. (2014) stated that the entry route for endophytic bacteria is generally through the roots and plant parts that are exposed to air directly, such as flowers, leaves (through stomata), and cotyledons. There were 27 isolates of endophytic bacteria that were successfully isolated from fig tissue in this study.[21]

The identification of endophytic bacterial isolates of figs microscopically was carried out by gram staining. Of the 27 isolates observed, 22 bacterial isolates were gram-negative bacteria and 5 bacterial isolates were gram-positive bacteria. Identification results can be seen on Table 2.

Based on microscopic observations on endophytic bacterial isolates of figs, 19 bacterial isolates were in the form of bacilli, and 8 isolates were in the form of cocci. The difference in color between gram-positive and negative bacterial cells indicates that there were differences in cell wall structure between the two types of bacteria (Lay, 1994). The shape and color of the endophytic bacterial cells of figs could be seen in Figure 2.

Antibacterial Activity

The test results showed that of the 27 isolates of endophytic bacteria, 19 had antibacterial activity: isolates TH1, TH2, TH3, TH4, TH5, TH6, TH7, TH8, TH9, TH10, TH11, TH12, TH13, TH14, TH15, TH16, TH19, TH23, and TH24. There weres9 isolates able to inhibit the growth of *P. aeruginosa*, 13 isolates were able to inhibit the growth of *K. pneumoniae*, and 12 isolates were able to inhibit the growth of *S. lugdunensis* (activity graph can be seen in Figure 1).

The selection process of potential isolates was carried out by selecting isolates that became the largest and clearest inhibition zones on the tested bacteria. The TH10 isolate was chosen because it showed inhibition against K. pneumoniae (2.5 mm) and S. lugdunensis (2.65 mm). The inhibition zone was formed when the testing of the antibacterial activity of TH10 isolates against S. lugdunensis could be seen in Figure 2.

An asocial relation in which bacteria cannot coexist with other bacteria is called an antagonistic relation. One species produces poison in another species. The growth of other species is disrupted due to substances produced by antagonistic species: antibiotic substances (Rifai *et al*.[15] Potential endophytic bacterial isolates were then analyzed using molecular techniques to identify the species. Based on the results of the antibacterial activity, there were three selected isolates (TH9, TH10, and TH11).

Table 1: Results of macroscopic characterization of endophytic bacterial isolates from figs (*Ficus carica* L.)

No.	Isolate Code	Colony shape	Edge	Elevation	Color
	TH1	Irregular	Undulate	Flat	Beige
	TH2	Rhizoid	Rhizoid	Flat	Beige
	TH3	Irregular	Irregular	Flat	Brown
	TH4	Irregular	Undulate	Flat	Beige
	TH5	Irregular	Rhizoid	Flat	Beige
	TH6	Irregular	Smooth	Flat	Brown
	TH7	Filamentous	Filiform	Flat	White
	TH8	Irregular	Undulate	Flat	White
	TH9	Rhizoid	Branching	Flat	White
	TH10	Rhizoid	Lobate	Flat	Beige
	TH11	Circular	Smooth	Raised	White
	TH12	Irregular	Lobate	Flat	White
	TH13	Complex	Irregular	Flat	White
	TH14	Filamentous	Ciliate	Flat	White
	TH15	Irregular	Undulate	Flat	White
	TH16	Circular	Wooly	Raised	Yellow
	TH17	Circular	Wooly	Raised	White
	TH18	Circular	Smooth	Flat	White
	TH19	Circular	Smooth	Raised	White
	TH20	Circular	Smooth	Flat	White
	TH21	Circular	Smooth	Raised	Yellow
	TH22	Irregular	Lobate	Flat	Transparent
	TH23	Circular	Undulate	Raised	Oranges
	TH24	Circular	Smooth	Raised	Yellow
	TH25	Circular	Smooth	Flat	Light yellow
	TH26	Rhizoid	Irregular	Flat	White
	TH27	Irregular	Undulate	Flat	Brown

Table 2: Results of microscopic characterization of fig endophytic bacterial isolates (*Ficus carica* L.)

Isolate	Shape	Gram	Isolate	Shape	Gram
TH1	Bacilli	Negative	TH15	Coccus	Negative
TH2	Bacilli	Negative	TH16	Coccus	Positive
TH3	Bacilli	Negative	TH17	Coccus	Negative
TH4	Bacilli	Negative	TH18	Bacilli	Negative
TH5	Bacilli	Negative	TH19	Coccus	Positive
TH6	Bacilli	Negative	TH20	Bacilli	Negative
TH7	Bacilli	Negative	TH21	Coccus	Negative
TH8	Bacilli	Negative	TH22	Coccus	Negative
TH9	Bacilli	Positive	TH23	Coccus	Negative
TH10	Bacilli	Negative	TH24	Bacilli	Negative
TH11	Coccus	Positive	TH25	Bacilli	Negative
TH12	Bacilli	Positive	TH26	Bacilli	Negative
TH13	Bacilli	Negative	TH27	Bacilli	Negative
TH14	Bacilli	Negative			

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

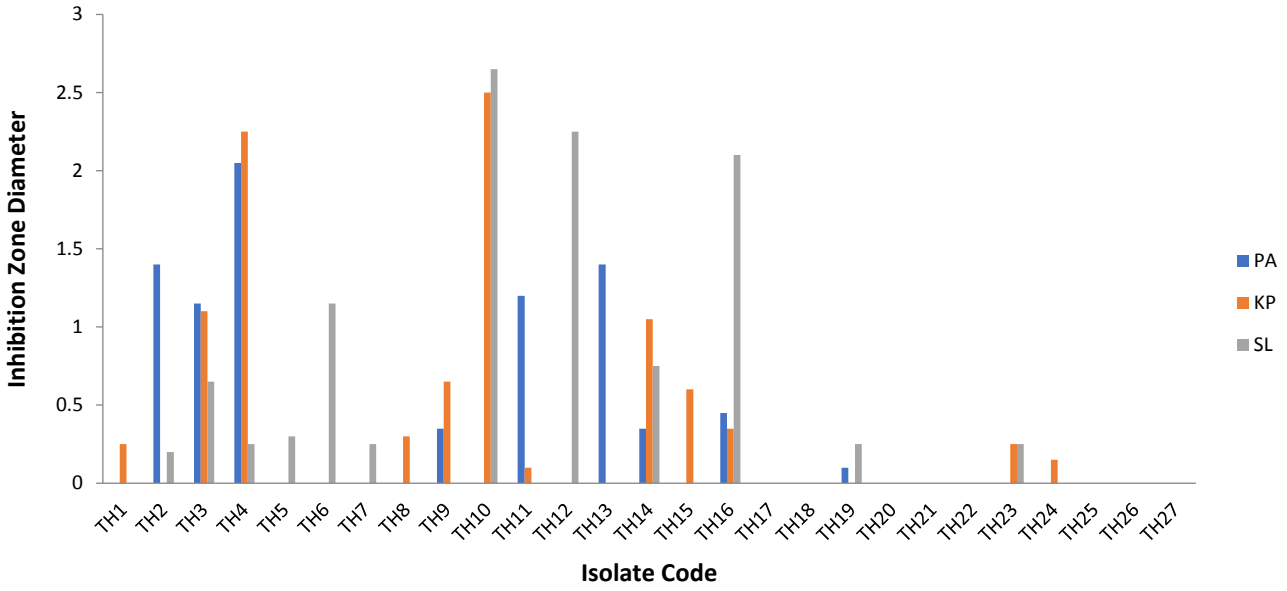

Figure 1: Graph of the measurement of the inhibition zone of antibacterial activity of the endophytic bacterial isolate of fig fruit (*Ficus carica* L.)

Table 3: BLAST results of potential isolates of fig endophytic bacteria (*Ficus carica* L.)

Isolate		Query Cover	Max Score	Total Score	Perc. Identity
TH9	*Bacillus velezensis* strain NM374 16S ribosomal RNA gene partial sequence	100%	2615	2615	99.86%
TH10	*Stutzerimonas stutzeri* strain CCUG 11256 16S ribosomal RNA gene partial sequence	100%	2599	2599	99.93%
TH11	*Staphylococcus warneri* strain CG10 16S ribosomal RNA gene partial sequence	100%	2630	2630	99.93%

Figure 2: Inhibition zones formed on the antibacterial activity of TH10 isolates against *S. lugdunensis*

Molecular analysis

Based on the antibacterial activity, potential isolates were selected, and then analyzed molecularly. There were three isolates selected: TH9, TH10, and TH11. The DNA of the three isolates was isolated and amplified using a polymerase chain reaction (PCR) machine. The PCR amplification results of the three isolates then proceed to the sequencing process. It was carried out at PT Indonesian Science Genetics. The sequencing results of isolates TH9, TH10, and TH11 could be seen in Table 3.

Based on table 3, the sequencing results of the 16S rRNA gene from isolates TH9, TH10, and TH11 showed that isolate TH9 totaled 1422 base pairs, isolate TH10 totaled 1410 base pairs, and isolate TH11 totaled 1427 base pairs. The number of nucleotides had been obtained, and then their homology was analyzed to determine the name of the bacterial isolate.

DNA sequencing of fig endophytic bacteria was analyzed using Basic Local Alignment Search Tool (BLAST). Through the analysis of BLAST results, it is known that which organisms or bacteria have similarities to the DNA sequence of the sample so that it can be used to identify bacteria. The results of BLAST analysis of the endophytic bacterial isolates TH9, TH10, and TH11 can be seen in Table 4.

Homology analysis through the BLAST program provided information on similarities between the isolates tested and the data available at the NCBI Genbank. Based on table 4., it can be seen that the three isolates came from different genera and species. TH9 has 99.86% homology with Bacillus velezensis strain NM374, TH10 isolate has 99.93% homology with Stutzerimonas stutzeri strain CCUG 11256 and TH11 isolate has 99.93% homology with Staphylococcus warneri strain CG10.

According to Stackebrandt and Goebel (1994), in the samples of microorganisms using 16S rRNA markers, they were said to be identical (similar) at the species level if the "percentage identity" value was above 97.5%, and at the genus level if the "percentage identity" value was above 95%. This indicates that the 16S rRNA gene marker used was considered capable of identifying endophytic bacterial isolates of fig

fruit down to the species level. Then a phylogenetic tree analysis was carried out to see which bacteria might have a relation with the isolates TH9, TH10 and TH11.

The construction of the phylogenetic tree was based on the alignment of the 16S rRNA gene sequences which were similar to isolates TH9, TH10 and TH11. These isolates were known from the results of homology analysis through the BLAST program. Phylogenetic analysis was performed using the MEGA-X application through Multiple Sequence Alignment in the ClustalW program.

Multiple Sequence Alignment was done by bootstrap 1000 repetitions. The distance matrix was obtained based on the differences in nucleotide sequences for each species. These values were used to construct a phylogenetic tree. The phylogenetic tree aimed to show the relation from each species based on the molecular characteristics between species and between strains within the same species. The phylogenetic tree can be seen in Figure 5.

Based on Figure 5, it could be seen that the TH9 isolate was a type of bacteria from the genus *Bacillus* and had a relation with the species *Bacillus subtilis* and *Bacillus amyloliquefaciens*. TH11 isolate came from the genus *Staphylococcus* and was related to S. warneri strain BPB1. TH10 isolate was a bacterium from the genus *Stutzerimonas* and had a relation with the bacterium *Pseudomonas stutzeri* ATCC.

Based on PCR amplification and sequence analysis with the 16S rRNA gene, each isolate had a sequence length of 1422, 1410, and 1427 base pairs. The three isolates had a number of base pairs of 1500 bp, which would then be aligned with the 16S rRNA gene sequence to determine the type of species and their relation. Based on the results of sequencing, BLAST, and phylogenetic analysis, it was found that the three isolates came from different genera.

As seen in Table 4, it was found that the TH9 isolate had a 99.86% similarity with the Bacillus velezensis strain NM374. This is in accordance with research conducted by Abid *et al*. 2022. They succeeded in isolating nine bacterial strains from dried figs, and the genus Bacillus was found to be the most frequently detected. Among them, isolate IC1 has 100% homology with Bacillus australimaris, isolate IC4 has 99% homology with Bacillus subtilis IAM 12118, and isolate DC7 has 100% homology with Bacillus bataviensis NBRC. Ye, M. et al. (2018) have investigated that Bacillus velezensis had the potential to inhibit pathogenic fungi and bacteria and became a biocontrol agent. At present, several dominant Bacillus strains, such as B. velezensis, have been introduced into biopesticide applications for plant diseases and registered as biological fungicides abroad for the control of powdery mildew, gray mildew, sheath blight, sclerotia, and late blight.[22]

The TH10 isolate has a 99.93% similarity with *Stutzerimonas stutzeri* strain CCUG 11256 and has a very close relation with the genus *Pseudomonas* (Figure 5). This is in accordance with Gomila *et al*. (2022) who stated that the genus *Stutzerimonas* was recently proposed within the family Pseudomonas and includes species previously ascribed to the genus *Pseudomonas* in the phylogenetic group of *Pseudomonas stutzeri*, known as the *P. stutzeri* complex[23]. The genus *Pseudomonas* is an endophytic bacteria that found in almost all plant samples. This is because this bacterium is easy to grow and has potential as a biocontrol agent[24]. *Pseudomonas* is known as an antagonistic bacteria because it produces secondary metabolites in the form of antibiotics against plant pathogenic

Conclusion

Based on the results of the study, it can be concluded that 27 isolates of endophytic bacteria can be isolated from fig or fig plants (*Ficus carica* L.) The TH10 isolate is the most potential isolate to produce antibacterial activity against two types of MDRO bacteria, each of which produced an inhibition zone against *K. pneumoniae* (2.5 mm) and *S. lugdunensis* (2.65 mm). The other two isolates selected were TH9 and TH11. From the results of morphological and molecular characteristics, it was found that TH10 isolate had similarity with *Stutzerimonas stutzeri* CCUG strain (99.93%), TH9 isolate (99.86%) with *Bacillus velezensis* strain NM374 and TH11 isolate (99.93%) with *Staphylococcus warneri* strain CG10. Hopefully, this research can be continued in subsequent studies detailing the biochemical activity and enzymatic activity capabilities of the endophytic fig bacteria that have been isolated, so that the implementation can be carried out in a wider field of science.

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.

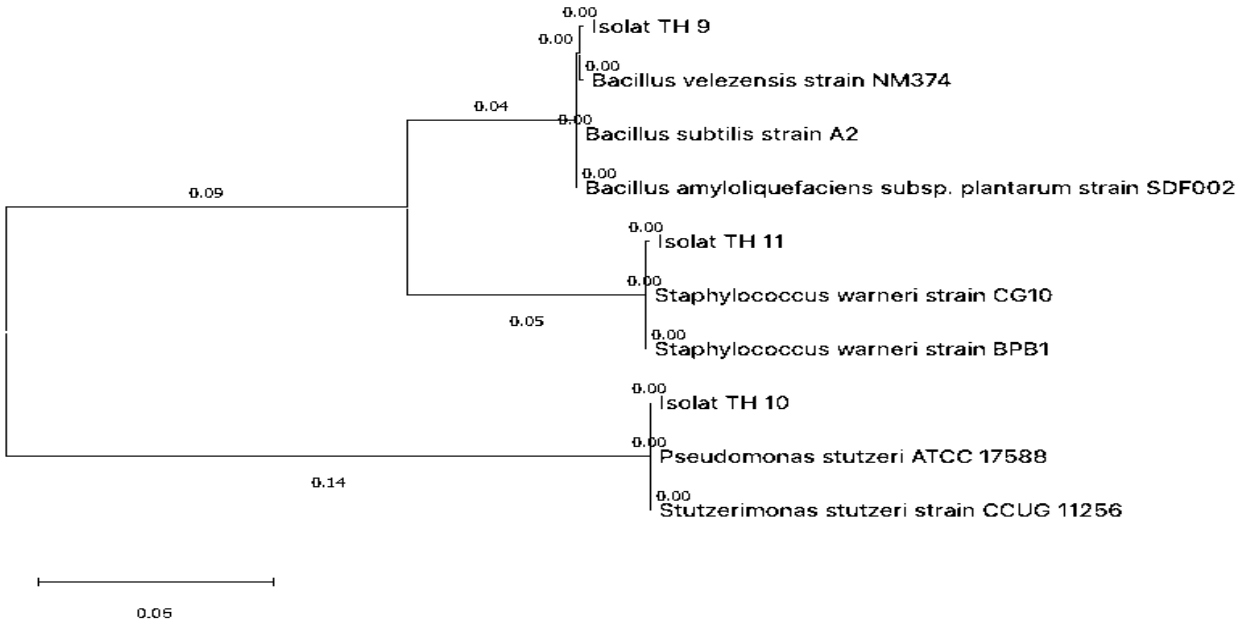

Figure 3: Results of phylogenetic analysis of potential isolates of fig endophytic bacteria (*Ficus carica* L.)

Table 4: Sequence of TH9, TH10 dan TH11

Code	Sequens
TH9	Sequences Assembly 1422 bp 1 GCAAGTCGAG CGGACAGATG GGAGCTTGCT CCCTGATGTT AGCGGCGGAC GGGTGAGTAA 61 CACGTGGGTA ACCTGCCTGT AAGACTGGGA TAACTCCGGG AAACCGGGGC TAATACCGGA 121 TGGTTGTTTG AACCGCATGG TTCAGACATA AAAGGTGGCT TCGGCTACCA CTTACAGATG 181 GACCCGCGGC GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCGAC GATGCGTAGC 241 CGACCTGAGA GGGTGATCGG CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG 301 GCAGCAGTAG GGAATTTTTC CGCAATGGAC GAAAGTCTGA CGGAGCAACG CCGCGTGAGT 361 GATGAAGGTT TTCGGATCGT AAAGCTCTGT TGTTAGGGAA GAACAAGTGC CGTTCAAATA 421 GGGCGGCACC TTGACGGTAC CTAACCAGAA AGCCACGGCT AACTACGTGC CAGCAGCCGC 481 GGTAATACGT AGGTGGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGGGC TCGCAGGCGG 541 TTTCTTAAGT CTGATGTGAA AGCCCCCGGC TCAACCGGGG AGGGTCATTG GAAACTGGGG 601 AACTTGAGTG CAGAAGAGGA GAGTGGAATT CCACGTGTAG CGGTGAAATG CGTAGAGATG 661 TGGAGGAACA CCAGTGGCGA AGGCGACTCT CTGGTCTGTA ACTGACGCTG AGGAGCGAAA 721 GCGTGGGGAG CGAACAGGAT TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGAGTGCTA 781 AGTGTTAGGG GGTTTCCGCC CCTTAGTGCT GCAGCTAACG CATTAAGCAC TCCGCCTGGG 841 GAGTACGGTC GCAAGACTGA AACTCAAAGG AATTGACGGG GGCCCGCACA AGCGGTGGAG 901 CATGTGGTTT AATTCGAAGC AACGCGAAGA ACCTTACCAG GTCTTGACAT CCTCTGACAA 961 TCCTAGAGAT AGGACGTCCC CTTCGGGGGG AGAGTGACAG GTGGTGCATG GTTGTCGTCA 1021 GCTCGTGTCG TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTTG ATCTTAGTTG 1081 CCAGCATTCA GTTGGGCACT CTAAGGTGAC TGCCGGTGAC AAACCGGAGG AAGGTGGGGA 1141 TGACGTCAAA TCATCATGCC CCTTATGACC TGGGCTACAC ACGTGCTACA ATGGACAGAA 1201 CAAAGGGCAG CGAAACCGCG AGGTTAAGCC AATCCCACAA ATCTGTTCTC AGTTCGGATC 1261 GCAGTCTGCA ACTCGACTGC GTGAAGCTGG AATCGCTAGT AATCGCGGAT CAGCATGCCG

	1321 CGGTGAATAC GTTCCCGGGC CTTGTACACA CCGCCCGTCA CACCACGAGA GTTTGTAACA 1381 CCCGAAGTCG GTGAGGTAAC CTTTATGAGC CAGCCGCCGA AG
TH10	Sequences Assembly 1410 bp 1 GCAAGTCGAG CGGATGAGTG GAGCTTGCTC CATGATTCAG CGGCGGACGG GTGAGTAATG 61 CCTAGGAATC TGCCTGGTAG TGGGGGACAA CGTTTCGAAA GGAACGCTAA TACCGCATAC 121 GTCCTACGGG AGAAAGTGGG GGATCTTCGG ACCTCACGCT ATCAGATGAG CCTAGGTCGG 181 ATTAGCTAGT TGGTGAGGTA AAGGCTCACC AAGGCGACGA TCCGTAACTG GTCTGAGAGG 241 ATGATCAGTC ACACTGGAAC TGAGACACGG TCCAGACTCC TACGGGAGGC AGCAGTGGGG 301 AATATTGGAC AATGGGCGAA AGCCTGATCC AGCCATGCCG CGTGTGTGAA GAAGGTCTTC 361 GGATTGTAAA GCACTTTAAG TTGGGAGGAA GGGCAGTAAG TTAATACCTT GCTGTTTTGA 421 CGTTACCAAC AGAATAAGCA CCGGCTAACT TCGTGCCAGC AGCCGCGGTA ATACGAAGGG 481 TGCAAGCGTT AATCGGAATT ACTGGGCGTA AAGCGCGCGT AGGTGGTTCG TTAAGTTGGA 541 TGTGAAAGCC CCGGGCTCAA CCTGGGAACT GCATCCAAAA CTGGCGAGCT AGAGTATGGC 601 AGAGGGTGGT GGAATTTCCT GTGTAGCGGT GAAATGCGTA GATATAGGAA GGAACACCAG 661 TGGCGAAGGC GACCACCTGG GCTAATACTG ACACTGAGGT GCGAAAGCGT GGGGAGCAAA 721 CAGGATTAGA TACCCTGGTA GTCCACGCCG TAAACGATGT CGACTAGCCG TTGGGATCCT 781 TGAGATCTTA GTGGCGCAGC TAACGCATTA AGTCGACCGC CTGGGGAGTA CGGCCGCAAG 841 GTTAAAACTC AAATGAATTG ACGGGGGCCC GCACAAGCGG TGGAGCATGT GGTTTAATTC 901 GAAGCAACGC GAAGAACCTT ACCAGGCCTT GACATGCAGA GAACTTTCCA GAGATGGATT 961 GGTGCCTTCG GGAACTCTGA CACAGGTGCT GCATGGCTGT CGTCAGCTCG TGTCGTGAGA 1021 TGTTGGGTTA AGTCCCGTAA CGAGCGCAAC CCTTGTCCTT AGTTACCAGC ACGTTAAGGT 1081 GGGCACTCTA AGGAGACTGC CGGTGACAAA CCGGAGGAAG GTGGGGATGA CGTCAAGTCA 1141 TCATGGCCCT TACGGCCTGG GCTACACACG TGCTACAATG GTCGGTACAA AGGGTTGCCA 1201 AGCCGCGAGG TGGAGCTAAT CCCATAAAAC CGATCGTAGT CCGGATCGCA GTCTGCAACT 1261 CGACTGCGTG AAGTCGGAAT CGCTAGTAAT CGTGAATCAG AATGTCACGG TGAATACGTT 1321 CCCGGGCCTT GTACACACCG CCCGTCACAC CATGGGAGTG GGTTGCTCCA GAAGTAGCTA

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

	1381 GTCTAACCTT CGGGGGACGG TTACCACGGA
TH11	Sequences Assembly 1427 bp 1 CAAGTCGAGC GAACAGATAA GGAGCTTGCT CCTTTGACGT TAGCGGCGGA CGGGTGAGTA 61 ACACGTGGAT AACCTACCTA TAAGACTGGG ATAACTTCGG GAAACCGGAG CTAATACCGG 121 ATAACATATT GAACCGCATG GTTCAATAGT GAAAGGCGGC TTTGCTGTCA CTTATAGATG 181 GATCCGCGCC GTATTAGCTA GTTGGTAAGG TAACGGCTTA CCAAGGCAAC GATACGTAGC 241 CGACCTGAGA GGGTGATCGG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG 301 GCAGCAGTAG GGAATCTTCC GCAATGGGCG AAAGCCTGAC GGAGCAACGC CGCGTGAGTG 361 ATGAAGGTCT TCGGATCGTA AAATTCTGTT ATCAGGGAAG AACAAATGTG TAAGTAACTG 421 TGCACATCTT GACGGTACCT GATCAGAAAG CCACGGCTAA CTACGTGCCA GCAGCCGCGG 481 TAATACGTAG GTGGCAAGCG TTATCCGGAA TTATTGGGCG TAAAGCGCGC GTAGGCGGTT 541 TTTTAAGTCT GATGTGAAAG CCCACGGCTC AACCGTGGAG GGTCATTGGA AACTGGAAAA 601 CTTGAGTGCA GAAGAGGAAA GTGGAATTCC ATGTGTAGCG GTGAAATGCG CAGAGATATG 661 GAGGAACACC AGTGGCGAAG GCGACTTTCT GGTCTGTAAC TGACGCTGAT GTGCGAAAGC 721 GTGGGGATCA AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GAGTGCTAAG 781 TGTTAGGGGG TTTCCGCCCC TTAGTGCTGC AGCTAACGCA TTAAGCACTC CGCCTGGGGA 841 GTACGACCGC AAGGTTGAAA CTCAAAGGAA TTGACGGGGA CCCGCACAAG CGGTGGAGCA 901 TGTGGTTTAA TTCGAAGCAA CGCGAAGAAC CTTACCAAAT CTTGACATCC TTTGACCGCT 961 CTAGAGATAG AGTTTTCCCC TTCGGGGGAC AAAGTGACAG GTGGTGCATG GTTGTCGTCA 1021 GCTCGTGTCG TGAGATGTTG GGTTAAGTCC CGCAACGAGC GCAACCCTTA AGCTTAGTTG 1081 CCATCATTAA GTTGGGCACT CTAAGTTGAC TGCCGGTGAC AAACCGGAGG AAGGTGGGGA 1141 TGACGTCAAA TCATCATGCC CCTTATGATT TGGGCTACAC ACGTGCTACA ATGGACAATA 1201 CAAAGGGCAG CTAAACCGCG AGGTCAAGCA AATCCCATAA AGTTGTTCTC AGTTCGGATT 1261 GTAGTCTGCA ACTCGACTAC ATGAAGCTGG AATCGCTAGT AATCGTAGAT CAGCATGCTA 1321 CGGTGAATAC GTTCCCGGGT CTTGTACACA CCGCCCGTCA CACCACGAGA GTTTGTAACA 1381 CCCGAAGCCG GTGGAGTAAC CATTTATGGA GCTAGCCGTC GAAGGTG

References

1. Nwinyi O, Chinedu SN, Ajani, Olayinka O, Chinwe IO, Ogunniran KO. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineens* on *Escherichia coli* and *Staphylococcus aureus*. Afric J. F Sci. 2009; 3 (3):22-25.
2. World Health Organization. "Epidemic-Prone and Pandemic-Prone Acute Respiratory Diseases: Infection Prevention and Control in Health-Care Facilities. Who. Indonesia Partner in Development. 2008; 53 (2):8–25.
3. Bacon CW, Hinton D. Endophytes: the endophytic Niche, its occupants and its utility. in plant-associated bacteria. Springer. Dordrecht. 2007; 155-194
4. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003; 67(4):491-502.
5. Tan R X, Zou WX. Endophytes: a rich source of functional metabolites. Nat Prod Rep. 2001; 18(4):448-459.
6. Aristina RF, Astuti W, Pratiwi DR. Screening and phytochemicals test of extract endhophytes bacteria from stem of pacing (*Costus* sp.). J Atomik. 2019; 4(1):21-24.
7. Aryani P, Kusdiyantini E, Suprihadi, A. Isolasi bakteri endofit daun alang-alang (*Imperata cylindrica*) dan metabolit sekundernya yang berpotensi sebagai antibakteri. J Akad Biol. 2020;*9*(2):20-28.
8. Joseph B and Raj SJ. Pharmacognostic and phytochemical properties of *Ficus carica* Linn–an overview. Inter J pharmtech research. 2011;*3*(1):8-12.
9. Ramadhan MR, Pratiwi IDP K, Arihanta NMIH. Uji daya hambat ekstrak buah Tin (*Ficus racemosa* Linn) terhadap pertumbuhan *Staphylococcus aureus* ATCC 25923. J Ilm dan Teknol Pang. 2020;9(1):38-45
10. Qomaruddin M, Riana D, Anton A. Segmentasi K-means citra daun tin dengan klasifikasi ciri gray level Co occurance matrix. J Sist dan Tekn Inform. 2021; 9(2):223-233.
11. Rau CH, Yudistira A, Simbala HEI. Isolasi, identifikasi secara molekuler menggunakan Gen 16s rRNA dan uji aktivitas antibakteri dari bakteri endofit alga *Padina* sp. Pharmacon, 2018; *7*(2):53–61.
12. Alvarado-Marchena L, Schmidt-Durán A, Alvarado-Ulloa C, Chacón-Cerdas R, Flores-Mora D. Molecular characterization of the endophytic bacteria found in the fig crops (*Ficus carica* var. Brown Turkey) in Costa Rica. J Agric Biol Sci. 2016; 11(7):290-297.
13. Linelejan YT, Umboh SD, Tallei TE. Identifikasi bakteri endofit daun *Ficus Minahassae* Miq. berdasarkan gen 16s rRNA. J. MIPA Unsrat Online. 2018;7(2):16-19
14. Leonita S, Bintang M, Pasaribu FH. Isolation and identification of endophytic bacteria from *Ficus variegata* blume as antibacterial compounds producer. J Cur Biochem. 2016;*2*(3):116-128.
15. Oktiansyah, R.E. Elfita, H. Widjajanti, A. Setiawan, M. Mardiyanto, and Nasution SS. Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from The Leaves of Sungkai (*Peronema canescens*): Trop J. of Nat Prod Res. 2023; 7(3): 2596-2600
16. Jilali, S. B., I. Rachid, B. Ghada, M. Tarik, R. Sanae, and K. Abderrazzak. Effect of Isolation Techniques on the Quantity, Quality, and Antimicrobial Activity of Lavandula Dentata Essential Oils:. Trop J. of Nat Prod Res. 2023; 7(4):2713-2717.
17. Ismail YS, Yulvizar C, Putriani P. Isolasi, karakterisasi dan uji aktivitas antimikroba bakteri asam laktat dari fermentasi biji kakao (*Theobroma cacao* L.). J Bioleuser. 2017;1(2).
18. Jabeen R , Iftikhar T, Batool H. Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. oryzae causing BLB disease in rice. Pak J. Bot. 2012; 44(1):261-265.
19. Oktavia N, dan Pujiyanto S. Isolasi dan uji antagonisme bakteri endofit tapak dara (*Catharanthus roseus* L.) terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus.* J. Berk Bioteknol. 2018; 1(1): 6-12.
20. Hallman J, Hallmann AQ, Mahaffee W, Kloepper JW. Bacterial endophytes in agricultural crops. Can J. Microbiol. 1997; 43: 895-914.
21. Purwanto UMS, Fachriyan HP, Maria B. Isolasi bakteri endofit dari tanaman sirih hijau (*Piper betle* L.) dan potensinya sebagai penghasil senyawa antibakteri. J. Cur Biochem. 2014;1(1): 51-57.
22. Nam MH, Park MS, Kim HG, Yoo SJ. Biological control of strawberry Fusarium wilt caused by *Fusarium oxysporum* f. sp. fragariae using *Bacillus velezensis* BS87 and RK1 formulation. J. Microbiol Biotechnol. 2009;19:520-524.
23. Gomila M, Mulet M, García Valdés E, Lalucat J. Genome-based taxonomy of the genus Stutzerimonas and proposal of *S. frequens* sp. nov. and *S. degradans* sp. nov. and Emended descriptions of *S. perfectomarina* and S. *chloritidismutans*. Microorganisms, 2022; *10*(7):1363.
24. Miller KI, Qing C, Sze DM, Roufogalis BD, Neilan BA. Culturable endophytes of medicinal plants and the genetic basis for their bioactivity. Microb Ecol. 2012; 64: 431-449