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Original Research Article

Metagenomic Analysis of Fig (*Ficus carica* L.) Endophytic Bacteria as a Source of Flavonoids

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ABSTRACT

The improper usage of antibacterials causes pathogenic bacteria to develop resistance to antibacterials. Secondary metabolites represent an alternative in the look for potential antibacterial bioactive compounds. Figs, as a source of flavonoids, exhibit antibacterial action owing to the presence of endophytic bacteria that inhabit the internal tissues of fig plants. Researching the diversity of microorganisms, including endophytic bacteria, is challenging because 99 percent of environmental microorganisms are not cultivable. For this reason, metagenomics—an analysis that can examine the diversity of endophytic bacteria without cultivation—is required. This study aimed to assess the diversity of endophytic bacteria with potential antibiotic activity using a metagenomic approach. The research methodology comprises metagenomic analysis using protocols for DNA extraction from fig tissues, amplification of the 16s rRNA gene, electrophoresis, Next Generation Sequencing, and phylogenetic tree construction. The acquired data will be descriptively interpreted and analyzed via the QIIME Operational Taxonomic Unit software to yield results in the form of fig endophytic bacterial species data. The species-level diversity of endophytic bacteria identified in the Iraqi and Blue Giant types of figs (*Ficus carica* L.) includes *Weissella ghanensis*, *Weissella paramesenteroides*, *Ralstonia pickettii*, *Leuconostoc citreum*, *Pantoea stewartii*, *Gluconobacter cerinus*, and *Lactococcus lactis*. This study's results demonstrate that the metagenomic method utilizing the 16S rRNA gene can identify endophytic bacteria in figs, offering the expanded potential for discovering beneficial chemicals from natural sources to combat antibacterial resistance.

***Keywords*:** Fig, Endophytic Bacteria, antibacterial, Flavonoid, Metagenomic

Introduction

Infectious diseases caused by bacteria are one of the main causes of death worldwide, making them a serious problem that must be handled[1]. Treatment of infections caused by bacteria is usually carried out by administering antibacterial agent drug therapy. Inappropriate use of antibacterials currently causes the problem of pathogenic bacteria's resistance to antibacterials. Resistance is the level of susceptibility of bacteria to antibacterial compounds, which are classified as susceptible, intermediate, or resistant. The rapidly growing case of bacterial resistance currently demands the discovery of natural ingredients with antibacterial activity. Secondary metabolites are one answer in the search for potential antibacterial bioactive compounds.
Flavonoids are the phenol group's largest secondary metabolites, which have antibacterial properties[2]. The mechanism of flavonoid antibacterial activity is associated with its chemical structure and ability to influence bacterial cell membranes[3]. Chalcone synthase (CHS) is a key gene in the formation and biosynthesis of flavonoids[4].

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Flavonoid biosynthesis occurs through the phenylpropanoid pathway, where phenylalanine is converted into 4-coumaroyl-CoA as the main precursor of the flavonoid formation pathway, where all flavonoids are derivatives of chalcone[5]. Research conducted by[6] revealed that testing the antibacterial activity of chalcone using the MIC method was proven to be able to inhibit the growth of Staphylococcus aureus and Escherichia coli bacteria, making it potential as an antibacterial agent. Medicinal plants are an alternative natural ingredient in searching for potential antibacterial compounds, especially Chalcone synthase, in forming flavonoids.
A medicinal plant that can grow in Indonesia but is still not widely used by the public is fig (*Ficus carica* L.) from the Moraceae family. This plant can grow and be cultivated in subtropical and tropical areas. In Indonesia, several varieties of figs can grow well and reproduce, such as red Israeli, brown Turkish, panache, green Jordan, purple Jordan, and conadria[7]. The prospect of demand for fig fruit plants will continue to increase in the future because of their benefits in the health sector, so they are widely sought after by the public even though the price is still relatively high[8]. Figs have been used worldwide to treat various diseases caused by bacteria. Fig fruit extract contains high amounts of phenolic compounds, especially flavonoids, which have potential as antibacterial drug formulations because they can inhibit the growth of pathogenic bacteria[9-10].The various health benefits of figs, such as antibacterial activity, cannot be separated from the presence of living microorganisms called endophytic bacteria in the plant tissue.
Endophytic bacteria are beneficial microorganisms without causing external disturbance or damage when interacting with plants[11]. Endophytic bacteria can produce the same bioactive compounds as their host plants[12], so there is no need to take the host plant, which takes a

long time to plant. In recent years, research on endophytic bacteria has attracted world attention because of their ability to produce the same or even more secondary metabolites than their hosts. Modern genomic tools have revealed the interactions of plant and bacterial endophytes that act as reservoirs of secondary metabolites of flavonoids, tetralones, tanones, quinones, steroids, and other subclasses[13]. The use of endophytic bacteria in searching for potential antibacterial bioactive compounds is based on the ease of molecular multiplication, limited sample efficiency, or relatively expensive prices.

Endophytic bacteria can obtain the transfer of entire gene clusters from the host plant through Horizontal Gene Transfer (HTG), which allows endophytic bacteria to be able to synthesize bioactive compounds originally from the host plant[14]. The research results[15] identified 17 genes annotated as Chalcone synthase genes in flavonoid synthesis with the help of genes originating from endophytic bacteria and fungi in Ginkgo biloba roots. Research on endophytic bacteria found in figs has been reported by [16], who determined that the endophytic bacteria obtained were Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Firmicutes, and Bacteriodetes. As it is already known that fig plants (*Ficus carica* L.) have antibacterial potential, the endophytic bacteria that reside on figs also have the same ability as their host plants in synthesizing antibacterial compounds.

Studying the diversity of microorganisms, including endophytic bacteria, is not easy because 99% of microorganisms in the environment are types that cannot be cultured in the laboratory, some of which even grow very slowly for years [17]. Therefore, analysis is needed to study the diversity of endophytic bacteria without cultivation, known as metagenomics. This modern technique can be a solution for studying culturable and unculturable bacterial communities. The metagenomic principle is based on direct DNA analysis of plant samples and then identified using a phylogenetic marker gene, namely 16s rRNA [18-19]. Metagenomic study uses Next Generation Sequencing (NGS), the latest informatics-based tracking technology, which provides information more effectively and efficiently in genome mapping, annotation (naming), gene identification sequences, and interactions between genes[20]. Based on this, this research aims to identify the diversity of endophytic bacteria that have the potential to act as antibacterials in fig fruit plants using metagenomic analysis based on the 16s rRNA.

Materials and Methods

Collection and Preparation of Figs (Ficus carica L.*)*

Samples of figs fruit variety red Israeli were obtained from Helvetia District, Medan City, North Sumatra. Sample was submitted to the Herbarium Bandungense, Institute Technology Bandung of Indonesia, Bandung for its identification by Arifin Surya Dwipa, Ph.D, and the voucher specimen was 3750/II.CO2.2/PL/2022. Samples were selected considering their fresh condition, characterized by a dark red color, a texture that was not too soft, and fruit stalks that were not hard. The figs chosen are fresh fruit because the plant tissue has a relatively high nucleic acid content. The sample surface was sterilized using sterile water once rinsed and then washed using 75% ethanol for 30 seconds. Then, the sample was dried using absorbent paper. Prepare an ice box, fill it with aluminum foil on the bottom surface, fill it with ice gel on the second layer, cover it with bubble wrap, and put the fig samples into the ice box. After that, bubble wrap and aluminum foil were placed on top of the sample, and ice gel was added and then covered with aluminum foil. Then, the ice box is packed with extra bubble wrap. The samples were then sent to Genetic Science Indonesia for the following research stage.

Sample preparation

The fig's surface was sterilized with absolute ethanol for one minute. Remove the fig skin using a sterile scalpel. A small portion of the fruit inside (flesh and seeds) was cut to as much as 150 mg for DNA extraction. Then, the sample was ground with liquid nitrogen, and the DNA extraction steps were continued.

DNA Extraction

The ground samples were placed into ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm). Then 750 µl of ZymoBIOMICS™ lysis solution was added to the tube and closed tightly. The tube was inserted into a beat beater equipped with a 2 ml tube holder assembly and processed using MP Fastprep®-24 for 1 minute at maximum speed and 5 minutes rest. The cycle is repeated five times. ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) were then centrifuged in a microcentrifuge machine at a speed of ≥ 10,000 x g for 1 minute. Then 400 µl of the supernatant was transferred into a collection tube with a Zymo-Spin ™ III-F Filter and centrifuged at 8,000 x g for 1 minute. The Zymo-Spin™ III-F filter was then discarded. Add 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer to the filtrate in the Collection Tube, then stir thoroughly. Transferred 800 µl of the mixture from the previous step to the IICR Zymo-Spin™ column tube in a collection tube and centrifuged at 10,000 x g for 1 minute. Then, discard the flow from the Collection Tube and repeat the steps.

Then, add 400 µl of ZymoBIOMICS ™ DNA Wash Buffer to the ZymoSpin ™ IICR Column in a new collection tube and centrifuge at 10,000 x g for 1 minute, then discard to the flowing stream. Added 700 µl of ZymoBIOMICS™ DNA Wash Buffer II to the ZymoSpin™ IICR Column in the Collection Tube and centrifuged at 10,000 x g for 1 minute. Add 200 µl of ZymoBIOMICS™ DNA Wash Buffer II to the ZymoSpin™ IICR Column in the Collection Tube and centrifuge at a speed of 10,000 x g for 1 minute. Transfer the IICR ZymoSpin™ Column to a clean 1.5 ml microcentrifuge tube and add 50 µl ZymoBIO MICS™ DNase/RNase Free Water directly to the column matrix and incubate for 1 minute, then centrifuge at 10,000 x g for 1 minute to elute the DNA. Place the Zymo-Spin™ III-HRC Filter in a new Collection Tube and add 600 µl of ZymoBIOMICS™ HRC Prep Solution, then centrifuge at 8,000 x g for 3 minutes. Transfer the eluted DNA into the Zymo-Spin™ III-HRC Filter, prepared in a clean 1.5 ml microcentrifuge tube and centrifuge at 16,000 x g for 3 minutes. The filtered DNA is now ready for PCR and other applications.

Amplification of the 16S rRNA Gene

The DNA extraction results from figs were amplified using the Polymerase Chain Reaction (PCR) method of 16s rRNA specific for Hypervariable V3-V4 bacteria primers 341Fn(CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT). PCR reaction using PCR Product with primer 16S V3-V4 using (2x) My TaqHS Red Mix Bioline (BIO-25048). Amplification was carried out with a 50 µL reaction consisting of 3 µL template DNA, one µL of each primer (forward and reverse), 25 µL MyTaq HS Red Mix, and 20 µL ddH2O. The stages of the amplification cycle are as follows: predenaturation is carried out for 1 minute at 95°C, denaturation for 15 seconds at 95°C, annealing for 15 seconds at 55°C, and extension for 10 seconds at 72°C. This process was carried out for 35 cycles and ended with a final extension for 5 minutes at 72°C.

Electrophoresis

Electrophoresis aims to see the amplification results in the form of DNA bands (bands) for further analysis. 1x loading buffer (containing agarose / green gel) is mixed with the PCR product and then detected using a 1% agarose gel for electrophoresis. Samples in the 400 bp bright band were selected and mixed at the same density ratio and purified using the ZymoBIOMICSTM Gel Electrophoresis Extraction Kit. The results were then sent to Novogen China for sequencing using the Illumina HiSeq 2500 (Illumina, San Diego, California, AS).

Next Generation Sequencing

Sequencing using an automatic DNA sequencing machine aims to determine the nucleotide sequence of DNA fragments detected from the visualization of DNA amplified in the PCR process. The sequencing process was carried out at Novogene Beijing, China. After getting the bacterial DNA sequencing results, the DNA sequencing results were then analyzed using bioinformatic tools (bioedit) and matched with the data at www.ncbi.nih.nlm.gov via the Basic Local Alignment Search Tool (BLAST) program to look for similarities in the nucleotide sequence of the 16S rRNA gene in determine the molecular characterization of bacteria related to the synthesis of bioactive compounds using 16S rRNA markers.

Phylogenetic Tree Construction
Phylogenetic tree construction using the Molecular Evolutionary Genetics Analysis (MEGA) program version 11.0 Neighbor-Jointing Method.

Data Analysis
Data from Next Generation Sequencing using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following recommendations and additional index code. Library quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The amplicon on the Illumina HiSeq2500 produces raw data with special processing, namely, Paired-end reading with unique barcodes and primers cut, then paired-ends are combined using FLASH V1.2.7 analysis tools, which are fast and accurate for combining DNA, the DNA splicing sequence obtained is called with raw data (http://ccb.jhu.edu/software/FLASH/). Filtering is performed on the raw data using specific filtering conditions of the QIIME (V1.7.0 http://qiime.org/index.html) quality control process. After that, it is compared with a reference database (Gold database, http://drive5.com/uchime/uchime_download.html UCHIME algorithm (UCHIME Algorithm http://www.drive5.com/usearch/manual/uchime_algo.html to detect chimeric sequences then The chimeric sequences are removed to obtain clean sequence data.
Operational Taxonomic Unit analysis using UPARSE-OTU and UPARSE-OTUref algorithm software. Sequences with similarity ≥ 97% and representative of each OTU classifier for annotation of taxonomic information. Observed Alpha Diversity, Chao1 estimates species abundance, Shannon and Simpson index. The data obtained will be interpreted and analyzed descriptively.

Results and Discussion

DNA Extraction Figs (Ficus carica L.)
The DNA extraction process led to the acquisition of both quantitative and qualitative data. The concentration value of the DNA extraction was expressed through quantitative data, while qualitative data was used to indicate the purity value of the extracted DNA. As per the findings in Table 2, the Iraki variety of figs had the highest concentration value of 8 ng/µL in this study, and its DNA extraction had the best purity value. The purity value of DNA extraction results was determined by examining the nanospectrometer's absorbance at the A260/280 wavelength. Wardoyo et al. (2020) have reported good purity results in the range of 1.7-2.0. Based on the purity values obtained in this investigation, it can be established that they exceeded the typical range of values for DNA purity. Contaminants such as protein, phenol, triazole, guanidine thiocyanate, and guanidine HCL have been identified as potential causes.atoms. The ^{1}H NMR spectrum of compound 1 revealed signals for phenolic compound, with three doublets of aromatic proton signals at δ = 7.59, 6.87, and 7.59 ppm, identified as H-2, H-3, and H-6, respectively. Furthermore, the ^{1}H NMR spectra showed the distinctive signal at δ = 3.89 ppm for a methoxy group. The above-mentioned data and published literature [19] confirmed compound 1 as vanillic acid.
Compound 2 exhibited phenolic compound indications in its ^{1}H NMR spectra, with three doublets of aromatic proton signals at δ = 7.60, 6.99, and 7.72 for H-2, H-3, and H-6, respectively and a typical signal at δ = 3.98 ppm for a methoxy group. Based on the aforementioned data and comparison with the literature,[20] compound 2 was identified as isovanillic acid, an isomer of vanillic acid.

PCR Amplification and Electrophoresis
The 16S rRNA V3-V4 primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATTCTAAT) (Table 1) were explicitly used for polymerase chain reaction amplification. The visualization results acquired using an automated capillary electrophoresis device on both samples showed that PCR product size values were between 400 and 500 bp (Figure 1). This PCR product's size conforms to the predicted range of the 16S rRNA gene region V3 - V4. Next Generation Sequencing (NGS) is used to sequence the data from this PCR product.

Next-Generation Sequencing Results
The amplicons were subjected to sequencing using an Illumina HiSeq2500 paired-end platform, generating paired-end raw reads (Raw PE). These raw reads were subsequently merged and subjected to pre-processing to get Clean Tags. The presence of chimeric sequences, which are not of high quality, is identified and subsequently eliminated from the Clean Tag. This process generates an Effective Tag that may be utilized for subsequent analytical purposes. The base (nt) refers to the fundamental numerical value of the storage capacity for Effective Tag. Tables 3 and 4 display the following summary, which was obtained during the data processing steps
For figs (*Ficus carica L.)* of the Iraqi and Blue Giant kinds, raw PE is raw paired-end data collected straight from the Illumina Hiseq2500 sequencing platform, without any processing, totaling 183501 and 185558 sequences. To create raw tags, primary base sequences and unique Illumina Hiseq2500 identifier codes for primers 341F and 806R, which add up to 182532 and 184520 sequences, are concatenated and pre-processed.
The outcome of performing quality control (QC) on the 180143 and 181954 sequences is clean data. By deleting sequencing adapters and low-quality sequences, an effective tag (no chime) is a result that works without the use of chimeric sequences. No chime may be used to analyze the 165333 and 178086 sequences using OTUs (Operational Taxonomic Units). AvgLen (nt) is the average Effective Tag sequence length of 418.50 and 406.01, corresponding to the target region V3-V4. The GC percentage is the percentage of Guanine and Cytosine contained in the sequence, which is considered very good, namely 52.57% and 55.61%. The GC content influences DNA stability because the bond between Guanine and Cytosine has three hydrogen bonds, making molecular cohesion stronger. The percentage of Q20 and Q30 is the base with a quality score equal to or higher than Q20 (error rate <1%) and Q30 (error rate <0.1%). The effective percentage is the number of effective tags obtained from the total Raw Tags of 90.10% and 95.97%.

Table 1: Primer Sequences Used

Primer	Sekuen (5'-3')	References
341F	CCTAYGGGRBGCASCAG	(Novogene, 2020)
806R	GGACTACNNGGGTATCTAAT	(Novogene, 2020)

Taxonomic Annotation of Fig Fruit Endophytic Bacteria (Ficus carica L.)
The diversity of the microbial population was examined by utilizing Operational Taxonomic Units (OTU) to classify and identify 97% of the sample's total Effective Tag sequences (142348). The OTU clustering strategy is predicated on the hypothesis that some bacteria will have target gene sequences that resemble GenBank data. Sequencing mistakes will also be reduced in cluster-level sequences or OTU data. Appendices 1 and 2 provide the 24 OTUs that were obtained from the OTU analysis using Qiime1. Information about the relative abundance of fig endophytic bacterial species at the kingdom, phylum, class, order, level, family, genus, and species was produced based on the OTU annotation results and sample characteristics.
Relative Abundance of Fig Fruit Endophytic Bacteria (*Ficus carica L.)*
A barplot representing the distribution of relative abundance of taxa was created by selecting the top 10 taxa from each taxonomic rank (Phylum, Class, Order, Family, Genus, and Species) based on the results of taxonomic annotation. This enables the proportions of taxa at various categorization levels and those with higher relative abundance to be displayed for every sample. The following illustration shows the relative abundance of taxa based on data from figures 1 and 2
Endophytic bacterial abundance in Iraqi and Blue Giant types of figs (*Ficus carica L.)* is displayed using taxonomic barplot's in Figures 1 and 2. At the Phylum level, the Iraki variation's abundance of fig endophytic bacteria is classified into four groups: Cyanobacteria,

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Firmicutes, Proteobacteria, and Actinobacteria. In contrast, the Iraqi variant lacks Actinobacteria. The Iraqi and Blue Giant types of Classis taxonomic classifications yield Cyanobacteria, Bacilli, Alphaproteobacteria, Gammaproteobacteria, Unidentified Actinobacteria, and Others. The Iraqi and Blue Giant varieties' taxonomic levels yield the same taxa: Chloroplast, Lactobacillales, Rickettsiales, Enterobacterales, Acetobacterales, Burkholderiales, and Others. The Blue Giant variety differs from the Iraqi one in that Micrococcales, Corynebacteriales, and Dongiales are present.

Both the Iraqi and Blue Giant varieties, at the family taxonomic level, yield Unidentified Chloroplast, Lactobacillaceae, Mitochondria, Erwiniaceae, Acetobacteraceae, Burkholderiaceae, and Others; however, the Blue Giant variety contains different forms of Streptococcaceae, Micrococcaceae, Corynebacteriaceae, and Dongiaceae than the Iraqi variety. Taxon level: The Iraqi and Blue Giant varieties of the same genus produce unidentified Mitochondria, Weissella, Leuconostoc, Tatumella, Acetobacter, Ralstonia, and Others. However, the Blue Giant variety differs from the Iraqi variety in terms of Pantoea, Gluconobacter, and Lactococcus. Taxon level of species: Weissella ghanensis, Weissella paramesenteroides, Ralstonia pickettii, and others are produced by both the Iraqi and Blue Giant varieties; however, Leuconostoc citreum, Pantoea stewartii, Gluconobacter cerinus, and Lactococcus lactis are present in the Blue Giant variety but not in the Iraqi variety. The phylum-level identification of cyanobacteria was achieved. The order of Micrococcales and Rickettsiales was determined. Streptococcaceae was found at the family level. Acetobacter was determined to be a specific genus. Cluster Abundance of Fig Fruit Endophytic Bacteria (*Ficus carica L.*)

To determine whether or not the samples with similarity processing grouped, a heatmap was made using the abundance data of the top 35 genera in the samples. The observational results are displayed in the picture below (Figure 3)

Table 2: Concentration and Purity Results of DNA Extraction from Figs (Ficus carica L.)

Name of Sample	Sample Code	Volume (µl)	Concentration (ng/µL)	A260/280
Figs Iraki Variety	114-1	50	8,0	3,14
Figs Blue Giant Variety	114-2	50	6,1	3,33

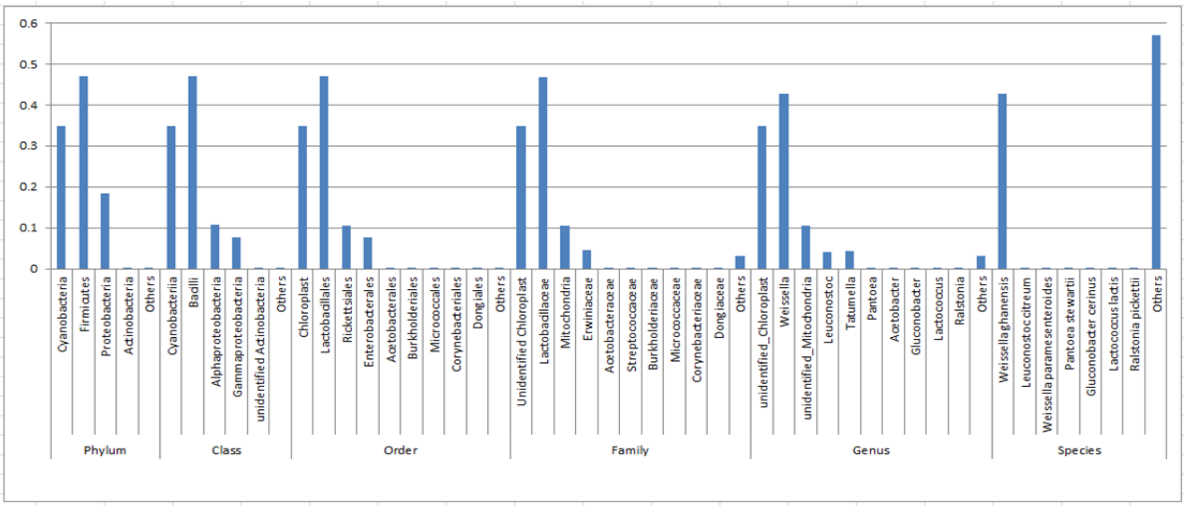

Figure 1: Relative Abundance Taxa of Endophytic Bacteria of Fig Iraqi Variety

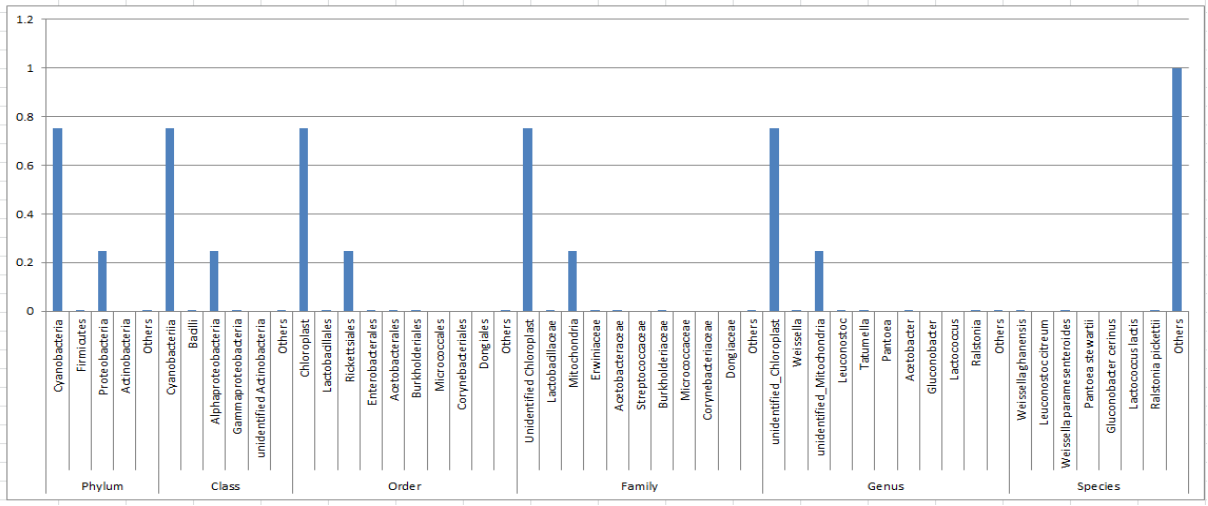

Figure 2: Relative Abundance Taxa of Endophytic Bacteria of Fig *Blue Giant* Variety

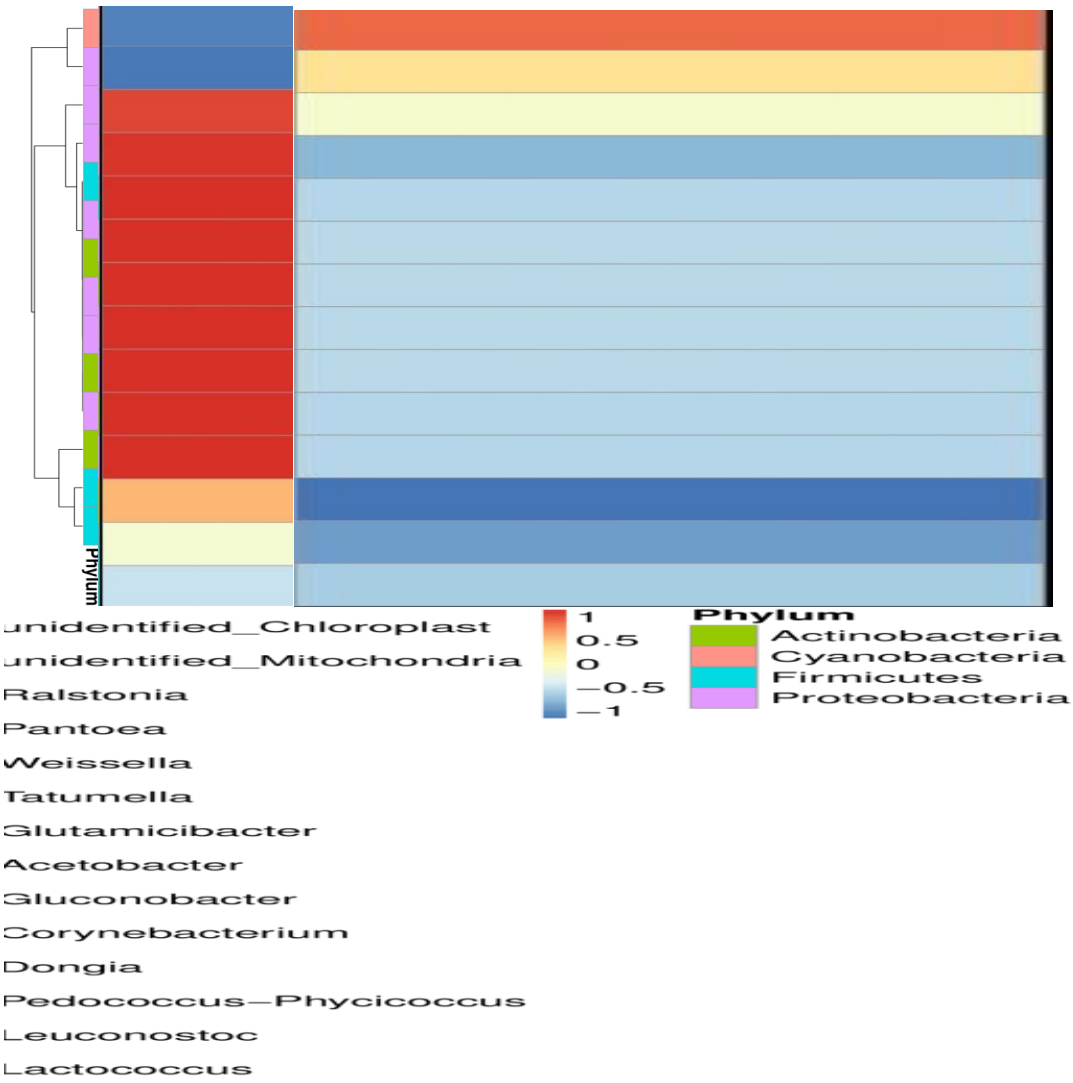

Figure 3: Heatmap of Relative Abundance of Fig Endophytic Bacteria

Table 3: NGS Statistical Data of Fig Fruit (*Ficus carica* L.) Iraqi Variety

Sample code	Raw PE	Raw Tags	Clean Tags	Effective Tag	Base (nt)	AvgLen (nt)	GC (%)	Q20 (%)	Q30 (%)	Effective %
14-1	183501	182532	180143	165333	69191448	418.50	52.57	98.61	95.22	90.10

Table 4: NGS Statistical Data of Fig Fruit (*Ficus carica* L.) Varietas *Blue Giant*

Sample code	Raw PE	Raw Tags	Clean Tags	Effective Tag	Base (nt)	AvgLen (nt)	GC (%)	Q20 (%)	Q30 (%)	Effective %
114-2	185558	184520	181954	178086	72304052	406.01	55.61	98.40	94.75	95.97

Alpha Diversity Index of Fig Fruit Endophytic Bacteria (Ficus carica L.)

By examining the diversity of one sample, one may determine the richness and diversity of the microbial community in each sample, as well as species accumulation, bar plots, and biodiversity curves. This technique is the alpha diversity study of microbial community diversity in samples. Sequence identity OTUs produced using ASV or clustering are generally considered homologous within a species. The alpha diversity statistical index can be summed up using. Table 5 showing alpha diversity index of fig fruit endophyti bacteria.

The variety of microorganisms in the sample is displayed using an abundance rank curve, also known as a rarefaction curve, which is created based on this data. The volume of sequence data that makes sense can be directly reflected in rarefaction curves. If the slope is steep,

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it indicates that many species are still undiscovered. A reasonable target number of endophytic bacteria has been reached if the curve flattens out. The variety Blue Giant (green) and Iraki (blue) figs *(Ficus carica L.*) diversity index is displayed in the following turn:

Shannon-Winner

The Shannon-Winner index is an index that is suitable for calculating the level of species diversity21. The rarefaction curve is in Figure 4. produces a flat curve that shows the diversity in the number of credible endophytic bacterial targets obtained or that many species have been discovered. The Shannon-Winner index values of 1.987 and 1.395 indicate that the level of diversity of the endophytic bacterial community of figs varieties Blue Giant and Iraki is very good.

Simpson

The Simpson index, sometimes called the dominance index, displays the probability that two randomly selected individuals will belong to distinct species. The range of the dominance index is 0 to 1, with a lower value indicating less dominance of a particular species and a higher value indicating more dominance of a specific species. Good levels of community diversity were suggested by the Simpson index values of 0.681 and 0.548 for the Blue Giant variety of fig endophytic bacteria (Ficus carica L.) and Iraki, respectively. The rarefaction curve presented in Figure 5 displays a flat curve that illustrates the diversity of plausible endophytic bacterial targets. Several species are identified.

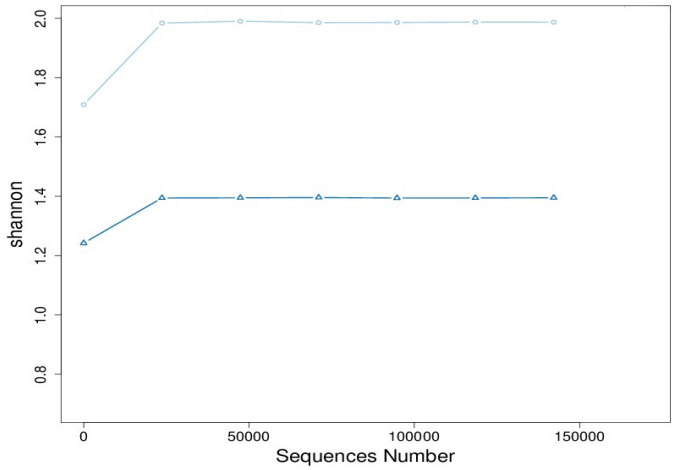

Figure 4: Index Visualisation Shannon Winner of Fig Endophytic Bacteria

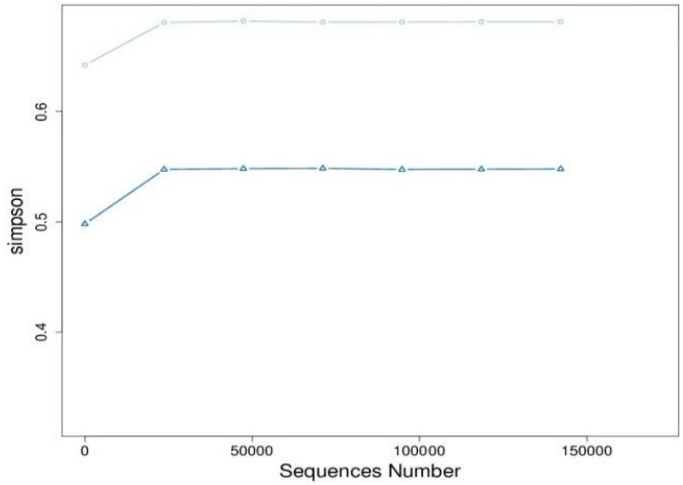

Figure 5: Index Simpson Visualisation of Figs Endophyte Bacteria

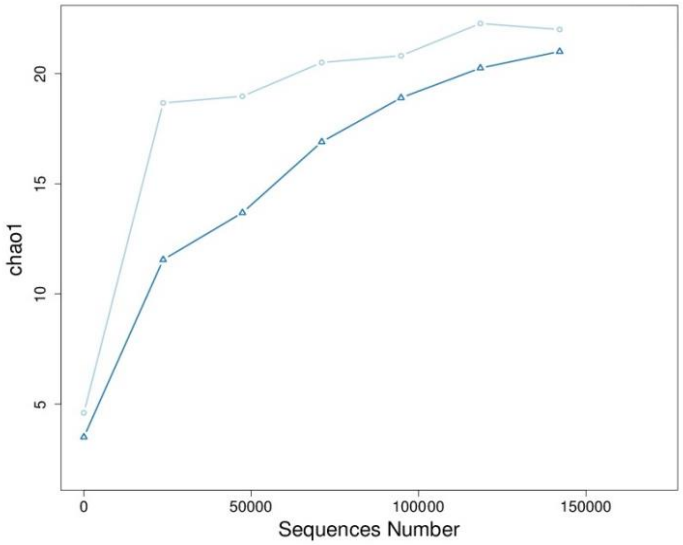

Figure 6: Visualization of the Chao1 Index of Fig Fruit Endophyte Bacteria

Chao1

The number of individuals in a sample that belong to a specific species is indicated by the Chao1 alpha diversity index as showing in Figure 6. The amounts of variety in the endophytic bacterial communities of figs (Ficus carica L.) of the Blue Giant and Iraqi types were quite good, as indicated by the Chao1 index values for these varieties (22,000 and 21,000, respectively). After adding proton pump inhibitors (PPI) in the case of gastroesophageal reflux disease (GERD), the Chao1 alpha diversity index of 12,500 results in a considerable diversity of bacteria.

Phylogenetic Tree of Figs Endofit Bacteria

Figure 7 of the phylogenetic tree. The genetic relationships between the endophytic bacterial strains isolated from the Blue Giant and Iraki kinds of fig plants are depicted by the circular shape that joins the 16S rRNA gene sequence. Between the Blue Giant variation (red), the Iraki variety (orange), and the Green Yordan variant (green), the circular tree's color pattern of the bacterial strain identifier draws attention to the source of separation. According to the phylogenetic tree, the three samples' endophytic bacteria, which belong to the Phylum Cyanobacteria, Firmicutes, Proteobacteria, and Actinobacteria, are genetically related to one another. Figs of the Blue Giant, Iraki, and Green Yordan types are included in the phylum Cyanobacteria. Figs belonging to the phylum Firmicutes include the Blue Giant and Iraki types. Figs of the Blue Giant, Iraki, and Green Yordan kinds are included in the phylum Proteobacteria. Only the phylum taxon level of the Actinobacteria phylum was recognized.

Ternary Plot Bakteri Endofit Fig Fruit (Ficus carica L.)

A data composition in the shape of an equilateral triangle displaying three endophytic bacterial data points is called a ternary diagram or plot. The Blue Giant variety is represented by each BT.1 data set, the Iraki variety by BT.2 data set, and the Green Yordan variety by BT.3 data set. When these three varieties are joined together, the result is 100%. Three endophytic bacterial species—Chloroplast, Lactobacilliales, and Rickettsiales—represent the highest percentage of endophytic bacteria at the taxon order level in Ternary Plot Figure 8. According to the Chloroplast data, the percentages are 20% for BT.1, 38% for BT.2, and 42% for BT.3. The percentages for Lactobacilliales were 18% at BT.1, 42% at BT.2, and 40% at BT.3. According to the findings, the percentage of Rickettsiales is 0% at BT.1, 20% at BT.2, and 80% at BT.3.

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Table 5: Alpha Diversity Index

Sample	Observed Species	Shannon	Simpson	Chao1
BT. Iraki	21	1,987	0.681	22,000
BT. Blue Giant	16	1.395	0.548	21.000

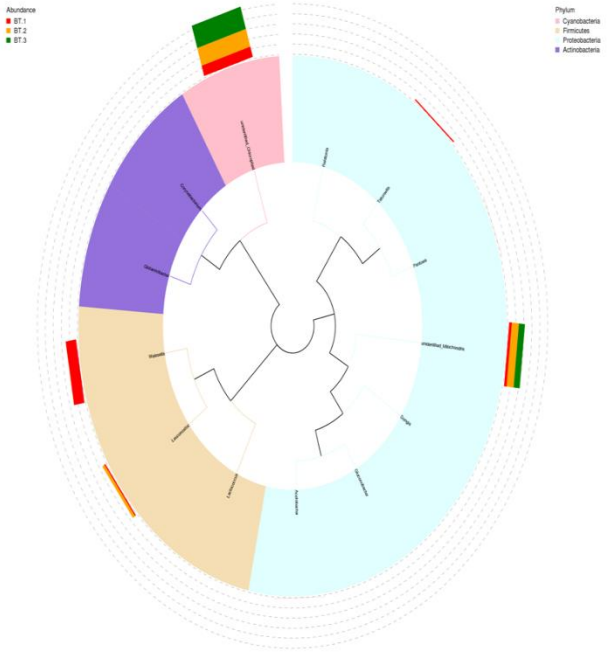

Figure 7: Evolutionary Tree of Figs Endofit Bacteria

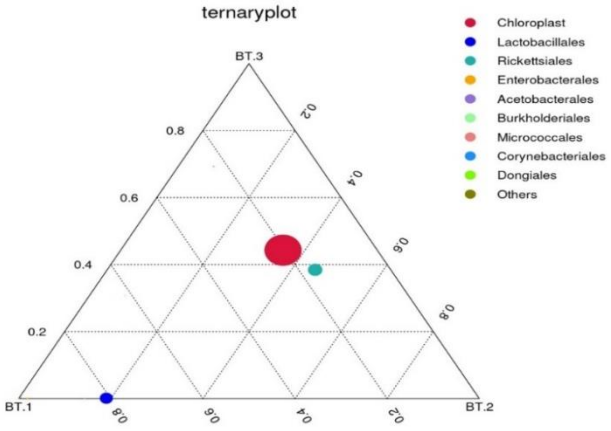

Figure 8: Evolutionary Tree of Figs Endofit Bacteria

Conclusion

The understanding of microbial diversity in fig fruit has been substantially expanded by the application of NGS technology to describe microbial diversity. Based on these findings, the microbial community in fig fruit may be extensively affected by the usage of the V3 and V4 domains, which demonstrate the hypervariable region and conservation of this NGS technique. Shorter sequences (400–500 bp) will yield more reads from the Miseq Illumina platform. Although the length of the generated sequence is not as long as the one obtained from conventional sequencing, it is still extremely practical to utilize for taxonomic research. The microbiological diversity found in fig fruit, both in the Iraki and Blue Giant varieties, includes the following species: *Gluconobacter cerinus, Weissella ghanensis, Weissella paramesenteroides, Ralstonia pickettii, Leuconostoc citreum, Pantoea stewartii,* and *Lactococcus lactis.* This finding suggests that endophytic bacteria in figs can be an alternative in the search for new effective antibacterials. The metagenomic approach has also proven effective in revealing the diversity of microorganisms that are difficult to culture, thus providing wider opportunities for exploring bioactive compounds from natural sources in overcoming antibacterial resistance.

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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