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Research Paper

Metagenomics analysis of microbial communities in Turmeric rhizomes plant (*Curcuma domestic* Val)

Fenti Fatmawati[1*], Ira Adiyati Rum[2], Titin Haryati[3]
[1]*(Faculty of Pharmacy Universitas Bhakti Kencana, Indonesia)*
[2]*(Faculty of Pharmacy Universitas Bhakti Kencana, Indonesia)*
[3]*(Research Center for Applied Microbiology, National Research and Innovation Agency, Indonesia)*
Corresponding Author: fenti.fatmawati@bku.ac.id

ABSTRACT: *The aim of this research is to obtain DNA sequences from all microbes using the nanopore metagenome analysis method so that all species of endophytic bacteria found in the turmeric rhizome can be identified. Thalassoporum komareki was shown to be the most prevalent species in the microbial community on the rhizomes of turmeric plants (Curcuma domestic Val) based on the results of metagenomic study.*
KEYWORDS: *Curcuma, Metagenomic, Microbial, Nanopore*

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I. INTRODUCTION

The zingiberaceae family is known to contain endophytic bacteria which have various bioactivities that are useful in the pharmaceutical field such as antifungal, antifungal [1] and antibacterial activity [2]. Isolation and identification of endophytic bacterial species still use conventional methods (16S). With this conventional method, only bacteria that can be cultured can be isolated, while the number of bacteria that cannot be cultured is much greater. To overcome this, a reliable isolation technique is needed to obtain information on all endophytic bacteria in turmeric (*Curcuma domestic* Val). The method used in this research is metagenome with nanopores. The aim of this research is to obtain DNA sequences from all microbes using the nanopore metagenome analysis method so that all species of endophytic bacteria found in the turmeric rhizome can be identified.

Metagenomics is a tool to explore the genetically rich resources of uncultivated/cultured microbiota. This metagenomics does not use conventional culture methods and is based on the principle of direct isolation of DNA from complex environmental samples containing diverse microbiota to reveal the true microbial composition of the environment. This technique can be used to obtain information on all DNA from microbial communities in an ecosystem. In this way, researchers can find out the total type of organisms contained in the sample [3]. Species-level genetic characterization of complex bacterial communities has important clinical applications in both diagnosis and treatment. Nanopore technology uses thin, nanopore-sized membranes to detect changes in potential when charged biological molecules smaller than the nanopore pass through holes. This technique is useful for analyzing single molecule amino acids, DNA, RNA [4]. This nanopore metagenomic sequencing method is a solution in obtaining complete 16S DNA sequences from the entire bacterial community which was previously carried out conventionally (16S sequencing) for culturable microbes. The metagenomic sequencing method, especially for microbiological diagnostic purposes, has been hampered by the difficulty of the sequencing process, quite large costs and the length of time required. The advantages of this technique are better resolution in distinguishing taxa, accurate and sensitive representation of the composition of bacterial samples, capable of producing DNA sequencing without theoretical reading limits as in conventional 16S rRNA sequencing.

II. METHODS

Turmeric rhizome was used as the research material in this study. ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300) was used to extract genomic DNA. The concentration of DNA was measured with a Qubit fluorometer and two NanoDrop spectrophotometers. The 16S primer used is 27F – 1492R. 2μL PCR of

amplicon were assessed by electrophoresis with 1% TBE agarose. Oxford Nanopore Technology kits were used for library preparations.

Version 22.05.7 of the MinKNOW software was used to operate the nanopore sequencing. Using a high-accuracy model, basecalling was carried out with Guppy version 6.1.5. NanoFilt was used to do quality filtering, and NanoPlot was used to visualize the quality of FASTQ files [5][6]. Centrifuge classifier was used for read classification[7]. Using the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/), an index of bacteria and archaea was created. RStudio running R version 4.2.0 (https://www.R-project.org/), Krona Tools (https://github.com/marbl/Krona), and Pavian (https://github.com/fbreitwieser/pavian) were used for downstream analysis and visualizations.

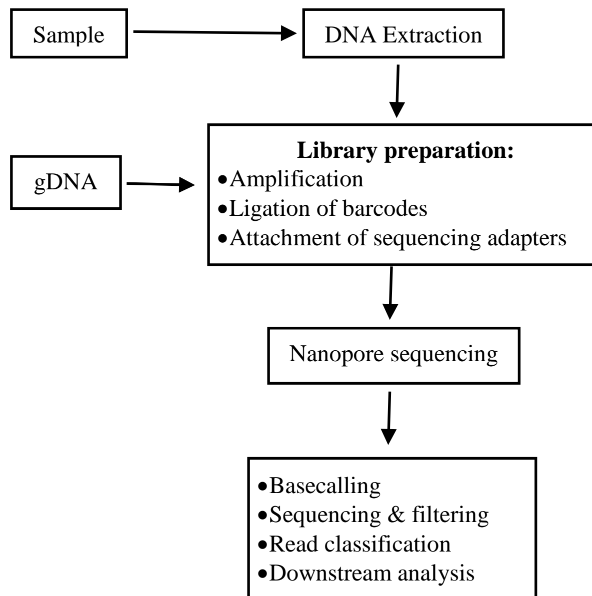

Diagram 1: Workflow

III. RESULTS AND DISCUSSION

It can be seen that the genomic DNA was successfully extracted and amplified. gDNA was visualized by agarose gel electrophoresis, as shown in Figure 1 below.

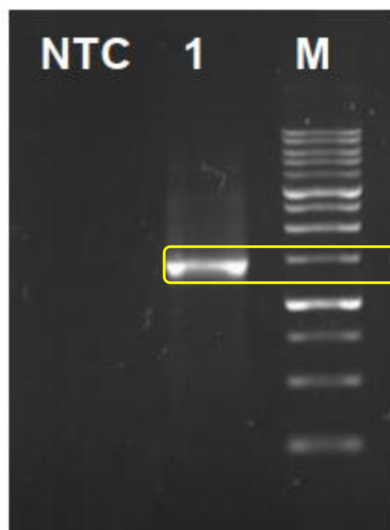

Figure 1 : gDNA

DNA purity measurements obtained an A260/280 value of 1.88. This shows that the DNA sample is relatively pure and has little protein contamination. This ratio is within the range considered good for pure DNA (Table 1).

Table 1: DNA purity measurement results

Sample	**Volume (µl)**	**NanoDrop Reading**			**Qubit Reading**
		gDNA Concentration (ng/µL)	**A260/280**	**A260/230**	**gDNA Concentration (ng/µL)**
	50	19	1.88	0.97	6.42

The A280 measures light absorption by proteins at a wavelength of 280 nm. The A260 measures light absorption by nucleic acids (DNA/RNA) at a wavelength of 260 nm, and the A230 measures light absorption by impurity compounds, such as salts, phenols, and carbohydrates, at 230 nm. The A260/230 ratio of the DNA sample was 0.97, indicating contamination by compounds that absorb at 230 nm. This suggests that the sample contains salt, phenol, or other compounds that can affect the purity and quality of the DNA (Table 1).

The Qubit fluorometer measures the amount of DNA in sample and provides information about that concentration. When measuring DNA concentration, Qubit uses fluorescent dyes that bind directly to DNA, yielding more precise results than spectrophotometric techniques, particularly in samples with low quantities. The top 10 relative abundances from each taxonomic level (Genus) are compared among the samples in the barplot (Figure 2).

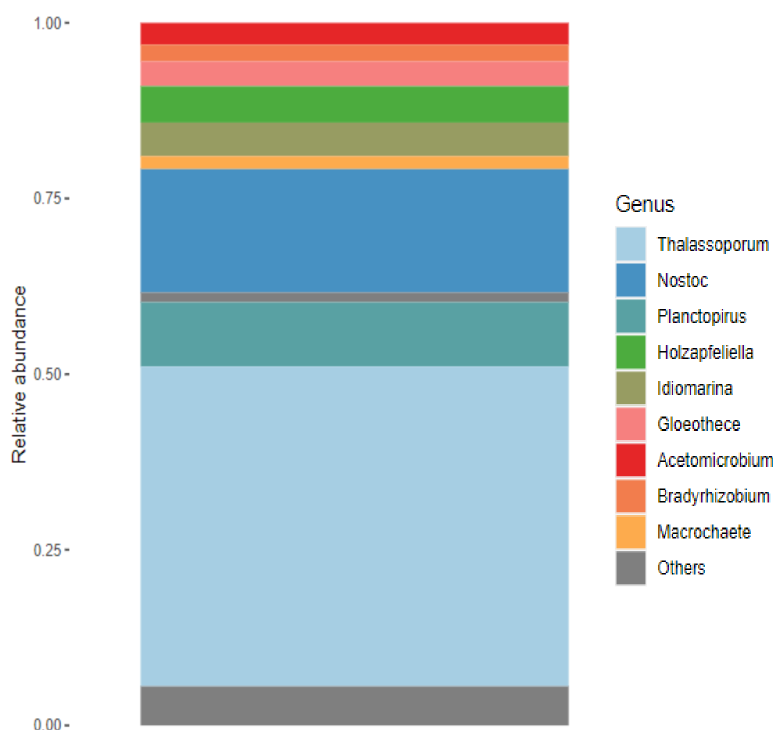

Figure 2: Abundance of Genus

Krona visualization allows us to intuitively explore the relative abundances within the complex hierarchies of metagenomic classifications (Figure 3). Sankey diagrams are used to visualize microbial species in samples. In this diagram, the arrow width is proportional to the quantity depicted to depict changes over time in the hierarchy between nodes (Figure 4). The most abundant species was found, namely *Thalassoporum komareki.*

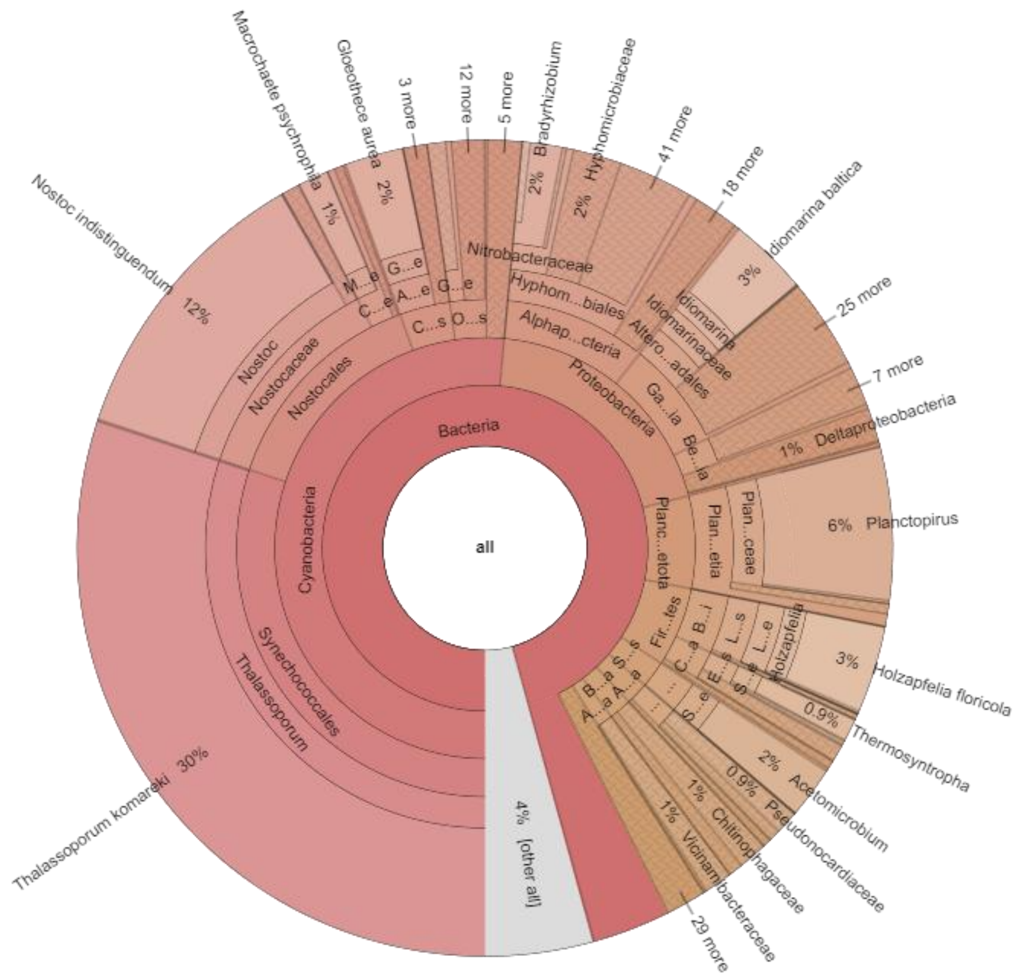

Figure 3: relative abundances within the complex hierarchies

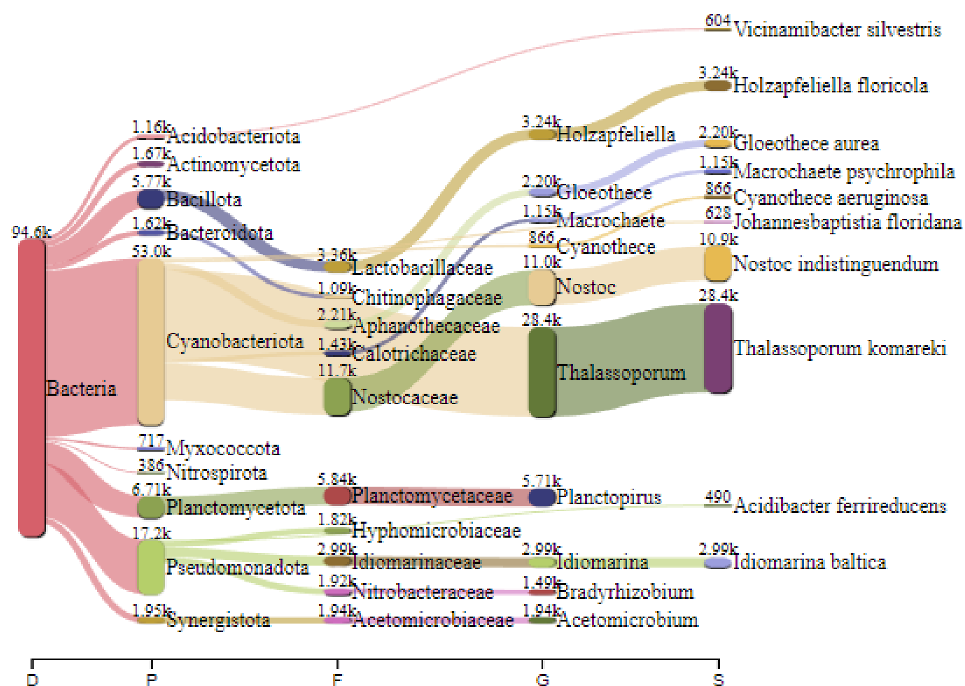

Figure 4: Sankey diagrams

*Corresponding Author: Fenti Fatmawati

IV. CONCLUSION

Thalassoporum komareki was shown to be the most prevalent species in the microbial community on the rhizomes of turmeric plants (Curcuma domestic Val) based on the results of metagenomic study.

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