Design Analysis and Assessment of Bacterial Diversity on Beeswax Coated Banana Bark Food Containers and Packaging using Oxford Nanopore Platform

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Abstract

As one of the largest banana producers, Indonesia is expected not only to produce banana fruits and processed food as the main commodity, but also to process waste produced by post-harvesting process of banana plantations. The large amount of banana bark as the post-harvest waste from banana plantations is currently used as animal feed. This multidisciplinary research continues the previous research related to banana plants, focusing on the assessment of bacterial diversity through sequencing of 16S ribosomal RNA (16S rRNA) genes using Oxford Nanopore Technology (ONT) on beeswax-coated food container/packaging products made from banana bark with heat press technology. Genomic DNA from each samples were isolated using Genomic DNA Mini Kit Plant from Geneaid. DNA concentration was determined using NanoDrop spectrophotometers and Qubit fluorometer. The research results may provide in more progressive development in food packaging made of banana bark as biodegradable and non-toxic material. Thus, the results of NGS 16S rRNA V3-V4 profiling, bioinformatics analysis using MOTHUR, and functional profile prediction using Piphillin. From the beeswax-coated banana bark samples we found 1721 bacteria, based on their relative abundance consisting of 20% Synechococcus sp JA-3-3Ab, 7%Pseudanabaena sp PCC 7367, 5%Chrooccocidiopsis therminalis, 4% each of Prestia megaterium and Amnonifex degensii, 3% each of Brevundimonas sp. Bb-A & Burkholderiales and 54% other bacteria < 3%. Meanwhile from the control (the beeswax-non coated banana bark samples), we found 1693 bacteria which relative abundance consists of 10% Pantoea sp At-9b, 7% Escherichia coli, 6% each of Klebsiela pneumonia, pantoea vagana, and Synechococcus sp JA-3-3Ab, 4% each of Salmonella enterica and Pantoea sp SO10, and 3% each of Serretia, Burkholderiales, and Alphaproteobacteria and 55% other bacteria < 3%. We found that 30% of bacterial in beeswax-coated banana bark samples was also found in the beeswax-non coated banana bark samples.

1 Introduction

The need for biodegradable packaging products is urgently needed as a substitute for plastic and other harmful materials. This research aims to provide further information on banana bark as a smart material in product design. This research activity is a part of cross-institutional Banana Smart Village (BSV)

multidisciplinary research program since 2018 aiming to empower the local community through the application of sustainable, zero waste based appropriate technology in banana cultivation and village revitalization. This study uses design approach and assessment of bacterial diversity through sequencing of n16S ribosomal RNA (16S rRNA) genes to analyses the chances of beeswax coated banana bark as a food container or packaging material.

2 Methods

2.1 Genomic DNA

Genomic DNA from each sample were isolated using Genomic DNA Mini Kit Plant from Geneaid. DNA concentration was determined using NanoDrop spectrophotometers and Qubit fluorometer. Library preparations were conducted using Kits from Oxford Nanopore Technology (ONT) that provides the long reads sequencing that cover the full-length sequence of 16S rRNA gene (V1-V9 regions) through a fast, cheap, and high throughput process. Since all the informative sites of 16S rRNA genes are considered, the full length 16S rRNA sequences offer a higher level of taxonomic and phylogenetic resolution for bacterial identification [1]. Further method used in this research is qualitative design thinking approach for an in-depth exploration to analyze the product design aspects of beeswax-coated food container and packaging made of banana bark to complement previous research in processing and optimizing this material for handicraft and functional products, specifically acoustic absorber unit [2].

2.2 16s rRNA Sequencing

The experimental workflow for the bacterial assessment on banana bark sample is consisting of : Genomic extraction, amplification of gDNA with 16s primers using rapid attachment chemistry, attachement of rapid ID sequencing adapters, Priming and loading to Gridion sequencer and sequencing.

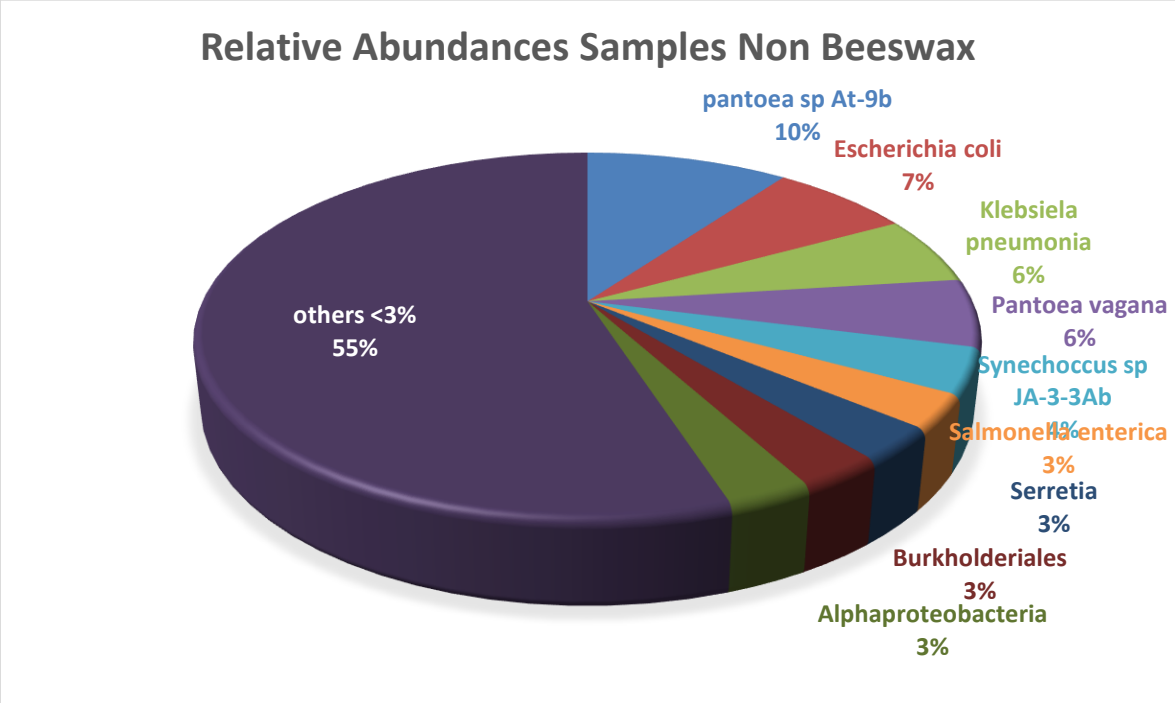
3 Experimental Results

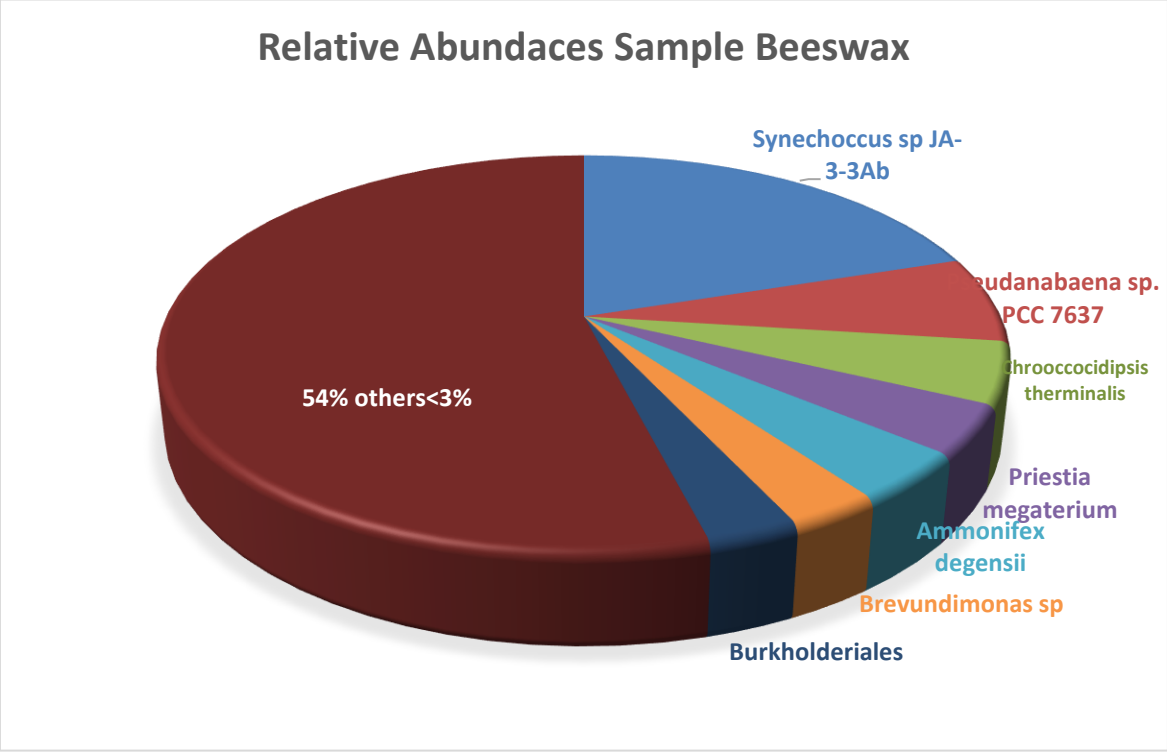
3.1 Sequencing results

Sample	Mean read length	Number of reads	Total Bases
beeswax-coated banana bark samples	1,531.0	131,995.0	202,090,361
beeswax-non coated banana bark samples	1,537.6	122,874.0	188,926,678

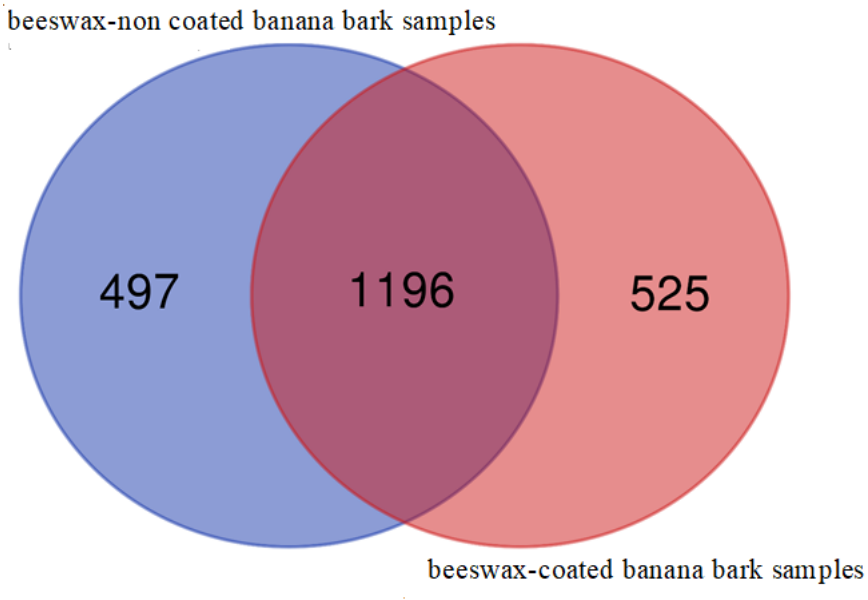
3.2 Relative abundances

Below is the venn diagram illustrating the logical relationship between samples:





3.3 Diagram venn



4 Discussions

For.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

These authors have contributed equally to this works.

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9 References

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