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Variant alpha and beta biodiversity of the genus in dadiah through deep sequencing 16S Ribosomal RNA genes

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Abstract. Dadiah is a traditional spontaneous fermentation of buffalo milk from West Sumatra that doesn't go through pasteurized stages. The aim of the study is to find out the biodiversity of α and β diversity of the microbiota in the level of the genus in dadiah. The V3-V4 hypervariable region of the 16 S rRNA deep sequencing was used to the detected genus of bacteria. The available data were analyzed by Alpha Diversity (richness and evenness) and Beta Diversity (Taxonomy Chart Heatmaps). These studies revealed that the most divergent sample is appropriate to C3 with Shannon diversity Index 2.014; meanwhile, the most evenness fit in to B4 with the Simpson diversity index of 0.573. Further, the highest value of the heatmap taxonomic chart on the B4 sample has its place in Lactococcus. There were differences in dadiah microbiota composition among the samples based on different areas. Each sample was found Lactococcus and Lactobacillus with the largest populations.

Keywords: Dadiah, microbiota, Alpha biodiversity, Beta Diversity, 16S rRNA Deep Sequencing

1. Introduction

Dadiah is traditional fermented buffalo milk from West Sumatra that occurs spontaneously fermented. The milk is stored in a bamboo tube and covered by banana leaves on top, and then the milk is incubated at room temperature for one to two days or up to the desired consistency. Dadiah is very beneficial for health because, in dadiah, there are lactic acid bacteria (BAL). Consumption of foods containing BAL can improve health because BAL can breed in the gastrointestinal tract. The presence of these bacteria can cause changes in the balance of bacteria in the gastrointestinal tract that provide health effects for humans [1]. But it does not close the possibility that there are variations of microbiota in dadiah.



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In the process of dadiah processing itself, there are several methods. The usual way of making dadiah is to pour buffalo milk directly into bamboo tubes that have been prepared in advance and continued with the incubation process for 2-4 days at room temperature. But lately, some farmers reuse dadiah that has been so before as a starter to shorten the incubation period to 18 - 24 hours. But there is a unique process of making dadiah found in Alahan Panjang makes dadiah by inserting buffalo milk little by little every day, which needs longer bamboo tubes. This process is unique if compared to other cities.

This raises the possibility of differences in the microbiota dadiah in each layer, either the upper layer exposed to oxygen or the lower layer that is anaerobic. Microbiota is microorganisms found in specific locations and are symbiotic communities of commensalism and pathogenic found in certain organisms or objects. Dadiah is a probiotic food containing BAL, but as we know, there is no pasteurization process in the manufacture of dadiah and stored in bamboo tubes. This process does not cover the possibility of genus variations found in dadiah.

The variation of microorganisms found in dadiah such as *Lactococcus*, *Lactobacillus*, and *Leuconostoc* is among other dadiah microbiota. In addition, pathogenic bacteria such as *Klebsiella* and *Chryseobacterium* were found in dadiah samples [2]. It was then added by [3] microbiota variations in dadiah as follows *Lactococcus*, *Klebsiella*, *Lactobacillaceae*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Staphylococcus*.

Variations in the genus of microbiota in dadiah can be caused by bamboo tubes used, buffalo milk, fermentation temperature, and the time during spontaneous fermentation [2]. In addition, according to [4] factors that affect the composition of the microbiota in livestock milk can be influenced by the host and the environment. *Klebsiella*, a group of *Enterobacteriaceae* in dairy products, indicates the occurrence of contamination by feces in the product [5]. *Chryseobacterium* can be found in soil and the environment [6]. Variations of non-profit bacteria in dadiah can be caused by environmental contamination and unhygienic milking management and the absence of the standard operating procedure maintenance of dairy buffalo and pasteurization process in manufacture unhygienic dadiah.

Previous research that has been done only looked at the composition of the microbiota in dadiah but only focused on the potential and benefits of microorganisms for health without looking in more detail at the presence of non-profitable bacteria. Considering the interest in dadiah consumption in West Sumatra is high, and its benefits are good for health. This makes researchers want to explore the variation of microbiota in dadiah based on Alpha and Beta diversity dadiah with the title "Biodiversity Genus Bacteria In Dadiah With Deep Sequencing Method Gen 16s rRNA".

2. Methods

2.1. Sample collection

The samples were obtained from local areas: Batusangkar, Alahan Panjang, Padang Panjang and Agam in West Sumatra, Indonesia. Dadiah is a small-scale dairy product that is not produced continuously for a year. Dadiah processing industry is rarely found on a large scale of dairy products, and usually, dadiah products can be found on traditional markets in each region in West Sumatra, Indonesia. Samples collected from different local areas were produced from various raw buffalo milk sources. All the sample were collected and transported by air to Lembaga Ilmu Pengetahuan Indonesia (LIPI) laboratory.

2.2. Bacterial isolation from dadiah samples

Bacteria and bacterial DNA were prepared using a modification of [7] Dadiah samples were suspended in 35 ml of PBS used for isolation from bacteria. Aliquots (10-100 ml) of dadiah suspension spread over LB (Luria-Bertani) in order, MRS Agar, HA (Haloarcula) agar (20% NaCl, 2% MgSO₄·7H₂O, 0.05% CaCl₂, 0.0125% MnCl₂, 1% yeast extract, and 1.5% agar), and Schaeffer spore-forming agar [0.8% nutrient broth (Becton Dickinson Co., Franklin Lakes, NJ, USA), 0.025% MgSO₄·7H₂O, 0.1% KCl, 1 μM FeSO₄, 1mM Ca(NO₃)₂, 10 μM MnCl₂, and 1.5% agar]. Each plate was incubated at a temperature of 37°C for 2-14 days. The colonies seen on the plates are taken and streak on a new order of the same

type for purification. Single colonies are cultivated at 50 ml of liquid medium, and this liquid culture was used for chromosomal isolation of DNA using Genomic-Tip 5000G (Qiagen, Tokyo Japan).

2.3. Gen amplification 16S rRNA preparation

Amplification sequencing techniques were managed based on the magnification of small fragments of hypervariable regions at 16S rRNA. This process happened in the V3 – V4 region. Bacteria and bacterial DNA were prepared to be modified based on previous research [7]: [8]. DNA was purified by treatment with ribonuclease A (Wako) and followed by a 20% peg 600 (Nacalai Tesque) deposit at 2.5 NaCl. DNA pellets were rinsed using 75% ethanol and dissolved in TE buffers. Hypervariable V3-V4 from the bacterial gene 16S rRNA PCR is amplified using the primary set of universal barcode tags 341F-805R (341F 5'–CCTAGGGNGGCWGCAG- 3' and 805R 5'–GACTACHVGGGTATCTAATCC- 3') [9]. PCR amplification was carried out using the following programs: 95°C for 3 minutes, 25 cycles (95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds), 72°C for 30 seconds, then hold at 4°C Agilent 2100 Bioanalyzer (Agilent Technologies) used to determine the size (about 550 base pairs) of PCR fragments. PCR products are purified using Agencourt AMPure XP beads (Beckman Coulter). Nextera XT Index Kit (Illumina) is used for amplicon labels with different double barcodes.

2.4. Deep sequencing and data analysis

Deep paired-end sequencing was performed using the Illumina Miseq platform [10]. The V3-V4 hypervariable region of the 16 S rRNA deep sequencing was used to detect the genus of bacteria. The available data were analyzed by Alpha Diversity (richness and evenness) and Beta Diversity (Taxonomy Chart Heatmaps).

3. Result and discussion

The screening of dadiah bacterial was done by collecting 11 samples of dadiah samples from six bamboo tubes obtained from local areas in West Sumatra, Indonesia, to inspect the diversity of the microbiota in dadiah. This study has got the compositional differences of the microbiota in dadiah from different areas (Figure 1).

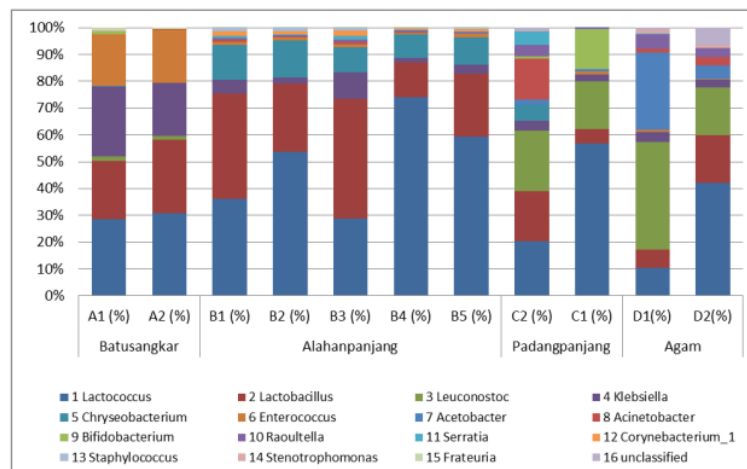


Figure 1. Percentage of the composition of the genus microbiota in dadiah.

The result show the difference between microbiota composition in dadiah from the different areas (Batusangkar, Alahan Pajang, Padang Panjang and Agam). The data recovered 16 genus microbiota such as *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Klebsiella*, *Chryseobacterium*, *Enterococcus*, *Acetobacter*, *Acinetobacter*, *Bifidobacterium*, *Raoultella*, *Serratia*, *Corynebacterium_1*, *Staphylococcus*, *Stenotrophomonas*, *Frateuria*, and the other is unclassified. All the samples of dadiah

showed convicted a highly abundant of *Lactococcus* and *Lactobacillus* as a fermentor in a fermentation dairy product. Besides *Lactococcus* and *Lactobacillus*, there is also detection of another lactic acid bacteria (BAL) that helps dadiah fermentation; it's *Leuconostoc*. However, *Leuconostoc* was found only in dadiah that was produced in Padang Panjang and Agam. Where *Leuconostoc* in other areas (Alahan Panjang and Batusangkar). Predominant composition of lactic acid bacteria (*Lactococcus*, *Lactobacillus*, and *Leuconostoc*) in dadiah showed that indicate of derived from buffalo milk, bamboo tubes, or banana leaves may be influenced and involved in the fermentation of dadiah even without starter culture [2]. The result of the dadiah sample in Padang Panjang revealed a similar composition of the genus bacteria in each dadiah's layer. Several genera obtained from microbiota can be categorized into profitable and non-profitable bacteria (Tabel 1.)

Tabel 1. The profitable and non-profitable genus of bacteria.

Genus of Bacteria	Profitable Bacteria	Non-Profitable Bacteria
<i>Lactococcus</i>	✓	
<i>Lactobacillus</i>	✓	
<i>Leuconostoc</i>	✓	
<i>Klebsiella</i>		✓
<i>Chryseobacterium</i>		✓
<i>Enterococcus</i>		✓
<i>Acetobacter</i>	✓	
<i>Acinetobacter</i>		✓
<i>Bifidobacterium</i>		✓
<i>Raoultella</i>		✓
<i>Serratia</i>		✓
<i>Corynebacterium_1</i>		✓
<i>Staphylococcus</i>		✓
<i>Stenotrophomonas</i>		✓
<i>Frateuria</i>		✓

Samples dadiah on the research unexpectedly revealed the composition of non-profitable bacteria. Those samples contained 11 genera of non-profitable bacteria, and it mostly showed in each sample highly contained *Klebsiella*, also high of *Corynebacterium_1* were present in sample dadiah from Agam (20%). Interestingly, even those samples contained non-profitable bacteria. It might have the potential to foodborne disease; however, there is no report of the outbreak or illness of foodborne disease because of consuming dadiah. And people in West Sumatra still consume dadiah as traditional food consumed daily or on specific occasions.

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