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Food Safety Aspects of Palm Sugar: The Authentic Local Sweetener from Baduy Tribe, Indonesia

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HIGHLIGHTS:
- The bacterial count in Baduy palm sugar was less than 30 Colony Forming Unit (CFU)/g.
- There was no growth of yeast or mold, and coliform in Baduy palm sugar.
- Two bacteria found in Baduy palm sugar were *Bacillus megaterium* and *Kocuria koreensis*.
- The heavy metals detected in Baduy palm sugar were under the limitation of the International Commission for Uniform Methods of Sugar Analysis for Pb and As.

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Acronyms and abbreviations
BGLB=Brilliant Green Lactose Broth
CFU=Colony Forming Unit
ICUMSA=International Commission for Uniform Methods of Sugar Analysis
MPN=Most Probable Number
SPC=Standard Plate Count
TPC=Total Plate Count
TYMC=Total Yeast and Mold Count

ABSTRACT

Background: Baduy is an Indonesian native tribe which still holds ancestral heritage, including food processing**.** Palm sugar is considered as an authentic local sweetener that is produced naturally by the Baduy individually and is frequently used as a souvenir for tourists. However, the data describing the safety of palm sugar in terms of microbial and heavy metal content have not been widely reported. This study aimed to analyze palm sugar's safety on the basis of microbial and heavy metal content.

Method: Palm sugars were obtained from 5 of 25 sugar makers in Kanekes village, Baduy using a purposive random sampling in April 2021. The analysis of Total Plate Count, Total Yeast and Mold Count, coliform, and heavy metal were conducted and their results were compared to the requirements of the International Commission for Uniform Methods of Sugar Analysis. Bacterial identification was conducted microscopically and molecularly using a conventional Polymerase Chain Reaction. The collected data were analyzed with the descriptive method.

Results: The results demonstrated that the bacterial count of Baduy palm sugar was less than 30 Colony Forming Unit (CFU)/g; no yeast and mold were observed; negative results for the coliform test; and detected heavy metals were under the limitation of the International Commission for Uniform Methods of Sugar Analysis standards except for copper. The two found bacteria are non-pathogenic, namely *Bacillus megaterium* and *Kocuria koreensis.*

Conclusion: The results of the research showed that Baduy palm sugar is safe in terms of microbiological and heavy metals**.** This research is expected to consist of useful information to the public based on the safety and quality of Baduy palm sugar, and consequently it can attract the attention of Indonesian tourists and even foreign tourists to visit Baduy.

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Introduction

…Baduy is an Indonesian native tribe located in the foothills of Kendeng mountain, Kanekes village, Leuwidamar district, Lebak regency, Banten province, Indonesia. The land area of Baduy is around 5,108 hectares, of which settlement area (0.48%), protected forest (48.85%), and farming area (50.67%) (Iskandar and Iskandar, 2021; Saleh et al., 2020). Palm tree classified into a family of palmae is one of the various plants found in the farming area. The sap of palm trees can be processed into palm sugar (or aren sugar) in the form of molded or granules (Ishak et al., 2013; Kurniawan et al., 2018). Baduy people make palm sugar as one of their livelihoods which is produced traditionally and sold to tourists as a souvenir. In addition, Baduy people use palm sugar as a sweetener for traditional food which is usually served during traditional ceremonies such as jojorong, apem putih, pasung, wajik, dodol, papais, and kue cincin. Palm sugar is generally favored since it has a distinctive taste and aroma and is not found in sugarcane or other sweeteners. Pyrazines, furans, ketones, fatty acids, and organic constitute the volatile components of palm sugar which produces states for its sweet, toasty, and caramel-like aroma (Hebbar et al., 2018).

…Authentic food is genuine food that comes from an area that is cooked in a unique way where each process demonstrates traditions, legends, stories, and symbols (Zhang et al., 2019). Baduy people made palm sugar by using simple tools, such as lodong (bamboo container to hold the sap), gonggo (the traditional stove which use firewood as fuel to heating the palm sap until thick), batok (the concenctrated sugar molding container), and kararas (the dried banana leaves to pack the palm sugar). Production of Baduy palm sugar is carried out by thermal processing through boiling and usually does not involve a purification process with additional chemicals (Victor and Orsat, 2018). The palm sap is tapped for one month and heated until it becomes caramelized, and concentrated. Then the concentrated palm liquid is cooled, molded, and packaged. Every tool and stage of the process has a philosophical meaning that is attractive for tourists who come and try to experience the local culture.

The quality of authentic food is important for tourists because it can demonstrate the high quality of processing and the safety of its consumption. However, it might be difficult to control the processes because of the difference in individual procedure techniques. Phaichamnan et al. (2010) expressed that the quality of palm sugar traditionally produced is difficult to define because it depends on personal hygiene, sanitary facilities, harvesting conditions, heating temperatures, heating time, and storage conditions. According to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA), the quality of sugar can be considered based on the maximum limit of microbial contaminant, namely 1×10^5 Colony Forming Unit (CFU)/g for Total Plate Count (TPC), 2 CFU/g for Total Yeast and Mold Count (TYMC), and 3 Most Probable Number (MPN)/g for coliform were; as well as the maximum limit of heavy metal on sugar should be less than 1.0 mg/kg copper (Cu), 0.5 mg/kg lead (Pb), and 0.5 mg/kg arsenic (As) (ICUMSA, 2014).

Unfortunately, there are few studies that specifically investigate the quality of authentic food produced by Indonesian native tribes, including Baduy palm sugar. Recently, various studies in Indonesia have only studied the chemical, physical, and microbiology properties evaluation in palm sap (Gunawan et al., 2020; Saputro et al., 2020) and food products with palm sugar (Nurhayati et al., 2022) which is usually produced by Indonesian Small and Medium-Sized Enterprises (SMEs). Baduy people were not informed about food safety which is appropriate for the quality of their palm sugar because of the inaccessibility of acquiring it all. Therefore, the analysis of the safety of Baduy palm sugar in terms of microbial contaminant and heavy metal content is urgent to investigate.

This study aimed to analyze the microbial contaminant and heavy metal content in Baduy palm sugar and compare them to ICUMSA. Identification of microbes in Baduy palm sugar was also conducted to determine the type of microbes in order to know the safety of Baduy palm sugar that is used as souvenirs for tourists.

Materials and methods

Sample and data collection

The samples of palm sugar were obtained from five of 25 palm sugar makers (G1, G2, G3, G4, and G5) in Kanekes Village with a purposive random sampling method in April 2021. The number of samples taken was 20% of the total palm sugar makers in Kanekes village. As much as 1 kg of palm sugar from each palm sugar maker was stored in a cooling box in order to prevent damage during the trip. The observation was also conducted directly at one of the palm sugar makers to obtain obvious pictures of the preparation and processing of raw materials until preparing procedures of goods, then it followed by direct interviews to obtain more in-depth information. The samples were then analyzed for microbial criteria and heavy metal content. All data were collected with three repetitions and analyzed using the descriptive method.

Microbial investigation

-TPC

TPC analysis is a quantitative method to determine the number of microbes present in a sample according to the

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Maturin and Peeler (2001) with the spread plate technique. Furthermore, the samples were grown using plate count agar media (Merck, Germany). The samples were incubated at 35 °C for 48 h, then the colonies were counted with Standard Plate Count (SPC) which is at least 25 to 250 colonies, several colonies are combined into a large collection of colonies where the number of colonies is difficult to count can be considered as one colony, then a series of colony chains that appear as a thick line is also counted as one colony. The results of the colony calculation were then compared to ICUMSA (2014) regarding the maximum limit of TPC in sugar, which was 10^5 CFU/g.

-TYMC

TYMC is a quantitative method to determine the amount of yeast and mold contamination in a sample according to the Tournas et al. (2001) with the spread plate technique. The samples were grown using Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Merck, Germany). The samples were incubated at room temperature for 5 days, then the colonies were counted using SPC which is at least 10-150 colonies. The results of the colony calculation were then compared with ICUMSA regarding the maximum limit of TYMC in palm sugar, which was 2 CFU/g (ICUMSA, 2014).

-Coliform analysis

Coliform analysis was conducted quantitatively by the MPN method based on Feng et al. (1998). The samples were grown using Lactose Broth (LB) media and Brilliant Green Lactose Broth (BGLB) media (Merck, Germany). Coliform analysis was divided into two steps, the first is a presumptive test where the presence of coliform is still a low probability level of presumption, and the second is a confirmed test where the presence of the suspected coliform was confirmed that are 99% coliform. The positive results demonstrated the color changing and gas-forming. For the presumptive test, make a serial dilution for 5 tubes containing LB with a sample until the concentration of 10^{-4} g/ml, then incubate at 35 °C for 24-48 h. Total positive tubes were then analyzed for confirmed test, i.e. inoculated to BGLB media and incubated at 35 °C for 48 h. The MPN value was determined by the total of positive tubes of BGLB media, then compared to ICUMSA regarding the maximum limit of coliform in palm sugar, which is less than 3 MPN/g (ICUMSA, 2014).

-Bacterial identification

Microorganisms were identified either microscopically with the Gram staining or molecularly with analysis of 16S rDNA method. The morphology was observed using a microscope (Leica DM 500, Germany). The bacterial staining procedure was carried out following the bacteriological analytical manual R32: Gram stain (FDA, 2001). The analysis procedure with 16S rDNA goes through several stages, i.e., extraction, amplification, and sequencing of bacterial DNA. Extraction of bacterial DNA was carried out following procedures of Presto™ Mini gDNA bacteria Kit (Geneaid, Taiwan). The amplification was done by Polymerase Chain Reaction (PCR) (Analytical Jena, US) to multiply the DNA nucleotide sequences, the forward primer used was 5'-AGAGTTTGATCCTGGTCAG-3', and the reverse primer used was 5'-GTTTACCTTGTTACGACTT-3'. DNA sequencing was carried out using the Sanger-Coulson method (Sanger et al., 1977) to determine the sequence of DNA nucleotides and then compare them with the National Center for Biotechnology Information (NCBI) database using a Basic Local Alignment Search Tool (BLAST) (Pradhan and Tamang, 2019). The phylogenetic tree was constructed by Geneious software, New Zealand, to analyze the similarity.

Heavy metal analysis

The heavy metals were investigated by following Association of Official Agricultural Chemists (AOAC) official method 2015.01 regarding heavy metals in food (AOAC, 2015). Samples were dried until to be ashed, then analyzed by Atomic Absorption Spectrophotometry (AAS; BEL Engineering UV-M51, Italy), at the wavelength of 283.3 nm for Pb, 283.3 nm for Cu, and 193.7 nm for As. The results of this test were compared to ICUMSA (2014) regarding the requirement of sugar quality.

Statistical analysis

Descriptive statistics were used to analyze, organize, and summarize all data using Microsoft Excel 2016 64-Bit edition for determining mean and standard deviation.

Results

Microbial investigation

-TPC

There was no bacterial growth found in samples G2, G4, and G5 while in samples G1 and G3 bacterial growth was found but only three colonies for sample G1 and one colony for sample G3. Because the number of colonies from all samples that grew did not comply with the SPC calculation, the results are considered clean or less than under 10^5 CFU/g (Table 1).

Two colonies from the sample were picked, and purified, as well as labeled as bacteria A and B (Figure 1). The purification of bacteria was conducted to obtain pure bacterial cultures, and can subsequently be applied for the next step, namely bacterial identification whether microscopically or molecularly using 16S rDNA analysis.

-TYMC

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TYMC has turned out to be a test determining the total amount of mold and yeast contamination in samples. Based on the results of TYMC on five samples of Baduy palm sugar, no mold and yeast growth was observed (Table 1). For not being yeast and mold growth, there was nothing to culture for subsequent identification.

-Coliform

The presumptive test showed a positive result only on sample G1 which is indicated to contain gas (Figure 2a), however after continuing with a confirmed test, the results uncovered to be negative (Figure 2b). The confirmed test is crucial to conduct for eliminating false positive results that can occur in the presumptive test. If none of the tubes contain gas, it can be perceived that the results have been negative and the samples are free from coliform. Generally, the final results of the coliform number analysis revealed a negative result or less than 3 MPN/g (Table 1).

Bacterial identification

Gram staining is a technique to identify whether a bacterial isolate is Gram-positive or Gram-negative and is also used to observe bacterial cell morphology. According to the result of Gram staining and observations under a microscope with a magnification of 1,000 times, it can be observed that both bacteria are Gram-positive. However, bacteria A has a morphology bacilli-shaped while bacteria B has a morphology coccus-shaped (Figure 3). Identification of molecular-based (16S rDNA sequencing) was conducted by comparing the obtained sequence from bacteria A and B to the GenBank Database (Table 2). The results manifested that bacteria A was related to *Bacillus megaterium* (MG786410) with 99% similarity (Figure 4) and bacteria B was related to *Kocuria koreensis* (MG557684) with 99% similarity (Figure 5).

Heavy metal analysis

The results of heavy metals analysis of Baduy palm sugar can be viewed in Table 3, showing that all samples have a low value, i.e less than 1 mg/kg for Pb and As, and more than 1 mg/kg for Cu. It indicated that the samples are free from lead and arsenic but it might be contaminated by copper.

Table 1: The results of the analysis of total plate count, total yeast and mold count, and coliform of palm sugars

Sample Code	TPC (CFU/g)	TYMC (CFU/g)	Coliform (MPN/g)
G1	$<3\times10^1\pm0.57$	0	0
G2	0	0	0
G3	$<3\times10^1\pm0.00$	0	0
G4	0	0	0
G5	0	0	0
ICUMSA (2014)	Max. 10^5	2	3

Note: 0 indicates there was no microbial growth
CFU=Colony Forming Unit; MPN=Most Probable Number; TPC=Total Plate Count; TYMC=Total Yeast and Mold Count

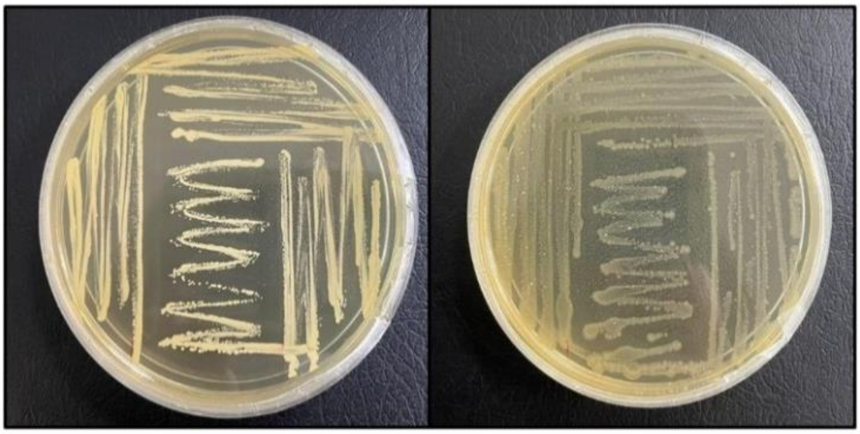

Figure 1: Colonies of bacteria: **A** (left) and **B** (right)

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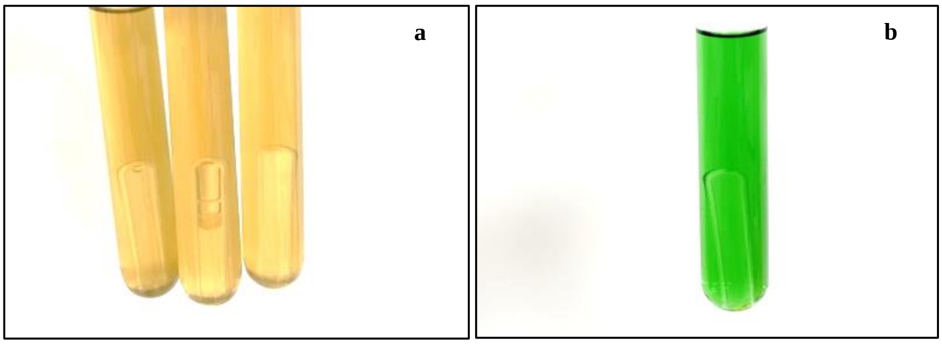

Figure 2: The results of coliform analysis in palm sugar sample G1: **(a)** presumptive test stage; **(b)** confirmed test stage

Table 2: Comparison of the bacterial sequence isolated from Baduy palm sugar with GeneBank Database based on 16S rDNA

16S rDNA sequences from isolates	Accesion number	Matches to 16S rDNA Sequences from GeneBank Database
Bacteria A-BML 1	MH197072.1	*Escherichia coli* NR_024570
	MF431748.1	*Bacillus* sp. strain SX2
	KX768284.1	*Bacillus megaterium* strain P1
	MF431767.1	*B. megaterium* strain WH13
	MG562498.1	*B. megaterium* strain MU2
	MG825091.1	uncultured *Bacillus* sp.
	AB066338.1	*Bacillus* sp.
	CP069397.1	*Priestia megaterium* strain CDC 2008724129
	CP069402.1	*P. megaterium* strain CDC 2008724129
	CP069606.1	*P. megaterium* strain CDC 2008724142
	CP069609.1	*P. megaterium* strain CDC 2008724142
	CP072473.1	*Priestia aryabhattai* strain LAD
	KF515667.1	*Bacillaceae bacterium* LJ17
	KY495205.1	*B. megaterium* strain CGAPGPBBS-034
	MF537086.1	*Bacillus* sp. strain CJKOP-41
	MF592275.1	*Bacillus* sp. strain K-661
	MF662219.1	*B. megaterium* strain HBUM06947
	MF974592.1	*B. megaterium* H-Cryo-23
	MG309333.1	*Bacillus* sp. strain 201705CJKOP-20
	MG309393.1	*Bacillus* sp. strain 201705CJKOP-80
	MG309418.1	*Bacillus* sp. strain 201705CJKOP-105
	MG489825.1	*B. megaterium* strain 15
	MG774438.1	*B. megaterium* strain CDK25
	MG786410.1	*B. megaterium* ICMP 20902
Bacteria B-BML 2	MH197072.1	*E. coli* NR_024570
	KM464080.1	Uncultured bacterium
	KM464070.1	Uncultured bacterium
	KM464338.1	Uncultured bacterium
	AY941087.1	*Kocuria* sp. CC-LTT-1
	KM464086.1	Uncultured bacterium
	KF984362.1	Uncultured *Kocuria sp.*
	KM464069.1	Uncultured bacterium
	AP022830.1	*Kocuria* sp. TGY1120_3
	FJ807674.1	*Kocuria* sp. CC-012508
	KM464072.1	Uncultured bacterium
	KM464082.1	Uncultured bacterium
	KM464068.1	Uncultured bacterium
	JX064596.1	Uncultured bacterium
	AP022830.2	*Kocuria* sp.
	JN013938.1	Uncultured bacterium
	KM464077.1	Uncultured bacterium
	KM464075.1	Uncultured bacterium
	NR_116745.1	*Rothia koreensis* strain P31
	LT223585.1	*Kocuria koreensis*
	KX242261.1	*K. koreensis* strain OS10.6
	AP022830.3	*Kocuria* sp.
	JN236225.1	Uncultured bacterium
	JX064592.1	Uncultured bacterium
	JX064590.1	Uncultured bacterium
	MG557684.1	*K. koreensis* strain G8-1

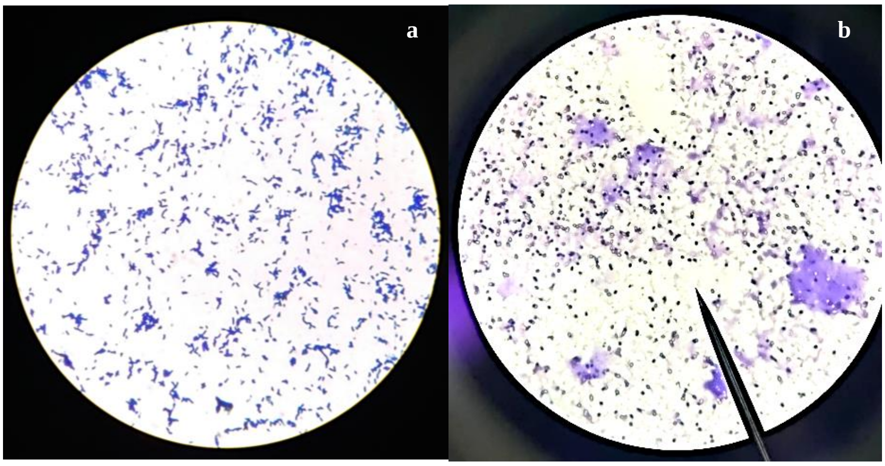

Figure 3: Bacterial identification based on Gram staining: a: Bacteria A: bacili-shaped, Gram-positive; b: Bacteria B: coccus-shaped, Gram-positive

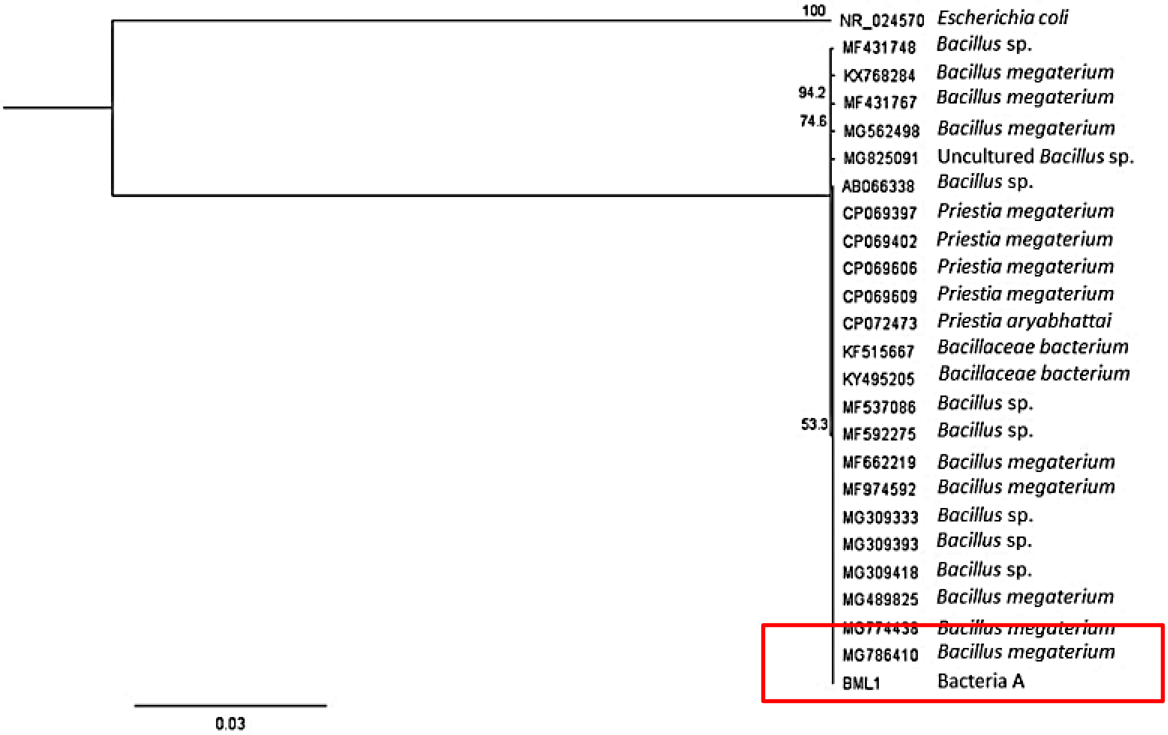

Figure 1: Phylogenetic tree showing the phylogenetic position of Bacteria A-BML 1 which has 99% similarity to *Bacillus megaterium*

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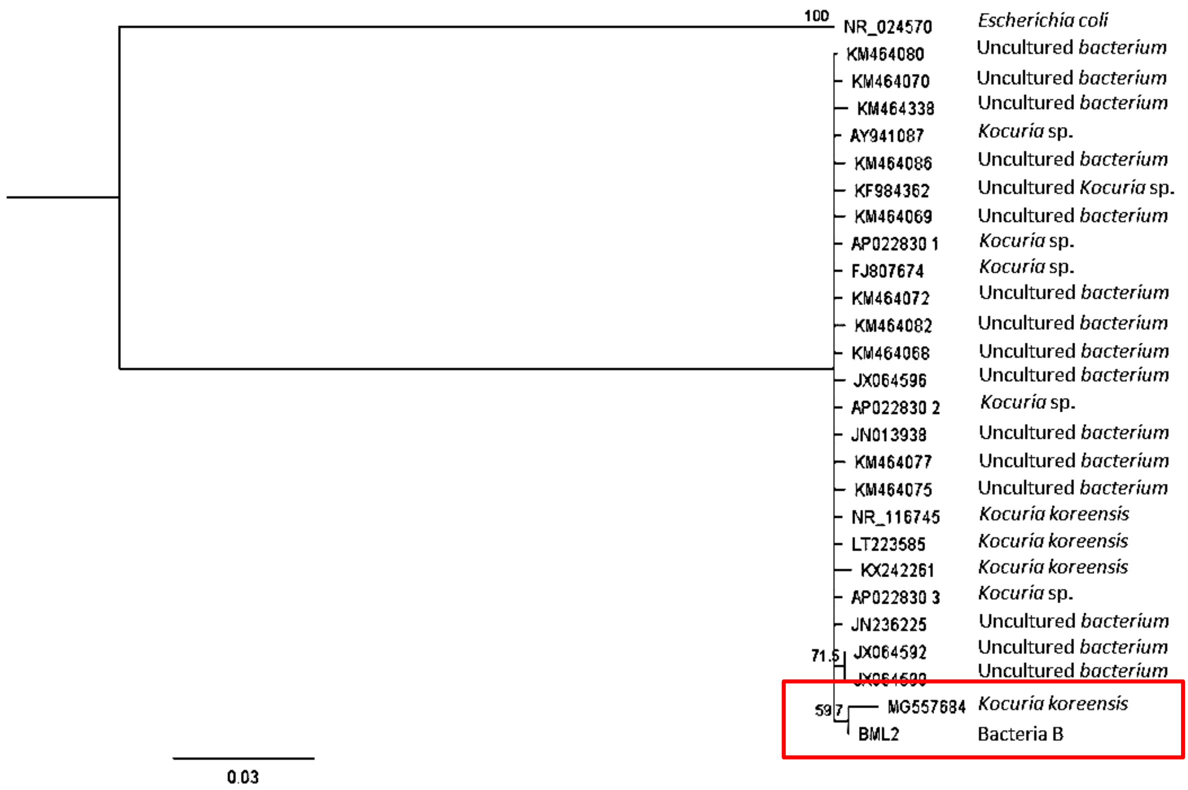

Figure 2: Phylogenetic tree indicating the phylogenetic position of bacteria B-BML 2 whichhas 99% similarity to *Kocuria koreensis*

Table 3: The results of heavy metal of Baduy palm sugar

Heavy Metals	ICUMSA Standard (mg/kg)	Samples				
		G1	G2	G3	G4	G5
Copper (Cu) (mg/kg)	Max 1.0	2.72±0.2	3.82±0.01	1.4±0.23	1.06±0.97	2.48±0.53
Lead (Pb) (mg/kg)	Max 0.5	<0.03±0.14	<0.03±1.32	<0.03±0.15	<0.03±0.21	<0.03±0.11
Arsenic (As) (mg/kg)	Max 0.5	<0.05±0.66	<0.05±0.75	<0.05±0.43	<0.05±0.79	<0.05±0.01

ICUMSA=International Commission for Uniform Methods of Sugar Analysis

Discussion

The quality of palm sugar can be influenced by several factors, one of which is the tapping process. Palm sap that is exposed to air in an open environment allows the presence of microbial contaminants and causes the palm sap to ferment. The microbes will consume the sucrose and convert it into various acids through three stages, firstly lactic acid fermentation, followed by alcoholic fermentation, and finally acetic acid fermentation (Sarkar et al., 2023). The usual microorganisms that grow in the palm sap are *Micrococcus, Streptococcus, Saccharomyces, Lactobacillus, Leuconostoc, Acetobacter*, and *Gluconobacter* (Gunawan et al., 2020; Hebbar et al., 2018).

Palm sugar originally contains mainly sucrose and amino acids (glutamine and asparagine) that play a role in the Maillard reaction and yield the brownish color in palm sugar after the heating process (Hebbar et al., 2018; Saputro et al., 2019). However, contaminated palm sap may not undergo much of a Maillard reaction when heated because the sucrose has already been consumed by the contaminating microbes which impact the quality of palm sugar in term of texture and color. In addition, the pH of palm sap will be below seven because of acid produced by microorganism activity and affects the sour taste which is irrelevant for palm sugar (Kurniawan et al., 2018).

TPC is a test to determine the number of microbial mesophilic organisms (pathogens and non-pathogens) that grow under aerobic conditions at moderate temperatures of 20-45 °C. This test contributes information about the sanitary and hygienic status of raw materials handling, processing conditions, storage conditions, and finishing goods. Furthermore, this test can determine the shelf life or sensory changes in food products (Mendonca et al., 2020). The palm sap of Baduy was tapped in lodong which is made of bamboo. In fact, bamboo may contribute to increase TPC value as Gunawan et al. (2020) declared in their report. It was expressed that TPC value in bamboo containers was greater than in stainless containers because of the difficulty in sterilizing of the bamboo containers, therefore preventing the growth of microbial contaminants was impossible. Besides that, the bacteria associated with bamboo contributed to the initial TPC value. Kumar et al. (2022) stated that the initial TPC value of bacteria associated with Bamboo was 6.55±0.91 to 7.86±1.21 log CFU/g with the majority from *Bacillus*, *Enterobacter*, and *Lactobacillus*. Phaichamnan et al. (2010) who analyzed the TPC value of Thai palm sugar declared as well that the tapping process in open conditions using bamboo permits contamination by various microorganisms. The results of his research manifested that the TPC value for Thai palm sugar was 10^3-10^6 CFU/g, in this case much higher than the maximum limit of Thai regulations, namely 500 CFU/g. Similar to Gunawan et al. (2020), Phaichamnan et al. (2010) also expressed that bamboo tubes sometimes are not considered to be cleaned before and after usages which leads to the high TPC value.

Contrary to the previous study, all samples in this research have TPC values below the ICUMSA limit, even, no bacterial growth was found in samples G2, G4, and G5, It represent that the raw materials, processing conditions, and storage conditions of Baduy palm sugar were hygienic and well sanitized even though processed using traditional tools and methods. Baduy palm sugar makers interpret the concept of food safety even though they never receive education about food safety. It might have happened because they really maintain the environment sustainably in accordance with the testament of their ancestors as written in the *Pikukuh* (Asteria et al., 2021; Sumawijaya et al., 2020), there should be no environmental changes caused by chemical, biological, and physical damage.

The results of TYMC of this research indicated there was no yeast and mold growth. In fact, even if the bamboo used for the tapping process is not cleaned before and after usage, the microbes present in the palm sap will be destroyed after heating, except for several osmophilic yeasts and fungi. Monisha and Mariyanancyarputha (2022) reported that there were molds in Indian palm sugar after process of heating, i.e., *Aspergillus flavus*, *A. niger*, and *A. fumigatus* that produced some mycotoxins, such as aflatoxin B1 and G1, citrinin, and ochratoxin. The presence of various molds in palm sugar is caused by the inverted sugar content which directly impacts the humidity conditions. In general, yeast and mold are more easily consumed the invert sugar than sucrose. The invert sugar attracts water from the outside which causes the humidity of the product to increase. The increased humidity causes mold to grow well (Monisha and Mariyanancyarputha, 2022). In the other hand, authors have already tested the sucrose content of the samples in previous research (Elfriede et al., 2023). Our previous research demonstrated that sucrose content in Baduy palm sugar samples was too high, ranged of 84-86% (w/w), it was far from the maximum limit of Indonesian standard which was only 77% (w/w). Therefore, this might be the reason for the absence of yeast and mold growth in our samples. Nevertheless, the sucrose content of our samples should be descended by increasing the heating time and maintaining the humidity in order to comply with the regulations.

The coliform analysis aims to determine the presence of coliform which is an indicator of the presence of other harmful pathogenic bacteria, such as *Salmonella*. Nevertheless, controlling the coliform can be performed by heating process only (MacArthur et al., 2021). Coliform is a bacterium that has the characteristics of rod-shaped, Gram-negative, facultative anaerobic, and ferments the lactose content in the media to produce acid and gas when incubated (Khasanah et al., 2021). MacArthur et al. (2021) declared that the presence of coliform in palm sugar might be due to the ineffectiveness of the processing methods used, poor package and storage conditions. According to the result of coliform analysis, all samples indicated negative results. Referring to the TPC results, it manifests the found bacteria in sample G1 was not coliform. The package of Baduy palm sugar was kararas which were made by dried banana leaves that protected it from the outside. Banana leaves have an antibacterial substance that can inhibit the growth of *Escherichia coli* which belongs to coliform, accordingly they are appropriate for bio-package of perishable food products (Egbuonu et al., 2016; Mostafa, 2021).

Two bacterial colonies found in TPC analysis were cultured for further investigation, namely bacterial identification based on Gram staining and 16S rDNA analysis. Bacteria A was *B. megaterium* which is a Gram-positive, bacilli-shaped, aerobic spore-forming, neutralophilic aerobic, grows at wide temperatures from 3 to 45° C, with an optimal temperature of around 30 °C, found in diverse habitats but commonly in soil (Goswami et al., 2018). *B. megaterium* is one of a large number of *Bacillus* that plays a satisfactory role force in the industrial production of enzymes, and other chemicals such as amylase, vitamin B12, and glucose dehydrogenase. It belongs to the class of probiotic bacteria and is non-pathogenic. It has volatile organic compounds metabolites which have antifungal properties and can suppress the growth of mycotoxins and colonies of several fungi including *A. flavus*, *Penicillium verrucosum*, and *Fusarium verticillioides* (Saleh et al., 2021), this can be one of the factors why the growth of yeast and molds with the TPC method indicates low yields. This bacteria produces bacteriocin such as megacin that can act as natural food preservatives, hence this compound is most likely to suppress bacterial growth which causes only a few bacterial colonies that grew in Baduy palm sugar.

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Moreover, Malanicheva et al. (2012) reported that *B. megaterium* has peptides antibiotic that is identical to bacimethrin which can inhibit the growth of bacterial contaminants such as *Leuconostoc mesenteroides*, *Pseudomonas aeruginosa*, and *E. coli.*

Bacteria B was *K. koreensis* which is a Gram-positive, non-motile, aerobic, and coccoid-shaped bacterium with a diameter of 1.0-1.5 µm. Optimal growth occurs at 30-37 °C (Park et al., 2010). This bacterium is a non-pathogenic bacterium due to its ubiquitous presence and as normal flora of the skin and mucous membranes in humans and animals (Kandi et al., 2016). However, its presence in Baduy palm sugar can be caused by the water used during the production or the final product that is in contact with Baduy palm sugar maker skin. Baduy people usually consume the water of Ciujung river which upstream is in a conserved area for their activities such as bathing, cooking, washing, or other activities. However, in their all activities, they never use chemical substances, therefore the whole environment is really still conserved.

Heavy metals can be dangerous for human health because they cause gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, and cancer. Heavy metals react with biological systems by losing electrons and forming metal cations that have an affinity to the vital macromolecules in various human organs (Balali-Mood et al., 2021). The contamination of heavy metals in food products can be found in the water and tools used during processing, kitchen, and storage, or in food plants given pesticides containing metals (Alengebawy et al., 2021; Morgan, 1999). As a matter of fact, the heavy metal content in food can be decreased by processing it in boiling water because the metal is soluble in water. Lee et al. (2019) pointed out that heavy metals in foods are water-soluble and can be decreased by processing them with water. However, problems will occur if the used water is contaminated with heavy metals, which will be absorbed into food components. In this study, the Pb, and As of 5 samples of Baduy palm sugar were under 1.0 mg/kg. Research by Dos Santos et al. (2018) reported that the Pb content in sugar cane was 0.043-0.200 mg/kg, which was not too different from this study. On the other research, Pb in raw sugar in Sudan was 6.74 mg/kg, Hg was 0.02 mg/kg, and As 2.73 mg/kg (Babeker et al., 2022), which was higher than in this study. It means that the palm sugars in this study are free from Pb and As. But, based on ICUMSA (2014) which said that the Cu in sugar should not be more than 1.0 mg/kg, the samples might be contaminated by Cu. However, in fact, the regulations of each country are different. Indonesia has a tolerant value for Cu of 10.0 mg/kg. It proved that either processing, kitchen and storage, or food plants of Baduy palm sugar were free from heavy metals.

The interesting issue about Baduy palm sugar is that although Baduy people do not have enough information or knowledge about food safety, they can make palm sugar safe for consumption with regard to microbial contaminants and heavy metals content. This is because they really keep the testament of their ancestors as written in the *Pikukuh*. In addition, according to Umarella et al. (2020), Baduy was featured in the 100 wonderfull Indonesia tourism event, which garnered considerable interest from both local and foreign tourist. Total number of tourists who visited Baduy always increased in the last three years, even reached the number of 42,228 in 2019 which consists of 42,174 local tourists and 54 foreign tourists. This is an opportunity for Baduy people to introduce globally their area which has palm sugar as an authentic local sweetener that is safe for consumption and can be brought by the tourists.

Conclusion

The five samples of Baduy palm sugar were proven to comply with the requirement of ICUMSA regarding the maximum limit of microbial contamination. The identified bacteria were *B. megaterium* and *K. koreensis* where both bacteria were non-pathogenic, therefore they did not interfere with the safety of Baduy palm sugar. All samples complied with the requirement of ICUMSA about the maximum limit of heavy metals in palm sugar for Pb and As, but not for Cu. The Pb and As content found in all samples were very low or nearly nothing, i.e. less than 1.0 mg/kg for Pb and As, whereas Cu was found in samples were 1.04-3.82 mg/kg. However, the regulations of each country are different. Indonesia has a tolerant value for Cu of 10.0 mg/kg. Due to this fact, it can be inferred that Baduy palm sugar was safe to consume. Although the number of samples evaluated in this study was limited, this research is expected to contain useful information to the public about the safety and quality of Baduy palm sugar, therefore it can attract the attention of Indonesian tourists and even foreign tourists to visit Baduy.

Author contributions

R.T.K.D. designed the research, took the samples, interpreted the data, wrote and translated the manuscript; D.P.E. designed the research, took the samples, and edited the manuscript; F. designed the research, and edited the manuscript; S.L. collected and recorded the data. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there was no conflict of interest in this study.

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