

Identification of lactic acid bacteria isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is inherited from ancestors who utilizes local food and that have distinctive tastes. The Rejang tribe is a native Bengkulu community who processes bamboo shoots into a fermented product known as *Lemea*, which is only found in Bengkulu province. *Lemea* is a source of indigenous lactic acid bacteria (LAB). This study aimed to isolate and identify lactic acid bacteria (LAB) strains in *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 hrs. Different types of bamboo shoots were expected to contain different types of LAB. Betung shoots (*Dendrocalamus asper* Schult) and yellow bamboo shoots (*Bambusa vulgaris* Schrad) have been used. Isolation of the LAB was performed through serial dilution of 10 g homogenized *Lemea* sample and spread on MRSA plates for each dilution series. Plates were incubated at 37°C for 48 hrs to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found four isolates from two types of *Lemea*. After identification, it was known that the four isolates were bacteria of the genus *Lactobacillus*. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots (*Bambusa vulgaris* Schrad), while *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult).

1. Introduction

The Rejang are the third-largest tribe in Bengkulu Province, after the Serawai and Basema. North Bengkulu, Central Bengkulu, Rejang Lebong, Lebong, and Kepahyang are the five districts where the Rejang people live. *Lemea* is an ethnic food from the Rejang tribe. Ethnic foods are meals that have their origins in an ethnic group's history and culture (Kwon, 2015). Bamboo shoots and river fish are fermented into *Lemea* by the Rejang people (Dewi *et al.*, 2014). Betung, Tabah, Mayan, and Seik bamboo are some of the most common bamboo varieties used by the Rejang to produce *Lemea*. Betok, kepala timah, and mujahir fish are the most common fish used. The odours and flavours are unique, and only the locals enjoy it. LAB has an impact on the flavour of fermented foods (Fox, 2011). Indigenous fermented foods have been extensively researched.

There are various fermented foods in the world, especially in Asia. Fermented foods made from bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus* (Kiran *et al.*, 2016).

Meakri, from Meghalaya Indian fermented bamboo shoots as a source of *Lactobacillus*, has characteristics suitable for probiotics (Das *et al.*, 2020). *Lactobacillus* is a group of LAB that are gram-positive bacteria. LAB have the potential to inhibit the infection and growth of 2 pathogenic microbes (Yang *et al.*, 2021). LAB isolated from fermented bamboo shoots are potential probiotic candidates that are beneficial for health (Mohamad *et al.*, 2020). LAB strains are selected for their decreased content of cholesterol, antioxidant activity, and antibacterial activity (Jitpakdee *et al.*, 2022). Isolation of lactic acid bacteria from indigenous fermented foods is very important (Mende *et al.*, 2022).

Bekasam is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional fermented food popular in Sumatera and Kalimantan (Desniar *et al.*, 2013). The sour taste in *Bekasam* is almost the same as in *Lemea*. The difference between these two products is the carbohydrate source and fermentation time. Carbohydrate sources are a source of nutrition for bacteria that play a role in the fermentation process. The

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source of carbohydrates used for rice in *Lemea* is bamboo shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes 10 days.

Isolates from several bamboo shoot products have been found. In pickled bamboo shoots, 88 isolates were found, and 3 of them had potential as probiotics (Wasis et al., 2019). A total of 180 LAB isolates have been isolated from Indonesian fermented foods (Sukmarini et al., 2014). Research on the effect of different types of bamboo shoots on strains of LAB produced during *Lemea* processing has not been carried out.

Therefore, this research is very important to be carried out at this time because there is still a scarcity of information. Studies on the molecular identification of *Lemea* isolates are still needed. This study aimed to identify and isolate LAB strains in fermented betung bamboo shoots (*Dendrocalamus Asper* Schult) and yellow bamboo shoots (*Bambusa Vulgaris* Schrad).

2. Materials and methods

2.1 Sample preparation

Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are thinly sliced and soaked for 30 hrs. Then they were washed, filtered, and weighed as much as 400 g and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for 48 hrs (Figure 1).

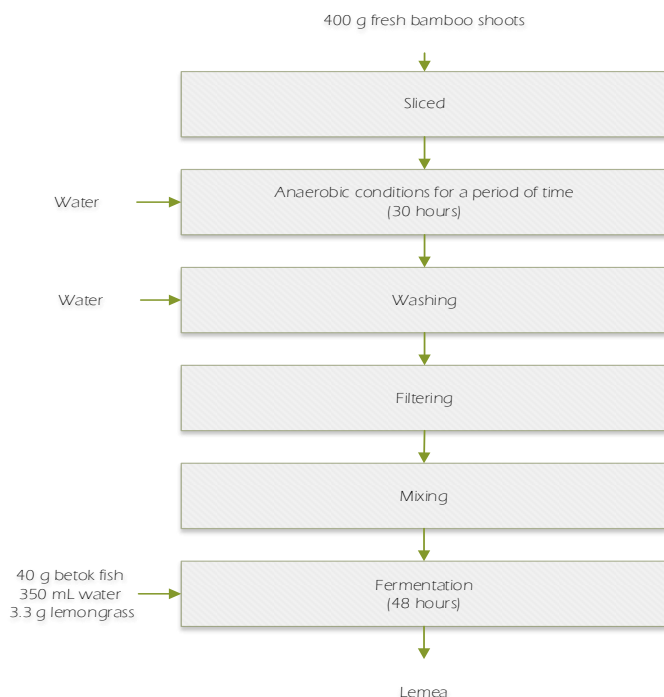


Figure 1. Fermented bamboo shoots making process

2.2 Lactic acid bacteria isolation

Isolation LAB was carried out using a device that was sterilized before hand and carried out aseptically

where 10 g of the *Lemea* sample was homogenized and then serially diluted with 1 percent sterile NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at 37°C for 48 hrs. The isolates obtained need to be purified and identified. Purification was carried out by the plate scratch method, which was repeated so that pure isolates were found. The purification process is perfect and will produce separate colonies between strokes. The selected colonies are then identified to determine the strain of the colonies obtained.

2.3 Molecular identification of lactic acid bacteria

Molecular identification was based on 16S rRNA gene amplification with genomic DNA isolation, DNA amplification, sequencing and analysis of nucleotide sequences in GenBank following the method by Veljovic et al. (2007).

2.3.1 Deoxyribonucleic acid isolation

DNA isolation was done using the Genomic DNA extraction with Presta™ Mini GDNA Bacteria Kit (Geneaid, GBB100). Stages of isolation based on the procedure kit.

2.3.2 Deoxyribonucleic acid amplification

DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with Zymoclean™ Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1 µL of the PCR product was assessed with 0.8% TBE agarose.

2.3.3 Deoxyribonucleic acid sequencing and phylogenetic analysis

Sequencing and analysis of nucleotide sequences in GenBank. Analysis of grouping arrangement performed by comparing obtained (inquiry) with those already in the Gene Bank, with the information base hunted on the NCBI webpage (<http://www.ncbi.nlm.nih.gov>) using Impact (Basic Local Alignment Search Tool). The size of the PCR amplification fragment was determined by comparing the position of the DNA marker size (Marker) with the sample fragment size.

3. Results and discussion

3.1 Isolation of lactic acid bacteria from Lemea

Isolation found four bacterial isolates from the two types of *Lemea* samples. *Lemea* made from betung bamboo shoots found two isolates and two isolates from yellow bamboo shoots. The isolates found were coded

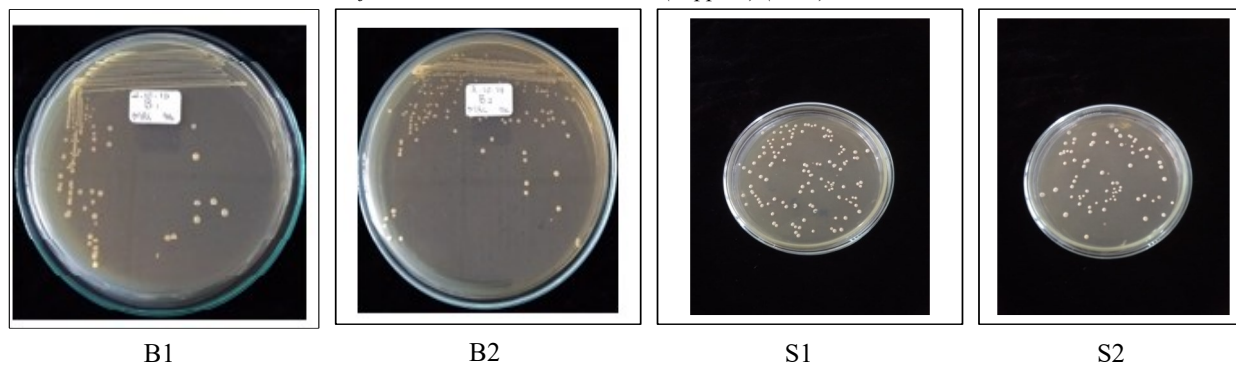


Figure 2. Isolate B1, B2, S1 and S2.

B1, B2, S1 and S2 (Figure 2). The bacterial isolates found were lactic acid bacteria because they were able to grow on MRSA specific media with cocci characteristics, a milky white color with a convex surface and smooth edges. The number of isolates obtained was less than that of *mesu*, *soidon*, *soibum*, and *soijon* but the same as unfermented bamboo shoots (Tamang *et al.*, 2008). The morphological characteristics of the isolates found in this study were almost the same as the previous findings isolated from *Lemea* produced by a cottage industry in Kepahyang Regency, Bengkulu (Kurnia *et al.*, 2020).

3.2 Identification of lemea isolates

The results of genomic DNA amplification of the 4 isolates can be seen in Figures 3a and 3b. Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size. Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434 bp for isolate B1 (Figure 4a), 1424 bp for B2 (Figure 4b). The nucleotide sequence S1 isolate was 882 bp (Figure 4c) and 849 bp for the S2 isolate (Figure 4d). The results of the analysis using the BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.

The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% like the partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of *L. plantarum* but not *L. fermentum*. Based on the phylogeny tree, isolate B1 was closely related to *L. plantarum* strain KK53 16S ribosomal RNA (Figure 5a), Isolate B2 with *L. plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b). S1 isolate was closely related to *L. plantarum* strain R4 16S ribosomal RNA (Figure 5c), isolate S2 was closely related to *L. fermentum* strain 8179 and 6567 16S ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was *L. plantarum* (Sukmarini *et al.*, 2014). All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis*, *Lactobacillus brevis*, and *L. plantarum* were found in dominating strains of Soidon fermented bamboo shoot food without salt from Indian Manipur (Jeyaram *et al.*, 2010). LAB strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) have been previously studied and isolated as 2 species, namely *L. plantarum* and *Lactobacillus rossiae* (Okfrianti *et al.*, 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *L. plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated

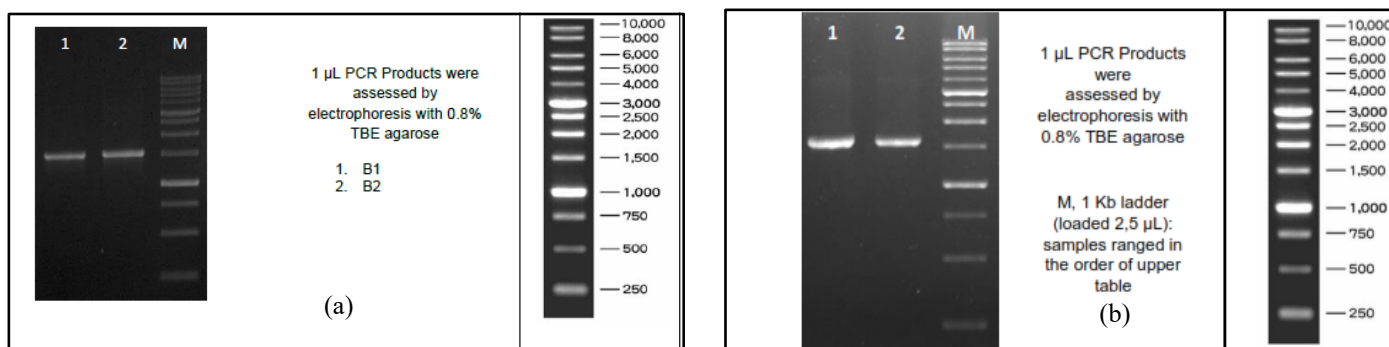


Figure 3. Genomic 16S rRNA amplification of (a) isolates B1 and B2 and (b) isolates S1 and S2. M: Kappa universal ladder.

Table 1. Comparison of homology levels of the 16S rRNA gene of LAB isolates with several sequences.

Types of Bamboo Shoots	Isolate Code	Species	GenBank Accession Number	Similarity (%)
Bamboo Betung	B1	<i>Lactobacillus plantarum</i>	MN37236.01	99
	B2	<i>Lactobacillus plantarum</i>	MN972325.1	99
Yellow Bamboo	S1	<i>Lactobacillus plantarum</i>	KM350169.1	99
	S2	<i>Lactobacillus fermentum</i>	MT538927.1	99



Figure 4. Nucleotide sequences of (a) B1, (b) B2, (c) S1 and (d) S2 isolates.

from different types of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the diversity of the bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria* and *Proteobacteria* (Li et al., 2021).

Lactobacillus fermentum was only found in *Lemea* made from yellow bamboo shoots and *L. plantarum* was found in yellow bamboo shoots and betung. Prebiotics in foodstuffs can trigger the growth of *Lactobacillus* (Macfarlane and Cummings, 1999). Oligosaccharides and fiber are prebiotics that promote the growth of specific bacteria found in the gut. Bamboo shoots are a good source of fibre (Felisberto et al., 2017). At 100 g of fresh weight, the yellow bamboo shoots contain 6.51 g of carbohydrates, 4.24 g of fibre while betung contained 4.90 g of carbohydrates and 3.54 g of fibre (Chongtham et al., 2011). Different bamboo species contained different macronutrients (Adebola et al., 2014). The differences in the content of bamboo shoots may affect the types of bacteria found in *Lemea* products.

4. Conclusion

A total of four *Lactobacillus* isolates were found in *Lemea* were from the *Lactobacillus* genus. *L. plantarum* and *L. fermentum* were isolates which were identified molecularly by 16S rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo

shoots while *L. plantarum* was found in yellow bamboo shoots and betung.

Conflict of interest

The authors declare no conflict of interest.

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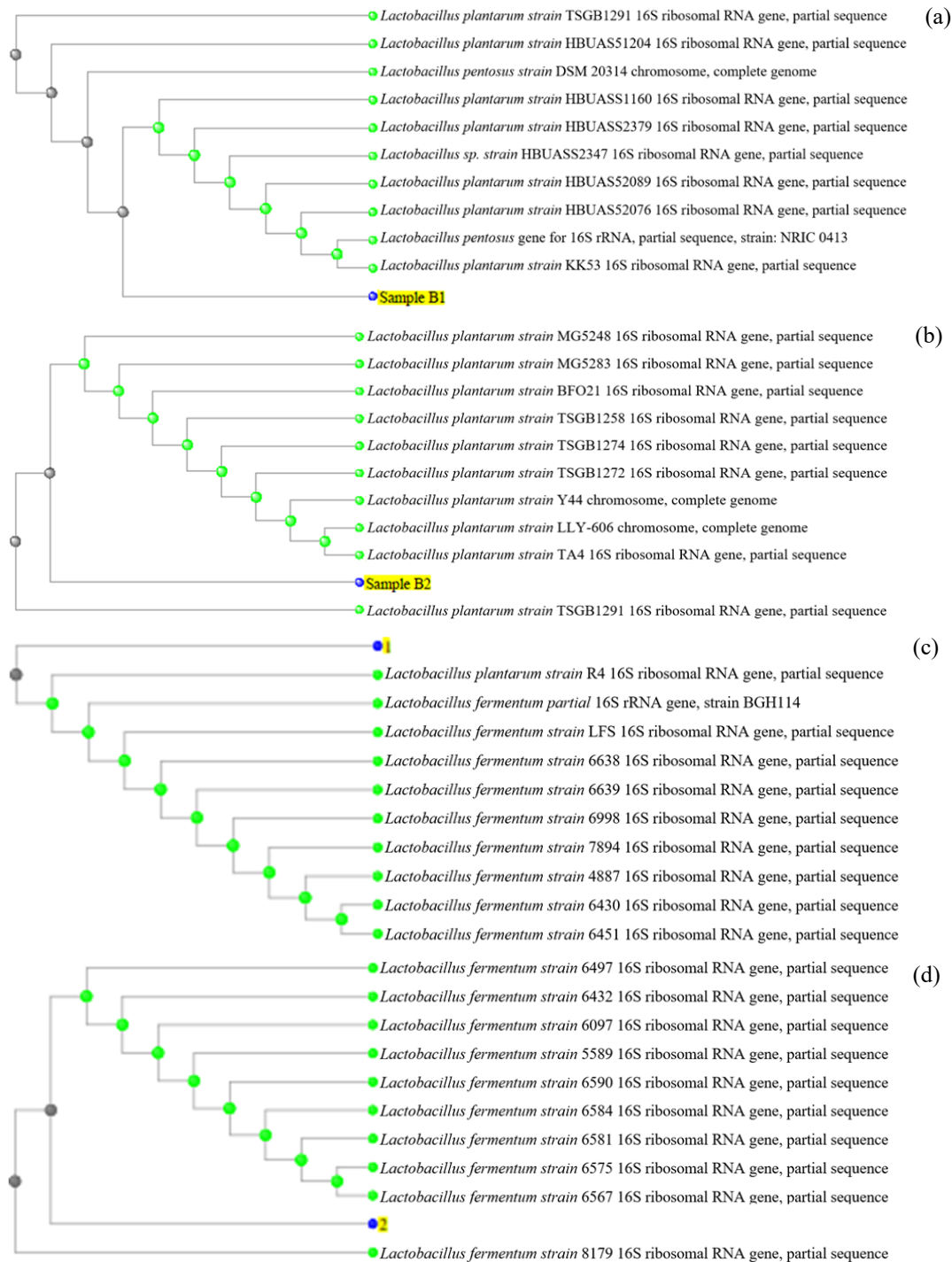


Figure 5. Phylogenetic tree of (a) B1, (b) B2, (c) S1 and (d) S2 isolates

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