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Evaluasi Riviewer 1

FOOD RESEARCH

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Date : 5th February 2022

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Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
1. Appropriateness of Contents			x		
2. Originality of Topic		x			
3. Manuscript Format			x		
4. Research Methodology				x	
5. Data Analysis				x	
6. Relevance to the Journal		x			

FOOD RESEARCH

(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS		(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
1.	**Title : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria** *It should reflect the article*: o The title does not very much reflect the contain of the article. o There is not any information and discussions about the ethnic food fermentation from Bengkulu in the results and discussion. o The content of this manuscript is isolation and identification of LAB from fermented bamboo shoot	
2.	**Abstract** *Background, Aim, Methodology and Conclusion*: o The background of the research is not clear. It is not stated explicitly and clearly o It is more appropriate if the study aim is to isolate and identified LAB from fermented Betung bamboo shoot, and yellow bamboo shoot o Methodology in the abstract is quite clear o Conclusion: the author wanted to find LAB in fermented bamboo shoots. Bacteria that has been found were gram positive bacteria and a group of lactic acid bacteria is not a conclusion	
3.	**Keywords: -** *Min. 3 and Max. 6*	
4.	**Introduction** *Concise with sufficient background* o Not enough information about this fermented betung bamboo shoots such as the raw materials and ingredients, the condition and time of fermentation process, and description of the product. o Related research and similar	

	traditional fermented product reported by other researchers are not evaluated. ○ The background is not clear. The purpose of the research is to investigate LAB strains in fermented Betung bamboo shoots, but the MM and results are isolation and identification of LAB	
5.	**Research design/Methodology** *Clearly described and reproducible*: ○ The research design/methodology is not described clearly ○ Sample preparation: the quantity of all the materials were not mentioned. ○ The time of fermentation in the MM is different from the diagram in figure 1, and also different from the one in the introduction ○ Isolation and identification steps need to be written clearly step by step	
6.	**Data Analysis** *Results well presented and discussed* ○ Results are not well presented and discussed ○ The authors do not explain how many isolates are obtained from MRSA. ○ There was not any discussion. ○ Only shows the facts, d anvery limited information	
7.	**Conclusion** *A clear summary of the study* ○ The conclusion written by the authors is not a conclusion since *L. plantarum* and *L. fermentum* are gram positive bacteria and LAB	
8.	**References** *References should follow the journal's format*	
9.	**English Proficiency:** Some grammatical errors	
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	reviewer about the article: ○ The article should be rewrite from abstract to conclusion for proper articles. ○ Results and discussion are very limited. They should be presented clearly, every step and discussed in more comprehensive supported by suitable references ○ There should be clear correlation among back ground, aim, MM, results and conclusion	

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8. Originality of Topic		v			
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12. Relevance to the Journal		v			

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11.	**Title** The title does not yet describe the method use d. There should be a word isolation of lactic a cid bacteria.	
12.	**Abstract** *It has not given the importance of isolating lactic acid b acteria from the product. Methods need to be summarize d*	
13.	**Keywords** *It should be added to the product name.*	
14.	**Introduction** *It has not yet described the importance of isolating lacti c acid bacteria from the product.*	
15.	**Research design/Methodology** *Method clearly described and reproducible.* .	
16.	**Data Analysis** *It is necessary to add a discussion about the differences in lactic acid bacteria obtained from differences in bamb oo types that are strengthened by reference.*	
17.	**Conclusion** *It should be associated with the source of lactic acid bac teria isolation*	

18.	**References** *Ok*	
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20.	**Additional comments/suggestions by the reviewer about the article**	

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Hasil Koreksi dari Riviewer 1

Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

Abstract

In order to avoid extinction, strains of lactic acid bacteria must be studied. This study aims to find Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus Asper* Schult) and Yellow bamboo shoots (*Bambusa Vulgaris* Schrad). Due to the ethnic diet of the rejang tribe, this fermented foodstuff is only found in Bengkulu province. Which was made by combining bamboo shoots with river fish and allowing it to stand for a few days until a distinct aroma emerges. The LAB identification of began with homogenizing 10 g of the samples, which then get serially diluted with 1 percent sterile NaCl and spreaded on MRSA media. The isolates were identified molecularly using 16S rRNA gene amplification, which included the procedures of isolating genomic DNA, amplification using DNA, sequencing, and nucleotide sequence analysis on GenBank. *Lactobacillus plantarum* strain B1 and *Lactobacillus plantarum* strain B2 were identified on fermented Betung bamboo shoots, while *Lactobacillus plantarum* S1 and *Lactobacillus fermentum* S2 on Yellow Bamboo shoots. *Lactobacillus plantarum* and *fermentum* isolated from fermented bamboo shoots were gram positive bacteria and a group of lactic acid bacteria.

Keywords: Lactic Acid Bacteria, Bamboo shoot, Ethnic Food Fermentation, PCR, 16S rRNA

1. Introduction

There is a great need to develop the traditional food which comprises the ethnic diet of a particular region. Moreover, it is also important to prevent the extinction of traditional foods such as fermented foods that contain lactic acid bacteria (Dewi *et al*., 2014). One of the tribes that has fermented food and needs to be maintained is the Rejang tribe, which is the largest tribe in Bengkulu (Dewi, 2015).

There are a variety of fermented foods in the world especially in Asia. Most of Asian fermented foods are non-dairy products featuring various other raw materials such as cereals, soybeans, fruit, vegetables and fish and other products (Rhee *et al*., 2011). Indonesia is rich in fermented foods including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al*., 2021).

River fish that are often used to make lemea are freshwater fish. One of the freshwater fish that can be used is betok fish, lemea containing lactic acid bacteria (Xu *et al*., 2021). Lactic acid bacteria consist of microbial strains that convert carbohydrates into lactic acid (Dinoto *et al*., 2020), LAB isolated results were identified based on phenotypic and genotypic characteristics (Lawalata *et al*., 2011). Genotypic LAB identification was carried out by PCR technique in the form of amplifying and sequencing the universal region of the 16S rRNA gene (Dinoto *et al*., 2020). Lactic Acid Bacteria (LAB) strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) has been previously studied. . The purpose of this study was to investigate Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus asper* Schult) and Yellow bamboo shoots (*Bambusa vulgaris* Schrad).

Commented [A1]: Please give the latin name of fish

2. Materials and methods

2.1 Sample Preparation

The process of processing fermented bamboo shoots begins with slicing thin samples and soaking them for 30 hours, then washing and filtering them. The next step is fermentation for 48 hours at room temperature by mixing sliced bamboo shoots, betok fish, lemongrass and salt and water (Figure 1)

2.2 LAB isolation

Isolation of LAB was carried out using a device that was sterilized beforehand and carried out aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1% NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at 37^{o}C for 48 hours. The isolates obtained need to be purified and identified

2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)

Molecular identification based on 16S rRNA gene amplification with genomic DNA isolation, DNA amplification, sequencing and analysis of nucleotide sequences in GenBank (Widodo *et al*., 2017). DNA isolation was Genomic DNA extraction with Presta TM Mini GDNA Bacteria Kit (Geneaid, GBB100). DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1uL of the PCR product was assessed with 0.8% TBE agarose.

3. Results and discussion/Results

Bacterial sequence homology based on 16S rRNA gene sequencing analysis. The types of lactic acid bacteria species on bamboo shoots of betung and yellow bamboo have 99% similarity. B1, B2, and S1 are closely related to *Lactobacillus plantarum*, this type of bacteria is known as lactic acid bacteria which has the potential as biopreservative because it can inhibit the growth of pathogens and destructive with greater inhibition than other lactic acid bacteria. (Azizah *et al*., 2019). *L. plantarum* is amylolytic which converts starch to lactic acid (Noor *et al*., 2018). S2 is closely related to *Lactobacillus fermentum*, this type of bacteria is known as lactic acid bacteria which belongs to heterofermentative species and can be found in the human intestinal tract where these bacteria can live at the pH of the digestive tract (Manin, 2010). B1 and B2 are isolate codes derived from fermented bamboo shoots. S1 and S2 codes for isolate fermented yellow bamboo shoots. (Table 1). Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of isolates **B**1, B2, S1 and **S**2 at 0.8% TBE agarose concentration using the Kappa Universal ladder (Figures 3a and 3b). hylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots contain more lactic acid bacteria in the form of *Lactobacillus fermentum.*

4. Conclusion

Lactobacillus plantarum and *fermentum* isolated from fermented bamboo shoots were found to be gram positive bacteria and belong to a group of lactic acid bacteria.

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the Poltekkes Kemenkes Bengkulu, Indonesia in 2020

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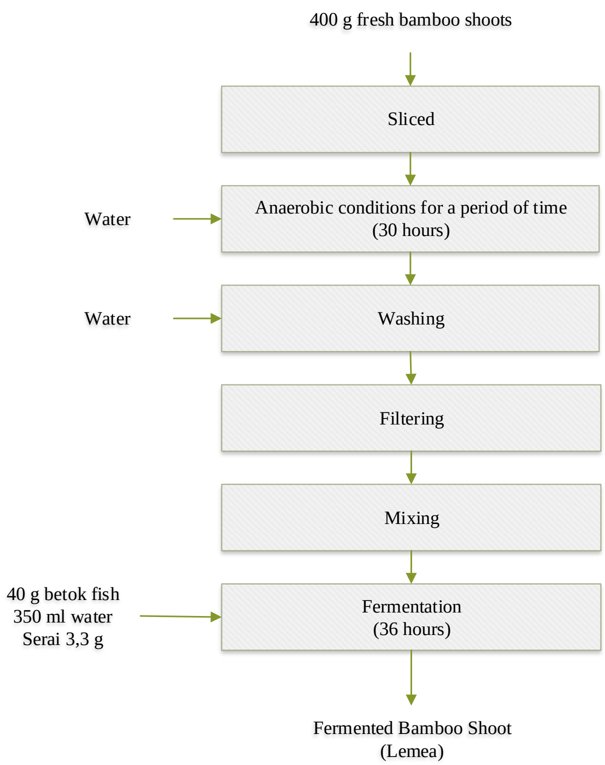
Xu, Y., Zang, J., Regenstein, J. M. and Xia, W. (2021). Technological Roles of Microorganisms in Fish Fermentation: a Review. *Critical Reviews in Food Science and Nutrition*, 61(6), 1000–1012. https://doi.org/10.1080/10408398.2020.1750342

Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

144 Figure 1. Fermented Bamboo shoot making process



145

Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

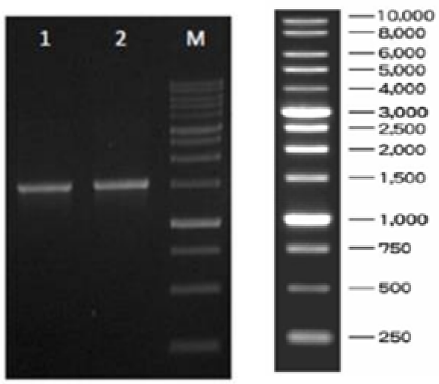


Figure 2b. Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

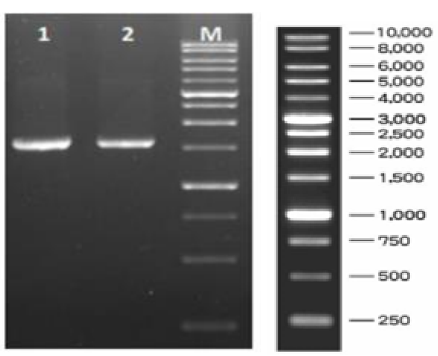


Figure 3a. Sequence assembly result – PCR product Isolate Code B1

Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 3b. Sequence assembly result – PCR product Isolate Code B2

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 3c. Sequence assembly result – PCR product Isolate Code S1

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 3d. Sequence assembly result – PCR product Isolate Code S2

Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
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Figure 4a. Phylogenetic tree Isolate Code B1

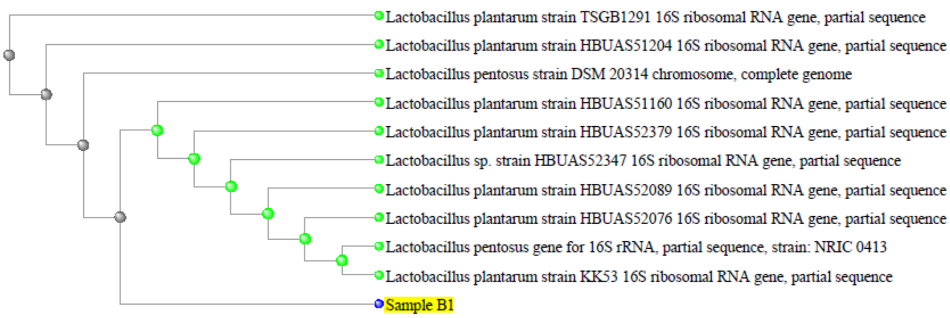


Figure 4b. Phylogenetic tree Isolate Code B2

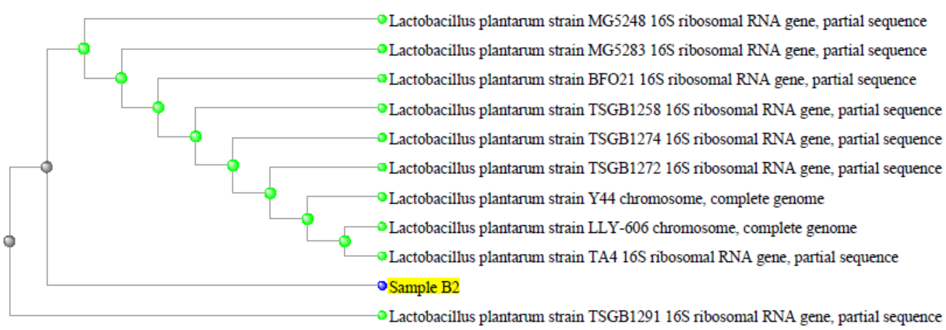


Figure 4c. Phylogenetic tree Isolate Code S1

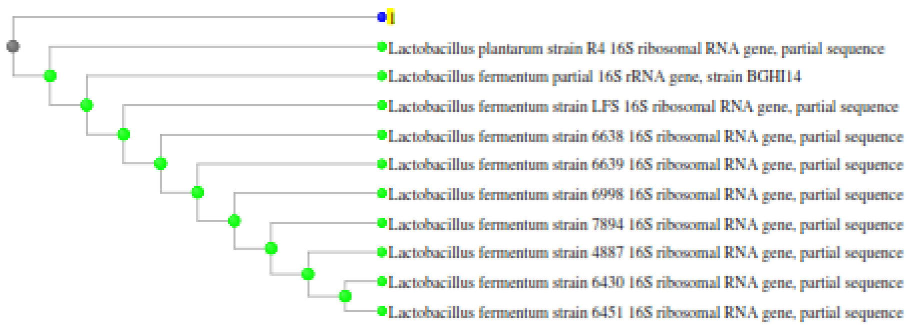
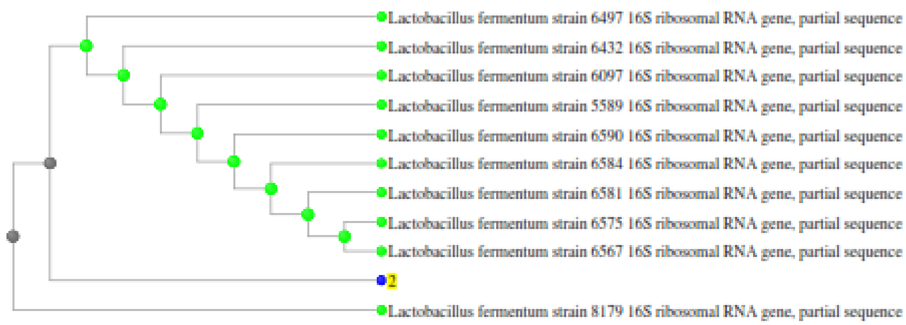


Figure 4d. Phylogenetic tree Isolate Code S2



Hasil Koreksian dari Riviewer 2

Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

Abstract

In order to avoid extinction, strains of lactic acid bacteria must be studied. This study aims to find Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus Asper* Schult) and Yellow bamboo shoots (*Bambusa Vulgaris* Schrad). Due to the ethnic diet of the rejang tribe, this fermented foodstuff is only found in Bengkulu province. Which was made by combining bamboo shoots with river fish and allowing it to stand for a few days until a distinct aroma emerges. The LAB identification of began with homogenizing 10 g of the samples, which then get serially diluted with 1 percent sterile NaCl and spreaded on MRSA media. The isolates were identified molecularly using 16S rRNA gene amplification, which included the procedures of isolating genomic DNA, amplification using DNA, sequencing, and nucleotide sequence analysis on GenBank. *Lactobacillus plantarum* strain B1 and *Lactobacillus plantarum* strain B2 were identified on fermented Betung bamboo shoots, while *Lactobacillus plantarum* S1 and *Lactobacillus fermentum* S2 on Yellow Bamboo shoots. *Lactobacillus plantarum* and *fermentum* isolated from fermented bamboo shoots were gram positive bacteria and a group of lactic acid bacteria.

Keywords: Lactic Acid Bacteria, Bamboo shoot, Ethnic Food Fermentation, PCR, 16S rRNA

5. Introduction

There is a great need to develop the traditional food which comprises the ethnic diet of a particular region. Moreover, it is also important to prevent the extinction of traditional foods such as fermented foods that contain lactic acid bacteria (Dewi *et al*., 2014). One of the tribes that has fermented food and needs to be maintained is the Rejang tribe, which is the largest tribe in Bengkulu (Dewi, 2015).

There are a variety of fermented foods in the world especially in Asia. Most of Asian fermented foods are non-dairy products featuring various other raw materials such as cereals, soybeans, fruit, vegetables and fish and other products (Rhee *et al*., 2011). Indonesia is rich in fermented foods

Commented [MOU2]: How many ethnic food fermentation in Bengkulu; If there are many ethnic fermented food and the authors only focus on the one type of fermented food better say so

Commented [MOU3]: Can we just study LAB to avoid extinction of these bacteria?

Commented [MOU4R3]:

Commented [MOU5]: To find or to isolate and identify?

Commented [MOU6]: Do you mean isolation

Commented [MOU7]: How many isolates were obtained from the samples?

Commented [MOU8R7]:

Commented [MOU9R7]:

Commented [MOU10]: L. plantarum and L. fermentum are already known as gram positive bacteria and belong to LAB group.

Commented [MOU11]: Could you please give the meaning or definition of traditional food and ethnic food including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al*., 2021).

Commented [MOU12]: Please explain more about Lemea, and other related subjects

River fish that are often used to make lemea are freshwater fish. One of the freshwater fish that can be used is betok fish, lemea containing lactic acid bacteria (Xu *et al*., 2021). Lactic acid bacteria consist of microbial strains that convert carbohydrates into lactic acid (Dinoto *et al*., 2020), LAB isolated results were identified based on phenotypic and genotypic characteristics (Lawalata *et al*., 2011). Genotypic LAB identification was carried out by PCR technique in the form of amplifying and sequencing the universal region of the 16S rRNA gene (Dinoto *et al*., 2020). Lactic Acid Bacteria (LAB) strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) has been previously studied. . The purpose of this study was to investigate Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus asper* Schult) and Yellow bamboo shoots (*Bambusa vulgaris* Schrad).

Commented [MOU13]: Is there any reference about lemea/fermented bamboo shoots?
Why did the authors want to isolated and identified LAB from fermented bamboo shoots?
Is there any reference about isolation of LAB from fermented bamboo shoots?

Commented [MOU14]: Explain more about this. What is the finding? such species, strain of LAB that were isolated from Tabah bamboo shoots.
You can use these findings for your discussion

Commented [MOU15]: Investigation or isolation?

Commented [MOU16]: Why did the authors choose fermented betung bamboo shoots and yellow bamboo shoots?

6. Materials and methods

2.1 Sample Preparation

Commented [MOU17]: Please describe more detail, such the quantity of each material, and the fermentation condition and time.

The process of processing fermented bamboo shoots begins with slicing thin samples and soaking them for 30 hours, then washing and filtering them. The next step is fermentation for 48 hours at room temperature by mixing sliced bamboo shoots, betok fish, lemongrass and salt and water (Figure 1)

Commented [MOU18]: The time of fermentation is different form the one in figure 1

2.2 LAB isolation

Isolation of LAB was carried out using a device that was sterilized beforehand and carried out aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1% NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at $37^{o}C$ for 48 hours. The isolates obtained need to be purified and identified

Commented [MOU19]: Please describe in more detail

Commented [MOU20]: Explain how to do purify the isokates

2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)

Molecular identification based on 16S rRNA gene amplification with genomic DNA isolation, DNA amplification, sequencing and analysis of nucleotide sequences in GenBank (Widodo *et al*., 2017). DNA isolation was Genomic DNA extraction with Presta TM Mini GDNA Bacteria Kit (Geneaid, GBB100). DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1uL of the PCR product was assessed with 0.8% TBE agarose.

7. Results and discussion/Results

Bacterial sequence homology based on 16S rRNA gene sequencing analysis. The types of lactic acid bacteria species on bamboo shoots of betung and yellow bamboo have 99% similarity. B1, B2, and S1 are closely related to *Lactobacillus plantarum*, this type of bacteria is known as lactic acid bacteria which has the potential as biopreservative because it can inhibit the growth of pathogens and destructive with greater inhibition than other lactic acid bacteria. (Azizah *et al*., 2019). *L. plantarum* is amylolytic which converts starch to lactic acid (Noor *et al*., 2018). S2 is closely related to *Lactobacillus fermentum*, this type of bacteria is known as lactic acid bacteria which belongs to heterofermentative species and can be found in the human intestinal tract where these bacteria can live at the pH of the digestive tract (Manin, 2010). B1 and B2 are isolate codes derived from fermented bamboo shoots. S1 and S2 codes for isolate fermented yellow bamboo shoots. (Table 1). Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration

Commented [MOU21]: Please show the results (which figure/table and then discuss in more comprehensive with support from references /findings from other researchers

Commented [MOU22]: Other species or strain?

Commented [MOU23]: Which lactic acid bacteria or L. plantarum that have amylolytic activity (stains??)

using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of isolates **B**1, B2, S1 and **S**2 at 0.8% TBE agarose concentration using the Kappa Universal ladder (Figures 3a and 3b). hylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots contain more lactic acid bacteria in the form of *Lactobacillus fermentum*.

Commented [MOU24]: What do you mean with yellow bamboo shoots contain more lactic acid bacteria in the form of L. fermentum.

8. Conclusion

Lactobacillus plantarum and *fermentum* isolated from fermented bamboo shoots were found to be gram positive bacteria and belong to a group of lactic acid bacteria.

Commented [MOU25]: This is not a conclusion. L. planatrum dan L. fermentum are gram positive bacteria and lactic and lactic acid bacteria

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the Poltekkes Kemenkes Bengkulu, Indonesia in 2020

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Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 1. Fermented Bamboo shoot making process

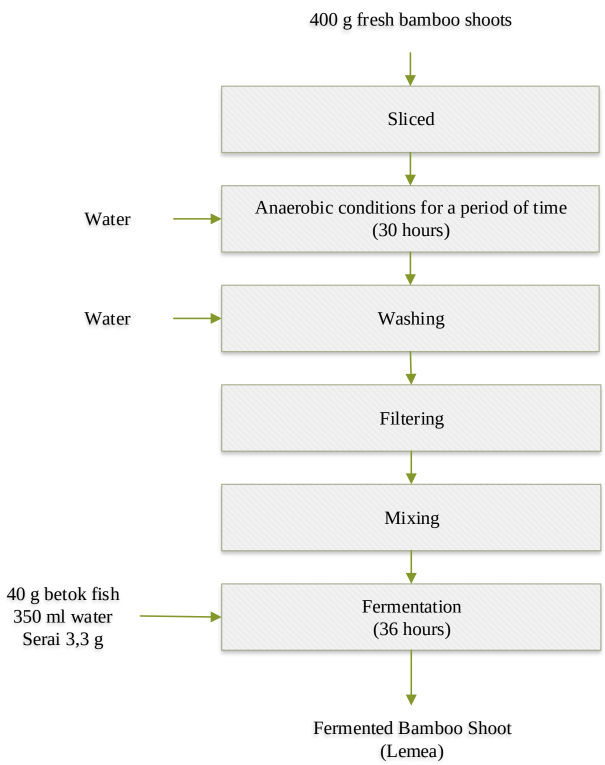

Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

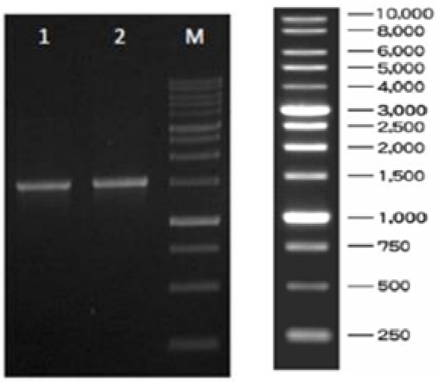

Figure 2b. Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

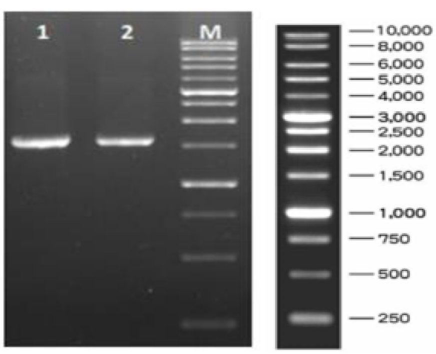

Figure 3a. Sequence assembly result – PCR product Isolate Code B1

Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 3b. Sequence assembly result – PCR product Isolate Code B2

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 3c. Sequence assembly result – PCR product Isolate Code S1

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 3d. Sequence assembly result – PCR product Isolate Code S2

Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
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Figure 4a. Phylogenetic tree Isolate Code B1

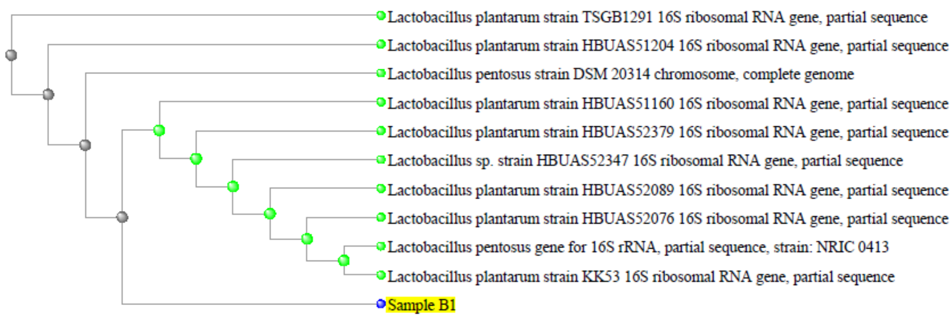


Figure 4b. Phylogenetic tree Isolate Code B2

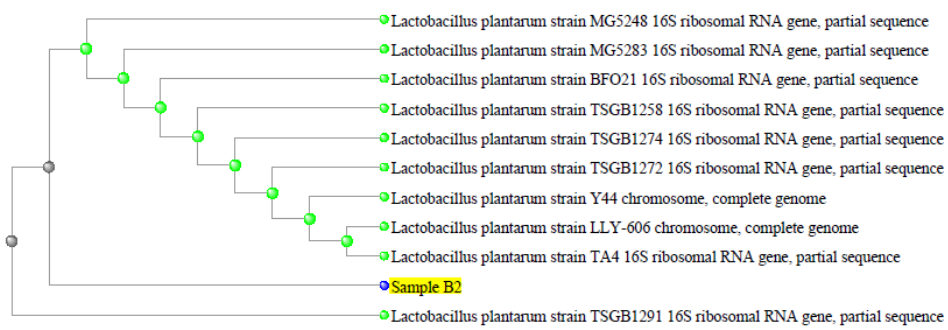


Figure 4c. Phylogenetic tree Isolate Code S1

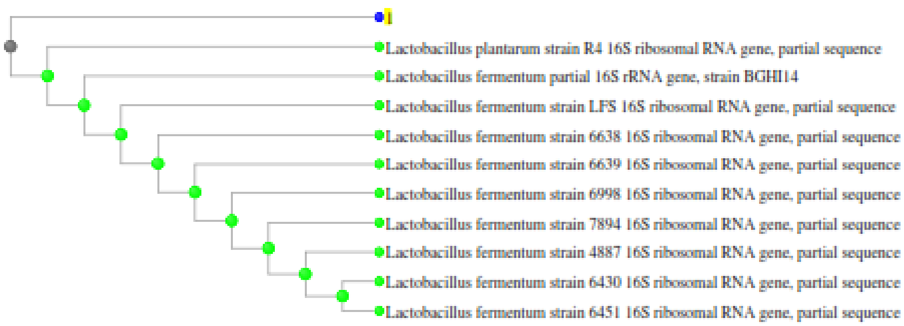
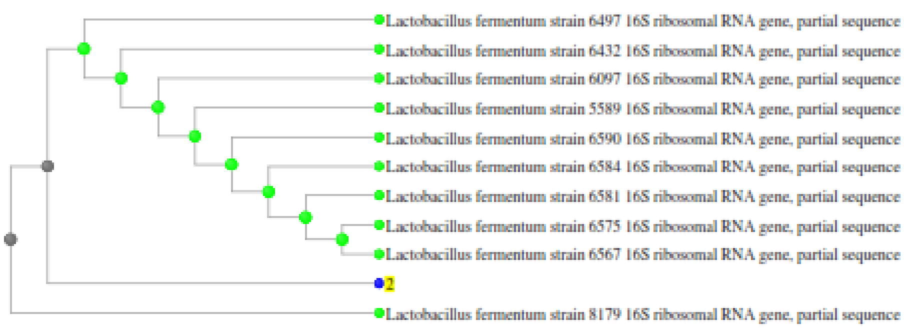


Figure 4d. Phylogenetic tree Isolate Code S2



MANUSCRIPT EVALUATION FORM

Respon Penulis terhadap evaluasi Riviwer 1

FOOD RESEARCH

Date	:	5th February 2022
Manuscript ID	:	FR-IFC-029
Please return by	:	5th March 2022
Title of Manuscript	:	Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

1. IF YOU CANNOT REVIEW THIS MANUSCRIPT OR MEET THE DEADLINE, PLEASE INFORM US WITHOUT DELAY.

2. Your review should consider the article's scholarly merit including originality of the research issue and/or methodology, adequacy and rigor of the research methodology and techniques used, quality and rigor of data analysis, comprehensiveness of literature review, and the readability and presentation of the article. Please provide detailed and specific comments to all items. Also, where appropriate please provide suggestions for revision.

COMMENT SHEET

Using item 2 in page 1 as a guideline, please indicate the reasons for your recommendations. Most author(s) will appreciate frankness, combined with a modicum of tact. Even if you recommend that the manuscript be accepted for publication, please provide some general comments to the author(s).

Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
13. Appropriateness of Contents			x		
14. Originality of Topic		x			
15. Manuscript Format			x		
16. Research Methodology				x	
17. Data Analysis				x	
18. Relevance to the Journal		x			

	(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS	(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
21.	**Title : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria** *It should reflect the article*: ○ The title does not very much reflect the contain of the article. ○ There is not any information and discussions about the ethnic food fermentation from Bengkulu in the results and discussion. ○ The content of this manuscript is isolation and identification of LAB from fermented bamboo shoot	The title has been corrected to: Identification of lactid acid bacteria isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia
22.	**Abstract** *Background, Aim, Methodology and Conclusion*: ○ The background of the research is not clear. It is not stated explicitly and clearly ○ It is more appropriate if the study aim is to isolate and identified LAB from fermented Betung bamboo shoot, and yellow bamboo shoot ○ Methodology in the abstract is quite clear ○ Conclusion: the author wanted to find LAB in fermented bamboo shoots. Bacteria that has been found were gram positive bacteria and a group of lactic acid bacteria is not a conclusion N	○ **The abstract** has been remade, focusing on the process of isolation and identification of lactic acid bacteria from lemea products. ○ **This study aims** to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. ○ **The methodology in the abstract has been clarified:** betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo (*Bambusa vulgaris Schrad*) have been used. ○ Furthermore, it was remotely analyzed on MRSA media and four LAB isolates were obtained. Molecular identification using the 16S rRNA method.
23.	**Keywords: -** *Min. 3 and Max. 6*	**Keywords:** *Lemea*, bamboo shoot, 16S rRNA, LAB
24.	**Introduction** *Concise with sufficient background* ○ Not enough information about this fermented betung bamboo shoots such as the raw materials and ingredients, the condition and time of fermentation process, and description of the product. ○ Related research and similar traditional fermented product	○ Information about the raw materials used has been added to the composition in 2.1 sample preparation ○ Research on a similar traditional fermented

	reported by other researchers are not evaluated. ○ The background is not clear. The purpose of the research is to investigate LAB strains in fermented Betung bamboo shoots, but the MM and results are isolation and identification of LAB	product has been reported, namely eksam and has been evaluated in the introduction ○ The title, objectives, background and conclusion have been adjusted according to the method
25.	**Research design/Methodology** *Clearly described and reproducible*: ○ The research design/methodology is not described clearly ○ Sample preparation: the quantity of all the materials were not mentioned. ○ The time of fermentation in the MM is different from the diagram in figure 1, and also different from the one in the introduction ○ Isolation and identification steps need to be written clearly step by step	○ The method has been described in more detail with Fig1 and 2 ○ The number of ingredients has been added ○ The fermentation time has been synchronized which is 48 hours in the sample preparation and Fig 1 ○ Isolation and identification steps have been added
26.	**Data Analysis** *Results well presented and discussed* ○ Results are not well presented and discussed ○ The authors do not explain how many isolates are obtained from MRSA. ○ There was not any discussion. ○ Only shows the facts, d anvery limited information	○ Results have been presented. Each Table and Figure has been explained in more detail ○ The resulting isolates were 4 isolates and supported by the image of the isolate in Fig 3 ○ Each result explanation has been added a discussion ○ Non-factual information has been added and explained in Results and discussion/Results
27.	**Conclusion** *A clear summary of the study* ○ The conclusion written by the authors is not a conclusion since *L. plantarum* and *L. fermentum* are gram positive bacteria and LAB	The conclusion has been corrected according to the reviewer's suggestion Four Lactobacillus isolates found in Lemea were from the Lactobacillus genus. Lactobacillus plantarum and Lactobacillus fermentum were isolates which were identified molecularly by 16S rRNA. *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*).
28.	**References** *References should follow the journal's format*	Reference has followed the format
29.	**English Proficiency:** Some grammatical errors	The grammar has been corrected.
30.	**Additional comments/suggestions by the reviewer about the article:** ○ The article should be rewrite from abstract to conclusion for proper articles. ○ Results and discussion are very limited. They should be presented clearly, every step and discussed in more comprehensive supported by suitable references ○ There should be clear correlation among back ground, aim, MM, results and conclusion	○ The article has been rewritten. ○ The results have been made clearer and supported with the latest references. ○ It has been correlated with the title, objectives, methods, results, and discussion.

Overall Evaluation

Please choose one.

Accept		**Major Revision**	**V**
Minor Revision		**Reject**	

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MANUSCRIPT EVALUATION FORM

Respon penuis terhadap evaluasi riviewer 2

FOOD
RESEARCH

Date	:	5th February 2022
Manuscript ID	:	FR-IFC-029
Please return by	:	5th March 2022
Title of Manuscript	:	Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

1. IF YOU CANNOT REVIEW THIS MANUSCRIPT OR MEET THE DEADLINE, PLEASE INFORM US WITHOUT DELAY.

2. Your review should consider the article's scholarly merit including originality of the research issue and/or methodology, adequacy and rigor of the research methodology and techniques used, quality and rigor of data analysis, comprehensiveness of literature review, and the readability and presentation of the article. Please provide detailed and specific comments to all items. Also, where appropriate please provide suggestions for revision.

COMMENT SHEET

Using item 2 in page 1 as a guideline, please indicate the reasons for your recommendations. Most author(s) will appreciate frankness, combined with a modicum of tact. Even if you recommend that the manuscript be accepted for publication, please provide some general comments to the author(s).

Evaluation Criteria	Grade				
	A (Excellent)	B	C	D	E (Worst)
19. Appropriateness of Contents		v			
20. Originality of Topic		v			
21. Manuscript Format			v		
22. Research Methodology			v		
23. Data Analysis			v		
24. Relevance to the Journal		v			

FOOD RESEARCH

	(REVIEWER'S SECTION) REVIEWER'S COMMENTS/SUGGESTIONS	(AUTHOR'S SECTION) AUTHOR'S ACTION/RESPONSE *NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below
31.	**Title** The title does not yet describe the method use d. There should be a word isolation of lactic a cid bacteria.	The title has been corrected to : Identification of lactid acid bacteria isolated from ethnic fermented bamboo shoot "*Lemea* " in Bengkulu, Indonesia
32.	**Abstract** *It has not given the importance of isolating lactic acid b acteria from the product. Methods need to be summarize d*	**The abstract** has been remade, focusing on the process of isolation and identification of lactic acid bacteria from lemea products.
33.	**Keywords** *It should be added to the product name.*	Product name has been added **Keywords:** *Lemea*, bamboo shoot, 16S rRNA, LAB
34.	**Introduction** *It has not yet described the importance of isolating lacti c acid bacteria from the product.*	**Introduction** The reason for isolation has been added to the introduction, namely looking for LAB strains from traditional foods
35.	**Research design/Methodology** *Method clearly described and reproducible.*	The methods of sample preparation, isolation and identification have been described in the isolation and sample preparation stages
36.	**Data Analysis** *It is necessary to add a discussion about the differences in lactic acid bacteria obtained from differences in bamb oo types that are strengthened by reference.*	The discussion on the differences in lactic acid bacteria obtained from different types of bamboo has been strengthened by adding the appropriate literature as a reference.
37.	**Conclusion** *It should be associated with the source of lactic acid bac teria isolation*	The conclusion has been corrected according to the reviewer's suggestion Four Lactobacillus isolates found in Lemea were from the Lactobacillus genus. Lactobacillus plantarum and Lactobacillus fermentum were

		isolates which were identified molecularly by 16S rRNA. *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*).
38.	**References** *Ok*	-
39.	**English Proficiency** Need to be checked again	English Proficiency has been checked according to the suggestion
40.	**Additional comments/suggestions by the reviewer about the article**	-

Overall Evaluation
Please choose one.

Accept		**Major Revision**	
Minor Revision	**v**	**Reject**	

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Commented [H26]: The article has been rewritten.
The results have been made clearer and supported with the latest references.
It has been correlated with the title, objectives, methods, results, and discussion.

Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 hours. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 grams of homogenized *Lemea* sample, then 1 ml is taken and 9 ml of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 hours to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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).

Keywords: *Lemea*, bamboo shoot, 16S rRNA, LAB

9. 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

Commented [H27]: The title has been corrected. The title describes the method used. The word "lactic acid bacteria isolation" has been added.

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Commented [H29]: **This study aims** to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*.

Commented [H30]: **The methodology in the abstract has been clarified:** betung shoots (*Dendrocalamus asper Schult*) and yellow ba mboo (*Bambusa vulgaris Schrad*) have been used.

Commented [H31]: **The abstract** has been remade, focusing on the process of isolation and identification of lactic acid bacteria from lemea products.

Commented [H32]: Identification

Commented [H33]: Furthermore, it was remotely analyzed o n MRSA media and four LAB isolates were obtained. Molecular ident ification using the 16S rRNA method.

Commented [H34]: Product name has been added

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 odor and flavour are unique, and only the locals enjoy it. LAB that have 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 (Tomar, 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 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 anti bacterial activity (Jitpakdee *et al*., 2022). Isolation of lactic acid bacteria from indegenous fermented foods is very important (Mende *et al*., 2022).

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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 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 aims to identify and isolate LAB strains in fermented betung bamboo shoots (*Dendrocalamus Asper* Schult) and yellow bamboo shoots (*Bambusa Vulgaris* Schrad).

10. 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 hours. Then they were washed, filtered, and weighed as much as 400 grams, and they added 40 g of betok fish, 350 ml of water, and 3.3 g of lemongrass, and fermented for 48 hours (Figure 1).

2.2 LAB 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 $37^{o}C$ for 48 hours. 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.

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Commented [H37]: The number of ingredients has been added

Commented [H38]: The fermentation time has been synchronized which is 48 hours in the sample preparation and Fig 1

Commented [H39]: The method has been described in more detail with Fig1 and 2

Commented [H40]: Isolation and identification steps have been added

(Figure 2).

a. Identification of LAB

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

Commented [H41]: identification steps have been added

i. DNA Isolation

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

ii. DNA amplification

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

iii. DNA 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.

11. Results and discussion/Results

3.1 Isolation of lactic acid bacteria from *Lemea*

Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded B1, B2, S1 and S2 (Figure 3). The bacterial isolates found were lactic acid bacteria

Commented [H42]: The resulting isolates were 4 isolates and supported by the image of the isolate in Fig 3

Commented [H43]: Results have been presented. Each Table and Figure has been explained in more detail 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 4a and 4b. 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 (Fig. 5a), 1424 bp for B2 (Fig. 5b). The nucleotide sequence S1 isolate was 882 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). 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% similar to the partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of *Lactobacillus plantarum* but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 6a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA. (Figure 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA. The type of isolate that was identified from Bekasam was *lactobacillus plantarum* (Sukmarini *et al*., 2014).All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus plantarum* were found in dominating

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Commented [H52]: Results have been presented. Each Table and Figure has been explained in more detail

Commented [H53]: Results have been presented. Each Table and Figure has been explained in more detail 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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al*., 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated 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).

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Lactobacillus fermentum was only found in lemea made from yellow bamboo shoots (Bambusa vulgaris Schrad) and Lactobacillus plantarum was found in yellow bamboo shoots (Bambusa vulgaris Schrad) and betung (Dendrocalamus asper Schult). Prebiotics are contained in foodstuffs that trigger the growth of Lactobacillus (Macfarlane & 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 fiber (Felisberto *et al*., 2017). At 100 g of fresh weight, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fiber, 4.90 g of D. Asper, and 3.54 g of fiber (Chongtham *et al*., 2011). Different bamboo species contain different macronutrients (Adebola *et al.,* 2014). Differences in the content of bamboo shoots affect the types of bacteria found in Lemea products.

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12. Conclusion

Four Lactobacillus isolates found in *Lemea* were from the Lactobacillus genus. Lactobacillus plantarum and Lactobacillus fermentum were isolates which were identified molecularly by 16S rRNA. *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*).

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The conclusion has been corrected according to the reviewer's suggestion
Four Lactobacillus isolates found in Lemea were from the Lactobacillus genus.

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the Poltekkes Kemenkes Bengkulu, Indonesia in 2020

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Figure 1. Fermented Bamboo shoot making process

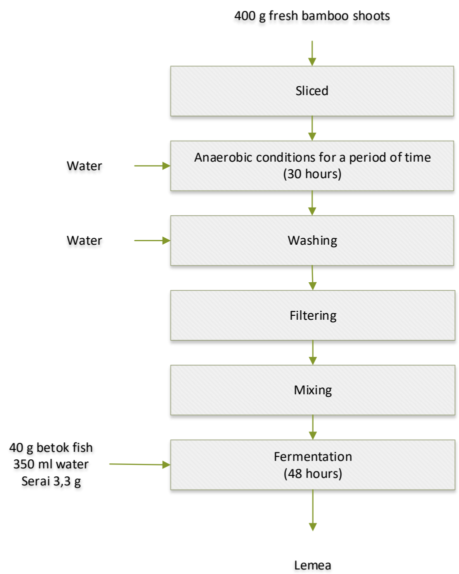


Figure 2. **Isolation Process**

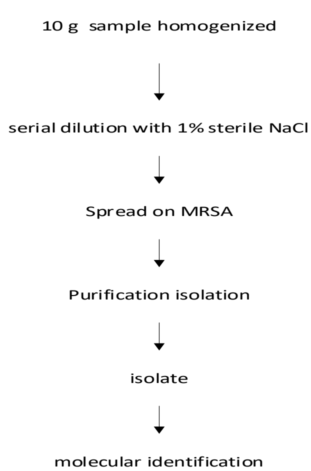
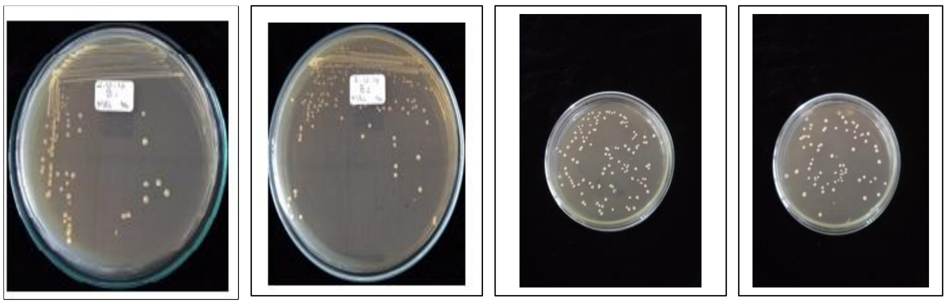


Figure 3. Isolate B1, B2,S1 dan S2



B1 B2 S1 S2

Figure 4a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

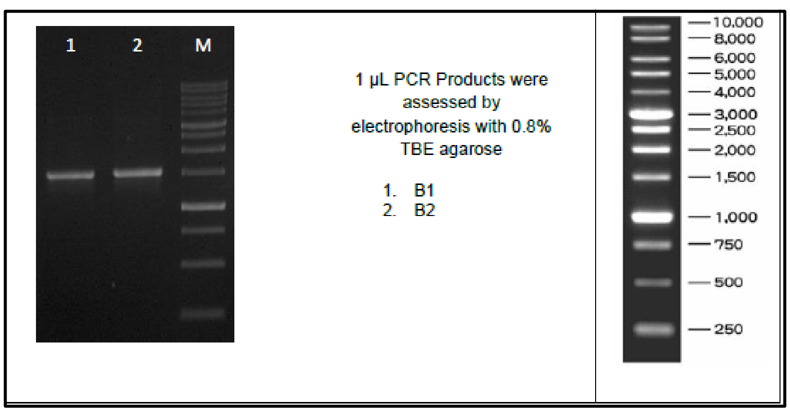


Figure 4b. Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

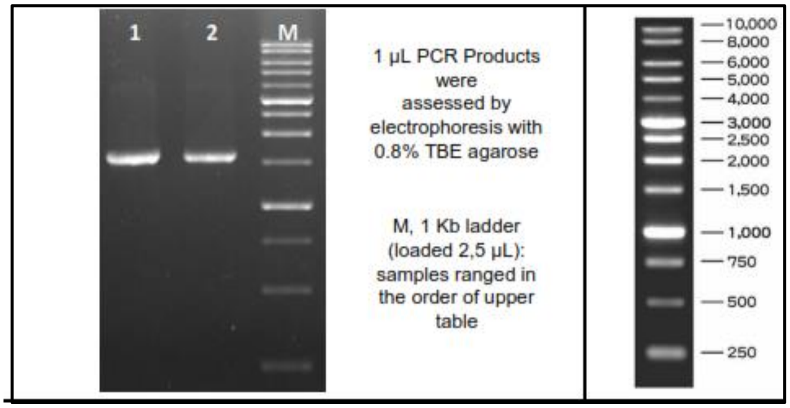


Figure 5a. Sequence assembly result – PCR product Isolate Code B1

Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 5b. Sequence assembly result – PCR product Isolate Code B2

Sequence Assembly 1424 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure **5**c. Sequence assembly result – PCR product Isolate Code S1

Sequence Result Reverse Primer 882bp

```
1      GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301    CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541    GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 5d. Sequence assembly result – PCR product Isolate Code S2

Sequence Result Reverse Primer 849bp

```
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301    GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841    CACTTCTCC
```

Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 6a. Phylogenetic tree Isolate Code B1

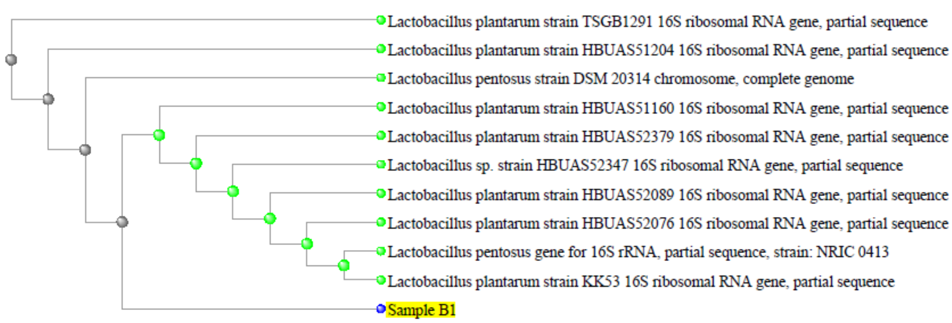


Figure 6b. Phylogenetic tree Isolate Code B2

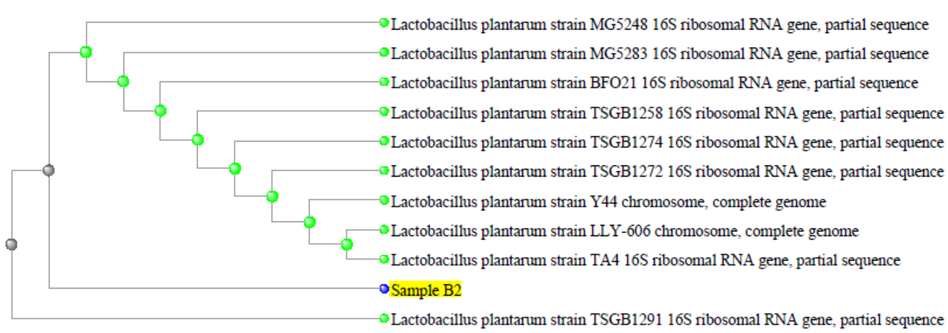


Figure 6c. Phylogenetic tree Isolate Code S1

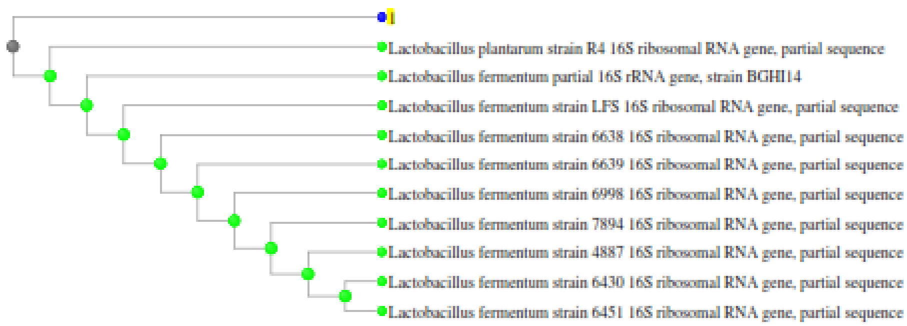
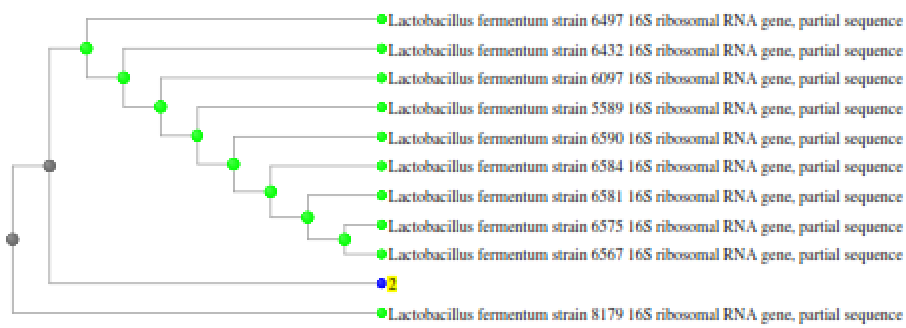


Figure 6d. Phylogenetic tree Isolate Code S2



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The manuscript has been revised according to the attached comments
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Formatting errors with respect to citations have been revised and the revised section is highlighted in yellow and listed in the reference section.

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Ethnic Food Fermentation From Bengkulu as a Source of Lactic Acid Bacteria

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Abstract

In order to avoid extinction, strains of lactic acid bacteria must be studied. This study aims to find Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus Asper* Schult) and Yellow bamboo shoots (*Bambusa Vulgaris* Schrad). Due to the ethnic diet of the rejang tribe, this fermented foodstuff is only found in Bengkulu province. Which was made by combining bamboo shoots with river fish and allowing it to stand for a few days until a distinct aroma emerges. The LAB identification of began with homogenizing 10 g of the samples, which then get serially diluted with 1 percent sterile NaCl and spreaded on MRSA media. The isolates were identified molecularly using 16S rRNA gene amplification, which included the procedures of isolating genomic DNA, amplification using DNA, sequencing, and nucleotide sequence analysis on GenBank. *Lactobacillus plantarum* strain B1 and *Lactobacillus plantarum* strain B2 were identified on fermented Betung bamboo shoots, while *Lactobacillus plantarum* S1 and *Lactobacillus fermentum* S2 on Yellow Bamboo shoots. *Lactobacillus plantarum* and *fermentum* isolated from fermented bamboo shoots were gram positive bacteria and a group of lactic acid bacteria.

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Keywords: Lactic Acid Bacteria, Bamboo shoot, Ethnic Food Fermentation, PCR, 16S rRNA

1. **Introduction**

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There is a great need to develop the traditional food which comprises the ethnic diet of a particular region. Moreover, it is also important to prevent the extinction of traditional foods such as fermented foods that contain lactic acid bacteria (Dewi *et al*., 2014). One of the tribes that has fermented food and needs to be maintained is the Rejang tribe, which is the largest tribe in Bengkulu (Dewi, 2015).

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There are a variety of fermented foods in the world especially in Asia. Most of Asian fermented foods are non-dairy products featuring various other raw materials such as cereals, soybeans, fruit, vegetables and fish and other products (Rhee *et al*., 2011). Indonesia is rich in fermented foods including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al*., 2021).

River fish that are often used to make lemea are freshwater fish. One of the freshwater fish that can be used is betok fish, lemea containing lactic acid bacteria (Xu *et al*., 2021). Lactic acid bacteria consist of microbial strains that convert carbohydrates into lactic acid (Dinoto *et al*., 2020), LAB isolated results were identified based on phenotypic and genotypic characteristics (Lawalata *et al*., 2011). Genotypic LAB identification was carried out by PCR technique in the form of amplifying and sequencing the universal region of the 16S rRNA gene (Dinoto *et al*., 2020). Lactic Acid Bacteria (LAB) strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) has been previously studied. . The purpose of this study was to investigate Lactic Acid Bacteria (LAB) strains in fermented Betung bamboo shoots (*Dendrocalamus asper* Schult) and Yellow bamboo shoots (*Bambusa vulgaris* Schrad).

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Commented [L8]: The research objectives have been clarified

2. Materials and methods

2.1 Sample Preparation

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The process of processing fermented bamboo shoots begins with slicing thin samples and soaking them for 30 hours, then washing and filtering them. The next step is fermentation for 48 hours at room temperature by mixing sliced bamboo shoots, betok fish, lemongrass and salt and water (Figure 1)

2.2 LAB isolation

Isolation of LAB was carried out using a device that was sterilized beforehand and carried out aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1% NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at 37°C for 48 hours. The isolates obtained need to be purified and identified

2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)

Molecular identification based on 16S rRNA gene amplification with genomic DNA isolation, DNA amplification, sequencing and analysis of nucleotide sequences in GenBank (Widodo *et al*., 2017). DNA isolation was Genomic DNA extraction with Presta TM Mini GDNA Bacteria Kit (Geneaid, GBB100). DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1uL of the PCR product was assessed with 0.8% TBE agarose.

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3. Results and discussion/Results

Bacterial sequence homology based on 16S rRNA gene sequencing analysis. The types of lactic acid bacteria species on bamboo shoots of betung and yellow bamboo have 99% similarity. B1, B2, and S1 are closely related to *Lactobacillus plantarum*, this type of bacteria is known as lactic acid bacteria which has the potential as biopreservative because it can inhibit the growth of pathogens and destructive with greater inhibition than other lactic acid bacteria. (Azizah *et al*., 2019). *L. plantarum* is amylolytic which converts starch to lactic acid (Noor *et al*., 2018). S2 is closely related to *Lactobacillus fermentum*, this type of bacteria is known as lactic acid bacteria which belongs to heterofermentative species and can be found in the human intestinal tract where these bacteria can live at the pH of the digestive tract (Manin, 2010). B1 and B2 are isolate codes derived from fermented bamboo shoots. S1 and S2 codes for isolate fermented yellow bamboo shoots. (Table 1). Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of isolates **B**1, B2, S1 and **S**2 at 0.8% TBE agarose concentration using the Kappa Universal ladder (Figures 3a and 3b). hylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots contain more lactic acid bacteria in the form of *Lactobacillus fermentum.*

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4. Conclusion

Lactobacillus plantarum and *fermentum* isolated from fermented bamboo shoots were found to be gram positive bacteria and belong to a group of lactic acid bacteria.

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the Poltekkes Kemenkes Bengkulu, Indonesia in 2020

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Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

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Figure 1. Fermented Bamboo shoot making process

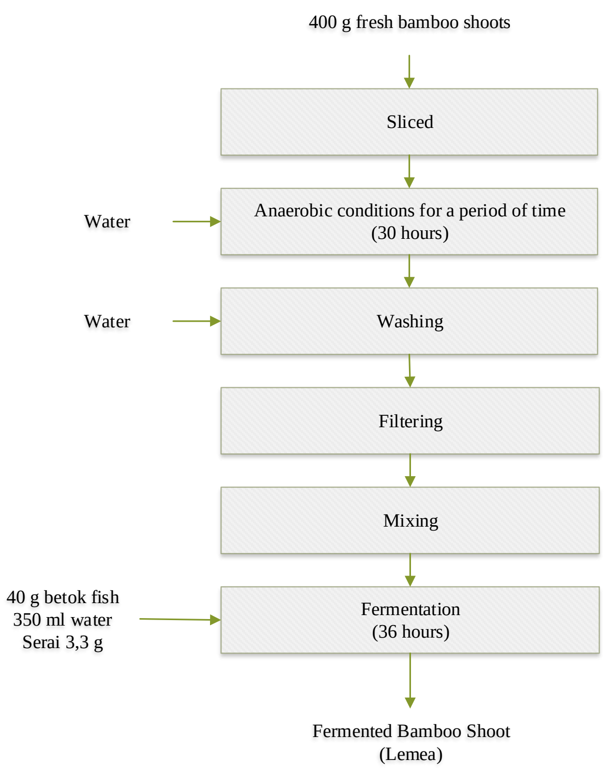

Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

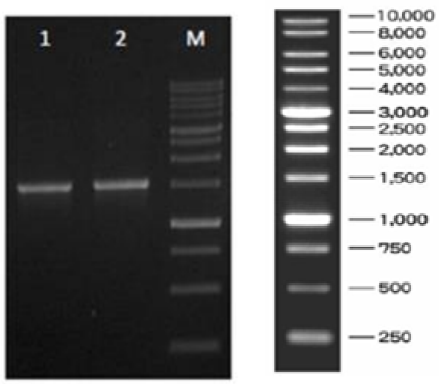

Figure 2b. Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

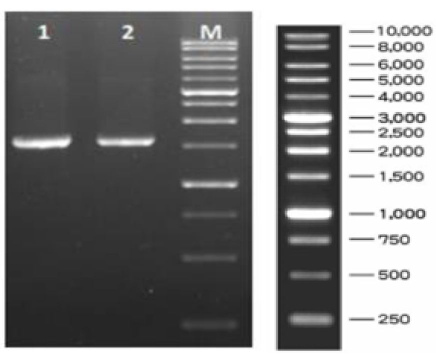

Figure 3a. Sequence assembly result – PCR product Isolate Code B1

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Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 3b. Sequence assembly result – PCR product Isolate Code B2

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 3c. Sequence assembly result – PCR product Isolate Code S1

Sequence Result Reverse Primer 882bp

```
1      GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301    CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541    GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 3d. Sequence assembly result – PCR product Isolate Code S2

Sequence Result Reverse Primer 849bp

```
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301    GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841    CACTTCTCC
```

Figure 4a. Phylogenetic tree Isolate Code B1

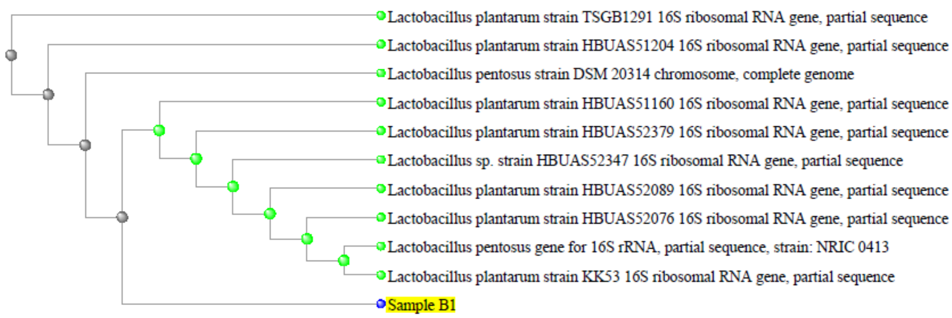


Figure 4b. Phylogenetic tree Isolate Code B2

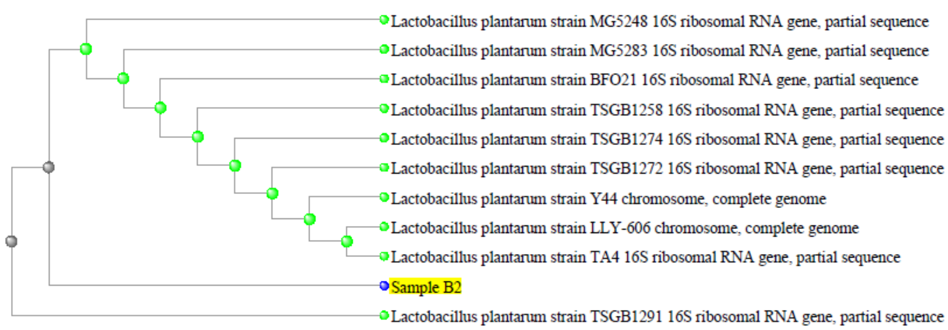


Figure 4c. Phylogenetic tree Isolate Code S1

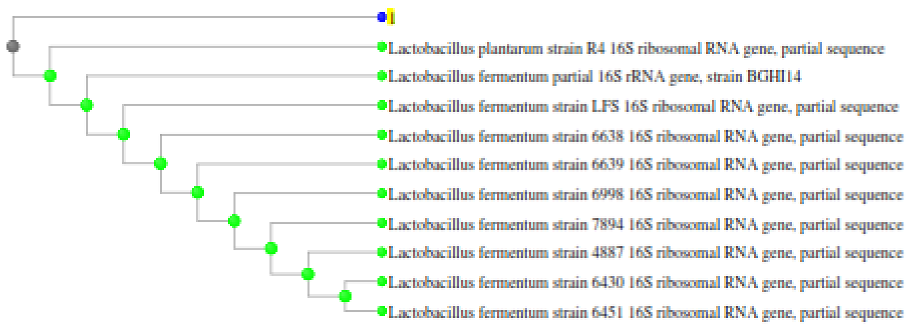
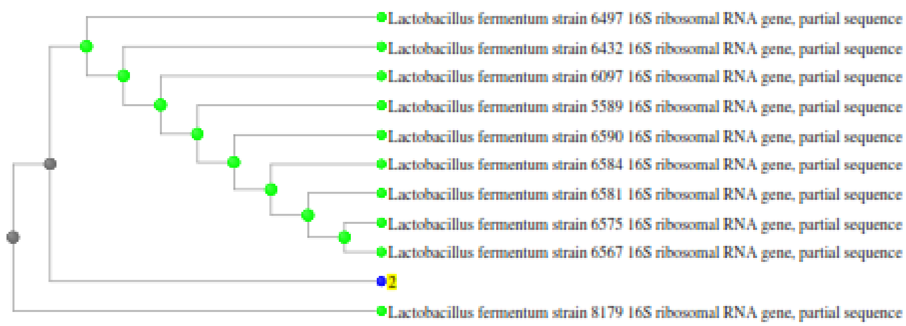


Figure 4d. Phylogenetic tree Isolate Code S2



Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 g of homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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*

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plantarum was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult).

Keywords: *Lemea*, Bamboo shoot, 16S rRNA, LAB

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). ~~(Kwon, 2015).~~ Bamboo shoots and river fish are fermented into *Lemea* by the Rejang people (Dewi *et al*., 2014). ~~(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 odour and flavour are unique, and only the locals enjoy it. LAB that have an impact on the flavour of fermented foods (Fox, 2011). ~~(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). ~~(Tomar, 2016).~~ *Meakri*, from Meghalaya Indian fermented bamboo shoots as a source of *Lactobacillus*, has characteristics suitable for probiotics (Das *et al*., 2020) ~~(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 pathogenic microbes (Yang *et al*., 2021). ~~(Yang *et al*., 2021).~~ LAB isolated from fermented bamboo shoots are potential probiotic candidates that are beneficial for health (Mohamad *et al*., 2020)~~(Mohamad *et al*., 2020).~~ LAB strains are selected for their decreased content of cholesterol, antioxidant activity, and anti-bacterial activity (Jitpakdee *et al*., 2022)~~(Jitpakdee *et al*.,~~

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~~2022)~~. Isolation of lactic acid bacteria from indegenous fermented foods is very important (Mende *et al*., 2022) ~~(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). ~~(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 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). ~~(Wasis *et al*., 2019).~~ A total of 180 LAB isolates have been isolated from Indonesian fermented foods (Sukmarini *et al*., 2014). ~~(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 aims 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 h. Then they were washed, filtered, and weighed as much as 400 g

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and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for 48 h (Figure 1).

2.2 LAB 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 h. 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.

2.2 Identification of LAB

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

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2.2.1 DNA Isolation

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

2.2.2 DNA amplification

DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA

Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1 uL of the PCR product was assessed with 0.8% TBE agarose.

2.2.3 *DNA sequenching 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 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded B1, B2, S1 and S2 (Figure 3). 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). ~~(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). ~~(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 4a and 4b. 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

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bp for isolate B1 (Figure 5a), 1424 bp for B2 (Figure 5b). The nucleotide sequence S1 isolate was 882 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). 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% similar to the partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of *Lactobacillus plantarum* but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 6a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA. (Figure 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA. The type of isolate that was identified from Bekasam was ~~*lactobacillus*~~ *Lactobacillus plantarum* (Sukmarini *et al*., 2014). ~~(Sukmarini *et al*., 2014).~~ All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus plantarum* were found in dominating strains of Soidon fermented bamboo shoot food without salt from Indian Manipur (Jeyaram *et al*., 2010). ~~(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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al.*, 2019). ~~(Okfrianti *et al*., 2019).~~ Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). ~~(Rahayu, 2003).~~ This research is expected to provide information on which LAB strains have been isolated 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). ~~(Li *et al*., 2021).~~

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Lactobacillus fermentum was only found in *Lemea*made from yellow bamboo shoots (*Bambusa vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in foodstuffs that trigger the growth of *Lactobacillus* ~~(~~(Macfarlane ~~&~~and Cummings, 1999). ~~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) ~~(Felisberto *et al*., 2017).~~ At 100 g of fresh weight, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of *D. asper*, and 3.54 g of fibre (Chongtham *et al*., 2011). ~~(Chongtham *et al*., 2011).~~ Different bamboo species contain different macronutrients (Adebola *et al*., 2014) ~~(Adebola *et al*., 2014).~~ Differences in the content of bamboo shoots affect the types of bacteria found in *Lemea* products.

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4. Conclusion

Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S rRNA. *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).

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Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

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Figure 1. Fermented bamboo shoot making process

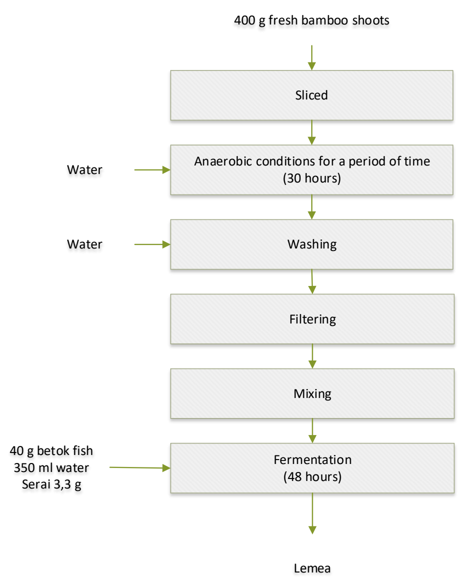


Figure 2. Isolation process

10 g sample homogenized

↓

serial dilution with 1% sterile NaCl

↓

Spread on MRSA

↓

Purification isolation

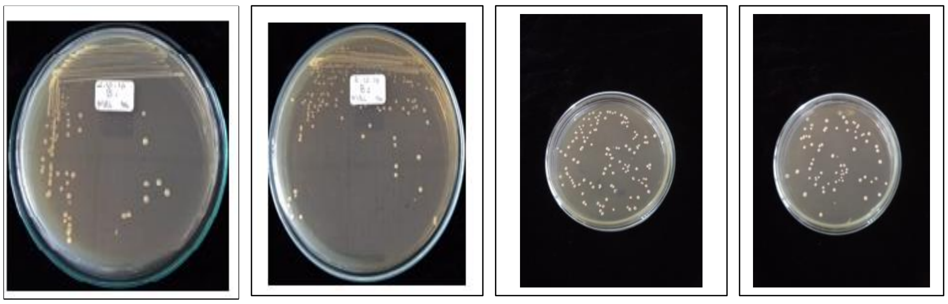
↓

isolate

↓

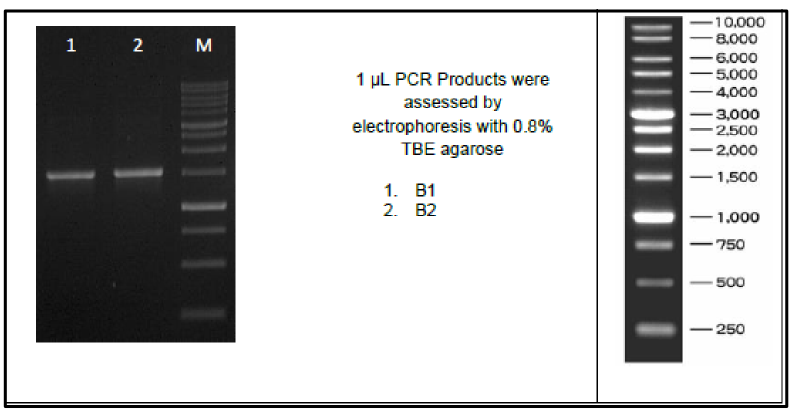
molecular identification

Figure 3. Isolate B1, B2,S1 dan S2



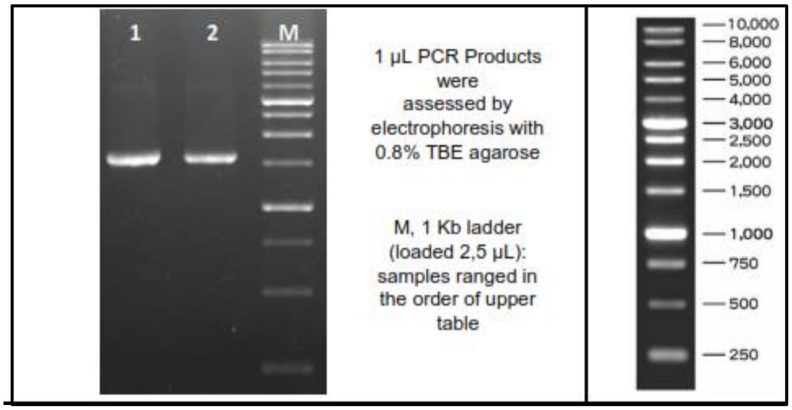
B1 B2 S1 S2

Figure 4a. Genomic DNA amplification of isolates B1 and B2



Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 4b. Genomic DNA amplification of isolates S1 and S2



Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 5a. The nucleotide sequence B1 isolate

Sequence Assembly 1434 bp

```
1     AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61    TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121   ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181   TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241   GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301   GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361   GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421   CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481   CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541   CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601   CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661   ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721   TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781   CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841   CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901   CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961   AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021  TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081  CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141  GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201  AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261  CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321  AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381  CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 5b. The nucleotide sequence B2 isolate

Sequence Assembly 1424 bp

```
1     AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61    TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121   ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181   TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241   GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301   GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361   GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421   CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481   CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541   CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601   CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661   ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721   TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781   CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841   CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901   CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961   AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021  TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081  CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141  GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201  AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261  CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321  AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381  CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 5c. The nucleotide sequence S1 isolate

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 5d. The nucleotide sequence S2

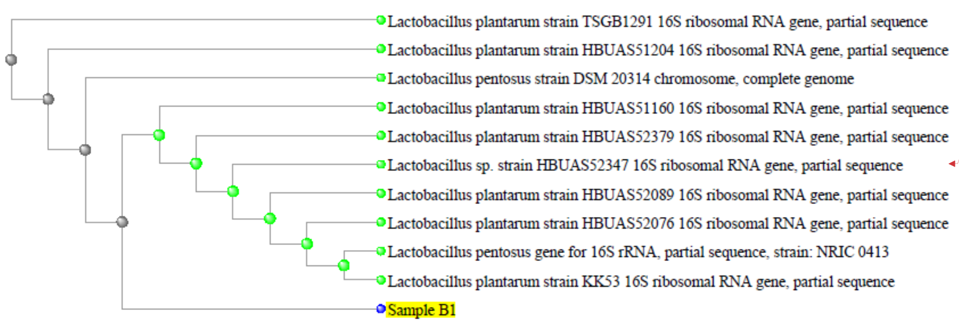
Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
```

Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 6a. Phylogenetic tree Isolate Code B1



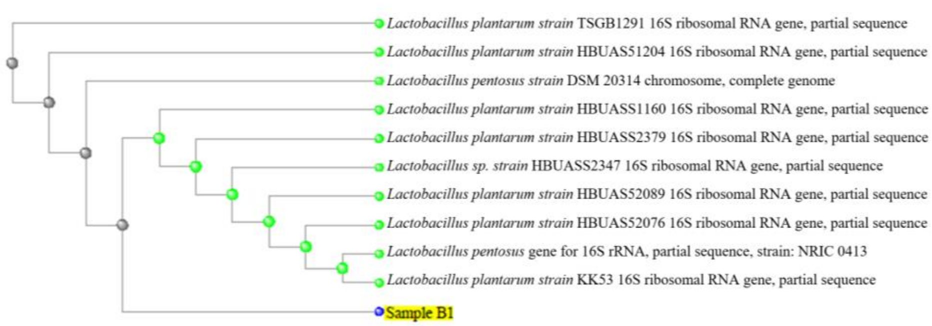


Figure 6b. Phylogenetic tree Isolate Code B2

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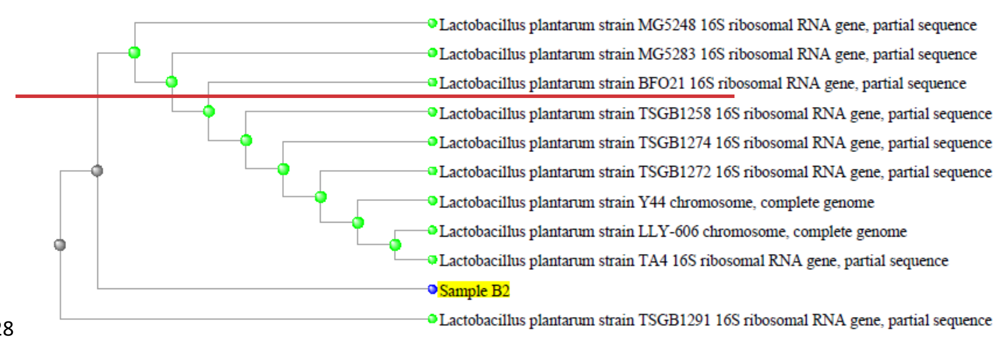
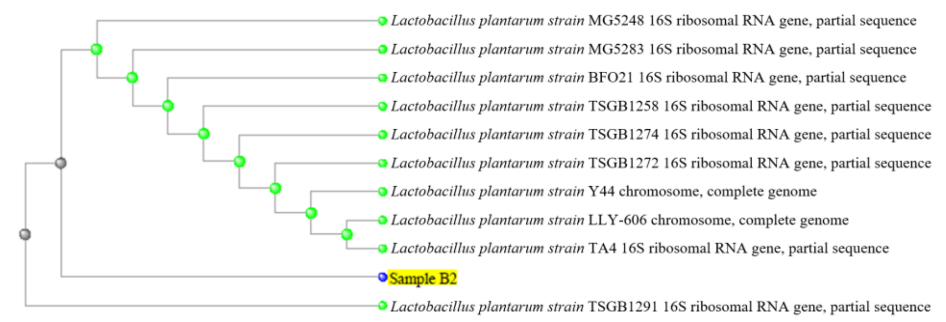

Lactobacillus plantarum strain MG5248 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain MG5283 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain BFO21 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1258 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1274 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1272 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain Y44 chromosome, complete genome
Lactobacillus plantarum strain LLY-606 chromosome, complete genome
Lactobacillus plantarum strain TA4 16S ribosomal RNA gene, partial sequence
Sample B2
Lactobacillus plantarum strain TSGB1291 16S ribosomal RNA gene, partial sequence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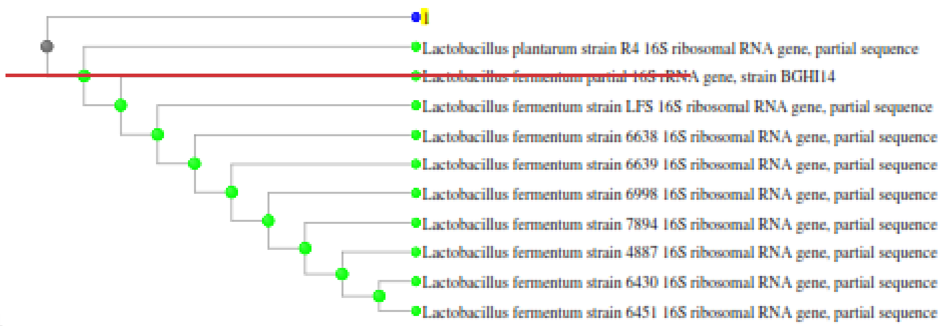
Lactobacillus plantarum strain MG5283 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain BFO21 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1258 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1274 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain TSGB1272 16S ribosomal RNA gene, partial sequence
Lactobacillus plantarum strain Y44 chromosome, complete genome
Lactobacillus plantarum strain LLY-606 chromosome, complete genome
Lactobacillus plantarum strain TA4 16S ribosomal RNA gene, partial sequence
Sample B2
Lactobacillus plantarum strain TSGB1291 16S ribosomal RNA gene, partial sequence

Figure 6c. Phylogenetic tree Isolate Code S1



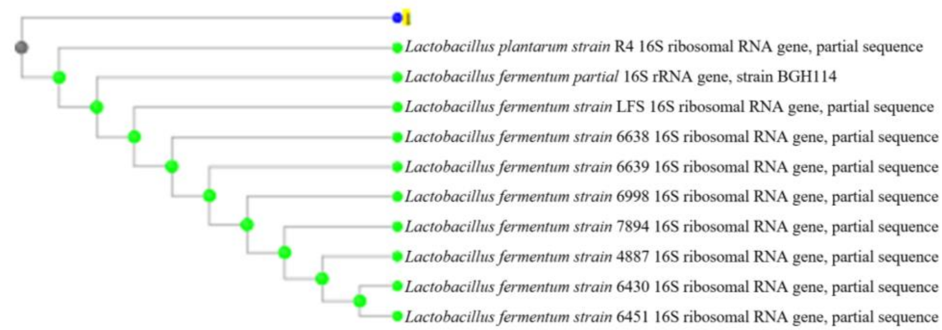


Figure 6d. Phylogenetic tree Isolate Code S2

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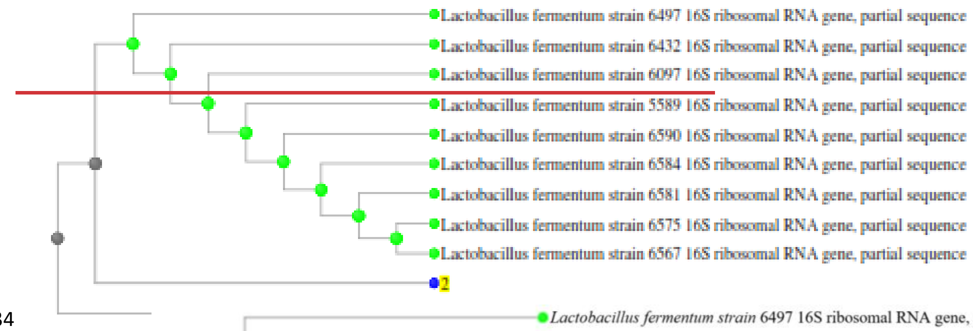

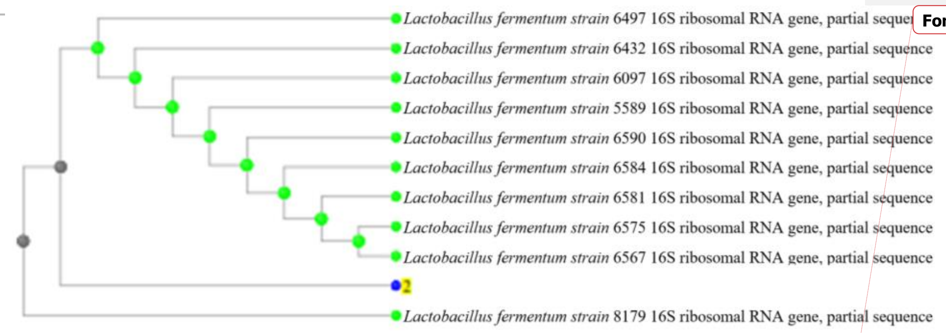

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Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 gof homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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).

Keywords: *Lemea*, Bamboo shoot, 16S rRNA, LAB

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 odour and flavour are unique, and only the locals enjoy it. LAB that have 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* ~~(Tomar, 2016)~~ (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 2pathogenic 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 anti-bacterial activity (Jitpakdee *et al*., 2022). Isolation of lactic acid bacteria from indegenous fermented foods is very important (Mende *et al*., 2022).

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Kiran, T., Rajani, C., Kumar, T.S., and Achun, P. (2016). Fermented Bamboo Shoots: A Riche Niche for Beneficial Microbes. Journal of Bacteriology &&& Mycology: Open Access, 2(4), 87–93. https://doi.org/10.15406/jbmoa.2016.02.00030

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Das, S., Mishra, B.K., and Hati, S. (2020). Techno-functional characterization of indigenous Lactobacillus isolates from the traditional fermented foods of Meghalaya, India. Current Research in Food

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Yang, L., Huang, W., Yang, C., Ma, T., Hou, Q., Sun, Z., and Zhang, H. (2021). Using PacBio sequencing to investigate the effects of treatment with lactic acid bacteria or antibiotics on cow endometritis. Electronic Journal of Biotechnology, 51, 67–78.

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Mohamad, N., Manan, H., Sallehhuddin, M., Musa, N., and Ikhwanuddin, M. (2020). Screening of Lactic Acid Bacteria isolated from giant freshwater prawn (Macrobrachium rosenbergii) as potential probiotics. Aquaculture Reports, 18(October 2019), 100523. https://doi.org/10.1016/j.aqrep.2020.100523

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Mende, S., Rohm, H., and Aros, D. (2022). Lactic Acid Bacteria: Exopolysaccharides. In Encyclopedia of Dairy Sciences (Vol. 4). Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.22982-x

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 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 aims 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 h. 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 h (Figure 1).

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Commented [LI12R11]: Already listed in the reference section. Wasis, N. O., Semadi Antara, N., and Wayan Gunam, I. B. (2019). Studi Viabilitas Isolat Bakteri Asam Laktat yang Diisolasi dari Asinan Rebung Bambu Tabah Terhadap pH Rendah dan Garam Empedu. Jurnal Rekayasa Dan Manajemen Agroindustri, 7(1), 1–10. https://doi.org/10.24843/jrma.2019.v07.i01.p01

2.2 LAB 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 h. 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.

2.2 Identification of LAB

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

2.2.1 DNA Isolation

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

2.2.2 DNA amplification

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

2.2.3 DNA sequenching 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 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded B1, B2, S1 and S2 (Figure 3). 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).

Commented [Editor13]: Not listed in the reference section?

Commented [LI14R13]: Already listed in the reference section. Kurnia, M., Amir, H., and Handayani, D. (2020). Isolasi Dan Identifikasi Bakteri Asam Laktat Dari Makanan Tradisional Suku Rejang Di Provinsi Bengkulu: "Lemea." Alotrop, 4(1), 25–32. https://doi.org/10.33369/atp.v4i1.13705

3.2 Identification of Lemea isolates

The results of genomic DNA amplification of the 4 isolates can be seen in Figures 4a and 4b. 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 5a), 1424 bp for B2 (Figure 5b). The nucleotide sequence S1 isolate was 882 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). 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% similar to the partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of

Lactobacillus plantarum but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 6a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA. (Figure 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA. The type of isolate that was identified from Bekasam was *lactobacillus plantarum* (Sukmarini *et al*., 2014).All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus 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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al*., 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated 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 (*Bambusa vulgaris* Schrad) and Lactobacillus plantarum was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in foodstuffs that 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, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of

D. asper, and 3.54 g of fibre (Chongtham *et al*., 2011). Different bamboo species contain different macronutrients (Adebola *et al.,* 2014). Differences in the content of bamboo shoots affect the types of bacteria found in *Lemea* products.

4. Conclusion

Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S rRNA. *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*).

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

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Yang, L., Huang, W., Yang, C., Ma, T., Hou, Q., Sun, Z., and Zhang, H. (2021). Using PacBio sequencing to investigate the effects of treatment with lactic acid bacteria or antibiotics on cow endometritis. *Electronic Journal of Biotechnology*, 51, 67–78. https://doi.org/10.1016/j.ejbt.2021.02.004

Figure 1. Fermented bamboo shoot making process

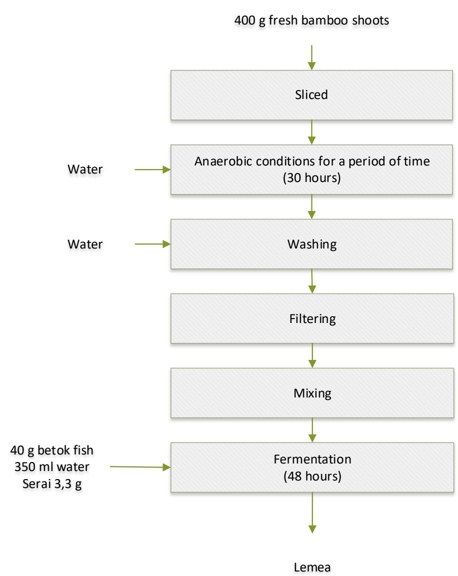


Figure 2. Isolation process

10 g sample homogenized

↓

serial dilution with 1% sterile NaCl

↓

Spread on MRSA

↓

Purification isolation

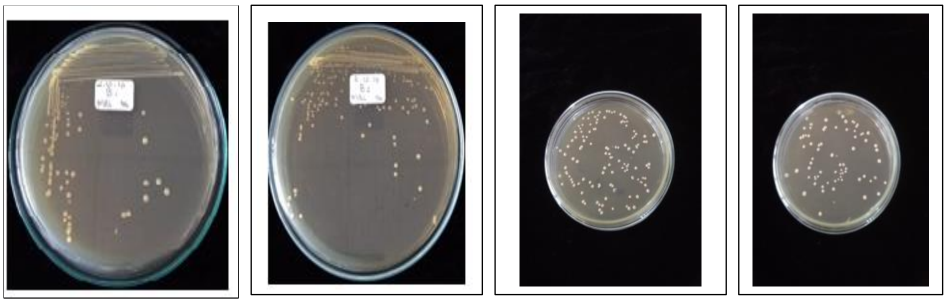
↓

isolate

↓

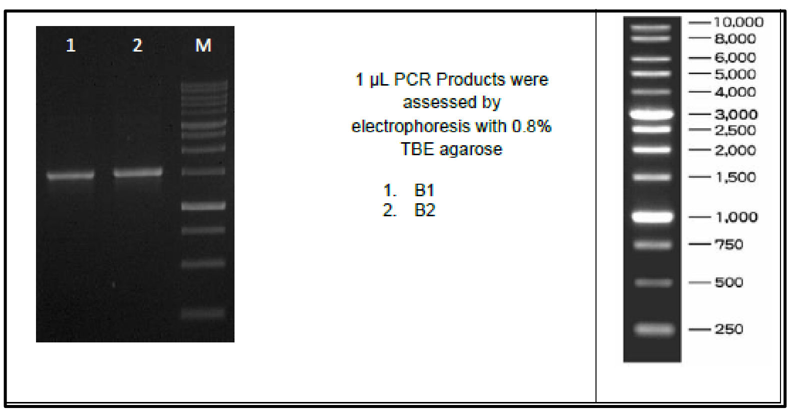
molecular identification

Figure 3. Isolate B1, B2,S1 dan S2



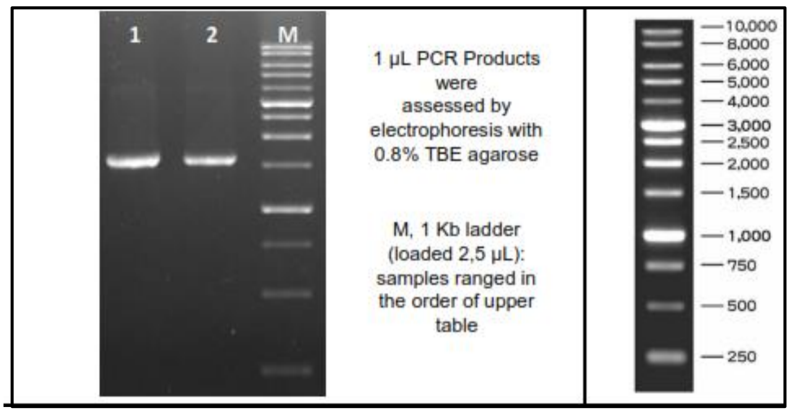
B1 B2 S1 S2

Figure 4a. Genomic DNA amplification of isolates B1 and B2



Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 4b. Genomic DNA amplification of isolates S1 and S2



Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 5a. The nucleotide sequence B1 isolate

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Sequence Assembly 1434 bp
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
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Figure 5b. The nucleotide sequence B2 isolate

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Sequence Assembly 1424 bp
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 5c. The nucleotide sequence S1 isolate

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 5d. The nucleotide sequence S2

Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
```

Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 6a. Phylogenetic tree Isolate Code B1

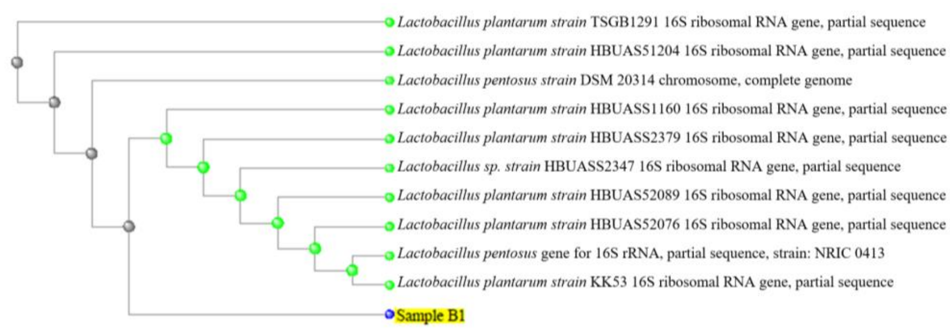

Figure 6b. Phylogenetic tree Isolate Code B2

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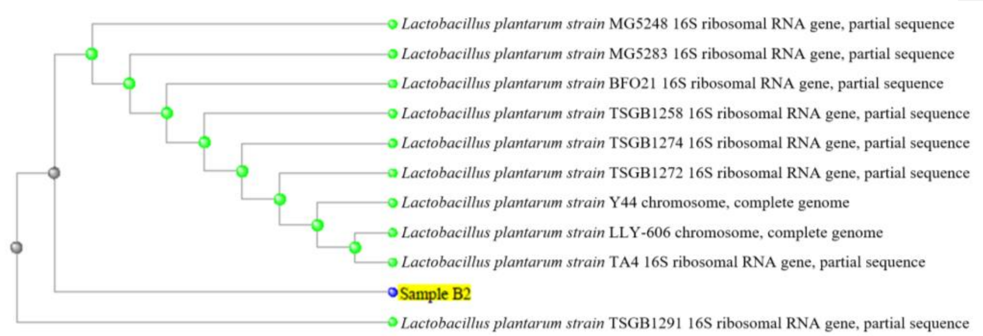

Figure 6c. Phylogenetic tree Isolate Code S1

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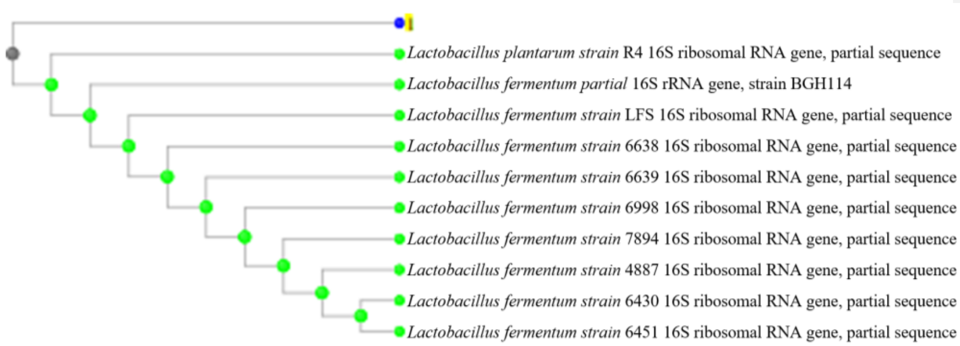

Figure 6d. Phylogenetic tree Isolate Code S2

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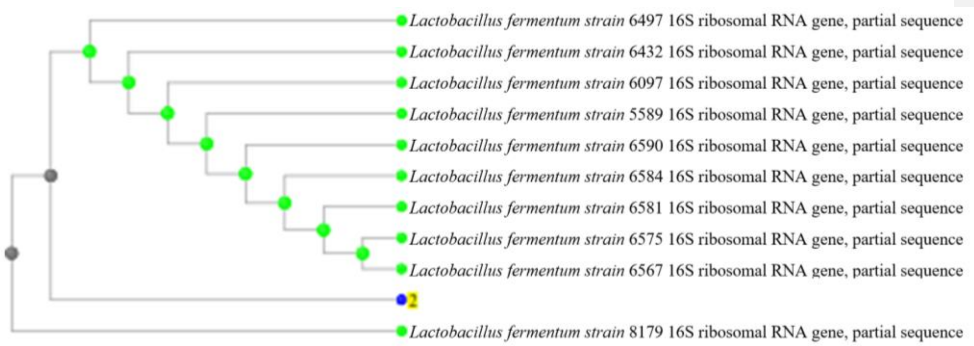

Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 gof homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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).

Keywords: *Lemea*, Bamboo shoot, 16S rRNA, Lactic acid bacteria

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 odour and flavour are unique, and only the locals enjoy it. LAB that have 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 anti-bacterial activity (Jitpakdee *et al*., 2022). Isolation of lactic acid bacteria from indegenous 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 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 aims 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 h. 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 h (Figure 1).

2.2 LAB 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.

2.2 Identification of LAB

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

2.2.1 DNA Isolation

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

2.2.2 DNA amplification

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

2.2.3 DNA sequenching 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 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded 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 *Lactobacillus plantarum* but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 5a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 5c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was *lactobacillus plantarum* (Sukmarini *et al*., 2014). All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus 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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al*., 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated 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 (*Bambusa vulgaris* Schrad) and Lactobacillus plantarum was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in foodstuffs that 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, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of *D. asper*, and 3.54 g of fibre (Chongtham *et al*., 2011). Different bamboo species contain different macronutrients (Adebola *et al.,* 2014). Differences in the content of bamboo shoots affect the types of bacteria found in *Lemea* products.

4. Conclusion

Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S rRNA. *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*).

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

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Figure 1. Fermented bamboo shoot making process

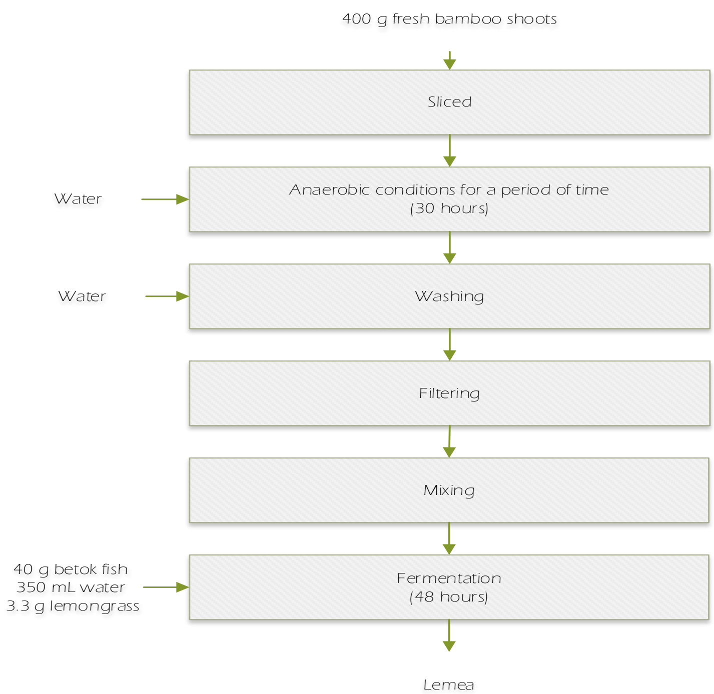
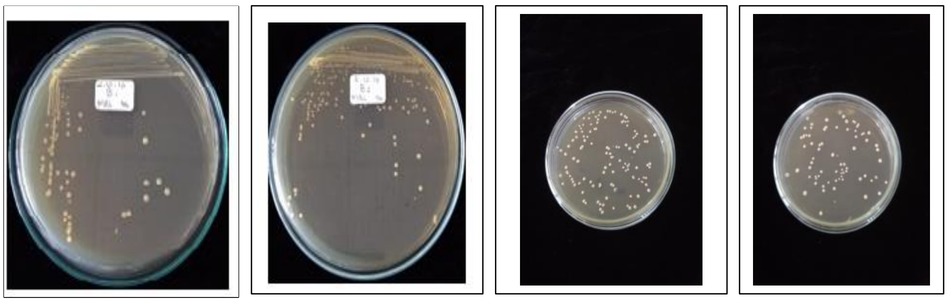
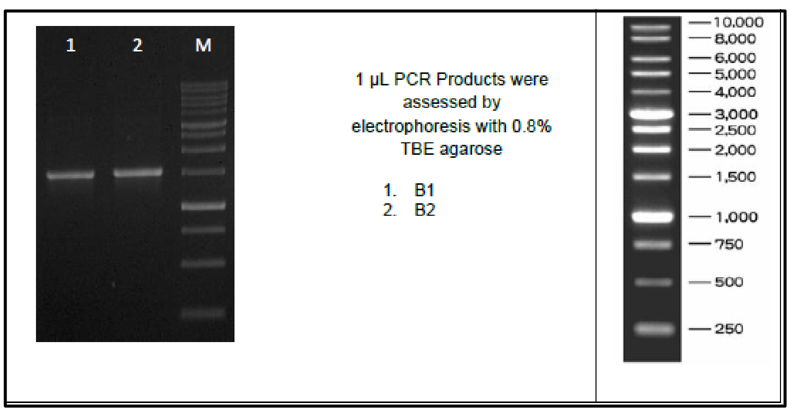


Figure 2. Isolate B1, B2,S1 and S2



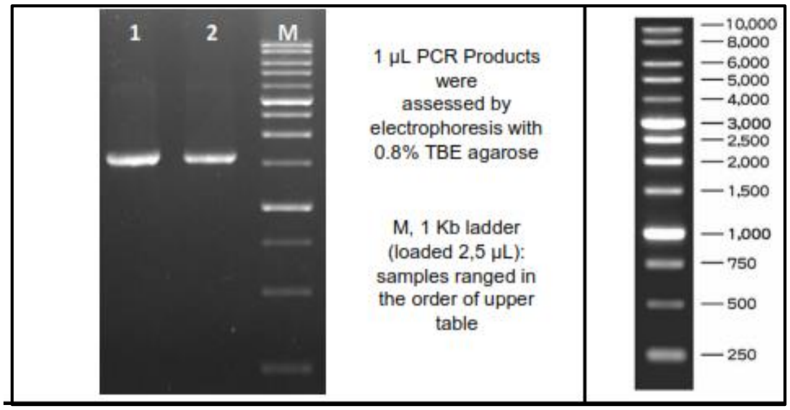
B1 B2 S1 S2

Figure 3a. Genomic DNA amplification of isolates B1 and B2



Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 3b. Genomic DNA amplification of isolates S1 and S2



Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 4a. The nucleotide sequence B1 isolate

Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 4b. The nucleotide sequence B2 isolate

Sequence Assembly 1424 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 4c. The nucleotide sequence S1 isolate

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 4d. The nucleotide sequence S2

Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
```

Table 1. Comparison of homology levels the 16S rRNA gene of Lactid Acid Bacteria isolates ~~BAL isolat~~ with several sequences

Types of Bamboo Shoots	Isolate Code	Species	~~Comparison Squen Access Number~~ GenBank Accession Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Commented [A1]: ?

Commented [A2R1]: Comparison of homology levels of the 16S rRNA gene of Lactid Acid Bacteria isolates with several sequences

Commented [A3]: ?

Commented [A4R3]: GenBank Accession Number

Figure 5a. Phylogenetic tree Isolate Code B1

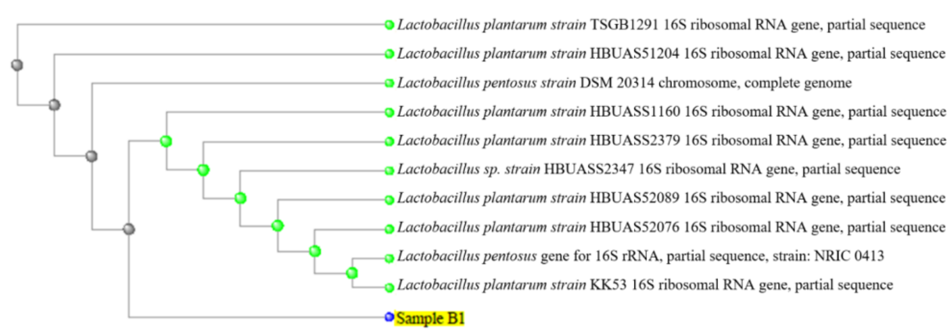


Figure 5b. Phylogenetic tree Isolate Code B2

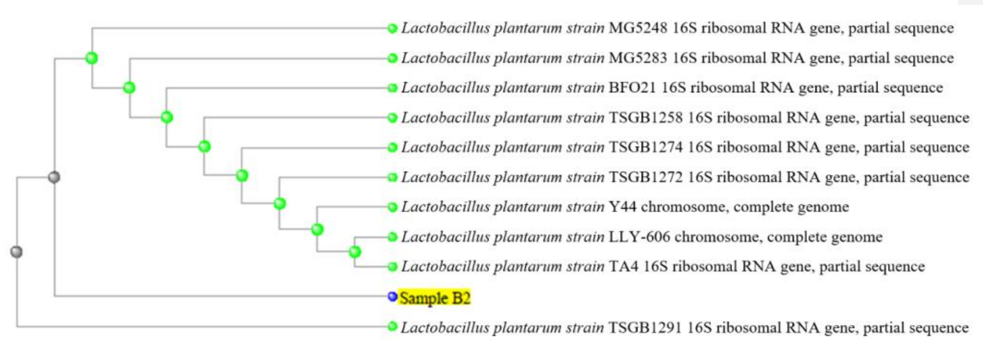


Figure 5c. Phylogenetic tree Isolate Code S1

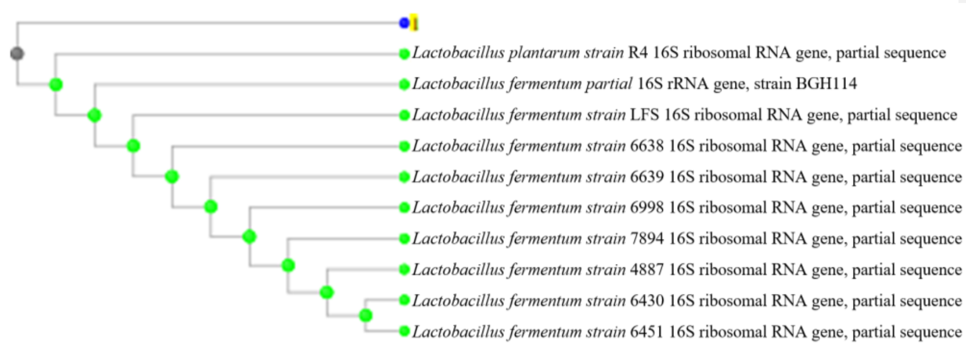
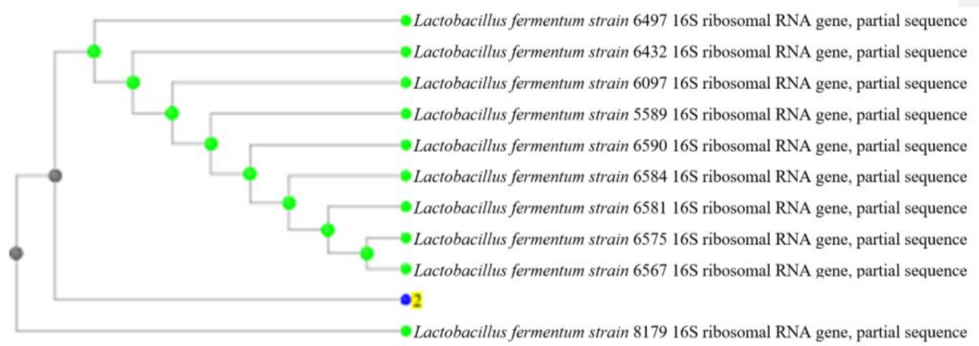


Figure 5d. Phylogenetic tree Isolate Code S2



Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 gof homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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).

Keywords: *Lemea*, Bamboo shoot, 16S rRNA, LAB

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 odour and flavour are unique, and only the locals enjoy it. LAB that have 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 anti-bacterial activity (Jitpakdee *et al*., 2022). Isolation of lactic acid bacteria from indegenous 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 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 aims 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 h. 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 h (Figure 1).

2.2 LAB 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^{o}C for 48 h. 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.

2.2 Identification of LAB

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

2.2.1 DNA Isolation

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

2.2.2 DNA amplification

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

2.2.3 DNA sequenching 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 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded 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

Lactobacillus plantarum but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 5a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 5c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was *lactobacillus plantarum* (Sukmarini *et al*., 2014). All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus 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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al*., 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated 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 (*Bambusa vulgaris* Schrad) and Lactobacillus plantarum was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in foodstuffs that 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, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of

D. asper, and 3.54 g of fibre (Chongtham *et al*., 2011). Different bamboo species contain different macronutrients (Adebola *et al.,* 2014). Differences in the content of bamboo shoots affect the types of bacteria found in *Lemea* products.

4. Conclusion

Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S rRNA. *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*).

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

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Figure 1. Fermented bamboo shoot making process

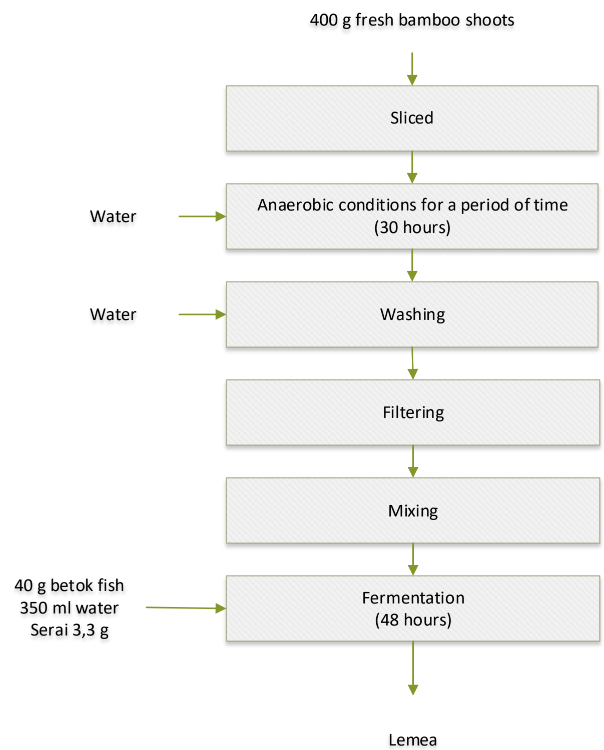
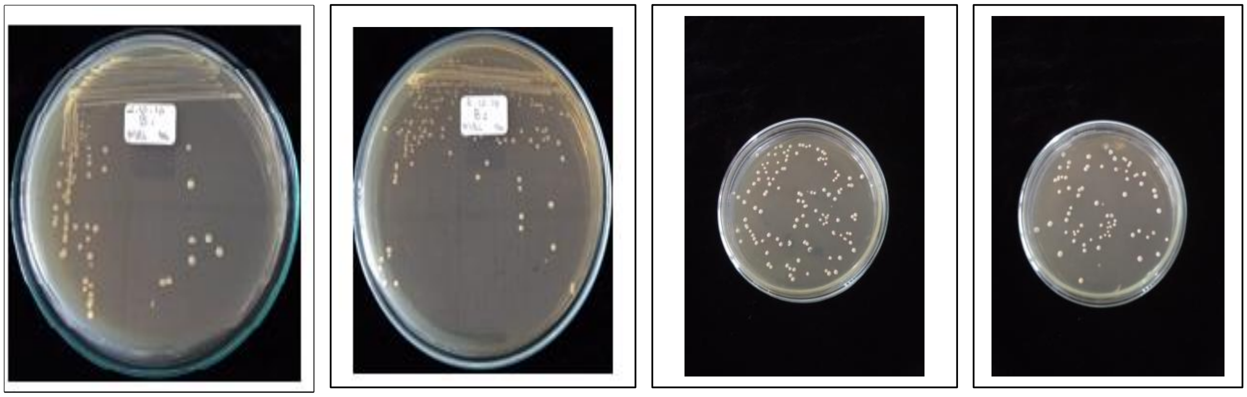
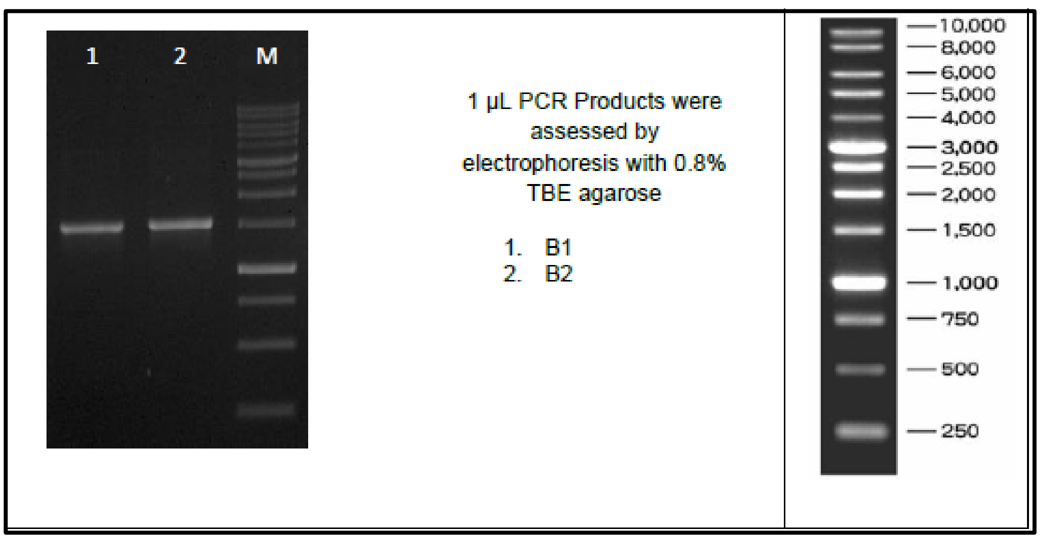


Figure 2. Isolate B1, B2,S1 and S2



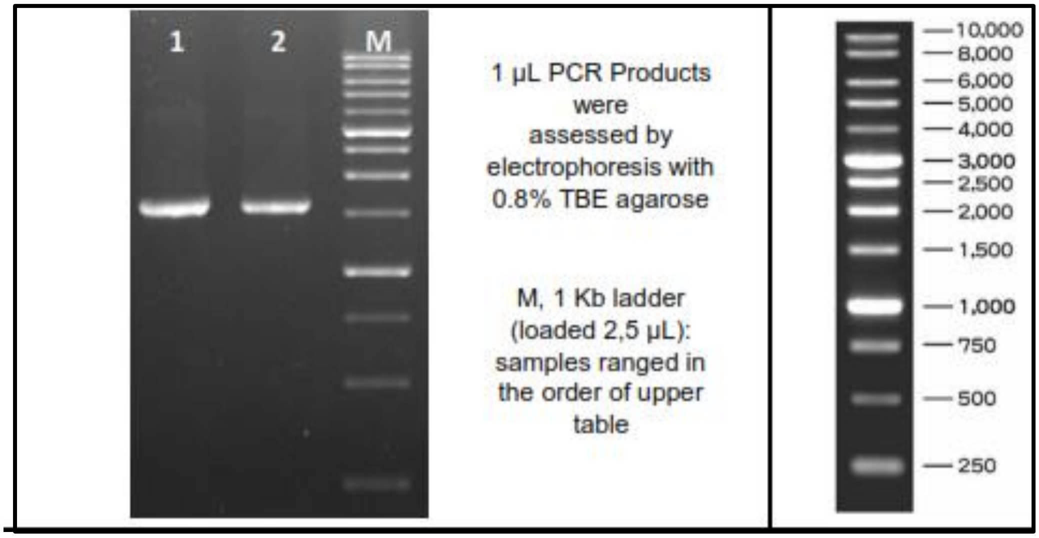
B1 B2 S1 S2

Figure 3a. Genomic DNA amplification of isolates B1 and B2



Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 3b. Genomic DNA amplification of isolates S1 and S2



Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 4a. The nucleotide sequence B1 isolate

```
Sequence Assembly 1434 bp
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 4b. The nucleotide sequence B2 isolate

```
Sequence Assembly 1424 bp
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 4c. The nucleotide sequence S1 isolate

Sequence Result Reverse Primer 882bp

```
1     GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61    CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121   TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181   AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241   GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301   CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361   AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421   ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481   TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541   GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601   TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661   GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721   AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781   ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841   GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 4d. The nucleotide sequence S2

Sequence Result Reverse Primer 849bp

```
1     ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61    TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121   CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181   CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241   CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301   GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361   GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421   ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481   AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541   GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601   TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661   ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721   TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781   CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841   CACTTCTCC
```

Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 5a. Phylogenetic tree Isolate Code B1

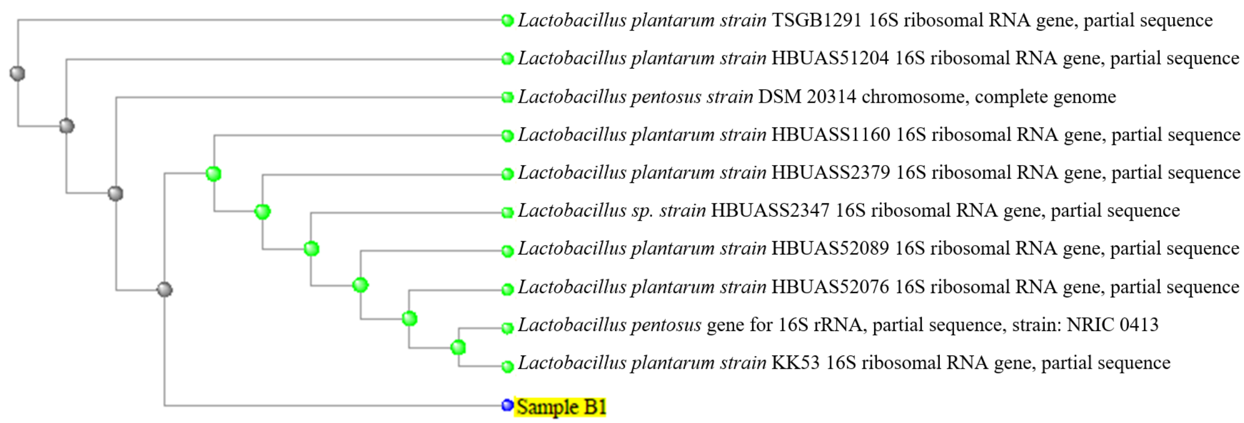


Figure 5b. Phylogenetic tree Isolate Code B2

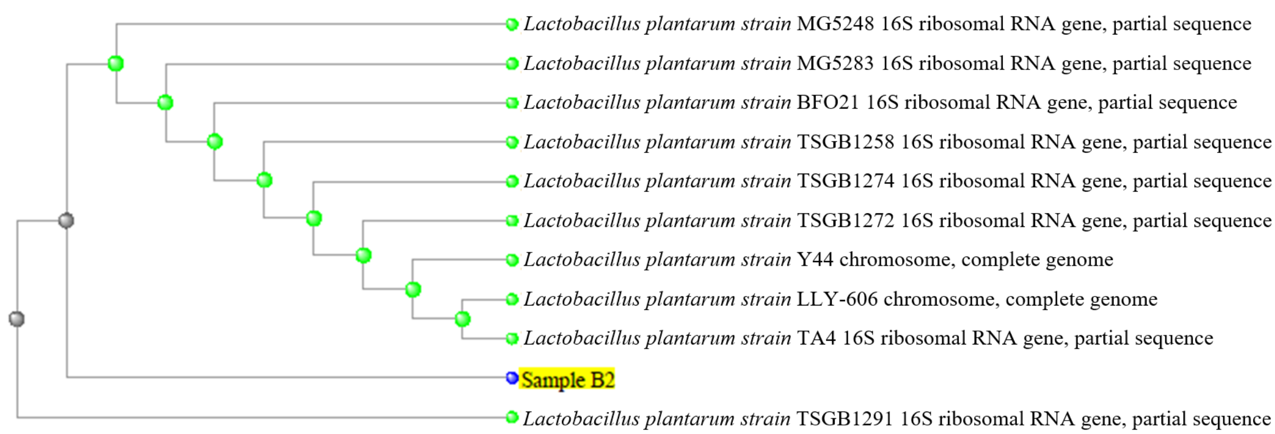


Figure 5c. Phylogenetic tree Isolate Code S1

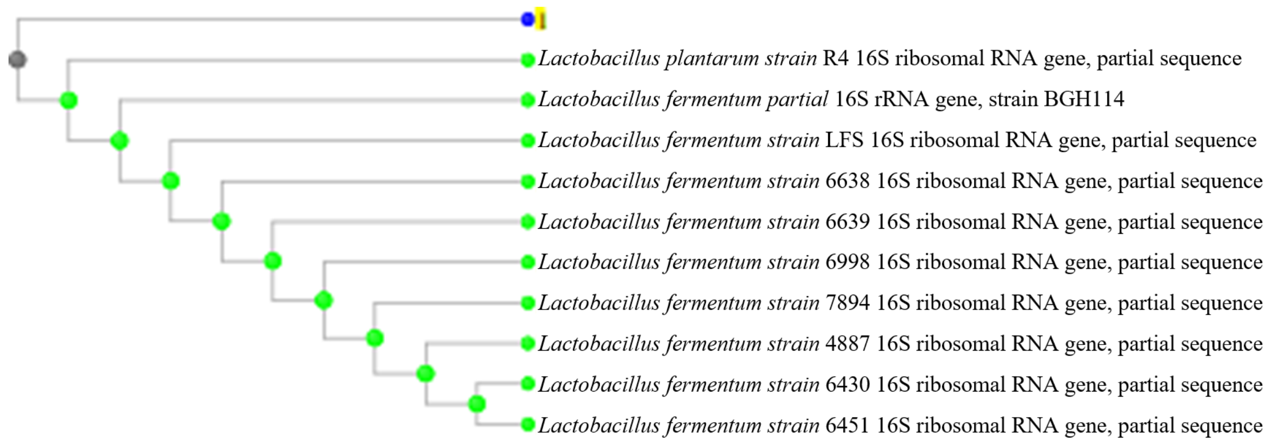
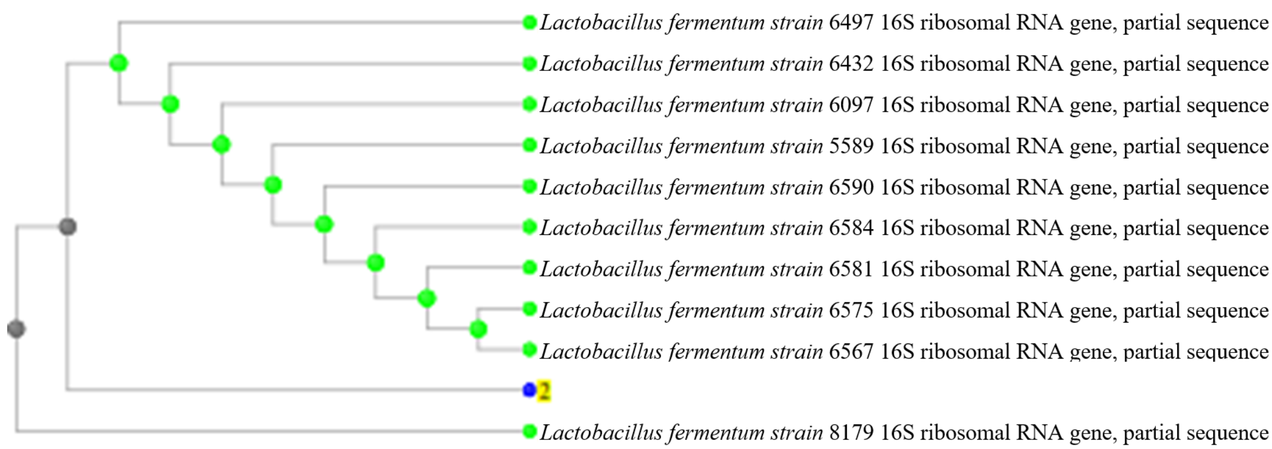


Figure 5d. Phylogenetic tree Isolate Code S2



FOOD
RESEARCH

27th June 2022

Dear Dr Okfrianti,

ACCEPTANCE LETTER

Food Research is pleased to inform you that the following manuscript has been accepted for publication in Food Research journal.

Manuscript Title	:	Identification of LAB isolated from ethnic fermented bamboo shoot "Lemea" in Bengkulu, Indonesia
Authors	:	Okfrianti, Y., Herison, C., Fahrurrozi and Budiyanto

We thank you for your fine contribution to the Food Research journal and encourage you to submit other articles to the Journal.

Yours sincerely,

Professor Dr. Son Radu
Chief Editor
Food Research

Identification of *LAB* isolated from ethnic fermented bamboo shoot "*Lemea*" in Bengkulu, Indonesia

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Abstract

Ethnic food is food inherited from ancestors whose process utilizes local food and 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 aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*. The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper Schult*) and yellow bamboo *(Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10 gof homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial dilutions were carried out starting from 10^{-1} to 10^{-7} and spread on MRSA media for each dilution series. Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA method. The results of the study found 4 isolates from 2 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).

Keywords: *Lemea*, Bamboo shoot, 16S rRNA, Lactic acid bacteria

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 odour and flavour are unique, and only the locals enjoy it. LAB that have 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 anti-bacterial activity (Jitpakdee *et al*., 2022). Isolation of lactic acid bacteria from indegenous 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 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 aims 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 h. 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 h (Figure 1).

2.2 LAB 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.

2.2 Identification of LAB

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

2.2.1 DNA Isolation

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

2.2.2 DNA amplification

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

2.2.3 DNA sequenching 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 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found were coded 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 *Lactobacillus plantarum* but not *Lactobacillus fermentum.* Based on the phylogeny tree, isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 5a), Isolate B2 *with Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure 5c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was *lactobacillus plantarum* (Sukmarini *et al*., 2014). All isolates have been homologous to the genus *Lactobacillus*. *Bacillus subtilis, Lactobacillus brevis*, and *Lactobacillus 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 *Lactobacillus plantarum and Lactobacillus rossiae* (Okfrianti *et al*., 2019). Lactic acid bacteria isolated from Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is expected to provide information on which LAB strains have been isolated 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 (*Bambusa vulgaris* Schrad) and Lactobacillus plantarum was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in foodstuffs that 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, bamboo shoots of B. vulgaris contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of *D. asper*, and 3.54 g of fibre (Chongtham *et al*., 2011). Different bamboo species contain different macronutrients (Adebola *et al.,* 2014). Differences in the content of bamboo shoots affect the types of bacteria found in *Lemea* products.

4. Conclusion

Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S rRNA. *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*).

Conflict of interest - Disclose potential conflicts of interest appropriately.

The authors declare no conflict of interest.

Acknowledgments

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Figure 1. Fermented bamboo shoot making process

Commented [A1]: Serai 3.3 g (comma change)
ml=mL

Commented [A2R1]: Is Serai = Lemon grass?

Commented [A3R1]: It has been fixed as suggested
350 mL water
3.3 lemongrass

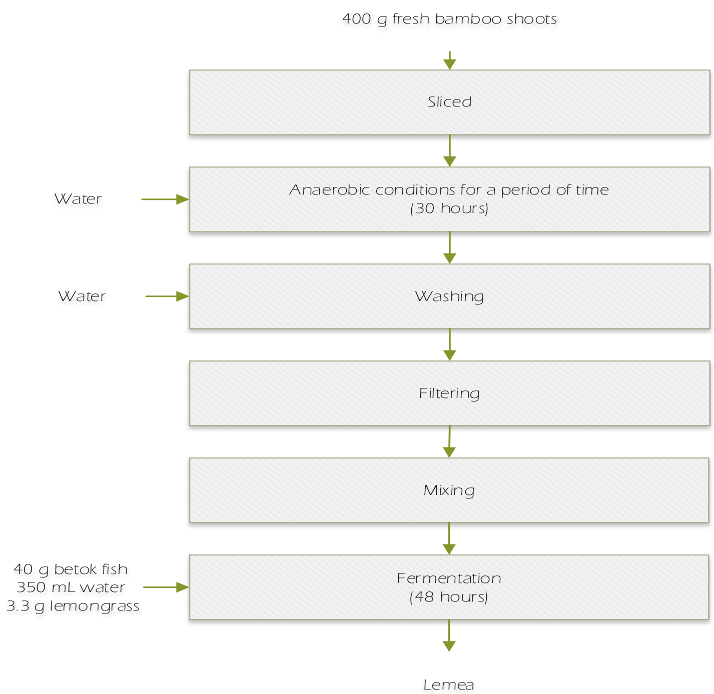
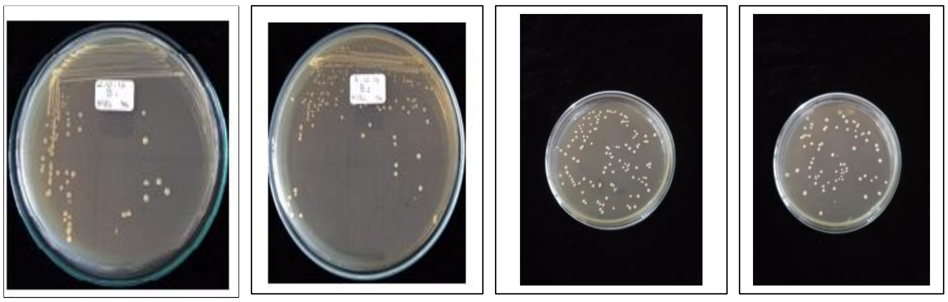
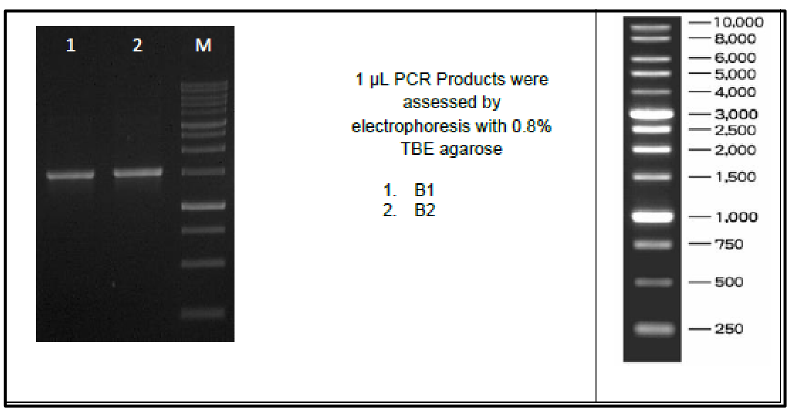


Figure 2. Isolate B1, B2,S1 and S2



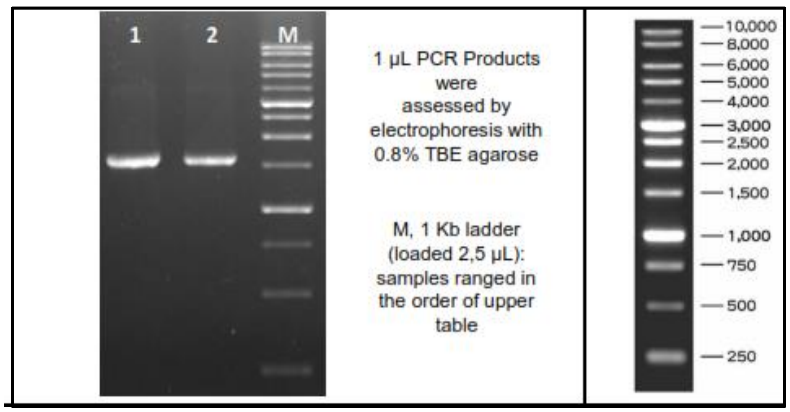
B1 B2 S1 S2

Figure 3a. Genomic DNA amplification of isolates B1 and B2



Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 3b. Genomic DNA amplification of isolates S1 and S2



Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder

Figure 4a. The nucleotide sequence B1 isolate

Sequence Assembly 1434 bp

```
1     AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61    TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121   ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181   TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241   GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301   GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361   GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421   CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481   CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541   CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601   CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661   ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721   TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781   CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841   CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901   CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961   AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021  TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081  CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141  GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201  AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261  CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321  AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381  CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
```

Figure 4b. The nucleotide sequence B2 isolate

Sequence Assembly 1424 bp

```
1     AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61    TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121   ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181   TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241   GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301   GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361   GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421   CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481   CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541   CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601   CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661   ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721   TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781   CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841   CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901   CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961   AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021  TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081  CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141  GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201  AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261  CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321  AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381  CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

Figure 4c. The nucleotide sequence S1 isolate

Sequence Result Reverse Primer 882bp

```
1       GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61      CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121     TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181     AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241     GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301     CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361     AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421     ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481     TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541     GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601     TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661     GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721     AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781     ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841     GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

Figure 4d. The nucleotide sequence S2

Sequence Result Reverse Primer 849bp

```
1       ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61      TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121     CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181     CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241     CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301     GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361     GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421     ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481     AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541     GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601     TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661     ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721     TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781     CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841     CACTTCTCC
```

Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

Types of Bamboo Shoots	Isolate Code	Species	Comparison Squen Access Number	Similarity (%)
Bamboo Betung	B1	*Lactobacillus plantarum*	M N37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

Figure 5a. Phylogenetic tree Isolate Code B1

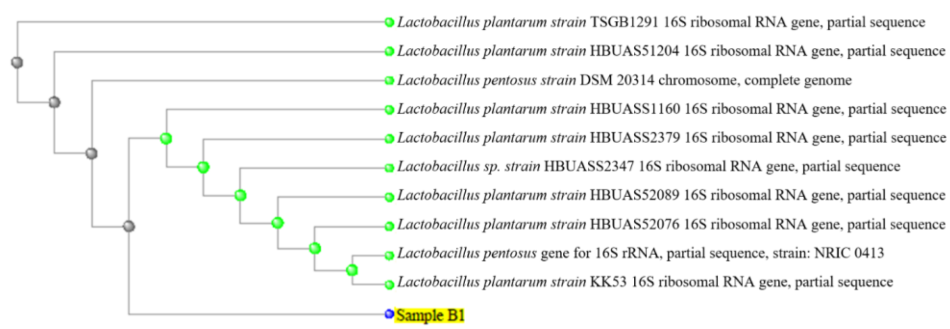


Figure 5b. Phylogenetic tree Isolate Code B2

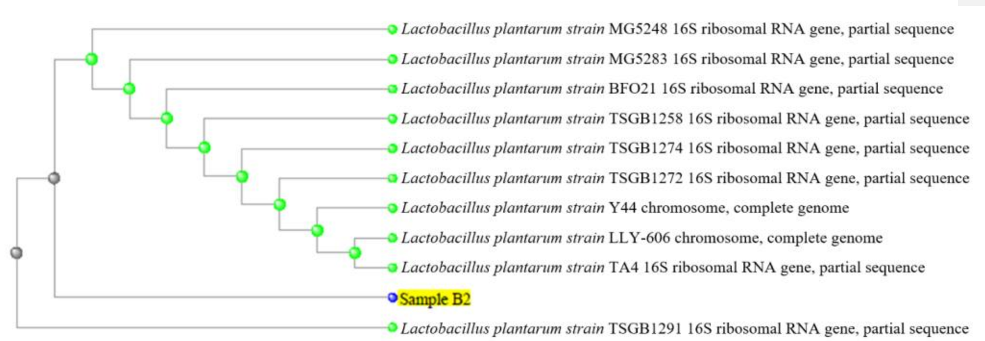


Figure 5c. Phylogenetic tree Isolate Code S1

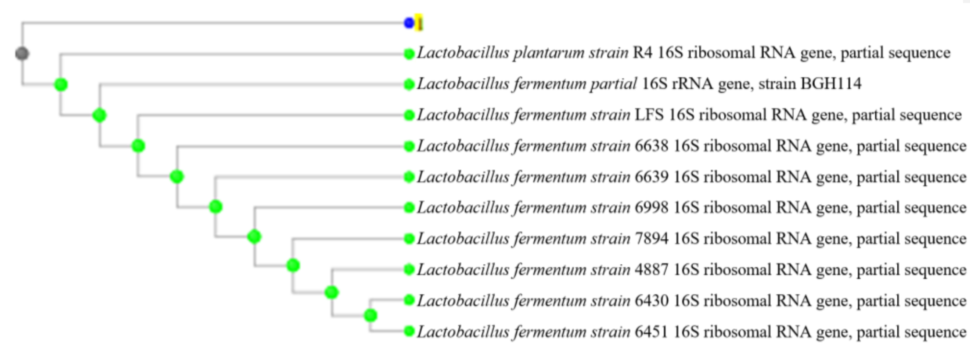
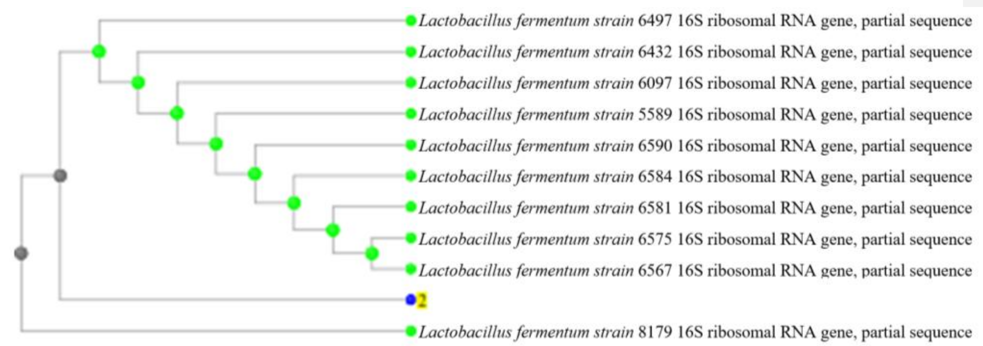


Figure 5d. Phylogenetic tree Isolate Code S2



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FOOD RESEARCH

FULL PAPER

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 anti-bacterial activity (Jitpakdee *et al.*, 2022). Isolation of lactic acid bacteria from indegenous 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).

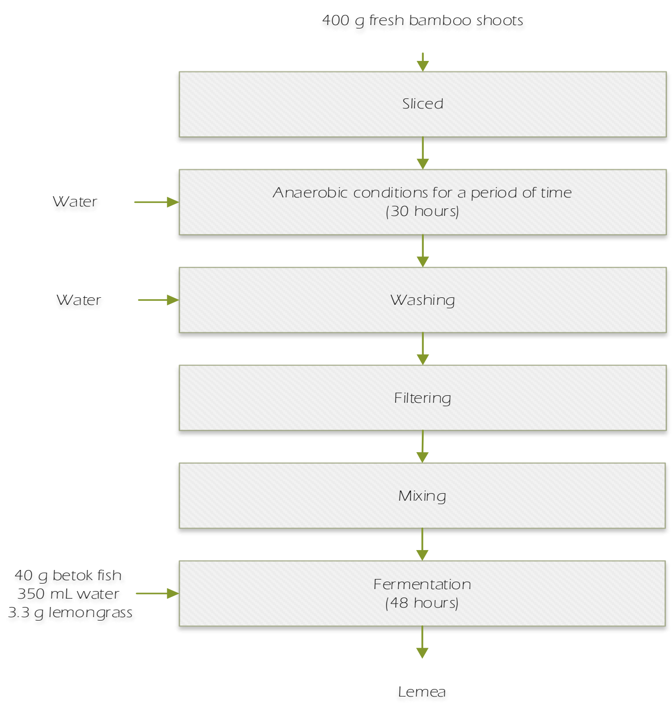


Figure 1. Fermented bamboo shoots making process

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 TM 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 ZymocleanTM 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.

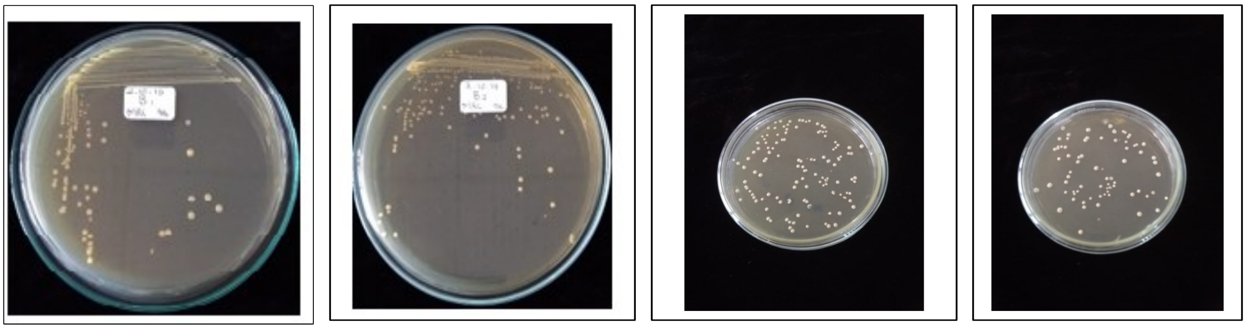
2.3.3 Deoxyribonucleic acid sequencing and phylogenetic analysis

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



B1 B2 S1 S2

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

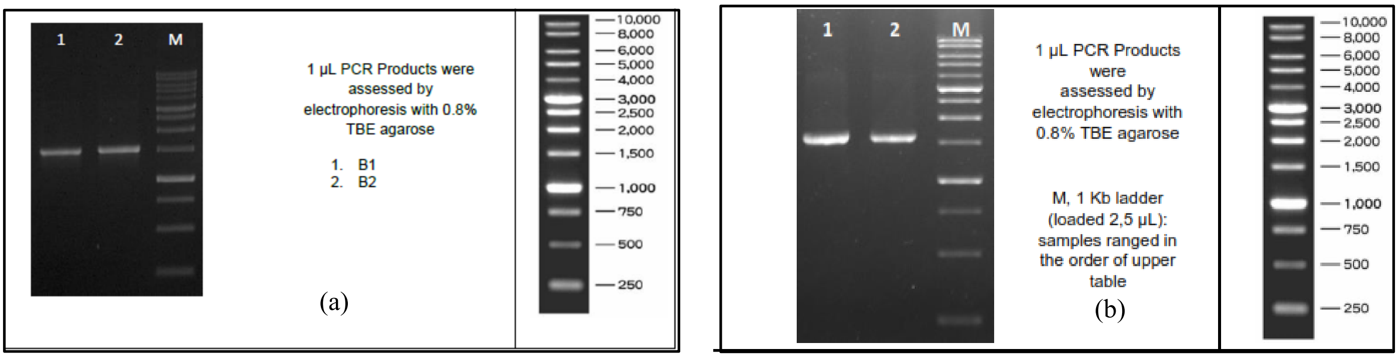


Figure 3. Genomic 16S rRNA amplification of (a) isolates B1 and B2 and (b) isolates S1 and S2. M: Kappa universal ladder.

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	*Lactobacillus plantarum*	MN37236.01	99
	B2	*Lactobacillus plantarum*	MN972325.1	99
Yellow Bamboo	S1	*Lactobacillus plantarum*	KM350169.1	99
	S2	*Lactobacillus fermentum*	MT538927.1	99

DOI

(a)

Sequence Assembly 1434 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTGCTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCG ACTT
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(b)

Sequence Assembly 1424 bp

```
1    AGGCGGCTGG TTCCTAAAAG GTTACCCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61   TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CGGCATGCTG ATCCGCGATT
121  ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181  TTAAGAGATT AGCTTACTCT CGCGAGTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241  GTGTAGCCCA GGTCATAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTTT
301  GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361  GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421  CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481  CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541  CCCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCGTACTC CCCAGGCGGA ATGCTTAATG
601  CGTTAGCTGC AGCACTGAAG GGCGGAAACC CTCCAACACT TAGCATTCAT CGTTTACGGT
661  ATGGACTACC AGGGTATCTA ATCCTGTTTG CTACCCATAC TTTCGAGCCT CAGCGTCAGT
721  TACAGACCAG ACAGCCGCCT TCGCCACTGG TGTTCTTCCA TATATCTACG CATTTCACCG
781  CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACTT
841  CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901  CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961  AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCCTTCTTCA CTCACGCGGC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCCT CCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCGCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
```

(c)

Sequence Result Reverse Primer 882bp

```
1    GTCCACCTTA GGCGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61   CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121  TCCGCGATTA CTAGCGATTC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181  AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241  GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301  CTCCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361  AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421  ATGCACCACC TGTCATTGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481  TGTCAAGACC TGGTAAGGTT CTTCGCGTAG CTTCGAATTA AACCACATGC TCCACCGCTT
541  GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGCG GTCGTACTCC CCAGGCGGAG
601  TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661  GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCGC TACCCATGCT TTCGAGTCTC
721  AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781  ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841  GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

(d)

Sequence Result Reverse Primer 849bp

```
1    ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61   TGGTGTGACG GGCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121  CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181  CGGTTTTAAG AGATTTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241  CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301  GGTTTGTCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361  GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACGACCATGC
421  ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481  AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAAACC ACATGCTCCA CCGCTTGTGC
541  GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601  TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCTCCA ACACCTAGCA CTCATCGTTT
661  ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721  TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781  CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841  CACTTCTCC
```

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.

Acknowledgments

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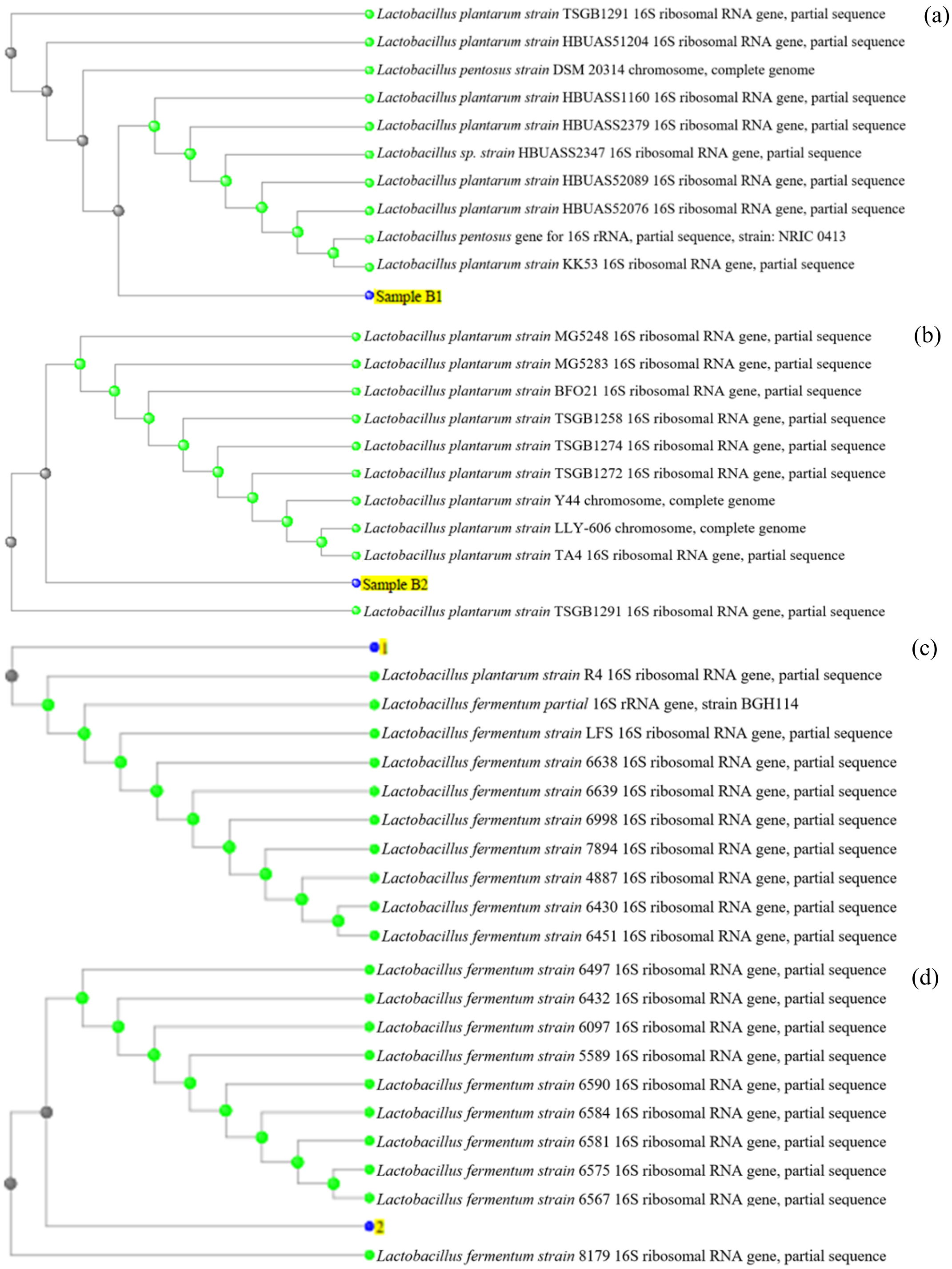

Figure 5. Phylogenetic tree of (a) B1, (b) B2, (c) S1 and (d) S2 isolates

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