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1. Appropriateness of Contents			x		
2. Originality of Topic		x			
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		<b>*NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below</b>	
1.	<b>Title : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria</b> <i>It should reflect the article:</i> <ul style="list-style-type: none"> <li>○ The title does not very much reflect the contain of the article.</li> <li>○ There is not any information and discussions about the ethnic food fermentation from Bengkulu in the results and discussion.</li> <li>○ The content of this manuscript is isolation and identification of LAB from fermented bamboo shoot</li> </ul>		
2.	<b>Abstract</b> <i>Background, Aim, Methodology and Conclusion:</i> <ul style="list-style-type: none"> <li>○ The background of the research is not clear. It is not stated explicitly and clearly</li> <li>○ It is more appropriate if the study aim is to isolate and identified LAB from fermented Betung bamboo shoot, and yellow bamboo shoot</li> <li>○ Methodology in the abstract is quite clear</li> <li>○ 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</li> </ul>		
3.	<b>Keywords: -</b> <i>Min. 3 and Max. 6</i>		
4.	<b>Introduction</b> <i>Concise with sufficient background</i> <ul style="list-style-type: none"> <li>○ 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.</li> <li>○ Related research and similar</li> </ul>		



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	<p>traditional fermented product reported by other researchers are not evaluated.</p> <ul style="list-style-type: none"> <li>○ 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</li> </ul>	
5.	<p><b>Research design/Methodology</b> <i>Clearly described and reproducible:</i></p> <ul style="list-style-type: none"> <li>○ The research design/methodology is not described clearly</li> <li>○ Sample preparation: the quantity of all the materials were not mentioned.</li> <li>○ The time of fermentation in the MM is different from the diagram in figure 1, and also different from the one in the introduction</li> <li>○ Isolation and identification steps need to be written clearly step by step</li> </ul>	
6.	<p><b>Data Analysis</b> <i>Results well presented and discussed</i></p> <ul style="list-style-type: none"> <li>○ Results are not well presented and discussed</li> <li>○ The authors do not explain how many isolates are obtained from MRSA.</li> <li>○ There was not any discussion.</li> <li>○ Only shows the facts, dan very limited information</li> </ul>	
7.	<p><b>Conclusion</b> <i>A clear summary of the study</i></p> <ul style="list-style-type: none"> <li>○ The conclusion written by the authors is not a conclusion since <i>L. plantarum</i> and <i>L. fermentum</i> are gram positive bacteria and LAB</li> </ul>	
8.	<p><b>References</b> <i>References should follow the journal's format</i></p>	
9.	<p><b>English Proficiency:</b> Some grammatical errors</p>	
10.	<p><b>Additional comments/suggestions by the</b></p>	

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8. Originality of Topic		v			
9. Manuscript Format			v		
10. Research Methodology			v		
11. Data Analysis			v		
12. Relevance to the Journal		v			

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11.	<b>Title</b> The title does not yet describe the method used. There should be a word isolation of lactic acid bacteria.	
12.	<b>Abstract</b> <i>It has not given the importance of isolating lactic acid bacteria from the product. Methods need to be summarized</i>	
13.	<b>Keywords</b> <i>It should be added to the product name.</i>	
14.	<b>Introduction</b> <i>It has not yet described the importance of isolating lactic acid bacteria from the product.</i>	
15.	<b>Research design/Methodology</b> <i>Method clearly described and reproducible.</i>  !	
16.	<b>Data Analysis</b> <i>It is necessary to add a discussion about the differences in lactic acid bacteria obtained from differences in bamboo types that are strengthened by reference.</i>	
17.	<b>Conclusion</b> <i>It should be associated with the source of lactic acid bacteria isolation</i>	

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18. <b>References</b> <i>ok</i>	
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1 **Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria**

2 **Abstract**

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

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

16 **1. Introduction**

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

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

24 including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West  
25 Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented  
26 Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular  
27 in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish  
28 and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-  
29 7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al.*, 2021).  
30 River fish that are often used to make lemea are freshwater fish. One of the freshwater fish that can  
31 be used is **betok fish**, lemea containing lactic acid bacteria (Xu *et al.*, 2021). Lactic acid bacteria consist  
32 of microbial strains that convert carbohydrates into lactic acid (Dinoto *et al.*, 2020), LAB isolated  
33 results were identified based on phenotypic and genotypic characteristics (Lawalata *et al.*, 2011).  
34 Genotypic LAB identification was carried out by PCR technique in the form of amplifying and  
35 sequencing the universal region of the 16S rRNA gene (Dinoto *et al.*, 2020). Lactic Acid Bacteria (LAB)  
36 strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) has been previously  
37 studied. . The purpose of this study was to investigate Lactic Acid Bacteria (LAB) strains in fermented  
38 Betung bamboo shoots (*Dendrocalamus asper* Schult) and Yellow bamboo shoots (*Bambusa vulgaris*  
39 Schrad).

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

## 40 2. Materials and methods

### 41 2.1 Sample Preparation

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

### 46 2.2 LAB isolation

47 Isolation of LAB was carried out using a device that was sterilized beforehand and carried out  
48 aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1%  
49 NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at  
50 37°C for 48 hours. The isolates obtained need to be purified and identified

### 51 2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)

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

### 59 3. Results and discussion/Results

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



71 using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of  
72 isolates B1, B2, S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder  
73 (Figures 3a and 3b). phylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by  
74 NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures  
75 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots  
76 contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots  
77 contain more lactic acid bacteria in the form of *Lactobacillus fermentum*.

#### 78 4. Conclusion

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

81

#### 82 Conflict of interest - Disclose potential conflicts of interest appropriately.

83 The authors declare no conflict of interest.

#### 84 Acknowledgments

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

86

87 **References**

- 88 Azizah, N., Suradi, K. and Gumilar, J. (2019). Effect of Concentration of Lactic Acid Bacteria *Lactobacillus*  
89 *Plantarum* and *Lactobacillus Casei* on Microbiological and Chemical Quality of Probiotic Mayonnaise.  
90 *Journal of Animal Science Padjadjaran University*, 18(2), 79–85.  
91 <https://doi.org/10.24198/jit.v18i2.19771>
- 92 Desniar, Rusmana, I., Suwanto, A. and Mubarik, R. (2013). Characterization of Lactic Acid Bacteria Isolated  
93 From an Indonesian Fermented Fish (Bekasam) and Their Antimicrobial Activity Against Pathogenic  
94 bacteria. 25(6), 489–494. <https://doi.org/10.9755/ejfa.v25i6.12478>
- 95 Dewi, KH. (2015). Raw Materials Inventory and Fermentation Process in Lemea Industry the Traditional  
96 Food of Rejang Tribe. *International Journal on Advanced Science, Engineering and Information*  
97 *Technology*, 5(3), 196–200. <https://doi.org/10.18517/ijaseit.5.3.512>
- 98 Dewi, KH, Silsia, D., Susanti, L. and Hasanuddin. (2014). Mapping of the “Lemea” Traditional Food Industry  
99 of the Rejang Tribe in Bengkulu Province. *Journal of AGRISEP*, 13(1), 60–66.  
100 <https://doi.org/10.31186/jagrisep.13.1.60-66>
- 101 Dinoto, A., Rosyidah, AL, Ria, A., Sari, P. and Julistiono, H. (2020). Isolation, Identification and Antimicrobial  
102 Activities of Lactic Acid Bacteria from Fruits of Wild Plants in Tambrauw Forest, West Papua,  
103 Indonesia. 21(7), 3391–3397. <https://doi.org/10.13057/biodiv/d210764>
- 104 Lawalata, HJ, Sembiring, L. and Rahayu, ES. (2011). Molecular Identification of Lactic Acid Bacteria  
105 Producing Antimicrobial Agents from Bakasang, An Indonesian Traditional Fermented Fish Product.  
106 *Indonesian Journal of Biotechnology*, 16(2), 93–99.
- 107 Manin, F. (2010). Potential of *Lactobacillus Acidophilus* And *Lactobacillus Fermentum* From Digestive  
108 Tracts Of Free-range Chickens From Peat As Sources Of Probiotics. *Scientific Journal of Animal*  
109 *Sciences Jambi University*, XIII(5), 221–228. <https://doi.org/10.22437/jiiip.v0i0.19>
- 110 Noor, Z., Cahyanto, MN, Indrati, R. and Sardjono, S. (2018). Screening of *Lactobacillus Plantarum* Produced  
111 Lactic Acid for Mocaf Fermentation. *Agritech*, 37(4), 437. <https://doi.org/10.22146/agritech.18821>
- 112 Rhee, SJ, Lee, JE and Lee, CH. (2011). Importance of Lactic Acid Bacteria in Asian Fermented Foods.  
113 *Microbial Cell Factories*, 10(SUPPL. 1), 1–13. <https://doi.org/10.1186/1475-2859-10-S1-S5>
- 114 Widodo, Wahyuningsih, TD, Nurrochmad, A., Wahyuni, E., Taufiq, TT, Anindita, NS, Lestari, S., Harsita, PA,

115 Sukarno, AS and Handaka, R. (2017). Local Strain Lactic Acid Bacteria "Isolation to Application as  
116 Probiotics and Milk Fermentation Starter. Yogyakarta: Gadjah Mada University Press.

117 Wirawati, CU, Sudarwanto, MB, Lukman, DW, Wientarsih, I. and Srihanto, EA. (2019). Diversity of Lactic  
118 Acid Bacteria in Dadih Produced by Either Back-Slopping or Spontaneous Fermentation From Two  
119 Different Regions of West Sumatra, Indonesia. *Veterinary World*, 12(6), 823–829.  
120 <https://doi.org/10.14202/vetworld.2019.823-829>

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

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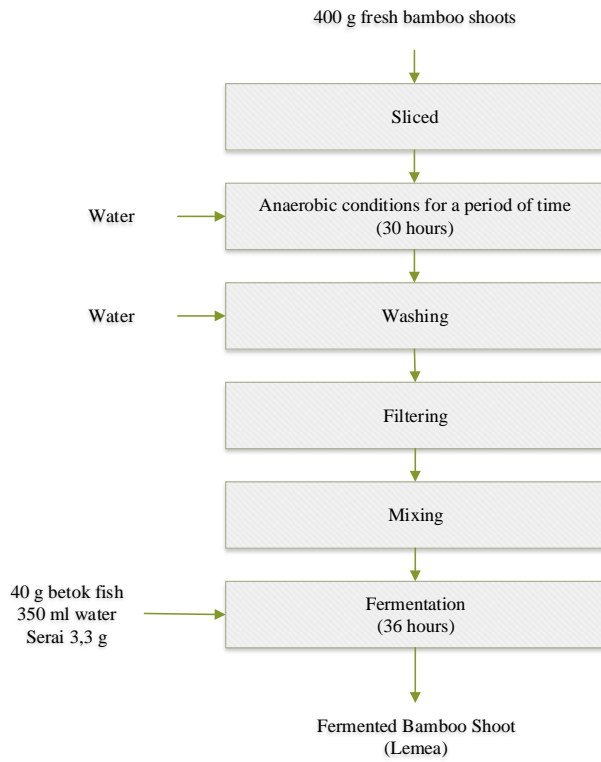
141 Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

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

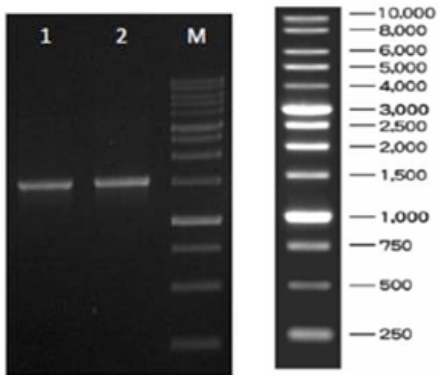
142

143

144 Figure 1. Fermented Bamboo shoot making process

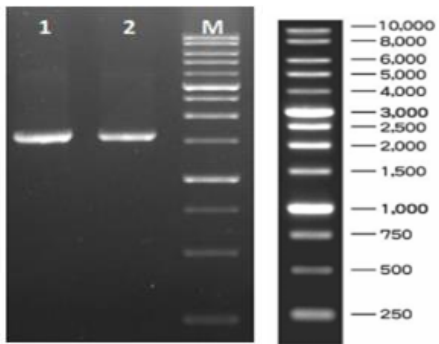


146 Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose  
147 concentration using the Kappa Universal ladder



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



152  
153  
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157 Figure 3a. Sequence assembly result – PCR product Isolate Code B1

**Sequence Assembly 1434 bp**

```

1   AGCGGGCTGG TTCCTAAAAG GTTACCCAC  CGACTTTGGG TGTACAAC  TCATGTTG
61  TGACGGGCGG TGTGTACAAG GCCCGGAAC  GTATTCACCG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC  T GAGAATGGCT
181 TTAAGAGATT AGCTTGTCT  CGCGAGITCG CAACTCGTIG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGTTTT
301 GTCACCGGCA GTCACCCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA  CATGCACCAC
421 CTGTATCCAT GTCCCGAAG  GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGCGTA GCTTCGAAT  AAAACCATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC  CCCAGGCGGA ATGCTTAAATG
601 CGTTAGCTGC AGCACTGAAG GGCAGAAACC CTCCACACT  TAGCATTCA  CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTITG  CTACCCATAC TTTCCAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC  TCGCCACTG  TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGT  TCCAGTTTC  CGATGCACCT
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAA  CGGCCTGCGC TGCTTTTACG
901 CCCAATAAAT CCGGACAAAG  CTTGCCACT  ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTCT
1021 TCTTAAACA  CAGAGTTTTA  CGAGCGAAA  CCTTCTTCA CTCAGCGGCG GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG  AAGATTCCT  ACTGCTGCT  CCCTAGGAG  TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT  TCAAGTCCGC  TACGTATCAT TGCCATGGTG
1201 AGCCGTATCC CCACCATCTA GCTAATACG  CGCGGACCA  TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTCA  AACTCGGACC  ATGCGGTCCA AGTGTATTG  CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT  GGGCAGGTT  CCCACGTGT  ACTCACAGT  TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACAA  TCAATACCAG AGTTGTTGCT ACTT

```

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

```

1   AGCGGGCTGG TTCCTAAAAG GTTACCCAC  CGACTTTGGG TGTACAAC  TCATGTTG
61  TGACGGGCGG TGTGTACAAG GCCCGGAAC  GTATTCACCG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC  T GAGAATGGCT
181 TTAAGAGATT AGCTTACTCT  CGCGAGITCG CAACTCGTIG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGTTTT
301 GTCACCGGCA GTCACCCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA  CATGCACCAC
421 CTGTATCCAT GTCCCGAAG  GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGCGTA GCTTCGAAT  AAAACCATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC  CCCAGGCGGA ATGCTTAAATG
601 CGTTAGCTGC AGCACTGAAG GGCAGAAACC CTCCACACT  TAGCATTCA  CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTITG  CTACCCATAC TTTCCAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC  TCGCCACTG  TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGT  TCCAGTTTC  CGATGCACCT
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAA  CGGCCTGCGC TGCTTTTACG
901 CCCAATAAAT CCGGACAAAG  CTTGCCACT  ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTCT
1021 TCTTAAACA  CAGAGTTTTA  CGAGCGAAA  CCTTCTTCA CTCAGCGGCG GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG  AAGATTCCT  ACTGCTGCT  CCCTAGGAG  TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT  TCAAGTCCGC  TACGTATCAT TGCCATGGTG
1201 AGCCGTATCC CCACCATCTA GCTAATACG  CGCGGACCA  TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTCA  AACTCGGACC  ATGCGGTCCA AGTGTATTG  CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT  GGGCAGGTT  CCCACGTGT  ACTCACAGT  TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACAA  TCAATACCAG AGTT

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

**Sequence Result Reverse Primer 882bp**

```

1      GTCCACCTTA GGGGGCTGGC TCCTAAAAGG TTACCCACC GACTTTGGGT GTTACAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGGCATTG CTAGCGGATC CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCACCTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCAATTGG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCCGGTAG CTTCGAATTA AACCCATATG TCCACCGCTT
541    GTGCGGGGCC CCGTCAATTC CTTTGAGTTT CAACCTTCCG GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAAACC TCCAACCTT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCCG TACCCATGCT TTAGGATCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCGCT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCACCCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACCTT TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
  
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166 Figure 3d. Sequence assembly result – PCR product Isolate Code S2

**Sequence Result Reverse Primer 849bp**

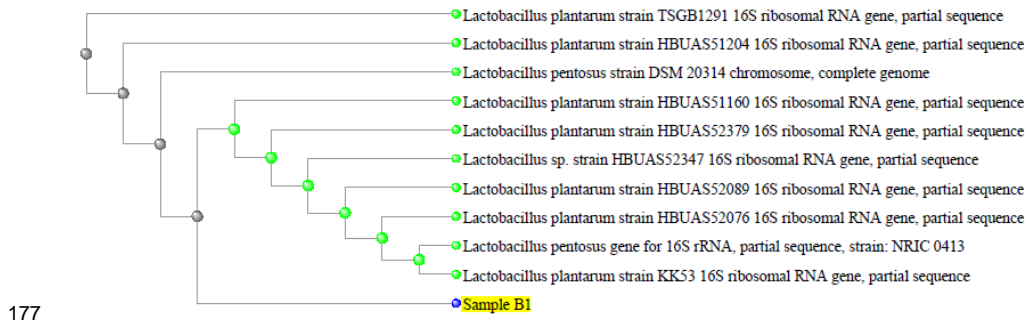
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1      ACCTTAGGGG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GGGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCTGTCAGG CGAGTTGCAG CTTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTTGCTT GOCCTCGCGA GTTCGGGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GOCOCAGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301    GGTTTGTAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTCGGCTCG TTGCGGGACT TAACCAACA TCTCAGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCCTCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCC AGTCTCAGCG
721    TCAGTTGACG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841    CACTTCTCC
  
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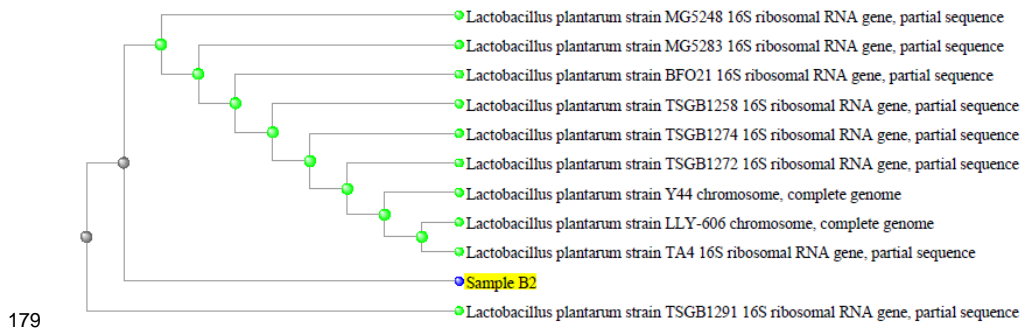
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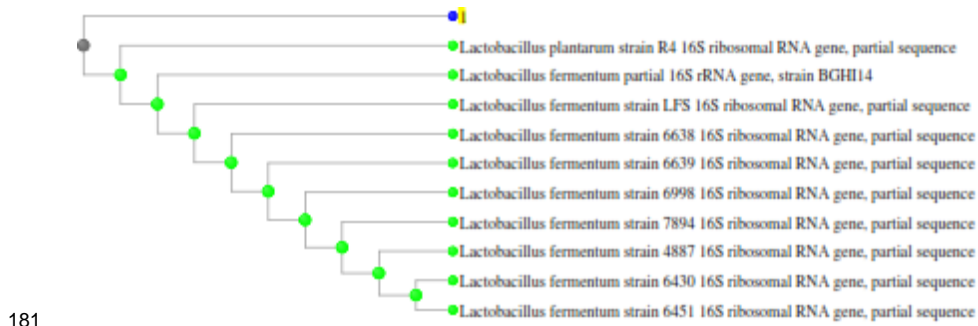
176 Figure 4a. Phylogenetic tree Isolate Code B1



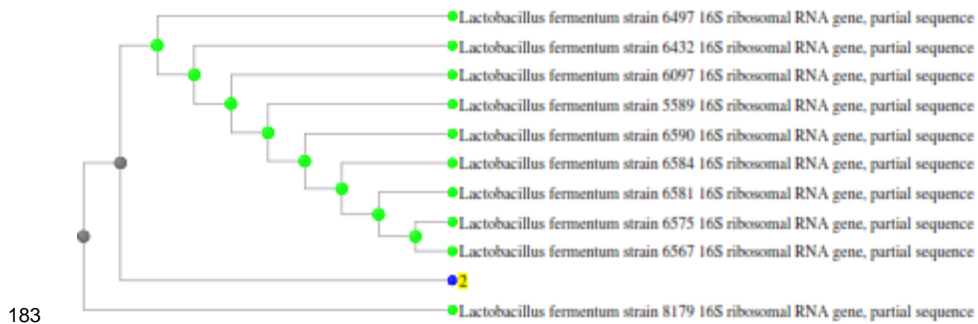
178 Figure 4b. Phylogenetic tree Isolate Code B2



180 Figure 4c. Phylogenetic tree Isolate Code S1



182 Figure 4d. Phylogenetic tree Isolate Code S2



## Hasil Koreksian dari Riviewer 2

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

#### 189 Abstract

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

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

#### 203 5. Introduction

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

208 There are a variety of fermented foods in the world especially in Asia. Most of Asian fermented foods  
209 are non-dairy products featuring various other raw materials such as cereals, soybeans, fruit,  
210 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

211 including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West  
212 Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented  
213 Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular  
214 in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish  
215 and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-  
216 7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al.*, 2021).

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

## 227 6. Materials and methods

### 228 2.1 Sample Preparation

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

### 233 2.2 LAB isolation

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

**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?

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

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

234 Isolation of LAB was carried out using a device that was sterilized beforehand and carried out  
235 aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1%  
236 NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at  
237 37°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

### 238 2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)

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

## 246 7. Results and discussion/Results

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

258 using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of  
259 isolates B1, B2, S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder  
260 (Figures 3a and 3b). phylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by  
261 NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures  
262 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots  
263 contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots  
264 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*.

## 265 8. Conclusion

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

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

## 269 Conflict of interest - Disclose potential conflicts of interest appropriately.

270 The authors declare no conflict of interest.

## 271 Acknowledgments

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

273

274 **References**

- 275 Azizah, N., Suradi, K. and Gumilar, J. (2019). Effect of Concentration of Lactic Acid Bacteria *Lactobacillus*  
276 *Plantarum* and *Lactobacillus Casei* on Microbiological and Chemical Quality of Probiotic Mayonnaise.  
277 *Journal of Animal Science Padjadjaran University*, 18(2), 79–85.  
278 <https://doi.org/10.24198/jit.v18i2.19771>
- 279 Desniar, Rusmana, I., Suwanto, A. and Mubarik, R. (2013). Characterization of Lactic Acid Bacteria Isolated  
280 From an Indonesian Fermented Fish (Bekasam) and Their Antimicrobial Activity Against Pathogenic  
281 bacteria. 25(6), 489–494. <https://doi.org/10.9755/ejfa.v25i6.12478>
- 282 Dewi, KH. (2015). Raw Materials Inventory and Fermentation Process in Lemea Industry the Traditional  
283 Food of Rejang Tribe. *International Journal on Advanced Science, Engineering and Information*  
284 *Technology*, 5(3), 196–200. <https://doi.org/10.18517/ijaseit.5.3.512>
- 285 Dewi, KH, Silsia, D., Susanti, L. and Hasanuddin. (2014). Mapping of the “Lemea” Traditional Food Industry  
286 of the Rejang Tribe in Bengkulu Province. *Journal of AGRISEP*, 13(1), 60–66.  
287 <https://doi.org/10.31186/jagrisep.13.1.60-66>
- 288 Dinoto, A., Rosyidah, AL, Ria, A., Sari, P. and Julistiono, H. (2020). Isolation, Identification and Antimicrobial  
289 Activities of Lactic Acid Bacteria from Fruits of Wild Plants in Tambrauw Forest, West Papua,  
290 Indonesia. 21(7), 3391–3397. <https://doi.org/10.13057/biodiv/d210764>
- 291 Lawalata, HJ, Sembiring, L. and Rahayu, ES. (2011). Molecular Identification of Lactic Acid Bacteria  
292 Producing Antimicrobial Agents from Bakasang, An Indonesian Traditional Fermented Fish Product.  
293 *Indonesian Journal of Biotechnology*, 16(2), 93–99.
- 294 Manin, F. (2010). Potential of *Lactobacillus Acidophilus* And *Lactobacillus Fermentum* From Digestive  
295 Tracts Of Free-range Chickens From Peat As Sources Of Probiotics. *Scientific Journal of Animal*  
296 *Sciences Jambi University*, XIII(5), 221–228. <https://doi.org/10.22437/jiiip.v0i0.19>
- 297 Noor, Z., Cahyanto, MN, Indrati, R. and Sardjono, S. (2018). Screening of *Lactobacillus Plantarum* Produced  
298 Lactic Acid for Mocaf Fermentation. *Agritech*, 37(4), 437. <https://doi.org/10.22146/agritech.18821>
- 299 Rhee, SJ, Lee, JE and Lee, CH. (2011). Importance of Lactic Acid Bacteria in Asian Fermented Foods.  
300 *Microbial Cell Factories*, 10(SUPPL. 1), 1–13. <https://doi.org/10.1186/1475-2859-10-S1-S5>
- 301 Widodo, Wahyuningsih, TD, Nurrochmad, A., Wahyuni, E., Taufiq, TT, Anindita, NS, Lestari, S., Harsita, PA,

302 Sukarno, AS and Handaka, R. (2017). Local Strain Lactic Acid Bacteria "Isolation to Application as  
303 Probiotics and Milk Fermentation Starter. Yogyakarta: Gadjah Mada University Press.

304 Wirawati, CU, Sudarwanto, MB, Lukman, DW, Wientarsih, I. and Srihanto, EA. (2019). Diversity of Lactic  
305 Acid Bacteria in Dadih Produced by Either Back-Slopping or Spontaneous Fermentation From Two  
306 Different Regions of West Sumatra, Indonesia. *Veterinary World*, 12(6), 823–829.  
307 <https://doi.org/10.14202/vetworld.2019.823-829>

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

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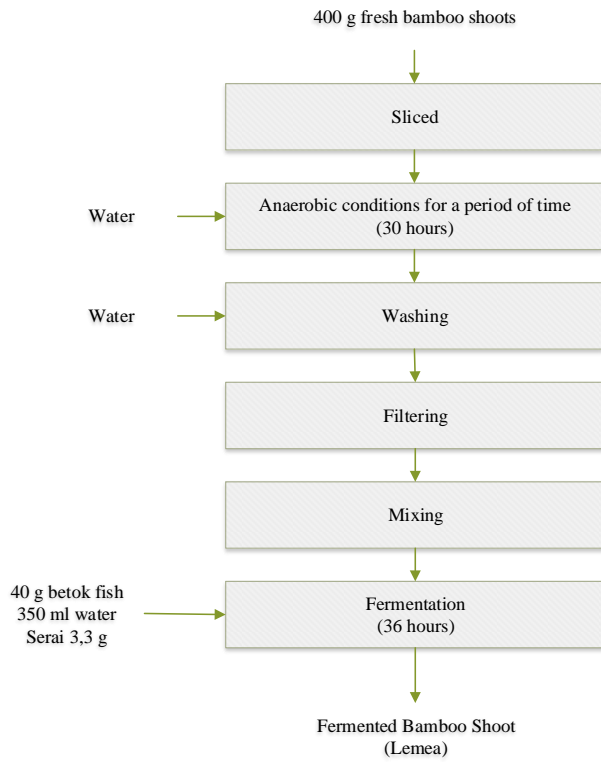
328 Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

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

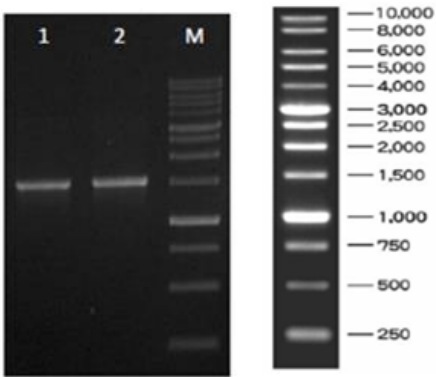
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331 Figure 1. Fermented Bamboo shoot making process

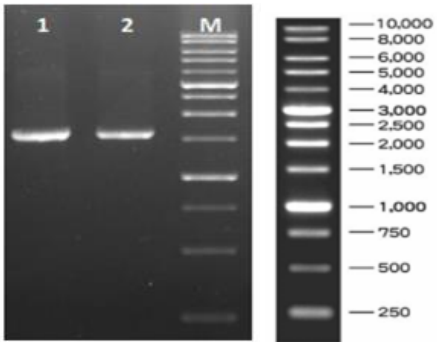


333 Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose  
334 concentration using the Kappa Universal ladder



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



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

Sequence Assembly 1434 bp  
1 AGCGGGCTGG TTCCTAAAAG GTTACCCAC CGACTTTGGG TGTACAAC TCATGTTG  
61 TGACGGGCGG TGTGTACAAG GCCCGGAAC GTATTACCG CGGCATGCTG ATCCGCGATT  
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC GAGAATGGCT  
181 TTAAGAGATT AGCTTGTCTT CCGGAGTTCC CAACTCGTTG TACCATCCAT TGTAGCACGT  
241 GTTAGGCCCA GGTCTAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGTTTT  
301 GTCACCGGCA GTCCTACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC  
361 GCTCGTTGGC GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA CATTGCAACC  
421 CTGTATCCAT GTCCCGAAG GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC  
481 CTGGTAAGGT TCTTGGCGTA GCTTCGAAT AAAACACATG CTCACCCGCT TGTGCGGGCC  
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC CCCAGGCGGA ATGCTTAAATG  
601 CGTTAGCTGC AGCACTGAAG GCGGAAACC CTCACACT TAGCATTCA CTTTTACGGT  
661 ATGGACTACC AGGGTATCTA ATCCGTITG CTACCCATAC TTTCCAGCCT CAGCGTCAGT  
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG  
781 CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGT TCCAGTTTC CGATGCACTT  
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAA CCGCCTGCGC TGCTTTTACG  
901 CCCAATAAAT CCGGACAAAG CTTGCCACTT ACGTATTACC GCGGCTGCTG GCACGTAGTT  
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT  
1021 TCTTAAACA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT  
1081 CAGACTTTCC TCCATTGTGG AAGATTCCTT ACTGCTGCTT CCGTAGGAG TTTGGGCCGT  
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAAGTCCGC TACGTATCAT TGCCATGGTG  
1201 AGCCGTATCC CCACCATCTA GCTAATACG CCGGGGACCA TCCAAAAGTG ATAGCCGAAG  
1261 CCATCTTCA AACTCGGACC ATGCGGTCCA AGTGTATTG CCGTATTAGC ATCTGTTTCC  
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACAGT TCGCCACTCA  
1381 CTCAAATGTA AATCATGATG CAAGCACAA TCAATACCAG AGTTGTTCCG ACTT

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346

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

1 AGCGGGCTGG TTCCTAAAAG GTTACCCAC CGACTTTGGG TGTACAAC TCATGTTG  
61 TGACGGGCGG TGTGTACAAG GCCCGGAAC GTATTACCG CGGCATGCTG ATCCGCGATT  
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC GAGAATGGCT  
181 TTAAGAGATT AGCTTACTCT CCGGAGTTCC CAACTCGTTG TACCATCCAT TGTAGCACGT  
241 GTTAGGCCCA GGTCTAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGTTTT  
301 GTCACCGGCA GTCCTACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC  
361 GCTCGTTGGC GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA CATTGCAACC  
421 CTGTATCCAT GTCCCGAAG GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC  
481 CTGGTAAGGT TCTTGGCGTA GCTTCGAAT AAAACACATG CTCACCCGCT TGTGCGGGCC  
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC CCCAGGCGGA ATGCTTAAATG  
601 CGTTAGCTGC AGCACTGAAG GCGGAAACC CTCACACT TAGCATTCA CTTTTACGGT  
661 ATGGACTACC AGGGTATCTA ATCCGTITG CTACCCATAC TTTCCAGCCT CAGCGTCAGT  
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG  
781 CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGT TCCAGTTTC CGATGCACTT  
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAA CCGCCTGCGC TGCTTTTACG  
901 CCCAATAAAT CCGGACAAAG CTTGCCACTT ACGTATTACC GCGGCTGCTG GCACGTAGTT  
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT  
1021 TCTTAAACA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT  
1081 CAGACTTTCC TCCATTGTGG AAGATTCCTT ACTGCTGCTT CCGTAGGAG TTTGGGCCGT  
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAAGTCCGC TACGTATCAT TGCCATGGTG  
1201 AGCCGTATCC CCACCATCTA GCTAATACG CCGGGGACCA TCCAAAAGTG ATAGCCGAAG  
1261 CCATCTTCA AACTCGGACC ATGCGGTCCA AGTGTATTG CCGTATTAGC ATCTGTTTCC  
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACAGT TCGCCACTCA  
1381 CTCAAATGTA AATCATGATG CAAGCACAA TCAATACCAG AGTT

348

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

**Sequence Result Reverse Primer 882bp**

```
1      GTCCACCTTA GGGGGCTGGC TCCTAAAAGG TTACCCACC GACTTTGGGT GTTACAACT
61     CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTG CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTGCG GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCACCTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCAATTGG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCCGGTAG CTTCGAATTA AACCCATGTC TCCACCGCTT
541    GTGCGGGGCC CCGTCAATTC CTTTGAGTTT CAACCTTCCG GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAAACC TCCAACCTT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCCG TACCCATGCT TTAGGATCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCGTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCACCCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACCTT TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
```

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352

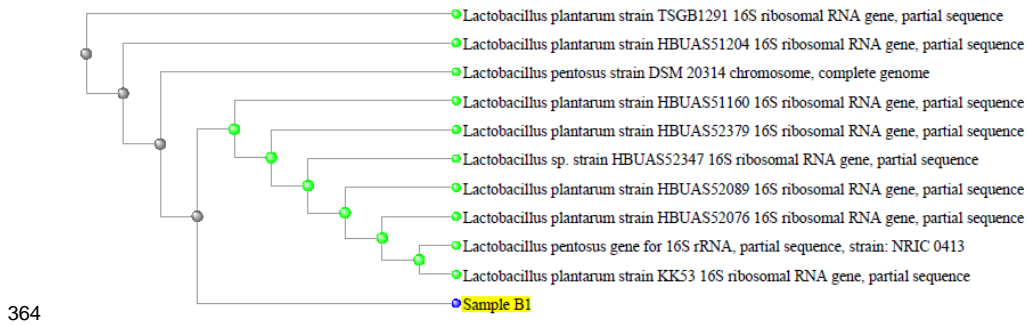
353 Figure 3d. Sequence assembly result – PCR product Isolate Code S2

**Sequence Result Reverse Primer 849bp**

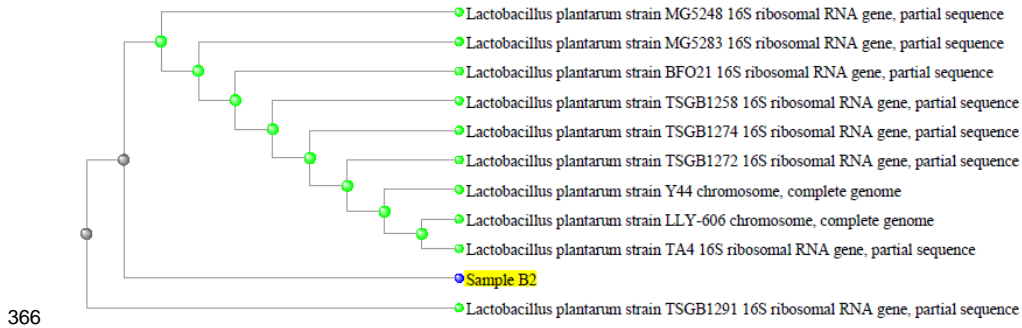
```
1      ACCTTAGGGG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GCGCGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCTGTCAGG CGAGTTGCGC CTTGCGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTTGCTT GOCCTCGCGA GTTCGGGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GOCOCAGTCA TAAGGGGCGT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301    GGTTTGTAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTCCGCTCG TTGCGGGACT TAACCAACA TCTCAGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTGTGAC ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCGATG
841    CACTTCTCC
```

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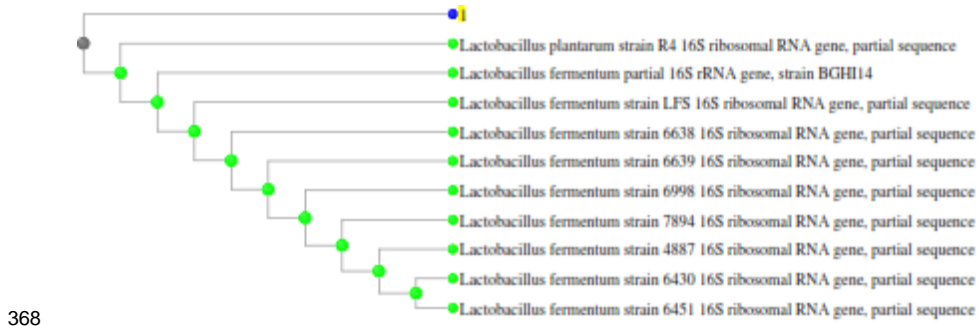
363 Figure 4a. Phylogenetic tree Isolate Code B1



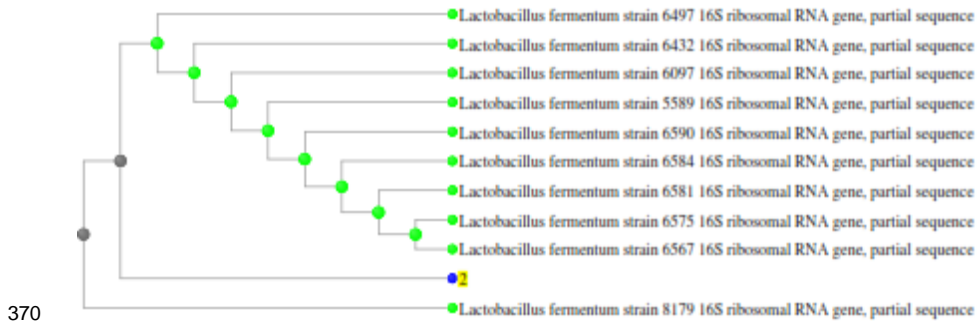
365 Figure 4b. Phylogenetic tree Isolate Code B2



367 Figure 4c. Phylogenetic tree Isolate Code S1



369 Figure 4d. Phylogenetic tree Isolate Code S2



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**Date** : 5<sup>th</sup> February 2022

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**Manuscript ID** : FR-IFC-029

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**Please return by** : 5<sup>th</sup> March 2022

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**Title of Manuscript** : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

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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			



<b>(REVIEWER'S SECTION)</b>  REVIEWER'S COMMENTS/SUGGESTIONS		<b>(AUTHOR'S SECTION)</b>  AUTHOR'S ACTION/RESPONSE	
		<b>*NOTE FOR AUTHOR: Please state your response to the reviewer's comments/suggestion below</b>	
21.	<p><b>Title : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria</b> <i>It should reflect the article:</i></p> <ul style="list-style-type: none"> <li>○ The title does not very much reflect the contain of the article.</li> <li>○ There is not any information and discussions about the ethnic food fermentation from Bengkulu in the results and discussion.</li> <li>○ The content of this manuscript is isolation and identification of LAB from fermented bamboo shoot</li> </ul>	<p>The title has been corrected to:</p> <p>Identification of lactid acid bacteria isolated from ethnic fermented bamboo shoot "<i>Lemea</i>" in Bengkulu, Indonesia</p>	
22.	<p><b>Abstract</b> <i>Background, Aim, Methodology and Conclusion:</i></p> <ul style="list-style-type: none"> <li>○ The background of the research is not clear. It is not stated explicitly and clearly</li> <li>○ It is more appropriate if the study aim is to isolate and identified LAB from fermented Betung bamboo shoot, and yellow bamboo shoot</li> <li>○ Methodology in the abstract is quite clear</li> <li>○ 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</li> </ul> <p>N</p>	<ul style="list-style-type: none"> <li>○ <b>The abstract</b> has been remade, focusing on the process of isolation and identification of lactic acid bacteria from lemea products.</li> <li>○ <b>This study aims</b> to find lactic acid bacteria (LAB) to isolate and identify strains of <i>Lemea</i>.</li> <li>○ <b>The methodology in the abstract has been clarified:</b> betung shoots (<i>Dendrocalamus asper Schult</i>) and yellow bamboo (<i>Bambusa vulgaris Schrad</i>) have been used.</li> <li>○ Furthermore, it was remotely analyzed on M RSA media and four LAB isolates were obtained. Molecular identification using the 16S rRNA method.</li> </ul>	
23.	<p><b>Keywords: -</b> <i>Min. 3 and Max. 6</i></p>	<p><b>Keywords:</b> <i>Lemea</i>, bamboo shoot, 16S rRNA, LAB</p>	
24.	<p><b>Introduction</b> <i>Concise with sufficient background</i></p> <ul style="list-style-type: none"> <li>○ 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.</li> <li>○ Related research and similar traditional fermented product</li> </ul>	<ul style="list-style-type: none"> <li>○ Information about the raw materials used has been added to the composition in 2.1 sample preparation</li> <li>○ Research on a similar traditional fermented</li> </ul>	

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	<p>reported by other researchers are not evaluated.</p> <ul style="list-style-type: none"> <li>○ 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</li> </ul>	<p>product has been reported, namely eksam and has been evaluated in the introduction</p> <ul style="list-style-type: none"> <li>○ The title, objectives, background and conclusion have been adjusted according to the method</li> </ul>
25.	<p><b>Research design/Methodology</b> <i>Clearly described and reproducible:</i></p> <ul style="list-style-type: none"> <li>○ The research design/methodology is not described clearly</li> <li>○ Sample preparation: the quantity of all the materials were not mentioned.</li> <li>○ The time of fermentation in the MM is different from the diagram in figure 1, and also different from the one in the introduction</li> <li>○ Isolation and identification steps need to be written clearly step by step</li> </ul>	<ul style="list-style-type: none"> <li>○ The method has been described in more detail with Fig1 and 2</li> <li>○ The number of ingredients has been added</li> <li>○ The fermentation time has been synchronized which is 48 hours in the sample preparation and Fig 1</li> <li>○ Isolation and identification steps have been added</li> </ul>
26.	<p><b>Data Analysis</b> <i>Results well presented and discussed</i></p> <ul style="list-style-type: none"> <li>○ Results are not well presented and discussed</li> <li>○ The authors do not explain how many isolates are obtained from MRSA.</li> <li>○ There was not any discussion.</li> <li>○ Only shows the facts, d anvery limited information</li> </ul>	<ul style="list-style-type: none"> <li>○ Results have been presented. Each Table and Figure has been explained in more detail</li> <li>○ The resulting isolates were 4 isolates and supported by the image of the isolate in Fig 3</li> <li>○ Each result explanation has been added a discussion</li> <li>○ Non-factual information has been added and explained in Results and discussion/Results</li> </ul>
27.	<p><b>Conclusion</b> <i>A clear summary of the study</i></p> <ul style="list-style-type: none"> <li>○ The conclusion written by the authors is not a conclusion since <i>L. plantarum</i> and <i>L. fermentum</i> are gram positive bacteria and LAB</li> </ul>	<p>The conclusion has been corrected according to the reviewer's suggestion</p> <p>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. <i>Lactobacillus fermentum</i> was only found in the fermentation process of yellow bamboo shoots (<i>Bambusa vulgaris Schrad</i>) while <i>Lactobacillus plantarum</i> was found in yellow</p>

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		bamboo shoots ( <i>Bambusa vulgaris Schrad</i> ) and betung ( <i>Dendrocalamus asper Schult</i> ).
28.	<b>References</b> <i>References should follow the journal's format</i>	Reference has followed the format
29.	<b>English Proficiency:</b> Some grammatical errors	The grammar has been corrected.
30.	<b>Additional comments/suggestions by the reviewer about the article:</b> <ul style="list-style-type: none"> <li>○ The article should be rewrite from abstract to conclusion for proper articles.</li> <li>○ Results and discussion are very limited. They should be presented clearly, every step and discussed in more comprehensive supported by suitable references</li> <li>○ There should be clear correlation among back ground, aim, MM, results and conclusion</li> </ul>	<ul style="list-style-type: none"> <li>○ The article has been rewritten.</li> <li>○ The results have been made clearer and supported with the latest references.</li> <li>○ It has been correlated with the title, objectives, methods, results, and discussion.</li> </ul>

### Overall Evaluation

*Please choose one.*

Accept		Major Revision	V
Minor Revision		Reject	

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## Respon penulis terhadap evaluasi reviewer 2

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**Date** : 5<sup>th</sup> February 2022

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**Manuscript ID** : FR-IFC-029

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**Please return by** : 5<sup>th</sup> March 2022

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**Title of Manuscript** : Ethnic food fermentation from Bengkulu as a source of lactic acid bacteria

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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			

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

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		isolates which were identified molecularly by 16S rRNA. <i>Lactobacillus fermentum</i> was only found in the fermentation process of yellow bamboo shoots ( <i>Bambusa vulgaris Schrad</i> ) while <i>Lactobacillus plantarum</i> was found in yellow bamboo shoots ( <i>Bambusa vulgaris Schrad</i> ) and betung ( <i>Dendrocalamus asper Schult</i> ).
38.	<b>References</b> <i>Ok</i>	-
39.	<b>English Proficiency</b> Need to be checked again	English Proficiency has been checked according to the suggestion
40.	<b>Additional comments/suggestions by the reviewer about the article</b>	-

**Overall Evaluation**

Please choose one.

<b>Accept</b>		<b>Major Revision</b>	
<b>Minor Revision</b>	<b>v</b>	<b>Reject</b>	

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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.

**Commented [H28]:** The abstract has been remade, focusing on the process of isolation and identification of lactic acid bacteria from lemea products.

**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 bamboo (*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 on MRSA media and four LAB isolates were obtained. Molecular identification using the 16S rRNA method.

**Commented [H34]:** Product name has been added

25 Rejang people live. *Lemea* is an ethnic food from the Rejang tribe. Ethnic foods are meals that have  
26 their origins in an ethnic group's history and culture (Kwon, 2015). Bamboo shoots and river fish are  
27 fermented into *Lemea* by the Rejang people (Dewi *et al.*, 2014). Betung, Tabah, Mayan, and Seik  
28 bamboo are some of the most common bamboo varieties used by the Rejang to produce *Lemea* .  
29 Betok, kepala timah, and mujahir fish are the most common fish used. The odor and flavour are  
30 unique, and only the locals enjoy it. LAB that have an impact on the flavour of fermented foods (Fox,  
31 2011). Indigenous fermented foods have been extensively researched.

32 There are various fermented foods in the world, especially in Asia. Fermented foods made from  
33 bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus*  
34 (Tomar, 2016). *Meakri*, from Meghalaya Indian fermented bamboo shoots as a source of  
35 *Lactobacillus*, has characteristics suitable for probiotics (Das *et al.*, 2020). *Lactobacillus* is a group of  
36 LAB that are gram-positive bacteria. LAB have the potential to inhibit the infection and growth of  
37 pathogenic microbes (Yang *et al.*, 2021). LAB isolated from fermented bamboo shoots are potential  
38 probiotic candidates that are beneficial for health (Mohamad *et al.*, 2020). LAB strains are selected  
39 for their decreased content of cholesterol, antioxidant activity, and anti bacterial activity (Jitpakdee  
40 *et al.*, 2022). Isolation of lactic acid bacteria from indigenous fermented foods is very important  
41 (Mende *et al.*, 2022).

42 *Bekasam* is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional  
43 fermented food popular in Sumatera and Kalimantan (Desniar *et al.*, 2013). The sour taste in *Bekasam*  
44 is almost the same as in *Lemea*. The difference between these two products is the carbohydrate  
45 source and fermentation time. Carbohydrate sources are a source of nutrition for bacteria that play  
46 a role in the fermentation process. The source of carbohydrates used for rice in *Lemea* is bamboo  
47 shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes 10 days.

**Commented [H35]:** The reason for isolation has been added to the introduction, namely looking for LAB strains from traditional foods



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

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

## 57 10. Materials and methods

### 58 2.1 Sample Preparation

59 Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are  
60 thinly sliced and soaked for 30 hours. Then they were washed, filtered, and weighed as much as  
61 400 grams, and they added 40 g of betok fish, 350 ml of water, and 3.3 g of lemongrass, and  
62 fermented for 48 hours (Figure 1).

### 63 2.2 LAB isolation

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

**Commented [H36]:** The methods of sample preparation, isolation and identification have been described in the isolation and sample preparation stages

**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

71 (Figure 2).

72 a. Identification of LAB

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

Commented [H41]: identification steps have been added

75 i. DNA Isolation

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

78 ii. DNA amplification

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

83 iii. DNA sequencing and phylogenetic analysis

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

90 11. Results and discussion/Results

91 3.1 Isolation of lactic acid bacteria from *Lemea*

92 Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung  
93 bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found  
94 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

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

### 101 3.2 Identification of *Lemea* isolates

102 The results of genomic DNA amplification of the 4 isolates can be seen in Figures 4a and 4b.  
103 Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size.  
104 Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434  
105 bp for isolate B1 (Fig. 5a), 1424 bp for B2 (Fig. 5b). The nucleotide sequence S1 isolate was 882  
106 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). The results of the analysis using the  
107 BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to  
108 *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.  
109 The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% similar to the  
110 partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain  
111 of *Lactobacillus plantarum* but not *Lactobacillus fermentum*. Based on the phylogeny tree,  
112 isolate B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure  
113 6a), isolate B2 with *Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA  
114 (Figure 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal  
115 RNA (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567  
116 16S ribosomal RNA. The type of isolate that was identified from Bekasam was *lactobacillus*  
117 *plantarum* (Sukmarini *et al.*, 2014). All isolates have been homologous to the genus *Lactobacillus*.  
118 *Bacillus subtilis*, *Lactobacillus brevis*, and *Lactobacillus plantarum* were found in dominating

**Commented [H44]:** Each result explanation has been added a discussion

**Commented [H45]:** Results have been presented. Each Table and Figure has been explained in more detail

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119 strains of Soidon fermented bamboo shoot food without salt from Indian Manipur (Jeyaram *et*  
120 *al.*, 2010). LAB strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz)  
121 have been previously studied and isolated as 2 species, namely *Lactobacillus plantarum* and  
122 *Lactobacillus rossiae* (Okfrianti *et al.*, 2019). Lactic acid bacteria isolated from Indonesian  
123 fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is  
124 expected to provide information on which LAB strains have been isolated from different types  
125 of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the diversity of the  
126 bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria* and  
127 *Proteobacteria* (Li *et al.*, 2021).  
128 *Lactobacillus fermentum* was only found in lemea made from yellow bamboo shoots (*Bambusa*  
129 *vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa*  
130 *vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in  
131 foodstuffs that trigger the growth of *Lactobacillus* (Macfarlane & Cummings, 1999).  
132 Oligosaccharides and fiber are prebiotics that promote the growth of specific bacteria found in  
133 the gut. Bamboo shoots are a good source of fiber (Felisberto *et al.*, 2017). At 100 g of fresh  
134 weight, bamboo shoots of *B. vulgaris* contain 6.51 g of carbohydrates, 4.24 g of fiber, 4.90 g of  
135 *D. Asper*, and 3.54 g of fiber (Chongtham *et al.*, 2011). Different bamboo species contain  
136 different macronutrients (Adebola *et al.*, 2014). Differences in the content of bamboo shoots  
137 affect the types of bacteria found in Lemea products.

Commented [H54]: Non-factual information has been added and explained in Results and discussion/Results

## 138 12. Conclusion

139 Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus*  
140 *plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S  
141 rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots

Commented [H55]: Each result explanation has been added a discussion

142 (*Bambusa vulgaris Schrad*), while *Lactobacillus plantarum* was found in yellow bamboo shoots

143 (*Bambusa vulgaris Schrad*) and betung (*Dendrocalamus asper Schult*).

144 **Conflict of interest - Disclose potential conflicts of interest appropriately.**

145 The authors declare no conflict of interest.

146 **Acknowledgments**

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149

**Commented [H56]:**

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

150 **References**

- 151 Adebola, O. O., Corcoran, O., & Morgan, W. A. (2014). Synbiotics: The impact of potential prebiotics inulin,  
152 lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of*  
153 *Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- 154 Chongtham, N., Bisht, M. S., & Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots: Potential  
155 and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and Food*  
156 *Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 157 Desniar, Rusmana, I., Suwanto, A., & Mubarik, D. N. R. (2013). Characterization of lactic acid bacteria  
158 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
159 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
160 <https://doi.org/10.9755/ejfa.v25i6.12478>
- 161 Dewi, K. H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P., & Universitas, P. (2014). Suku Rejang di Provinsi  
162 Bengkulu Industry Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province. *AGRISEP*,  
163 14(1), 61–69.
- 164 Felisberto, M. H. F., Miyake, P. S. E., Beraldo, A. L., & Clerici, M. T. P. S. (2017). Young bamboo culm:  
165 Potential food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
166 <https://doi.org/10.1016/j.foodres.2017.08.058>
- 167 Fox, P. F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
168 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- 169 Jeyaram, K., Romi, W., Singh, T. A., Devi, A. R., & Devi, S. S. (2010). Bacterial species associated with  
170 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
171 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
172 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- 173 Jitpakdee, J., Kantachote, D., Kanzaki, H., & Nitoda, T. (2022). Potential of lactic acid bacteria to produce  
174 functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160, 113269.  
175 <https://doi.org/10.1016/j.lwt.2022.113269>
- 176 Kwon, D. Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
177 <https://doi.org/10.1016/j.jef.2015.02.001>

- 178 Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M., & Gao, H. (2021). In vitro fecal fermentation  
179 characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry: X*, 11.  
180 <https://doi.org/10.1016/j.fochx.2021.100129>
- 181 Macfarlane, G. T., & Cummings, J. H. (1999). Probiotics and prebiotics: Can regulating the activities of  
182 intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.
- 183 Okfianti, Y., Darwis, & Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
184 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter-Professional Health  
185 Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-](https://doi.org/10.2991/icihc-18.2019.52)  
186 [18.2019.52](https://doi.org/10.2991/icihc-18.2019.52)
- 187 Rahayu, E. S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 75–84.  
188 <https://doi.org/10.22146/agritech.13515>
- 189 Sukmarini, L., Mustopa, A. Z., Normawati, M., & Muzdhalifa, I. (2014). Identification of Antibiotic-  
190 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of  
191 Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>
- 192 Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M., & Topisirovic,  
193 L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese. *Journal of  
194 Applied Microbiology*, 103(6), 2142–2152. <https://doi.org/10.1111/j.1365-2672.2007.03450.x>

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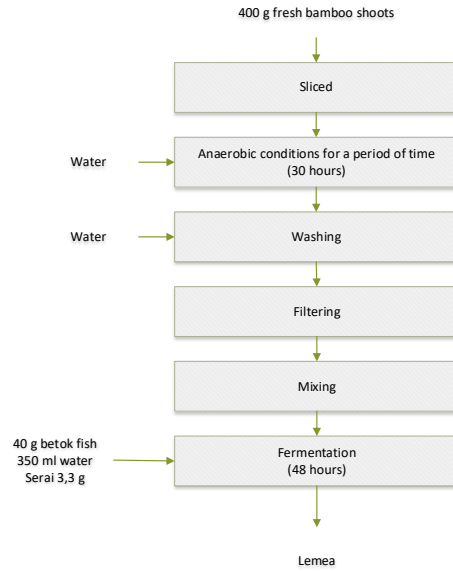
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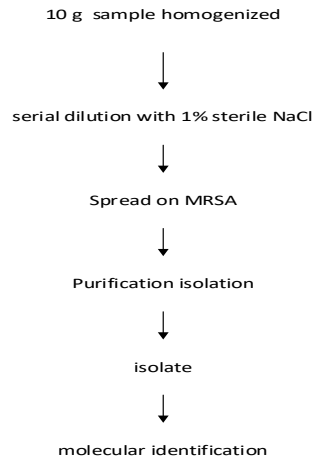
Figure 1. Fermented Bamboo shoot making process



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Figure 2. Isolation Process



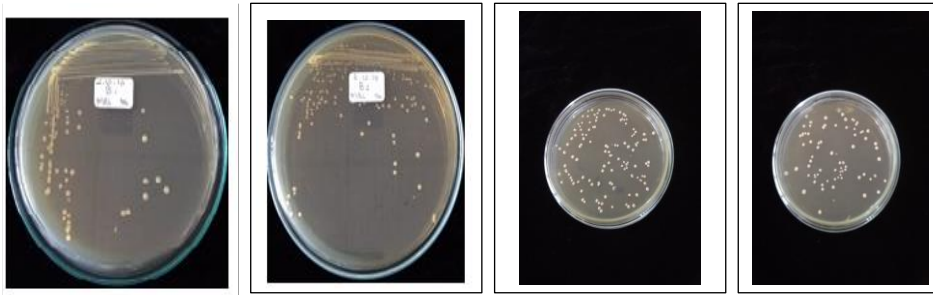
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Figure 3. Isolate B1, B2, S1 dan S2



B1

B2

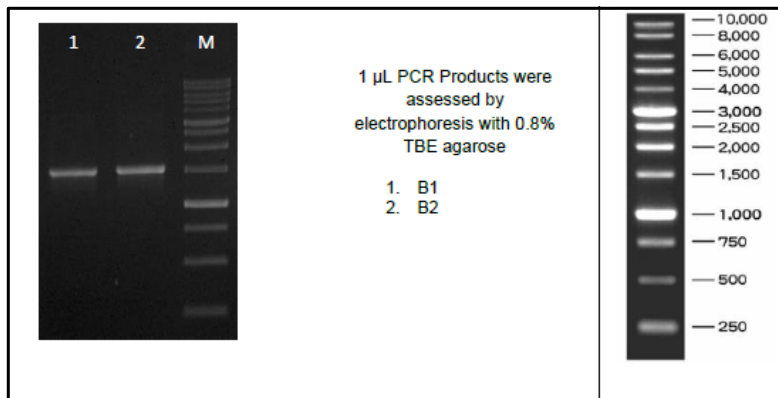
S1

S2

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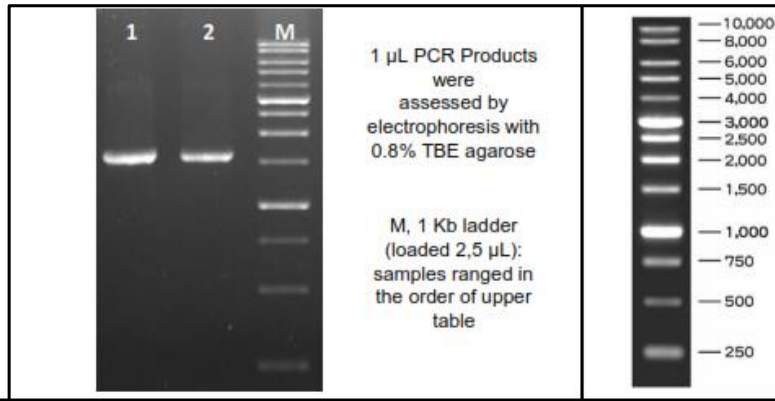
213 Figure 4a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose

214 concentration using the Kappa Universal ladder



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



218  
219

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

Sequence Assembly 1434 bp

```

1   AGGCGGCTGG TTCCTAAAG GTTACCCAC CGACTTTGGG TGTACAAAC TCTCATGGTG
61  TGACGGGCGG TGTGTACAAG GCGCGGAAC GTATTACCG GGGCATGCTG ATCCGGGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAATGGCT
181 TTAAGAGATT AGCTTGTCTT CCGGAGTTCC CACTCGTTG TACCATCCAT TGTAGCACGT
241 GTGTAGCCCA GGTCTAAGGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTIT
301 GTCACGGGCA GTCTCACCG AGTGCOCAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCCACAC
421 CTGTATCCAT GTCCCGGAAG GGAAGTCTA ATCTTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGTAAAGT TCTTCGGTA GCTTGAATT AAACCAATG CTCCACCGCT TGTGGGGGCC
541 CCGTCAATT CTTTTGAGTT TCAGCCTTGC GGCCTACTC CCGAGCGGA ATGTTAATG
601 CGTACTGCTG AGCACTGAAG GCGGGAACC CTCCAGACT TAGCATTCAI CGTTACGGT
661 ATGCACTACC AGGATCTTA ATCCTTTTG CTACCCATAC TTTGAGCCTI CAGGTCAGT
721 TACAGCCAG ACACCGGCTT TCGCCAGTGG TGTCTTCCA TATATCTAGC CAITTCACCG
781 CTACATATGG AGTTCACCTG TCCCTTCTG CACTCAAGTT TCCCASTTTC CGATGCACTT
841 CTTGGTITGA GCGGAAGGCT TTCACATCAG ACTTAAAAA CCGCCTGGCC TCGCTTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACTAGTIT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTCTI
1021 TCTTTAACAA CAGAGTTTAA CAGCGGAAA CCGTCTTCA CTCACGGGCG GTTGTCCAT
1081 CAGACTTTCG TCCATTGGGG AAGATTCCCT ACTGCTGCTI CCGGTAGGAG TTTGGGCGGT
1141 GTCTCAGTCC CAATGGGGCC GATTACCTC TCAGGTCGGC TACGTATCAI TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC GCGGGGACCA TCCAAAAGTG ATAGCGGAAG
1261 CCATCTTCCA AACTCGGACC ATGCGGTCCA AGTGTIATG CCGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCGCGTCTT GGGCAGGTTT CCGACGTGTT ACTCACCAAT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCC ACTT
  
```

221

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

```

Sequence Assembly 1424 bp
1  AGGCGGCTGG  TTCTAAAG  GTTACCCAC  CGACTTTGGG  TGTACAAAC  TCTCATGGTG
61  TGACGGGCGG  TGTGTACAAG  GCGCGGGAAC  GTATTCAACG  GGCATGCTG  ATCCGCGATT
121  ACTAGCGATT  CGACTTCAT  GTAGCGAGT  TCGAGCTAC  AATCCGACT  GAGATGGCT
181  TTAGAGATT  AGCTTACTCT  CGGAGTTG  CACTCGTGG  TACCATCCAT  TGTAGCACT
241  GTTAGCCCA  GGTCAAGG  GGCATGATGA  TTTAGCTCA  TCCCACCTT  CCTCCGTTT
301  GTACCGGCA  GTCTACCAG  AGTGCCCAAC  TTAATGCTGG  CACTGATAA  TAAAGGTTG
361  GCTCGTGGC  GACTTAACC  CAACATCTCA  CGACAGAGC  TGAGACAAC  CATGCACCAC
421  CTGTATCCAT  GTCCCCGAAG  GGAACGTCTA  ATCTTTAGA  TTTGCATAGT  ATGTCAAGAC
481  CTGGTAAGGT  TCTTCGGTA  GCTTCGAAAT  AAACCCATG  CCCCACCGCT  TGTGCGGGCC
541  CCGTCAATT  CTTTGGATT  TCAGCCTTGC  GCGCGTACT  CCCAGCGGA  ATGCTAATG
601  CGTTAGCTGC  AGCACTGAAG  GCGGGAACC  CTCCACACT  TAGCATTCAI  CTTTTACGT
661  ATGGACTACC  AGGGATCTA  ATCCGTITG  CACCCATAC  TTTGAGGCT  CAGCGCAGT
721  TACAGACAG  AGAGCCGCT  TCGCCACTGG  TGTCTTCCA  TATATCTAG  CATTTCACCG
781  CTACACAAG  AGTCCACTG  TCCCTTCTG  CACTCAAGT  TCCAGTTC  CGATGCACIT
841  CTTGGTIGA  GCGGAAGCT  TTCACATCA  ACTIAAAAA  CGGCTGGGC  TGGCTTACG
901  CCAATAAAT  CCGGACAAG  CTTGCCACT  AGTATTACC  GGGCTGGTG  GCACGATGT
961  AGCGGTGGCT  TTCTGGTAA  ATACCGTCA  TACCTGAACA  GTTACTCTCA  GATATGTCT
1021  TCTTTACAA  CAGAGTTTA  CGAGCCGAA  CCGTCTTCA  CTCACGGGC  GTTGCTCCAI
1081  CAGACTTTC  TCCATTGGG  AAGATTCCCT  ACTGTGCT  CCGTAGGAG  TTTGGCCGT
1141  GTCTAGTCC  CAATGGGCC  GATTACCTC  TCGGTGGC  TACGTATCAI  TGCCATGGTG
1201  AGCGGTTACC  CCACATCTA  GCTAATAGC  CGCGGGAACA  TCCAAAAGT  ATAGCCGAAG
1261  CCATCTTCA  AACTGGACC  ATGCGTCCA  AGTTGTTATG  CGGTATTAGC  ATCTGTTTCC
1321  AGGTGTTATC  CCGCGTCT  GGGCAGGTT  CCCAGTGT  ACTCACAGT  TGGCCACTCA
1381  CTCAAATGTA  AATCATGATG  CAAGCACC  TCAATACCAG  AGTT

```

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

```
Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGGGCTGGC TCCTAAAAGG TTACCCACCC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTG CACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTT GCTTGCCTC GCGAGTTCGC GACTCGTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301    CTCGGGTTTG TCACCGGCAG TCTACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAAACC AACATCTCAC GACAGGAGT GACGAGGACC
421    ATGCACACCC TGTCAATTGG TTCCCGAAGG AAAGCCCTA TCTTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCCGGTAG CTTCAATTA AACCATATG TCACCGGCTT
541    GTCGGGGCCC CCGTCAATTC CTTGAGTTT CAACCTTGGC GTGCTACTCC CCAGCGGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTAICTAA TCCTGTTCCG TACCATGCT TTGAGTCTC
721    AGGTCAGTGT GCAGACCAGG TAGCCGCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTAAAG CCGAAGGCTT TCACATCATA CT
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Figure 5d. Sequence assembly result – PCR product Isolate Code S2

```
Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GCGGTGTGT ACAAGGCCCG GGAACGTATT CACCGGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTGGCTT GGCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCC ACCTTCTCTC
301    GTTTTGTAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGGCTCG TTGCGGGACT TAACCCAACA TCTCAGGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCTTTT GAGTTTCAAC CTTGCGGTGC TACTCCCAG GCGGAGTGTCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCTTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGACG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCCTC TTCTGCACTC AAGTTATCCA GTTTCGATG
841    CACTTCTCC
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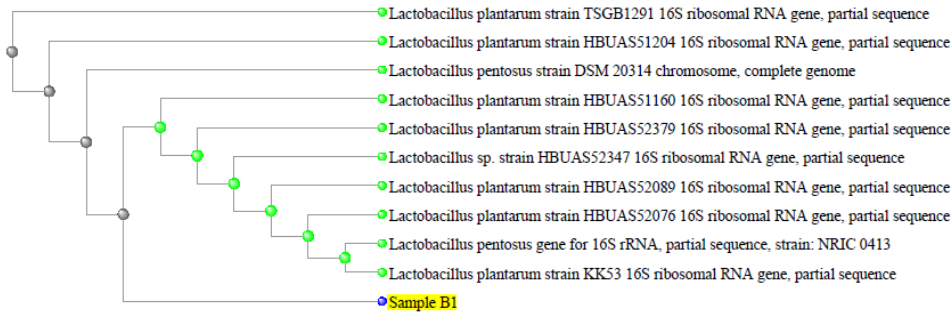
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231 Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

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

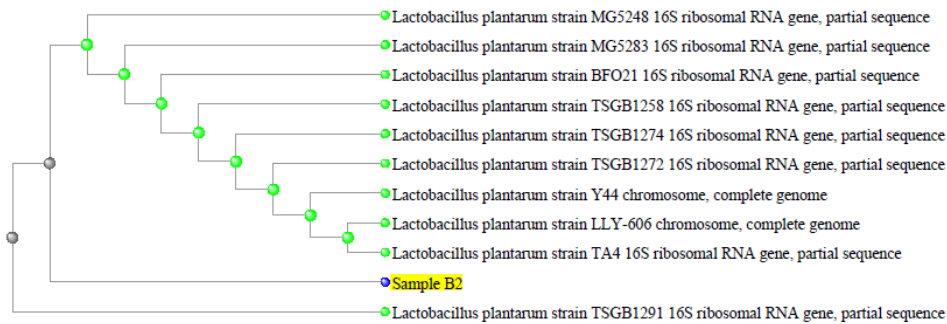
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233 Figure 6a. Phylogenetic tree Isolate Code B1



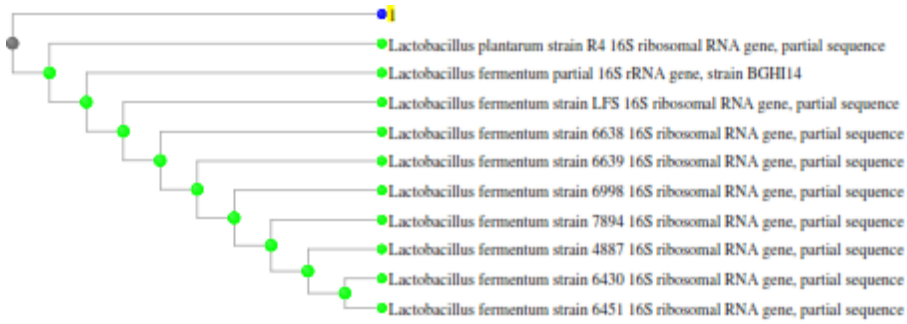
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235 Figure 6b. Phylogenetic tree Isolate Code B2



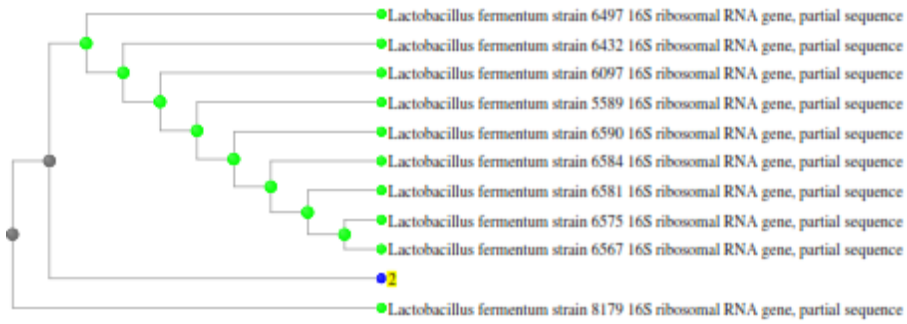
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237 Figure 6c. Phylogenetic tree Isolate Code S1



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239 Figure 6d. Phylogenetic tree Isolate Code S2



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5 Juni 2022 pukul 20.11

Dear Son Radu, PhD  
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The manuscript has been revised according to the attached comments  
Thank you. I hope this manuscript can be accepted.

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There were formatting errors in regards to the citations, coding appeared next to citations.  
Please also ensure to make changes Only on the manuscript provided previously because the Editor had already made minor editing on it and also the comments were given on that document.  
Your cooperation is much appreciated.

Best regards,  
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8 Juni 2022 pukul 02.16

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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.

Changes made to the manuscript are those that were previously rendered. Thank you

Best Regard  
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Figure 2 was not cited in the manuscript. If it is not needed please remove and revise figure labels.

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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

27 1. **Introduction**

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

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32 There are a variety of fermented foods in the world especially in Asia. Most of Asian fermented foods  
33 are non-dairy products featuring various other raw materials such as cereals, soybeans, fruit,  
34 vegetables and fish and other products (Rhee *et al.*, 2011). Indonesia is rich in fermented foods  
35 including Dadih, Bekasam and Lemea. Curd, fermented buffalo milk from Minangkabau, West  
36 Sumatra, various types of LAB isolated from curd (Wirawati *et al.*, 2019). Bekasam, a fermented  
37 Indonesian freshwater fish product that tastes sour and contains lactic acid bacteria (LAB) is popular  
38 in Central Java, South Sumatra and South Kalimantan (Desniar *et al.*, 2013). Lemea is a processed fish  
39 and bamboo shoots originating from Bengkulu, precisely the Rejang tribe, which is fermented for 3-  
40 7 days and generally uses freshwater fish with lactic acid bacteria as the main actor (Xu *et al.*, 2021).

41 River fish that are often used to make lemea are freshwater fish. One of the freshwater fish that can  
42 be used is betok fish, lemea containing lactic acid bacteria (Xu *et al.*, 2021). Lactic acid bacteria consist  
43 of microbial strains that convert carbohydrates into lactic acid (Dinoto *et al.*, 2020), LAB isolated  
44 results were identified based on phenotypic and genotypic characteristics (Lawalata *et al.*, 2011).

45 Genotypic LAB identification was carried out by PCR technique in the form of amplifying and  
46 sequencing the universal region of the 16S rRNA gene (Dinoto *et al.*, 2020). Lactic Acid Bacteria (LAB)  
47 strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) has been previously  
48 studied. . The purpose of this study was to investigate Lactic Acid Bacteria (LAB) strains in fermented

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49 Betung bamboo shoots (*Dendrocalamus asper* Schult) and Yellow bamboo shoots (*Bambusa vulgaris*  
50 Schrad).

Commented [L8]: The research objectives have been clarified

51 **2. Materials and methods**

52 **2.1 Sample Preparation**

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

57 **2.2 LAB isolation**

58 Isolation of LAB was carried out using a device that was sterilized beforehand and carried out  
59 aseptically where 10 g of the lemea sample was homogenized and then serially diluted with 1%  
60 NaCl. And each dilution series was spread on MRSA media and then petridish was incubated at  
61 37°C for 48 hours. The isolates obtained need to be purified and identified

62 **2.3 Identification of LAB Strain Fermented Bamboo Shoot (Lemea)**

63 Molecular identification based on 16S rRNA gene amplification with genomic DNA isolation, DNA  
64 amplification, sequencing and analysis of nucleotide sequences in GenBank (Widodo *et al.*, 2017).

65 DNA isolation was Genomic DNA extraction with Presta TM Mini GDNA Bacteria Kit (Geneaid,  
66 GBB100). DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red  
67 Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA Recovery Kit  
68 (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1uL of the  
69 PCR product was assessed with 0.8% TBE agarose.

70 **3. Results and discussion/Results**

71 Bacterial sequence homology based on 16S rRNA gene sequencing analysis. The types of lactic acid  
72 bacteria species on bamboo shoots of betung and yellow bamboo have 99% similarity. B1, B2, and  
73 S1 are closely related to *Lactobacillus plantarum*, this type of bacteria is known as lactic acid bacteria  
74 which has the potential as biopreservative because it can inhibit the growth of pathogens and

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75 destructive with greater inhibition than other lactic acid bacteria. (Azizah *et al.*, 2019). *L. plantarum*  
76 is amylolytic which converts starch to lactic acid (Noor *et al.*, 2018). S2 is closely related to  
77 *Lactobacillus fermentum*, this type of bacteria is known as lactic acid bacteria which belongs to  
78 heterofermentative species and can be found in the human intestinal tract where these bacteria can  
79 live at the pH of the digestive tract (Manin, 2010). B1 and B2 are isolate codes derived from  
80 fermented bamboo shoots. S1 and S2 codes for isolate fermented yellow bamboo shoots. (Table 1).  
81 Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration  
82 using the Kappa Universal ladder (Figure 2a and 2b). Visualization of 16 S rRNA gene amplification of  
83 isolates B1, B2, S1 and S2 at 0.8% TBE agarose concentration using the Kappa Universal ladder  
84 (Figures 3a and 3b). phylogenetic analysis using MEGA 7.0 with Neighbor-joining (unrooted tree) by  
85 NCBI tree method showed that B1, B2 and S1 were closely related to *Lactobacillus plantarum* (Figures  
86 4a, 4b and 4c). S2 is closely related to *Lactobacillus fermentum* (Figure 4d). Betung bamboo shoots  
87 contain lactic acid bacteria in the form of *Lactobacillus plantarum* while yellow bamboo shoots  
88 contain more lactic acid bacteria in the form of *Lactobacillus fermentum*.

#### 89 4. Conclusion

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

92

#### 93 Conflict of interest - Disclose potential conflicts of interest appropriately.

94 The authors declare no conflict of interest.

#### 95 Acknowledgments

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

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97 **References**

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- 98 Azizah, N., Suradi, K., & Gumilar, J. (2019). Effect of Concentration of Lactic Acid Bacteria *Lactobacillus*  
99 *Plantarum* and *Lactobacillus Casei* on Microbiological and Chemical Quality of Probiotic Mayonnaise.  
100 *Journal of Animal Science Padjadjaran University*, 18(2), 79–85.  
101 <https://doi.org/10.24198/jit.v18i2.19771>
- 102 Desniar, Rusmana, I., Suwanto, A., & Mubarik, R. (2013). Characterization of Lactic Acid Bacteria Isolated  
103 From an Indonesian Fermented Fish (Bekasam) and Their Antimicrobial Activity Against Pathogenic  
104 bacteria. 25(6), 489–494. <https://doi.org/10.9755/ejfa.v25i6.12478>
- 105 Dewi, KH. (2015). Raw Materials Inventory and Fermentation Process in Lemea Industry the Traditional  
106 Food of Rejang Tribe. *International Journal on Advanced Science, Engineering and Information*  
107 *Technology*, 5(3), 196–200. <https://doi.org/10.18517/ijaseit.5.3.512>
- 108 Dewi, KH, Silsia, D., Susanti, L., & Hasanuddin. (2014). Mapping of the “Lemea” Traditional Food Industry  
109 of the Rejang Tribe in Bengkulu Province. *Journal of AGRISEP*, 13(1), 60–66.  
110 <https://doi.org/10.31186/jagrisep.13.1.60-66>
- 111 Dinoto, A., Rosyidah, AL, Ria, A., Sari, P., & Julistiono, H. (2020). Isolation, Identification and Antimicrobial  
112 Activities of Lactic Acid Bacteria from Fruits of Wild Plants in Tambrau Forest, West Papua,  
113 Indonesia. 21(7), 3391–3397. <https://doi.org/10.13057/biodiv/d210764>
- 114 Lawalata, HJ, Sembiring, L., & Rahayu, ES. (2011). Molecular Identification of Lactic Acid Bacteria  
115 Producing Antimicrobial Agents from Bakasang, An Indonesian Traditional Fermented Fish Product.  
116 *Indonesian Journal of Biotechnology*, 16(2), 93–99.
- 117 Manin, F. (2010). Potential of *Lactobacillus Acidophilus* And *Lactobacillus Fermentum* From Digestive  
118 Tracts Of Free-range Chickens From Peat As Sources Of Probiotics. *Scientific Journal of Animal*  
119 *Sciences Jambi University*, XIII(5), 221–228. <https://doi.org/10.22437/jiiip.v0i0.19>
- 120 Noor, Z., Cahyanto, MN, Indrati, R., & Sardjono, S. (2018). Screening of *Lactobacillus Plantarum* Produced  
121 Lactic Acid for Mocaf Fermentation. *Agritech*, 37(4), 437. <https://doi.org/10.22146/agritech.18821>
- 122 Rhee, SJ, Lee, JE, & Lee, CH. (2011). Importance of Lactic Acid Bacteria in Asian Fermented Foods. *Microbial*  
123 *Cell Factories*, 10(SUPPL. 1), 1–13. <https://doi.org/10.1186/1475-2859-10-S1-S5>
- 124 Widodo, Wahyuningsih, TD, Nurrochmad, A., Wahyuni, E., Taufiq, TT, Anindita, NS, Lestari, S., Harsita, PA,

125 Sukarno, AS, & Handaka, R. (2017). Local Strain Lactic Acid Bacteria "Isolation to Application as  
126 Probiotics and Milk Fermentation Starter. Yogyakarta: Gadjah Mada University Press.

127 Wirawati, CU, Sudarwanto, MB, Lukman, DW, Wientarsih, I., & Srihanto, EA. (2019). Diversity of Lactic  
128 Acid Bacteria in Dadih Produced by Either Back-Slopping or Spontaneous Fermentation From Two  
129 Different Regions of West Sumatra, Indonesia. *Veterinary World*, 12(6), 823–829.  
130 <https://doi.org/10.14202/vetworld.2019.823-829>

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

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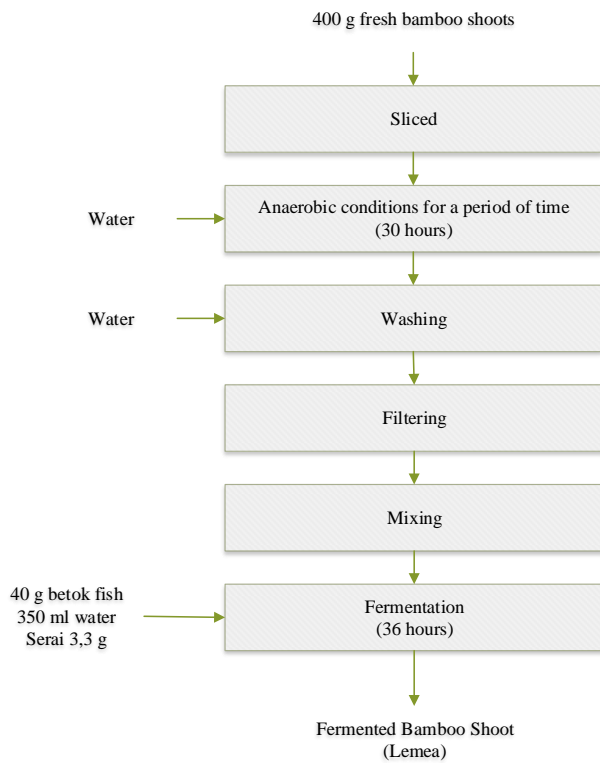
151 Table 1. Comparison of Homology Levels the 16S rRNA gene of BAL isolat with several sequences

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

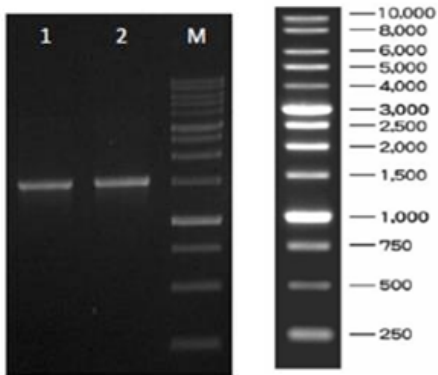
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152 Figure 1. Fermented Bamboo shoot making process



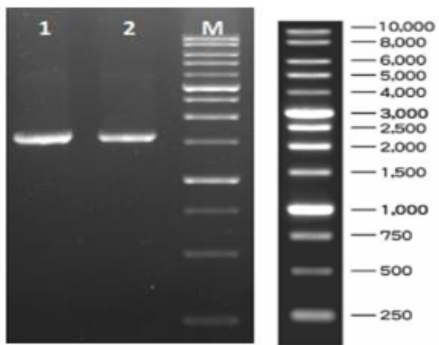
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154 Figure 2a. Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose  
155 concentration using the Kappa Universal ladder



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



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

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```
Sequence Assembly 1434 bp
1   AGCGGGCTGG TTCCIAAAG GTTACCCAC CGACTTTGGG TGTACAAC TCICATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTACCG CCGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC TGAATGGCT
181 TTAAGAGATT AGCTTGTCTT CCGGAGTTCC CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTIT
301 GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGGC GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA CATTGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAAACCAATG CTCACCCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGAAACC CTCACACTC TAGCATTCA TCGTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTITG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGT TCCAGTTTC CGATGCACCT
841 CTTCGGTIGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901 CCCAATAAAT CCGGACAAAG CTTGCCACTC ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTCTT
1021 TCTTAAACA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCAGCGGCG GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG AAGATTCCTT ACTGCTGCTT CCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAAGTCCGC TACGTATCAT TGCCATGGTG
1201 AGCCGTATCC CCACCATCTA GCTAATACGC CCGGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTCA AACTCGGACC ATGCGGTCCA AGTGTATTAG CCGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTGTTCCG ACTT
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167 Figure 3b. Sequence assembly result – PCR product Isolate Code B2

```
1   AGCGGGCTGG TTCCIAAAG GTTACCCAC CGACTTTGGG TGTACAAC TCICATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTACCG CCGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC TGAATGGCT
181 TTAAGAGATT AGCTTACTCT CCGGAGTTCC CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTCA TCCCCACCTT CCTCCGGTIT
301 GTCACCGGCA GTCTCACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGGC GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAA CATTGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGCGTA GCTTCGAATT AAAACCAATG CTCACCCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCITGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGAAACC CTCACACTC TAGCATTCA TCGTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTITG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGT TCCAGTTTC CGATGCACCT
841 CTTCGGTIGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGCGC TCGCTTTACG
901 CCCAATAAAT CCGGACAAAG CTTGCCACTC ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTCTT
1021 TCTTAAACA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCAGCGGCG GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG AAGATTCCTT ACTGCTGCTT CCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAAGTCCGC TACGTATCAT TGCCATGGTG
1201 AGCCGTATCC CCACCATCTA GCTAATACGC CCGGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTCA AACTCGGACC ATGCGGTCCA AGTGTATTAG CCGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTT
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169 Figure 3c. Sequence assembly result – PCR product Isolate Code S1

**Sequence Result Reverse Primer 882bp**

```
1      GTCCACCTTA GGGGGCTGGC TCCTAAAAGG TTACCCACC GACTTTGGGT GTTACAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCGGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGGATC CGACTTCGTG CAGCGGAGTT GCAGCCTGCA GTCCGAAGT
181    AGAACGGTTT TAAGAGATT GCTTGCCCTC GCGAGTTGCG GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGGC CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCATTGGC TTCCCGAAGG AAACGGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCCGGTAG CTTGCAATTA AACCACATGC TCCACCGCTT
541    GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTGGC GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAAACC TCCAACCTT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTGCG TACCCATGCT TTAGGATCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCCTT CGCCACTGGT GTTCTTCCAT ATATCTACGC
781    ATTCACCCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACCTT TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
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171 Figure 3d. Sequence assembly result – PCR product Isolate Code S2

**Sequence Result Reverse Primer 849bp**

```
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GCGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CTTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTTGCTT GGCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCTCCT
301    GGTTTGTAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGCTCG TTGCGGGACT TAACCCAACA TCTCAGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAC GGCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTG TACTCCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCCCTCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGTT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC GGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCTTC TTCTGCACTC AAGTTATCCA GTTTCGGATG
841    CACTTCTCC
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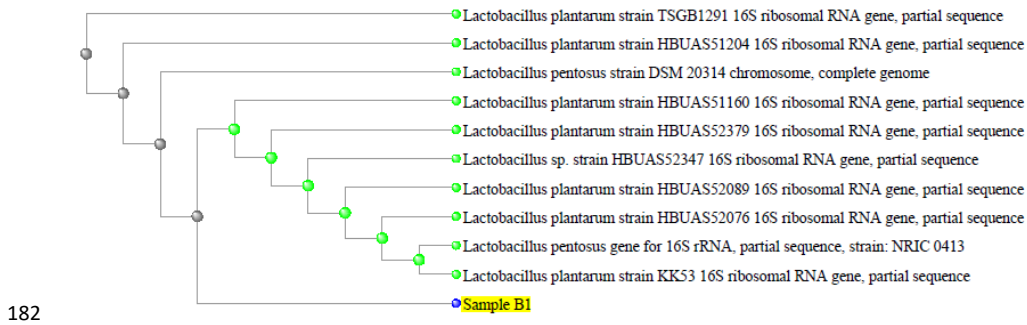
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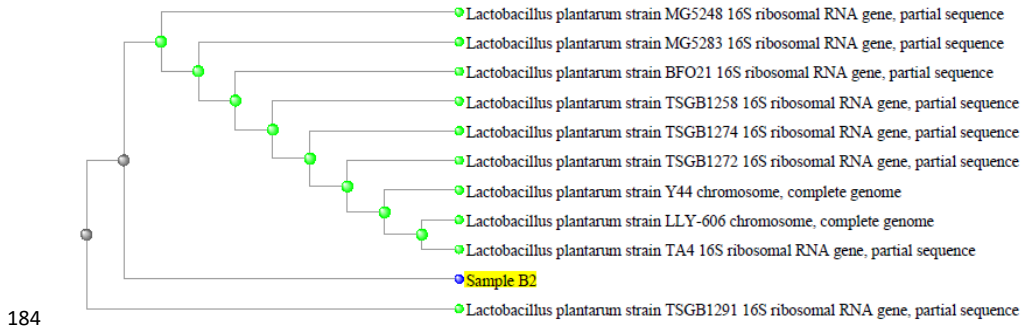
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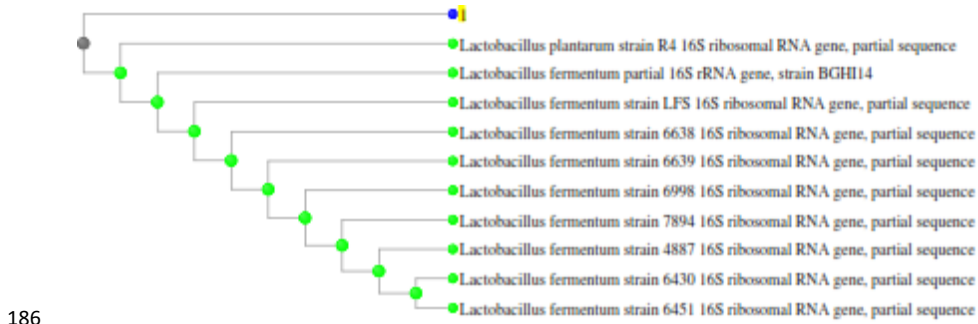
181 Figure 4a. Phylogenetic tree Isolate Code B1



183 Figure 4b. Phylogenetic tree Isolate Code B2

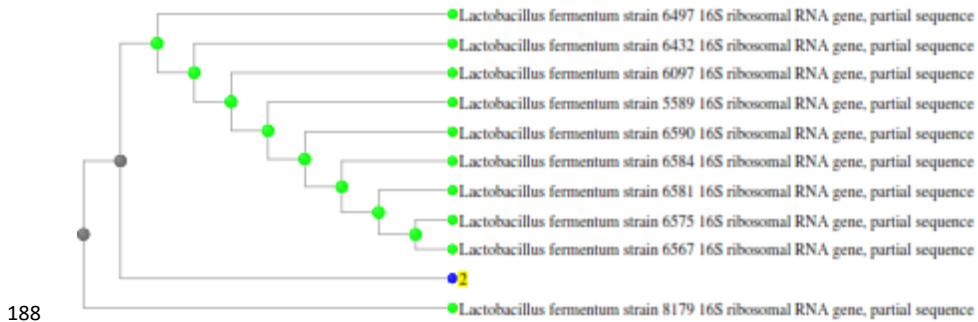


185 Figure 4c. Phylogenetic tree Isolate Code S1



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187 Figure 4d. Phylogenetic tree Isolate Code S2



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

3                                   <sup>1,2</sup>\*Okfrianti, Y., <sup>2</sup>Herison, C., <sup>2</sup>Fahrurrozi and <sup>2</sup>Budiyanto

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11                   **Abstract**

12  
13                   Ethnic food is food inherited from ancestors whose process utilizes local food and distinctive tastes. The  
14                   Rejang tribe is a native Bengkulu community who processes bamboo shoots into a fermented product  
15                   known as *Lemea*, which is only found in Bengkulu province. *Lemea* is a source of indigenous lactic acid  
16                   bacteria (LAB). This study aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*.  
17                   The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of  
18                   bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper*  
19                   *Schult*) and yellow bamboo (*Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10  
20                   g of homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial  
21                   dilutions were carried out starting from 10<sup>-1</sup> to 10<sup>-7</sup> and spread on MRSA media for each dilution series.  
22                   Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA  
23                   method. The results of the study found 4 isolates from 2 types of *Lemea*. After identification, it was known  
24                   that the four isolates were bacteria of the genus *Lactobacillus*. *Lactobacillus fermentum* was only found  
25                   in the fermentation process of yellow bamboo shoots (*Bambusa vulgaris* Schrad), while *Lactobacillus*  
26                   

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

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

### 30 1. Introduction

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

41 There are various fermented foods in the world, especially in Asia. Fermented foods made from  
42 bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus* (Kiran  
43 *et al.*, 2016). ~~(Fomar, 2016)~~. *Meakri*, from Meghalaya Indian fermented bamboo shoots as a source  
44 of *Lactobacillus*, has characteristics suitable for probiotics (Das *et al.*, 2020). ~~(Das et al., 2020)~~.  
45 *Lactobacillus* is a group of LAB that are gram-positive bacteria. LAB have the potential to inhibit the  
46 infection and growth of pathogenic microbes. (Yang *et al.*, 2021). ~~(Yang et al., 2021)~~. LAB isolated from  
47 fermented bamboo shoots are potential probiotic candidates that are beneficial for health  
48 (Mohamad *et al.*, 2020). ~~(Mohamad et al., 2020)~~. LAB strains are selected for their decreased content  
49 of cholesterol, antioxidant activity, and anti-bacterial activity (Jitpakdee *et al.*, 2022). ~~(Jitpakdee et al.,~~

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50 ~~2022~~). Isolation of lactic acid bacteria from indigenous fermented foods is very important (Mende *et*  
51 *al.*, 2022) ~~(Mende *et al.*, 2022)~~.

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52 *Bekasam* is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional  
53 fermented food popular in Sumatera and Kalimantan ~~(Desniar *et al.*, 2013)~~ ~~(Desniar *et al.*, 2013)~~.

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54 The sour taste in *Bekasam* is almost the same as in *Lemea*. The difference between these two  
55 products is the carbohydrate source and fermentation time. Carbohydrate sources are a source of  
56 nutrition for bacteria that play a role in the fermentation process. The source of carbohydrates used  
57 for rice in *Lemea* is bamboo shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes  
58 10 days.

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

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

## 68 2. Materials and methods

### 69 2.1 Sample preparation

70 Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are  
71 thinly sliced and soaked for 30 h. Then they were washed, filtered, and weighed as much as 400 g

72 and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for  
73 48 h (Figure 1).

74

75

## 76 2.2 LAB isolation

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

## 84 2.2 Identification of LAB

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

87 ~~(Veljovic *et al.*, 2007)~~

### 88 2.2.1 DNA Isolation

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

### 91 2.2.2 DNA amplification

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

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

### 96 2.2.3 DNA sequencing and phylogenetic analysis

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

## 103 3. Results and discussion

### 104 3.1 Isolation of lactic acid bacteria from Lemea

105 Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung  
106 bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found  
107 were coded B1, B2, S1 and S2 (Figure 3). The bacterial isolates found were lactic acid bacteria  
108 because they were able to grow on MRSA specific media with cocci characteristics, a milky white  
109 color with a convex surface and smooth edges. The number of isolates obtained was less than  
110 that of *mesu*, *soidon*, *soibum*, and *soijon* but the same as unfermented bamboo shoots (Tamang  
111 *et al.*, 2008). ~~(Tamang *et al.*, 2008).~~ The morphological characteristics of the isolates found in this  
112 study were almost the same as the previous findings isolated from *Lemea* produced by a cottage  
113 industry in Kepahyang Regency, Bengkulu. ~~(Kurnia *et al.*, 2020).~~ ~~(Kurnia *et al.*, 2020).~~

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### 114 3.2 Identification of Lemea isolates

115 The results of genomic DNA amplification of the 4 isolates can be seen in Figures 4a and 4b.  
116 Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size.  
117 Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434

118 bp for isolate B1 (Figure 5a), 1424 bp for B2 (Figure 5b). The nucleotide sequence S1 isolate was  
119 882 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). The results of the analysis using the  
120 BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to  
121 *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.

122 The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% similar to the  
123 partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of  
124 *Lactobacillus plantarum* but not *Lactobacillus fermentum*. Based on the phylogeny tree, isolate  
125 B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 6a),  
126 Isolate B2 with *Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA. (Figure  
127 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA  
128 (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S  
129 ribosomal RNA. The type of isolate that was identified from Bekasam was *lactobacillus*

130 *Lactobacillus plantarum* (Sukmarini *et al.*, 2014).~~(Sukmarini *et al.*, 2014).~~ All isolates have been  
131 homologous to the genus *Lactobacillus*. *Bacillus subtilis*, *Lactobacillus brevis*, and *Lactobacillus*  
132 *plantarum* were found in dominating strains of Soidon fermented bamboo shoot food without

133 salt from Indian Manipur (Jeyaram *et al.*, 2010).~~(Jeyaram *et al.*, 2010).~~ LAB strains in fermented  
134 Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz) have been previously studied and

135 isolated as 2 species, namely *Lactobacillus plantarum* and *Lactobacillus rossiae* (Okfrianti *et al.*,  
136 2019).~~(Okfrianti *et al.*, 2019).~~ Lactic acid bacteria isolated from Indonesian fermented foods are

137 dominated by *Lactobacillus plantarum* (Rahayu, 2003).~~(Rahayu, 2003).~~ This research is  
138 expected to provide information on which LAB strains have been isolated from different types

139 of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the diversity of the  
140 bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria* and

141 *Proteobacteria* (Li *et al.*, 2021).~~(Li *et al.*, 2021).~~

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142 *Lactobacillus fermentum* was only found in *Lemea* made from yellow bamboo shoots (*Bambusa*  
143 *vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa*  
144 *vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in  
145 foodstuffs that trigger the growth of *Lactobacillus* (Macfarlane\_&\_Cummings, 1999).  
146 ~~Macfarlane and Cummings, 1999~~. Oligosaccharides and fiber are prebiotics that promote the  
147 growth of specific bacteria found in the gut. Bamboo shoots are a good source of fibre.(Felisberto  
148 *et al.*, 2017) ~~(Felisberto et al., 2017)~~. At 100 g of fresh weight, bamboo shoots of *B. vulgaris*  
149 contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of *D. asper*, and 3.54 g of fibre.(Chongtham  
150 *et al.*, 2011). ~~(Chongtham et al., 2011)~~. Different bamboo species contain different  
151 macronutrients (Adebola *et al.*, 2014).~~(Adebola et al., 2014)~~. Differences in the content of  
152 bamboo shoots affect the types of bacteria found in *Lemea* products.

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#### 153 4. Conclusion

154 Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus*  
155 *plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S  
156 rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots  
157 (*Bambusa vulgaris* Schrad), while *Lactobacillus plantarum* was found in yellow bamboo shoots  
158 (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult).

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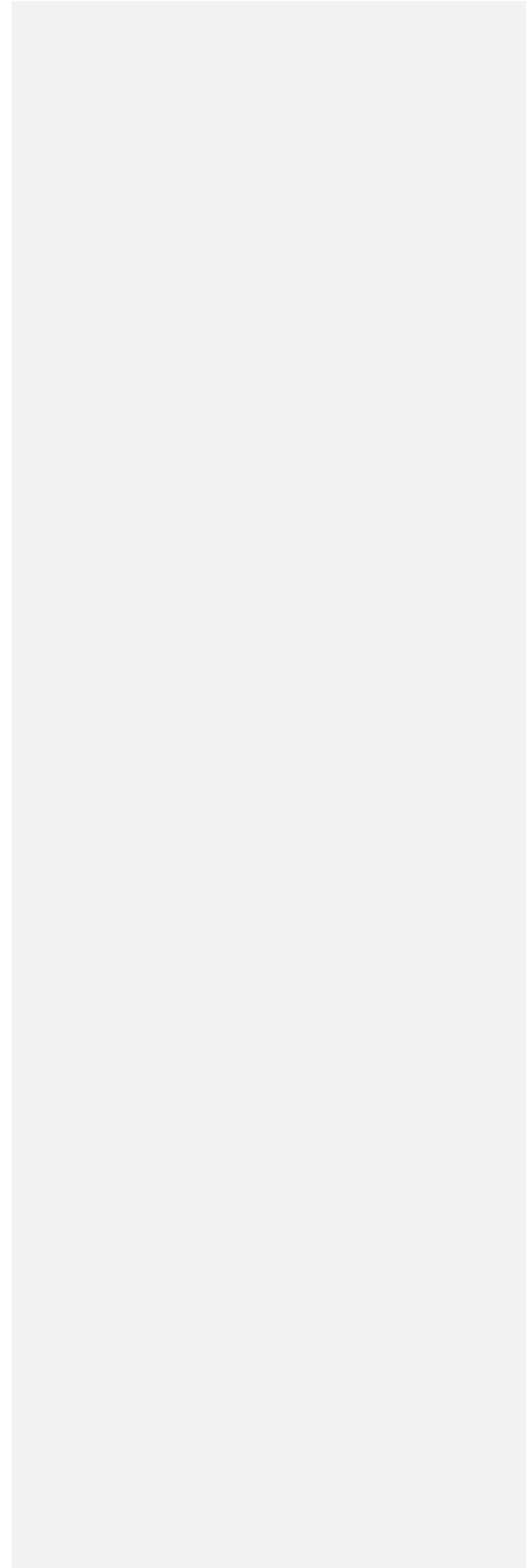
#### 159 Conflict of interest - Disclose potential conflicts of interest appropriately.

160 The authors declare no conflict of interest.

#### 161 Acknowledgments

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165 **References**

- 166 Adebola, O.-O., Corcoran, O., & Morgan, W.-A. (2014). Synbiotics: The impact of potential prebiotics  
167 inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of*  
168 *Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- 169 Chongtham, N., Bisht, M.-S., & Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots:  
170 Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science*  
171 *and Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 172 Das, S., Mishra, B.-K., & Hati, S. (2020). Techno-functional characterization of indigenous Lactobacillus  
173 isolates from the traditional fermented foods of Meghalaya, India. *Current Research in Food*  
174 *Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>
- 175 Desniar, Rusmana, I., Suwanto, A., & Mubarik, D.-N.-R. (2013). Characterization of lactic acid bacteria  
176 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
177 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
178 <https://doi.org/10.9755/ejfa.v25i6.12478>
- 179 Dewi, K.-H., Silsia, D., Susanti, L., & Hasanuddin. (2014). Suku Rejang di Provinsi Bengkulu Industry  
180 Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province. *AGRISEP*, 14(1), 61–69.  
181 <https://doi.org/https://doi.org/10.31186/jagrisep.13.1.60-66>
- 182 Felisberto, M.-H.-F., Miyake, P. S. E., Beraldo, A.-L., & Clerici, M.-T.-P.-S. (2017). Young bamboo culm:  
183 Potential food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
184 <https://doi.org/10.1016/j.foodres.2017.08.058>
- 185 Fox, P.-F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
186 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- 187 Jeyaram, K., Romi, W., Singh, T.-A., Devi, A.-R., & Devi, S.-S. (2010). Bacterial species associated with  
188 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
189 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
190 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- 191 Jitpakdee, J., Kantachote, D., Kanzaki, H., & Nitoda, T. (2022). Potential of lactic acid bacteria to  
192 produce functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160,  
193 113269. <https://doi.org/10.1016/j.lwt.2022.113269>

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- 194 Kiran, T., Rajani, C., Kumar, T.-S., & Achun, P. (2016). Fermented Bamboo Shoots: A Riche Niche for  
195 Beneficial Microbes. *Journal of Bacteriology & Mycology: Open Access*, 2(4), 87–93.  
196 <https://doi.org/10.15406/jbmoa.2016.02.00030>
- 197 Kurnia, M., Amir, H., & Handayani, D. (2020). Isolasi Dan Identifikasi Bakteri Asam Laktat Dari  
198 Makanan Tradisional Suku Rejang Di Provinsi Bengkulu: “Lemea.” *Alotrop*, 4(1), 25–32.  
199 <https://doi.org/10.33369/atp.v4i1.13705>
- 200 Kwon, D.-Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
201 <https://doi.org/10.1016/j.jef.2015.02.001>
- 202 Li, Q., Wu, W., Chen, H., Fang, X., & Han, Y. (2021). Food Chemistry : X In vitro fecal fermentation  
203 characteristics of bamboo shoot ( *Phyllostachys edulis* ) polysaccharide. *Food Chemistry: X*, 11, 1–  
204 9. <https://doi.org/10.1016/j.fochx.2021.100129>
- 205 Macfarlane, G.-T., & Cummings, J.-H. (1999). Probiotics and prebiotics: Can regulating the activities  
206 of intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.
- 207 Mende, S., Rohm, H., & Jaros, D. (2022). Lactic Acid Bacteria: Exopolysaccharides. In *Encyclopedia of*  
208 *Dairy Sciences* (Vol. 4). Elsevier. <https://doi.org/10.1016/b978-0-08-100596-5.22982-x>
- 209 Mohamad, N., Manan, H., Sallehuddin, M., Musa, N., & Ikhwanuddin, M. (2020). Screening of Lactic  
210 Acid Bacteria isolated from giant freshwater prawn (*Macrobrachium rosenbergii*) as potential  
211 probiotics. *Aquaculture Reports*, 18(October 2019), 100523.  
212 <https://doi.org/10.1016/j.aqrep.2020.100523>
- 213 Okfrianti, Y., Darwis, & Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
214 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter-Professional Health*  
215 *Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-](https://doi.org/10.2991/icihc-18.2019.52)  
216 [18.2019.52](https://doi.org/10.2991/icihc-18.2019.52)
- 217 Rahayu, E.-S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84.  
218 <https://doi.org/10.22146/agritech.13515>
- 219 Sukmarini, L., Mustopa, A.-Z., Normawati, M., & Muzdhalifa, I. (2014). Identification of Antibiotic-  
220 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of*  
221 *Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>

- 222 Tamang, B., Tamang, J.-P., Schillinger, U., Franz, C.-M.-A.-P., Gores, M., ~~&and~~ -Holzapfel, W.-H. (2008).  
223 Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented  
224 bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–  
225 40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>
- 226 Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M., ~~&and~~  
227 Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlatar  
228 cheese. *Journal of Applied Microbiology*, 103(6), 2142–2152. [https://doi.org/10.1111/j.1365-  
229 2672.2007.03450.x](https://doi.org/10.1111/j.1365-2672.2007.03450.x)
- 230 Wasis, N.-O., Semadi Antara, N., ~~&and~~ -Wayan Gunam, I.-B. (2019). Studi Viabilitas Isolat Bakteri Asam  
231 Laktat yang Diisolasi dari Asinan Rebung Bambu Tabah Terhadap pH Rendah dan Garam Empedu.  
232 *Jurnal Rekayasa Dan Manajemen Agroindustri*, 7(1), 1–10.  
233 <https://doi.org/10.24843/jrma.2019.v07.i01.p01>
- 234 Yang, L., Huang, W., Yang, C., Ma, T., Hou, Q., Sun, Z., ~~&and~~ -Zhang, H. (2021). Using PacBio sequencing  
235 to investigate the effects of treatment with lactic acid bacteria or antibiotics on cow endometritis.  
236 *Electronic Journal of Biotechnology*, 51, 67–78. <https://doi.org/10.1016/j.ejbt.2021.02.004>
- 237
- 238 ~~Adebola, O.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin,  
239 lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of  
240 Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>~~
- 241 ~~Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots:  
242 Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and  
243 Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>~~
- 244 ~~Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria  
245 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
246 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
247 <https://doi.org/10.9755/ejfa.v25i6.12478>~~
- 248 ~~Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di  
249 Provinsi Bengkulu Industry Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province.  
250 *AGRISEP*, 14(1), 61–69.~~

251 Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential  
252 food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
253 <https://doi.org/10.1016/j.foodres.2017.08.058>

254 Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
255 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>

256 Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and Devi, S.S. (2010). Bacterial species associated with  
257 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
258 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
259 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>

260 Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce  
261 functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160, 113269.  
262 <https://doi.org/10.1016/j.lwt.2022.113269>

263 Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
264 <https://doi.org/10.1016/j.jef.2015.02.001>

265 Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). In vitro fecal fermentation  
266 characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11,  
267 <https://doi.org/10.1016/j.fochx.2021.100129>

268 Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of  
269 intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.

270 Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
271 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter Professional Health*  
272 *Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-  
273 \*18.2019.52\*](https://doi.org/10.2991/icihc-18.2019.52)

274 Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 75–84.  
275 <https://doi.org/10.22146/agritech.13515>

276 Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-  
277 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of*  
278 *Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>

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279 ~~Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and~~  
280 ~~Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese.~~  
281 ~~*Journal of Applied Microbiology*, 103(6), 2142–2152. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.2007.03450.x)~~  
282 ~~[2672.2007.03450.x](https://doi.org/10.1111/j.1365-2672.2007.03450.x)~~

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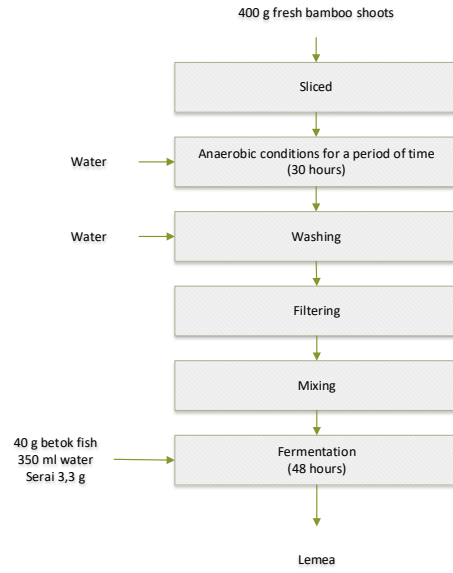
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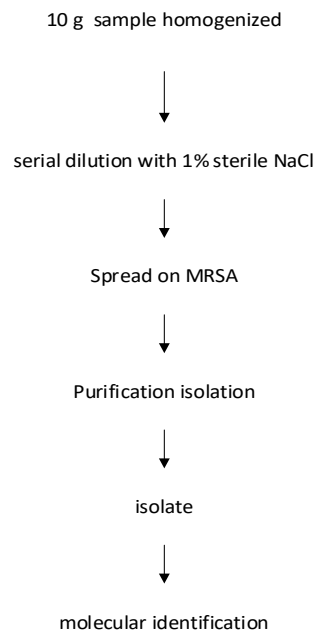
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Figure 1. Fermented bamboo shoot making process



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Figure 2. Isolation process



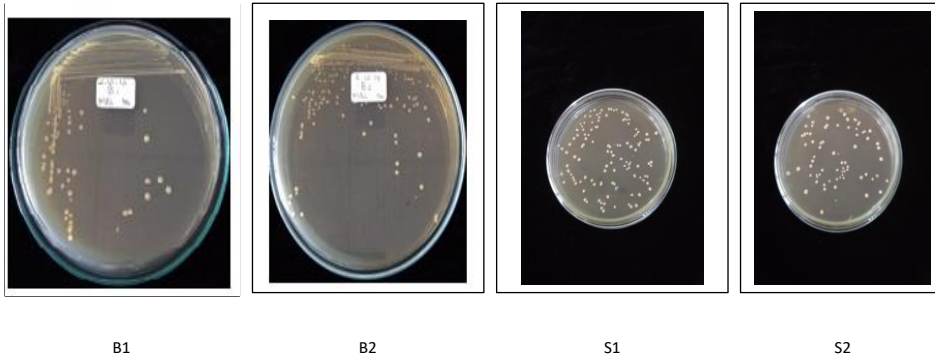
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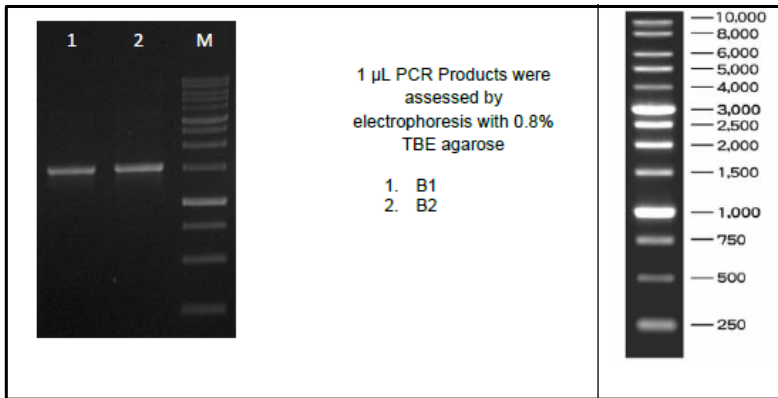
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Figure 3. Isolate B1, B2, S1 dan S2



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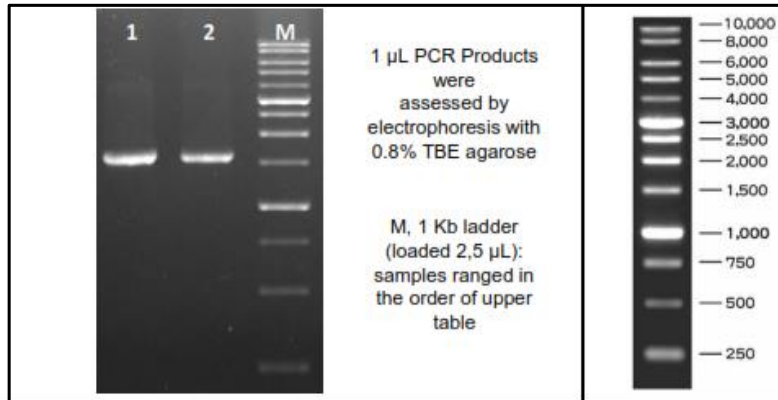
302 Figure 4a. Genomic DNA amplification of isolates B1 and B2



303

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

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



307

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

310

Figure 5a. The nucleotide sequence B1 isolate

```

Sequence Assembly 1434 bp
1  AGGCGGCTGG  TTCCTAAAAG  GTTACCCAC  CGACTTTGGG  TGTACAAAC  TCTCATGGTG
61  TGACGGGCGG  TGTGTACAAG  GCCCGGGAAC  GTATTACCG  CGGCATGCTG  ATCCGCGATT
121  ACTAGCGATT  CCGACTTCAT  GTAGGCGAGT  TGCAGCCTAC  AATCCGAAC  GAGAATGGCT
181  TTAAGAGATT  AGCTTGCTCT  CGCGAGTTCC  CAACTCGTTG  TACCATCCAT  TGTAGCACGT
241  GTGTAGCCCA  GGTCTAAAGG  GGCATGATGA  TTTGACGTCA  TCCCACCTT  CCTCCGGTTT
301  GTCACCGCCA  GTCTACCAG  AGTGCCCAAC  TTAATGCTGG  CAACTGATAA  TAAGGGTTGC
361  GCTCGTTGCG  GGCCTTAACC  CAACATCTCA  CGACACGAGC  TGACGACAA  CATGCACCAC
421  CTGTATCCAT  GTCCCCGAAG  GGAACGTCTA  ATCTCTTAGA  TTTGCATAGT  ATGTCAAGAC
481  CTGGTAAGGT  TCTTGGCGTA  GCTTGGAAAT  AAACCACATG  CTCCACCGCT  TGTGCGGGCC
541  CCGGTCAAAT  CCTTTGAGTT  TCAGCCTTGC  GGCCTACTC  CCCAGGCGGA  ATGCTTAATG
601  CGTTAGCTGC  AGCACTGAAG  GCGGGAAACC  CTCCAACACT  TAGCATTCA  CGTTACCGT
661  ATGGACTACC  AGGGTATCTA  ATCCTGTTTG  CTACCCATAC  TTTGAGCCT  CAGGTCAGT
721  TACAGACCAG  ACAGCCGCC  TCGCCACTGG  TGTCTTCCA  TATATCTACG  CATTCAACCG
781  CTACACATGG  AGTTCCACTG  TCTCTTCTG  CACTCAAGTT  TCCCAGTTT  CGATGCACAT
841  CTTCGGTTGA  GCGGAAGGCT  TTCACATCAG  ACTTAAAAA  CCGCCTGGCG  TCGCTTTACG
901  CCCAATAAAT  CCGGACAACG  CTTGCCACCT  ACGTATTACC  GCGGCTGCTG  GCACGTAGTT
961  AGCCGTTGGCT  TCTGTTTAA  ATACCGTCAA  TACCTGAACA  GTTACTCTCA  GATATGTTCT
1021  TCTTTAACAA  CAGAGTTTAA  CGAGCCGAAA  CCTTCTTCA  CTCACGCGGC  GTTGCTCCAT
1081  CAGACTTTCG  TCCATTGTGG  AAGATTCCCT  ACTGCTGCC  CCGTAGGAG  TTTGGCCCGT
1141  GTCTCAGTCC  CAATGTGGCC  GATTACCCCT  TCAGGTGCGC  TACGTATCAT  TGCCATGGTG
1201  AGCCGTTACC  CCAACATCTA  GCTAATACGC  CGCGGGAACA  TCCAAAAGTG  ATAGCCGAAG
1261  CCATCTTTCA  AACTCGGACC  ATGCGGTCCA  AGTTGTTATG  CGGTATTAGC  ATCTGTTTCC
1321  AGGTGTTATC  CCCCCTTCT  GGGCAGGTTT  CCCACGTGTT  ACTCACCACT  TCGCCACTCA
1381  CTCAAATGTA  AATCATGATG  CAAGCACCAA  TCAATACCAG  AGTTGCTTGG  ACTT

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311

312

Figure 5b. The nucleotide sequence B2 isolate

```

Sequence Assembly 1424 bp
1  AGGCGGCTGG  TTCCTAAAAG  GTTACCCAC  CGACTTTGGG  TGTACAAAC  TCTCATGGTG
61  TGACGGGCGG  TGTGTACAAG  GCCCGGGAAC  GTATTACCG  CGGCATGCTG  ATCCGCGATT
121  ACTAGCGATT  CCGACTTCAT  GTAGGCGAGT  TGCAGCCTAC  AATCCGAAC  GAGAATGGCT
181  TTAAGAGATT  AGCTTACTCT  CGCGAGTTCC  CAACTCGTTG  TACCATCCAT  TGTAGCACGT
241  GTGTAGCCCA  GGTCTAAAGG  GGCATGATGA  TTTGACGTCA  TCCCACCTT  CCTCCGGTTT
301  GTCACCGCCA  GTCTACCAG  AGTGCCCAAC  TTAATGCTGG  CAACTGATAA  TAAGGGTTGC
361  GCTCGTTGCG  GGCCTTAACC  CAACATCTCA  CGACACGAGC  TGACGACAA  CATGCACCAC
421  CTGTATCCAT  GTCCCCGAAG  GGAACGTCTA  ATCTCTTAGA  TTTGCATAGT  ATGTCAAGAC
481  CTGGTAAGGT  TCTTGGCGTA  GCTTGGAAAT  AAACCACATG  CTCCACCGCT  TGTGCGGGCC
541  CCGGTCAAAT  CCTTTGAGTT  TCAGCCTTGC  GGCCTACTC  CCCAGGCGGA  ATGCTTAATG
601  CGTTAGCTGC  AGCACTGAAG  GCGGGAAACC  CTCCAACACT  TAGCATTCA  CGTTACCGT
661  ATGGACTACC  AGGGTATCTA  ATCCTGTTTG  CTACCCATAC  TTTGAGCCT  CAGGTCAGT
721  TACAGACCAG  ACAGCCGCC  TCGCCACTGG  TGTCTTCCA  TATATCTACG  CATTCAACCG
781  CTACACATGG  AGTTCCACTG  TCTCTTCTG  CACTCAAGTT  TCCCAGTTT  CGATGCACAT
841  CTTCGGTTGA  GCGGAAGGCT  TTCACATCAG  ACTTAAAAA  CCGCCTGGCG  TCGCTTTACG
901  CCCAATAAAT  CCGGACAACG  CTTGCCACCT  ACGTATTACC  GCGGCTGCTG  GCACGTAGTT
961  AGCCGTTGGCT  TCTGTTTAA  ATACCGTCAA  TACCTGAACA  GTTACTCTCA  GATATGTTCT
1021  TCTTTAACAA  CAGAGTTTAA  CGAGCCGAAA  CCTTCTTCA  CTCACGCGGC  GTTGCTCCAT
1081  CAGACTTTCG  TCCATTGTGG  AAGATTCCCT  ACTGCTGCC  CCGTAGGAG  TTTGGCCCGT
1141  GTCTCAGTCC  CAATGTGGCC  GATTACCCCT  TCAGGTGCGC  TACGTATCAT  TGCCATGGTG
1201  AGCCGTTACC  CCAACATCTA  GCTAATACGC  CGCGGGAACA  TCCAAAAGTG  ATAGCCGAAG
1261  CCATCTTTCA  AACTCGGACC  ATGCGGTCCA  AGTTGTTATG  CGGTATTAGC  ATCTGTTTCC
1321  AGGTGTTATC  CCCCCTTCT  GGGCAGGTTT  CCCACGTGTT  ACTCACCACT  TCGCCACTCA
1381  CTCAAATGTA  AATCATGATG  CAAGCACCAA  TCAATACCAG  AGTTGCTTGG  AGTT

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313

314

315 Figure 5c. The nucleotide sequence S1 isolate

```
Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTG CCACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCGGAACG
181    AGAACGGTTT TAAGAGATTG GCTTGCCTC GCGAGTTCCG GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGGC CTCGTTGGG GACTTAAACC AACATCTCAC GACACGAGCT GACGAGGACC
421    ATGCACACAC TGTCAATTGG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTGGCGTAG CTTCAATTA AACACATGC TCCACGCTT
541    GTGCGGGCCC CCGTCAATTC CTTGAGTTT CAACCTTGGG GTCGACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTCGGGCA TGGACTACCA GGTATCTAA TCCTGTTCCG TACCATGCT TCGAGTCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCTT CGCCACTGGT GTTCTCCAT ATATCTACCG
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTCG ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTAAAG CCGAAGGCTT TCACATCATA CT
```

316

317

Figure 5d. The nucleotide sequence S2

```
Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGAAG GCGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGGGCCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CCGTTTTAAG AGATTGGCTT GGCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCACC ACCTTCTCTC
301    GGTGTTGCAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGCGCTG TTGCGGGACT TAACCCAACA TCTCAGGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GGCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCC TACTCCCAG GCGGAGTGTCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGGCG AAACCTTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCCT TCTGCACTC AAGTTATCCA GTTTCGATG
841    CACTTCTCC
```

318

319

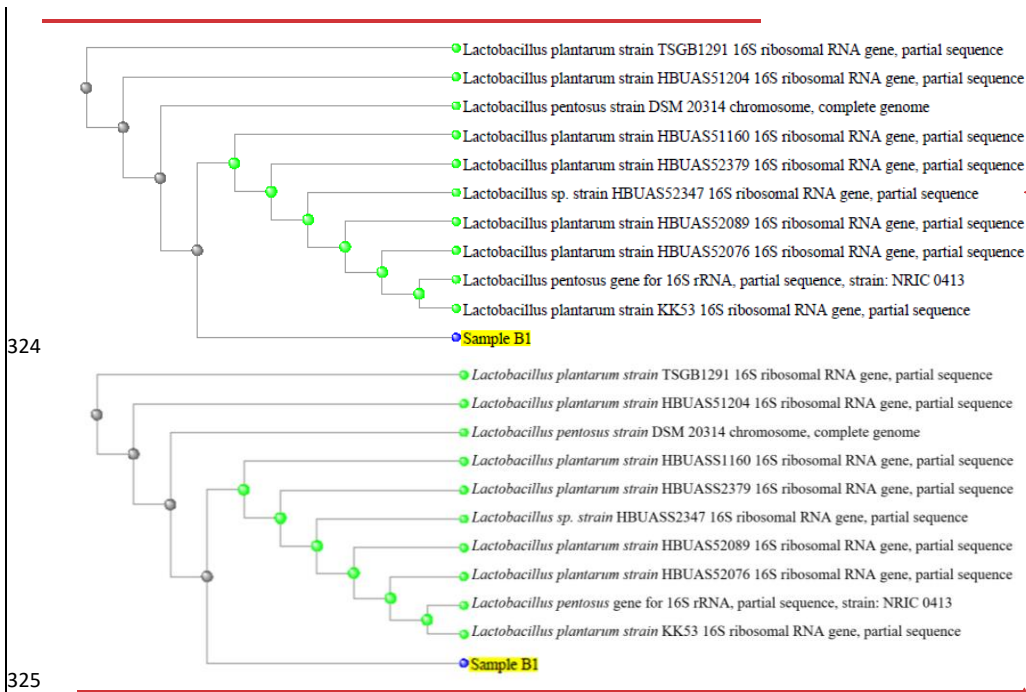
320

321 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	<i>Lactobacillus plantarum</i>	M N37236.01	99
	B2	<i>Lactobacillus plantarum</i>	MN972325.1	99
Yellow Bamboo	S1	<i>Lactobacillus plantarum</i>	KM350169.1	99
	S2	<i>Lactobacillus fermentum</i>	MT538927.1	99

322

323 Figure 6a. Phylogenetic tree Isolate Code B1



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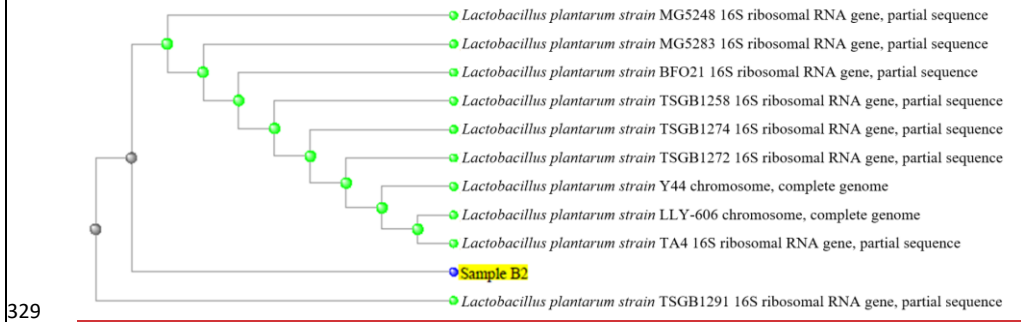
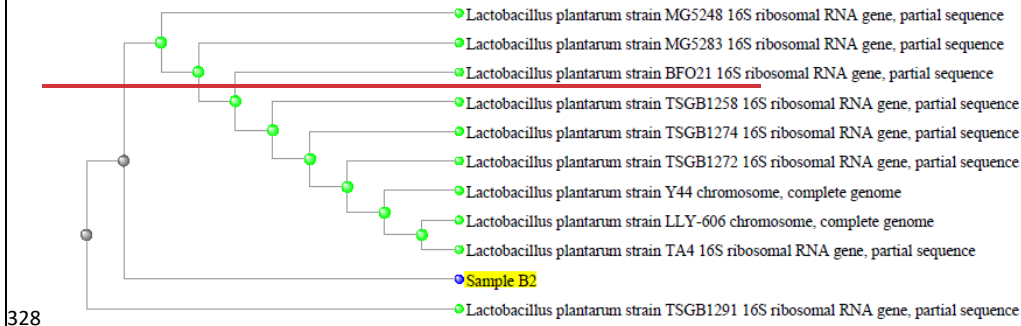
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326 Figure 6b. Phylogenetic tree Isolate Code B2

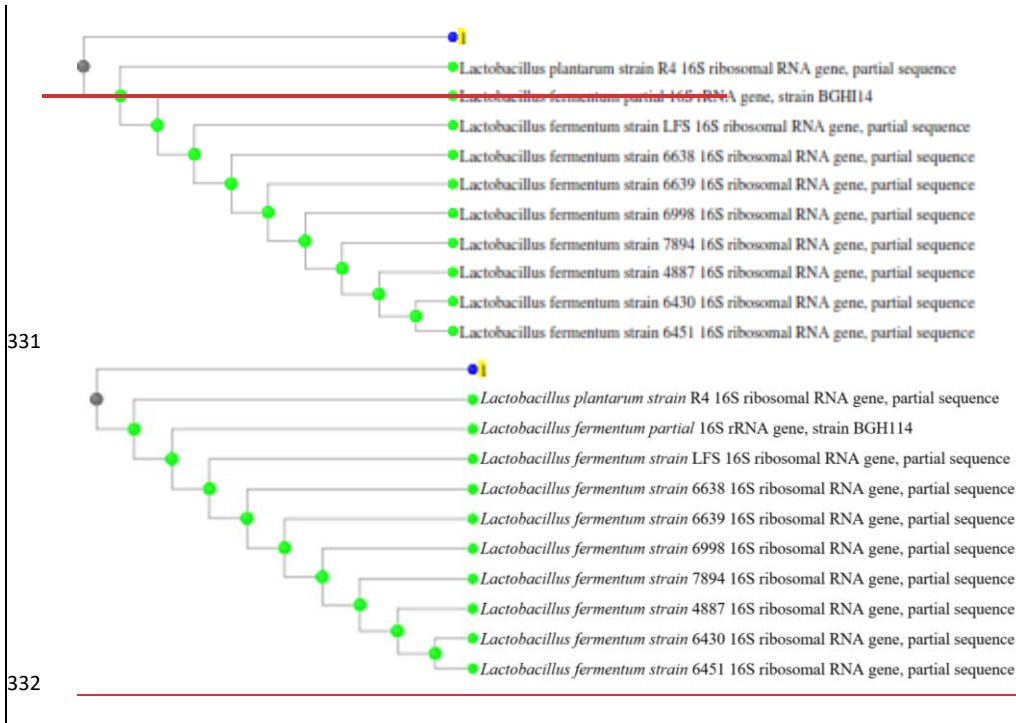
327

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330 Figure 6c. Phylogenetic tree Isolate Code S1



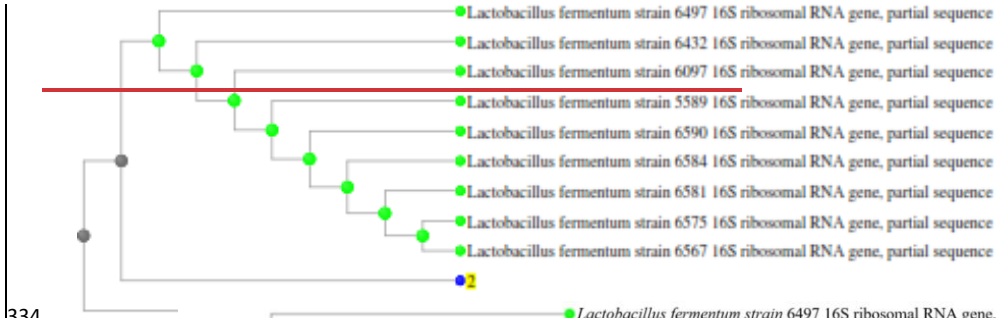
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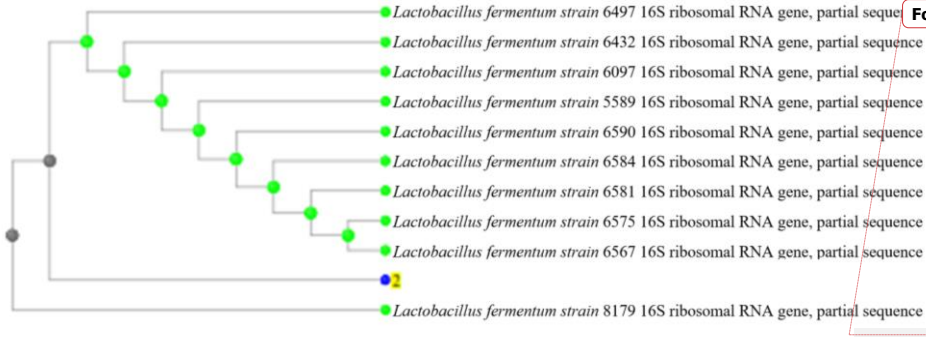
333 Figure 6d. Phylogenetic tree Isolate Code S2

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

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

### 30 1. Introduction

31 The Rejang are the third-largest tribe in Bengkulu Province, after the Serawai and Basema. North  
32 Bengkulu, Central Bengkulu, Rejang Lebong, Lebong, and Kepahyang are the five districts where the  
33 Rejang people live. *Lemea* is an ethnic food from the Rejang tribe. Ethnic foods are meals that have  
34 their origins in an ethnic group's history and culture (Kwon, 2015). Bamboo shoots and river fish are  
35 fermented into *Lemea* by the Rejang people (Dewi *et al.*, 2014). Betung, Tabah, Mayan, and Seik  
36 bamboo are some of the most common bamboo varieties used by the Rejang to produce *Lemea* .  
37 Betok, kepala timah, and mujahir fish are the most common fish used. The odour and flavour are  
38 unique, and only the locals enjoy it. LAB that have an impact on the flavour of fermented foods (Fox,  
39 2011). Indigenous fermented foods have been extensively researched.

40 There are various fermented foods in the world, especially in Asia. Fermented foods made from  
41 bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus*  
42 (Tomar, 2016) (Kiran *et al.*, 2016). *Meakri*, from Meghalaya Indian fermented bamboo shoots as a  
43 source of *Lactobacillus*, has characteristics suitable for probiotics (Das *et al.*, 2020). *Lactobacillus* is a  
44 group of LAB that are gram-positive bacteria. LAB have the potential to inhibit the infection and  
45 growth of 2pathogenic microbes (Yang *et al.*, 2021). LAB isolated from fermented bamboo shoots are  
46 potential probiotic candidates that are beneficial for health (Mohamad *et al.*, 2020). LAB strains are  
47 selected for their decreased content of cholesterol, antioxidant activity, and anti-bacterial activity  
48 (Jitpakdee *et al.*, 2022). Isolation of lactic acid bacteria from indogenous fermented foods is very  
49 important (Mende *et al.*, 2022).

**Commented [Editor1]:** Not listed in the reference section?

**Commented [LI2R1]:** An error occurred in the author's name should be: (Kiran *et al.*, 2016) 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/ibmoa.2016.02.00030>

Already listed in the reference section.

**Commented [Editor3]:** Not listed in the reference section?

**Commented [LI4R3]:** Already listed in the reference section.

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

**Commented [Editor5]:** Not listed in reference section?

**Commented [LI6R5]:** Already listed in the reference section. 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.

**Commented [Editor7]:** Not listed in the reference section?

**Commented [LI8R7]:** Already listed in the reference section. Mohamad, N., Manan, H., Sallehuddin, 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>

**Commented [Editor9]:** Not listed in the reference section?

**Commented [LI10R9]:** Already listed in the reference section. 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>

50 *Bekasam* is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional  
51 fermented food popular in Sumatera and Kalimantan (Desniar *et al.*, 2013). The sour taste in *Bekasam*  
52 is almost the same as in *Lemea*. The difference between these two products is the carbohydrate  
53 source and fermentation time. Carbohydrate sources are a source of nutrition for bacteria that play  
54 a role in the fermentation process. The source of carbohydrates used for rice in *Lemea* is bamboo  
55 shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes 10 days.

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

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

## 65 2. Materials and methods

### 66 2.1 Sample preparation

67 Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are  
68 thinly sliced and soaked for 30 h. Then they were washed, filtered, and weighed as much as 400 g  
69 and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for  
70 48 h (Figure 1).

71

72

**Commented [Editor11]:** Not listed in the reference section?

**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>

73      *2.2 LAB isolation*

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

81      *2.2 Identification of LAB*

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

84      *2.2.1 DNA Isolation*

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

87      *2.2.2 DNA amplification*

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

92      *2.2.3 DNA sequenching and phylogenetic analysis*

93            Sequencing and analysis of nucleotide sequences in GenBank. Analysis of grouping  
94            arrangement performed by comparing obtained (inquiry) with those already in the Gene  
95            Bank, with the information base hunted on the NCBI webpage

96 (http://www.ncbi.nlm.nih.gov) using Impact (Basic Local Alignment Search Tool). The size  
97 of the PCR amplification fragment was determined by comparing the position of the DNA  
98 marker size (Marker) with the sample fragment size.

### 99 3. Results and discussion

#### 100 3.1 Isolation of lactic acid bacteria from Lemea

101 Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung  
102 bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found  
103 were coded B1, B2, S1 and S2 (Figure 3). The bacterial isolates found were lactic acid bacteria  
104 because they were able to grow on MRSA specific media with cocci characteristics, a milky white  
105 color with a convex surface and smooth edges. The number of isolates obtained was less than  
106 that of *mesu*, *soidon*, *soibum*, and *soijon* but the same as unfermented bamboo shoots (Tamang  
107 *et al.*, 2008). The morphological characteristics of the isolates found in this study were almost  
108 the same as the previous findings isolated from *Lemea* produced by a cottage industry in  
109 Kepahyang Regency, Bengkulu (Kurnia *et al.*, 2020).

#### 110 3.2 Identification of *Lemea* isolates

111 The results of genomic DNA amplification of the 4 isolates can be seen in Figures 4a and 4b.  
112 Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size.  
113 Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434  
114 bp for isolate B1 (Figure 5a), 1424 bp for B2 (Figure 5b). The nucleotide sequence S1 isolate was  
115 882 bp (Figure 5c) and 849 bp for the S2 isolate (Figure 5d). The results of the analysis using the  
116 BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to  
117 *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.  
118 The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% similar to the  
119 partial sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of

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>



120 *Lactobacillus plantarum* but not *Lactobacillus fermentum*. Based on the phylogeny tree, isolate  
121 B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 6a),  
122 isolate B2 with *Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA. (Figure  
123 6b). S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA  
124 (Figure 6c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S  
125 ribosomal RNA. The type of isolate that was identified from Bekasam was *Lactobacillus*  
126 *plantarum* (Sukmarini *et al.*, 2014). All isolates have been homologous to the genus *Lactobacillus*.  
127 *Bacillus subtilis*, *Lactobacillus brevis*, and *Lactobacillus plantarum* were found in dominating  
128 strains of Soidon fermented bamboo shoot food without salt from Indian Manipur (Jeyaram *et*  
129 *al.*, 2010). LAB strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata* buse-kurz)  
130 have been previously studied and isolated as 2 species, namely *Lactobacillus plantarum* and  
131 *Lactobacillus rossiae* (Okfrianti *et al.*, 2019). Lactic acid bacteria isolated from Indonesian  
132 fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This research is  
133 expected to provide information on which LAB strains have been isolated from different types  
134 of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the diversity of the  
135 bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria* and  
136 *Proteobacteria* (Li *et al.*, 2021).  
137 *Lactobacillus fermentum* was only found in Lemeamade from yellow bamboo shoots (*Bambusa*  
138 *vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa*  
139 *vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in  
140 foodstuffs that trigger the growth of *Lactobacillus* (Macfarlane and Cummings, 1999).  
141 Oligosaccharides and fiber are prebiotics that promote the growth of specific bacteria found in  
142 the gut. Bamboo shoots are a good source of fibre (Felisberto *et al.*, 2017). At 100 g of fresh  
143 weight, bamboo shoots of *B. vulgaris* contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of

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

#### 147 **4. Conclusion**

148 Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus*  
149 *plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S  
150 rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots  
151 (*Bambusa vulgaris Schrad*), while *Lactobacillus plantarum* was found in yellow bamboo shoots  
152 (*Bambusa vulgaris Schrad*) and betung (*Dendrocalamus asper Schult*).

#### 153 **Conflict of interest - Disclose potential conflicts of interest appropriately.**

154 The authors declare no conflict of interest.

#### 155 **Acknowledgments**

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

157

158

159 **References**

- 160 Adebola, O.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin,  
161 lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of*  
162 *Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- 163 Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots:  
164 Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and*  
165 *Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 166 Das, S., Mishra, B.K., and Hati, S. (2020). Techno-functional characterization of indigenous *Lactobacillus*  
167 isolates from the traditional fermented foods of Meghalaya, India. *Current Research in Food*  
168 *Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>
- 169 Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria  
170 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
171 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
172 <https://doi.org/10.9755/ejfa.v25i6.12478>
- 173 Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di  
174 Provinsi Bengkulu Industry Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province.  
175 *AGRISEP*, 14(1), 61–69. <https://doi.org/https://doi.org/10.31186/jagrisep.13.1.60-66>
- 176 Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential  
177 food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
178 <https://doi.org/10.1016/j.foodres.2017.08.058>
- 179 Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
180 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- 181 Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and Devi, S.S. (2010). Bacterial species associated with  
182 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
183 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
184 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- 185 Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce  
186 functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160, 113269.

187 <https://doi.org/10.1016/j.lwt.2022.113269>

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

191 Kurnia, M., Amir, H., and Handayani, D. (2020). Isolasi Dan Identifikasi Bakteri Asam Laktat Dari Makanan  
192 Tradisional Suku Rejang Di Provinsi Bengkulu: “Lemea.” *Alotrop*, 4(1), 25–32.  
193 <https://doi.org/10.33369/atp.v4i1.13705>

194 Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
195 <https://doi.org/10.1016/j.jef.2015.02.001>

196 Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). In vitro fecal fermentation  
197 characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11, 1–  
198 9. <https://doi.org/10.1016/j.fochx.2021.100129>

199 Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of  
200 intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.

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

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

207 Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
208 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter-Professional Health*  
209 *Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-](https://doi.org/10.2991/icihc-2018.2019.52)  
210 [18.2019.52](https://doi.org/10.2991/icihc-2018.2019.52)

211 Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84.  
212 <https://doi.org/10.22146/agritech.13515>

213 Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-

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Commented [LI16R15]: 1–9.

Commented [Editor17]: Volume number missing?

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214 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of*  
215 *Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>

216 Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M., and Holzapfel, W.H. (2008).  
217 Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented  
218 bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–  
219 40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>

220 Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and  
221 Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese.  
222 *Journal of Applied Microbiology*, 103(6), 2142–2152. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.2007.03450.x)  
223 [2672.2007.03450.x](https://doi.org/10.1111/j.1365-2672.2007.03450.x)

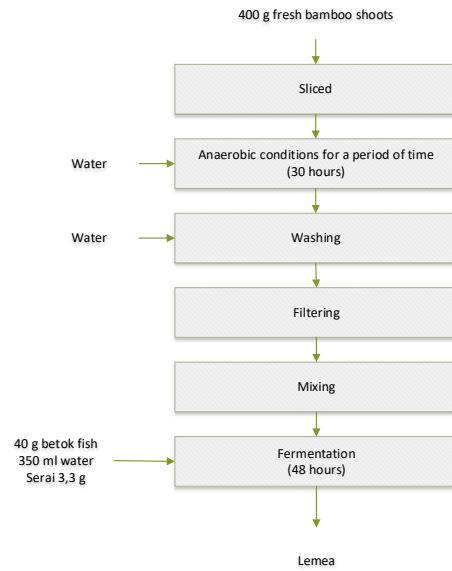
224 Wasis, N.O., Semadi Antara, N., and Wayan Gunam, I.B. (2019). Studi Viabilitas Isolat Bakteri Asam Laktat  
225 yang Diisolasi dari Asinan Rebung Bambu Tabah Terhadap pH Rendah dan Garam Empedu. *Jurnal*  
226 *Rekayasa Dan Manajemen Agroindustri*, 7(1), 1–10.  
227 <https://doi.org/10.24843/jrma.2019.v07.i01.p01>

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

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Figure 1. Fermented bamboo shoot making process

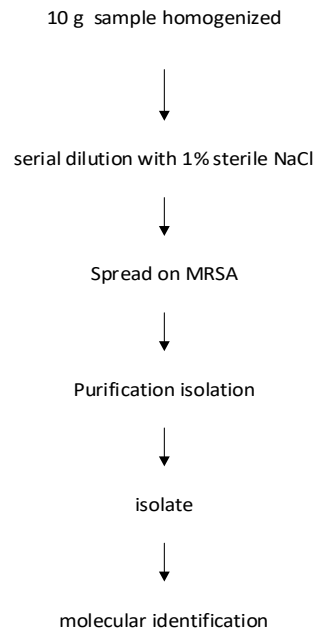


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Figure 2. Isolation process



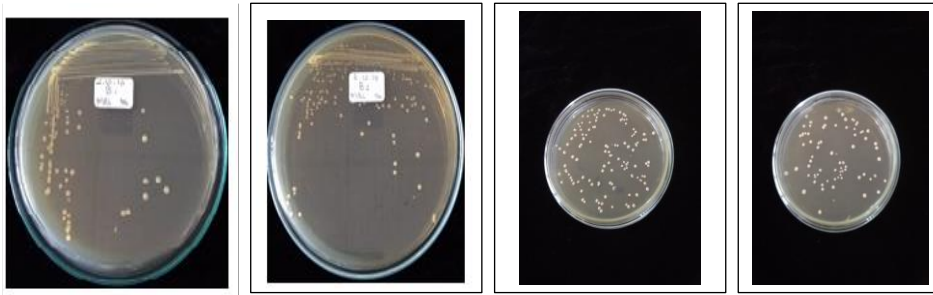
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Figure 3. Isolate B1, B2, S1 dan S2



B1

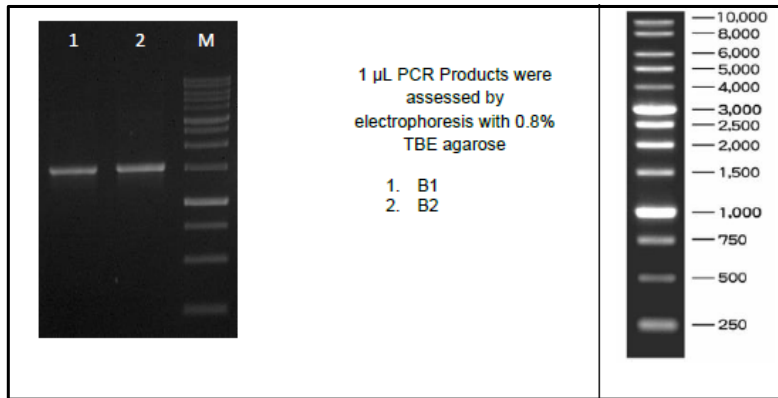
B2

S1

S2

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

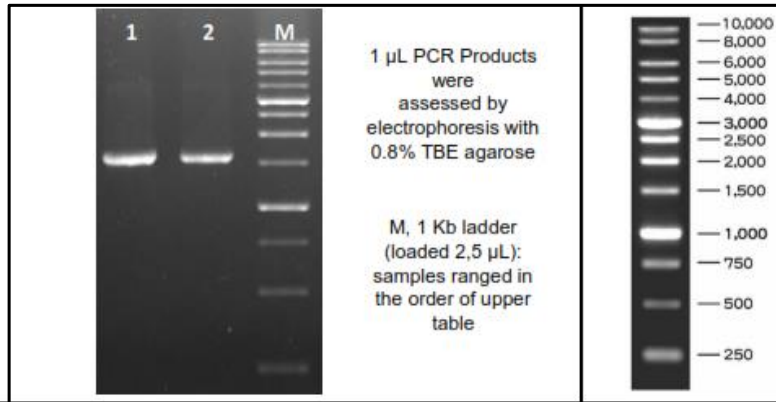


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251 Visualization of 16 S rRNA gene amplification of isolates B1 and B2 at 0.8% TBE agarose concentration  
252 using the Kappa Universal ladder



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



254

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

257

Figure 5a. The nucleotide sequence B1 isolate

```

Sequence Assembly 1434 bp
1   AGGCGGCTGG TTCCTAAAAG GTTACCCAC CGACTTTGGG TGTACAAAC TCTCATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCAACG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC T GAGAATGGCT
181 TTAAGAGATT AGCTTGCTCT CGCGAGTTCC CAACTCGITG TACCATCCAT TGTAGCAAGT
241 GTGTAGCCCA GGTCTAAAGG GGCATGATGA TTTGACGTCA TCCCACCTT CCTCCGGTIT
301 GTCACCGCCA GTCTACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GACTTAACC CAACATCTCA CGACACGAGC TGACGACAA C ATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTGGCGTA GCTTGGAAAT AAAACCATG CTCCAACGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACCC CTCCAACTC TAGCATTCA T CGTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTTTG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCCTCTCTG CACTCAAGTT TCCCAGTTT C GATGCACIT
841 CTTCGGTTGA GCGGAAGGCT TTCACATCAG ACTTAAAAA CCGCTGGCG TCGCTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGIT
961 AGCCGTTGGCT TTTGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTIA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCTC ACTGCTGCC TCCGTAGGAG TTTGGCCGCT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCAACATCTA GCTAATACGC CGCGGGAACA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCC ACTI

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Figure 5b. The nucleotide sequence B2 isolate

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Sequence Assembly 1424 bp
1   AGGCGGCTGG TTCCTAAAAG GTTACCCAC CGACTTTGGG TGTACAAAC TCTCATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCAACG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC T GAGAATGGCT
181 TTAAGAGATT AGCTTACTCT CGCGAGTTCC CAACTCGITG TACCATCCAT TGTAGCAAGT
241 GTGTAGCCCA GGTCTAAAGG GGCATGATGA TTTGACGTCA TCCCACCTT CCTCCGGTIT
301 GTCACCGCCA GTCTACCAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GACTTAACC CAACATCTCA CGACACGAGC TGACGACAA C ATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTGGCGTA GCTTGGAAAT AAAACCATG CTCCAACGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACCC CTCCAACTC TAGCATTCA T CGTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCGTTTG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCCTCTCTG CACTCAAGTT TCCCAGTTT C GATGCACIT
841 CTTCGGTTGA GCGGAAGGCT TTCACATCAG ACTTAAAAA CCGCTGGCG TCGCTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGIT
961 AGCCGTTGGCT TTTGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTIA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT
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1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCAACATCTA GCTAATACGC CGCGGGAACA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTCGTTCC AGTI

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262 Figure 5c. The nucleotide sequence S1 isolate

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Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGGGCTGGC TCCTAAAAGG TTACCCACC GACTTTGGGT GTTACAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTG CCACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCGGAACG
181    AGAACGGTTT TAAGAGATTG GCTTGCCTC GCGAGTTCCG GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGGC CTCGTTGGG GACTTAAACC AACATCTCAC GACACGAGCT GACGAGGACC
421    ATGCACACAC TGTCAATTGG TTCCCGAAGG AAAGCCCTTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTGGCGTAG CTTCAATTA AACACATGC TCCACGCTT
541    GTGCGGGCCC CCGTCAATTC CTTGAGTTT CAACCTTGGG GTCGACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTCAGGCA TGGACTACCA GGTATCTAA TCCTGTTCCG TACCATGCT TCGAGTCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCTT CGCCACTGGT GTTCTCCAT ATATCTACCG
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTCG ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
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Figure 5d. The nucleotide sequence S2

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Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGAGC GCGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTGGCTT GGCCTGCGCA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCACC ACCTTCCTCC
301    GGTGTTGCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGCGCTG TTGCGGGACT TAACCAACA TCTCAGGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GGCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCC TACTCCCAG GCGGAGTGTCT
601    TAATCGGTTA GCTCCGGCAC TGAAGGGCGG AAACCTTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCC AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCCT TCTGCACTC AAGTTATCCA GTTTCGATG
841    CACTTCTCC
```

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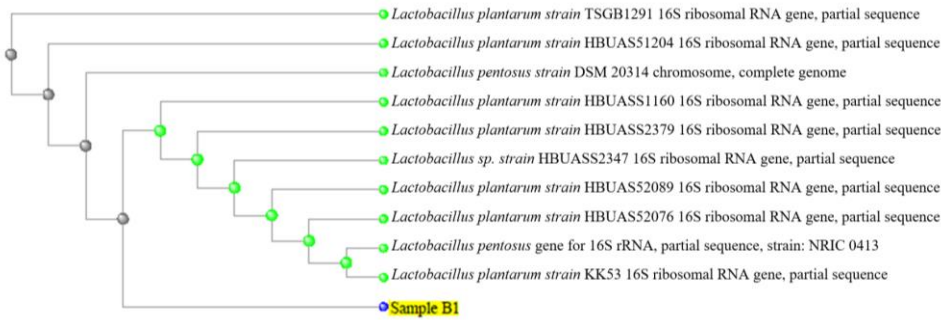
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268 Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

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

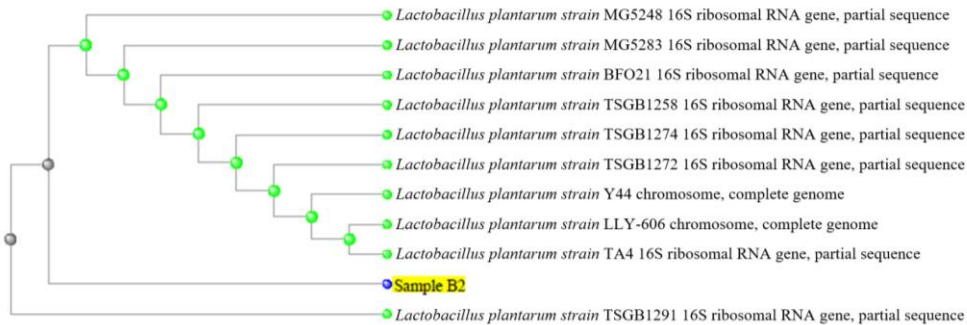
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270 Figure 6a. Phylogenetic tree Isolate Code B1



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272 Figure 6b. Phylogenetic tree Isolate Code B2

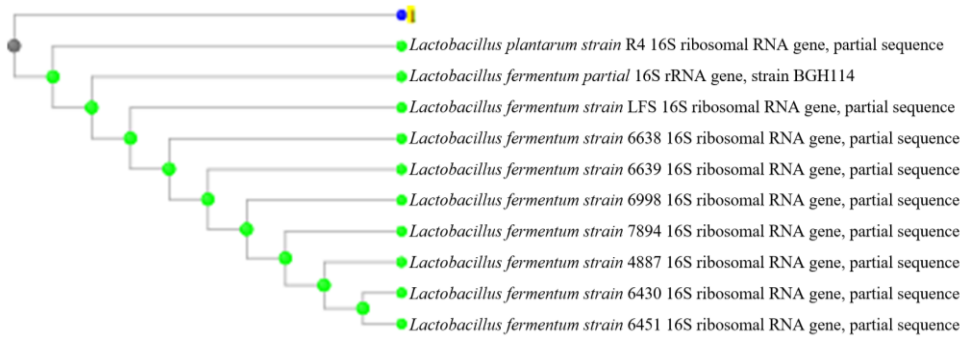


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274 Figure 6c. Phylogenetic tree Isolate Code S1

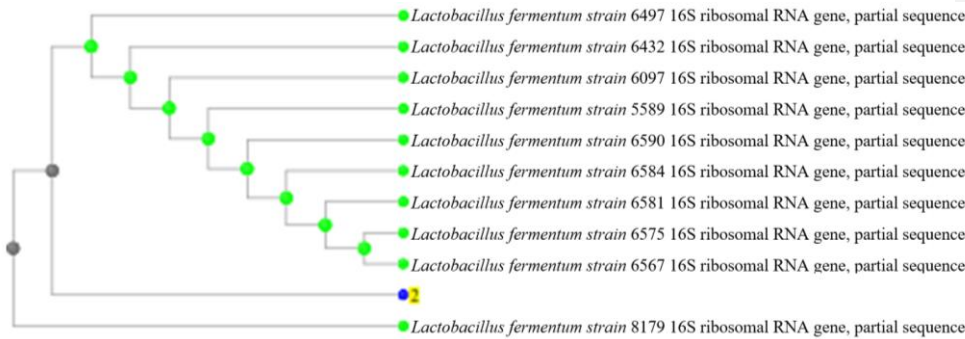


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276 Figure 6d. Phylogenetic tree Isolate Code S2



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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 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 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 indigenous fermented foods is very important (Mende *et al.*, 2022).

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

- Adebola, O.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 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 Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>
- Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494. <https://doi.org/10.9755/ejfa.v25i6.12478>
- Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di Provinsi Bengkulu Industry Mapping of "Lemea" Rejang Traditional Food in Bengkulu Province. *AGRISEP*, 14(1), 61–69. <https://doi.org/https://doi.org/10.31186/jagrisep.13.1.60-66> [In Bahasa Indonesia].
- Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential food as source of fiber and starch. *Food Research International*, 101, 96–102. <https://doi.org/10.1016/j.foodres.2017.08.058>
- Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. In Fuquay, J.W. (Ed.) *Encyclopedia of Dairy Sciences*. 2<sup>nd</sup> ed., p. 401–402. Elsevier E-Book. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and Devi, S.S. (2010). Bacterial species associated with traditional starter cultures used for fermented bamboo shoot production in Manipur state of India. *International Journal of Food Microbiology*, 143(1–2), 1–8. <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce functional fermented whey beverage with putative health promoting attributes. *LWT*, 160, 113269. <https://doi.org/10.1016/j.lwt.2022.113269>
- Kiran, T., Rajani, C., Kumar, T.S. and Achun, P. (2016). Fermented Bamboo Shoots: A Riche Niche for Beneficial Microbes. *Journal of Bacteriology and Mycology*, 2(4), 87–93. <https://doi.org/10.15406/jbmoa.2016.02.00030>
- 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> [In Bahasa Indonesia]
- Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.

<https://doi.org/10.1016/j.jef.2015.02.001>

- Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). *In vitro* fecal fermentation characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11, 100129. <https://doi.org/10.1016/j.fochx.2021.100129>
- Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.
- Mende, S., Rohm, H. and Aros, D. (2022). Lactic Acid Bacteria: Exopolysaccharides. In McSweeney, P.L.H. and McNamara, J.P. (Eds.) *Encyclopedia of Dairy Sciences*. Vol. 4, 3<sup>rd</sup> ed. Elsevier E-Book. <https://doi.org/10.1016/b978-0-08-100596-5.22982-x>
- Mohamad, N., Manan, H., Sallehuddin, 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, 100523. <https://doi.org/10.1016/j.aqrep.2020.100523>
- Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional Fermented Rejang Shoot “Lemea”. *Advances in Health Sciences Research*, 14, 237–240. <https://doi.org/10.2991/ichc-18.2019.52>
- Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84. <https://doi.org/10.22146/agritech.13515>
- Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>
- Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M. and Holzapfel, W.H. (2008). Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>
- Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese. *Journal of Applied Microbiology*, 103(6), 2142–2152. <https://doi.org/10.1111/j.1365-2672.2007.03450.x>
- 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> [In Bahasa Indonesia].
- 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

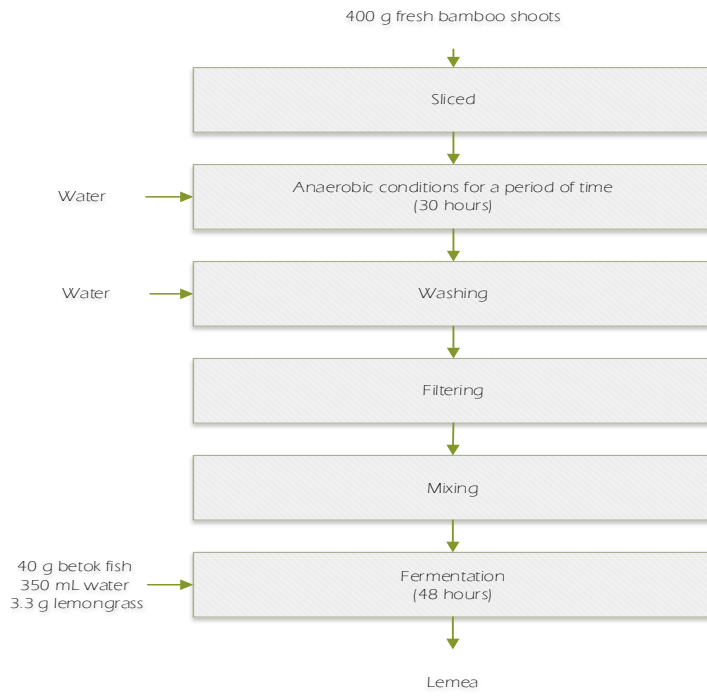




Figure 2. Isolate B1, B2, S1 and S2

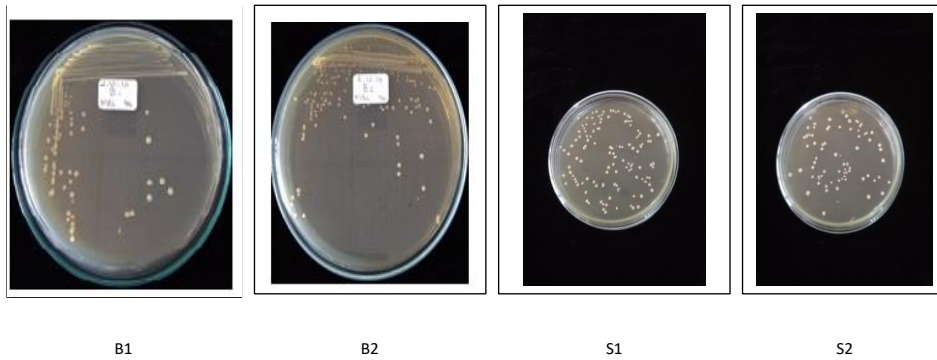
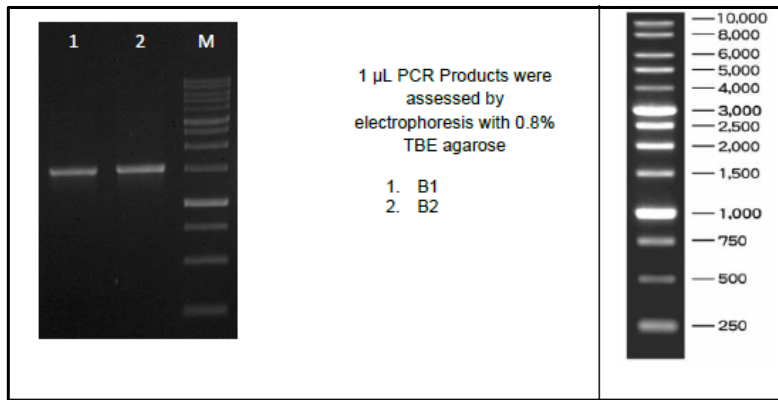
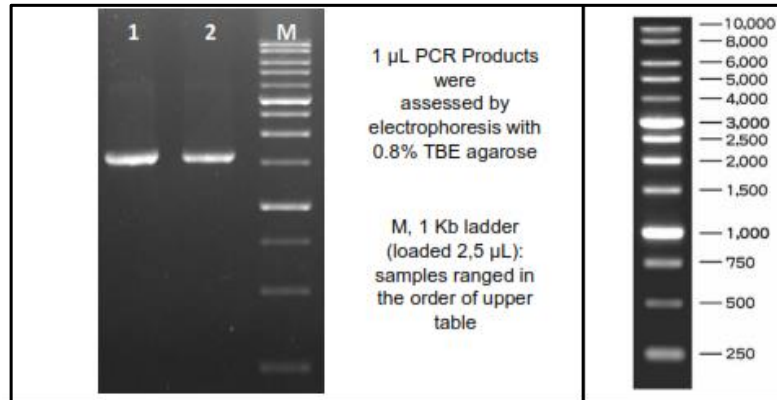


Figure 3a. Genomic DNA amplification of isolates B1 and B2



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   AGGGGGCTGG TTCTAAAAG GTTACCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61  TGACGGGGCGG TGTGTACAAG GCCCGGGAAC GTATTACCCG CGGCATGCTG ATCCGGGATT
121 ACTAGCGGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAAATGGCT
181 TTAAGAGATT AGCTTGTCTT CCGAGTTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCTAAAGG GGCATGATGA TTTGACGTCA TCCCACCTT CCTCCGGTTT
301 GTACCCGGCA GTCTCACCGA AGTGCACAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GCACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGGTA GCTTCGAATT AAACCAATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACC CTCCAACTC TAGCATTCAI CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCTGTTTG CTAACCCATC TTTGAGCCTC CAGCGTCAGT
721 TACAGACCAG ACAGCCGCCCT TCGCCACTGG TGTTCTTCCA TATACTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACIT
841 CTTCGGTTGA GCGGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGGCC TCGCTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACTC ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TCTGGTTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCTTCTTCCA CTCACCGGCC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCC CTCCGAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTAACCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTACC CCACCATCTA GCTAATACGC CCGGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCGACGTGTT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTGCTTGC ACTT

```

Figure 4b. The nucleotide sequence B2 isolate

```

Sequence Assembly 1424 bp
1   AGGGGGCTGG TTCTAAAAG GTTACCCAC CGACTTTGGG TGTTACAAAC TCTCATGGTG
61  TGACGGGGCGG TGTGTACAAG GCCCGGGAAC GTATTACCCG CGGCATGCTG ATCCGGGATT
121 ACTAGCGGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAACT GAGAAATGGCT
181 TTAAGAGATT AGCTTGTCTT CCGAGTTTCG CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTTAGGCCCA GGTCTAAAGG GGCATGATGA TTTGACGTCA TCCCACCTT CCTCCGGTTT
301 GTACCCGGCA GTCTCACCGA AGTGCACAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGCG GCACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGGTA GCTTCGAATT AAACCAATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACC CTCCAACTC TAGCATTCAI CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCCTGTTTG CTAACCCATC TTTGAGCCTC CAGCGTCAGT
721 TACAGACCAG ACAGCCGCCCT TCGCCACTGG TGTTCTTCCA TATACTACG CATTTCACCG
781 CTACACATGG AGTTCACCTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACIT
841 CTTCGGTTGA GCGGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGGCC TCGCTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACTC ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TCTGGTTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCTTCTTCCA CTCACCGGCC GTTGCTCCAT
1081 CAGACTTTCG TCCATTGTGG AAGATTCCCT ACTGCTGCC CTCCGAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTAACCTC TCAGGTCGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTACC CCACCATCTA GCTAATACGC CCGGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CGGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCGACGTGTT ACTCACCACT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTGCTTGC ACTT

```

Figure 4c. The nucleotide sequence S1 isolate

```
Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGGGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGGGATTA CTAGCGATTG CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACTG
181    AGAACGGTTT TAAGAGATTG GCTTGCCTC GCGAGTTCCG GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCGAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACTTC
301    CTCGGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGGC CTCGTTGGGG GACTTAAACC AACATCTCAC GACACGAGCT GACGAAGACC
421    ATGCACCACC TGTCAATTGG TTCCCGAAGG AAAGCCCTTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTGGCGTAG CTTCAATTA AACCCATGC TOCACGGCTT
541    GTCGGGGGCC CGGTCAATTC CTTGAGTTT CAACCTTGGG GTCGTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGTATCTAA TCCTGTTCCG TACCATGCT TTCGAGTCTC
721    AGGTCAGTGT GCAGACCAGG TAGCCGCCCT CGCCACTGGT GTTCTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTAAAG CCGAAGGCTT TCACATCATA CT
```

Figure 4d. The nucleotide sequence S2

```
Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGAAG GCGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGGGCCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CCGTTTTAAG AGATTGGCTT GGCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCTCTC
301    GGTGTTGCAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGCGCTG TTGGCGGACT TAACCCAACA TCTCAGGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GGCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCTTTT GAGTTTCAAC CTTGCGGTGC TACTCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCTTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTT TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCCT TTCTGCACTC AAGTTATCCA GTTTCGATG
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 Accession Number GenBank Accession Number	Similarity (%)
Bamboo Betung	B1	<i>Lactobacillus plantarum</i>	M N37236.01	99
	B2	<i>Lactobacillus plantarum</i>	MN972325.1	99
Yellow Bamboo	S1	<i>Lactobacillus plantarum</i>	KM350169.1	99
	S2	<i>Lactobacillus fermentum</i>	MT538927.1	99

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

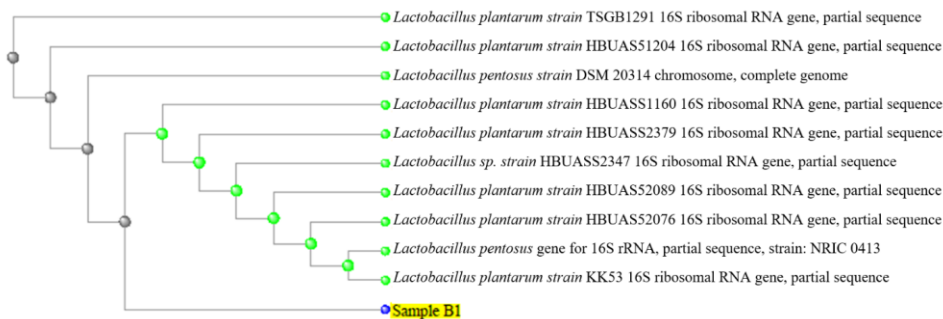


Figure 5b. Phylogenetic tree Isolate Code B2

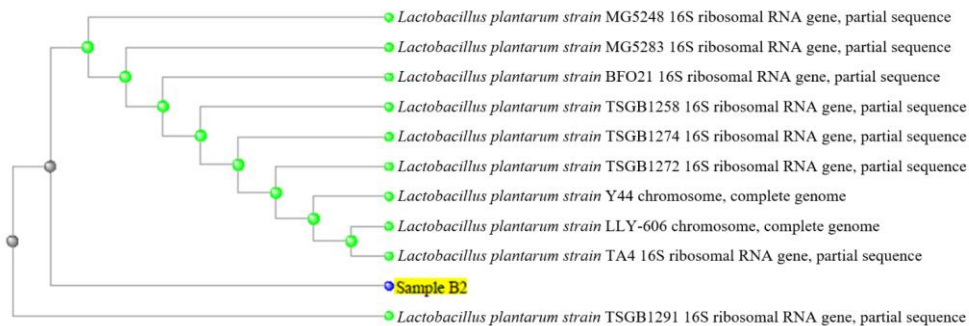


Figure 5c. Phylogenetic tree Isolate Code S1

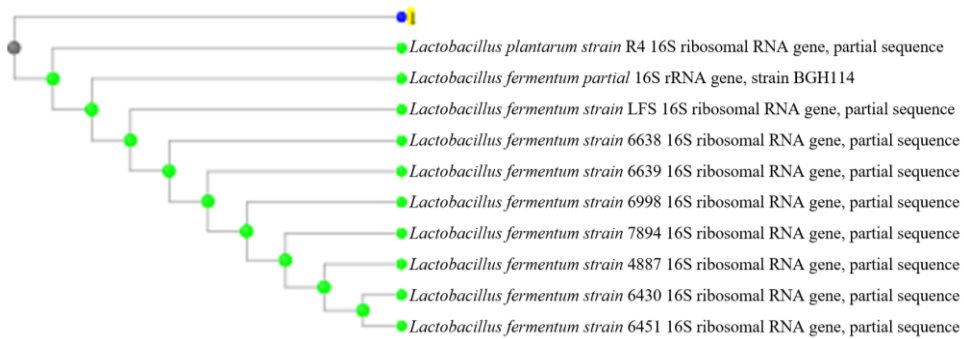
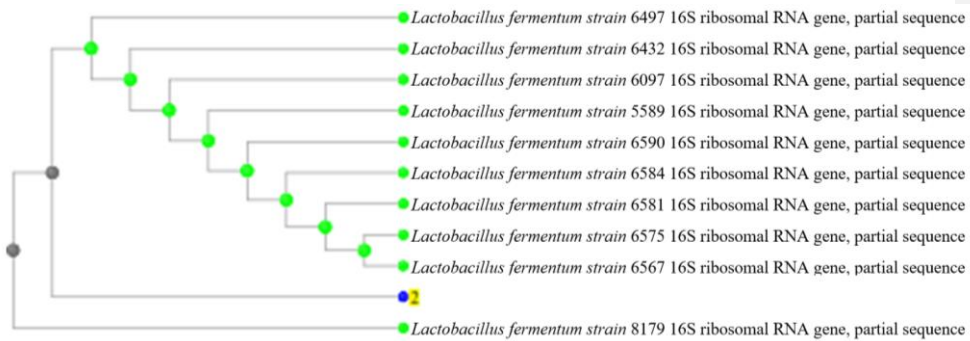


Figure 5d. Phylogenetic tree Isolate Code S2





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

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

## 30 1. Introduction

31 The Rejang are the third-largest tribe in Bengkulu Province, after the Serawai and Basema. North  
32 Bengkulu, Central Bengkulu, Rejang Lebong, Lebong, and Kepahyang are the five districts where the  
33 Rejang people live. *Lemea* is an ethnic food from the Rejang tribe. Ethnic foods are meals that have  
34 their origins in an ethnic group's history and culture (Kwon, 2015). Bamboo shoots and river fish are  
35 fermented into *Lemea* by the Rejang people (Dewi *et al.*, 2014). Betung, Tabah, Mayan, and Seik  
36 bamboo are some of the most common bamboo varieties used by the Rejang to produce *Lemea* .  
37 Betok, kepala timah, and mujahir fish are the most common fish used. The odour and flavour are  
38 unique, and only the locals enjoy it. LAB that have an impact on the flavour of fermented foods (Fox,  
39 2011). Indigenous fermented foods have been extensively researched.

40 There are various fermented foods in the world, especially in Asia. Fermented foods made from  
41 bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus* (Kiran  
42 *et al.*, 2016). *Meakri*, from Meghalaya Indian fermented bamboo shoots as a source of *Lactobacillus*,  
43 has characteristics suitable for probiotics (Das *et al.*, 2020). *Lactobacillus* is a group of LAB that are  
44 gram-positive bacteria. LAB have the potential to inhibit the infection and growth of 2 pathogenic  
45 microbes (Yang *et al.*, 2021). LAB isolated from fermented bamboo shoots are potential probiotic  
46 candidates that are beneficial for health (Mohamad *et al.*, 2020). LAB strains are selected for their  
47 decreased content of cholesterol, antioxidant activity, and anti-bacterial activity (Jitpakdee *et al.*,  
48 2022). Isolation of lactic acid bacteria from indigenous fermented foods is very important (Mende *et*  
49 *al.*, 2022).



50 *Bekasam* is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional  
51 fermented food popular in Sumatera and Kalimantan (Desniar *et al.*, 2013). The sour taste in *Bekasam*  
52 is almost the same as in *Lemea*. The difference between these two products is the carbohydrate  
53 source and fermentation time. Carbohydrate sources are a source of nutrition for bacteria that play  
54 a role in the fermentation process. The source of carbohydrates used for rice in *Lemea* is bamboo  
55 shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes 10 days.

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

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

## 65 **2. Materials and methods**

### 66 *2.1 Sample preparation*

67 Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are  
68 thinly sliced and soaked for 30 h. Then they were washed, filtered, and weighed as much as 400 g  
69 and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for  
70 48 h (Figure 1).

71

72

73        *2.2 LAB isolation*

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

81        *2.2 Identification of LAB*

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

84        *2.2.1 DNA Isolation*

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

87        *2.2.2 DNA amplification*

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

92        *2.2.3 DNA sequenching and phylogenetic analysis*

93            Sequencing and analysis of nucleotide sequences in GenBank. Analysis of grouping  
94            arrangement performed by comparing obtained (inquiry) with those already in the Gene  
95            Bank, with the information base hunted on the NCBI webpage

96 (http://www.ncbi.nlm.nih.gov) using Impact (Basic Local Alignment Search Tool). The size  
97 of the PCR amplification fragment was determined by comparing the position of the DNA  
98 marker size (Marker) with the sample fragment size.

### 99 3. Results and discussion

#### 100 3.1 Isolation of lactic acid bacteria from Lemea

101 Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung  
102 bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found  
103 were coded B1, B2, S1 and S2 (Figure 2). The bacterial isolates found were lactic acid bacteria  
104 because they were able to grow on MRSA specific media with cocci characteristics, a milky white  
105 color with a convex surface and smooth edges. The number of isolates obtained was less than  
106 that of *mesu*, *soidon*, *soibum*, and *soijon* but the same as unfermented bamboo shoots (Tamang  
107 *et al.*, 2008). The morphological characteristics of the isolates found in this study were almost  
108 the same as the previous findings isolated from *Lemea* produced by a cottage industry in  
109 Kepahyang Regency, Bengkulu (Kurnia *et al.*, 2020).

#### 110 3.2 Identification of Lemea isolates

111 The results of genomic DNA amplification of the 4 isolates can be seen in Figures 3a and 3b.  
112 Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size.  
113 Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434  
114 bp for isolate B1 (Figure 4a), 1424 bp for B2 (Figure 4b). The nucleotide sequence S1 isolate was  
115 882 bp (Figure 4c) and 849 bp for the S2 isolate (Figure 4d). The results of the analysis using the  
116 BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to  
117 *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.  
118 The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% like the partial  
119 sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of

120 *Lactobacillus plantarum* but not *Lactobacillus fermentum*. Based on the phylogeny tree, isolate  
121 B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 5a),  
122 Isolate B2 with *Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b).  
123 S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure  
124 5c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S  
125 ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was  
126 *Lactobacillus plantarum* (Sukmarini *et al.*, 2014). All isolates have been homologous to the genus  
127 *Lactobacillus*. *Bacillus subtilis*, *Lactobacillus brevis*, and *Lactobacillus plantarum* were found in  
128 dominating strains of Soidon fermented bamboo shoot food without salt from Indian Manipur  
129 (Jeyaram *et al.*, 2010). LAB strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata*  
130 buse-kurz) have been previously studied and isolated as 2 species, namely *Lactobacillus*  
131 *plantarum* and *Lactobacillus rossiae* (Okfrianti *et al.*, 2019). Lactic acid bacteria isolated from  
132 Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This  
133 research is expected to provide information on which LAB strains have been isolated from  
134 different types of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the  
135 diversity of the bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria*  
136 and *Proteobacteria* (Li *et al.*, 2021).

137 *Lactobacillus fermentum* was only found in *Lemeamade* from yellow bamboo shoots (*Bambusa*  
138 *vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa*  
139 *vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in  
140 foodstuffs that trigger the growth of *Lactobacillus* (Macfarlane and Cummings, 1999).  
141 Oligosaccharides and fiber are prebiotics that promote the growth of specific bacteria found in  
142 the gut. Bamboo shoots are a good source of fibre (Felisberto *et al.*, 2017). At 100 g of fresh  
143 weight, bamboo shoots of *B. vulgaris* contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of

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

#### 147 **4. Conclusion**

148 Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus*  
149 *plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S  
150 rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots  
151 (*Bambusa vulgaris Schrad*), while *Lactobacillus plantarum* was found in yellow bamboo shoots  
152 (*Bambusa vulgaris Schrad*) and betung (*Dendrocalamus asper Schult*).

#### 153 **Conflict of interest - Disclose potential conflicts of interest appropriately.**

154 The authors declare no conflict of interest.

#### 155 **Acknowledgments**

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157

158

159 **References**

- 160 Adebola, O.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin,  
161 lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of*  
162 *Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- 163 Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots:  
164 Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and*  
165 *Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 166 Das, S., Mishra, B.K., and Hati, S. (2020). Techno-functional characterization of indigenous Lactobacillus  
167 isolates from the traditional fermented foods of Meghalaya, India. *Current Research in Food*  
168 *Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>
- 169 Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria  
170 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
171 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
172 <https://doi.org/10.9755/ejfa.v25i6.12478>
- 173 Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di  
174 Provinsi Bengkulu Industry Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province.  
175 *AGRISSEP*, 14(1), 61–69. <https://doi.org/https://doi.org/10.31186/jagrissep.13.1.60-66>
- 176 Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential  
177 food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
178 <https://doi.org/10.1016/j.foodres.2017.08.058>
- 179 Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
180 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- 181 Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and Devi, S.S. (2010). Bacterial species associated with  
182 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
183 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
184 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- 185 Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce  
186 functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160, 113269.

187 <https://doi.org/10.1016/j.lwt.2022.113269>

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

191 Kurnia, M., Amir, H., and Handayani, D. (2020). Isolasi Dan Identifikasi Bakteri Asam Laktat Dari Makanan  
192 Tradisional Suku Rejang Di Provinsi Bengkulu: “Lemea.” *Alotrop*, 4(1), 25–32.  
193 <https://doi.org/10.33369/atp.v4i1.13705>

194 Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
195 <https://doi.org/10.1016/j.jef.2015.02.001>

196 Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). In vitro fecal fermentation  
197 characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11. 1–  
198 9.<https://doi.org/10.1016/j.fochx.2021.100129>

199 Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of  
200 intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.

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

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

207 Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
208 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter-Professional Health*  
209 *Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-](https://doi.org/10.2991/icihc-18.2019.52)  
210 [18.2019.52](https://doi.org/10.2991/icihc-18.2019.52)

211 Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84.  
212 <https://doi.org/10.22146/agritech.13515>

213 Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-

214 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of*  
215 *Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>

216 Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M., and Holzapfel, W.H. (2008).  
217 Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented  
218 bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–  
219 40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>

220 Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and  
221 Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese.  
222 *Journal of Applied Microbiology*, 103(6), 2142–2152. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.2007.03450.x)  
223 [2672.2007.03450.x](https://doi.org/10.1111/j.1365-2672.2007.03450.x)

224 Wasis, N.O., Semadi Antara, N., and Wayan Gunam, I.B. (2019). Studi Viabilitas Isolat Bakteri Asam Laktat  
225 yang Diisolasi dari Asinan Rebung Bambu Tabah Terhadap pH Rendah dan Garam Empedu. *Jurnal*  
226 *Rekayasa Dan Manajemen Agroindustri*, 7(1), 1–10.  
227 <https://doi.org/10.24843/jrma.2019.v07.i01.p01>

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

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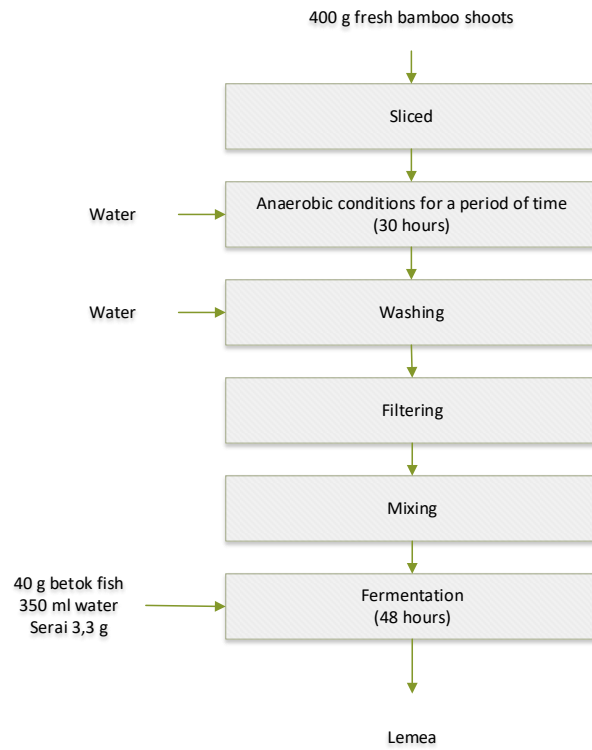
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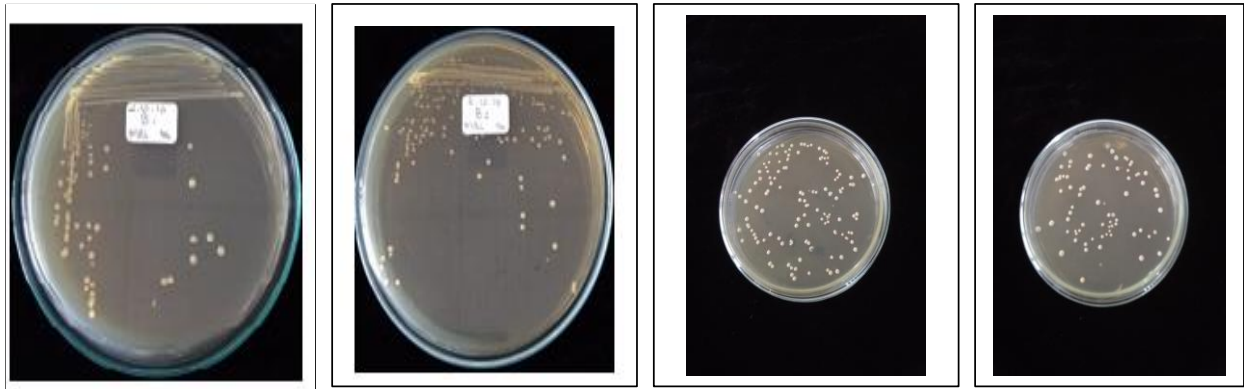
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Figure 1. Fermented bamboo shoot making process



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



B1

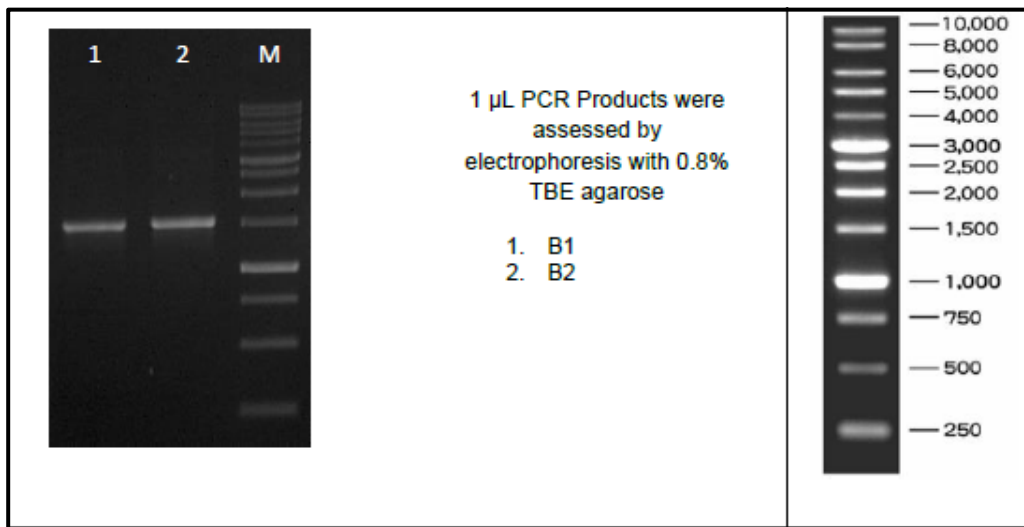
B2

S1

S2

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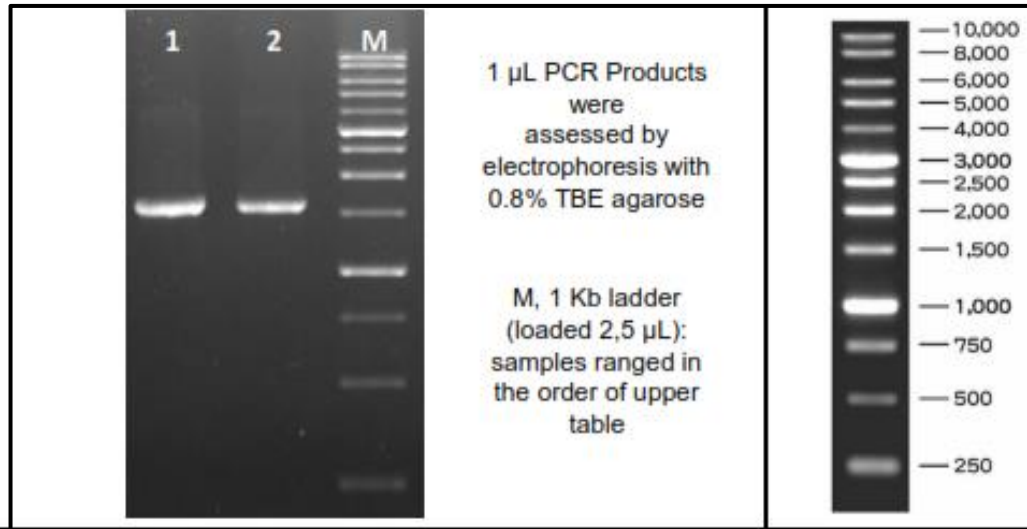
245 Figure 3a. Genomic DNA amplification of isolates B1 and B2



246

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

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



250

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

253 Figure 4a. The nucleotide sequence B1 isolate

```

Sequence Assembly 1434 bp
1   AGGCGGCTGG TTCTAAAAG GTTACCCAC CGACTITGGG TGTTACAAC TCTCATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCCGGAAC GTATTACCG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC TACAATGGCT
181 TTAAGAGATT AGCTTGTCTT CGCGAGTTGG CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTGTAGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTC TCCCCACCTT CCTCCGGTIT
301 GTCACCGGCA GTCTCACAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGGG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGGCTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACC CTCCAACACT TAGCATTTCAT CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCTGTITG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACCT
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGGGC TCGCTTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG AAGATTCCCT ACTGCTGCC TCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAGGTGGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CCGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTGCTTGG ACTT

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254

255 Figure 4b. The nucleotide sequence B2 isolate

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Sequence Assembly 1424 bp
1   AGGCGGCTGG TTCTAAAAG GTTACCCAC CGACTITGGG TGTTACAAC TCTCATGGTG
61  TGACGGGCGG TGTGTACAAG GCCCCGGAAC GTATTACCG CGGCATGCTG ATCCGCGATT
121 ACTAGCGATT CCGACTTCAT GTAGGCGAGT TGCAGCCTAC AATCCGAAC TACAATGGCT
181 TTAAGAGATT AGCTTGTCTT CGCGAGTTGG CAACTCGTTG TACCATCCAT TGTAGCACGT
241 GTGTAGCCCA GGTCAAAAGG GGCATGATGA TTTGACGTC TCCCCACCTT CCTCCGGTIT
301 GTCACCGGCA GTCTCACAG AGTGCCCAAC TTAATGCTGG CAACTGATAA TAAGGGTTGC
361 GCTCGTTGGG GGACTTAACC CAACATCTCA CGACACGAGC TGACGACAAC CATGCACCAC
421 CTGTATCCAT GTCCCCGAAG GGAACGTCTA ATCTCTTAGA TTTGCATAGT ATGTCAAGAC
481 CTGGTAAGGT TCTTCGGCTA GCTTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGCC
541 CCGTCAATT CCTTTGAGTT TCAGCCTTGC GGCCTACTC CCCAGGCGGA ATGCTTAATG
601 CGTTAGCTGC AGCACTGAAG GCGGGAACC CTCCAACACT TAGCATTTCAT CGTTTACGGT
661 ATGGACTACC AGGGTATCTA ATCTGTITG CTACCCATAC TTTGAGCCT CAGCGTCAGT
721 TACAGACCAG ACAGCCGCC TCGCCACTGG TGTCTTCCA TATATCTACG CATTTCACCG
781 CTACACATGG AGTTCCACTG TCCTCTTCTG CACTCAAGTT TCCCAGTTTC CGATGCACCT
841 CTTCGGTTGA GCCGAAGGCT TTCACATCAG ACTTAAAAAA CCGCCTGGGC TCGCTTTACG
901 CCCAATAAAT CCGGACAACG CTTGCCACCT ACGTATTACC GCGGCTGCTG GCACGTAGTT
961 AGCCGTGGCT TTCTGGTTAA ATACCGTCAA TACCTGAACA GTTACTCTCA GATATGTTCT
1021 TCTTTAACAA CAGAGTTTTA CGAGCCGAAA CCTTCTTCA CTCACGCGGC GTTGTCCAT
1081 CAGACTTTCC TCATTGTGG AAGATTCCCT ACTGCTGCC TCCGTAGGAG TTTGGGCCGT
1141 GTCTCAGTCC CAATGTGGCC GATTACCCCT TCAGGTGGGC TACGTATCAT TGCCATGGTG
1201 AGCCGTTACC CCACCATCTA GCTAATACGC CGCGGGACCA TCCAAAAGTG ATAGCCGAAG
1261 CCATCTTTCA AACTCGGACC ATGCGGTCCA AGTTGTTATG CCGTATTAGC ATCTGTTTCC
1321 AGGTGTTATC CCCCCTTCT GGGCAGGTTT CCCACGTGTT ACTCACCAGT TCGCCACTCA
1381 CTCAAATGTA AATCATGATG CAAGCACCAA TCAATACCAG AGTTGCTTGG AGTT

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257

258 Figure 4c. The nucleotide sequence S1 isolate

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Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGGCTGGC TCCTAAAAGG TTACCCACCC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGCGGT GTGTACAAGG CCCGGGAACG TAITCACCGC GGCATGCTGA
121    TCCGCGATTA CTAGCGATTG CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCCGAACGT
181    AGAACGGTTT TAAGAGATTT GCTTGCCCTC GCGAGTTCGC GACTCGTTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCAG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACCTTC
301    CTCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCAACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT GACGACGACC
421    ATGCACCACC TGTCAATGCG TTCCCGAAGG AAACGCCCTA TCTCTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTCCGCTAG CTTTCAATTA AACCACATGC TCCACCGGTT
541    GTGCGGGCCC CCGTCAATTC CTTTGAGTTT CAACCTTCGG GTCGTAATCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGGTATCTAA TCCTGTTCCG TACCCATGCT TTCGAGTCTC
721    AGCGTCAGTT GCAGACCAGG TAGCCGCTTT CGCCACTGGT GTTCTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTGC ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTTAAG CCGAAGGCTT TCACATCATA CT
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259

260 Figure 4d. The nucleotide sequence S2

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Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGACG GCGGCTGTGT ACAAGGCCCG GGAACGTATT CACCGCGGCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CGGTTTTAAG AGATTGCTT GCCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCCCC ACCTTCCTCC
301    GGTTTGTAC CGGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGCCTCG TTGCGGGACT TAACCCAACA TCTCAGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GCCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GCGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCCTTT GAGTTTCAAC CTTGCGGTCG TACTCCCAG GCGGAGTGCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTT TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG
841    CACTTCTCC
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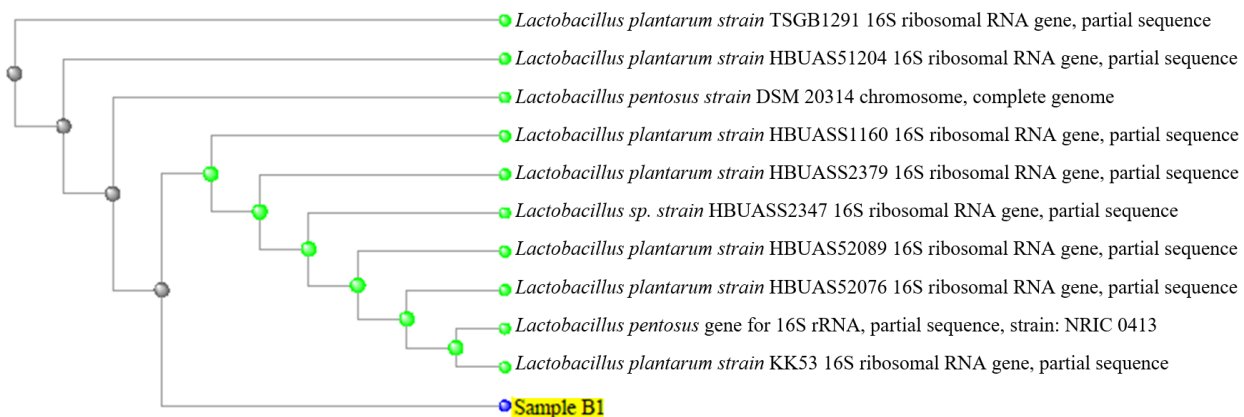
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264 Table 1. Comparison of homology levels the 16S rRNA gene of BAL isolat with several sequences

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

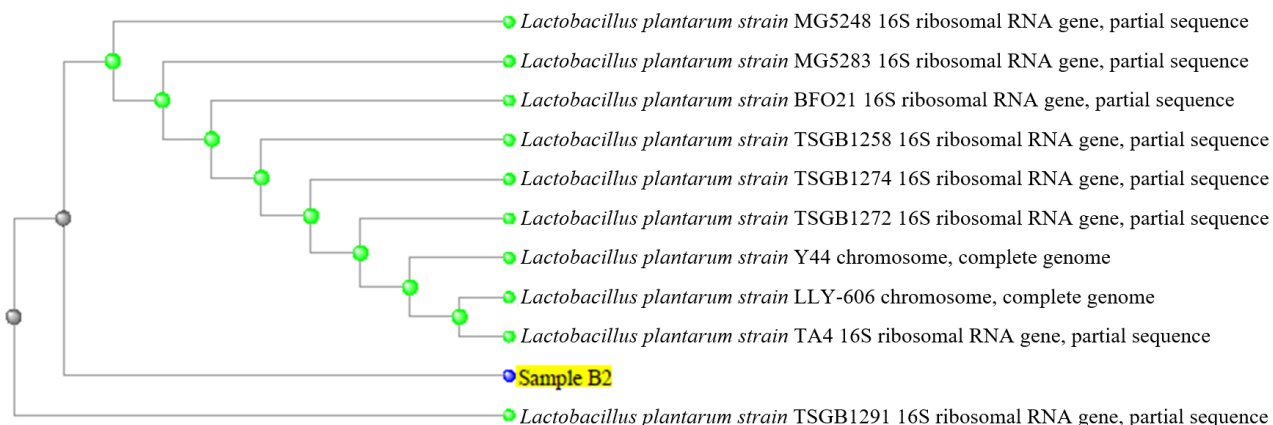
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266 Figure 5a. Phylogenetic tree Isolate Code B1



267

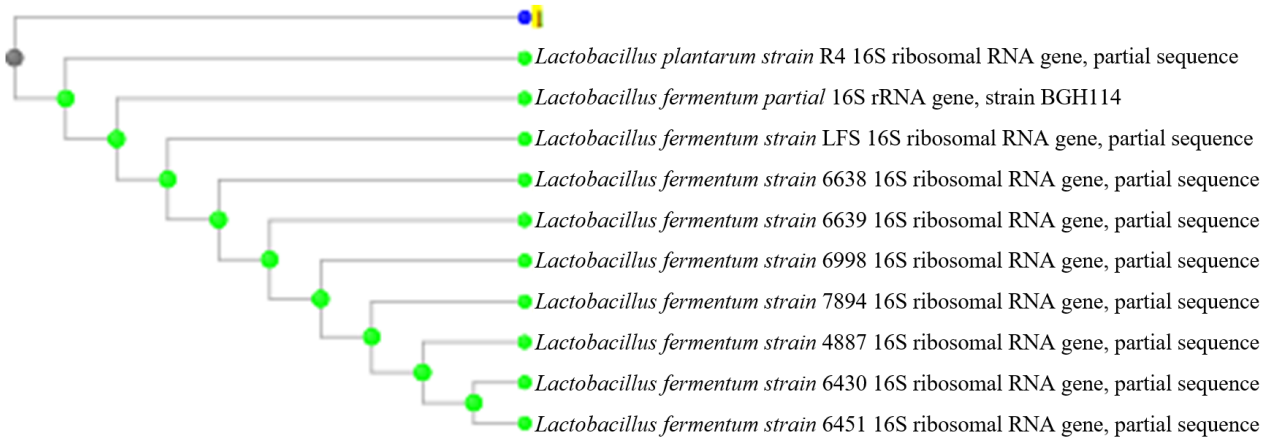
268 Figure 5b. Phylogenetic tree Isolate Code B2



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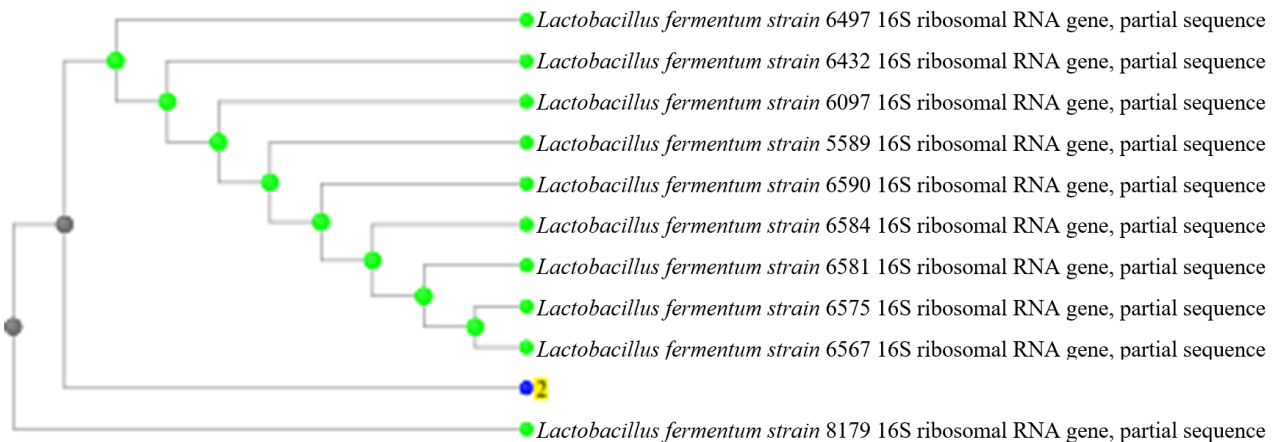


270 Figure 5c. Phylogenetic tree Isolate Code S1



271

272 Figure 5d. Phylogenetic tree Isolate Code S2



273

27<sup>th</sup> 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



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

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17

18 **Abstract**

19 Ethnic food is food inherited from ancestors whose process utilizes local food and distinctive tastes. The

20 Rejang tribe is a native Bengkulu community who processes bamboo shoots into a fermented product

21 known as *Lemea*, which is only found in Bengkulu province. *Lemea* is a source of indigenous lactic acid

22 bacteria (LAB). This study aims to find lactic acid bacteria (LAB) to isolate and identify strains of *Lemea*.

23 The bamboo shoots with betok fish (*Anabas testudineus*) were fermented for 48 h. Different types of

24 bamboo shoots are expected to provide different types of LAB. Betung shoots (*Dendrocalamus asper*

25 *Schult*) and yellow bamboo (*Bambusa vulgaris* Schrad) have been used. The isolation stage begins with 10

26 g of homogenized *Lemea* sample, then 1 mL is taken and 9 mL of sterile 1% NaCl is added. Then serial

27 dilutions were carried out starting from  $10^{-1}$  to  $10^{-7}$  and spread on MRSA media for each dilution series.

28 Incubate at 37 °C for 48 h to obtain isolates. The isolates were identified molecularly using the 16S rRNA

29 method. The results of the study found 4 isolates from 2 types of *Lemea*. After identification, it was known  
30 that the four isolates were bacteria of the genus *Lactobacillus*. *Lactobacillus fermentum* was only found  
31 in the fermentation process of yellow bamboo shoots (*Bambusa vulgaris* Schrad), while *Lactobacillus*  
32 *plantarum* was found in yellow bamboo shoots (*Bambusa vulgaris* Schrad) and betung (*Dendrocalamus*  
33 *asper* Schult).

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

### 35 1. Introduction

36 The Rejang are the third-largest tribe in Bengkulu Province, after the Serawai and Basema. North  
37 Bengkulu, Central Bengkulu, Rejang Lebong, Lebong, and Kepahyang are the five districts where the  
38 Rejang people live. *Lemea* is an ethnic food from the Rejang tribe. Ethnic foods are meals that have  
39 their origins in an ethnic group's history and culture (Kwon, 2015). Bamboo shoots and river fish are  
40 fermented into *Lemea* by the Rejang people (Dewi *et al.*, 2014). Betung, Tabah, Mayan, and Seik  
41 bamboo are some of the most common bamboo varieties used by the Rejang to produce *Lemea* .  
42 Betok, kepala timah, and mujahir fish are the most common fish used. The odour and flavour are  
43 unique, and only the locals enjoy it. LAB that have an impact on the flavour of fermented foods (Fox,  
44 2011). Indigenous fermented foods have been extensively researched.

45 There are various fermented foods in the world, especially in Asia. Fermented foods made from  
46 bamboo shoots from India, Indonesia, and Taiwan are a source of LAB, especially *Lactobacillus* (Kiran  
47 *et al.*, 2016). *Meakri*, from Meghalaya Indian fermented bamboo shoots as a source of *Lactobacillus*,  
48 has characteristics suitable for probiotics (Das *et al.*, 2020). *Lactobacillus* is a group of LAB that are  
49 gram-positive bacteria. LAB have the potential to inhibit the infection and growth of 2 pathogenic  
50 microbes (Yang *et al.*, 2021). LAB isolated from fermented bamboo shoots are potential probiotic  
51 candidates that are beneficial for health (Mohamad *et al.*, 2020). LAB strains are selected for their

52 decreased content of cholesterol, antioxidant activity, and anti-bacterial activity (Jitpakdee *et al.*,  
53 2022). Isolation of lactic acid bacteria from indigenous fermented foods is very important (Mende *et*  
54 *al.*, 2022).

55 *Bekasam* is an Indonesian fermented food that is similar to *Lemea*. *Bekasam* is a traditional  
56 fermented food popular in Sumatera and Kalimantan (Desniar *et al.*, 2013). The sour taste in *Bekasam*  
57 is almost the same as in *Lemea*. The difference between these two products is the carbohydrate  
58 source and fermentation time. Carbohydrate sources are a source of nutrition for bacteria that play  
59 a role in the fermentation process. The source of carbohydrates used for rice in *Lemea* is bamboo  
60 shoots. Fermentation time for *Lemea* is 2-3 days, while *Bekasam* takes 10 days.

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

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

## 70 **2. Materials and methods**

### 71 *2.1 Sample preparation*

72 Bamboo shoot samples were obtained from Lebong Regency. The peeled bamboo shoots are  
73 thinly sliced and soaked for 30 h. Then they were washed, filtered, and weighed as much as 400 g

74 and they added 40 g of betok fish, 350 mL of water, and 3.3 g of lemongrass, and fermented for  
75 48 h (Figure 1).

76

77

## 78 2.2 LAB isolation

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

## 86 2.2 Identification of LAB

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

### 89 2.2.1 DNA Isolation

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

### 92 2.2.2 DNA amplification

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

97           2.2.3 *DNA sequencing and phylogenetic analysis*

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

104   **3. Results and discussion**

105    3.1 *Isolation of lactic acid bacteria from Lemea*

106          Isolation found 4 bacterial isolates from 2 types of *Lemea* samples. *Lemea* made from betung  
107          bamboo shoots found 2 isolates and 2 isolates from yellow bamboo shoots. The isolates found  
108          were coded B1, B2, S1 and S2 (Figure 2). The bacterial isolates found were lactic acid bacteria  
109          because they were able to grow on MRSA specific media with cocci characteristics, a milky white  
110          color with a convex surface and smooth edges. The number of isolates obtained was less than  
111          that of *mesu*, *soidon*, *soibum*, and *soijon* but the same as unfermented bamboo shoots (Tamang  
112          *et al.*, 2008). The morphological characteristics of the isolates found in this study were almost  
113          the same as the previous findings isolated from *Lemea* produced by a cottage industry in  
114          Kepahyang Regency, Bengkulu (Kurnia *et al.*, 2020).

115    3.2 *Identification of Lemea isolates*

116          The results of genomic DNA amplification of the 4 isolates can be seen in Figures 3a and 3b.  
117          Isolates B1, B2 produced 1500 bp amplicons and S1, S2 amplicons with 1400 bp size.  
118          Visualization of PCR results by electrophoresis on 0.8% agarose. Nucleotide sequence at 1434  
119          bp for isolate B1 (Figure 4a), 1424 bp for B2 (Figure 4b). The nucleotide sequence S1 isolate was  
120          882 bp (Figure 4c) and 849 bp for the S2 isolate (Figure 4d). The results of the analysis using the

121 BLAST algorithm on other isolates showed that isolates B1, B2 and S1 have been close to  
122 *Lactobacillus plantarum* while isolates S2 had *Lactobacillus fermentum*.  
123 The 16S rRNA gene sequences of each isolate B1, B2, S1 and S2 have been 99% like the partial  
124 sequences of the comparison isolates (Table 1). The bacteria were found to be a strain of  
125 *Lactobacillus plantarum* but not *Lactobacillus fermentum*. Based on the phylogeny tree, isolate  
126 B1 was closely related to *Lactobacillus plantarum* strain KK53 16S ribosomal RNA (Figure 5a),  
127 Isolate B2 with *Lactobacillus plantarum* strain TA4 and TSGB1291 16S ribosomal RNA (Figure 5b).  
128 S1 isolate was closely related to *Lactobacillus plantarum* strain R4 16S ribosomal RNA (Figure  
129 5c), isolate S2 was closely related to *Lactobacillus fermentum* strain 8179 and 6567 16S  
130 ribosomal RNA (Figure 5d). The type of isolate that was identified from Bekasam was  
131 *lactobacillus plantarum* (Sukmarini *et al.*, 2014). All isolates have been homologous to the genus  
132 *Lactobacillus*. *Bacillus subtilis*, *Lactobacillus brevis*, and *Lactobacillus plantarum* were found in  
133 dominating strains of Soidon fermented bamboo shoot food without salt from Indian Manipur  
134 (Jeyaram *et al.*, 2010). LAB strains in fermented Tabah bamboo shoots (*Gigantochloa nigrociliata*  
135 buse-kurz) have been previously studied and isolated as 2 species, namely *Lactobacillus*  
136 *plantarum* and *Lactobacillus rossiae* (Okfrianti *et al.*, 2019). Lactic acid bacteria isolated from  
137 Indonesian fermented foods are dominated by *Lactobacillus plantarum* (Rahayu, 2003). This  
138 research is expected to provide information on which LAB strains have been isolated from  
139 different types of bamboo shoots. Bamboo Shoot Polysaccharide fermentation increases the  
140 diversity of the bacterial community by increasing the abundance of *Firmicutes*, *Actinobacteria*  
141 and *Proteobacteria* (Li *et al.*, 2021).  
142 *Lactobacillus fermentum* was only found in *Lemeamade* from yellow bamboo shoots (*Bambusa*  
143 *vulgaris* Schrad) and *Lactobacillus plantarum* was found in yellow bamboo shoots (*Bambusa*  
144 *vulgaris* Schrad) and betung (*Dendrocalamus asper* Schult). Prebiotics are contained in

145 foodstuffs that trigger the growth of *Lactobacillus* (Macfarlane and Cummings, 1999).  
146 Oligosaccharides and fiber are prebiotics that promote the growth of specific bacteria found in  
147 the gut. Bamboo shoots are a good source of fibre (Felisberto *et al.*, 2017). At 100 g of fresh  
148 weight, bamboo shoots of *B. vulgaris* contain 6.51 g of carbohydrates, 4.24 g of fibre, 4.90 g of  
149 *D. asper*, and 3.54 g of fibre (Chongtham *et al.*, 2011). Different bamboo species contain  
150 different macronutrients (Adebola *et al.*, 2014). Differences in the content of bamboo shoots  
151 affect the types of bacteria found in *Lemea* products.

#### 152 4. Conclusion

153 Four *Lactobacillus* isolates found in *Lemea* were from the *Lactobacillus* genus. *Lactobacillus*  
154 *plantarum* and *Lactobacillus fermentum* were isolates which were identified molecularly by 16S  
155 rRNA. *Lactobacillus fermentum* was only found in the fermentation process of yellow bamboo shoots  
156 (*Bambusa vulgaris Schrad*), while *Lactobacillus plantarum* was found in yellow bamboo shoots  
157 (*Bambusa vulgaris Schrad*) and betung (*Dendrocalamus asper Schult*).

#### 158 Conflict of interest - Disclose potential conflicts of interest appropriately.

159 The authors declare no conflict of interest.

#### 160 Acknowledgments

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162

163

164 **References**

- 165 Adebola, O.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin,  
166 lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of*  
167 *Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- 168 Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots:  
169 Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and*  
170 *Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- 171 Das, S., Mishra, B.K., and Hati, S. (2020). Techno-functional characterization of indigenous Lactobacillus  
172 isolates from the traditional fermented foods of Meghalaya, India. *Current Research in Food*  
173 *Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>
- 174 Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria  
175 isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against  
176 pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494.  
177 <https://doi.org/10.9755/ejfa.v25i6.12478>
- 178 Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di  
179 Provinsi Bengkulu Industry Mapping of " Lemea " Rejang Traditional Food in Bengkulu Province.  
180 *AGRISEP*, 14(1), 61–69. <https://doi.org/https://doi.org/10.31186/jagrisep.13.1.60-66>
- 181 Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential  
182 food as source of fiber and starch. *Food Research International*, 101(July), 96–102.  
183 <https://doi.org/10.1016/j.foodres.2017.08.058>
- 184 Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. *Encyclopedia of Dairy Sciences:*  
185 *Second Edition*, 70(1996), 401–402. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>
- 186 Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and Devi, S.S. (2010). Bacterial species associated with  
187 traditional starter cultures used for fermented bamboo shoot production in Manipur state of India.  
188 *International Journal of Food Microbiology*, 143(1–2), 1–8.  
189 <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- 190 Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce  
191 functional fermented whey beverage with putative health promoting attributes. *Lwt*, 160, 113269.



192 <https://doi.org/10.1016/j.lwt.2022.113269>

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

196 Kurnia, M., Amir, H., and Handayani, D. (2020). Isolasi Dan Identifikasi Bakteri Asam Laktat Dari Makanan  
197 Tradisional Suku Rejang Di Provinsi Bengkulu: “Lemea.” *Alotrop*, 4(1), 25–32.  
198 <https://doi.org/10.33369/atp.v4i1.13705>

199 Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1.  
200 <https://doi.org/10.1016/j.jef.2015.02.001>

201 Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). In vitro fecal fermentation  
202 characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11. 1–  
203 9.<https://doi.org/10.1016/j.fochx.2021.100129>

204 Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of  
205 intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.

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

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

212 Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional  
213 Fermented Rejang Shoot “Lemea.” *1st International Conference on Inter-Professional Health*  
214 *Collaboration (ICIHC 2018) Identification*, 14(Icihc 2018), 237–240. [https://doi.org/10.2991/icihc-](https://doi.org/10.2991/icihc-2019.52)  
215 [18.2019.52](https://doi.org/10.2991/icihc-2019.52)

216 Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84.  
217 <https://doi.org/10.22146/agritech.13515>

218 Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-

219 Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of*  
220 *Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>

221 Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M., and Holzapfel, W.H. (2008).  
222 Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented  
223 bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–  
224 40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>

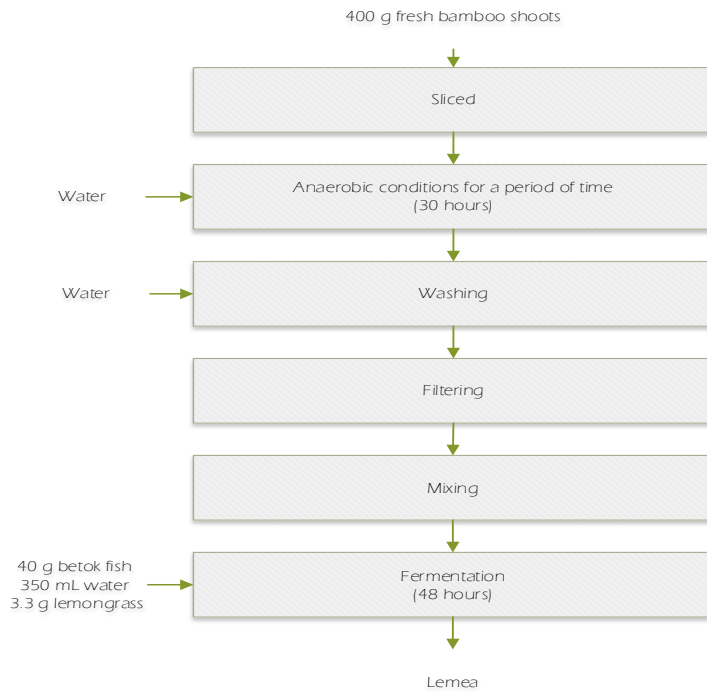
225 Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and  
226 Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlata cheese.  
227 *Journal of Applied Microbiology*, 103(6), 2142–2152. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2672.2007.03450.x)  
228 [2672.2007.03450.x](https://doi.org/10.1111/j.1365-2672.2007.03450.x)

229 Wasis, N.O., Semadi Antara, N., and Wayan Gunam, I.B. (2019). Studi Viabilitas Isolat Bakteri Asam Laktat  
230 yang Diisolasi dari Asinan Rebung Bambu Tabah Terhadap pH Rendah dan Garam Empedu. *Jurnal*  
231 *Rekayasa Dan Manajemen Agroindustri*, 7(1), 1–10.  
232 <https://doi.org/10.24843/jrma.2019.v07.i01.p01>

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

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Figure 1. Fermented bamboo shoot making process

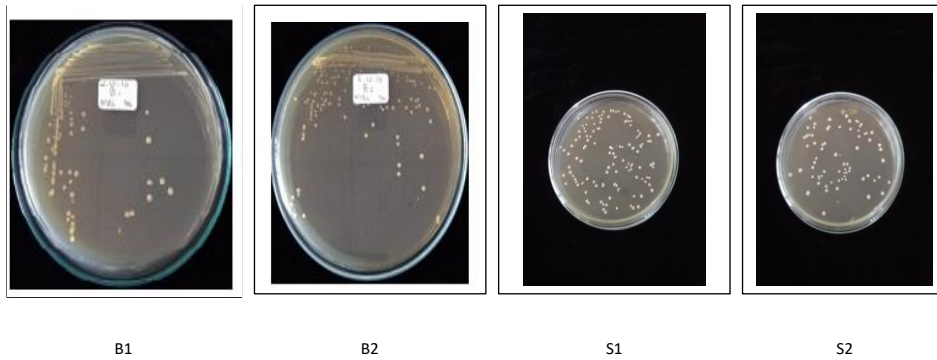


**Commented [A1]:** Serai 3.3 g (comma change) ml=mL

**Commented [A2R1]:** Is Serai = Lemon grass?

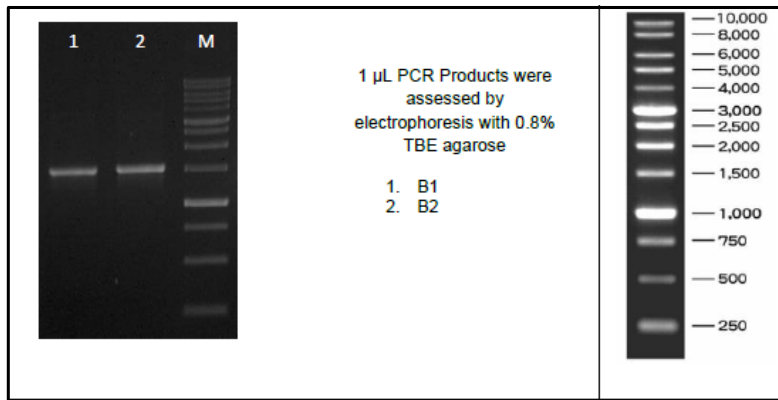
**Commented [A3R1]:** It has been fixed as suggested  
350 mL water  
3.3 lemongrass

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



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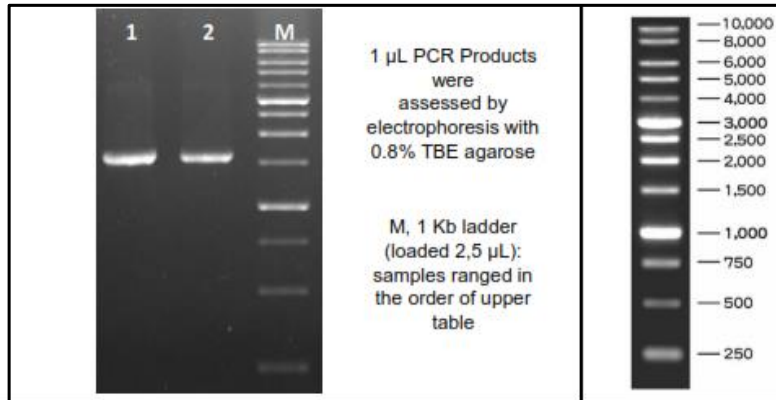
262 Figure 3a. Genomic DNA amplification of isolates B1 and B2



263

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

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



267

268 Visualization of 16 S rRNA gene amplification of isolates S1 and S2 at 0.8% TBE agarose concentration

269 using the Kappa Universal ladder

270 Figure 4a. The nucleotide sequence B1 isolate

```
Sequence Assembly 1434 bp
1   AGGGGGCTGG  TTCTAAAAG  GTTACCCAC  CGACTTTGGG  TGTACAAAC  TCTCATGGTG
61  TGACGGGGCG  TGTGTACAAG  GCCCGGGAAC  GTATTACCCG  CGGCATGCTG  ATCCGGGATT
121  ACTAGCGGATT  CCGACTTCAT  GTAGGCGAGT  TGCAGCCTAC  AATCCGAACT  GAGAAATGGCT
181  TTAAGAGATT  AGCTTGTCT  CGCGAGTTCC  CAACTCGTTG  TACCATCCAT  TGTAGCACGT
241  GTTAGGCCCA  GGTCTAAAGG  GGCATGATGA  TTTGACGTCA  TCCCACCTT  CCTCCGGTTT
301  GTACCCGGCA  GTCTACCAG  AGTCCCAAC  TTAATGCTGG  CAACTGATAA  TAAGGGTTGC
361  GCTCGTTGCG  GACTTAAACC  CAACATCTCA  CGACACGAGC  TGACGACAAC  CATGCCACC
421  CTGTATCCAT  GTCCCCGAAG  GGAACGTCTA  ATCTCTTAGA  TTTGCATAGT  ATGTCAAGAC
481  CTGGTAAGGT  TCTTCGGTA  GCTTCGAATT  AAACCATG  CTCCACCGCT  TGTGCGGGCC
541  CCGTCAATT  CCTTTGAGTT  TCAGCCTTGC  GGCCTACTC  CCCAGGCGGA  ATGCTTAATG
601  CGTTAGCTGC  AGCACTGAAG  GCGGGAACC  CTCCACACT  TAGCATTCA  CGTTTACGGT
661  ATGGACTACC  AGGGTATCTA  ATCCTGTTTG  CTACCCATAC  TTTGAGCCT  CAGCGTCAGT
721  TACAGACCAG  ACAGCCGCC  TCGCCACTGG  TGTCTTCCA  TATACTACG  CATTTCACCG
781  CTACACATGG  AGTTCACCTG  TCTCTTCTG  CACTCAAGTT  TCCCAGTTTC  CGATGCACIT
841  CTTCGGTTGA  GCGGAAGGCT  TTCACATCAG  ACTTAAAAA  CCGCCTGGCC  TCGCTTACG
901  CCCAATAAAT  CCGGACAACG  CTTGCCACT  ACGTATTACC  GCGGCTGCTG  GCACGTAGTT
961  AGCCGTGGCT  TTCTGGTTAA  ATACCGTCAA  TACCTGAACA  GTTACTCTCA  GATATGTTCT
1021  TCTTAAACA  CAGAGTTTTA  CGAGCCGAAA  CCTTCTTCA  CTCACGCGCC  GTTGTCCAT
1081  CAGACTTTCG  TCCATTGTGG  AAGATTCCCT  ACTGCTGCC  CCGTAGGAG  TTTGGGCCGT
1141  GTCTCAGTCC  CAATGTGGCC  GATTAACCTC  TCAGGTCGGC  TACGTATCAT  TGCCATGGTG
1201  AGCCGTACC  CCACCATCTA  GCTAATACGC  CCGGGACCA  TCCAAAAGTG  ATAGCCGAAG
1261  CCATCTTCA  AACTCGGACC  ATGGGTCCA  AGTTGTTATG  CGGTATTAGC  ATCTGTTTCC
1321  AGGTGTTATC  CCCCCTTCT  GGGCAGGTTT  CCCCAGTGT  ACTCACCACT  TCGCCACTCA
1381  CTCAAATGTA  AATCATGATG  CAAGCACCAA  TCATACCAG  AGTTGTTTCC  ACTT
```

271

272 Figure 4b. The nucleotide sequence B2 isolate

```
Sequence Assembly 1424 bp
1   AGGGGGCTGG  TTCTAAAAG  GTTACCCAC  CGACTTTGGG  TGTACAAAC  TCTCATGGTG
61  TGACGGGGCG  TGTGTACAAG  GCCCGGGAAC  GTATTACCCG  CGGCATGCTG  ATCCGGGATT
121  ACTAGCGGATT  CCGACTTCAT  GTAGGCGAGT  TGCAGCCTAC  AATCCGAACT  GAGAAATGGCT
181  TTAAGAGATT  AGCTTGTCT  CGCGAGTTCC  CAACTCGTTG  TACCATCCAT  TGTAGCACGT
241  GTTAGGCCCA  GGTCTAAAGG  GGCATGATGA  TTTGACGTCA  TCCCACCTT  CCTCCGGTTT
301  GTACCCGGCA  GTCTACCAG  AGTCCCAAC  TTAATGCTGG  CAACTGATAA  TAAGGGTTGC
361  GCTCGTTGCG  GACTTAAACC  CAACATCTCA  CGACACGAGC  TGACGACAAC  CATGCCACC
421  CTGTATCCAT  GTCCCCGAAG  GGAACGTCTA  ATCTCTTAGA  TTTGCATAGT  ATGTCAAGAC
481  CTGGTAAGGT  TCTTCGGTA  GCTTCGAATT  AAACCATG  CTCCACCGCT  TGTGCGGGCC
541  CCGTCAATT  CCTTTGAGTT  TCAGCCTTGC  GGCCTACTC  CCCAGGCGGA  ATGCTTAATG
601  CGTTAGCTGC  AGCACTGAAG  GCGGGAACC  CTCCACACT  TAGCATTCA  CGTTTACGGT
661  ATGGACTACC  AGGGTATCTA  ATCCTGTTTG  CTACCCATAC  TTTGAGCCT  CAGCGTCAGT
721  TACAGACCAG  ACAGCCGCC  TCGCCACTGG  TGTCTTCCA  TATACTACG  CATTTCACCG
781  CTACACATGG  AGTTCACCTG  TCTCTTCTG  CACTCAAGTT  TCCCAGTTTC  CGATGCACIT
841  CTTCGGTTGA  GCGGAAGGCT  TTCACATCAG  ACTTAAAAA  CCGCCTGGCC  TCGCTTACG
901  CCCAATAAAT  CCGGACAACG  CTTGCCACT  ACGTATTACC  GCGGCTGCTG  GCACGTAGTT
961  AGCCGTGGCT  TTCTGGTTAA  ATACCGTCAA  TACCTGAACA  GTTACTCTCA  GATATGTTCT
1021  TCTTAAACA  CAGAGTTTTA  CGAGCCGAAA  CCTTCTTCA  CTCACGCGCC  GTTGTCCAT
1081  CAGACTTTCG  TCCATTGTGG  AAGATTCCCT  ACTGCTGCC  CCGTAGGAG  TTTGGGCCGT
1141  GTCTCAGTCC  CAATGTGGCC  GATTAACCTC  TCAGGTCGGC  TACGTATCAT  TGCCATGGTG
1201  AGCCGTACC  CCACCATCTA  GCTAATACGC  CCGGGACCA  TCCAAAAGTG  ATAGCCGAAG
1261  CCATCTTCA  AACTCGGACC  ATGGGTCCA  AGTTGTTATG  CGGTATTAGC  ATCTGTTTCC
1321  AGGTGTTATC  CCCCCTTCT  GGGCAGGTTT  CCCCAGTGT  ACTCACCACT  TCGCCACTCA
1381  CTCAAATGTA  AATCATGATG  CAAGCACCAA  TCATACCAG  AGTTGTTTCC  AGTT
```

273

274

275 Figure 4c. The nucleotide sequence S1 isolate

```
Sequence Result Reverse Primer 882bp
1      GTCCACCTTA GCGCGCTGGC TCCTAAAAGG TTACCCCACC GACTTTGGGT GTTACAAACT
61     CTCATGGTGT GACGGGGGGT GTGTACAAGG CCCGGGAACG TATTCACCGC GGCATGCTGA
121    TCCGGGATTA CTAGCGATTG CGACTTCGTG CAGGCGAGTT GCAGCCTGCA GTCGGAACG
181    AGAACGGTTT TAAGAGATTG GCTTGCCTC GCGAGTTCCG GACTCGTGT ACCGTCCATT
241    GTAGCACGTG TGTAGCCCGG GTCATAAGGG GCATGATGAT CTGACGTCGT CCCCACTTC
301    CTCGGTTTG TCACCGGCAG TCTCACTAGA GTGCCCACT TAATGCTGGC AACTAGTAAC
361    AAGGGTTGGC CTCGTTGGG GACTTAAACC AACATCTCAC GACACGAGCT GACGAGGACC
421    ATGCACCACC TGTCAATTGG TTCCCGAAGG AAAGCCCTA TCTTAGGGT TGGCGCAAGA
481    TGTCAAGACC TGGTAAGGTT CTTGGCGTAG CTTCAATTA AACACATGC TCCACGGCTT
541    GTGCGGGCCC CGGTCAATC CTTGAGTTT CAACCTTGGG GTGCTACTCC CCAGGCGGAG
601    TGCTTAATGC GTTAGCTCCG GCACTGAAGG GCGGAAACCC TCCAACACCT AGCACTCATC
661    GTTTACGGCA TGGACTACCA GGTATCTAA TCCTGTTCCG TACCATGCT TTCGAGTCTC
721    AGGTCAGTTC GCAGACCAGG TAGCCGCTT CGCCACTGGT GTTCTCCAT ATATCTACGC
781    ATTCCACCGC TACACATGGA GTTCCACTAC CCTCTTCTG ACTCAAGTTA TCCAGTTTCC
841    GATGCACTTC TCCGGTAAAG CCGAAGGCTT TCACATCATA CT
```

276

277 Figure 4d. The nucleotide sequence S2

```
Sequence Result Reverse Primer 849bp
1      ACCTTAGGCG GCTGGCTCCT AAAAGGTTAC CCCACCGACT TTGGGTGTTA CAAACTCTCA
61     TGGTGTGAAG GCGGGTGTGT ACAAGGCCCG GGAACGTATT CACCGGGCCA TGCTGATCCG
121    CGATTACTAG CGATTCCGAC TTCGTGCAGG CGAGTTGCAG CCTGCAGTCC GAACTGAGAA
181    CCGTTTTAAG AGATTGGCTT GGCCTCGCGA GTTCGCGACT CGTTGTACCG TCCATTGTAG
241    CACGTGTGTA GCCCAGGTCA TAAGGGGCAT GATGATCTGA CGTCGTCACC ACCTTCTCTC
301    GGTTTGTAC CCGCAGTCTC ACTAGAGTGC CCAACTTAAT GCTGGCAACT AGTAACAAGG
361    GTTGGCGCTG TTGCGGGACT TAACCCAACA TCTCAGGACA CGAGCTGACG ACGACCATGC
421    ACCACCTGTC TTTGCGTTCC CGAAGGAAAC GGCCTATCTC TAGGGTTGGC GCAAGATGTC
481    AAGACCTGGT AAGGTTCTTC GGTAGCTTC GAATTAACC ACATGCTCCA CCGCTTGTGC
541    GGGCCCCCGT CAATTCTTTT GAGTTTCAAC CTTGCGGTGC TACTCCCAG GCGGAGTGTCT
601    TAATGCGTTA GCTCCGGCAC TGAAGGGCGG AAACCTTCCA ACACCTAGCA CTCATCGTTT
661    ACGGCATGGA CTACCAGGGT ATCTAATCCT GTTCGCTACC CATGCTTTCG AGTCTCAGCG
721    TCAGTTGCAG ACCAGGTAGC CGCCTTCGCC ACTGGTGTTC TTCCATATAT CTACGCATTC
781    CACCGCTACA CATGGAGTTC CACTACCCCT TCTGCACTC AAGTTATCCA GTTTCGATG
841    CACTTCTCC
```

278

279

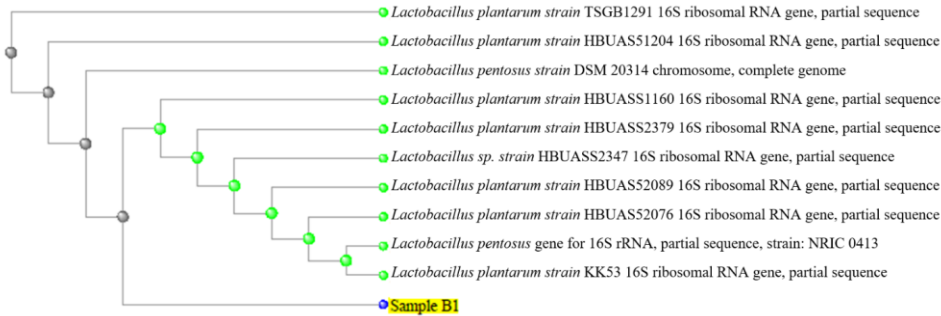
280

281 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	<i>Lactobacillus plantarum</i>	M N37236.01	99
	B2	<i>Lactobacillus plantarum</i>	MN972325.1	99
Yellow Bamboo	S1	<i>Lactobacillus plantarum</i>	KM350169.1	99
	S2	<i>Lactobacillus fermentum</i>	MT538927.1	99

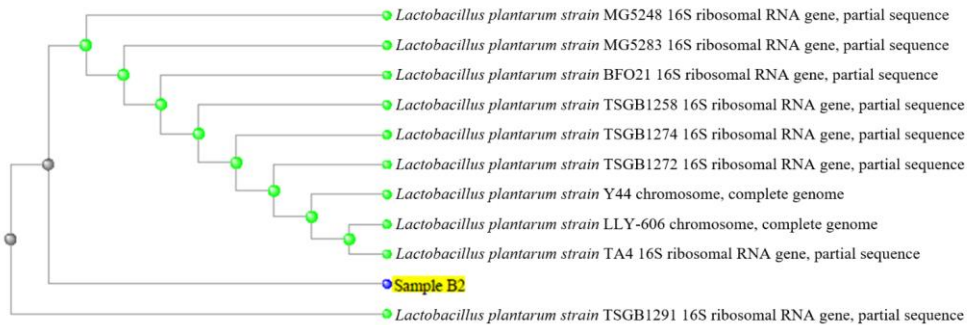
282

283 Figure 5a. Phylogenetic tree Isolate Code B1



284

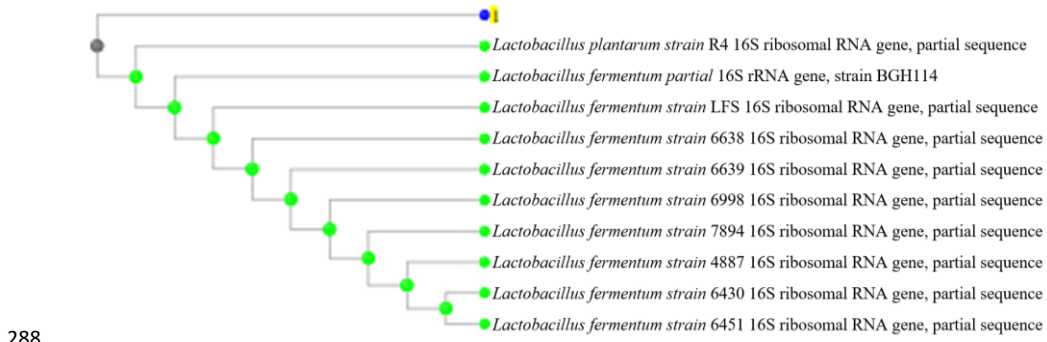
285 Figure 5b. Phylogenetic tree Isolate Code B2



286

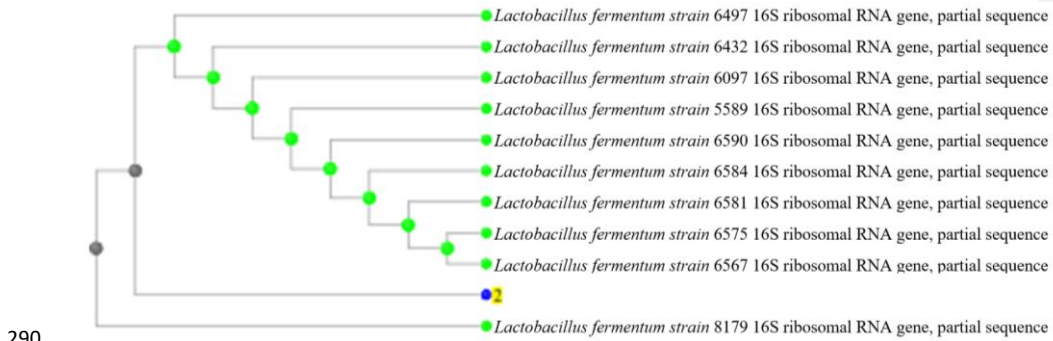


287 Figure 5c. Phylogenetic tree Isolate Code S1



288

289 Figure 5d. Phylogenetic tree Isolate Code S2



290



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Manuscript Title: Identification of LAB isolated from ethnic fermented bamboo shoot "Lemea" in Bengkulu, Indonesia

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

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### Abstract

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

## 1. Introduction

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

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

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

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

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

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

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

## 2. Materials and methods

### 2.1 Sample preparation

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

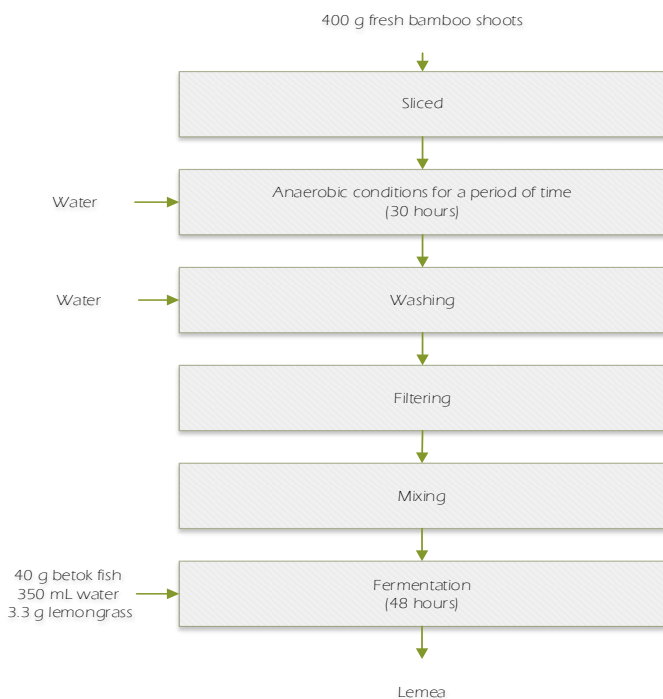


Figure 1. Fermented bamboo shoots making process

### 2.2 Lactic acid bacteria isolation

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

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

### 2.3 Molecular identification of lactic acid bacteria

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

#### 2.3.1 Deoxyribonucleic acid isolation

DNA isolation was done using the Genomic DNA extraction with Presta 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 Zymoclean™ Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1 µL of the PCR product was assessed with 0.8% TBE agarose.

#### 2.3.3 Deoxyribonucleic acid sequencing and phylogenetic analysis

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

## 3. Results and discussion

### 3.1 Isolation of lactic acid bacteria from Lemea

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



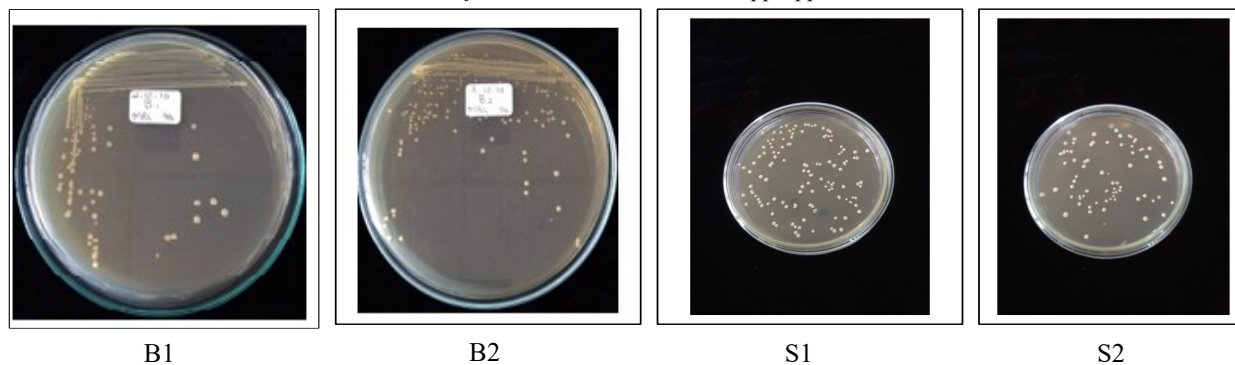


Figure 2. Isolate B1, B2, S1 and S2.

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

### 3.2 Identification of lemea isolates

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

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

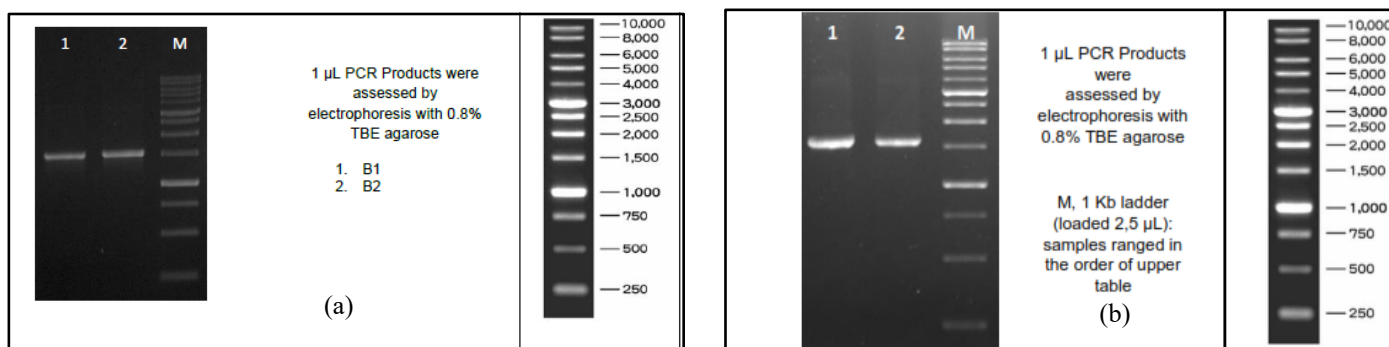


Figure 3. Genomic 16S rRNA amplification of (a) isolates B1 and B2 and (b) isolates S1 and S2. M: Kappa universal ladder.

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

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



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

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

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

#### 4. Conclusion

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

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

#### Conflict of interest

The authors declare no conflict of interest.

#### Acknowledgments

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#### References

- Adebola, A.O., Corcoran, O. and Morgan, W.A. (2014). Synbiotics: The impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of Functional Foods*, 10, 75–84. <https://doi.org/10.1016/j.jff.2014.05.010>
- Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and Food Safety*, 10(3), 153–168. <https://doi.org/10.1111/j.1541-4337.2011.00147.x>
- Das, S., Mishra, B.K. and Hati, S. (2020). Techno-functional characterization of indigenous *Lactobacillus* isolates from the traditional fermented



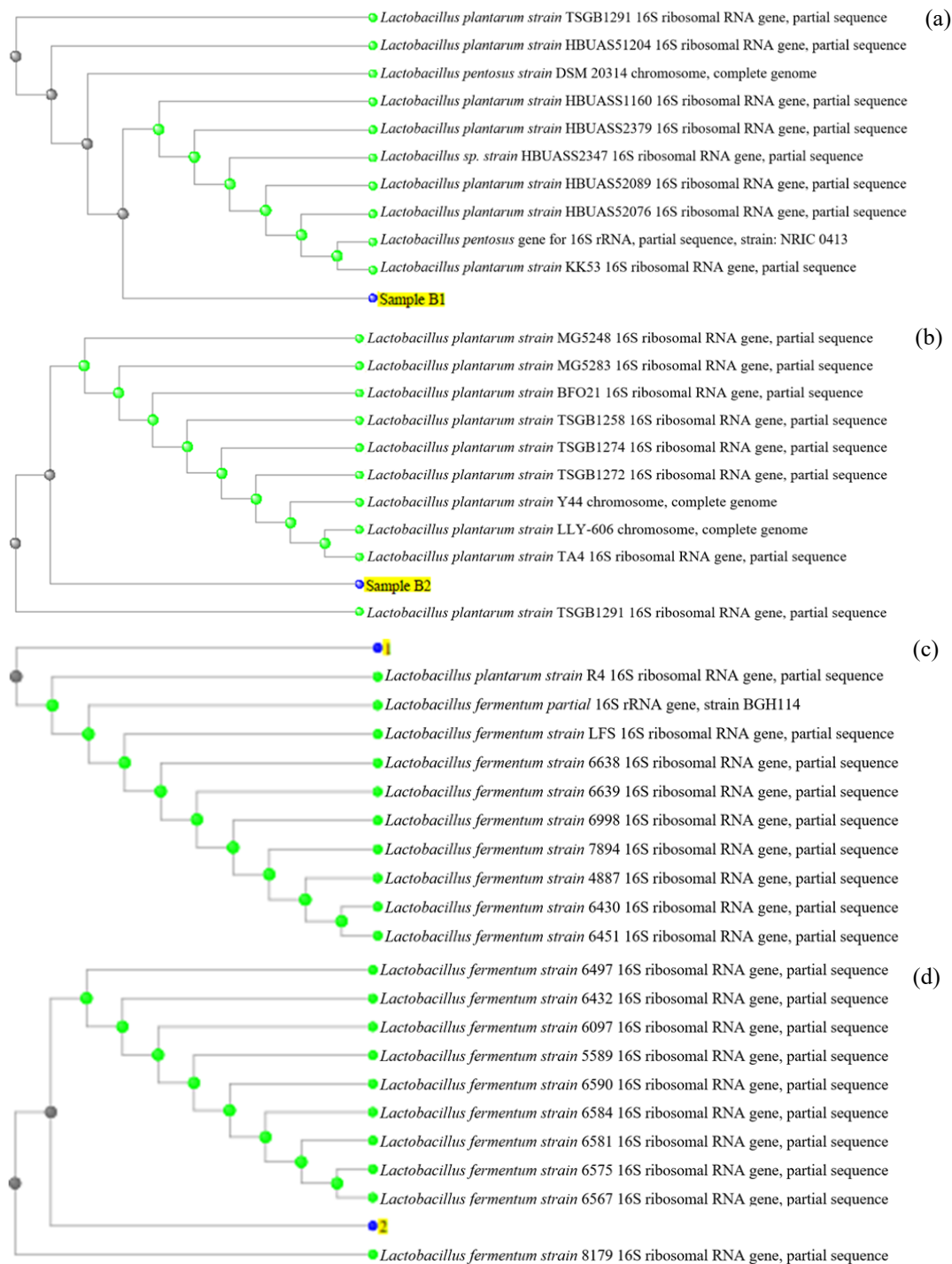


Figure 5. Phylogenetic tree of (a) B1, (b) B2, (c) S1 and (d) S2 isolates

foods of Meghalaya, India. *Current Research in Food Science*, 3, 9–18. <https://doi.org/10.1016/j.crfs.2020.01.002>

Desniar, Rusmana, I., Suwanto, A. and Mubarik, D.N.R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489–494. <https://doi.org/10.9755/efja.v25i6.12478>

Dewi, K.H., Silsia, D., Susanti, L., Teknologi, J., Fakultas, P. and Universitas, P. (2014). Suku Rejang di Provinsi Bengkulu Industry Mapping of "Lemea" Rejang Traditional Food in Bengkulu Province.

*AGRISEP*, 14(1), 61–69. <https://doi.org/https://doi.org/10.31186/jagriseip.13.1.60-66> [In Bahasa Indonesia].

Felisberto, M.H.F., Miyake, P.S.E., Beraldo, A.L. and Clerici, M.T.P.S. (2017). Young bamboo culm: Potential food as source of fiber and starch. *Food Research International*, 101, 96–102. <https://doi.org/10.1016/j.foodres.2017.08.058>

Fox, P.F. (2011). Bacteria, Beneficial: Lactic Acid Bacteria: An Overview. In Fuquay, J.W. (Ed.) *Encyclopedia of Dairy Sciences*. 2<sup>nd</sup> ed., p. 401–402. Elsevier E-Book. <https://doi.org/10.1016/B978-0-12-374407-4.00046-7>

Jeyaram, K., Romi, W., Singh, T.A., Devi, A.R. and

- Devi, S.S. (2010). Bacterial species associated with traditional starter cultures used for fermented bamboo shoot production in Manipur state of India. *International Journal of Food Microbiology*, 143(1–2), 1–8. <https://doi.org/10.1016/j.ijfoodmicro.2010.07.008>
- Jitpakdee, J., Kantachote, D., Kanzaki, H. and Nitoda, T. (2022). Potential of lactic acid bacteria to produce functional fermented whey beverage with putative health promoting attributes. *LWT*, 160, 113269. <https://doi.org/10.1016/j.lwt.2022.113269>
- Kiran, T., Rajani, C., Kumar, T.S. and Achun, P. (2016). Fermented Bamboo Shoots: A Riche Niche for Beneficial Microbes. *Journal of Bacteriology and Mycology*, 2(4), 87–93. <https://doi.org/10.15406/jbmoa.2016.02.00030>
- 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> [In Bahasa Indonesia]
- Kwon, D.Y. (2015). What is ethnic food? *Journal of Ethnic Foods*, 2(1), 1. <https://doi.org/10.1016/j.jef.2015.02.001>
- Li, Q., Wu, W., Chen, H., Fang, X., Han, Y., Xie, M. and Gao, H. (2021). *In vitro* fecal fermentation characteristics of bamboo shoot (*Phyllostachys edulis*) polysaccharide. *Food Chemistry X*, 11, 100129. <https://doi.org/10.1016/j.fochx.2021.100129>
- Macfarlane, G.T. and Cummings, J.H. (1999). Probiotics and prebiotics: Can regulating the activities of intestinal bacteria benefit health? *Western Journal of Medicine*, 171(3), 187–191.
- Mende, S., Rohm, H. and Aros, D. (2022). Lactic Acid Bacteria: Exopolysaccharides. In McSweeney, P.L.H. and McNamara, J.P. (Eds.) *Encyclopedia of Dairy Sciences*. Vol. 4, 3<sup>rd</sup> ed. Elsevier E-Book. <https://doi.org/10.1016/b978-0-08-100596-5.22982-x>
- Mohamad, N., Manan, H., Sallehuddin, 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, 100523. <https://doi.org/10.1016/j.aqrep.2020.100523>
- Okfrianti, Y., Darwis and Pravita Sari, A. (2019). Identification of Lactic Acid Bacteria in Traditional Fermented Rejang Shoot “Lemea”. *Advances in Health Sciences Research*, 14, 237–240. <https://doi.org/10.2991/icihc-18.2019.52>
- Rahayu, E.S. (2003). Lactic Acid bacteria in Fermented Foods of Indonesian Origin. *Agritech*, 23(2), 75–84. <https://doi.org/10.22146/agritech.13515>
- Sukmarini, L., Mustopa, A.Z., Normawati, M. and Muzdhalifa, I. (2014). Identification of Antibiotic-Resistance Genes from Lactic Acid Bacteria in Indonesian Fermented Foods. *HAYATI Journal of Biosciences*, 21(3), 144–150. <https://doi.org/10.4308/hjb.21.3.144>
- Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M. and Holzapfel, W.H. (2008). Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 121(1), 35–40. <https://doi.org/10.1016/j.ijfoodmicro.2007.10.009>
- Veljovic, K., Terzic-Vidojevic, A., Vukasinovic, M., Strahinic, I., Begovic, J., Lozo, J., Ostojic, M. and Topisirovic, L. (2007). Preliminary characterization of lactic acid bacteria isolated from Zlatar cheese. *Journal of Applied Microbiology*, 103(6), 2142–2152. <https://doi.org/10.1111/j.1365-2672.2007.03450.x>
- 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> [In Bahasa Indonesia].
- 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>