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

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Abstract

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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).

Meakri, from Meghalaya Indian fermented bamboo

shoots as a source of Lactobacillus, has characteristics

suitable for probiotics (Das et al., 2020). Lactobacillus is

a group of LAB that are gram-positive bacteria. LAB

have the potential to inhibit the infection and growth of 2

pathogenic microbes (Yang et al., 2021). LAB isolated

from fermented bamboo shoots are potential probiotic

candidates that are beneficial for health (Mohamad et al.,

2020). LAB strains are selected for their decreased

content of cholesterol, antioxidant activity, and anti-

bacterial activity (Jitpakdee et al., 2022). Isolation of

lactic acid bacteria from indegenous fermented foods is

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

Carbohydrate sources are a source of nutrition for

bacteria that play a role in the fermentation process. The

Bekasam is an Indonesian fermented food that is

carbohydrate source and fermentation time.

very important (Mende et al., 2022).

the

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).

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

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

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

2. Materials and methods

2.1 Sample preparation

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

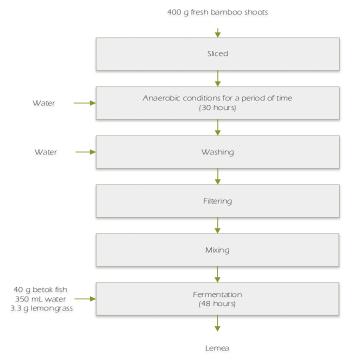


Figure 1. Fermented bamboo shoots making process

2.2 Lactic acid bacteria isolation

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

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

2.3 Molecular identification of lactic acid bacteria

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

2.3.1 Deoxyribonucleic acid isolation

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

2.3.2 Deoxyribonucleic acid amplification

DNA amplification 16S rDNA sequence amplification was performed using MyTaqHS Red Mix (Bioline, BIO-25047). PCR Products were purified with ZymocleanTM Ge; DNA Recovery Kit (Zymo Research, D4001). The PCR results were visualized by electrophoresis as much as 1 μ L 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

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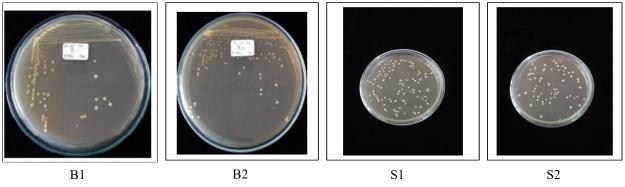


Figure 2. Isolate B1, B2, S1 and S2.

B1, B2, S1 and S2 (Figure 2). The bacterial isolates found were lactic acid bacteria because they were able to on MRSA specific media with grow 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

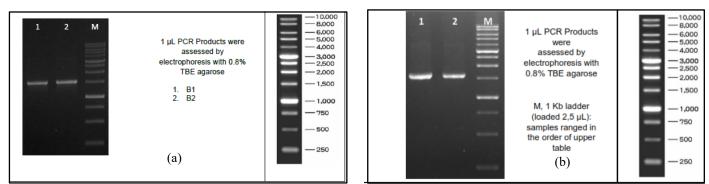


Figure 3. Genomic 16S rRNA amplification of (a) isolates B1 and B2 and (b) isolates S1 and S2. M: Kappa universal ladder.

Table 1. Comparison of homology levels of the 16S	<i>rRNA</i> gene of LAB isolates with several sequences.
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Types of Bamboo Shoots	Isolate Code	Species	GenBank Accession Number	Similarity (%)
Bamboo Betung	B1	Lactobacillus plantarum	MN37236.01	99
	B2	Lactobacillus plantarum	MN972325.1	99
Yellow Bamboo	S 1	Lactobacillus plantarum	KM350169.1	99
	S2	Lactobacillus fermentum	MT538927.1	99

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1 61						TGTTACAAAC CGGCATGCTG	
121						AATCCGAACT	
181						TACCATCCAT	
241	GTGTAGCCCA						
301	GTCACCGGCA						
361	GCTCGTTGCG						
421 481	CIGIAICCAT					CTCCACCGCT	
541	CCCGTCAATT						
601	CGTTAGCTGC					TAGCATTCAT	
661						TTTCGAGCCT	
721 781	TACAGACCAG						
781 841	CTACACATGG CTTCGGTTGA						CGATGCACTT
901	CCCAATAAAT						
961						GTTACTCTCA	
	TCTTTAACAA						
	CAGACTITCG						
	GTCTCAGTCC AGCCGTTACC						TGCCATGGTG
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					ICARIACCAS	AGTICGTICG	(c)
Sequ	ence Result R	everse Pr	imer 8	82bp			(c)
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721							TATATCTACG	
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Sequ 1 61 121 181 241 301 361	ence Result ACCTTA ACCTTA ACCTTA TGGTGT CGATTA CGGTTT CACGTG GTTGCG	Reve GGCG GACG CTAG IAAG IGTA ICAC CTCG	ACCOUNT OF THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT OF THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT OF THE CONTRACT. THE CONTRACT OF THE CONTRACT OF	CT AAAA GT ACAA AC TTCG TT GCCC CA TAAG TC ACTA CT TAAC	GGTTAC GGCTAC GGCCCG TGCAGG TCGCGA GGGCAT GAGTGC CCAACA	CCCACCGAC CCCACCGAC GCAACGTAT GCGACGTGCAC GTCGCGAC GATGATCTG CCAACTTAN A TCTCACGACJ	ACTOROCAGT AGIT TIGGGTGTTA CACCGCGGGA CCTGCAGTCC CCTGCAGTCC CGTGGCAACT A CGAGCTGACG	CAAACTCT TGCTGATCI GAACTGATCI GAACTGAG TCCATTGTJ ACCTTCCTI AGTAACAAI ACGACCATU
Sequ 1 61 121 181 241 301 361 421	ence Result ACCTTA ACCTTA ACCTTA TGGTGT CACCTC GGTTT GGTTTG GTTGCG ACCACC	Reve GGCG GACG CTAG CTAG CTAG CTAG CTAG CTCG CTC	SCHETTET CATGATG SCHEGETG GCCGGCTG GCCGGCTG GCCAGGT CGATTCCC GCCCAGGT CGCCAGGT TTGCCGGA TTTGCCGGA	er 849bp CT AAAA GT ACAA GT ACAA CT TCG TT GCCC CA TAAG TC ACTA CT TAAC CC CGAA	GGTTAC GGTTAC GGCCCG TGCAGG TCGCGA GGGCAT GAGTGC CCAACA GGAAAC	CCCACCGAC CCCACCGAC GGACGTAC GGACGTTGCA GTTGCGCAC CCCACCTAC CCCACCTAC CCCACCTAC CCCACCTAC CCCACCTAC CCCACCTAC	CTCACCAGT AGT TTGGGTGTTA CACCGCGGGA CCTGCAGTCC CGTGGCAGTCC GCTGGCAACT A CGAGCTGACG TAGGGTTGGC	CAAACTCT TGCTGATCI GAACTGAGI TCCATTGT ACTTACTT AGTAACAAI ACGACCAT(GCAAGATG)
Sequ 1 381 1 381 1 61 1 21 1 81 2 41 3 01 3 61 4 21 4 81	AGGTGTTATC CTCAAATGT CTCAAATGT ACCTTA TGGTGT CGATTA CGGTTT CACGTG GGTTGC GTTGCG ACCACC AAGACC	Reve GGCG GACG CTAG IAAG ICAC CTCG ICGT ICGT	SCHETTET CATGATG SCTGGCTG GCCGGCTG GCCGGCTG CGATTCCC AGATTGC GCCCAGGT CGGCAGTC TTGCGGGA TTGCGGGA	CCACCAC CAAGCA CAAGCA CTAAAA GT ACAA GT ACAA GT ACAA CTTCG CCA TAAG TC ACTA CT TAAC CC CGAA TC GCGT	GGTTAC GGCCCG GGCCCG TCGCAG GGGCAT GAGTGC CCAACA GGAAAC AGCTTC	CCCACCGACT CCCACCGACT GGAACGTAT CGAGTGGCACT GATGGCACTAAT CCCACCTAAT A TCTCACGCACT CCCAACTTAAT A TCTCACCACT CCCAACTTAAT CCCACCGACTAAT	ACTCACCAGT AGIT TTGGGTGTTA CACCGCGGGA CCTGCAGTCC CCTGCAGTCC CCTGCACTC CCTGCACT CCTGCACCC CCTGCACC CCTGCCACC CCACCTGACC CACGCTCGC ACATGCTCCA	CAAACTOT TGGCGATCI GAACTGATCI GAACTGAGI TCCATTGTI ACTATCATA ACTAACAAI ACGACAATGI CCGCTTGTI
Sequ 1 381 1 381 1 61 1 21 1 81 2 41 3 01 3 61 4 21 4 81 5 41	AGGTGTTATC CTCAAATGT ACCTTA ACCTTA TGGTGT CGATTA CGGTTT CACCTG GTTGCG ACCACC AAGACC GGGCCC	Reve GGCG GACG TAAG TCAC TCG TCG TCG TCG TCGT CCGT	SCALTON CATGALAN CONTROL CONTROL CONTROL CONTROL COCALGET COCALGET TTGCGGGA TTTGCGGT AAGGTTCT CANTCCT	CCACCAC CAAGCA CAAGCA CAAGCA CT AAAA CT ACAA CT ACAA CT ACAA CT AAAG CT AAAG CT AAAG CT AAAG CT AAAC CC CGAA TC GCGT. TT GAGT	GGTTAC GGCCCG GGCCCG TCGCAG GGGCAT GAGTGC CCAACA GGAAAC AGCTTC TTCAAC	CCCACCGAC CCCACCGAC GGAACGTAT GGAACGTAT GGAGTGCGA GTCGCGAC GATGATCTG GAACTTAA TCTCACGAC GCCTATCT GGATTAAACC CCATTAAACC CCTTGCGGTCC	r TTGGGTGTTA CACCGGGGGA CCTGCGGGGCA CCTGCGGCGCC CGTGGTACCG CGTGGCACCC CGAGCTGACG CAGCCGACG A CAAGCTGCCC A CAAGCTGCCC A CAAGCTCCCAG TACCTCCCCAG	CAAACTCTC TGCTGATCC GAACTGAG TCCATTGT/ ACCTCCTC AGTACAAC ACGACCATC GCCAGTGTC CCGCTTGTC GCGGAGGTGC
Sequ 1321 1381 1 61 121 181 241 301 361 421 481 541 601	ACCITAT CICAAATGI ACCITA TGGTGT CGATTA CGGTTT CACGTC GGTTTG GGTTGG ACCACC AAGACC AAGACC TAATGC	Reve GGCG GACG TAAG TCAC CTCG TCGT CCGT TCTC TCGT TCCGT	RSC Prime GGCGGCG GGCGGCG GGCGATC CGATTCCC AGATTCCC GCCAGGT TTGCGGA TTGCGGA AAGGTTCT CAATTCCT GCTCCGGC	r 849bp CT AAAA GT ACAA GT ACAA GT ACAA GT ACAA GT ACAA CT TCG CA TAAG CT TCACTA CC CGAA TC GCGT. TT GAGT	GGTTAC GGCCCG GGCCCG TGCAGG TGCGGA GGGCAT GAGTGC CCAACA GGAAAC AGCTTC TCCAAC GGGCGG	C CCCACCGAC C CCCACCGAC G GGAACGTAT C GGATGCGAC G GGACGTAT C GAGGTGCGAC G GTCGCGAC C CCACCTACT G GCCTACTG G GCCTATCT G GCCTATCTC G GATTAAACC C CTGCGGTCC AAACCCTCCJ	ACTCACCAGT AGIT TTGGGTGTTA CACCGCGGCA CCTGCAGTCC CGTGTACCG CGTGGCAACT CGTGGCAACT CGTGGCGACG CGTGGCGACG CACGTCGCCA CACGTCCCAG A CACCCTAGCA	CAAACTCTC TGCTGATCC GAACTGAG TCCATTGTJ ACCTTCCTC AGTAACAAC ACGACCATC GCAGATGT CCGCTGTC CCGCTGTC
Sequ 1 381 1 381 1 61 1 21 1 81 2 41 3 01 3 61 4 21 4 81 5 41	ence Result ACCTTA ACCTTA TGGTGT CGGTT CACGTG GGTTG GTTGCG ACCACC ACCACC ACCACC ACCACC ACCACC ACCACC	Reve GGCG GACG TAGG TCAC CTCG TGTC TCGT CCGT TTTA TGGA	RECTION CATGATO CONTINUE CONTI	r 849bp CT AAAA GT ACAA GT ACAA GT ACAA GT ACAA GT ACAA CT CACA CC TAAG CC CGAA TC GCGT. TT GAGT AC TGAA GT ATCT.	GGTTAC GGTTAC GGCCGG GGCCGG TGCAGG GGGCAT GAGTGC CCAACA GGAAAC AGCTTC TTCAAC GGGCGG AATCCT	C CCCACCGACT CAATACCAG C CCCACCGACT G GGAACGTAT C CGATTGCA G GTCGCGACT G GATGATCTG/ C CCACTTAAT C CCACCTACTG G GCCTATCTG G GCCTATCAGACJ C GCCCTATCTG G GATTAAACC C CTTGCGGTCC AAACCCTCCJ T GTTCGCTACC	r TTGGGTGTTA CACCGGGGGA CCTGCGGGGCA CCTGCGGCGCC CGTGGTACCG CGTGGCACCC CGAGCTGACG CAGCCGACG A CAAGCTGCCC A CAAGCTGCCC A CAAGCTCCCAG TACCTCCCCAG	CAAACTCT(TGCTGATC(GAACTCAG) ACCTTCCT(AGTACAAC ACGACCAT(GCAAGATG) CCGCTTGT(GCGAAGATG) CCGCTTGT(GCGGATGT(CTCATCGT)

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

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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.

CACCGCTACA CATGGAGTTC CACTACCCTC TTCTGCACTC AAGTTATCCA GTTTCCGATG

Conflict of interest

CACTTCTCC

The authors declare no conflict of interest.

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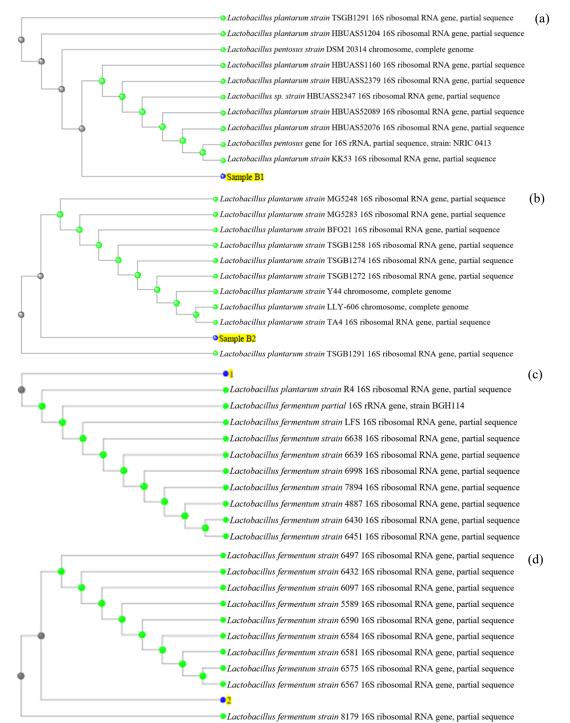


Figure 5. Phylogenetic tree of (a) B1, (b) B2, (c) S1 and (d) S2 isolates

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