

Quantity Evaluation of Lactic Acid Bacteria Using the FncC 0027 and C410li Gene on Different Storage of Acid Bamboo

Putri Widelia Welkriana^{1*} and Halimatussa'diah²

^{1,2} Department of Medical Laboratory Technology, Poltekkes Kemenkes Bengkulu, Indonesia

Indra Giri Nomor 3 kelurahan Padang Harapan kecamatan Gading Cempaka

*Email : putriwidelia5@gmail.com

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ABSTRACT

*The fiber content in bamboo shoots is higher than other tropical vegetables. The water content in saw bamboo shoots is quite high, causing the bamboo shoots to have a shelf life of only 2 days. The fermentation process is characterized by the conversion of carbohydrates into lactic acid by bacteria. This study aimed to calculate LAB with FNCC 0027 and C410LI genes in tamarind shoots with different storage periods. The method used in this study is a descriptive method to describe the total plate number of microbes, LAB examination and molecular identification of bacteria found in tamarind shoots with different storage times. The results showed that all samples showed the presence of microbial TPC, examination. As for molecular identification, it was found that samples 1, 2, and 3 were identified as LAB with the C410LI gene, while the FNCC0027 gene was not detected. Concluded that the total plate number values with different storage times of tamarind shoots, namely 0, 24, and 48 hours were 2.5×10^5 CFU/g, 5.4×10^5 CFU/g, and 7.3×10^5 CFU/g. In samples 1, 2, and 3, there were lactic acid bacteria of the type *Bacillus plantarum* C401LI.*

INTRODUCTION

The fermentation process is characterized by the conversion of carbohydrates into lactic acid by bacteria which are very influential in the industrial world. Some lactic acid bacteria (LAB) fermented from powdered milk are affected by the safety and quality of food which involve microbial agents. Some of the bacteria that cause pathogens in this product are *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* have been studied (Cintas et al. 2001; De Vuyst and Leroy 2007). In addition, several types of these bacteria are found in the digestive tract. In the digestive tract, LAB bacteria cause bacteriocins and prevent the growth and infection of pathogenic bacteria (Castro et al. 2011; Ghanbari et al. 2013; Parada et al. 2007; Ahmed et al. 2013; Mahrous et al. 2013). Bacteriocins are found in two main classes, namely lactibiotics such as Nisin (Class I), and nonlantibiotics such as Pediocin and PlantaricinEF (Class II) (Noda et al. 2015). In recent years, BAL has produced bacteriocins (Leroy and De Vuyst 2004; Molloy et al. 2011; Verma et al. 2014).

This microorganism produces lactic acid as its main product by the anaerobic metabolic pathway and sequencing plays an important role in the accurate identification of *Lactobacillus* (Ortolani et al. 2010). The different types are affected by the type of milk and cheese. Based on the selection of strains in the form of *Lactobacillus* spp. What is isolated from Iranian milk can improve the quality of fermented products, besides that it can be used

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for further metabolism so that it plays a role in fermented food product technology. LAB acts as a starter, probiotic, produces nutraceuticals (Naeem et al., 2012; Emerenini et al., 2013; Ruiz Rodríguez et al., 2017a). Several recent studies have described the isolation of LAB using various techniques (Cagno et al., 2009, 2013; Endo and Salminen, 2013; Olofsson et al., 2014). LAB found in flowers, fruits and vegetables has a high carbohydrate and low protein composition with an acidic pH (Naeem et al., 2012). The growth of these bacteria is influenced by intrinsic factors (physical and nutritional conditions) and extrication (environment) (Naeem et al., 2012; Di Cagno et al., 2013; Garcia et al., 2016).

Based on research by Delfahedah et al. (2013) lactic acid bacteria are capable of producing various antimicrobial components such as lactic acid, hydrogen peroxide (H₂O₂) and bacteriocin. This component is able to inhibit the growth of Gram positive and Gram negative bacteria as indicated by the formation of clear zones in the antimicrobial test. According to Siregar (2013), the genera of lactic acid bacteria include Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus and Propionibacterium.

Currently the application of LAB is not only used for fermenting dairy products, but also for other products such as fruit juices which are good as a medium for the growth of lactic acid bacteria. One LAB that can be used for fruit juice fermentation is *L. plantarum*. According to Gilliland (1986), *L. plantarum* is classified as a gram-positive bacterium, is homofermentative, catalase negative, grows optimally at 30-37°C, and has potential as a probiotic. The use of *L. plantarum* FNCC-0027 in fruit juice was carried out by Retnowati and Kusnadi (2014), using date palm juice with the result that LAB can grow optimally at pH 3.85 with a total LAB of 4.90 x 10¹⁵ CFU/ml. Granato et al., (2010) stated that fruit juice can be a good growing medium for lactic acid bacteria.

In the previous research, it was found that fermented lemea with the basic ingredients of betok fish contained LAB of 1.7x10⁸ colonies/g with the types of bacteria obtained, namely *Lactobacillus plantarum* C410L1 and *Lactobacillus rosii* LS6. Previous research was to prove lemea has potential as a fermented food native to the Rejang tribe as a producer of anti-hypertensive angiotensin converting enzyme. However, in this study, it has not been studied in vitro to prove whether lemea is capable of being a probiotic. So it is necessary to investigate the resistance of LAB in lemea to temperature, low pH and bile salts. Several species of LAB are good probiotics because they can survive the low gastric pH and attach to or colonize the intestine. As a result, bad bacteria in the intestine will decrease because they cannot compete with LAB (Ls & Bile, 2018).

The activity of lactic acid bacteria with different lengths of storage of bamboo shoots can be observed by soaking and boiling which is the most common method of processing bamboo shoots to reduce the cyanide content of bamboo shoots. Both of these processes do not really extend the shelf life of bamboo shoots. It is necessary to develop processing processes to minimize cyanide levels and extend the shelf life of bamboo shoots. One alternative that can be applied in processing bamboo shoots is the process of fermenting vegetables. Kimchi is a processed fermented vegetable made by adding salt, spices or other ingredients and originates from Korea. According to Kim & Chun (2005), kimchi is made by mixing various types of vegetables so that the fiber content in kimchi is quite high but low in calories. Some vegetables that are good for health, including chilies, garlic, onions, are made into kimchi, so that the bioactive components contained in kimchi are determined based on the use of raw materials in processing. High phosphorus and calcium are obtained by adding red chili powder, fish sauce and oyster sauce, while red chili will give kimchi a distinctive red color (W.-C. Lee & Huang, 2000).

Fermentation time is a variable related to the microbial growth phase during the fermentation process so that it will affect the fermentation results. Previous studies have shown that fermentation time affects the characteristics of lactic acid fermented rice drinks. Based on the research of Wongkhalaung et al., (2000), showed that the fermentation time in rice milk used *Lactobacillus acidophilus* IFRPD 2013 and *Lactobacillus casei* subsp. *rhamnosus* IFRPD 2020 for 24 hours at 37 °C produced the best rice yogurt product with a lactic acid content of 0.8%, a total LAB of 1×10^8 CFU/g, and a pH of 3.58. According to Yunus et al. (2015) lactic acid fermentation time that is too short will cause the growth of lactic acid bacteria is not optimal and the number of population is less to be categorized as probiotics, while the fermentation time that is too long will produce a too sour taste in the product and also cause a decrease in the number of lactic acid bacteria populations. due to depletion of nutrients in the substrate and accumulation of toxic metabolites such as ethanol produced by heterofermentative lactic acid bacteria. Based on this background, it is necessary to conduct research to determine the effect of fermentation time on sour bamboo shoots. This study aims to calculate LAB with the FNCC 0027 and C410LI genes in tamarind bamboo shoots with different storage times.

MATERIALS/METHOD

This research was conducted from June to October 2022 at the Integrated Laboratory of the Bengkulu Ministry of Health Polytechnic. Samples were taken randomly at markets spread across the city of Bengkulu. Samples were stored for 0, 24 hours, and 48 hours and each had three repetitions. The method used is descriptive. Especially to know the total plate numbers. Furthermore, identification of the type of LAB that grows.

According to Sambrook (2001) 1.5 mL of bacterial culture was centrifuged at 8000 rpm for 10 minutes, then the pellet was washed with STE buffer (0.3 M sucrose; 25 mM Tris-HCL; 25 mM EDTA.2Na pH 8), then centrifuged at 8000 rpm for 10 minutes. The pellets were washed 3 times repeatedly. The pellet was added with 200 L of STE buffer and 45 L of lysozyme (20 mg/mL) then incubated at 55 °C for 1 hour until protoplasts were formed. 20 L of proteinase-K (20 mg/mL) was added to the mixture and incubated at 55 °C for 60 minutes. Then 400 L of 10% CTAB was added in 0.7 M NaCl solution and then incubated at 65 °C for 30 minutes and put into a solution of phenol:chloroform (25:24), then centrifuged at 12000 rpm for 10 minutes. The clear phase was put into a new tube and added 0.6 times the volume of isopropanol and 20 L of sodium acetate, then incubated at -20 °C overnight. Then centrifuged at 12000 rpm for 10 minutes. The pellet was washed using 1 mL of 70% alcohol. DNA was dried for 1 hour and then dissolved in 50 L of sterile ddH₂O, then isolated DNA was stored at 4°C or -20°C.

The 16S rRNA gene from genomic DNA was amplified by Polymerase Chain Reaction (PCR) machine using a prokaryotic specific primer [18], namely forward primer 63f (5'-CAG GCC TAA CAC ATG CAAGTC-3') and reverse primer 1387r. (5'-GGG CGG WGT GTA CAA GGC-3'). The PCR conditions used were pre-denaturation, denaturation, annealing, elongation and post-PCR for a total of 30 cycles. Separation of PCR product DNA using 1% agarose at 75 volts for 45 minutes. DNA visualization was carried out on a UV transilluminator.

The sorted data was then tidied up and assembled using the ChromasPro version 1.5 program. Data was performed by BLAST on genomic data registered by the NCBI/National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>). The data were analyzed again by sequence alignment using the MEGA 5.0 program and phlogenetic tree construction was carried out to show the degree of kinship between Xyl_22 isolates with

actinomycetes and non-actinomycetic microbes using the Neighbor Joining Tree method with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

The results of the total LAB plate number of tamarind bamboo shoots with a storage time of 0, 24, and 48 hours are as follows.

Table 1. Plate Numbers of Total LAB from Tamarind Shoots with Different Storage Periods

Storage Time (Hours)	Number of Colonies (10 ⁵)
0	2,5
24	5,4
48	7,3

The sequencing results of the 3 samples with different storage times can be seen in Table 2 below.

Table 2. Sequencing of BAL Isolates from Tamarind Shoots with Different Storage Periods

Sample (Hours)	FNCC- 0027	C410LI
0	0 %	99,17%
24	0 %	99,35%
48	0 %	99,73%

Based on the results of the study showed that the total microbial plate count at 0, 24, and 48 hours of the first week for samples had a total microbial plate count (TPC) of 2.5x10⁵ CFU/g, 5.4x10⁵ CFU/g, and 7.3x10⁵ CFU/ g, each of which can be seen in Table 1. Meanwhile, all samples at 0.24 h and 48 h showed almost the same morphological characteristics, including white colony color, convex elevation, smooth edges, and stem shape (Table 1).

The next factor is due to the different duration of the fermentation process, between 0 hours and 48 hours, which accumulates acid obtained from the breakdown of sugar, thereby lowering the pH of sour shoots. This is in accordance with Apridani's statement (2013) that the longer the fermentation time, the greater the amount of sugar converted to lactic acid. The sugar content in fruit juice also affects the decrease in the number of LAB. The higher the sugar content in fruit juice, the more acid is produced from the fermentation process so that it can lower the pH. These bacteria are proteolytic which can convert protein compounds into simpler compounds such as lactic acid (Rostini, 2007). so that the production of lactic acid is not only the result of the breakdown of carbohydrates but also the result of the breakdown of glycoproteins contained in egg whites. A similar study using *L. plantarum* bacteria in bekasem fish experienced an increase in total titrated acid from 0.02% to 0.18% during 9 days of fermentation (Wikandari et al., 2011), as well as fermentation of *L.*

helveticus bacteria in skim milk experienced an increase in titration ie from 0.18% to 1.24 for 30 hours of fermentation (Sun et al., 2009). The difference in the percentage of total acid for each food ingredient is determined by the ability of microbes to decompose the constituent components of the food. Utilization of *L. plantarum* bacteria can increase the acidity of the substrate by 1.5 to 2.0% (Rostini, 2007).

Identification of LAB found in tamarind bamboo shoots was carried out genetically at a representative sample for each storage period. This was done because the colony characteristics for each sample were similar. As for molecular identification, PCR amplification for sample 1, sample 2 and sample 3 showed a band size of 132 bp. This is according to the size of the primer used. Furthermore, the results of the sequencing and construction of the phylogenetic tree showed that samples 1, 2, and 3 were identified as *L. plantarum* C410LI partial 16S rRNA gene isolates (Table 2). *Bacillus plantarum* is a Gram-positive, rod-shaped, facultative aerobic bacterial species that is widely distributed in various countries (Marchlewicz, 2016). Several studies have shown that *Bacillus plantarum* is a prebiotic. These bacteria have a wider environmental coverage due to their facultative aerobic nature (Hou, 2021). Therefore, there is a possibility that this bacteria is present in sour bamboo shoots. Apart from that, these bacteria are also found in lettuce, kimbap, and spinach which are sold in South Korea (Wei, 2019). Several previous studies have also reported that this bacterium has been detected in foods such as milk, fresh fruits and grains (Chiu, 2016). Furthermore, *Bacillus cereus* is a rod-shaped bacterium, and is commonly found in some food retailers in Nigeria (Adesetan, 2020). While molecular identification showed that samples 1, 2, and 3 were identified as isolates of the partial 16S rRNA BD17-E12 *Bacillus plantarum* gene.

CONCLUSIONS

This study concluded that the total plate number values with different storage times of tamarind shoots, namely 0, 24, and 48 hours were 2.5×10^5 CFU/g, 5.4×10^5 CFU/g, and 7.3×10^5 CFU/g. In samples 1, 2, and 3, there were lactic acid bacteria of the type *Bacillus plantarum* C401L1.

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