Identification of Lactic Acid Bacteria in Traditional Rejang Fermented Food (Lemea)

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Abstract*—Lemea is a traditional fermented Rejang food, Lemea potential can be used as a functional food to support health. The process of processed lemea by fermentation produces several types of lactic acid bacteria (BAL) which can be used as probiotics. The purpose of this study is to determine the type of BAL found in the final product of Lemea. Identification of types of lactic acid bacteria using Taba bamboo shoots and betok fish (Anabas testudineus) obtained from Rejang Lebong District in Bengkulu Province. Analysis of the type of lactic acid bacteria was applied using PCR (Polymerase Chain Reaction), followed by analysis of fragment sequences of the 16S rRNA gene base sequence and then compared with DNA registered in the gene bank in the BLAST program. Sequencing results showed that there were two types of BAL isolates found in lemea fermentation products (610.R.4530 A and 610.R.4530 B), namely Lactobacillus plantarum strain C410L1 and Lactobacillus rossiae strain LS6 with query values of 100% and 99% respectively. Based on the above results it was concluded that lemea has the potential as a probiotic food because it contains two types of lactic acid bacteria in its fermentation.

***Keywords*—Lemea, Lactobacillus Plantarum strain C410L1, and Lactobacillus rossiae strain LS6**

I. INTRODUCTION

Lactic acid bacteria are gram-positive bacteria which belong to GRAS *(Generally Recognized As Safe)*, play an essential role in food fermentation processes, and provide health benefits (Kumar, 2013; Lawalata & Satiman, 2015). This bacterial growth occurs spontaneously in the process of food fermentation or can also be inoculated by starter culture (Widyastuti & Febrisiantosa, 2014). The nature and metabolism of lactic acid bacteria formed during fermentation play an essential role as probiotics (Hayek & Ibrahim, 2013).

Lemea is one of the traditional fermented foods of the Rejang Tribe in Bengkulu Province, Indonesia, which has the potential to be used as a probiotic. Caused by Lemea fermentation which utilizes some lactic acid bacteria.

The essential ingredients for making lemea are using young bamboo or called bamboo shoots which are then chopped and mixed with fish from freshwater while stirring evenly. Furthermore, the mixture of bamboo shoots and fish is fermented for several days to produce lemea products (Dewi, K, Zuki.M, 2012; Dewi, 2015).

Research shows that fermented foods from fish such as one of the lemea products contain some BAL which can potentially be used as probiotics. Lemea fermentation which is almost the same as Indonesian traditional food from fish namely bakasang found two isolates of lactic acid bacteria found in bakasang namely *pediocccous* B3.5 and *Pediococcus* B9.7. This lactic acid bacterial strain has been further identified using Amplified Ribosomal DNA Restriction Analysis (ARDRA) and obtained lactic acid bacteria found in the tub have similarities with *Pedioccus acidilactici*. These bacteria have strong proteolytic abilities and can degrade fish protein into bioactive peptides so that they can become probiotics and ACE inhibitors. (Lawalata & Satiman, 2015).

The same research was also proven (Wikandari et al., 2012), by using the potential of traditional Indonesian fermented food from fish, Bekasam. In his study found BAL as many as six strains isolated from the scam, including *Lactobacillus Plantarum* B1765, *Lactobacillus Plantarum* T256, *Lactobacillus Plantarum* N2352, *Lactobacillus Plantarum* B1465, *Lactobacillus pentosus* B2555, and *Pedioccous pentosaseus* B1661. During the fermentation process, six strains of lactic acid bacteria grew well and showed an increase in the number of peptides and an increase in ACE inhibitors. Several studies of fermented foods and beverages containing some BAL have been proven in vitro and in vivo can be used as ACE inhibitors (LM Beltran, A. Hernandez, MJ Torres, AF Gonzalez, 2016).

Based on the explanation above, this study aims to identify the types of acid lakat bacteria found in traditional fermented foods of the Rejang "lemea" tribe which could potentially be probiotics.

II. METHODS

In this study using ingredients namely Taba Bamboo Shoot and Betok Fish (*Anabas testudineus)* obtained from Rejang Lebong District in Bengkulu Province, while for chemical reagents used Mann Ragosa Sharpe (MRS) medium gram dye (crystal violet, iodine, safranin alcohol),SDS (*sodium dodecyl sulphate* 10%), 10 mg / ml proteinase K, PCl solution (phenol, chloroform, isoamil alcohol) with a ratio of 25: 24: 1, absolute ethanol, 70% ethanol, buffer Te (Treis EDTA), STE buffer (*Saline Tris Edta*), ddH2O, DNAEnzyme *Polymerase*, forward and reverse primer, dNTP containing dTTP, dCTP, PCR buffer, MgCI, EtBr solution, TAE buffer (Tris Acetate EDTA), 1 kb DNA marker This research is a type of experiment carried out at the Bogor Saraswanti Laboratory. Sample preparation in this study were bacterial isolates that had been isolated from lemea using Mann Ragosa Sharpe (MRS) media and gram grading was carried out. Stages Making Phase Lemea.

Bamboo shoots Taba that have been obtained are then cleaned using running water, then peeled and thinly sliced or chopped. Then, the Taba Bamboo shoot was fermented by soaking it with 500 ml of water for 30 hours. The results of the bamboo shoot fermentation were taken as much as 150 grams. Add 50 grams of fish and stir evenly. Mix 50 ml lemongrass spice water (made by roughly 1 cm sliced lemongrass and soaked with water for 30 minutes) to add flavor to lemea. Finally, fermentation of lemea for 48 hours with a temperature of 27°C in a prepared container that is in this study using a plastic container.

DNA extraction

Bacterial isolates obtained from the preparation were taken as much as 10 ml, in 1000 µl eppendorf tubes separated by centipede for 2 minutes at a speed of 1000 rpm. The supernatant formed was discarded, while the precipitate was suspended using 500µL of STE buffer. Then, using a vortex, the solution was homogenized and separated again using a centrifuge for 2 minutes. The pellet solution was resuspended with 500 mL TE buffer and 100 mL SDS 10%, then homogenized and proteinase K 10 mL added. Incubation for 1 hour at 37°C. After incubation, add 500 mL PCl solution. Then, the solution was separated by centrifuge for 10 minutes at a speed of 10000 rpm. The top solution was taken as much as 400 µl and transferred to a sterile eppendorf tube. Add an absolute cold ethanol solution of 800 µl and cool for 30 minutes at 4°C. A centrifuge separates the cooled Eppendorf tube for 10 minutes at a speed of 10000 rpm. The deposits obtained were resuspended using 70% ethanol. Separate the solution with a centrifuge with a speed of 10000 rpm for 10 minutes. Finally, the sediment that has been obtained is dried using an evaporator. Resuspension by adding 50 mL ddH2O in a dry precipitate.

Preparation of Agrosa Gel Solution

of 0.4 gram of granulated powder in 4 ml of TAE buffer and heated using a microwave for 2 minutes. Next, pour the solution into the *chamber* electrophoresis, after the hardened solution adds TAE buffer until the gel is submerged.

DNA Separation by Agrosa Gel Electrophoresis

A total of 3 µl loading buffers were prepared on parafilm sheets. The results of the isolated DNA samples were added as much as 7 µl into the loading buffer. The DNA marker is
inserted into the most end hole in the agrosa gel. Next, do 30 minutes of electrophoresis with a voltage of 5 V / cm. Then, do the coloring process by soaking the gel with EtBr solution for 10 minutes. DNA observation using a UV transilluminator lamp.

16S rRNA gene amplification

Combine 5 µl of molded DNA samples which were isolated with 25 µl multi mix PCR reagent, primer, *forward* 5 µl of5 µl of reverse primer andddH210 µl ofO. Then put into a PCR tube using a pre-temperature of 95^{O}separationC for 5 minutes, separating at 95^{O}C for 30 seconds, attaching the primer with a temperature of 60temperature of^{O}C for 30 minutes and extending the chain with a72^{O}C for 1 minute. Then, purification of the Amplification Product with Agrosa Gel was carried out. The results of the amplication were taken as many as 30 µl and put into Eppendorf. Next, addddH270 µl ofO and a binding buffer of 500 µl. The mixture was separated using a centrifuge for 1 minute with a speed of 10000 rpm. Deposits obtained were added with 500 µl of washing buffer. Moreover, separated again using centrifuges. Washing using buffers is done twice. The separation tube is inserted into the Eppendorf tube. AddddH225 µl ofO and centrifuge it again. Next, separate the solution obtained with the agrosa gel.

DNA sequencing

The purification solution of the amplification product was analyzed using the 16S rRNA gene fragment sequence using a forward primer. Fragment Analysis of 16S rRNA Genes from Identified Bacterial Isolates Nucleotide sequences from the results of 16S rRNA gene sequencing were analyzed using the

BLAST program uploaded on the website www.ncbi.nlm.nih.gov, the results obtained were then made phylogeny tree, with double alignment *(multiple alignments),* then visualizing the kinship using Neighbor-joining trees with 1000x Bootstrap on MEGA software (6.06) (Akhmetsadykova et al., 2015)

III. RESULT

Types of lactic acid bacteria found in the traditional food of the Rejang tribe, namely lemea, with the essential ingredients of bristles and beta fish can be known by using the initial stages of DNA extraction to separate the DNA genome from other molecules in the cell, with the way the cell wall is solved, removal of protein and RNA, and the deposition of DNA. Furthermore, the results of the DNA extract were carried out by an electrophoresis test. This electrophoresis test is used to determine the size of DNA and the quality of bacterial isolation obtained from isolates of lemea samples. The results obtained from DNA separation on lemea samples using agrose gel electrophoresis measuring 250-10000 bp were continued with amplification using PCR.

Gene amplification using PCR was carried out in 25 cycles. Temperature settings are carried out for every 25 cycles. The first cycle begins with the initiation stage. This initial stage serves to denaturate the perfect DNA chain. The second stage is carried out again to refine the long chain of DNA further. Furthermore, to separate the double chain into a single chain is carried out the denaturation stage by breaking bonds. The final stage is that in single chain DNA the primary attachment and extension of the DNA chain are carried out (Ashmaig et al., 2009). The results of amplification can be seen in Fig 1 below.

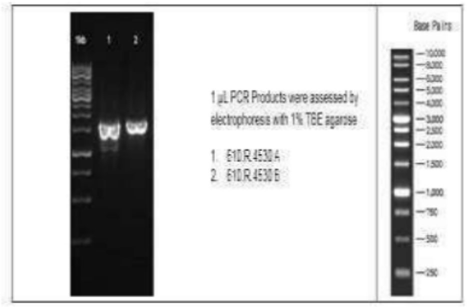

Fig 1. Results of Photo-PCR Product Gel on Lemea Base Loads of Taba Bamboo Shoots and Betok Head Fishes

In Figure 1. above we can see that there are two DNA migration pathways obtained from samples of isolates from Lemea. The first band is at 2500 bp which shows the existence of molecules with a size of 2500 bp. While the second band shows that there are 3000 bp molecules in DNA isolates. Based on the electrophoresis results there were 2 PCR products found namely 610.R.4530.A and 610.R.4530.B which were then tested for sequencing of 16S rRNA gene fragments. The process of amplification of 16S rRNA fragments in this lemea isolate was to obtain more copies of the 16S rRNA gene used for DNA sequencing processes, making it easier to identify the types of lactic acid bacteria isolates found in lemea (Perdana, 2011). Sequencing of DNA isolates from Lemea isolates made from taba bamboo and parrot fish, based on the sequence of nucleotide bases for the 610.R.4530.A and 610.R.4530.B groups can be seen in table 1 below:

TABLE I. RESULTS OF PAIRED SAMPLES T-TEST IN THE YELLOW WATERMELON JUICE TREATMENT GROUP

al Isolates	Species	Cover	tity	Accession No.
610.R.4530.A	*Lactobacillus plantarum* strain C410L1	0%	0%	CP017954.1
610.R.4530. B	*illus rossiae* strain LS6	%	0%	JN68078.1

IV. DISCUSSION

Based on Table 2 above, it is known that the types of lactic acid bacteria found in isolate lemea were matched with the gene bank contained in the BLAST program, namely *Lactobacillus plantarum* strain C410L1 with a *query* or similarity of 100% and *Lactobacillus rossiae* strain LS6 with query 99%, the identity obtained in each strain is 100%. Bacterial species are said to be similar if the query results and identity are more than 95% (Akhmetsadykova et al., 2015; Adeyemo & Onilude, 2014).

bacterial species *Lactobacillus Plantarum* commonly found in fermented foods from meat and poultry and dairy products. Wikandari et al. (2012), found four strains of *Lactobacillus Plantarum* found in a scam, namely *Lactobacillus Plantarum* B1765, *Lactobacillus Plantarum* T2565, *Lactobacillus Plantarum* N2352, *and Lactobacillus Plantarum* B1465. Bekasam is a traditional fermented food from fish which is almost the same as lemea. *Lactobacillus Plantarum* is a homofermentative lactic acid bacteria and is a gram-positive type. These bacteria are rod-shaped and purplish blue. Identification of lactic acid bacteria using PCR is a very appropriate and effective method to find out the type of BAL in food products (Adeyemo & Onilude, 2014). These bacteria can grow well during the Lemea fermentation process, which is influenced by pH, lactic acid content and moisture content (Mangalisu et al., 2015).

Based on sources obtained from Banaay et al. (2013), found BAL of the type of *Lactobacillus plantarum* in several traditional fermented food products from Philippine sourced from fish and processed fish products namely balao-balao, burong-isda, and burong talangka. Fermented foods from fish are often used as complementary foods, pasta sauces, and spices in Philippine people's food. *Lactobacillus Plantarum* in various studies has also been proven to be a probiotic that can improve health, so that consumption of foods containing bacteria of this type is highly recommended to help improve individual health status. In addition to the bacteria *Lactobacillus Plantarum*, Lemea fermentation also produces bacteria such as *Lactobacillus rossiae* strain LS6. The

bacteria *Lactobacillus rossiae* LS6 strain in lemea products are also found in traditional Korean foods from wheat, namely sourdough. According to Aslam et al., (2006), using the gene! 6S rRNA phylogenetic analysis, found BAL of the type *Lactobacillus rossiae* DSM 15814T with a query value of 98% in sourdough wheat isolates of South Korean specialties. This bacterium is the type of lactic acid bacteria are gram-positive, non-spore, non-motile, and can grow at a temperature of 30°C and is heterofermentative and anaerobic fermentation process lemea in anaerobic very helpful at the growth of bacterial species *Lactobacillus rossiae* on products end of lemea.

Research by Galanis et al. (2015), the PCR method is very accurate and useful for detecting molecular form identification of BAL types. In his research found 23 lactic acid bacteria with different strains of *Lactobacillus Plantarum* 2035 and *Lactobacillus Plantarum* ACA-DC 2640 isolated from feta cheese. The working principle of PCR is that it can multiply a nucleotide sequence which is then followed by DNA sequences and see the suitability of DNA of lactic acid bacteria using gene banks in the BLAST program.

The probiotic abilities of *Lactobacillus plantarum* and *Lactobacillus rossiae* which are beneficial for health have been widely studied by experts (Retnowati & Kusnadi, 2014). The benefits of this bacterium as a probiotic are its role in maintaining the health of the digestive tract. According to RP

Goddess, Adirestuti & Anggraeni (2015), *Lactobacillusplantarum* has antibacterial activity plays an essential role in maintaining the health of the digestive tract, as evidenced in the results of his research in vivo that the activity of *Lactobacillus plantarum* can inhibit the growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella* typhimurium, often interfere with digestion in humans.

Other health benefits that can be caused by probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus rossiae*, one of which can reduce the risk of degenerative diseases such as coronary heart disease. Sumarno et al. (2011), proven by LAB of *Lactobacillus plantarum* isolated from noni juice in vivo, can reduce serum LDL *(Low-Density Lipoprotein), triglyceride,* levels and increase HDL *(High-Density Lipoprotein)* levels in Wistar rats with a confidence level ($p < 0.01$). Aside from being a probiotic, lactic acid bacteria such as *Lactobacillus rossiae* can also increase the nutritional value of a food product. According to Angelis et al., (2014), the growth of *Lactobacillus rossiae* can increase the production of essential micronutrients, namely complex B vitamins such as cobalamin, riboflavin, and folic acid. This vitamin cannot be synthesized naturally by humans but can be obtained from food sources that contain vitamin B complex.

V. CONCLUSION

This study concludes that two types of lactic acid bacteria exist in the Lemea fermentation end product made from taba bamboo and betok fish which can be used as probiotic foods to support health. Sequencing of 16S rRNA gene fragments and compared with gene banks in the BLAST program identified *Lactobacillus plantarum* strains C410L1 and *Lactobacillus rossiae* strains LS6 found in lemea products with query values of 100% and 99%, respectively. These bacteria are often found in fermented meat and dairy foods. These bacteria grow well in anaerobic conditions. Lemea fermentation process that utilizes anaerobic conditions greatly helps the growth of these two types of bacteria.

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