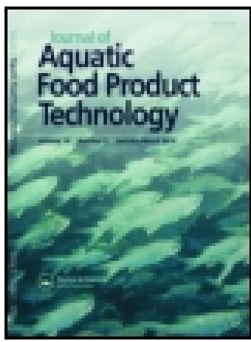

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Moderate Halophilic Lactic Acid Bacteria from *Jambal roti*: A Traditional Fermented Fish of Central Java, Indonesia

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ABSTRACT

Jambal roti is a traditional salted-fermented product made from *Arius thalassinus*, which is a popular fish in Central Java Province, Indonesia. This research aimed to identify the moderate halophilic lactic acid bacteria isolated from *jambal roti*. The study began with enrichment culture (with MRS media and 5% NaCl) and isolation of lactic acid bacteria (LAB) using streak plate method. After the isolation, the isolate was identified using API 50 CHL kit (for phenotypic characteristic) and 16s rRNA gene sequence analysis (for genotypic characteristic). Eight pure isolates were identified from the fermented fish product. Most isolates (87.5%) were cocci (tetrad and pair), while the rest (1 isolated) was *Lactobacillus* (rod-shaped). All of which were moderate halophilic LAB. The isolates were selected based on their high proteolytic activity (quantitative) and R-value (qualitative). The analysis using API 50 CHL kit showed that all isolates were identified as *Pediococcus pentosaceus* 2 (99.9%), while further identification by repetitive PCR revealed three types of band profiles. In the 16s rRNA gene sequence analysis, three isolates were identified as *Pediococcus acidilactici* (strain no 306, 315, and 332), four isolates were identified as *Pediococcus pentosaceus* (strain no 312, 326, 328, and 339), and the other was identified as *Lactobacillus plantarum* (strain no 307). Moderate halophilic lactic acid bacteria isolated from *Jambal roti* were *Pediococcus acidilactici, Pediococcus pentosaceus*, and *Lactobacillus plantarum*.

KEYWORDS
Jambal roti; salted fish; moderate halophilic; lactic acid bacteria; 16s rRNA

Introduction

Lactic acid bacteria (LAB) are a large and diverse group of Gram positive bacteria, non-spore-forming bacteria, negative catalase, and lactic acid as the main product of fermentation. LAB species can be identified based on morphological, biochemical, physiological, and genotypic characterization (16s rRNA gene sequence analysis). Morphological characters include cell form, cell size, cell arrangement, Gram staining, catalase reaction, and spore formation (Pelinescu et al., 2019; Rahayu 2003). Various classifications have been proposed for halophilic bacteria, based on their requirement of salt. Moderate halophilic bacteria is a bacteria that grows in the presence of concentration of salt ranging from 3% to 15% (Gibbons 1969). The isolation of such bacteria uses MRS media with 1% $CaCO_3$ and 5% NaCl.

Lactic acid bacteria offers a tremendous number of advantages for health. *Chao* (a fermented fish typical of South Sulawesi, Indonesia) is produced using indigenous LAB with antimicrobial constituents that serve as a bio preservative (Nurkhikmayani et al., 2019). Indonesian fermented buffalo milk (*dadih*) prepared by LAB fermentation contains high concentration of folate (Purwandhani et al. 2018). *Lactobacillus* sp.OL-5, *Lactobacillus plantarum* Mut-7, and *Lactobacillus plantarum* Dad-13

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induce intracellular uricase activities (Handayani et al. 2018). *Bekasam*, another traditional fermented fish, has antihypertensive properties (Wikandari et al. 2012). LAB exhibits proteolytic activity on fermented fish and produces a bioactive compound as an ACE inhibitor (Fujita et al., 1999; Itou and Akahane, 2004; Je et al. 2005; Kim et al. 2016; Okamoto, 1995).

Fish is a high-quality protein and unsaturated fatty acid source and has favorable taste. However, fish is a perishable product, so it is easily contaminated by spoilage microorganisms. To overcome these problems, fish is often preserved as a fermented product. Drying and salting are common methods to preserve fish. Indigenous microorganisms change the composition of fish in a natural condition and produce specific color, texture, and flavor (Zhu, 2016). Fishermen in several countries have practiced traditional fermentation processes to preserve fish that impart unique characteristics in the final product (Nuraida 2015).

Jambal roti is a traditional fermented fish, originally from Rembang, Central Java Province, Indonesia. This salted fish is quite famous in the province. The term *jambal roti* refers to the texture after frying, which is similar to that of bread crumbs. It is made by incubating the salted fish (30% sea salt) for 3 days, allowing fermentation to set, and sun-drying for another 3 days. Recently, there have been various studies analyzing the microbial diversity of some fermented fish products, such as *bekasam* (Wikandari et al., 2012), *bekasang* (Lawalata and Satiman, 2015), *plassom* (Hwanhlem et al. 2011), fish sauce (Udomsil et al., 2010), *chao* (Matti et al. 2019), *plaa-som* (Kopermsub and Yunchalab, 2010), and *plajom* (Miyashita et al., 2012). The current research aimed to isolate and identify the moderate halophilic lactic acid bacteria, which have proteolytic properties, from *jambal roti*. The identification was performed by morphological, biochemical, and phenotypic characterization and 16s rRNA gene sequence. LAB isolated from indigenous products like fermented fish have proteolytic activity and produce bioactive compounds acting as ACE inhibitors with antihypertensive properties.

Material and method

Raw material (sampling)

Jambal roti was obtained from a traditional producer in Rembang, Central Java, Indonesia. The sample was taken on the first day to the sixth day of fermentation. *Jambal roti* is prepared by fermenting fish with 30% salt (per fish weight) for 3 days and then sun-drying for another 3 days. The samples were placed in ice tubes in a cooler-box and transported to the Microbiology Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. Before the analysis, the sample was preserved at −20°C.

Isolation of lactic acid bacteria

Lactic acid bacteria were isolated in de Man Rogosa and Sharpe (MRS, Merck, Germany) broth media, which was enriched according to the modified technique proposed by Karami et al. (2017). Ten grams of the sample was transferred into a flask containing MRS broth, fish extract (20%), NaCl (5%) (Merck), and Natrium Aside (50 ppm) were added, and then incubated at 37°C for 48 h. The isolation of lactic acid bacteria was performed by modifying the method of Purwandhani et al. (2018). After the enrichment (48 h), the culture was placed on a petri dish and covered with MRS agar supplemented with 1% $CaCO_3$, fish extract, NaCl (5%), and incubated at 37°C for another 48 h. The colonies, which formed clear zones as a result of the reaction to $CaCO_3$, were enumerated. Two to five randomly selected white colonies were purified and then isolated by streak plate procedure using the same medium.

Morphological and biochemical characterization

The morphological and biochemical characterizations of lactic acid bacteria were performed following the procedure explained in Rahayu (2003). After 2–3 days of incubation with MRS broth, the cell

morphology and cell arrangement were characterized. The bacteria were subjected to Gram staining and catalase test. The Gram staining employed a general method. For the catalase test, the bacterial isolates were introduced to H_2O_2 and observed for the production of CO_2.

Proteolytic activity was qualitatively analyzed based on the technique proposed by Prihanto et al. (2013) and detected using skim milk agar (SMA). The SMA (2% skim milk and 2% agar) was prepared in a petri dish with a hole on agar media. The culture (75 µl) was placed using micropipette in the hole and incubated at 35ºC for 24 h. The proteolytic activities were represented by the formation of a clear zone around the colonies. The diameter of the clear zone was measured with a ruler. The clear zone was calculated by reducing the diameter of the clear zone with the hole diameter. The analysis was done twice.

Afterwards, the proteolytic activity was detected quantitatively based on Baehaki et al. (2011). The isolates were grown in MRS broth media with 1% casein at 37°C for 24 h. Then, the culture was centrifuged at 3500 rpm for 15 min and analyzed by extracellular protease digestion; the supernatant was then sampled. Protease analysis was carried out by mixing 100 µl supernatant with 200 µl casein Hammerstein (50 ppm) and 300 µl phosphate buffer (pH 7). The mixture was incubated at 37°C for 60 min in a water bath for the proteolytic reaction. Then, 400 µl of trichloroacetic acid (TCA) was added to inactivate the enzyme. The supernatant was incubated for 30 min at room temperature and then separated by centrifugation at 3500 rpm for 10 min. A total of 100 µl of supernatant was diluted five times with phosphate buffer (pH 7). The absorbance of the mixture was then gauged using a UV-VIS spectrophotometer at 275 nm, with a standard curve for tyrosine measurement. One protease activity unit indicates the number of enzymes needed to produce 1 ml of tyrosine for 1 min of reaction. Protease enzyme activity was determined by the formula: enzyme activity = ([tyrosine]/Mr tyrosine) x (v/(pxq)) x fp, where v = sample volume (ml); *p* = number of enzymes (ml); q = time of incubation (s); fp = dilution factor.

Physiological characteristic of LAB

The physiological characteristics of LAB were determined from the ability of these bacteria to grow in various levels of salt concentration, pH, and temperature. Rahayu (2003) performed this test with modified MRS broth. The salt concentrations applied to the MRS broth media were 2%, 6%, 12%, 18%, and 20%. The pH levels varied from 4.6, 5.65, 6.4, to 10.65. The analysis used MRS broth media at 37°C for 24 h. Also, LAB was exposed to different temperatures, namely 37°C and 50°C.

Phenotypic characterization

The phenotypic characterization was carried out using the API 50 CHL kit (Biomeriux, France) by carbohydrate assimilation and fermentation of 49 types of carbon source (one as a treatment control). The lactic acid bacteria were inoculated in MRS broth media with 5% NaCl at 37°C for 24 h. Bacterial cells were centrifuged at 3500 rpm for 30 min and rinsed with 0.85% NaCl, then suspended in 5 ml of 0.85% sterilized NaCl. One ml of the solution cell was suspended in an API CHL 50 medium, and 600 µl of the solution was transferred onto the strips containing 49 types of carbon sources. The strip was covered with paraffin and incubated at 37°C for 48 h. A positive (+) result is represented by change in color, i.e., from dark blue to yellow (LAB can be used as a carbon source for metabolism), while a negative result (-) is marked by no change in the color (dark blue, LAB cannot be used as a carbon source for metabolism). The bacteria species in the results were identified using the Apiweb program (https://apiweb.biomerieux.com).

Genotypic characterization

DNA extraction

The DNA extraction was performed according to the modified procedure from Suhartatik et al. (2012) and Matti et al. (2019). The LAB isolates were inoculated in MRS broth (6 ml) and incubated at 37°C

for 18–24 h. The samples were then centrifuged at 13.000 rpm and 4°C to collect the cell pellet. The cells were suspended in 50 µl of a lysis buffer, mixed with vortex, and then incubated for 20 s. Then, 20 µl proteinase K (20 mg/ml) was added to the solution, mixed using vortex, and incubated for 20 s to accelerate the destruction of the cells. The solution was added with 40 µl lysozyme (100 mg/ml), mixed with vortex, and incubated at 55°C for 30 s. The supernatant was separated by centrifugation at 13000 rpm for 15 s. It was transferred into a new microtube (2 ml) and phenol 1:1 (v/v) was added. The solution was mixed in a vortex and incubated using a shaker for 30 s.

The supernatant was separated by centrifugation at 13000 rpm for 10 s. The upper solution was transferred into a new microtube (2 ml) and a mixture of cold chloroform and phenol (1:1) was added. The mixture was mixed using a vortex, and incubated for 20 s. The supernatant was separated by centrifugation at 13000 rpm for 10 s. The upper solution was transferred into a new microtube (1.5 ml) and cold ethanol 1:1 (v/v) was added.

The DNA genome was separated by centrifugation at 13000 rpm for 10 s and 500 µl of cold ethanol (70%) was added. The solution was centrifuged at 13000 rpm for 5 s to collect pure DNA genome (pellet). Afterwards, the DNA genome was dried for 2 h, 50 µl TE buffer and 3 µl RNAse was added, and incubated at 37°C for 1 h before analysis.

Amplification of repetitive polymerase chain reaction (PCR)

The amplification of repetitive PCR used a technique modified from Suhartatik et al. (2014). Twenty-five microliters of PCR mixture contained 9.5 µl Non-Free Water, 12.5 µl Mix PCR (MyTaq HS Red Mix, 2x from Bioline Germany), 1 µl Primer 27 F (-AGAGTTTGATCCTGGCTCAG-), 1 µl Primer 1492 R (-GTTACCTTGTTACGACTT-), and 1 µl template DNA. The equipment program required an initial denaturation step at 96°C for 4 min, 30 cycles of 94°C for 1 min (denaturation), annealing for 1 min 30 s at 51.5°C, an extension for 8 min at 68°C, and then final extension at 68°C for 10 m. Agarose gel electrophoresis (1%) was performed to examine the purity of the 16s rRNA with SYBR^{TM} safe (DNA Gel Stain) 8 µl and 10 µl of PCR product. Electrophoresis was run for 60 min, and the absorbance of the result was read using a UV-vis spectrophotometer (Hoefer's double vision).

16s rRNA gene sequence analysis

The 16s rRNA gene sequence analysis with PCR method was performed according to Zhu et al. (2012). For strain identification, the 16s rRNA gene (1500 bp) was amplified using universal primers for LAB 27 F: -AGAGTTTGATCCTGGCTCAG- and 1492 R: -GTTACCTTGTTACGACTT-

DNA sequence and phylogenetic analysis

The 16s rRNA gene sequence was analyzed for its similarity with the 16s rRNA nucleotide base sequence of other bacteria listed in the GenBank database organized by the National Center of Biotechnology Information (NCBI). This analysis was processed in the BLAST program (https://blast.ncbi.nlm.nih.gov). For the phylogenetic analysis sequence, the phylogenetic tree was constructed in the CLUSTALW program. The similarity between the nucleotide base sequences was analyzed by the neighbor-joining method with 1000x bootstrap in MEGA5 software.

Result and discussion

Isolation of moderate halophilic lactic acid bacteria

The *jambal roti* in this study was obtained from a traditional producer in Rembang, Central Java, Indonesia. *Jambal roti* was made of *Arius thalassinus* fish and prepared by salting (30%) and then sun-drying. The samples were physically collected on each day of fermentation (i.e., salting and drying process). Lactic acid bacteria were isolated using MRS media, which had 5% NaCl and 20% fish extract added, and left in an anaerobic condition. A total of eight pure isolates were obtained from three types

of samples (Table 1): day 1 salting (F1), day 2 of salting (F2), and product (after drying). All isolates were identified as lactic acid bacteria based on their morphological characteristics (rod, cocci, cocci tetrad, and cocci pair) and biochemical properties (Gram staining, catalase reaction, and type of fermentation). The characterization showed that all isolates were Gram-positive, rod-shaped, cocci-shaped (tetrad and in pair), and homofermentative and tested negative for catalase.

The morphological and biochemical characteristics of lactic acid bacteria

Based on the Gram staining analysis, all isolates were Gram-positive bacteria. According to the cell shapes, seven isolates were cocci and tetrad or pair (87.5%), and only one isolate was rod-shaped (Table 1). In other words, cocci in pairs and tetrads were the dominant bacteria in *jambal roti*. The biochemical characterization revealed that all isolates did not produce catalase (negative) and were fermented in a homofermentative nature. All isolates were qualitatively tested for protease using SMA, as performed by Prihanto et al. (2010) with modification. The proteolytic activity was measured from the diameter of the clear zone (R-value), which determines whether or not the observed isolate secretes protease. The test results showed that all pure isolates formed clear zones with varying R-values (Table 1), i.e., between 2.23 and 3.86 cm. Meanwhile, the quantitative analysis of protease had been performed by Baehaki and Rinto (2011). The results yielded values between 43.07 and 67.77 ppm. These figures indicate that all isolates can degrade protein from the product and potentially produce bioactive peptide.

The physiological characteristics of lactic acid bacteria

The results of LAB growth at various levels of pH, temperature, and NaCl are presented in Table 1. The isolates grew in media containing 2% and 6% NaCl but did not grow in media with higher NaCl concentrations (12%, 18%, and 20%), signifying moderate halophilic bacteria in the isolates. Udomsil et al. (2010) reported that halophilic lactic acid bacteria play an essential role in fish sauce fermentation. Since these isolates can grow in media containing 2% and 6% NaCl, they are categorized as potential probiotics for salty products.

These isolates could grow in the MRS media at various levels of pH such as 4.5, 5.65, and 6.45, while some can grow in media with pH 10.65 (strain number 307, 312, 328, 339). Rahayu (2003) stated that the genus *Pediococcus* survives at pH 8.5 and 4.2. Since all isolates grew at pH 4.6, 5.65, and 6.45, they are categorized as members of *Pediococcus*. While some selected isolates grew at a temperature of 37°C, others survived at 50°C (strain number 307, 315, and 332). These isolates belong to the Genus *Pediococcus*, and the ability to grow at 37°C categorizes them as lactic acid bacteria.

The phenotypic characteristics of isolates were determined using the API 50 CHL kit. Based on the quantitative and qualitative analysis of the protease activity, the isolates indicated as *Pediococcus pentosaceus 2* had 99.9% matched identity. All isolates promoted fermentation in various sugar sources, such as L-arabinose, D-ribose, D-galactose, D-glucose, D-mannose, D-fructose, L-rhamnose, N-acetyl glucosamine, Amygdalin, Arbutin, Esculin, Salicin, D-cellobiose, D-maltose, D-melibiose, D-saccharose, D-trehalase, D-raffinose, Gentobiose, D-tagatose, and 2-ketogluconate.

The phenotypic characteristics of lactic acid bacteria

It is not easy to differentiate the species among the Pediococci. The species were Gram-positive and catalase-negative tetrad cocci. They did not produce gas from glucose. They formed approximately equal quantities of L-(1)- and D-(2)-lactic acid, carbohydrate fermentation patterns, such as those of lactose, maltose, trehalose, and D-xylose. In addition, they could not be identified to the species level based on a phenotypic characteristic (Cai, 1999).

Phenotypic procedures are limited to differentiate easily and clearly the species of Pediococci, particularly *Pediococcus acidilactici* and *Pediococcus pentosaceus*. The phenotypic procedures are time-

Table 1. Characteristics of Lactic Acid Bacteria from *Jambal roti*.

	Strain no							
Characteristic	306	307	312	315	326	328	332	339
Origin	F1	F1	F2	F2	*Jambal roti*	*Jambal roti*	*Jambal roti*	*Jambal roti*
Cel form	cocci	rod	cocci	cocci	cocci	cocci	cocci	cocci
Cell arrangement	Pair/ tetrad	Single/chain	Pair/ tetrad	Pair/ tetrad	Pair/ tetrad	Pair/ tetrad	Pair/ tetrad	Pair/ tetrad
Catalase reaction	-	-	-	-	-	-	-	-
Type fermentation	Ho[1)]	Ho[1)]	Ho[1)]	Ho[1)]	Ho[1)]	Ho[1)]	Ho[1)]	Ho[1)]
Gram staining	+	+	+	+	+	+	+	+
Growth at 2% NaCl	+	+	+	+	+	+	+	+
Growth at 6% NaCl	+	+	+	+	+	+	+	+
Growth at 12% NaCl	-	-	-	-	-	-	-	-
Growth at 37°C	+	+	+	+	+	+	+	+
Growth at 50°C	-	+	-	+	-	-	+	-
Growth at pH 4.6	+	+	+	+	+	+	+	+
Growth at pH 5.65	+	+	+	+	+	+	+	+
Growth at pH 6.4	+	+	+	+	+	+	+	+
Growth at pH 10.65	-	+	+	-	-	+	-	+
Qualitative	3.86	3.03	2.83	2.26	3.10	3.23	3.03	2.23
Proteolytic activity (R value) (cm)	± 0.06	± 0.06	± 0.15	± 0.21	± 0.10	± 0.15	± 0.21	± 0.06
Quantitative Proteolytic activity(ppm)	53.73 ± 0.422	48.74 ± 0.106	62.62 ± 0.528	67.77 ± 0.633	63.97 ± 0.739	48.22 ± 0.211	45.61 ± 0.739	43.07 ± 0.317
Acid from carbohydrate [2)]								
Negative for	Glycerol. D-arabinose. D-xylose, D-mannitol. D-sorbitol, D-melezitose, starch, D-xylose, gluconate, xylitol							
Negative for	L-arabinose, D-ribose, D-galactose, D-glucose, D-mannose, D-fructose, L-rhamnose, N-acetyl glucosamine, Amygdalin, Arbutin, Esculin, Salicin, D-cellobiose, D-maltose, D-melibiose, D-saccharose, D-trehalase, D-raffinose, Gentobiose, D-tagatose, and 2-ketogluconate.							
Suspected genus/species	*Pediococcus pentosaceus*	*Lactobacillus*	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*

Note: [1)] Homofermentatif: [2)] analyzed using API 50 CHL kit

consuming, laborious, and imprecise, and this has hampered distinction of field isolates for taxonomic purposes. Therefore, although detailed taxonomic studies on Pediococci date back to 1884 from Garvie (1986), there is still a lack of precise and rapid procedures for clearly identifying an isolate belonging to this genus to the species level (Nigatu et al. 1998).

The amplification of repetitive PCR

Repetitive element sequence-based PCR (rep-PCR) is a new typing method that differentiates microbes by using primers complementary to interspersed repetitive consensus sequences that enable amplification of diverse-sized DNA fragments consisting of sequences between the repetitive elements. The repetitive PCR generates DNA fingerprints, which allow the identification of bacterial strains. Repetitive PCR refers to the general methodology that involves the use of oligonucleotide primer based on the short repetitive sequence element dispersed through the bacterial genome (Spigaglia and Mastrantonio 2003).

The results of the repetitive PCR amplification (Figure 1) showed three types of bands, meaning that the eight isolates belong to three types of band profiles. The band of strain number 306 was the same as that of strain number 315 and 332 (Rep PCR type 1), indicating that these isolates come from the same group of bacteria. The same case applied to the strain number 312 that shared the same band as strain number 326, 328, and 339 (Rep PCR type 2). All isolates were identified using 16s rRNA gene sequence.

According to the identification using morphological, biochemical, and phenotypic characterization, all isolates were *Pediococcus pentosaceus*. However, the identification using 16s rRNA gene sequence showed that three isolates were *Pediococcus acidilactici*, four isolates were *Pediococcus pentosaceus*, and one isolate was *Lactobacillus plantarum*. The result showed that the molecular identification (using 16s rRNA gene sequence) was more sensitive to identify the strain of microorganism because it was based on the DNA profile.

The 16s rRNA gene sequence and phylogenetic analysis

The PCR product of the isolates showed the formation of a single band (Figure 1), which indicates that the 16s rRNA gene sequence has successfully amplified PCR at 1500 bp. The results of the sequencing of all isolates were compared with the 16s rRNA gene sequence listed in the GenBank database organized by NCBI (Table 2).

Table 2 shows that strain number 307 had a genetic similarity of 99.86% with *Lactobacillus plantarum* strain JCM 1149, and the relationship between them is described in the phylogenetic trees (Figure 2). This isolate is in the same branch as *Lactobacillus plantarum* strain JCM1149. The morphological, biochemical, and physiological characteristic analysis (Table 1) showed that the isolate strain number 307 was suspected as *Lactobacillus*.

Strains 306, 315, and 332 shared a genetic similarity with *Pediococcus acidilactici* strain DSM 20284 at 99.65% (306), 99.21% (315), and 99.44% (332). Morphological, biochemical, phenotypic, and physiological characteristic analysis (Table 1) showed that the isolate strain was suspected as *Pediococcus acidilactici*. The result of identification was different because the identification using 16s rRNA gene sequence was more sensitive and valid compared to the morphological, biochemical, and

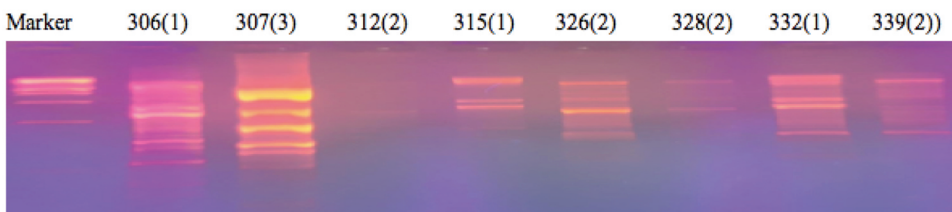

Figure 1. DNA band-profile produced by repetitive PCR amplification selected LAB.

Table 2. Identification of selected LAB from *Jambal roti* by 16s rRNA gene sequence.

Strain no	Reference from NCBI data base	Similarity %	Reference
306 (Rep PCR type 1)	*Pediococcus acidilactici* strain DSM 20284	99.65%	NR 042057.1
307(Rep PCR type 3)	*Lactobacillus plantarum* strain JCM 1149	99.86%	NR 115605.1
312 (Rep PCR type 2)	*Pediococcus pentosaceus* strain DSM 20336	99.44%	NR 042058.1
315 (Rep PCR type 1)	*Pediococcus acidilactici* strain DSM 20284	99.21%	NR 042057.1
326 (Rep PCR type 2)	*Pediococcus acidilactici* strain DSM 20284	99.44%	NR 042057.1
328 (Rep PCR type 2)	*Pediococcus pentosaceus* strain DSM 20336	99.65%	NR 042058.1
332 (Rep PCR type 1)	*Pediococcus pentosaceus* strain DSM 20336	99.51%	NR 042058.1
339(Rep PCR type 2)	*Pediococcus pentosaceus* strain DSM 20336	99.49%	NR 042058.1

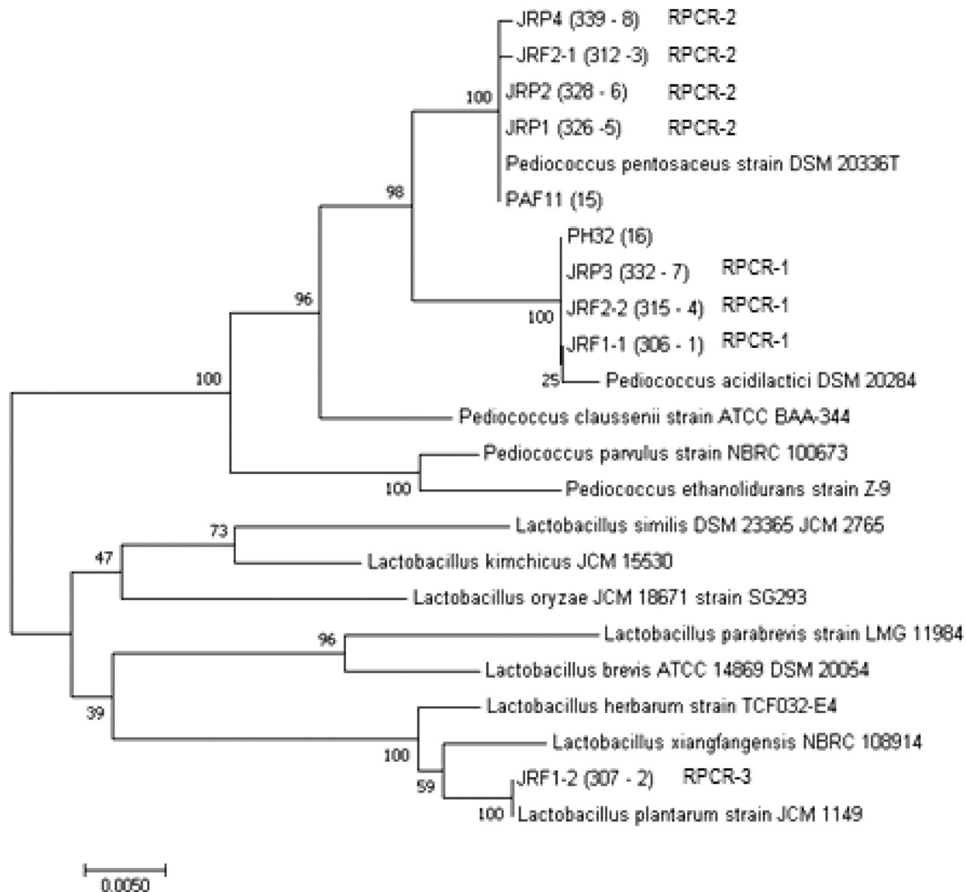

Figure 2. Neighbor-joining phylogenetic tree of selected LAB based on 16s rRNA gene sequence.

phenotypic identification. The identification using 16s rRNA was based on DNA profile of microorganism.

Strains 312, 326, 328, and 339 had a genetic similarity with *Pediococcus pentosaceus* strain DSM 20336 at 99.44% (312), 99.56% (326), 99.51% (328), and 99.49% (339). The result was the same for morphological, biochemical, phenotypic, and physiological characteristic analysis (Table 1).

Pediococcus acidilactici plays a role in the fermentation of *bekasang* (Lawalata and Dan Satiman 2015), while *Lactobacillus plantarum* in the fermentation of *plaa-som* (Kopermsub and Yunchalard, 2010) and *bekasam* (Wikandari et al. 2012).

Identification using API 50 CHL kit (phenotypic characterization) relies on the growth of isolates in 49 types of carbon source. According to Suhartatik (2014), basic molecular identification is more accurate than morphological and biochemical tests and analysis of isolate growth on 49 sources of carbon.

There have been many studies on LAB identification in fermented fish. For instance, *Tetragenococcus halophillus* in fish sauce (Udomsil et al. 2010), *Lactococcus garvieae, Pediococcus pentosaceus, Lactobacillus plantarum*, and *Lactobacillus fermentum* in *plaa-som* (Kopermsub and Dan Yunchalard 2010), *Lactobacillus plajomi* sp. nov. in *pla-jom* (Miyashita et al., 2015), and *Lactobacillus* and *Pediococcus* in *momone*, fermented fish from Ghana (Anihouvi et al., 2012). Wikandari et al. (2012) successfully identified indigenous LAB from *bekasam, Lactobacillus plantarum, Lactobacillus pentosus*, and *Pediococcus pentosaceus*, all of which have inhibitory properties against angiotensin-converting enzyme (ACE) and are useful for antihypertensive treatment. Wikandari and Yuanita (2014) claimed that *L. plantarum* B1765 extracted from *bekasam* is a culture starter that has a high potential as antihypertensive (as tested *in-vivo* in hypertensive rats). They also explained that the higher the concentration of peptide, the more significant the reduction of systolic blood pressure. Karparvar et al. (2019) identified two indigenous lactic acid bacteria in *mahyaveh* (fermented fish from Iran), *Lactobacillus plantarum* and *Enterococcus faecium*, and concluded that since the LAB strains are prevalent in *mahyaveh*, they act as a starter in *mahyaveh* production. Rahayu (2003) suggested that *Lactobacillus, Pediococcus, Streptococcus, Enterococcus*, and *Leuconostoc* can be found in salted fish in Indonesia. According to this research, the indigenous LAB isolated from *jambal roti* are *Lactobacillus plantarum, Pediococcus acidilactici*, and *Pediococcus pentosaceus*.

Conclusions

There are three types of lactic acid bacteria identified as indigenous moderate halophilic in *jambal roti*. Those isolates have high proteolytic activities. The identification was performed based on morphological, biochemical, phenotypic, and physiological characteristic analysis and 16s rRNA gene sequence. The isolates are *Lactobacillus plantarum, Pediococcus acidilactici*, and *Pediococcus pentosaceus*, which are all moderate halophilic. Considering their proteolytic activity, these isolates have potential to be applied as bioactive peptides in *jambal roti* and other fermented fish.

Disclosure statement

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