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Characteristics of Lactic Acid Bacteria isolated from traditional fermented fish

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Abstract. *Novia R, Ardiningsih P, Adhityawarman, Sarwiyati. 2022. Characteristics of Lactic Acid bacteria isolated from traditional fermented fish. Biodiversitas 23: 5662-5669.* Lactic acid bacteria (LAB) are commonly involved in most food fermentation and contribute to the process's quality and safety. The study investigates the characteristics of LAB isolated from *buduk*, *pekasam*, and *pekasam ale-ale*. Twelve colonies each sample were isolated and identified based on morphology and biochemical test. The *pekasam ale-ale* isolates were identified as *Pediococcus* sp. strain A1 and *Pediococcus* sp. strain A3; *Enterococcus* sp. strain A2 and *Enterococcus* sp. strain A4. The LAB in *buduk* were identified as *Streptococcus* sp. strain B1, *Pediococcus* sp. strain B2 and *Pediococcus* sp. strain B4, *Enterococcus* sp. strain B3. The *pekasam* isolates were identified as *Pediococcus* sp. strain P1, *Streptococcus* sp. strain P2 and *Streptococcus* sp. strain P4, *Lactobacillus* sp. strain P3. The LAB showed antimicrobial activities while *Lactobacillus* sp. strain P3 and *Streptococcus* sp. strain P4 could grow at pH 1. Two LAB including *Enterococcus* sp. strain A2 and *Pediococcus* sp. strain P1 showed amylase activity while *Pediococcus* sp. strain A3, *Streptococcus* sp. strain B1, *Pediococcus* sp. strain B2, *Enterococcus* sp. strain B3 showed protease activity. All LAB produced organic acids with varying concentrations at different incubation times. Furthermore, those isolated from various fish fermentations showed different genera and characteristics. *Lactobacillus* sp. strain P3 was the best to initiate food fermentation and a probiotic candidate based on their characteristics.

Keywords: *Buduk*, fish fermentation, Lactic acid bacteria, *pekasam*, *pekasam ale-ale*

INTRODUCTION

Indonesia is an archipelagic country with high marine biodiversity and fish abundance. Compared to other foodstuffs, seafood products can be preserved by fermentation, which is popular in Southeast Asian countries such as Indonesia. Fermented fish foods are made in the country using various foodstuffs and traditional formulas. These include chao, wadi, peda, *pekasam ale-ale*, *buduk* and *pekasam* (Nofiani et al. 2010; Nofiani and Ardiningsih 2018; Nofiani et al. 2019; Nurhikmayani et al. 2019).

Traditional fermented food, such as cheese, fish sauce, tempoyak, *buduk*, cincalok, usually contains lactic acid bacteria (LAB), which produce various compounds that are probably important for fermentation quality and health. Lactic acid and other organic acids produced by LAB during fermentation can decrease pH, which limits the spoilage microflora. Furthermore, bacteriocins were produced to inhibit or kill food spoilage or pathogenic microorganisms in addition to pH (Thomas 2018). Antifungal or anti-mycotoxigenic compounds produced by LAB can inhibit fungal growth or absorb mycotoxins (Dalié et al. 2010). Therefore, LAB can be used as bio preservative for meat products (Barcenilla et al. 2022). Some compounds produced by its metabolism contribute to the development of flavour fermentation, such as diacetyl, acetoin, acetaldehyde, or acetic acid (Jobby et al. 2020) as baijiu (Pang et al. 2021), artisanal white cheese (Albayrak and Duran 2021). A hydrolytic enzyme such as amylase secreted by these bacteria, is beneficial in the gastrointestinal tract of chickens and mammals (pigs, horses, rabbits, and humans). It can also be applied in the industry, including medical, clinical fields and chemistry (Padmavathi et al. 2018). Some LAB exhibit probiotics or other activities, for example reducing cholesterol, that confer a health benefit on the host (Jobby et al. 2020). Conversely, LAB can probably produce biogenic amine compounds that can be toxic to humans (Barbieri et al. 2019).

Bacteria producing lactic acid as the primary or sole product of fermentative metabolism are grouped as LAB (Orla-Jensen 1919; Nikita and Hemangi 2012). Homofermentative LAB produce only lactic acid, while the heterofermentative produces other acids as the end product in metabolisms (Endo and Dicks 2014). Other LAB characteristics are Gram-positive, catalase-negative, usually non-motile, non-sporulating bacteria, and no gas production from glucose. Furthermore, they can be grown at different temperatures (i.e. 15ºC and 45ºC), and different NaCl concentrations (i.e. 2%, 4% and 6.5%) can be used to distinguish different genera (Garvie 1984; Devriese et al. 1995). Based on these characteristics, LAB are a heterogeneous group consisting of different genera such as *Aerococcus*, *Atopobium*, *Bifidobacteria*, *Brochothrix*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Weissella*, *Vagococcus* (Kalschne et al. 2015).

Genus and characteristic LAB contained in the food fermentation vary depending on foodstuff flora and process conditions. *Buduk*, *pekasam* and *pekasam ale-ale* were traditional fish fermentations that contain different LAB genera and characteristics. *Buduk* is a sauce prepared by fermenting fresh small fish, sugar, roasted rice, vinegar, or wine (optional) for seven days at room temperature (Nofiani et al. 2019). *Pekasam* is prepared by fermenting various small freshwater fish with salt in a certain ratio and incubated at room temperature for 7-10 days. Furthermore, *pekasam ale-ale* or ale-ale is prepared through fermentation of fresh ale-ale (*Meritrix meritrix*) flesh with a certain concentration of salt, porridge rice or angkak for seven days (Nofiani et al. 2010). Therefore, this study aims to investigate the genus and characteristics of the LAB isolated from *buduk*, *pekasam* and *pekasam ale-ale*. The identification was conducted by comparing the morphological and biochemical characteristics between the LAB isolates and the standard based on Bergey's Manual of Systemic Bacteriology (Holt et al. 1994). The isolates were characterized namely enzyme activities, antimicrobial activities, acid tolerance (AT), acid production ability, biogenic amine formation.

MATERIALS AND METHODS

Sampling

The *buduk* and ale-ale samples were obtained from a traditional market, Flamboyan, Pontianak City. West Kalimantan, Indonesia. *Pekasam* sample was collected from a home industry at Jungkat, Mempawah District, West Kalimantan, Indonesia.

Isolation and Identification of the LAB

Using a blender, a suspension sample was prepared by homogenizing the sample and buffer saline. Furthermore, 100 µL of it was spread onto peptone yeast glucose agar (PYGA) medium and then incubated using an anaerobic incubator at room temperature for four days. Each colony was transferred into a new medium until a pure form was obtained. The isolates predicted as LAB were identified based on Bergey's Manual of Systemic Bacteriology (Holt et al. 1994). Each bacterial strain was determined by Gram staining, catalase, motility, growth tests with different NaCl concentrations of 2%, 4% and 6.5%, growth tests with varying temperatures of 15ºC and 45ºC, and gas CO_2 - production from glucose. Gram staining was performed based on Gram's procedure (Harley and Prescott 2002). The catalase test, each LAB colony was suspended and smeared the suspension on a clean slide, then dripped with 3% of hydrogen peroxide, as much as 1-2 drops (Harley and Prescott 2002). The bubbles on the colony surface showed a positive catalase. Each LAB was stabbed into a MRS semisolid slant agar and incubated using an anaerobic incubator at 30°C for 48 hrs (Kajikawa et al. 2016). The colony growing and spreading on the surface of the medium indicate that the bacteria were motile. Each LAB was inoculated into a MRS semisolid slant agar supplemented with different NaCl concentrations of 2%, 4% and 6.5% and incubated using an anaerobic incubator at 37°C for 24 h (Harley and Prescott 2002). They were also inoculated into a MRS semisolid slant agar and incubated at different temperatures at 15ºC and 45ºC (Nikita and Hemangi 2012). The colony that appeared for both conditions showed NaCl and temperature tolerance. The gas production from glucose was tested by inoculating each isolate to a tube containing MRS broth supplemented with 1% glucose which was placed in an inverted Durham tube and incubated at 30°C for five days (Kajikawa et al. 2016). The positive test showed a bubble on the top of the Durham tube.

Enzyme activities

Enzyme activities of each LAB were determined on MRS agar supplemented with 1% amylum for an amylase activity test, MRS agar supplemented with 1% of casein for protease activity test, and MRS agar supplemented with tributyrin 2% for lipase activity test (Jini et al. 2011). Furthermore, they were incubated under an anaerobic condition at 37°C for four days. A clear zone around the colonies showed enzyme activities.

Antimicrobial activity

Cell-free supernatant (CFS) was prepared by inoculating the LAB colony into MRS broth medium. The bacteria culture was centrifuged after incubating using an anaerobic incubator at 37°C for 48 hrs to remove its cells and filtered using a 0.22 µm filter. Each bacterial strain was inoculated into 10 mL of nutrient broth and incubated at 200 rpm, 37°C, for 12-14 hrs. A 0.5 mL bacterial culture test was thoroughly mixed with 15 mL of warm nutrient agar medium and then poured into a 9 cm diameter Petri dish. The medium was punched with a tool of 5 mm diameter after solidification to make a well. A 50 µL of the CFS was dispensed slowly into the well, and a laminar flow was let in until the solvent evaporated, then incubated at 37°C for 24 hrs. A clear zone around the well indicated positive antimicrobial activity was measured for diameter.

Acid tolerance

Acid tolerance was tested following the procedure by Gotcheva et al. (2002). A colony of LAB was inoculated into MRS broth, incubated using an anaerobic incubator at 37°C for 24 hrs and harvested by centrifugation to collect the cells which were washed three times using phosphate buffer saline (PBS [phosphate buffer saline consisting of phosphate buffer 0.1 M, NaCl 0.8%, pH 7,2). The cells were resuspended with a ratio of 1: 100 in PBS with various pH of 1, 2, and 3 then incubated at different times 2 hrs and 4 hrs at 37ºC.

Acid production ability

Acid production ability was measured by decreasing the pH culture of the culture. Each LAB colony was inoculated into MRS broth at pH 6 then incubated using an anaerobic incubator for 24 hrs to obtain a starter culture. The starter culture was inoculated into MRS broth (pH 6.5) to obtain an optical density at 600 nm of 0.05 and then incubated. Furthermore, the pH was measured using a pH meter at hours 4, 6, 8, 12, 24 and 48. The data were presented as mean ± standard deviation, and the variance between the

group was analysed using a Tuckey with a 95% ($p<0.05$) confidence level. Finally, its analysis was analysed using the IBM® Statistics 26.

Screening of biogenic amine formation

Biogenic amine formation was screened using an improved medium from Bover-Cid and Holzapfel's procedure (Bover-Cid and Holzapfel 1999). A colony of each LAB was inoculated on the improved medium and incubated at 47°C for four days. Finally, positive LAB produced biogenic amines when the medium colour changed from yellow to violet.

RESULTS AND DISCUSSION

Isolation and characterization of Lactic Acid Bacteria

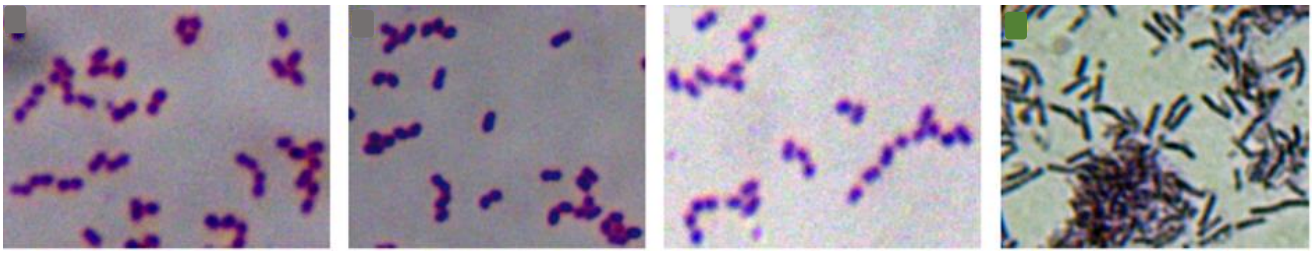
Most fish fermentations involve LAB such as peda which contain *Lactobacillus* and *Pediococcus*; terasi containing *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus*; salted fish and shrimp containing *Lactobacillus* and *Streptococcus*; wadi with *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus* (Endang 2018). This study screened *buduk*, *pekasam ale-ale* and *pekasam* were screened for LAB candidates using a selective medium, such as PYGA supplemented with $CaCO_3$. Ninety-eight LAB candidates appeared on the medium with a clear zone around each colony and were isolated (Figure 1). Each colony was selected based on the morphology and position in the medium, Gram staining and catalase test. Those with Gram-positive and catalase-negative characteristics were indicated as LAB colonies. Four from each sample of *pekasam ale-ale* [coded "A"], *buduk* [coded "B"] and *pekasam* [coded "P"]) were further characterized to identify their genus.

The morphological and biochemical characteristics of LAB were Gram-positive and catalase-negative, no motile, had good growth at 45°C, 2% and 4% of NaCl, and produced CO_2 from glucose and homofermentative fermentation (Holt et al. 1994; Nikita and Hemangi 2012). *Enterococcus* sp. strain A2, *Enterococcus* sp. strain A4, *Enterococcus* sp. strain B3, *Pediococcus* sp. strain A3, and *Pediococcus* sp. strain B2 could grow at 15°C and in 6.5% of NaCl (Table 1). Gram staining results of each isolate were observed for the morphological cells of each isolate. Eleven of twelve isolated LAB showed different coccus shapes; and only one was a rod shape. Finally, the shape of the coccus was chain-like, short-chain, diplococci, and tetrad cocci (Figure 2).

The cell shapes of the genus *Enterococcus* are pair and short-chain cocci similar to *Lactococcus* (Du Toit et al. 2014). Genus *Enterococcus* can grow up to 6.5% of NaCl, while *Lactococcus* generate approximately 4% (Schillinger and Lücke 1987; Du Toit et al. 2014). Furthermore, it can distinguish between the genera *Enterococcus* and *Lactococcus*. Isolates A2, A3, A4, B2 and B3 showed pair and small chain cocci and could grow up to 6.5% NaCl (Figure 2; Table 1). Therefore, isolates A2, A3, A4, B2 and B3 were identified as *Enterococcus* sp. strain A2, *Enterococcus* sp. strain A3, *Enterococcus* sp. strain A4, *Enterococcus* sp. strain B2 and *Enterococcus* sp. strain B3.

A **B** **C** **D**

Figure 1. A. *Buduk*; B. *Pekasam*. C. *Pekasam ale-ale*. D. LAB colonies in PYGA



A **B** **C** **D**

Figure 2. Cell shapes of the LAB colonies were observed under the light microscope with 1000 x magnification. A. Diplococci and short-chain shape of isolate P2; B. Diplococci and tetrad shape of isolate B2; C. Diplococci and short-chain shape isolate B3; D. Rod shape of isolate P3

Table 1. Characteristics of the LAB isolates

Isolate	Gram staining	Cell shape	Catalase	Motility	Growth at different temp.		Grow in NaCl			Gas production from glucose	Fermentation
					15 °C	45 °C	2%	4%	6.5%		
Pediococcus sp. strain A1	+	c	-	-	±	+	+	+	-	-	m
Enterococcus sp. strain A2	+	c	-	-	+	+	+	+	+	-	m
Pediococcus sp. strain A3	+	c	-	-	+	-	+	+	+	-	m
Enterococcus sp. strain A4	+	c	-	-	+	+	+	+	+	-	m
Streptococcus sp. strain B1	+	c	-	-	+	+	+	+	-	-	m
Pediococcus sp. strain B2	+	c	-	-	+	-	+	+	+	-	m
Enterococcus sp. strain B3	+	c	-	-	+	+	+	+	+	-	m
Pediococcus sp. strain B4	+	c	-	-	+	+	+	+	-	-	m
Pediococcus sp. strain P1	+	c	-	-	+	+	+	+	-	-	m
Streptococcus sp. strain P2	+	c	-	-	+	+	+	+	-	-	m
Lactobacillus sp. strain P3	+	r	-	-	+	+	+	+	-	-	m
Streptococcus sp. strain P4	+	c	-	-	+	+	+	+	-	-	m

Note: c. coccus; r. rod; ±. Slow growth; +. Positive; -. negative. m. homofermentative fermentation.

Genus *Streptococcus* is Gram-positive, catalase-negative cocci, grow under facultatively aerobic conditions, produce L-(+)- lactic acid, and undergo homofermentative fermentation, hence, they do not produce CO_2 from glucose (Du Toit et al. 2014). Isolates B1, P2 and P4 showed similar characteristics to the genus *Streptococcus* (Table 1). Furthermore, their morphology showed diplococci and chain-forming cocci, which indicated the genus *Streptococcus* (Figure 2A). Therefore, isolates B1, P2, and P4 were probably identified as *Streptococcus* sp. strain B1, *Streptococcus* sp. strain P2, and *Streptococcus* sp. strain P4.

Isolates A1, A3, B2, B4 and P1 showed diplococci and tetrad shapes. The tetrad shape is a specific characteristic of LAB from the genus *Pediococcus* (Du Toit et al. 2014). Its other characteristics were Gram-positive, catalase-negative, oxidase-negative, grow under facultatively aerobic to microaerophilic conditions, produce lactic acid, lack of CO_2 production from glucose, and inability to reduce nitrate (Weiss 1992; Franz et al. 2014). Furthermore, *Pediococcus* sp. can detect food fermentation such as tape (Du Toit et al. 2014). Therefore, isolates A1, A3, B2, B4 and P1 were probably identified as *Pediococcus* sp. strain A1, *Pediococcus* sp. A3, *Pediococcus* sp. strain B2, *Pediococcus* sp. strain B4 and *Pediococcus* sp. strain P1.

The cell shapes of the genus *Enterococcus* are pair and short-chain cocci similar to *Lactococcus* (Du Toit et al. 2014). Genus *Enterococcus* can grow up to 6.5% of NaCl, while genus *Lactococcus* can grow NaCl to approximately 4% (Schillinger and Lücke 1987; Du Toit et al. 2014). Growth in different NaCl concentrations can distinguish between the genera *Enterococcus* and *Lactococcus.* Isolate A2, A3, A4, B2 and B3 showed pair and small chain cocci and could grow to 6.5% of NaCl. Therefore, they were identified as *Enterococcus* sp. strain A2, *Enterococcus* sp. strain A3, *Enterococcus* sp. strain A4, *Enterococcus* sp. strain B2 and *Enterococcus* sp. strain B3.

The genus *Streptococcus* characteristics are Gram-positive, catalase-negative cocci, grow under facultatively aerobic conditions, produce L-(+)- lactic acid, homofermentative, hence, they do not produce CO_2 from glucose (Du Toit et al. 2014). Isolates B1, P2 and P4 showed similar characteristics and shapes, including diplococci and chain-forming cocci, as well as a similar shape to *Streptococcus* standard (Table 1). Therefore, they were probably identified as *Streptococcus* sp. strain B1, *Streptococcus* sp. strain P2, and *Streptococcus* sp. strain P4.

Isolates A1, A3, B2, B4 and P1 showed diplococci and tetrad shapes. The tetrad shape is a specific morphology of a LAB from the genus *Pediococcus* (Du Toit et al. 2014). The other characteristics of *Pediococcus* sp. were Gram-positive, catalase-negative and oxidase-negative, facultatively aerobic to microaerophilic conditions, lactic acid production, lack of CO_2 production from glucose, and cannot reduce nitrate (Franz et al. 2014). Therefore, isolates A1, A3, B2, B4 and P1 were identified as *Pediococcus* sp. strain A1, *Pediococcus* sp. strain A3, *Pediococcus* sp. strain B2, *Pediococcus* sp. B4 and *Pediococcus* sp. strain P1. *Pediococcus* sp. can detect on food fermentation such as tape (Du Toit et al. 2014).

Enzyme activities

Enzyme activities are important to decrease fermentation time in certain food fermentation. α-Amylases (EC 3.2.1.1) are starch-degrading enzymes capable of hydrolyzing α-1,4 glycosidic bonds of polysaccharides including starch fermentation by LAB (Padmavathi et al. 2018). Lipase is an enzyme that hydrolyze triacylglyceride to diacylglyceride, monoglycerides, fatty acid and glycerol. Lipase can develop a unique aroma during food fermentation, such as fermented milk (Shojaei Zinjanab et al. 2021), a fish fermented, *shuanzhayu* (Jiang et al. 2021). Protease activity contribute to protein degradation by causing flesh breakdown in fish fermentation. Some products such as fermented fish sauce and *buduk* need protease to break down protein from flesh. Meanwhile, some fish fermentation does not allow fish flesh degradation such as cincalok and *pekasam ale-ale*. The fermentation of silver carp inoculated with *Lactobacillus plantarum* can increase aspartic acid, glutamic acid, and alanine, resulting in umami and sweet taste (Yang et al. 2016).

The enzyme activities tested in this study were amylase, lipase and protease. This study detected no lipase activity on the LAB (Table 2). *Enterococcus* sp. strain A2 from *pekasam ale-ale* and *Pediococcus* sp. strain P1 from *pekasam* showed amylase activity. Three and one of four LAB isolated from *pekasam* showed protease activity, respectively, exhibited protease activity.

Antimicrobial activities

Antimicrobial activities can be used against enteropathogens in the intestine. Most LAB produce organic acids, hydrogen peroxide, bacteriocins, and other compounds contributing to antimicrobial activity (Rachmawati et al. 2006; Ayantola and Oladunmoye 2015). Those with this parameter have an important characteristic of a probiotic bacterial candidate (Vantsawa et al. 2017). Based on the well-diffusion method, the LABs showed antimicrobial activities against 13 of the tested microbes. *Enterococcus* sp. strain A2, *Enterococcus* sp. strain B3, *Lactobacillus* sp. strain P3, *Pediococcus* sp. strain P1 and *Streptococcus* sp. strain P4 exhibited the best result against the microbial test (Table 3). Finally, the LAB isolated from the *pekasam* dominated as the best antimicrobial producer.

Acid tolerance

The acid tolerance of LAB is caused by various mechanisms such as central metabolic pathways (arginine dihydrolase system (ADS), amino acid decarboxylation, malolactic fermentation), proton pump, changes in cell membrane composition and cell density, DNA and protein damage repair, and neutralization processes (Grandgirard et al. 2002; Wang et al. 2018). The ADS can catalyze the conversion of arginine into ornithine, ammonia and carbon dioxide. The ammonia neutralizes protons in the cell to maintain intracellular pH homeostasis. LAB, including *Lactobacillus lactis, Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus rhamnosus* secrete high F1-F0-Atpase concentration to adapt acid tolerance. LAB membrane fluidity and proportion of unsaturated fatty acid are higher to enhance acid tolerance, such as in *Lactobacillus casei* (Wu et al. 2012). Their survival was determined using an acid tolerance test under low pH gastric juice conditions before reaching the intestinal tract. The pH value used in this study was from 1 to 3 because stomach acidity varies among individuals. Twelve isolates survived at pH 7.2 (control) and 3 (Table 4). *Streptococcus* sp. strain P2, *Streptococcus* sp. strain P4 and *Lactobacillus* sp. strain P3 survived at pH 1 after 2 hrs of incubation. However, all LAB survived after 4 hrs of incubation except *Enterococcus* sp. strain B3 dan *Streptococcus* sp. strain P2 (Table 4). The best LAB-resistant acid was *Lactobacillus* sp. strain P3 is based on the survival LAB total at pH 1, 2, and 3. The LAB that survived in the acid condition indicated a resistant acidic and probably considered probiotic candidates. *Leuconostoc citreum* 344, *Lactobacillus brevis* 183, *Leuconostoc mesenteroides* 348 and *Lactobacillus plantarum* 327 showed resistant acid condition (Grosu-Tudor and Zamfir 2012).

Table 2. Enzyme activities of LAB isolates

Isolate	Enzyme activities		
	Amylase	**Lipase**	**Protease**
Pediococcus sp. strain A1	-	-	-
Enterococcus sp. strain A2	+	-	-
Pediococcus sp. strain A3	-	-	+
Enterococcus sp. strain A4	-	-	-
Streptococcus sp. strain B1	-	-	+
Pediococcus sp. strain B2	-	-	+
Enterococcus sp. strain B3	-	-	+
Pediococcus sp. strain B4	-	-	-
Pediococcus sp. strain P1	+	-	-
Streptococcus sp. strain P2	-	-	-
Lactobacillus sp. strain P3	-	-	-
Streptococcus sp. strain P4	-	-	-

Table 3. Antimicrobial activities of the LAB CFS (50 µL/well)

Isolate	Diameter of a clear zone (mm)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Pediococcus sp. strain A1	11.22	-	5.95	5.37	-	3.89	7.24	-	8.99	7.64	3.12	-	8.07
Enterococcus sp. strain A2	5.39	7.49	12.11	7.81	11.01	7.31	15.89	11.88	4.09	9.54	6.62	6.91	8.91
Pediococcus sp. strain A3	6.85	-	5.71	4.93	-	4.22	17.62	-	8.73	9.06	5.89	-	8.96
Enterococcus sp. strain A4	7.39	-	5.84	7.46	8.81	6.16	15.99	15.03	14.84	9.39	6.01	6,67	10.69
Streptococcus sp. strain B1	4.64	-	-	9.09	-	4.41	19.09	-	4.41	9.93	4,59	-	8.03
Pediococcus sp. strain B2	8.55	-	9.18	9.05	-	6.56	12.34	8.77	12.43	6.65	6.45	1.57	6.78
Enterococcus sp. strain B3	7.26	7.49	9.33	7.54	7.35	6.69	18.76	11.79	6.78	7.49	6.21	5.32	8.71
Pediococcus sp. strain B4	4.76	-	8.29	8.82	-	4.55	13.41	-	10.89	6.39	6.09	-	5.37
Pediococcus sp. strain P1	7.95	4.11	7.09	10.75	8.69	7.03	10.84	7.72	13.68	6.19	5.83	5.42	8.01
Streptococcus sp. strain P2	9.09	-	5.84	12.08	-	6.14	16.33	14.81	12.14	8.12	7.09	8.34	9.83
Lactobacillus sp. strain P3	6.29	7.76	11.01	9.72	11.14	7.75	21.09	9.76	7.67	7.67	7.56	4.09	9.49
Streptococcus sp. strain P4	6.32	7.10	9.40	8.47	9.94	10.08	16.61	10.34	5.01	7.39	6.24	6.34	9.29

Note: 1. *A. hydropila;* 2. *Enterobacter* sp.; 3. *S. aureus*; 4. *B. cereus*; 5. *V. cholerae*; 6. *Bacillus* sp.; 7. *K. pneumoniae*; 8. *L. monocytogene*; 9. *C. freundii*; 10. *C. albicans*; 11. *B. subtilis*; 12. *E.* coli; 13. *Salmonella* sp.; -. Inactive.

Table 4. Acid tolerance characteristics of the LAB

Isolate	Colony average, log CFU/g							
	pH 7.2		pH 1		pH 2		pH 3	
	2 hours	4 hours	2 hours	4 hours	2 hours	4 hours	2 hours	4 hours
Pediococcus sp. strain A1	9.670	9.571	0	9.019	9,339	8,637	9,396	9,548
Enterococcus sp. strain A2	8.884	9.820	0	7.637	5,608	0	9,753	9,498
Pediococcus sp. strain A3	9.396	9.538	0	7.283	8,941	7,760	7,212	5,155
Enterococcus sp. strain A4	8,900	9.169	0	6.155	8,928	4,456	9,373	8,997
Streptococcus sp. strain B1	9.394	9.458	0	9.735	0	1,155	9,592	9,349
Pediococcus sp. strain B2	9.684	9.673	0	9.050	1,269	7,479	9,656	9,784
Enterococcus sp. strain B3	9.533	9.509	0	0	0	0	9,085	8,894
Pediococcus sp. strain B4	9.419	9.501	0	7.155	5,274	4,791	9,481	9,776
Pediococcus sp. strain P1	9.206	9.503	0	5.854	7,765	0	9,623	8,900
Streptococcus sp. strain P2	9.349	9.605	9.651	0	9,830	7,263	9,698	8,256
Lactobacillus sp. strain P3	9.813	9.728	5.850	9.136	7,964	9,677	9,543	9,738
Streptococcus sp. strain P4	9.269	9.046	3.632	7.465	8,530	9,528	8,746	9,104

Table 5. pH during LAB growth

Strain	pH					
	4 hours	6 hours	8 hours	12 hours	24 hours	48 hours
Pediococcus sp. strain A1	5.22 ± 0.13^{a}	5.01 ± 0.06^{ab}	4.75 ± 0.06^{bc}	4.48 ± 0.05^{c}	4.32 ± 0.13^{cd}	4.12 ± 0.14^{d}
Enterococcus sp. strain A2	5.30 ± 0.09^{a}	4.89 ± 0.03^{b}	4.63 ± 0.07^{c}	4.40 ± 0.05^{d}	4.08 ± 0.07^{e}	3.89 ± 0.08^{f}
Pediococcus sp. strain A3	5.08 ± 0.07^{a}	4.71 ± 0.13^{b}	4.57 ± 0.09^{bc}	4.42 ± 0.03^{c}	4.09 ± 0.06^{d}	4.01 ± 0.06^{d}
Enterococcus sp. strain A4	5.12 ± 0.04^{a}	4.74 ± 0.10^{b}	4.56 ± 0.07^{c}	4.44 ± 0.01^{cd}	4.10 ± 0.06^{d}	3.93 ± 0.08^{d}
Streptococcus sp. strain B1	5.15 ± 0.02^{a}	4.74 ± 0.09^{b}	4.59 ± 0.09^{bc}	4.41 ± 0.09^{c}	4.19 ± 0.10^{d}	3.97 ± 0.04^{e}
Pediococcus sp. strain B2	5.20 ± 0.10^{a}	4.78 ± 0.06^{b}	4.56 ± 0.05^{c}	4.43 ± 0.02^{c}	4.11 ± 0.05^{d}	3.84 ± 0.07^{e}
Enterococcus sp. strain B3	5.16 ± 0.06^{a}	4.77 ± 0.09^{b}	4.58 ± 0.06^{c}	4.42 ± 0.02^{c}	4.08 ± 0.07^{d}	3.99 ± 0.06^{d}
Pediococcus sp. strain B4	5.27 ± 0.18^{a}	4.75 ± 0.08^{b}	4.57 ± 0.07^{b}	4.45 ± 0.03^{b}	4.11 ± 0.06^{c}	3.99 ± 0.06^{c}
Pediococcus sp. strain P1	5.26 ± 0.08^{a}	4.74 ± 0.11^{b}	4.60 ± 0.10^{b}	4.41 ± 0.05^{b}	4.02 ± 0.09^{d}	3.93 ± 0.01^{d}
Streptococcus sp. strain P2	5.15 ± 0.12^{a}	4.73 ± 0.12^{b}	4.58 ± 0.10^{bc}	4.46 ± 0.01^{c}	4.09 ± 0.08^{d}	3.90 ± 0.03^{d}
Lactobacillus sp. strain P3	5.21 ± 0.06^{a}	4.77 ± 0.15^{b}	4.58 ± 0.10^{bc}	4.42 ± 0.04^{c}	4.09 ± 0.09^{d}	3.88 ± 0.05^{d}
Streptococcus sp. strain P4	5.22 ± 0.16^{a}	4.79 ± 0.12^{b}	4.60 ± 0.10^{bc}	4.43 ± 0.04^{c}	4.13 ± 0.05^{d}	3.99 ± 0.04^{d}

Note: The value was presented as mean ± standard deviation. The different lowercase letters in a row indicated a significantly different value (p <0.05) based on Tuckey HSD. n=3.

Table 6. Screening of biogenic amine production produced by the LAB

Isolate	Control	Amino acids/biogenic amines			
		Histidine/histamine	Tyrosine/tyramine	Lysin/cadaverine	Arginine/agmatine
Pediococcus sp. strain A1	-	+	+	-	+
Enterococcus sp. strain A2	-	+	-	-	+
Pediococcus sp. strain A3	-	-	-	-	+
Enterococcus sp. strain A4	-	-	+	-	+
Streptococcus sp. strain B1	-	-	-	-	+
Pediococcus sp. strain B2	-	-	+	-	+
Enterococcus sp. strain B3	-	-	+	-	+
Pediococcus sp. strain B4	-	-	-	-	+
Pediococcus sp. strain P1	-	-	+	-	+
Streptococcus sp. strain P2	-	-	-	-	+
Lactobacillus sp. strain P3	-	-	-	-	+
Streptococcus sp. strain P4	-	-	+	-	+

Organic acid production ability

Organic acid production abilities such as acetate, citrate, formate, lactate and succinate measured by pH culture, are specific characteristics of LAB (Endo and Dicks 2014; Thomas 2018). Heterofermentative LAB produce various organic acids such as acetate, citrate, formate, and lactate, while homofermentative LAB only produces lactate (Abedi and Hashemi 2020). The lactate produced by the bacteria is secreted into the medium. Furthermore, accumulated lactate decrease pH in the medium. pH culture from 14 LAB decreased significantly from 6.3 to 5 in the first 4 hours and continued to decrease slowly until around 3.9 (Table 5). Low pH probably reduced the growth and viability of the LAB, as seen in *Lactobacilli* (Hassanzadazar et al. 2012).

Biogenic amine production

Most food fermentations contain biogenic amines, such as agmatine, tyramine, histamine, putrescine, and cadaverine produced as microorganism activities through substrate-specific decarboxylase enzymes from amino acid decarboxylation. Histamine, cadaverine, tyramine, and agmatine are derived from histidine, lysine, tyrosine, and arginine decarboxylation. High concentrations of biogenic amines in the body may induce headaches, respiratory distress, heart palpitation, hyper or hypotension, and several allergenic disorder (Comas-Basté et al. 2019). Histamine and tyramine can cause "scombroid fish poisoning" and "cheese reaction," respectively (Barbieri et al. 2019). The biogenic amine production can be produced by LAB such as *Enterococci*, *Carnobacteria* and some strains of *Lactobacilli*, particularly *Lactobacillus curvatus*, *Lactobacillus brevis* and *Lactobacillus buchneri* (Bover-Cid and Holzapfel 1999).

The biogenic amines in this study were screened using four different amino acids namely histidine, tyrosine, lysine, and arginine. Some LAB could produce histamine and tyramine (Table 5). Furthermore, *Lactobacillus* sp. strain P3, *Pediococcus* sp. strain B4, *Pediococcus* sp. strain A3, *Streptococcus* sp. strain B1, *Streptococcus* sp. strain P2 was relatively safe compared to the other isolate because it only produced a biogenic amine, agmatine.

In conclusion, four different LAB were isolated successfully from traditional fish fermentation namely *buduk*, *pekasam*, and *pekasam ale-ale* and identified as *Lactobacillus* sp., *Enterococcus* sp., *Pediococcus* sp., and *Streptococcus* sp. The isolates showed different characteristics, such as antimicrobial activities, biogenic amine production, organic acid production, AT, and enzyme activities (amylase and protease). However, the best LAB based on this characteristic was *Lactobacillus* sp. strain P3 due to inhibiting all tests, no histamine, tyramine and cadaverine production; resistance at pH 1 and 2.

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REFERENCES

Abedi E, Hashemi SMB. 2020. Lactic acid production - producing microorganisms and substrates sources-state of art. Heliyon 6(10): e04974. DOI: 10.1016/j.heliyon.2020.e04974.

Albayrak ÇB, Dura M. 2021. Isolation and characterization of aroma producing lactic acid bacteria from artisanal white cheese for multifunctional properties. Lwt 150: 112053-112063. DOI: 10.1016/j.lwt.2021.112053.

Ayantola KJ, Oladunmoye MK. 2015. Antibacterial activity of *Lactobacillus* species isolated from poultry waste (droppings) against poultry pathogens. Curr Res Poult Sci 6 (1): 7-12. DOI: 10.3923/crpsaj.2016.7.12.

Barbieri F, Montanari C, Gardini F, Tabanelli G. 2019. Biogenic amine production by lactic acid bacteria: A review. Foods 8 (17): 1-27. DOI: 10.3390/foods8010017.

Barcenilla C, Ducic M, López M, Prieto M, Álvarez-Ordóñez A. 2022. Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. Meat Sci 183: 10861-10873. DOI: 10.1016/j.meatsci.2021.108661.

Bover-Cid S, Holzapfel WH. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. Intl J Food Microbiol 53 (1): 33-41. DOI: 10.1016/S0168-1605(99)00152-X.

Comas-Basté O, Latorre-Moratalla ML, Sánchez-Pérez S, Veciana-Nogués, MT, Vidal-Carou MC. 2019. Histamine and other biogenic amines in food. From scombroid poisoning to histamine intolerance. In: Proestos C (eds) Biogenic Amines. IntechOpen Limited, London. DOI: 10.5772/intechopen.84333.

Dalié DKD, Deschamps AM, Richard-Forget F. 2010. Lactic acid bacteria - Potential for control of mould growth and mycotoxins: A review. Food Control 21 (4): 370-380. DOI: 10.1016/j.foodcont.2009.07.011.

Devriese LA, Pot B. 1995. The genus enterococcus. In The genera of lactic acid bacteria. Springer, Boston, MA. DOI: 10.1007/978-1-4615-5817-0_10.

Du Toit M, Huch M, Cho GS, Franz CMAP. 2014. The genus *Streptococcus*. In: Holzapfel WH, Wood BJ (eds.). Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, India. DOI: 10.1002/9781118655252.ch28.

Endang SR. 2018. Lactic acid bacteria in fermented food of Indonesian origin. AgriTech 23 (2): 75-84.

Endo A, Dicks LMT. 2014. Physiology of the LAB. In: Holzapfel WH Wood BJ (eds.). Lactic acid bacteria: Biodiversity and taxonomy. Wiley Blackwell, India. DOI: 10.1002/9781118655252.ch2.

Franz CM, Endo A, Abriouel H, Reenen CA, Gálvez A, Dicks LM. 2014. The genus Pediococcus. Lactic acid bacteria: Biodiversity and taxonomy. DOI: 10.1002/9781118655252.ch21.

Garvie EI. 1984. 5 Separation of Species of the Genus *Leuconostoc* and Differentiation of the Leuconostocs from Other Lactic Acid Bacteria. In: Methods in microbiology. Academic Press. DOI: 10.1016/S0580-9517(08)70390-8.

Gotcheva V, Hristozova E, Hristozova T, Guo M, Roshkova Z, Angelov A. 2002. Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. Food Biotechnol 16 (3): 211-225. DOI: 10.1081/FBT-120016668.

Grandgirard J, Poinsot D, Krespi L, Nénon JP, Cortesero AM. 2002. Costs of secondary parasitism in the facultative hyperparasitoid *Pachycrepoideus dubius*: Does host size matter? Entomol Exp Appl 103 (3): 239-248. DOI: 10.1046/j.1570-7458.2002.00982.x.

Grosu-Tudor SS, Zamfir M. 2012. Probiotic potential of some lactic acid bacteria isolated from Romanian fermented vegetables. Ann Romanian Soc Cell Biol 17 (1): 234-239.

Harley JP, Prescott LM. 2002. Laboratory exercises in microbiology. The McGraw-Hill, New York.

Hassanzadazar H, Ehsani A, Mardani K, Hesari J. 2012. Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. Vet Res For 3 (3): 181-185.

Jiang C, Liu M, Yan X, Bao R, Ji C, Zhang S, Lin X, Liu A, Wang W, Zhang Z, Liang H. 2021. Lipase addition promoted the growth of proteus and the formation of volatile compounds in suanzhayu, a traditional fermented fish product. Foods 10 (11): 2529-2539. DOI: 10.3390/foods10112529.

Jini R, Swapna HC, Rai AK, Vrinda R, Halami PM, Sachindra NM, Bhaskar N. 2011. Isolation and characterization of potential lactic acid bacteria (LAB) from freshwater fish processing wastes for application in fermentative utilisation of fish processing waste. Braz J Microbiol 42 (4): 1516-1525. DOI: 10.1590/S1517-83822011000400039.

Jobby R, Flora Y, Bora A, Jha P, Kawalkar H, Desai N. 2020. Exploring Probiotic Activity of Lactobacillus sp. Isolated from Indigenous Breeds of Cattle Milk and Fecal Samples in Bhatan Village, MH. Curr Microbiol 77 (7): 1184-1190. DOI: 10.1007/s00284-020-01910-x.

Kajikawa A, Midorikawa E, Masuda K, Kondo K, Irisawa T, Igimi S, Okada S. 2016. Characterization of flagellins isolated from a highly motile strain of *Lactobacillus agilis*. BMC Microbiol 16 (1): 1-8. DOI: 10.1186/s12866-016-0667-x.

Kalschne DL, Womer R, Mattana A, Sarmento CMP, Colla LM, Colla E. 2015. Characterization of the spoilage lactic acid bacteria in "sliced vacuum-packed cooked ham. Braz J Microbiol 46 (1): 173-181. DOI: 10.1590/S1517-838246120130019.

Nikita C, Hemangi D. 2012. Isolation, identification and characterization of lactic acid bacteria from dairy sludge sample. J Environ Res Dev 7: 1-11.

Nofiani R, Ardiningsih P. 2018. Physicochemical and microbiological profiles of commercial cincalok from West Kalimantan. Jurnal Pengolahan Hasil Perikanan Indonesia 21 (2): 243-249. DOI: 10.17844/jphpi.v21i2.22851. [Indonesia]

Nofiani R, Elminah E, Ardiningsih P. 2019. Chemical and microbiological properties of *buduk*, A commercial fish sauce from West Kalimantan. Jurnal Pengolahan Hasil Perikanan Indonesia 22 (3): 601-608. DOI: 10.17844/jphpi.v22i3.29230. [Indonesia]

Nofiani R, Kalbar M, Ardiningsih, P. 2010. Chemical characteristics and microbiological safety of commercial fermented ale-ale from West Kalimantan, Indonesia. J Fish Aquat Sci 5 (6): 483-493. DOI: 10.3923/jfas.2010.483.493.

Nurhikmayani R, Daryono BS, Retnaningrum E. 2019. Isolation and molecular identification of antimicrobial-producing lactic acid bacteria from chao, South Sulawesi (Indonesia) fermented fish product. Biodiversitas 20 (4): 1063-1068. DOI: 10.13057/biodiv/d200418.

Orla-Jensen. 1919. The lactic acid bacteria. Fred HInost and Son, Copenhagen.

Padmavathi T, Bhargavi R, Priyanka PR, Niranjan NR, Pavitra PV. 2018. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. J Gen Eng Biotechnol 16 (2): 357-362. DOI: 10.1016/j.jgeb.2018.03.005.

Pang XN, Chen C, Huang XN, Yan YZ, Chen JY, Han BZ. 2021. Influence of indigenous lactic acid bacteria on the volatile flavor profile of light-flavor Baijiu. Lwt 147: 111540. DOI: 10.1016/j.lwt.2021.111540.

Rachmawati I, Suranto SR. 2005. Uji antibakteri bakteri asam laktat asal asinan sawi terhadap bakteri patogen. Bioteknologi 2 (2): 43-48. DOI: 10.13057/biotek/c020202. [Indonesian]

Schillinger U, Lücke FK. 1987. Identification of Lactobacilli from meat and meat products. Food Microbiol 4 (3): 199-208. DOI: 10.1016/0740-0020(87)90002-5.

Shojaei Zinjanab M, Golmakani MT, Eskandari MH, Toh M, Liu SQ. 2021. Natural flavor biosynthesis by lipase in fermented milk using in situ produced ethanol. J Food Sci Technol 58 (5): 1858-1868. DOI: 10.1007/s13197-020-04697-8.

Thomas B. 2018. Lactic acid bacteria: Their applications in foods. J Bacteriol Mycol 6 (2): 89-94. DOI: 10.15406/jbmoa.2018.06.00182.

Vantsawa PA, Maryah UT, Bulus T. 2017. Isolation and identification of lactic acid bacteria with probiotic potential from fermented cow milk (Nono) in Unguwar Rimi Kaduna State Nigeria. Am J Mol Biol 07 (02): 99-106. DOI: 10.4236/ajmb.2017.72008.

Wang C, Cui Y, Qu X. 2018. Mechanisms and improvement of acid resistance in lactic acid bacteria. Arch Microbiol 200 (2): 195-201. DOI: 10.1007/s00203-017-1446-2.

Weiss J, Elsbach P, Shu C, Castillo J, Grinna L, Horwitz A, Theofan G. 1992. Human bactericidal/permeability-increasing protein and a recombinant NH2-terminal fragment cause killing of serum-resistant gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria. J Clin Investig 90 (3): 1122-1130. DOI: 10.1172/JCI115930.

Wu C, Zhang J, Wang M, Du G, Chen J. 2012. *Lactobacillus casei* combats acid stress by maintaining cell membrane functionality. J Ind Microbiol Biotechnol 39 (7): 1031-1039. DOI: 10.1007/s10295-012-1104-2.

Yang F, Xia WS, Zhang XW, Xu YS, Jiang QX. 2016. A comparison of endogenous and microbial proteolytic activities during fast fermentation of silver carp inoculated with *Lactobacillus plantarum*. Food Chem 207: 86-92. DOI: 10.1016/j.foodchem.2016.03.049.