Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X

Volume 31 | No. 2 | **August** | 2025

Original article

Isolation and molecular identification of 16s rRNA of lactic acid bacteria with the most probiotics potential from 'lawar ikan', a traditional fermented typical food from East Nusa Tenggara

Adelya Irawan Manalu[1]*, Lukas Pardosi[1], Kristoforus W. Kia[2]

[1]Department of Biology, Faculty of Agriculture, Science and Health, University of Timor, East Nusa Tenggara, Indonesia
[2]Department of Animal Husbandry, Faculty of Agriculture, Science and Health, University of Timor, East Nusa Tenggara, Indonesia

Abstract

'Lawar ikan' is a traditional fermented food typical of East Nusa Tenggara made from fresh anchovies. Fermented foods generally contain lots of lactic acid bacteria (LAB). This research aims to isolate lactic acid bacteria which have probiotic potential from 'lawar ikan'. The research method used is the isolation of LAB by first making samples of 'lawar ikan', then carrying out serial dilutions, growing them in specific media, the colonies that grow will be selected for morphological and biochemical characterization. Furthermore, to determine whether the isolates produced have probiotic potential, resistance tests were carried out against low pH, bile salts and antibacterial tests against the pathogenic bacteria *E. coli* and *S. aureus*. The isolation results showed that 11 isolates had different characteristics, and were gram-positive bacilli and 1 isolate was a gram-positive cocci. The resulting isolate was heterofermentative and catalase negative. The results of the BAL resistance test to low pH, namely pH 2, were isolate L03 with a final viability percentage of 61.75%, at pH 3, namely isolate L04 with a final viability percentage of 51.75%. Testing for resistance to bile salts obtained isolates that were resistant at a concentration of 0.3%, namely isolates L07, L05 and L04. Testing against pathogenic bacteria found 2 potential isolates, namely L04 with an inhibitory zone diameter of 5 mm against *E.coli* and 13 mm against *S. aureus*, while isolate L11 with an inhibitory zone diameter of 1 mm against E.coli and equal to 21 mm against *S. aureus.* The best isolate was identified based on the 16S rRNA gene, the identification result of isolate L04 was *Pediococcus pentosaceus* with a similarity percentage of 100%.

Keywords: probiotic, lactat acid bacteria, fermentation, antimicrobial, lawar ikan
Received: July 14, 2024 Revised: January 30, 2025 Accepted: April 10, 2025

Introduction

Indonesia has a variety of traditional fermented foods, including 'lawar ikan' which is a traditional food typical of East Nusa Tenggara. 'Lawar ikan' is fermented fish using fresh anchovies as raw materials which are processed with the addition of acid or lime, salt, chilies, onions and other spices which are then left for 1 (one) hour before consumption so that it goes through an anaerobic fermentation process. The addition of lime in making 'lawar ikan' aims to minimize the growth of pathogens so that the food is safe to consume even without cooking. Fermented products tend to contain potentially probiotic bacteria. Probiotics are good microorganisms that are beneficial for the health of their hosts and can even increase immunity if consumed in sufficient quantities.

Fermented fish products are generally rich in lactic acid bacteria and therefore have probiotic potential. Lactic acid bacteria (LAB) are natural microbes found in basic food ingredients and play an important role in the fermentation process of traditional foods. Lactic acid bacteria can function as bacteriocins which are very beneficial for health (Ramesh *et al*., 2015). Lactic acid bacteria are also called probiotics (Emmawati *et al*., 2015). Probiotics are living organisms that are capable of providing beneficial effects on the health of their hosts when consumed in sufficient quantities. Probiotics must be able to withstand acidic conditions and the presence of bile salts, have the ability to attach to the intestine and have activity against pathogenic bacteria (Rizal *et al*., 2016).

Probiotics have an important role for health. Lactic Acid Bacteria (LAB) are mostly potential probiotics. LAB in food not only plays a role in fermentation but also has a role as a probiotic, namely replacing the activity of natural microbiota in the intestine or regulating immune reactivity which is beneficial for health. Although historically fermented foods with LAB were milk-based foods, this decade a lot of research has led to the exploration of LAB from traditional foods as potential probiotics. Several studies show that traditional fermented fish products contain various types of lactic acid bacteria which have probiotic potential, including dekke naniura, a traditional food typical of North Sumatra (Haro *et al*., 2020; Hang, 2021) lemea typical of Rejang (Okfrianti *et al*., 2018), fermented tilapia fish typical of South Sumatra (Nurnaafi *et al*., 2015), fermented budu fish typical of West Sumatra (Soemarie *et al*., 2021) the group of bacteria produced is usually from the genus Lactobacillus. This information is initial information that allows for the probiotic potential of lactic acid bacteria isolated from the 'lawar ikan' typical of East Nusa Tenggara.

* Corresponding Author:

Adelya Irawan Manalu
Department of Biology, Faculty of Agriculture, Science and Health, University of Timor, East Nusa Tenggara, Indonesia
Phone: -
E-mail: adelyamanalu@unimor.ac.id

http://dx.doi.org/10.23869/bphjbr.31.2.20252
Published by © PBI East Java. Open Access www.berkalahayati.org

Methods

Research materials

The sample used was lawar ikan. The materials used for bacterial isolation are MRS (de Man Rogosa Sharpe) Broth- HiMedia™ (peptone 10 g/L, dextrin 20 g/L, glucose 20 g/L, lactic acid 0.5 g/L, ammonium nitrate (NH_4NO_3) 2 g/L, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 0.1 g/L, Potassium Phosphate (K_2HPO_4) 0.5 g/L, Sodium Chloride (NaCl): 5g/L)), distilled water, agar bacteriological, $CaCO_3$. The materials used for bacterial characterization are Gram stain, Triple Sugar Iron Agar (TSIA) media- Oxoid™ (peptone 10 g/L, calcium carbonate ($CaCO_3$) 0.3 g/L, lactose 10 g/L, sucrose 10 g/L, glucose 1 g/L, sodium thiosulfate ($Na_2S_2O_3$): 0.3 g/L, senosolfthalein 0.025 g/L, agar 15 g/L), Simmon Citrate Agar (SIM) media- Oxoid™ (sodium citrate 2 g/L, ammonium dihydrogen phosphate ($NH_4H_2PO_4$) 1 g/L, magnesium sulfate ($MgSO_4$) 0.2 g/L, sodium chloride (NaCl) 5 g/L, dipotassium phosphate (K_2HPO_4) 0.2 g/L, bromothymol blue 0.08 g/L, agar 15 g/L), HCl, and bile salt Oxoid™. The antimicrobial test materials used were MHA media- Oxoid™, blank discs Oxoid™, pathogenic microorganisms (*E. coli* ATCC 8739 and *S. aureus* ATCC 6538).

Research tool

The tools used in this research are Laminar Air Flow (LAF), vortex, autoclave, oven, incubator, centrifuge, spectrophotometer, microscope, laboratory glassware, micropipette, object and cover glass, hockey stick, eppendorf tube, tube needle, volumetric pipette, caliper, sprayer, bunsen.

Sample preparation

Clean 100 grams of fresh anchovies, then mix with spices such as lime, salt, chili, onions and basil leaves, then leave for 48 hours in a container that is not tightly closed.

Bacterial isolation

Isolation of LAB from 'lawar ikan' was carried out using a serial dilution technique on MRSB media. A total of 1 g of lawar was dissolved in 9 mL of MRSB. The suspension was homogenized using a vortex and then incubated for 24- 48 hours at 37ºC. The incubation results were diluted with serial dilutions 10^{-1} to 10^{-7}. A total of 1 ml of each dilution was inoculated into a 15 mL MRSA+ 1% $CaCO_3$ petri dish then spread with a spreader containing it and incubated for 24- 48 hours at 37ºC. The isolate colonies that grew were considered LAB, so the colonies were transferred to petri dishes containing NA media using the streak plate methode, carried out three times at different times to obtain pure isolates.

Characterization of bacteria

Bacterial isolates were characterized morphologically and biochemically. Morphological characterization was carried out by observing colonies, Gram staining, while biochemical tests included the TSIA test, SCA test, catalase test and fermentation type test.

The TSIA test is carried out by taking 1 dose of bacterial isolate which is then inoculated by scratching and pricking on TSIA slant media and incubating at 27 ºC for 48 hour. The SCA test is carried out by taking 1 dose of bacterial isolate which is then inoculated by scratching on the SCA slant media then incubated for 24 hours at 37°C. The catalase test is carried out by taking a small amount of bacterial inoculum from pure culture using an inoculation loop and placing the bacteria on the surface of a glass slide, then dropping a 3% hydrogen peroxide solution on the bacterial colonies on the slide, then observing to see if there are any bubbles. In the fermentation test, the bacterial isolate was inoculated into MRS broth media, then inserted into a Durham tube in an inverted position. Incubate for 24 hours and then observe whether bubbles form in the Durham tube.

LAB resistance test to low pH

LAB isolates were cultured into MRSB media with varying incubation times of 0, 6, 12, 18 and 24 hours at a pH of 2, 3, 4, 5 and 6. Then the number of LAB was calculated by measuring the OD on a spectrophotometer and then calculating the CFU (Colony Forming Unit) per ml using the formula Log (CFU/ml)= *m x absorbance + b*, *m* is the slope of the line *b* is the intercept on the y-axis. The greater the decrease in cell number after incubation, the less resistant the bacteria are to low pH. Viability Percentage is calculated using the formula: *((initial viability-final viability)/ initial viability) x 100%*.

LAB resistance test to bile salts

One ml (10^6 CFU/ml) of culture that had been refreshed in MRSB was incubated for 24 hours in bile salt with concentration variants of 0%, 0.3%, 0.6%, and 0.9%. Then the amount of LAB was calculated by measuring the OD on a spectrophotometer and then calculating the CFU (Colony Forming Unit) per ml using the formula Log (CFU/ml)= *m x absorbance + b*, *m* is the slope of the line *b* is the intercept on the y-axis. The more the number of incubation cells decreases, the less resistant the bacteria are to NaCl.

Antibacterial test

Antibacterial testing was carried out against the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method using MHA media. The test bacteria were suspended following the 0.5 Mac Farlane standard, then swapped using a sterile cotton swap. The paper discs were soaked in LAB suspension (following the 0.5 Mac Farlane standard) then inoculated into a petri dish containing the test bacteria. The clear zone formed is measured using a ruler and then converted in mm. The inhibition zone diameter is obtained using the yield formula: reduction of vertical diameter and diameter filter paper is added to the results reduction of horizontal diameter and diameter

filter paper then divided in half. Interpretation of inhibitory power is determined according to categories: Resistant if the inhibition zone is ≤ 14 mm, Intermediate if the inhibition zone 15-18 mm, Susceptible if zone of inhibition ≥ 19 mm (CSLI, 2013).

Molecular identification of 16S rRNA

Isolation of potential probiotic LAB DNA obtained from 'lawar ikan' was carried out using a hot and cold process. A total of 1.5 mL of pure bacterial culture aged 24 hours was taken and centrifuged for 10 minutes. The supernatant was discarded and then 100 µL of distilled water was added under aseptic conditions. The cell suspension was frozen at -10ºC until the solution crystallized and then thawed at 90ºC for 10 minutes. Then the sample was sent to Macrogen for 16S rRNA identification.

Figure 1. Sample of Lawar Fish

Results

Sample preparation

Fresh anchovies are mixed with spices, then left for 1 hour, after the 'lawar ikan' has been prepared, leave it again for 48 hours so that the fish ferments. A sample of 'lawar ikan' can be seen in Figure 1.

Isolation and morphological characterization

The results of the isolation of lactic acid bacteria (LAB) from lawar ikan obtained 11 isolates (Figure 2). The isolate was characterized morphologically and by Gram staining, the results of which can be seen in Table 1.

Table 1. Characteristics of LAB Isolates from Lawar

No	Isolate Code	Character				Gram Staining
		Color	Shape	Edge	Elevation	
1	L01	white	round	flat	entire	positive bacili
2	L02	white	round	flat	entire	positive bacili
3	L03	white	irregular	flat	entire	positive bacili
4	L04	white	round	flat	entire	positive cocci
5	L05	white	round	undulate	entire	positive bacili
6	L06	white	round	flat	entire	positive bacili
7	L07	white	round	flat	entire	positive bacili
8	L08	white	round	flat	entire	positive bacili
9	L09	white	round	flat	entire	positive bacili
10	L10	white	round	flat	entire	positive bacili
11	L11	white	round	flat	entire	positive bacili

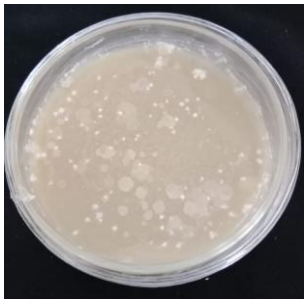

Figure 2. LAB isolate from lawar ikan

Based on morphological observations, the characteristics observed include color, shape, edges and elevation. It was discovered that all the LAB isolates obtained had morphological characteristics of white colonies, there was 1 isolate with an irregular shape, 1 isolate with wavy edges, and 11 isolates with flat elevations. Based on gram staining, it was found that there were 10 isolates in the form of bacilli which were Gram positive bacteria and 1 isolate was a Gram positive cocci. The appearance of positive bacilli and negative bacilli isolates can be seen in Figure 3.

Eleven isolates obtained were subjected to biochemical testsThe results of the biochemical tests for

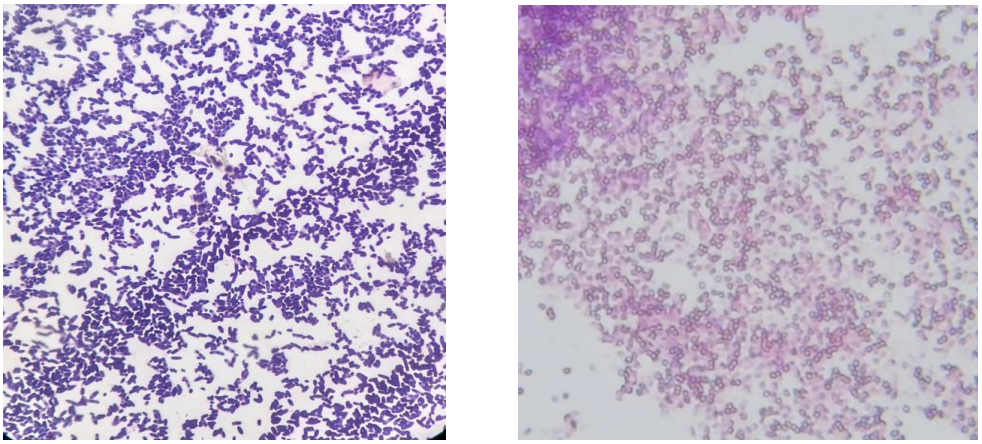

Figure 3. Appearance of LAB under a microscope with 100x magnification, a) bacilli; b) cocci

LAB isolates, namely the TSIA test, SCA test, catalase test, and fermentation type test can be seen in Table 2.

Table 2. Biochemical Test Results

No	Isolate Code	Test Result			
		TSIA	SCA	Katalase	Type of Fermentation
1	L01	A/A	Negative	Negative	Heterofermentative
2	L02	A/A	Negative	Negative	Heterofermentative
3	L03	A/A	Negative	Negative	Heterofermentative
4	L04	K/K	Negative	Negative	Heterofermentative
5	L05	A/A	Negative	Negative	Heterofermentative
6	L06	A/A	Negative	Negative	Heterofermentative
7	L07	K/K	Positive	Negative	Heterofermentative
8	L08	K/K	Negative	Negative	Heterofermentative
9	L09	K/K	Positive	Negative	Heterofermentative
10	L10	K/K	Negative	Negative	Heterofermentative
11	L11	K/K	Negative	Negative	Heterofermentative

In the TSIA test, it was discovered that of the 11 BAL isolates there were 5 isolates with test results which were characterized by a change in TSIA color, namely the color changed to yellow on the slant and butt (A/A), code K/K if there is no change in media color. The five isolates are L01, L02, L03, L05, and L06. Meanwhile, in the SCA test on these 11 bacterial isolates, there were 2 positive isolates which were indicated by a change in media color from green to blue. The two isolates are isolates L07 and L09. In the catalase test, all isolates did not form bubbles, and the fermentation type was heterofermentative, characterized by the formation of gas bubbles in the Durham tube.

LAB resistance test to low pH

The LAB resistance test to low pH was carried out by varying the acidity of the pH of the media, namely pH 2, 3, 4, 5, and 6. The results can be seen in Figure 4

Based on the data above, the LAB isolate from ‘awar ikan showed a decrease in number at the 6th hour but

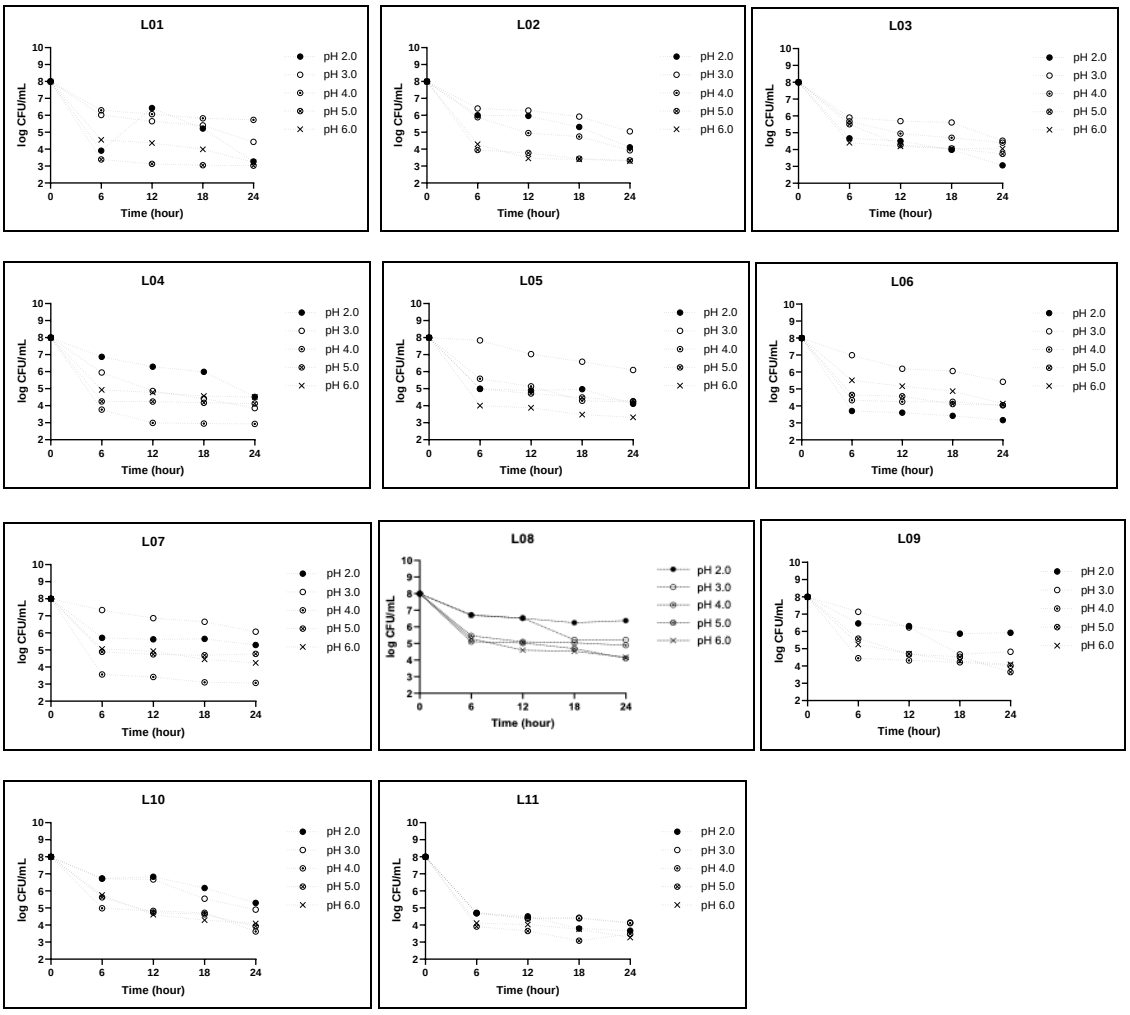

Figure 4. Resistance of LAB isolates to PH variant

was still alive at the 24th hour. The final viability was calculated to see the percentage of bacterial viability at the 24th hour with the pH variant tested (Figure 5).

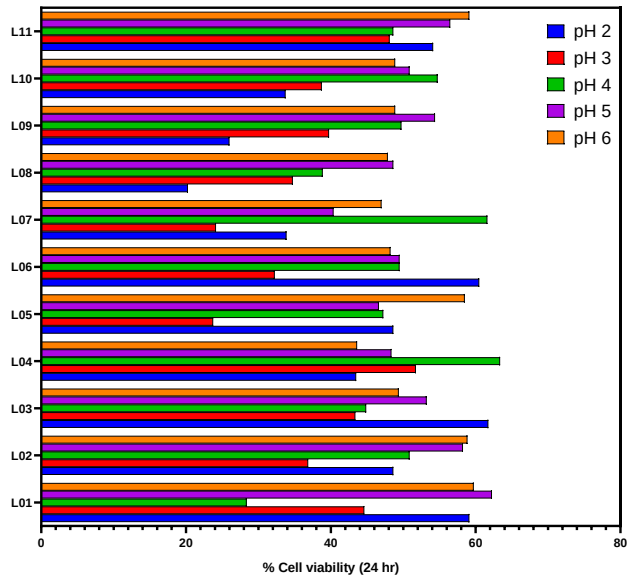

Figure 5. Final viability of LAB isolates

The percentage of final viability of isolates shows that the most resistant isolate to grow in the 24th hour at low pH (pH 2) is isolate L03 with a percentage of 61.75%, at pH 3 is isolate L04 with a percentage of 51.75%, at pH 4 is isolate L04 with a percentage of 63.38%, at pH 5 is isolate L01 with a percentage of 62.25%, and at pH 6 is isolate L01 with a percentage of 59.75%.

LAB resistance test to bile salts

LAB resistance to bile salts can be known by looking at the decrease in the number of isolates when given variant concentrations of bile salts. The final viability of isolates with concentration variants can be seen in Figure 6.

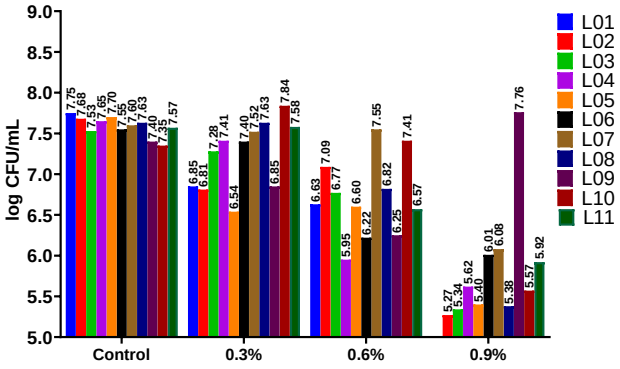

Figure 6. Final viability of LAB isolates with variant concentrations of Bile Salt

In Figure 5, the higher the concentration of bile salts, the more the growth of isolates decreased. The most viable isolates at 0.3% bile salt concentration were isolates L07, L05 and L04, at 0.6% bile salt concentration was isolate L07, while at 0.9% bile salt concentration was isolate L09.

Antibacterial test of LAB isolates against pathogenic bacteria

Antibacterial test results of LAB isolates against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* can be seen in Table 3.

Table 3. Antibacterial Test Results against Pathogenic Bacteria

No	Isolate Code	Inhibition Zone Diameter (mm)	
		Escherichia coli	*Staphylococcus aureus*
1	L01	10	11
2	L02	6	10
3	L03	10	10
4	L04	5	13
5	L05	-	1
6	L06	-	1
7	L07	-	1
8	L08	-	5
9	L09	1	12
10	L10	-	5
11	L11	1	21

The results of antibacterial tests of 11 isolates against *Escherichia coli* and *Staphylococcus aureus* bacteria showed that some isolates formed inhibition zones. Isolates tested against *Staphylococcus aureus* that have a large inhibition zone are isolate L11 with an inhibition zone diameter of 21 mm with susceptible category, isolate L04 diameter of 13 mm with resistant category while isolates tested against *Escherichia coli* that have inhibition zones are isolates L01 and L03 with an inhibition zone diameter of 1 mm, L02 of 6 mm and L04 of 5 mm with resistant category.

Molecular identification of 16S rRNA

Identification was carried out to see the similarity or kinship of LAB isolates from 'lawar ikan' with identified species. The results of species identification of L04 isolates based on the 16S rRNA gene can be seen in Figure 4 and Table 3.

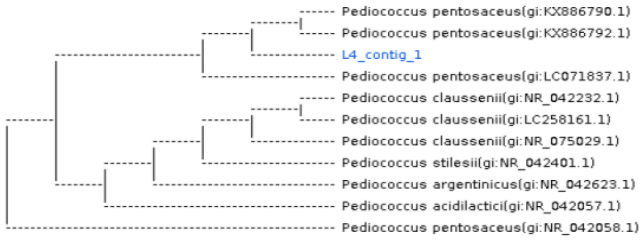

Figure 4. Phylogenetic tree of isolate L04

Table 3. Isolate L04 identification results based on 16S rRNA gene

Accesion	Description	Coverage	Total Score	e Value	Identity %
KX886792.1	*Pediococcus pentosaceus*	98	1504	0.0	100.00

Discussion

Isolation and morphological characterization

Isolates obtained from isolation from 'lawar ikan' have similar characters but some have variations. Research by Candra et al. (2007), LAB isolated from fermented products have varied characters. LAB isolated from Bekasam had a round shape, white, yellow and cream color, smooth edges, raised and convex alevation.

The TSIA test is a method for identifying bacteria based on sugar fermentation, gas production and H_2S production. In this reseach, the media in 5 isolates changed color to yellow on the slant and butt (A/A), it mean all sugar fermentation (glucose, lactose and sucrose), the bacteria produce acid, and no H_2S. Lactic acid bacteria usually ferment sugars by producing lactic acid, they tend to produce acidic conditions (yellow) in TSIA. While the SCA test, the media on 2 isolates experienced a color change from green to blue, which means that the isolates use citrate as one of the carbon sources in the metabolic process. Bacteria that can utilize sodium citrate as a carbon source will convert citrate into basic compounds (ammonia or ammonium compounds). In general, lactic acid bacteria such as Lactobacillus, Streptococcus, and Enterococcus cannot metabolize citrate (Ljungh & Wadström, 2006). Heterofermentative lactic acid bacteria ferment carbohydrates, producing several products, namely lactic acid, acetic acid, and gas (CO_2) as a by-product. heterofermentative lactic acid bacteria usually *Pediococcus sp, Lactobacillus sp, Leuconostoc sp*.

Gram-positive bacteria are identified with a purple color which indicates that the bacteria are able to retain crystal violet dye, this is because these bacteria have a thick peptidoglycan content and conversely a low lipid layer in their cell walls. The peptidoglycan layer is known to be able to absorb crystal violet dye which also does not fade its color by alcohol (Candra et al., 2007; Emmawati et al., 2015).

Lactic acid bacteria are a group of bacteria that are very common in fermented foods, including fish products. Its play a role in reducing the pH of fermented products through the production of lactic acid, which can help preserve the product and give it its characteristic sour taste. The bacteria that are often found in traditional fermented fish products are lactic acid bacteria (LAB) of the *Lactobacillus* sp, *Steptococcus* sp, and *Pediococcus* sp groups. Chandra et al. (2007) obtained 5 LAB isolates from Bekasam which were thought to be Lactobacillus bacteria. Hang (2021) obtained 6 LAB isolates from Naniura thought to be from the Lactobacillus and Steptococcus groups.

LAB resistance test to low pH

LAB from 'lawar ikan' experienced a decrease in growth but still survived at the 24th hour with a predetermined pH variant. This shows that the isolate has resistance to low pH which is one of the characteristics of probiotics. The percentage of LAB viability at the pH variant was measured to see how capable the bacteria can survive at a predetermined pH condition. The greater the percentage of final viability, the more able the bacteria are to survive in media with predetermined pH conditions. Bacterial growth at low pH has different growth conditions due to physiological factors of the bacteria. As a probiotic candidate, LAB must be able to withstand gastric acid pH 2-3. The LAB resistance test to low pH in this study was carried out for up to 24 hours to see how far LAB could survive in acidic conditions. Some LAB are able to survive at low pH conditions because LAB have a system that simultaneously transports lactic acid and protons to the outside of the cell (Ramirez-Chavarin et al., 2013).

LAB resistance test to bile salts

Resistance to bile salts is indicated by a small decrease in the number of isolates. The character of isolates tested as probiotics is resistant to bile salts of the small intestine so that they can survive in the large intestine (Halim & Zubaidah, 2013). Lactic acid bacteria cells that are resistant to bile salts when incubated in 0.3% bile salts still grow and do not lyse, only experiencing a slight leakage of intracellular material (Surono, 2004). Bile salts are toxic to living cells, therefore microbes in the digestive tract must have a defense mechanism to protect themselves from the activity of these toxins. The presence of probiotics, namely LAB, in the digestive tract will provide benefits, namely the deconjugation of bile salts by the enzyme bile salt hydrolase (BSH), the production of short-chain fatty acids, the assimilation of cholesterol into the bacterial cell membrane, and the conversion of cholesterol by hydrogenation into the poorly absorbed sterol coprostanol. The potentially probiotic Lactobacillus group has a high rate of bile salt deconjugation (Hernández-Gómez et al., 2021). *P. pentosaceus* isolates in this study were resistant at pH 2 for 2-6 hour with significant loss in viable cells subsequently. This was This is in accordance with previous research Vidhyasagar & Jeevaratnam (2013), where *P. pentosaceus* can survive at pH 2 for 2 hours. ***Pediococcus pentosaceus*** produces lactic acid which can lower intestinal pH and inhibit the growth of pathogens (Qi et al., 2020).

Antibacterial test of LAB isolate against pathogenic bacteria

Antibacterial test of LAB against pathogenic bacteria showed the presence of inhibition zone. The formation of inhibition zone indicates that the isolate has antibacterial activity. LAB isolates have secondary metabolites that can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. The ability of probiotic LAB to suppress pathogenic growth is due to its ability to produce antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide and bacteriocins. In line with previous research, LAB isolated from bekasam produced several antimicrobial compounds in the form of organic compounds, hydrogen peroxide and peptides, which were dominated by organic acids, namely lactic acid (Halim & Zubaidah, 2013). Another study also reported that lactic acid bacteria of the Lactobacillus group have the ability to inhibit pathogenic bacteria Salmonella tiphii (Hang, 2021). BAL of *P. pentosaceus* produces bacteriocins which function to inhibit the growth of bacteria, especially pathogenic bacteria *Listeria monocytogenes* (Vidhyasagar & Jeevaratnam, 2013).

The molecular identification of 16SrRNA

The molecular identification of LAB isolate with code L04 from 'lawar ikan', phylogentic analysis shows the isolate is a species of *Pediococcus pentosaceus*. The advantage of identification technology using the 16S rRNA gene can only be done if the nucleotide sequence information of the targeted bacterial nucleotide is known in advance (Jiang et al., 2021). Research (Iman Hidayat, 2020) reported that from the results of LAB identification from tape and tempeh samples in Bali province, there were 3 species of Pediococcus pentosaceus bacteria. *Pediococcus pentosaceus* bacteria are commonly found in traditional fermented foods and show probiotic potential because they are resistant to stomach acid, resistant to bile salts and have BSH (bile salt hydrolase) activity, and have antibacterial activity (Qi et al., 2021).

Conclusion

The isolation results obtained 11 isolates that have different characters and are Gram-positive bacilli and 1 isolate is a Gram-positive cocci. The isolates produced are heterofermentative and catalase negative. LAB resistance test results to low pH, namely pH 2 is isolate L03 with a final viability percentage of 61.75%, at pH 3 is isolate L04 with a final viabitlity percentage of 51.75%. Resistance testing against bile salts obtained isolates that are resistant at a concentration of 0.3%, namely isolates L07, L05 and L04. Testing against pathogenic bacteria there are 2 potential isolates, namely L04 with an inhibition zone diameter of 5 mm against *E.coli* and 13 mm against *S. aureus*, while isolate L11 with an inhibition zone diameter of 1 mm against *E.coli* and 21 mm against *S. aureus*. LAB isolates have probiotic potential because they can survive at low pH and bile salts. The result of molecular identification of probiotic LAB candidate, isolate L04, is *Pediococcus pentosaceus*.

Acknowledgement

Particular thanks are due to the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology (DRTPM) in 2023 for providing financial assistance in the implementation of Research activities for Beginner Lecturers (PDP) with Contract Number 174/E5/PG.02.00.PL/2023. Furthermore, thanks to LPPM University of Timor for facilitating the administration of research activities.

References

Candra, J. I., Zahiruddin, W., & Desniar. (2007). Isolasi dan Karakterisasi Bakteri Asam Laktat dari Produk Bekasam Ikan Bandeng (*Chanos chanos)*. *Buletin Teknologi Hasil Perikanan*, *X*(2), 14–24.

Clinical Laboratory Standart Institute. (2013). Performance Standart for Antimicrobial Susceptibility Testing; Twentieth Information Supplement. USA

Emmawati A, Laksmi B, Nuraida L.(2015). Characterization of Lactic Acid Bacteria Isolates from Mandai Function as Probiotic. *Jurnal Agritech* 35: 146.

Halim, C. N., & Zubaidah, E. (2013). Probiotic Assay of Lactic Acid Bacteria as High Exopolysaccharides Producer from Sour Pickled Mustard (Brassica juncea). *Jurnal Pangan Dan Argoindustri*, *Vol. 1*(1), 129–137.

Hang, M. (2021). The probiotic potential of lactic acid bacteria (LAB) isolated from naniura (a traditional Batak food). *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, *4*(1), 68–74.

Haro, G., Iksen, I., & Nasri, N. (2020). Identification, characterization and antibacterial potential of probiotic lactic acid bacteria isolated from naniura (A traditional batak fermented food from carp) against Salmonella typhi. *Rasayan Journal of Chemistry*, *13*(1), 464–468.

Hernández-Gómez, J. G., López-Bonilla, A., Trejo-Tapia, G., Ávila-Reyes, S. V., Jiménez-Aparicio, A. R., & Hernández-Sánchez, H. (2021). In vitro bile salt hydrolase (Bsh) activity screening of different probiotic microorganisms. *Foods*, *10*(3), 1–10.

Iman Hidayat. (2020). Identifikasi molekuler Bakteri Asam Laktat dari Tempe dan Tape Berdasarkan Sekuen Gen 16S rRNA. *Majalah Ilmiah Biologi Biosfera : A Scientific Journal*, *37*(2), 69–77.

Jiang, S., Cai, L., Lv, L., & Li, L. (2021). *Pediococcus pentosaceus*, a future additive or probiotic candidate. *Microbial Cell Factories*, *20*(1), 1–14.

Ljungh, Å., & Wadström, T. (2006). *Lactic acid bacteria: Microbiological and functional aspects*. *Food Research International*, *39*(10), 1189-1200.

Nurnaafi, A., Setyaningsih, I., & -, D. (2015). Probiotic Potential of Bekasam Lactic Acid Bacteria of Tilapia Fish. *Jurnal Teknologi Dan Industri Pangan*, *26*(1), 109–114.

Okfrianti, Y., Darwis, D., & Pravita, A. (2018). Bakteri Asam Laktat Lactobacillus Plantarum C410LI dan Lactobacillus Rossiae LS6 yang Diisolasi dari Lemea Rejang terhadap Suhu, pH dan Garam Empedu Berpotensi sebagai Prebiotik. *Jurnal Ilmu Dan Teknologi Kesehatan*, *6*(1), 49–58.

Qi, Y., Huang, L., Zeng, Y., Li, W., Zhou, D., Xie, J., Xie, J., Tu, Q., Deng, D., & Yin, J. (2021). Pediococcus pentosaceus: Screening and Application as Probiotics in Food Processing. *Frontiers in Microbiology*, *12*(December).

Ramesh, C, & Ray, DM. (2015). *Food Biology Series*. 108–109. CRC Press, Boca Raton, Florida.

Ramirez-Chavarin, M. L., Wacher, C., Eslava-Campos, C. A., & Perez-Chabela, M. L. (2013). Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. *International Food Research Journal*, *20*(2), 991–1000.

Rizal, S., Erna, M., & Nurainy, F. (2016). Karakteristik Probiotik Minuman Fermentasi Laktat Sari Buah Nanas dengan Variasi Jenis Bakteri Asam Laktat Probiotic Characteristic of Lactic Fermentation Beverage of Pineapple Juice with Variation of Lactic Acid Bacteria (LAB) Types mengonsumsi minuman. *Indonesian Journal of Applied Chemistry*, *18*(1), 63–71.

Soemarie, Y. B., Milanda, T., & Barliana, M. I. (2021). Fermented foods as probiotics: A review. *Journal of Advanced Pharmaceutical Technology and Research*, *12*(4), 335–339.

Surono, I., 2004. *Probiotik Susu Fermentasi dan Kesehatan*, Vol. 7, Ed.2, PT. Zitri Cipta Karya, Jakarta.

Vidhyasagar, V., & Jeevaratnam, K. (2013). Evaluation of Pediococcus pentosaceus strains isolated from Idly batter for probiotic properties in vitro. *Journal Of Functional Foods* 5 (2013) 235 – 243.