BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 897-903 | DOI: 10.14421/biomedich.2025.142.897-903 ISSN 2540-9328 (online)

Isolation, Identification, and Evaluation of Antimicrobial of the LAB from Bekasam: The Traditional Fermented Fish in Indonesia

Nur Rohman[1], Muhammad Bachrun Alim[2]*
[1]Agricultural Product Technology Program, Faculty of Agroindustry, Universitas Mercu Buana Yogyakarta, Indonesia
Jl. Raya Wates-Jogjakarta, Bantul, Yogyakarta 5575, Telp. 0274-6498212, Fax. 0274-6498213, Indonesia.
[2]Department of Marine Biology, Faculty of Marine Science, King Abdulaziz University, Saudi Arabia
Idara st, Jeddah, Telp. +966 12 6952043, Saudi Arabia.

Corresponding author*
Mmuhyanto@stu.kau.edu.sa

Manuscript received: 29 July, 2025. Revision accepted: 10 October, 2025. Published: 24 October, 2025.

Abstract

Lactic acid bacteria (LAB) are frequently utilized in fermented foods and can incresed shelf life for the products through their secondary metabolites includes carbon dioxide, hydrogen peroxide, lactic acid and bacteriocins. Beside increasing the shelf life, LAB also affect taste, smelt, and texture. Thus, this study aimed to isolate, identify and evaluate antibacterial LAB strains from Indonesian traditional fermented fish (Bekasam). Gram staining, the catalase assay, and motility assays were used to initially characterize the presumed isolates phenotypically following primary isolation on De Man, Rogosa, and Sharper (MRS) agar. 14 isloates were determined to be presumed LAB by preliminary phenotypic testing. One species that has the highest antibacterial activity is confirmed by a 16S rRNA sequencing study. *Lactobacillus plantarum* CP-134 was identified from the bekasam. In the biochemical characterization all isolates were catalase-negative and non-motile. According to our research, these LAB strains may have applications in fermented foods due to their probiotic properties, which include antibacterial activity. They might be used as natural substitutes for additives and antibiotics, but more in vivo or in silico research is required to verify their potential and effectiveness. A typical traditional food preservation technique for enhancing shelf life, food safety and nutrition and sensory qualities is fermentation. Bekasam is a traditional fermented food from Indonesia.

Keywords: Bekasam; fermentation; lactic acid bacteria; 16S rRNA.

Abbreviations: LAB: Lactic acid bacteria; MRS: De Man, Rogosa, and Sharper; CFU:Colony Forming Unit; TPC: Total Plate Count.

INTRODUCTION

One of the most popular traditional fermented fish food from Indonesia is Bekasam. Bekasam is one of the fermented fish product that is processed by adding salt and sugar. Fish fermentation procedures often involve salting, adding species or chemicals, and maintaining anaerobic conditions, which promote the growth of certain protechnological microbes, and distinct flavour, texture, and nutritional values. Generally, the ex-fermentation process occurs spontaneously by utilizing salt as a microbial selector of decomposer (Mudoor Sooresh et al., 2023). In addition to salt, the sugar added is used as an energy source for the growth of bacteria that ferment fish (Setiarto & Herlina, 2024). Salt is a traditional and effective method for preserving fish, resulting in a salt adapted microbiota. The addition of salt causes physicochemical change in fish tissue, including a reduction in the moisture content and water activity through salt diffusion and increasing the solubility of denatured protein (known as salting-in). Bekasam is a local Indonesian food, popular in Centra Java, South Kalimantan and South Sumatra. In general LAB is the dominant bacterium found in fermented products. Traditional fermented foods are primarily produced at the household level using largely uncontrolled spontaneous inoculation methods in which microorganisms associated with the raw food material and processing environment serve as inoculants.

Many studies have characterized spontaneous fermentation. The role of the starter in the fermentation process is too important. Despite the importance of microorganisms in fish fermentation, discrepancies have been found in the LAB colony during fermentation and the impact of starter culture on fermented fish. The starter cultures used in fermented fish and the microbial diversity involved during this process, and an in-depth discussion of the methods used to identify these microorganisms.

LAB are bacteria that are found in many fermented fish products. The metabolism of LAB includes carbon dioxide, hydrogen peroxide, lactic acid, and bacteriocins, which can inhibit the growth of spoilage bacteria, thereby increasing the shelf life of the products. Besides

increasing the shelf life, LAB also affect taste, flavour, and texture. This type survives at salt level of 3%-6% including *Lactobacillus plantarum*, *Lactobacillus sakei* (Li et al., 2023), *Lactobacillus acidophilus* CM1 and *Lactobacillus delbrueckii* OS1 could survived at 4% and 6% NaCl level significantly (Khushboo et al., 2023). This study aims to determine the type of LAB originating from bekasam which is processed with different salt levels for 14 days which are spontaneously fermented.

MATERIALS AND METHODS

Study Area

This study was conducted in September 2024 at Tropical Marine Biotechnology Laboratory, Universitas Diponegoro, Central Java, Indonesia.

Sample of Bekasam

The fermented food sample used in this study includes bekasam that is processed with salt at different levels (Melia et al., 2019)

pH measurement

A digital pH meter was used to determine pH. The electrode was dipped into the sample until the pH value was shown (Greulich et al., 2024).

Titratable acidity

The titration method was used to measure the amount of lactic acid. Ten grams of the bekasam samples have been mashed, and the filtrate is then taken and placed in an erlenmeyer flask, followed by the addition of 2-3 drops of 1% PP and 10 mL of aquadest (Greulich et al., 2024). The following formula was used to determine the lactic acid % result:

$$\text{lactic acid (\%)} = \frac{\text{mL NaOH x N NaOH x 0,009}}{\text{Sample weight}} \text{ x } 100\%$$

Enumeration of LAB in bekasam

The total bacterial count was calculated using the TPC (Total Plate Count) method (Greulich et al., 2024). The overall bacterial was count determined using the following formula:

$$\text{Total (CFU/mL)} = \frac{\text{Total Colony}}{\text{Spread Volume x dilution factor}}$$

Isolation and identification of LAB

The LAB from Bekasam were cultured in MRS broth media (Merck, Germany) and incubated at 37ºC in an anaerobic jar for 48 hours. Additionally, morphological (form and color) and biochemical (gram staining, catalase test, motility test) features were determined (Madushanka et al., 2025).

Gram staining

Pure culture smears that had been air-dried and heat-fixed were successively stained with safranin, crystal violet, gram iodine, and a decolorizing agent. Each staining step was followed by a gentle wash. After being blot-dried with absorbent paper, the slides were viewed under an oil immersion microscope (El Ahmadi et al., 2025).

Catalase assays

A sterile inoculating loop was used to transmit a tiny portion of a recently isolated colony onto a microscope slide. After that, a drop of 3% H_2O_2 was applied. The quick development of air bubbles demonstrated that the catalase test was positive (Joos et al., 2025).

Motility test

In the middle of the coverslip was a loopful of freshly made broth culture that had been developed overnight. In order to make a depression over the culture drop, a cavity slide was then carefully inverted and placed over the coverslip. The drop was allowed to dangle freely in the cavity after the slide was carefully inverted. To determine if there was movement or not. The cells were viewed in low light and at medium strength (×40) (Rizwan & Masoodi, 2025).

Antimicrobial activity test

To perform an antibacterial test, 14 colonies from bekasam were cultured in MRS broth at 37ºC for 48 hours. Antibacterial activity was tested on strains associated with food poisoning, including *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity of each isolates components was determined using the paper disc method (Park et al., 2024). A paper disc was inoculated with 50 µL of LAB suspension containing 10^{-7} CFU/mL obtained from bekasam. The paper disc were incubated at 37ºC for 48 hours, and antibacterial activity was measured by the diameter of the clear zones. We chose one top-performing LAB based on our findings.

Genomic analysis of LAB and the construction of a phylogenetic tree for identified strains with strong antimicrobial activity

LAB isolates have been grown in MRS broth at 37ºC for 24 hours. Genomic DNA was extracted using the Promega kit (USA). A single colony of LAB from MRS broth (Merck, Germany) was pipetted up to 1000 µL and added to a new eppendorf tube. Centrifuged at 14000 rpm for two minutes. The supernatant is then removed, and keeping only the pellet. Added 840 µL of 50 mM EDTA. Then, 120 µL lysozyim was applied. Next, incubate in a 37ºC water bath for 60 minutes. Centrifuge for 2 minutes at 14000 rpm, then remove the supernatant and take the pellet. Applied 600 µL of nuclei lysis solution. After incubating at 80ºC for 5 minute, let it cool

to room temperature. Add 3 µL of Rnase solution and incubate in water bath at 37ºC for 60 minutes. Add 200 µL of protein precipitation solution, then vortex. Approximately 600 µL of isopropanol was added. Centrifugated at 14000 rpm for 2 minute, then pellets were collected and supernatant have been eliminated. Add 600 µL of 70% ethanol and homogenized. Centrifuged at 14000 rpm for 2 minutes, then pellets were collected and the supernatant eliminated. To rehydrate pellet DNA, add 10-100 µL of rehydration solution and incubate for 30 minutes at 65ºC. General primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGTTAACCTTGTTACGACTT-3') generated (concentration 10 Pm) take 90 µL dH_2O + (primary R and F). (primary R and F in TE buffer, concentration 100 µM). PCR cocktail in eppendorf (Master Mix 12,5 µL, Primary F 1 µL, Primary R 1 µL, Template DNA 1 µL, ddH_2O 9,5 µL) with PCR denaturation 95ºC 45 second, annealing 56ºC 45 second, extension 72ºC 1 minute 40 second, and final extension 72ºC 10 minute. Electrophoresis of a 10 µL sample in to wall agar, inserting 4 µL of DNA ladder. Set at 100 volts for 45 minutes. The gel was placed in a container with TBE until submerged. The gel was then examined under a UV lamp. The 16S rRNA gene sequences of the isolates were submitted to NCBI for a BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) comparison to database records. Phylogenetic trees were generated through the neighbor-joining method (https://www.megasoftware.net/).

Data analysis

Three separate experiments were conducted, and the statistical package for the social science (SPSS) was used to analyze all data using a one-way ANOVA. Duncan's multiple range was used to determine whether mean differences were significant ($P<0.05$).

RESULTS AND DISCUSSION

Results

Characteristics of the isolated LAB

Table 1 shows the total of LAB colony from bekasam made with three different salt levels (15%, 20% and 25%).

Table 1. result of pH and number of LAB in bekasam.

Sample Code	pH	% Lactic Acid	Total colony of LAB (CFU/mL)
B1	5.3	4.3	1.7×10^5
B2	5	4.1	1.1×10^5
B3	5.3	4.7	1.5×10^5

Note. B1: salt level 15%, B2: 20%, B3: 25%

Isolation and Morphological Characterisation of the LAB

Table 2. result of LAB morphological and biochemical test

Code	Number of Isolates				
	Gram Stain	Motility	Catalase	Morphology	
				Cocci	Rod
Bal 1	+	-	-		+
Bal 2	+	-	-		+
Bal 3	+	-	-		+
Bal 4	+	-	-		+
Bal 5	+	-	-	+	
Bal 6	+	-	-		+
Bal 7	+	-	-		+
Bal 8	+	-	-	+	
Bal 9	+	-	-	+	
Bal 10	+	-	-	+	+
Bal 11	+	-	-		+
Bal 12	+	-	-	+	
Bal 13	+	-	-		+
Bal 14	+	-	-		+

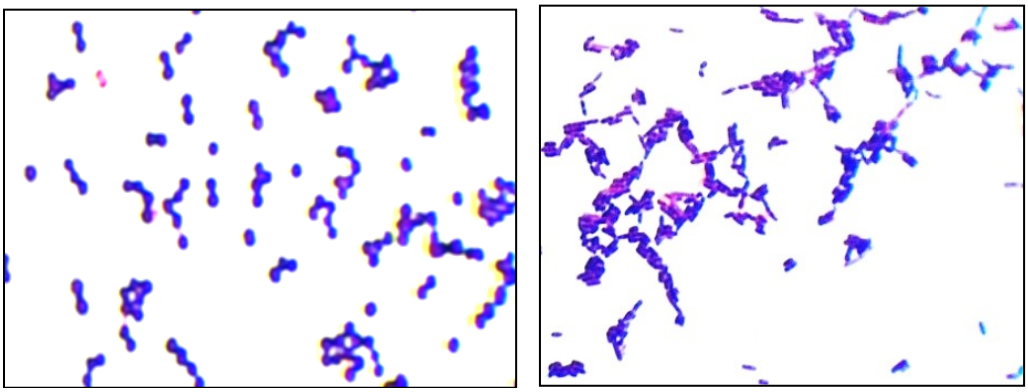

Figure 1. morphological characteristic of gram-positive of LAB (a. shows cocci-shaped bacteria, b. shows rod-shaped bacteria).

Antimicrobial activity

The antimicrobial activity test demonstrated that LAB inhibited the development of *Staphylococcus aureus* FNCC-0047 and *Escherichia coli* FNCC-0091, but the inhibition zone was relatively modest in size. The diameter of the inhibition zone was measured after incubation for 24 hours and repeated three times.

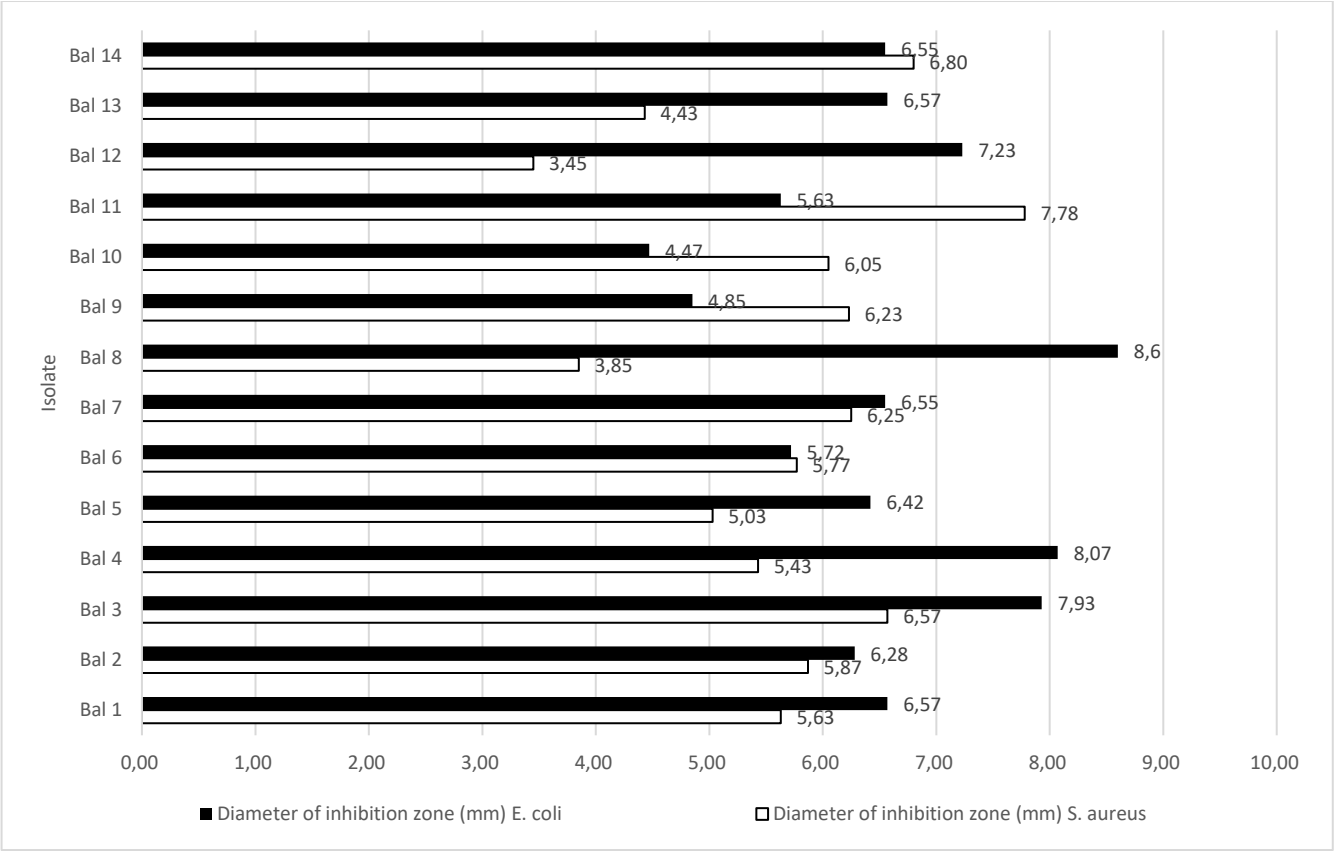

Figure 2. Antimicrobial activity test of LAB isolate (mm).

Identification strain with strong antimicrobial activity using 16S rRNA

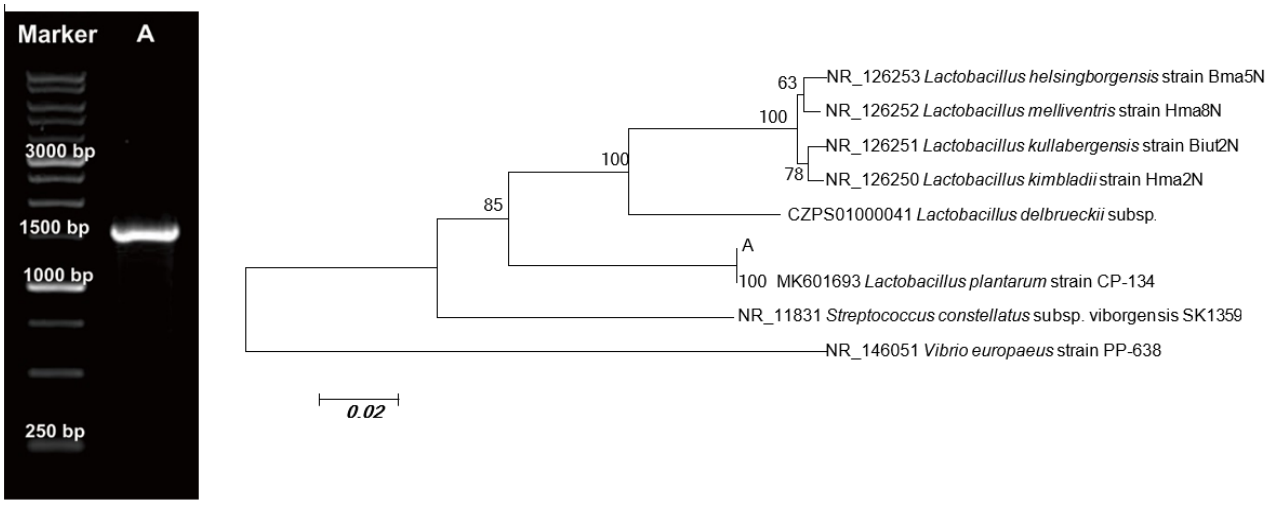

Figure 3. A gel with agarose electrophoresis of 16S rRNA gene amplification by PCR revealed and phylogenetic tree of lactobacilli strains isolated from bekasam on the 16S rRNA gene.

Discussions

Characteristic of isolated LAB

The bekasam sample has a pH between 5.0 and 5.3, which means that the provision of different salt levels in bekasam processing does not have a significant effect on the pH. Similarly, the three samples of bekasam showed no substantial changes in total lactic acid levels. The number of LAB colonies of bekasam from the three samples ranged between 1.1 x 10^5 CFU/mL to 1.7 x 10^5 CFU/mL. The outcome demonstrates a relationship between the pH value and the number of LAB. LAB can thrive in low pH and high salt environment conditions, Ter et al., (2024) reported that, the optimal conditions for LAB growth are with a pH of 5.0 – 6.2. On the other hand, bacterial metabolism, which produces lactic acid, modulates the pH, causing it to become acidic (Zhou et al., 2025). Because the development and metabolic activites of LAB produce acid and lower pH of the fermented fish (bekasam), their presence in the bekasam is crucial. The growth of the harmful bacteria may be inhibited by this acidic environment.

Isolation and morphological characterisation of LAB

The bekasam was fermented for up to 7 days to produce more fermented liquid. The liquid was thought to contain the most bioactive antimicrobial compounds because it had the largest clear zone of inhibition in our early investigation. This also revealed that the liquid already had an excess of LAB. The liquid was streaked in quadrants across the MRS medium. The opaque color media is the product of $CaCO_3$ precipitation in the MRS medium. Calcium carbonate dissolve when a colony creates lactic acid molecules (Sariyanti et al., 2025). A single colony with a clear zone was considered to be LAB, thus it became isolated and characterized.

Fourteen isolates exhibited gram-positive, non-motile, and catalase-negative, with 9 rod morphologies and only 5 cocci respectively (Table. 2) and (Figure. 1), these were standard criteria for targeted strains (Alharbi & Alsaloom, 2021). The study found that bekasam was the primary source of LAB, concurring with Desniar et al., (2013) who successfully identified of LAB from eight types of fish bekasam from eight areas in Indonesia included *Lactobacillus* sp., *Pediococcus* spp., and *Aerococcus* spp. In other study also report that LAB from Plara (traditional fermented fish from Thailand) like bekasam was successfully collected, which are *Enterococcus avium*, *Aerococcus viridans*, *E. faecalis*, *E. thailandicus*, *E. hirae*, *L. lactis*, *L. plantarum*, *Pedicoccus pentosaceus*, *L. paracasei*, *P. acidilactici*, *W.cibaria*, *Tetragenococcus halophilus*, *W. paramesenteroides*, *W. confusa*, and *W. viridescens* (Miyashita et al., 2012).

Antimicrobial activity

The antimicrobial activity of the LAB was assessed using the paper disk method. Antimicrobial activity testing is required to determined the inhibitory potential of LAB microorganisms against pathogenic bacteria. Figure 2 demonstrate the results of antimicrobial testing with the diameter of the inhibitory clear zone of numerous isolates. The inhibitory band of every isolate ranged around 4.47 mm to 8.07 mm against *Escherichia coli* FNCC-0091and 3.45 mm to 7.78 mm against *Staphylococcus aureus* FNCC-0047.

LAB produce antimicrobial chemicals and exhibit considerable antagonistic action against several pathogenic bacteria. LAB can produce considerable amounts of antimicrobial metabolites, including as lactic acid, bacteriocin, diacetyl, and hydrogen peroxide, which

inhibit the growth of several harmful bacteria (Santos et al., 2025) The existence of clean zone indicates antimicrobial action against pathogenic microorganisms (Apenteng-takyiako et al., 2025). In the examination of antimicrobial activity against *Escherichia coli* FNCC-0091and *Staphylococcus aureus* FNCC-0047, the findings of measuring the diameter if the inhibition zone indicates moderate results. The isolate that generated the largest diameter of the inhibition zone was Bal8 code.

(Bhattacharjya et al., 2024) classified antimicrobial activity into three categories; no inhibition (negative), moderate (<9 mm), and strong (>9 mm). Based on this criteria, it can be conclude that the majority of the isolate LAB had modest inhibition against *Escherichia coli* FNCC-0091. The isolate with a code Bal8 has the largest zone of inhibition, measuring.

Identification of a strain with strong antimicrobial activity using 16S rRNA

By using the BLAST algorithm to compare homology with 16S rRNA gene sequences in GeneBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequencing findings were utilized to determine genetic LAB from bekasam isolates. Figure 3 showed the LAB PCR sequencing result from bekasam sample. Figure 3 displayed the LAB phylogenetic tree from bekasam sample.

According to the findings, *Lactobacillus plantarum* strain CP134 and LAB from sample of bekasam were 100% same. According to (Najeeb et al., (2025), isolates were deemed to be of the same species if their 16S rRNA sequences were more than 97% identical. On the other hand, 93%-97% of sequence similarities were regarded as belonging to different species but the same genus.

CONCLUSIONS

This study's main goals were to screen bekasam for the types and presence of lactic acid bacteria (LAB) at various salt levels during fermentation and demonstrate that bekasam, a traditional fermented fish, may contain lactic acid bacteria, offering several health benefits associated with probiotic consumption. From the examined bekasam samples, *Lactobacillus plantarum* CP-134 were detected. The isolation exhibited antibacterial activity against certain food-borne pathogens and was tolerant of low pH and high salt levels. According to this study, lactic acid bacteria (LAB), which are essential for fermentation, are present in bekasam and may have antibacterial qualities. The prevalence of LAB indicates their role in the fermented fish potential health advantages and microbiological stability. Regarding food safety, the study emphasizes how LAB has antagonistic effects on foodborne pathogens, suggesting that they may lower the risk of contaminations and improve microbiological safety. Their capacity to produce bacteriocins, organic acids, and other antimicrobial substances may help extend shelf life and prevent spoilage. Certain LAB strains may be used as indicators to evaluate the microbiological integrity of bekasam. Additionally, this work creates new opportunity to obtain probiotics from natural and unconventional sources like bekasam.

Acknowledgements: The authors would like to thank to PT. Indofood Sukses Makmur, Tbk for funding this research under Indofood Riset Nugraha Program.

Authors' Contributions: Nur Rohman designed the study, carried out the laboratory work, analyzed the data, wrote the manuscript, Muhammad Bachrun Alim review and editing the manuscript, all authors read and approved the final version of the manuscript.

Competing Interests: The authors declare no conflict of interest

Funding: The authors would like to thank to PT. Indofood Sukses Makmur, Tbk for funding this research under Indofood Riset Nugraha Program.

REFERENCES

Alharbi, N. K., & Alsaloom, A. N. (2021). Characterization of Lactic Bacteria Isolated from Raw Milk and Their Antibacterial Activity against Bacteria as the Cause of Clinical Bovine Mastitis. *Journal of Food Quality*, *2021*, 12–16. https://doi.org/10.1155/2021/6466645

Apenteng-takyiako, S., Afua, C., Takyi, A., Chama, M. A., Osei, M., Donkor, E. S., & Futagbi, G. (2025). *In vitro antibacterial activity of Moringa oleifera leaf and seed extracts against antibiotic-resistant bacteria and clinical isolates*. *6*.

Bhattacharjya, S., Zhang, Z., & Ramamoorthy, A. (2024). LL-37: Structures, Antimicrobial Activity, and Influence on Amyloid-Related Diseases. *Biomolecules*, *14*(3). https://doi.org/10.3390/biom14030320

Desniar, Rusmana, I., Suwanto, A., & Mubarik, D. N. R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food and Agriculture*, *25*(6), 489–494. https://doi.org/10.9755/ejfa.v25i6.12478

El Ahmadi, K., Haboubi, K., El Allaoui, H., El Hammoudani, Y., Bouhrim, M., Eto, B., Shahat, A. A., & Herqash, R. N. (2025). Isolation and preliminary screening of lactic acid bacteria for antimicrobial potential from raw milk. *Frontiers in Microbiology*, *16*(March), 1–13. https://doi.org/10.3389/fmicb.2025.1565016

Greulich, O., Duedahl-Olesen, L., Mikkelsen, M. S., Smedsgaard, J., & Bang-Berthelsen, C. H. (2024). Fourier Transform Infrared Spectroscopy Tracking of Fermentation of Oat and Pea Bases for Yoghurt-Type Products. *Fermentation*, *10*(4). https://doi.org/10.3390/fermentation10040189

Halder, D., Mandal, M., Chatterjee, S. S., Pal, N. K., & Mandal, S. (2017). Indigenous probiotic Lactobacillus isolates presenting antibiotic like activity against human pathogenic bacteria.

Biomedicines, *5*(2), 1–11. https://doi.org/10.3390/biomedicines5020031

Joos, M., Van Ginneken, S., Villanueva, X., Dijkmans, M., Coppola, G. A., Pérez-Romero, C. A., Vackier, T., Van der Eycken, E., Marchal, K., Lories, B., & Steenackers, H. P. (2025). EPS inhibitor treatment of Salmonella impacts evolution without selecting for resistance to biofilm inhibition. *Npj Biofilms and Microbiomes*, *11*(1), 1–15. https://doi.org/10.1038/s41522-025-00693-y

Khushboo, Karnwal, A., & Malik, T. (2023). Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1170725

Li, S., Tang, S., Mo, R., Li, J., & Chen, L. (2023). Effects of NaCl curing and subsequent fermentation with Lactobacillus sakei or Lactobacillus plantarum on protein hydrolysis and oxidation in yak jerky. *Lwt*, *173*(August 2022), 114298. https://doi.org/10.1016/j.lwt.2022.114298

Madushanka, D., Vidanarachchi, J. K., Kodithuwakku, S., Nayanajith, G. R. A., Jayatilake, S., & Priyashantha, H. (2025). Isolation and characterization of probiotic lactic acid bacteria from fermented traditional rice for potential applications in food and livestock production. *Applied Food Research*, *5*(1). https://doi.org/10.1016/j.afres.2025.100865

Melia, S., Purwati, E., Kurnia, Y. F., & Pratama, D. R. (2019). Antimicrobial potential of pediococcus acidilactici from Bekasam, fermentation of sepat rawa fish (Tricopodus trichopterus) from Banyuasin, South Sumatra, Indonesia. *Biodiversitas*, *20*(12), 3532–3538. https://doi.org/10.13057/biodiv/d201210

Miyashita, M., Malimas, T., Sugimoto, M., & Yoshino, M. (2012). 16S rRNA gene sequence analysis of lactic acid bacteria isolated from fermented foods in Thailand. *Microbial Culture Collection*, *28*, 1–9.

Mudoor Sooresh, M., Willing, B. P., & Bourrie, B. C. T. (2023). Opportunities and Challenges of Understanding Community Assembly in Spontaneous Food Fermentation. *Foods*, *12*(3). https://doi.org/10.3390/foods12030673

Najeeb, M., Cameron, G., Grimard-conea, M., Matthews, S., Brodeur, J., Cadieux, G., & Pierre, A. (2025). *Comparative genome analysis investigation of nosocomial and*. *13*(7).

Park, J. M., Moon, J. W., Zhang, B. Z., & An, B. K. (2024). Antioxidant Activity and Other Characteristics of Lactic Acid Bacteria Isolated from Korean Traditional Sweet Potato Stalk Kimchi. *Foods*, *13*(20). https://doi.org/10.3390/foods13203261

Rizwan, D., & Masoodi, F. A. (2025). Isolation and characterization of Lactic acid Bacteria from spontaneously fermented kohlrabi pickle of Jammu and Kashmir, India. *Applied Food Research*, *5*(2), 100973. https://doi.org/10.1016/j.afres.2025.100973

Santos, C., Raymundo, A., Moreira, J. B., & Prista, C. (2025). Exploring the Potential of Lactic Acid Bacteria Fermentation as a Clean Label Alternative for Use in Yogurt Production. *Applied Sciences (Switzerland)*, *15*(5). https://doi.org/10.3390/app15052686

Sariyanti, M., Adri, P. M., Wibowo, R. H., Nasution, A. A., Hidayah, T., & Sipriyadi. (2025). Molecular identification and antimicrobial activities of Bacillus subtilis MS-01 isolated from Indonesian ethnic food lemea. *Biodiversitas*, *26*(3), 1069–1074. https://doi.org/10.13057/biodiv/d260305

Setiarto, R. H. B., & Herlina, V. T. (2024). Exploring bekasam, an indigenous fermented fish product of Indonesia: original South Sumatra region. *Journal of Ethnic Foods*, *11*(1). https://doi.org/10.1186/s42779-024-00230-w

Ter, Z. Y., Chang, L. S., Babji, A. S., Zaini, N. A. M., Fazry, S., Sarbini, S. R., Peterbauer, C. K., & Lim, S. J. (2024). A review on proteolytic fermentation of dietary protein using lactic acid bacteria for the development of novel proteolytically fermented foods. *International Journal of Food Science and Technology*, *59*(3), 1213–1236. https://doi.org/10.1111/ijfs.16888

Zhou, T., Lei, X., Luo, Y., Ou, Y., Tian, S., Xie, Y., Zheng, Y., Sun, H., & Yang, F. (2025). High temperatures and antibacterial plant additives change the fermentation quality, free amino acids and lactic acid bacteria fermentation type in Caragana Korshinskii silage. *BMC Microbiology*, *25*(1). https://doi.org/10.1186/s12866-025-04153-7

THIS PAGE INTENTIONALLY LEFT BLANK