**JOPS: *Journal of Pharmacy and Science***
p-ISSN: 2622-9919 | e-2615-1006
Homepage: http://jurnal.univrab.ac.id/index.php/jops

J Pharm & Sci Vol. 8, No. 1 (Dec 2024), pp. 29-35

**ORIGINAL RESEARCH**

# Isolation and Characterization of Bacteria from Bekasam-Fermented Fish (*Rasbora sp*) and Their Antibacterial Potential

**M. Rifqi Efendi[1*], Yunita Safitri[2], Rustini[3], Mesa Sukmadani Rusdi[4]**
[1]*Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia*
[2]Department of Pharmacy, Universitas Dharma Andalas, Padang, West Sumatera, Indonesia
[3]*Department of Pharmacy, Faculty of Pharmacy, Universitas Andalas, West Sumatera, Indonesia*
[4]*Department of Pharmacy, Poltekkes Kemenkes Jambi, Jambi, Indonesia*

ABSTRACT

Bekasam is a traditional fermented fish product. This study aimed to isolate and identify the bacteria involved in the fermentation of Bekasam from Riau and evaluate the antibacterial potential of their ethyl acetate extracts. Bacterial isolation was conducted using the pour plate method with selective media (MRS agar-$CaCO_3$ 0.5%). Antibacterial activity was tested against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the agar diffusion method, with chloramphenicol as a positive control. Four bacterial isolates were identified: *Lactobacillus sp1* (BAC1), *Lactobacillus sp2* (BAC2), *Bacillus sp1* (BAC3), and *Bacillus sp2* (BAC4). All ethyl acetate extracts from these isolates demonstrated antibacterial activity. The highest activity was observed from the *Lactobacillus sp1* extract against *E. coli*, with an inhibition zone diameter of 16.1 mm.

**Keywords**: Bekasam, fermentation, *Lactobacillus sp*., *Bacillus sp*., antibacterial

## Introduction

Fermented foods result from controlled microbial growth and the transformation of food components through enzymatic action. Traditionally, the purposes of fermentation include food preservation and enhancement of sensory qualities. This process occurs spontaneously in a conducive environment (Melini et al., 2019), allowing native lactic acid bacteria (LAB) to thrive optimally in the fermented food. Numerous studies have demonstrated that microorganisms present in fermented foods confer health benefits and can reduce disease risk, making them viable as probiotics. Microorganisms isolated from fermented foods can also reduce enzymes, antibiotics, vitamins, and bioactive metabolites (Qian et al., 2020).

Bekasam is a traditional fermented fish product that is widely popular in various regions of Indonesia. Each region has its distinctive type of bekasam, determined by the type of freshwater fish used. The production process involves using salt and roasted rice, which stimulate the spontaneous growth of LAB. LAB and substrates play crucial roles in the fermentation process, leading to changes in aroma and texture and contributing to preservation, thus extending the final product's shelf life (Haryo et al., 2024).

Bioactive compounds derived from bacteria in fermented foods exhibit diverse bioactivities, particularly those sourced from LAB. For instance, *Lactobacillus plantarum* isolated from spontaneously fermented goat milk has demonstrated probiotic properties, including antimicrobial effects against several pathogenic bacteria (Moreno-Montoro et al., 2018). Another study identified antimicrobial compounds such as bacteriocin from bacteria isolated from Kimchi(Yi and Kim, 2023). (Li et al., 2021) reported that Lactobacillus isolated from fermented soybean milk produced antioxidant compounds.

Isolation and bioactivity testing of metabolite compounds from bacteria isolated from bekasam have been performed. Wikandari et al., 2013 demonstrated that *L. plantarum* isolated from bekasam exhibited angiotensin-converting enzyme (ACE) inhibitory activity. Additionally, (Dewanti et al., 2017) reported that

*Corresponding Author: M. Rifqi Effendi
[1]*Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia*
Email: *rifqi.efendi09@gmail.com*

*Lactobacillus acidophilus* isolated from bekasam produced bioactive components such as lovastatin and bioactive peptides capable of inhibiting cholesterol synthesis. *Pediococcus acidilactici* isolated from bekasam of Sepat fish showed antibacterial activity against several pathogenic bacteria (Melia et al., 2019).

Based on the literature review, there is limited information regarding the bacteria involved in fermented products, particularly in Riau's bekasam. Therefore, further research on this product is necessary, especially on the acid-producing bacterial isolates from bekasam and their antibacterial potential

## Materials and Methode

### Materials

Bekasam purchased from Pasar Jumat, Muara Lembu village, Singingi sub-district, Kuantan Singingi district, Riau province; 0.9% NaCl, De Man–Rogosa–Sharpe (MRS) agar (Merck®), Mueller Hinton Agar (MHA) (Oxoid®), CaCO3, sterile distilled water, ethanol 96% (Brathachem®), ethyl acetate (Brathachem®), disk paper.

### Equipment

Analytical balance (KERN®), laminar airflow (INNOTECH-BIOBASE®), autoclave (HIRAYAMA®), Erlenmeyer flask (AGC Iwaki®), test tubes (Borosil®), Petri dishes (Normax®), inoculating loop, beaker glass (AGC Iwaki®), micropipette (Socorex Acura®), incubator (Memmert®), Microscope (Olympus®), Hotplate (IKA®) were used in this study.

### Methods

1. Isolation of Bacteria from Traditional Fermented Food (Bekasam)

The bacteria were isolated from bekasam using the pour plate method. One gram of bekasam sample was ground in a sterile mortar to obtain a homogeneous sample. The homogenized sample was diluted with 0.9% NaCl to a total volume of 10 ml, resulting in a $10^{-1}$ dilution. From the $10^{-1}$ dilution, 1 ml was taken and added to a test tube containing 9 ml of NaCl, resulting in a $10^{-2}$ dilution, and so on until a $10^{-5}$ dilution was obtained. From the $10^{-4}$ and $10^{-5}$ dilutions, 1 ml each was taken and inoculated onto Petri dishes containing MRS agar medium (supplemented with 0.5% $CaCO_3$). The Petri dishes were incubated at 37°C for 48 hours. Different bacterial colonies or those showing a clear zone were aseptically taken with an inoculating loop and streaked onto MRS agar medium. They were incubated in an incubator at 37°C for 48 hours. Purification was repeated until pure bacterial isolates were obtained (Zahra et al., 2018).

2. Identification and Characterization of Bacterial Isolates from Traditional Fermented Food (Bekasam)

Bacterial isolates were identified and characterized macroscopically and microscopically at the Bukittinggi Veterinary Center through organoleptic observation, Gram staining, catalase, motility, and endospore tests.

3. Fermentation and Extraction of Secondary Metabolites from Bacterial Isolates

Two inoculating loops of bacterial isolates were inoculated into 25 ml of sterile distilled water and homogenized. The prepared bacterial suspension was inoculated into 225 ml of previously sterilized NB medium and then placed on a rotary shaker at 120 rpm at room temperature for 48 hours. The bacterial fermentation product was mixed with ethyl acetate in a 1:1 ratio, stirred, and left for 24 hours. The ethyl acetate phase was separated using a separating funnel and evaporated using a rotary evaporator at 60°C. The extract was weighed and added with 0.5 ml of dimethyl sulfoxide (DMSO) (Handayani et al., 2015).

4. Antibacterial Activity Test of Ethyl Acetate Extract

The method used for testing the antibacterial activity of the ethyl acetate extract of bacterial isolates was the agar diffusion method using the MHA agar medium. The test samples (0.2 g/mL) were ethyl acetate extracts of bekasam bacterial isolates dissolved in DMSO. The test bacteria used were Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, and Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial suspension was prepared in a sterile 0.9% NaCl solution with

turbidity corresponding to McFarland standard 0.5. Ten µl of the sample suspension (ethyl acetate extract of bekasam bacterial isolate, chloramphenicol (positive control), DMSO (negative control) was dropped onto sterile disk paper, and each disk was placed on the medium of each test bacterium. Each test was performed in triplicate. The Petri dishes were incubated at 37°C for 48 hours. The clear zone was measured using a caliper (Efendi et al., 2022).

## Data Analysis

Antibacterial activity was measured by observing the diameter of the inhibition zones on the disk paper, and the data were analyzed descriptively.

## Result and Discussion

The bekasam product used in this study was purchased from Pasar Jumat, Muara Lembu village, Riau province. The study began with bacterial isolation by cultivating bacteria on MRS agar, a selective medium for lactic acid bacteria (LAB) growth. Isolation was performed using the pour plate method, adding $CaCO_3$ to the medium to distinguish acid-producing bacteria (LAB) from non-acid-producing bacteria. This differentiation is indicated by clear zones around the bacterial growth. This occurs because $CaCO_3$ reacts with the acid produced by LAB, forming soluble calcium lactate, which results in clear zones around the growing colonies, as similarly observed by Olivia (Putri and Kusdiyantini, 2018). Generally, the isolated bacteria appeared round and cream-white, with spreading growth.

Based on macroscopic observation and the formation of clear zones in their growth media, visually distinct isolates were re-inoculated onto other media and repeatedly purified until pure isolates were obtained. Purity was confirmed by observing single colonies that did not overlap. Four pure isolates were obtained and coded as BAC1, BAC2, BAC3, and BAC4, which were then subcultured on slant MRS agar. Each bacterial isolate was subsequently characterized macroscopically and microscopically at the Bukittinggi Veterinary Laboratory (Reference No.: 29014/PK.301/F4B.1/12/2021). The identification results for each isolate are presented in Table 1.

**Table 1.** Identification results of bacterial isolates derived from bekasam

| Code of Bacteria | Isolate of Bacteria | Bacteria |
|---|---|---|
| BAC1 |  | *Lactobacillus sp1* |
| BAC2 |  | *Lactobacillus sp2* |
| BAC3 |  | *Bacillus sp2* |

BAC4  *Bacillus sp1*

Several studies have linked microbes resulting from fish fermentation to various microorganisms, including lactic acid bacteria, *Staphylococcus spp.*, *Bacillus spp.*, and yeasts(Belleggia and Osimani, 2023). This supports previous findings that the four bacterial isolates identified belong to the genera *Lactobacillus sp.* and *Bacillus sp*. Research on these microorganisms is primarily centered on their fermentative capabilities and probiotic benefits. Additionally, they are known for producing valuable metabolites that are applied in nutraceuticals, pharmaceuticals, commodity chemicals, and flavor and aroma compounds. Furthermore, during fermentation, lactic acid bacteria can generate antimicrobial agents such as organic acids, hydrogen peroxide, and bacteriocins, which effectively inhibit the proliferation of spoilage and pathogenic bacteria (Sharma et al., 2019).

The bacterial cultures underwent extraction using ethyl acetate in a 1:1 ratio. Ethyl acetate, a semi-polar solvent with no antimicrobial properties, effectively dissolves both polar and non-polar compounds. Subsequently, the ethyl acetate filtrate was evaporated using a rotary evaporator at 60°C to obtain the extracts. The concentrations of these extracts were ranked in descending order as BAC2, BAC3, BAC4, and BAC1, depicted in Figure 1.

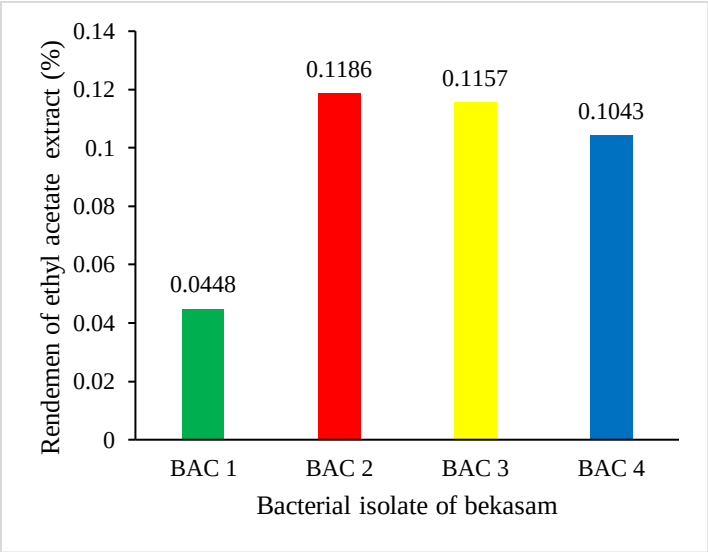



**Figure 1.** Yield of ethyl acetate extracts from bacterial isolates of Bekasam
Note: BAC1: *Lactobacillus sp1;* BAC2: *Lactobacillus sp2*; BAC3: *Bacillus sp2*; BAC4: *Bacillus sp1*

The antibacterial activity of the bacterial extracts was tested using the agar diffusion method with disk paper (Kirby & Bauer method). The positive control was 3 mg/ml chloramphenicol, a broad-spectrum antibiotic effective against Gram-positive and Gram-negative bacteria. Antibacterial activity was categorized based on the diameter of the inhibition zones: weak activity for diameters of 7-9 mm, moderate activity for 9-12 mm, and strong activity for ≥12 mm (Sharma et al., 2009). Four bacterial extracts exhibited antibacterial activity to *Staphylococcus aureus*. One isolate showed weak activity with an 8.1 mm diameter (BAC4), and Three isolates showed strong activity with diameters of 15.75 mm (BAC1), 13.45 mm (BAC2), and 12.2 mm (BAC3). Four extracts showed antibacterial activity to *E. coli*. Two isolates had strong activity with diameters of 16.1 mm (BAC1) and 15.7 mm (BAC2). BAC3 exhibited moderate activity with a 12.05 mm diameter, while BAC4 showed no antibacterial activity. Three extracts exhibited antibacterial activity to *Pseudomonas aeruginosa*. One isolate had moderate activity with a 9.65 mm diameter (BAC3), and two

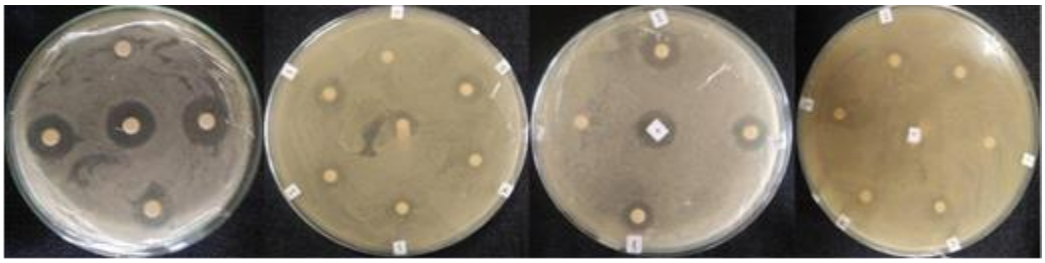
isolates had strong activity with diameters of 13.1 mm (BAC1) and 13.45 mm (BAC2). BAC4 showed no antibacterial activity. Two extracts showed antibacterial activity against *Enterococcus faecalis*. Two isolates had moderate activity with diameters of 13.15 mm (BAC1) and 11.5 mm (BAC2). BAC3 and BAC4 showed no antibacterial activity.

Several studies have underscored the capability of *Lactobacillus sp*. to mitigate bacterial infections following oral administration in mice. Bacteriocins produced by probiotics are characterized as small, cationic, hydrophobic, and heat-stable peptides. Moreover, bacteriocins are primarily recognized for their potent antimicrobial activity against pathogens (Qian et al., 2020). These peptides operate through two antibacterial mechanisms: either by binding to lipid II to inhibit cell wall synthesis, thereby inducing cell death or by utilizing lipid II as a docking molecule to initiate membrane insertion and pore formation, resulting in swift cell death (Cotter et al., 2005).

**Table 2.** Antibacterial activity of ethyl acetate extracts from bacterial isolates

| Ethyl Acetate Extract | Average Inhibition Zone Diameter (mm) | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *P.aeruginosa* | *E.faecalis* |
| BAC1 | 15.75 | 16.1 | 13.1 | 13.15 |
| BAC2 | 13.45 | 15.7 | 13.45 | 11.5 |
| BAC3 | 12.2 | 12.05 | 9.65 | - |
| BAC4 | 8.1 | - | - | - |
| K + | 16.65 | 16.4 | 13.6 | 16.85 |
| K - | - | - | - | - |

Note: BAC1: *Lactobacillus sp1;* BAC2: *Lactobacillus sp2*; BAC3: *Bacillus sp2*; BAC4: *Bacillus sp1*



**1** **2** **3** **4**

**Figure 2.** Antibacterial activity testing of ethyl acetate extracts from bacterial isolates of Bekasam
Note: 1: *Staphylococcus aureus*, 2: *Escherichia coli*, 3: *Pseudomonas aeruginosa*, and 4: *Enterococcus faecalis*

## Conclusion

This research derived four bacterial isolates from fermented fish products (Bekasam). Two isolates were identified as members of the *Lactobacillus sp.* group (BAC1 and BAC2), while the other two were identified as belonging to the *Bacillus sp*. group (BAC3 and BAC4). All ethyl acetate extracts from these isolates demonstrated antibacterial activity, with the most potent antibacterial effect observed in the ethyl acetate extract from *Lactobacillus sp1*.

## Acknowledgement

We would like to express our sincere gratitude to Universitas Dharma Andalas for their invaluable support and assistance throughout this research.

## References

Belleggia, L., Osimani, A., 2023. Fermented fish and fermented fish-based products, an ever-growing source of microbial diversity: A literature review. Food Research International 172, 113112. https://doi.org/10.1016/J.FOODRES.2023.113112

Cotter, P.D., Hill, C., Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3, 777–788. https://doi.org/10.1038/NRMICRO1273

Dewanti, R., Yasni, S., Thenawidjaja Suhartono, M., Author, C., 2017. Novel HMG-CoA Reductase Inhibitor Peptide from Lactobacillus acidophilus Isolated from Indonesian fermented food Bekasam. Journal of Pharmaceutical, Chemical and Biological Sciences 5, 195–204.

Efendi, M.R., Rusdi, M.S., Dinda, A., 2022. Antibacterial activity of ethyl acetate extracts of fungal endophytes isolated from leaf gambir leaves (Uncaria gambir (Hunter) Roxb). Journal of Pharmaceutical Science 19, 17–23. https://doi.org/10.12928/mf.v19i1.20483

Handayani, D., Sandrawaty, N., Murniati, M., Regina, R., 2015. Screening of Endophytic Bacteria Isolated from Marine Sponge Haliclona fascigera for Inhibition against Clinical Isolates of Methicillin Resistant Staphylococcus aureus (MRSA). J Appl Pharm Sci 5, 139–142. https://doi.org/10.7324/JAPS.2015.50926

Haryo, R., Setiarto, B., Tresnadiana Herlina, V., 2024. Exploring bekasam, an indigenous fermented fish product of Indonesia: original South Sumatra region. Journal of Ethnic Foods 2024 11:1 11, 1–14. https://doi.org/10.1186/S42779-024-00230-W

Li, C., Fan, Y., Li, S., Zhou, X., Park, K.Y., Zhao, X., Liu, H., 2021. Antioxidant Effect of Soymilk Fermented by Lactobacillus plantarum HFY01 on D-Galactose-Induced Premature Aging Mouse Model. Front Nutr 8, 667643. https://doi.org/10.3389/FNUT.2021.667643

Putri, O., Kusdiyantini, E., 2018. Isolasi dan identifikasi bakteri asam laktat dari pangan fermentasi berbasis ikan (Inasua) yang diperjualbelikan di Maluku-Indonesia. Jurnal Biologi Tropika 1, 6–12. https://doi.org/10.14710/JBT.1.2.6-12

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, 3532–3538. https://doi.org/10.13057/BIODIV/D201210

Melini, F., Melini, V., Luziatelli, F., Ficca, A.G., Ruzzi, M., 2019. Health-Promoting Components in Fermented Foods: An Up-to-Date Systematic Review. Nutrients 11. https://doi.org/10.3390/NU11051189

Moreno-Montoro, M., Navarro-Alarcón, M., Bergillos-Meca, T., Giménez-Martínez, R., Sánchez-Hernández, S., Olalla-Herrera, M., 2018. Physicochemical, Nutritional, and Organoleptic Characterization of a Skimmed Goat Milk Fermented with the Probiotic Strain Lactobacillus plantarum C4. Nutrients 10. https://doi.org/10.3390/NU10050633

Qian, Z., Zhao, D., Yin, Y., Zhu, H., Chen, D., 2020. Antibacterial Activity of Lactobacillus Strains Isolated from Mongolian Yogurt against Gardnerella vaginalis. Biomed Res Int 2020. https://doi.org/10.1155/2020/3548618

Sharma, A., Kaur, J., Lee, S., Park, Y.S., 2019. Tracking of Intentionally Inoculated Lactic Acid Bacteria Strains in Yogurt and Probiotic Powder. Microorganisms 2020, Vol. 8, Page 5 8, 5. https://doi.org/10.3390/MICROORGANISMS8010005

Sharma, M.C., Nigam, V.K., Behera, B., Kachhawa, 2009. Antimicrobial Activity of Aqueous Extract of Holoptelea Integrifolia (Roxb.) Leaves: an In vitro Study. Pharmacologyonline 1, 155–159.

Wikandari, P.R., Suparmo, S., Marsono, Y., Rahayu, E.S., 2013. Potensi Bakteri Asam Laktat yang Diisolasi Dari Bekasam sebagai Penghasil Angiotensin Converting Enzyme Inhibitor pada Fermentasi "Bekasam-Like" Product. agriTECH 32, 2012. https://doi.org/10.22146/AGRITECH.9616

Yi, E.J., Kim, A.J., 2023. Antimicrobial and Antibiofilm Effect of Bacteriocin-Producing Pediococcus inopinatus K35 Isolated from Kimchi against Multidrug-Resistant Pseudomonas aeruginosa. Antibiotics 12. https://doi.org/10.3390/ANTIBIOTICS12040676

Zahra, Z., Amaliah, N., Bahri, S., Amelia, P., 2018. Isolasi dan karakterisasi bakteri asam laktat dari limbah cair rendaman kacang kedelai. Jurnal Fitofarmaka Indonesia 5, 253–257.