# SCREENING AND IDENTIFICATION OF GABA-PRODUCING BACTERIA FROM LEMEA, A TRADITIONAL FERMENTED FOOD

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**Abstract.** Introduction: Gamma-aminobutyric acid (GABA) is a functional bioactive compound known for its health-promoting properties and is commonly found in fermented foods. Although numerous studies have investigated GABA production from various fermentation substrates, Lemea, a traditional fermented food from Indonesia, remains an underexplored source of GABA-producing bacteria. Objective: This study aimed to quantify GABA production by bacterial isolates obtained from Lemea and to identify the most potent GABA-producing strain through molecular characterization. Methods: Six bacterial isolates previously screened for GABA production were rejuvenated and cultured in de Man, Rogosa, and Sharpe Broth (MRSB) supplemented with glutamic acid for 36 hours. GABA concentrations in the culture supernatants were quantified using an ultra-performance liquid chromatography–photodiode array (UPLC–PDA) method (18-5-167/MU/SMM-SIG), with analyses performed in duplicate. The isolate exhibiting the highest GABA production was identified by 16S rRNA gene sequencing.Results: The results showed that GABA production among the isolates ranged from 40.09 ± 1.17 mg/L to 113.03 ± 0.15 mg/L. The highest GABA-producing isolate was molecularly identified as *Staphylococcus epidermidis*, exhibiting a 100% sequence similarity coefficient. Conclusion: These findings demonstrate that Lemea is a promising source of GABA-producing bacteria. The identification of *Staphylococcus epidermidis* as a high GABA-producing strain suggests its potential application in the development of functional fermented food products.

**Keywords**: Bacteria; Fermentation; GABA; Lemea; *Staphylococcus epidermidis*

## 1 Introduction

Indonesia has a wide diversity of traditional fermented foods that can be developed into functional food products. Several fermented foods originating from Indonesia include bekasam made from fish, curd and dangke derived from milk, gatot and growol produced from cassava, lemea made from bamboo shoots, mandai from cempedak fruit peel, peda from marine fish, and tempoyak from durian. These fermented foods are produced through the activity of diverse microorganisms that contribute to their nutritional value, safety, and functional properties [1].

Lemea is a traditional fermented bamboo shoot product that contains lactic acid bacteria (LAB) as well as other Gram-positive bacteria. These microorganisms provide various health benefits, including maintaining digestive tract health through probiotic activity and contributing to food preservation via the production of antimicrobial compounds [1–3,5]. In

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addition, microorganisms involved in fermentation are capable of producing bioactive compounds that enhance the functional value of fermented foods.

One important bioactive compound produced during fermentation is gamma-aminobutyric acid (GABA), a non-protein amino acid found in bacteria, plants, and animals [6,7]. In bacteria, particularly LAB, GABA is synthesized through the activity of the enzyme glutamate decarboxylase (GAD), which catalyzes the decarboxylation of L-glutamic acid into GABA and carbon dioxide [8]. LAB are Gram-positive bacteria that play a major role in food fermentation and have been widely reported as effective GABA producers [9,10]. However, fermentation of Lemea does not involve LAB exclusively; other microbial groups may also be present and potentially contribute to GABA production.

Despite the growing interest in GABA-producing bacteria, studies focusing on the identification and characterization of GABA-producing microorganisms from Lemea remain limited. Most previous research has emphasized LAB isolated from other fermented foods, while the genetic diversity and functional potential of bacteria associated with Lemea have not been thoroughly explored. Understanding the microbial composition and identifying specific GABA-producing bacteria from Lemea are therefore essential to enhance its functional value.

Genetic diversity analysis using molecular markers is widely applied to identify and characterize microorganisms. The 16S rRNA gene is one of the most commonly used PCR-based molecular markers for bacterial identification due to its conserved and variable regions, which allow accurate determination of genetic relationships among bacterial species [12,14].

Several studies have reported GABA production by different bacterial species, such as *Lacticaseibacillus rhamnosus, Lacticaseibacillus paracasei, Levilactobacillus brevis*, and *Lactococcus lactis*, isolated from various fermented foods [10,15,16]. These findings indicate that fermented foods represent promising sources of GABA-producing bacteria. However, information regarding GABA-producing bacteria from Lemea is still scarce.

Therefore, this study aimed to quantify GABA production by bacterial isolates obtained from Lemea and to identify the most potent GABA-producing strain using 16S rRNA gene sequencing. The results of this study are expected to provide scientific evidence supporting the development of Lemea as a functional fermented food and to increase public interest in its production and consumption.

## 2 Methods

### 2.1 Sample Collection

Lemea samples were collected from a traditional fermentation producer in the Rejang ethnic community, Bengkulu Province, Indonesia. The samples were transported to the laboratory under refrigerated conditions and stored at 4 °C prior to microbial isolation and analysis.

### 2.2 GABA Quantification by UPLC–PDA

GABA quantification was performed using an ultra-performance liquid chromatography–photodiode array (UPLC–PDA) method (18-5-167/MU/SMM-SIG). The test sample was dissolved in distilled water in a 50 mL volumetric flask until homogeneous and subsequently filtered through a 0.2 µm GHP membrane filter. An aliquot of 500 µL of the filtrate was mixed with a 2.5 mM α-aminobutyric acid (AABA) internal standard.

For derivatization, 10 µL of the mixture was reacted with AccQ•Tag™ Fluorine Borate Buffer and AccQ•Tag™ Reagent 2A, followed by heating at 60 °C and cooling to room

temperature. The derivatized solution was then injected into the UPLC system for amino acid profiling and GABA quantification. All analyses were performed in duplicate.

### 2.3 Molecular Identification of Bacteria

Molecular identification of the bacterial isolate exhibiting the highest GABA production was conducted using 16S rRNA gene analysis. Genomic DNA was extracted from bacterial cultures, and the 16S rRNA gene was amplified by polymerase chain reaction (PCR). The amplified PCR products were purified and verified by agarose gel electrophoresis prior to DNA sequencing.

The obtained nucleotide sequences were analyzed for similarity using the Basic Local Alignment Search Tool (BLAST) against reference sequences available in public databases to determine the closest phylogenetic affiliation of the isolate.

## 3 Results and discussion

This study was conducted by taking isolates and obtained 6 bacterial isolates found in local Indonesian fermentation products (lemea). The isolates obtained will be identified using the 16S rRNA method, and the GABA production produced will be seen using the UPLC-PDA method.

### 3.1 GABA Levels in Bacterial Isolates from Lemea

Measurement of GABA levels in bacterial isolates was carried out using the UPLC-PDA method, and it was found that 6 bacterial isolates had different GABA contents (Tabel 1).

Tabel.1. GABA levels in bacterial isolates from lemea using the UPLC-PDA method

| Kode Isolat | Rerata Kadar GABA (g/mL) |
|---|---|
| 1 | 40,09±1,17 |
| 2 | 44,71±0,08 |
| 3 | 52,06±0,24 |
| 4 | 55,55±0,56 |
| 5 | 54,39±0,45 |
| 6 | 113,03±0,15 |

Based on the image above, it can be seen that 6 bacterial isolates from lemea can produce GABA, with the highest GABA production in isolates 6 and 4, namely 113.03 ± 0.15 g/mL and 55.55 ± 0.24 g/mL. This is in line with [25], which state that bacteria can produce antioxidant compounds, namely gamma-aminobutyric acid (GABA). GABA concentration can be influenced by temperature, fermentation time, and the media used [26, 27]. The fermentation time can affect the concentration of isolates and GABA production. Lemea in Bengkulu province is fermented for 48–120 hours so that the lactic acid bacteria produced can produce GABA.

Lactic acid bacteria are one of the bacteria found in fermented foods, including lemea, and have the ability to produce GABA. Research by [28], succeeded in isolating GABA-producing lactic acid bacteria from fermented bean foods, fermented beans (white lobia), khoji (processed yam), and idli batter. In addition to lactic acid bacteria, GABA can also be produced by other bacteria; besides that, GABA can also be sourced from plants [18]. Lemea isolates that are proven to contain GABA are then identified to determine the type of bacteria in lemea that are able to produce GABA.

### 3.2 Identification of Bacteria in Lemea

The genetic identification method for 6 bacterial isolates is based on polymerase chain reaction (PCR) with the 16S rRNA method. Of the 6 bacterial isolates, 2 bacteria were found in the lemea that were not included in lactic acid bacteria but other types of gram-positive bacteria (Fig.1).

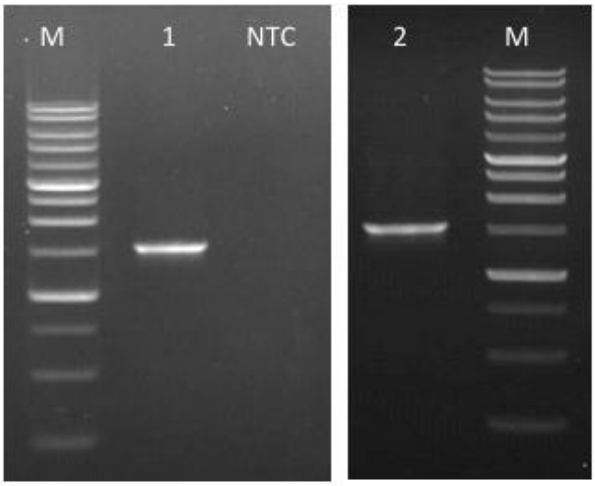


**Fig. 1.** Visualization of bacterial 16S rRNA markers in isolates 4 and 6

Based on the primers used, it can detect genetic differences between the genus and species of bacteria. Based on the results of the study, it is known that bacteria in isolates 1, 2, 3, and 5 are lactic acid bacteria, while isolates 4 and 6 are other gram-positive bacteria that have similarities that can be seen from the visual results of the 16S rRNA marker to see the level of similarity of bacteria in isolates 4 and 6. Where isolates 4 and 6 are obtained from lemea with the addition of tilapia, the manufacture of lemea can be added to fish, spices, and rice [19].

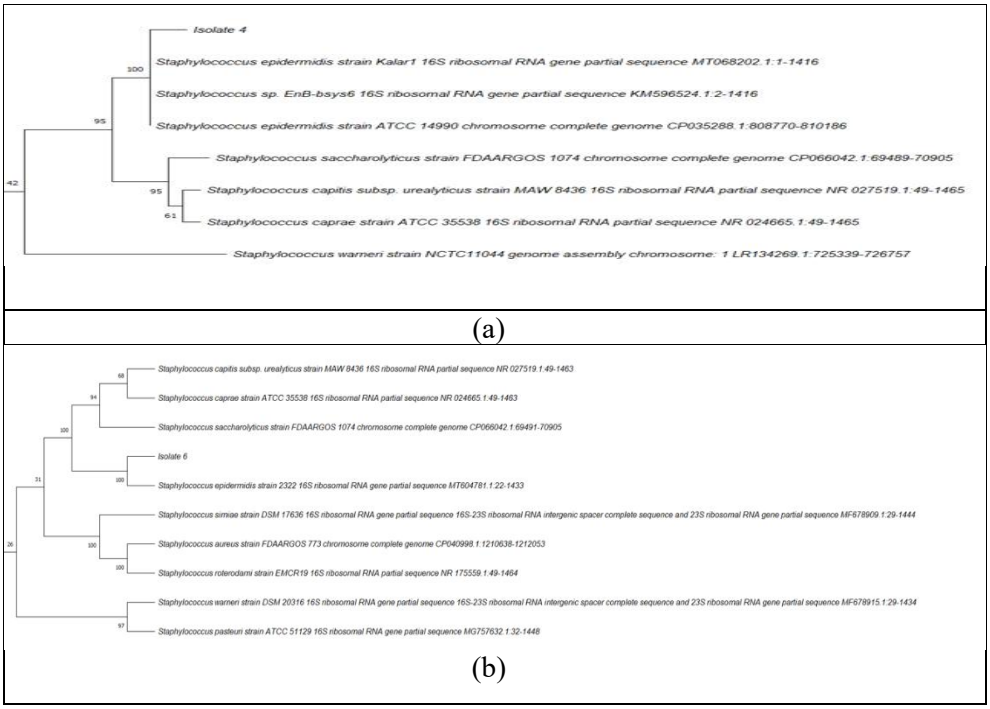


**Fig. 2.** Bacterial phylogenetic tree (a) isolate 4 (b) isolate 6

Based on the phylogenetic tree above (Fig.2), it can be seen that isolate 4 and isolate 6 bacteria are included in *Staphylococcus* epidermidis bacteria, which are gram-positive bacteria with a similarity coefficient of 100% using the 16S rRNA marker. For microorganism samples, if using the 16S rRNA marker, it is said to be identical (similar) at the species level if the "percentage identity" value is above 97.5%, and at the genus level if the "percentage identity" value is above 95% [20], so it can be said that the microbes in isolate 4 and isolate 6 are one species with Staphylococcus epidermidis.

In the production of lemea, the microbes that are generally found are lactic acid bacteria in the form of *Lactobacillus*, but it is not impossible that other microbes can help in the fermentation process in lemea. Isolates 1, 2, 3, and 5 were identified as lactic acid bacteria, confirming that lactic acid bacteria are dominant microorganisms in bamboo shoot fermentation. Nevertheless, the involvement of other bacterial groups in the fermentation process remains possible.

This is in line with [22], where one type of microbe that can be found in fermented foods is Staphylococcus epidermidis, Listeria monocytogenes, and Vibrio parahaemolyticus. Staphylococcus bacteria are included in bacteria that are often found both in the environment and in the body, both in plants and animals, including on human skin [23]. Staphylococcus bacteria are able to produce GABA [24]. Likewise, lactic acid bacteria have the ability to produce GABA at different concentrations [16, 15, 10].

## 4 Conclusion

This study demonstrates that lemea, a traditional fermented food from Bengkulu, Indonesia, is a promising source of GABA-producing bacteria. Quantitative analysis using the UPLC–PDA method showed that all six bacterial isolates were capable of producing GABA, with

Isolate 6 and Isolate 4 exhibiting the highest production levels of 113.03 ± 0.15 mg/L and 55.55 ± 0.24 mg/L, respectively. A key outcome of this study is the identification of *Staphylococcus epidermidis* as a potent GABA-producing bacterium, indicating that GABA production in lemea is not limited to lactic acid bacteria alone and highlighting a broader microbial diversity involved in the fermentation process. The identification of high-yield GABA-producing bacteria from lemea provides a scientific basis for the development of functional fermented foods with enhanced bioactive properties, including potential applications as natural stress-relief or antihypertensive food ingredients. Although this study confirms the presence of non-LAB GABA-producing bacteria in lemea, further research is necessary to optimize fermentation parameters such as temperature, pH, and incubation time to maximize GABA production. In addition, comprehensive safety assessment and evaluation of probiotic potential of the isolated S. epidermidis strains are required prior to their application in large-scale food production. Overall, this research contributes to the field of food microbiology by emphasizing the untapped potential of traditional Indonesian fermented foods as valuable sources of functional bioactive compounds.

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