BIODIVERSITAS
Volume 25, Number 11, November 2024
Pages: 4190-4198

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251118

Metagenomic analysis of the bacterial community and gamma-aminobutyric acid (GABA) synthesis by lactic acid bacteria from *seredele*, a Balinese fermented soybean product

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Manuscript received: 13 June 2024. Revision accepted: 13 November 2024.

Abstract. *Kusumaningsih P, Jatmiko YD, Kusumawati IGA, Nursini NW, Yogeswara IBA. 2024. Metagenomic analysis of the bacterial community and gamma-aminobutyric acid (GABA) synthesis by lactic acid bacteria from* seredele*, a Balinese fermented soybean product. Biodiversitas 25: 4190-4198. Seredele* is a fermented Balinese soybean product that undergoes spontaneous fermentation for two days at room temperature. This fermented food, commonly consumed as a condiment, has a distinctive smell similar to Japanese natto. The bacterial dynamics during fermentation using a metagenomic approach have not been reported. This study aimed to reveal bacterial dynamics during *seredele* fermentation and to obtain gamma-aminobutyric acid (GABA)-producing lactic acid bacteria from *seredele*. The bacterial dynamics of *seredele* were investigated using 16S rRNA sequencing. Lactic acid bacteria (LAB) were also isolated and screened for their ability to produce GABA. The metagenomics analysis showed that *Weissella*, *Streptococcus*, and *Enterococcus* are the most prevalent bacterial genera during fermentation. The relative abundance of bacteria at the species level indicates that *W. confusa*, *W. paramesenteroides*, *E. faecium*, *S. lutetiensis*, and *E. durans* are the most abundant bacteria at all stages of fermentation. Consistent with the metagenomic analysis, two strains, *Limosilactobacillus fermentum* A7304 and *Enterococcus lactis* A7303, produced 60 mg/L and 65 mg/L of GABA, respectively, in de Man Rogosa (MRS) medium enriched with 1% monosodium glutamate (MSG). This work opens opportunities to understand the bacterial dynamics in *seredele* and its potential as a source of GABA-producing bacteria.

Keywords: Bacterial dynamics, gamma-aminobutyric acid, lactic acid bacteria, metagenomics, *seredele*

INTRODUCTION

Fermented foods have been a part of the Indonesian diet for centuries, mainly consisting of legumes, fruits, cassava, and meat. Indigenous fermented foods have shown multiple health benefits, primarily due to metabolites produced by microorganisms (Marco et al. 2017). *Seredele* is a traditional Indonesian food made from soybeans, originating from Sukawati Village, Gianyar Regency, Bali. Its production involves two stages, i.e. boiling soybeans for three hours, followed by spontaneous fermentation for two days, and mixing with distinctive Balinese spices. *Seredele* has a unique taste and aroma and can be consumed raw. It is often referred to as Bali's natto due to its similarity to Japanese natto and is usually consumed raw or as a condiment. Unlike industrialized fermentation processes, the artisanal preparation of seredele allows for the cultivation of a diverse microbial community, influenced by environmental factors and traditional practices. This unique fermented soybean product is commonly produced at the cottage level and requires a special technique to achieve its distinctive taste and flavor (Suparthana et al. 2018).

Gamma-aminobutyric acid (GABA) has been a primary focus for food scientists among the released bioactive compounds due to its physiological functions. GABA is a non-proteinaceous amino acid that plays a role in neurotransmission and acts as an antihypertensive and antidepressant (Abe et al. 1995; Seo et al. 2012). The demand for natural GABA sources has spurred interest in functional foods enriched with this bioactive compound. Studies have shown that certain strains of lactic acid bacteria (LAB), isolated from traditional fermented foods, can synthesize GABA during fermentation, thus enhancing the health-promoting properties of these foods (Komatsuzaki et al. 2005; Seo et al. 2012). GABA is synthesized through the α-decarboxylation of L-glutamate by glutamate decarboxylase (GAD). Several studies have reported that lactic acid bacteria (LAB) species/strains, such as *Lacticaseibacillus brevis*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and *Lactobacillus futsaii* exhibit GAD activity and produce high GABA levels during food fermentations (Shi and Li 2011; Sanchart et al. 2017; Lyu et al. 2019; Yogeswara et al. 2021). In addition, most of these GABA-producing LAB were obtained from fermented traditional foods, such as kimchi (Lu et al. 2008), fish (Komatsuzaki et al. 2005), fermented shrimp

(Sanchart et al. 2017), soybean (Yogeswara et al. 2018), and meat (Ratanaburee et al. 2013).

Scientific investigation on *seredele*, particularly regarding the bacterial community during fermentation is poorly reported and their functional properties. Current research primarily focuses on its nutritional composition. Spontaneously fermented soybeans often involve the breakdown of protein-rich substances to produce peptides, essential amino acids, and alkaline conditions, contributing to the umami taste and volatile compounds (D'Este et al. 2018; Yongsawas et al. 2023). Spontaneously fermented soy-based foods widely found in East and Southeast Asia include *cheonggukjang* (Nam et al. 2012), *da-jiang* (Wu et al. 2013), *doenjang* (Jeong et al. 2014), *kinema* (Kharnaior and Tamang 2021), and *natto* (Afzaal et al. 2022). These fermented foods are considered safe and have been consumed for centuries to enhance the flavor and nutritional content of food. The bacterial dynamics in soy fermentation products vary depending on the region and sodium content (Wu et al. 2013). According to Xie et al. (2020), the genera *Leuconostoc*, *Enterococcus*, and *Lactobacillus* are most dominant in *da-jiang* fermentation. Soy fermentation products largely contain *Bacillus* species and lactic acid bacteria (LAB) capable of releasing bioactive compounds.

We hypothesized that using various spices can cause bacterial diversity in *seredele*. Due to the limited information on bacterial diversity in *seredele*, in this study, we performed metagenomic analysis. Metagenomic analysis is considered a cutting-edge technology in genomic sequencing by providing a culture-independent approach. This high throughput sequencing contributes to determining species identification methods without cloning or 16S rRNA amplification (Xie et al. 2020; Afzaal et al. 2022). Additionally, the ability of LAB isolated from *seredele* as GABA producers was also investigated. Therefore, this study opens opportunities to understand the bacterial dynamics in *seredele* and its potential as a source of GABA-producing bacteria that add functional properties to food systems.

MATERIALS AND METHODS

Research materials

Seredele was obtained from local household manufacturer located in Sukawati Village, Gianyar Regency, Bali, Indonesia. Generally, the *seredele* fermentation process is carried out spontaneously. The soybeans are washed clean and boiled for 3-5 hours. The boiled soybeans are transferred into bamboo storage containers, the water is drained, and they are covered using perforated bamboo plugs, and left to ferment spontaneously for two days at room temperature before spices are added. Samples were taken periodically every 12 hours (Suparthana et al. 2018). Traditionally, *seredele* producers never replace their bamboo containers with new ones (Figure 1).

Procedures

DNA extraction

About 250 mg of samples were ground in liquid nitrogen for further DNA extraction. Genomic DNA extraction from raw samples was performed using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). The DNA extraction was carried out according to the manufacturer's instructions. The DNA concentration was determined using a NanoDrop spectrophotometer and a Qubit fluorometer (ThermoFisher Scientific). The extracted DNA was stored at -20°C before metagenomic analysis.

Metagenomic analysis

The bacterial succession was monitored by amplifying the 16S rRNA gene using the forward primer 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and reverse primer 1492R: 5'- GGTTACCTTGTTACGACTT -3' (Sumarmono et al. (2023). The PCR mixtures were performed in a volume of 50 µL consisting of 38.5 µL deionized water, 7.5 µL MyTaq HS Red Mix 2X (Bioline, BIO-25048), 1 µL of each primer, and 2 µL of DNA template. The PCR conditions were initial denaturation at 96°C for 5 minutes and followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes. After PCR amplification, the PCR Barcoding Expansion Kit 1-96 (Nanopore, Oxford, UK) was used for barcoding the PCR process, and the products were purified with AMPure XP. The products were collected in a tube to create the DNA library. The DNA repair PCR was carried out to prevent DNA damage according to the protocol provided by the barcoding kit. Subsequently, the PCR product obtained after DNA repair was purified using AMPure XP, and the adapter-binding PCR process was started. The adapter ligation was performed using the Ligation Sequencing Kit (Nanopore, Oxford, UK) and followed according to the manufacturer's instructions. The purified product was loaded into the instrument, and Nanopore sequencing was performed. After the loading process, a sequencing protocol was operated by MinKNOW software version 22.05.7 (Nanopore), which was already installed on the Flow Cell MinIon (Nanopore) device. The pH of the samples was measured using a pH meter (Ohaus, NJ, USA).

Figure 1. *Seredele* in the bamboo container

Isolation and identification of GABA-producing LAB

A total of 5 g of *seredele* was serially diluted in 0.85% NaCl and inoculated on MRS agar supplemented with 1% monosodium glutamate (MSG) (Ajinomoto) and bromocresol purple, followed by incubation at 37°C for 24-48 hours. Clear zones formed around the colonies were considered as LAB. Isolates were purified by streaking onto the same medium. To obtain LAB capable of producing GABA, isolates were grown in MRS medium containing 1% MSG and incubated at 37°C for 24 hours. The culture broth was then centrifuged at 8000 x g for 15 minutes at 4°C, and the supernatant was filtered using a 0.45 µm membrane filter. The resulting supernatant was further used to determine the concentration of GABA.

Molecular identification of GABA-producing LAB was conducted by isolating genomic DNA using the GeneJET DNA kit (ThermoFisher, CA, USA) according to the manufacturer's instructions. The extracted DNAThe 16S rRNA gene was amplified using universal primers corresponding to 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The PCR conditions were as follows: initial denaturation at 95°C for 7 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 90 seconds, and final extension at 72°C for 5 minutes. The partial sequences of 16S rRNA of LAB isolated from *seredele* were compared with the Gene Bank (NCBI) database, and a phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) 4.

GABA quantification

GABA concentrations were measured using Ultra Performance Liquid Chromatography (UPLC Acquity H-Class, Waters Corporation, Milford, MA, USA) equipped with a PDA detector and a C18 column. Samples were hydrolyzed using 6 N HCl followed by derivatization of the samples and GABA standards. Derivatization was performed using the Ultra Derivatization Tag kit (Waters, Milford, MA, USA) according to the manufacturer's instructions. The derivatized samples were then injected into the Acquity UPLC H class. The mobile phase consisted of three eluents, i.e. AccQ. Tag Ultra Eluent A 100%, AccQ. Tag Ultra Eluent B (Aquabides 90:10), and Aquabides Eluent C; AccQ. Tag Ultra Eluent B 100%. The system was operated at a flow rate of 0.5 mL/min, a temperature of 49°C, and a wavelength of 260 nm.

Data analysis

The DNA sequences were processed for downstream analysis using several software. Raw data containing low-quality reads were filtered to obtain high-quality reads. Filtered reads were classified using Centrifuge classifier and the indexing scheme based on the Ferragina-Manzini (FM) index (Kim et al. 2016). The Centrifuge classification engine processes millions of reads from high-throughput DNA sequencing within minutes. After sequencing, the results in fast5 format were converted using Basecalling with Guppy version 6.1.5 to translate electrical signals into nucleotide sequences using a high-accuracy model (Carafa et al. 2019; Wick et al. 2019). The quality of the FASTQ files was visualized using NanoPlot and quality filtering and trimming were conducted using NanoFilt (De Coster et al. 2018; Nygaard et al. 2020). Sequences were demultiplexed and aligned using Mothur software, leading to the formation of Operation Taxonomic Units (OTUs) through clustering reads with more than 99% similarity, using a similarity matrix to calculate distances between them. Pearson rank correlation was performed using SR online plots (Tang et al. 2023) to determine the correlation between top genera.

Alpha diversity was determined by calculating the Shannon and Simpson's diversity indices as well as the Chao1 and rarefaction estimators. Weighted and unweighted calculations were conducted prior to Principal Coordinate Analysis (PCoA). The Venn diagram was generated using RStudio (v. 4.2.0) to visualize shared and unique species or OTU's during fermentation. The bacteria and archaea index was built using the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/). Downstream analysis and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian), and RStudio using R version 4.2.0 (https://www.R-project.org/). A phylogenetic tree is constructed using the molecular evolutionary analysis Neighbor-joining method (Kharnaior and Tamang 2023).

RESULTS AND DISCUSSION

Bacterial diversity in *seredele* during fermentation

A total of 396,983 high-quality reads were obtained after classification using the Centrifuge classifier. OTU reads were classified into 9 phyla, 3 classes, 5 orders, 8 families, 13 genera, 13 species, and unclassified groups throughout the fermentation period (Figures 2.A and 2.B). A bar plot was used to visualize the abundance of each classification to observe bacterial dynamics during fermentation. Bacterial sequencing reads were analyzed at the phylum and genus levels. At the phylum level, *Bacillota* were dominant in the early stages of fermentation at 0 h (98.50%) and 12 h (99.89%) (S0 and S12) (Figure 2.A).

The abundance of *Bacillota* was surpassed by the presence of *Pseudomonadota* at 36 h (56.9%) and 48 h (46.3%) of fermentation (S36 and S48). At the genus level, 12 genera were found in *seredele* during fermentation (Figure 2.B). *Weissella* was dominant at the beginning of fermentation at 68.5%, further increased at 12 h of fermentation (88%), and decreased at 24 h (45%), when it was replaced by other genera, such as *Enterococcus* (27.30%), *Streptococcus* (28%), *Providencia* (28%), and *Bacillus* (11%) after 48 h of fermentation. The correlation among the 15 dominant genera was assessed based on the Pearson rank correlation (Figure 2.C).

At the species level, *Weissella paramesenteroides* was dominant at 24 h of fermentation (43.8%) and *W. confusa* was abundant at 12 h of fermentation (76.2%), followed by *Enterococcus* sp. at the end of fermentation (Figure 3.A), along with the emergence of distinctive species including *Leuconostoc citreum* and *Vagococcus martis* (Figure 3.B).

At lower abundances, we also found potential pathogenic genera, such as *Klebsiella* (3.12%) and *Cronobacter* (6.3%). The development of these bacterial phyla is consistent with findings in the fermentation of *doenjang* and *thua-nao* under dry and wet conditions. Initially, bacteria from the phylum Firmicutes/Bacillota were found in these fermentations, but the second-most abundant bacterial phylum varied slightly, with Actinobacteria being predominant (Jung et al. 2016; Wongsurawat et al. 2023). At the genus level, *Weissella* spp. developed at 6, 12, and 18 h. The abundance of *Streptococcus* increased at 18 h, began to decrease at 24 h, and was not found at 30 h. The most abundant bacteria developing between 18-30 h were *Enterococcus*, *Bacillus*, *Proteus*, *Providencia*, *Vagococcus*, *Klebsiella*, *Enterobacter*. Kwofie et al. (2020) stated that *Bacillus* plays an important role in the fermentation of soy products. In *seredele* fermentation, *Bacillus* began to develop at 12 h and continued to grow at 24-48 h (Figure 2A). This increased growth of *Bacillus* overtakes the presence of *Weissella*. In this study, *Weissella* spp. developed significantly at the beginning of fermentation (S0) (Figure 2B). Fessard and Remize (2017) found that in naturally fermented foods without starter bacteria, using grains or plants as the base material, *Weissella* thrives at the start of fermentation. *Weissella* can adapt to the environment, reproduce quickly, and has excellent prospects as a starter culture for fermented food. The genus *Weissella* is commonly found in fermented foods like *douchi* and *doenjang*, which use soybeans as the base material for fermentation (Fusco et al. 2015).

The diversity of genera from *seredele* fermentation obtained in this study is consistent with Wongsurawat et al. (2023) on the isolation of fermentation bacteria from *thua-nao*, as well as the bacterial identification results using 16S rRNA by Suparthana et al. (2018), which found nine bacterial species from the genera *Bacillus*, *Weissella*, *Acinetobacter*, *Proteus*, and *Klebsiella*. The diversity of bacterial communities that grow differently from spontaneous soy-based fermentation samples is influenced by several factors such as geographic location, temperature, humidity, and preparation techniques (Kharnaior and Tamang 2021).

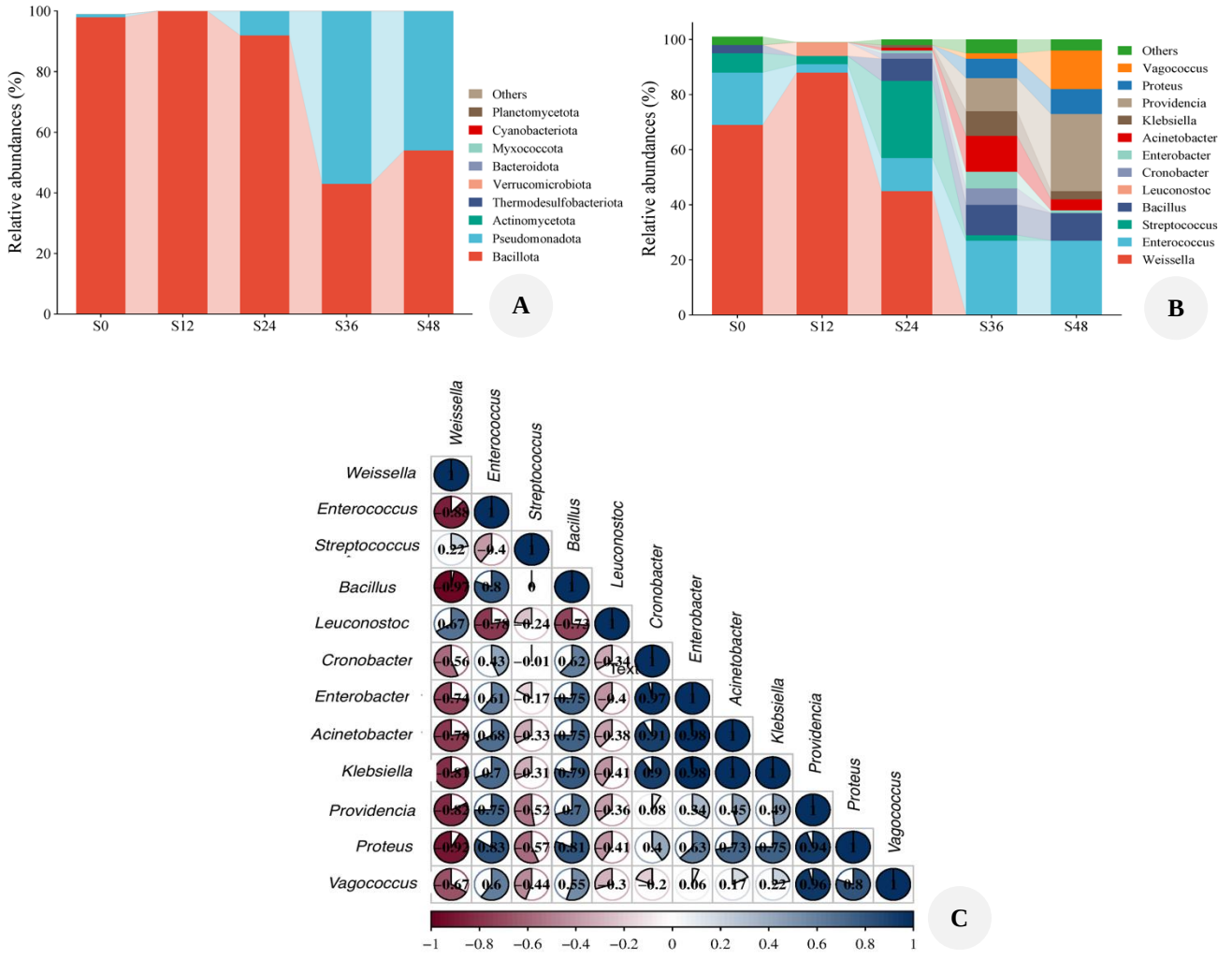


Figure 2. Bacterial communities during *seredele* fermentation at: A. phylum level; B. genus level; C. Correlation matrix among the top 15 genera using Pearson's rank correlation with the coefficient range from 1 to -1. Higher correlation coefficients closer to +1 (positive correlation) or -1 (negative correlation). The x axis at (A) and (B) refers to fermentation time

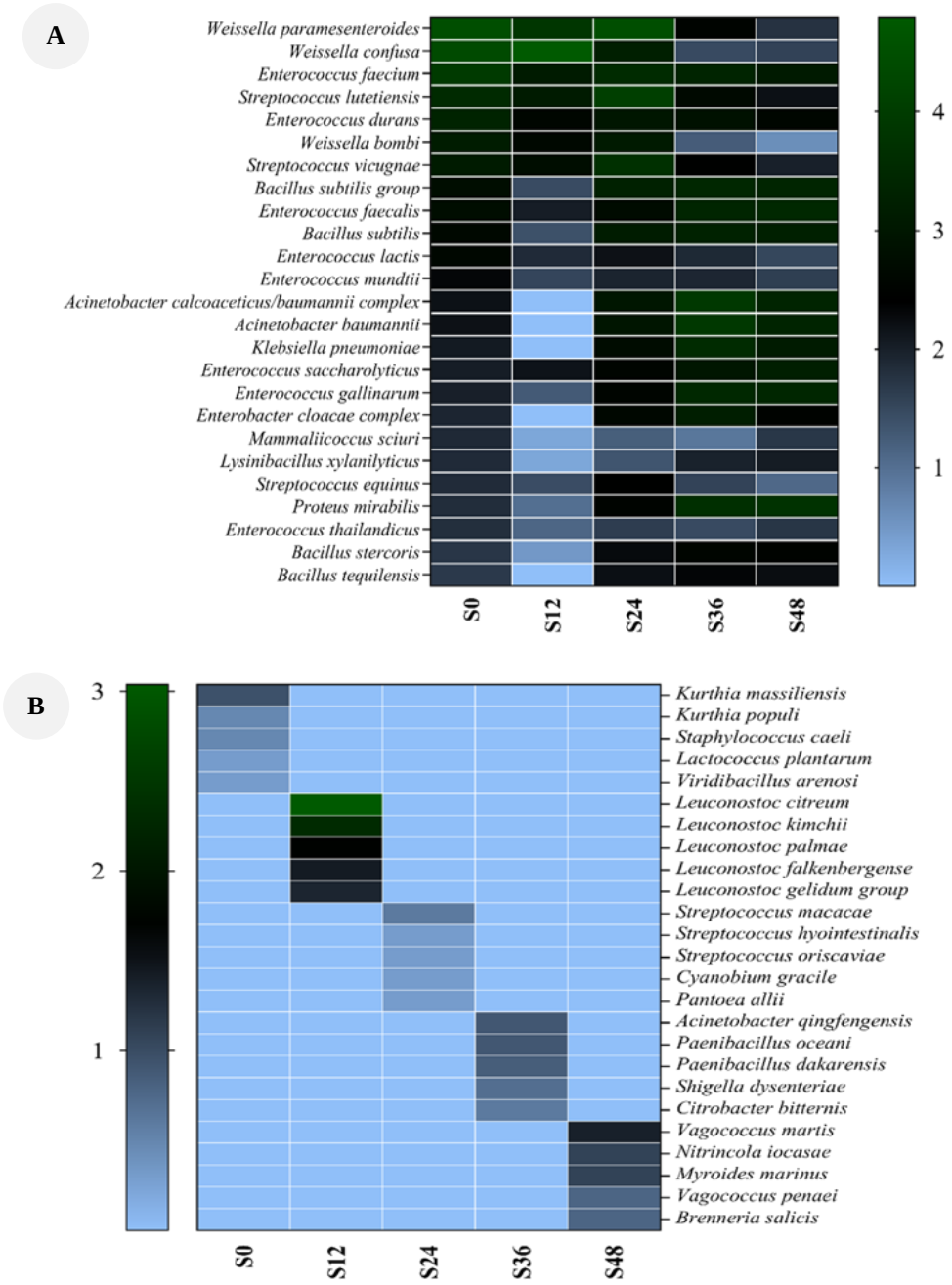


Figure 3. Heatmap profile during *seredele* fermentation of: A. Bacterial species; B. Distinctive species. The x axis refer to fermentation time (S0-S48) and y axis refer to the name of the bacterial species

The genus *Weissella* is negatively correlated with most genera, such as *Enterococcus*, *Bacillus*, *Klebsiella*, *Proteus*, *Cronobacter*, *Enterobacter*, *Acinetobacter*, *Providencia*, and *Vagococcus*. *Enterococcus* positively correlated with *Bacillus*, *Cronobacter*, *Klebsiella*, *Enterobacter*, *Proteus*, *Acinetobacter*, *Providencia*, and *Vagococcus*, and negatively correlated with *Streptococcus* and *Leuconostoc*. A similar result is presented by Mandhania et al. (2019) that *Weissella* was the most abundant genus during idli fermentation and showed a negative correlation with most other genera. This suggests that the genus *Weissella* contributes to the early phase of fermentation.

Diversity analysis

Alpha diversity refers to the species diversity within a single habitat or community (Sumarmono et al. 2023). Alpha diversity was determined to show the richness of the bacterial community in *seredele*. Alpha diversity analysis, including dominance (Simpson), equity (Shannon), ACE (observed OTUs), and singleton (Chao1), showed significant differences during fermentation (Table 1). The highest bacterial diversity was found at 36 h of fermentation, as indicated by four diversity indices: Simpson (0.96), Shannon (3.87), ACE (1304), and Chao1 (1394). The lowest diversity was shown at 12 h of fermentation (Group 2) with Simpson (0.48), Shannon (1.32), ACE (561), and Chao1 (512). It is

suggested that fermentation at 36 h (Group 3) has the most diverse bacteria among the other groups. The reason for this is probably the ability of propagation of a variety of bacteria during fermentation at a certain time. Nutrient competition and metabolic products may affect the adaption of some species and disappearance of some bacterial species (Xie et al. 2020).

Unique and shared OTUs during fermentation were visualized in a Venn diagram (Figure 4). According to the Venn diagram, specific OTUs at all stages of fermentation were 39 (0 h), 71 (12 h), 74 (24 h), 238 (36 h), and 215 (48 h). Meanwhile, across all fermentation stages, 88 OTUs were found with 626 OTUs evenly distributed across all samples. These results indicate significant differences in the species and abundance of bacterial communities during fermentation. This can be influenced by environmental conditions and nutritional composition, such as carbohydrates, proteins, fats, sodium, fiber, and sugars (Nam et al. 2012; Xie et al. 2020). Other factors such as the hygienic conditions of the *seredele* producers and sanitation conditions also have a significant impact on the diversity of bacteria during fermentation.

Principal Coordinate Analysis (PCoA) of both unweighted and weighted UniFrac was performed to analyze the beta diversity of samples. The unweighted PCoA for the first and second main components accounted for 71.8% and 18.7%, respectively (Figure 5). All taxa were distributed across all quadrants during 0-24 h of fermentation (Groups 1, 2, and 3), while taxa in Groups 4 and 5 (fermentation at 36 and 48 h) were clustered in the first quadrant. This indicates that the microbial communities at 36 and 48 h of fermentation show a high similarity. The weighted UniFrac PCoA analysis accounted for 71.8% and 6.07%, respectively. This analysis shows that the microbial communities during 24 and 36 h (Groups 3 and 4) of fermentation are spread in the second quadrant. The microbial communities during 0-48 h (Groups 1, 2, and 5) are located in the first, third, and fourth quadrants. The unweighted UniFrac PCoA analysis measures the similarity between microbial species/OTUs while ignoring their relative abundances, whereas weighted UniFrac takes into account the abundance of taxa when assessing dissimilarities among microbial communities (Joishy et al. 2019).

Abiotic factors such as pH may affect microbial structures in *seredele* (Table 2). Most fermented soybean products, such as *kinema* of India or Nepal and *lanna* of Thailand, have alkaline pH during fermentation. Alkaline conditions allow the breakdown of soy protein into peptides and amino acids, resulting in ammonia and an increase in pH. These reactions lead to the development of an ammonia smell and give distinct organoleptic profiles (taste, texture, and flavor) (Kharnaior and Tamang 2021; Yongsawas et al. 2023).

Screening and identification of GABA-producing bacteria

Thirty-two LAB isolates were obtained and screened for GABA production. The UPLC method was used to quantitatively screen GABA production in all isolates. The study results showed that 14 isolates were considered GABA-producing LAB (Figure 6.A). Among them, two isolates produced the highest GABA levels during 24 hours of incubation. Strains A7303 and A7304 produced 60 mg/L and 65 mg/L of GABA, respectively, during incubation in MRS medium supplemented with 1% MSG. This result was also found on *Enterococcus faecium* B5 (Sabna et al. 2021). Other studies also found that *Limosilactobacillus fermentum* L18 isolated from human gut showed potential in producing GABA (Kaur et al. 2023). Two of isolates that produce GABA were A7303 and A7304 on the phylogenetic tree, showed sample A7303 (acc. number PQ432825) have similarity 99% the DNA composition with *E.faecium* strain DMPUV3 and *E. faecium* strain RO.141.1., from nucleotide GenBank (NCBI). Meanwhile, sample A7304 (acc. number PQ432826) based on phylogenetic tree showed 99% similarity of three DNA compositions compared with *Limosilactobacillus fermentum* strain P3, *L. fermentum* strain YEM E and *L. fermentum* strain SRI (Figure 6.B). Moreover, whole-genome sequencing results (data not shown) using Nanopore sequencing (Oxford Nanopore Technology) confirmed that strains A7303 and A7304 exhibit 98% ANI (Average Nucleotide Identity) compared to reference strains (data not shown).

GABA has shown beneficial effects and is involved in various physiological processes, including the inhibition of nervous system activity (Diez-Gutiérrez et al. 2020), regulation of anxiety and stress (Hepsomali et al. 2020), blood pressure regulation (Nakamura et al. 2009), enhancing sleep quality by reducing brain activity, and anti-obesity (Hong et al. 2016). Traditional fermented foods are considered excellent sources of GABA-producing LAB. Several GABA-producing LAB have been isolated from fermented foods such as *L. brevis* from kimchi (Cho et al. 2007), *L. futsaii* from *kung-som* (Sanchart et al. 2017), *L. plantarum* (Siragusa et al. 2007; Yogeswara et al. 2018), *L. otakiensis* from fermented dairy products (Ribeiro et al. 2018), and *Lactococcus lactis* from Mexican artisanal cheese (Franciosi et al. 2015). Furthermore, their ability to produce GABA varies depending on the species/strain. Our findings suggest that the strains *L. fermentum* A7304 and *E. lactis* A7303 can be used as functional starter cultures, improving the quality of traditional fermented soybean products enriched with GABA.

Table 1. Diversity index of bacterial dynamics

Sample	Diversity index							
	Chao1	**se.chao1**	**ACE**	**se.ACE**	**Shannon**	**Simpson**	**InvSimpson**	**Fisher**
S0	651.50	53.42	672.07	15.09	2.27	0.80	4.95	54.60
S12	512.75	75.43	561.78	15.36	1.32	0.48	1.91	28.27
S24	959.84	69.02	1003.49	18.99	2.74	0.83	5.99	82.98
S36	1394.33	90.42	1304.84	20.02	3.87	0.96	25.88	126.87
S48	1293.08	80.46	1289.99	20.49	3.55	0.95	18.29	118.62

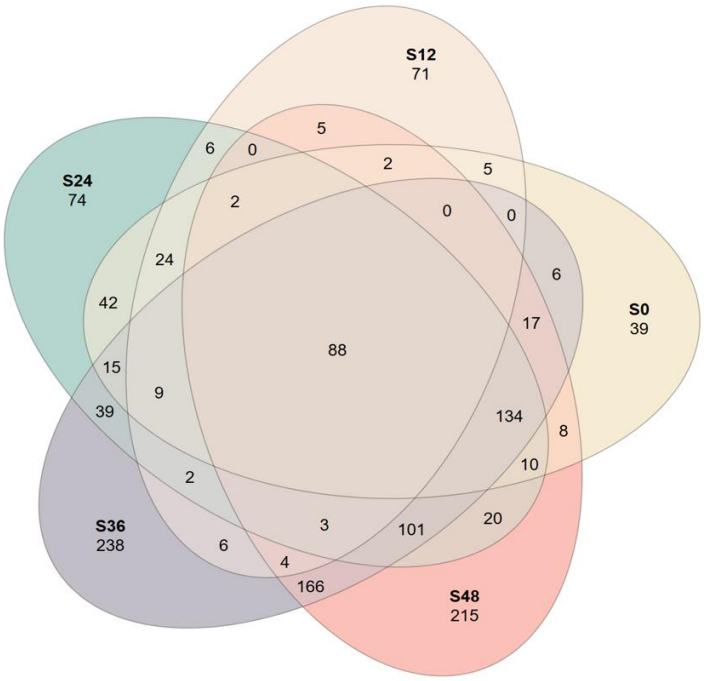
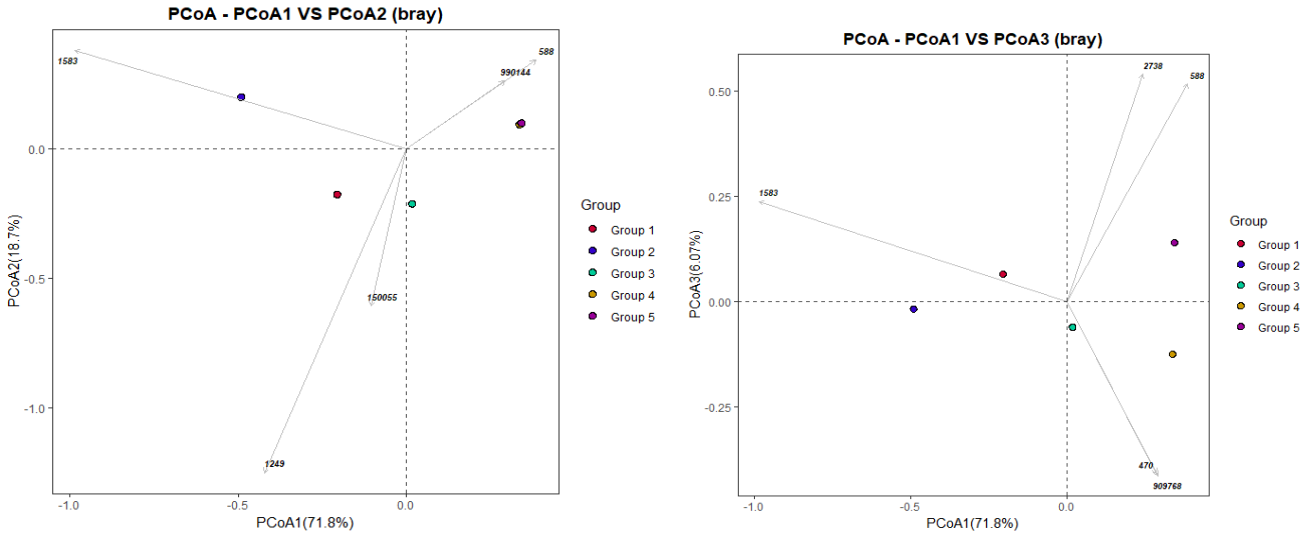


Figure 4. Venn diagram of bacterial diversity during *seredele* fermentation

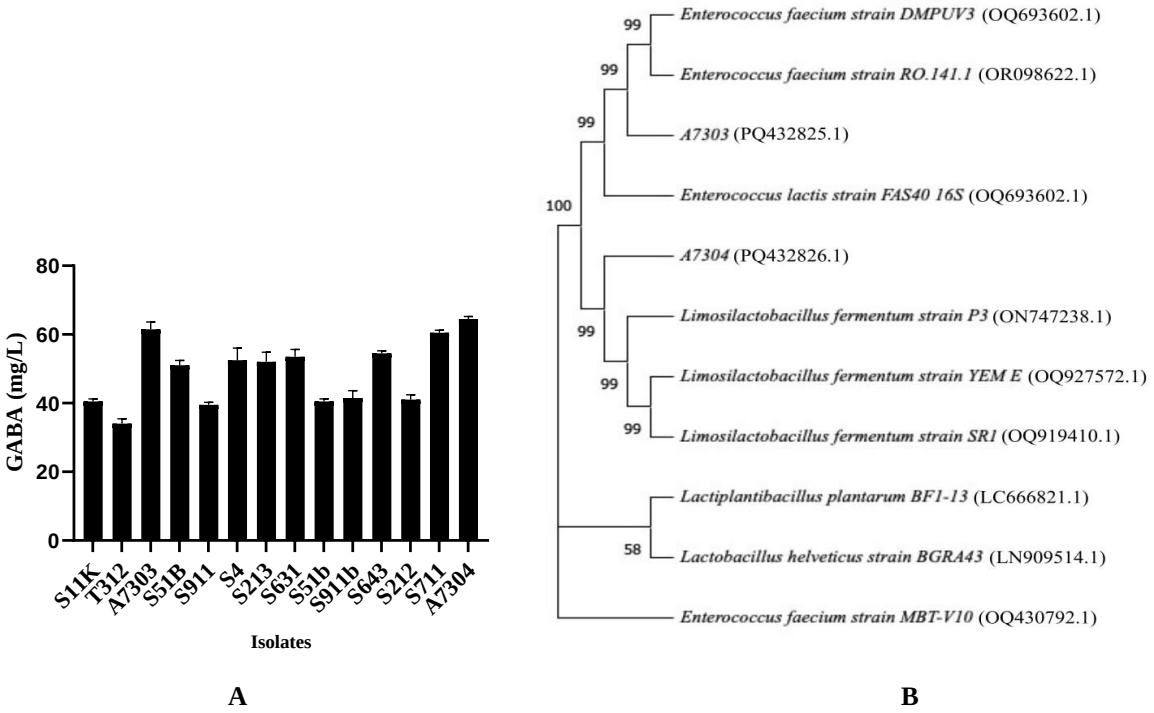


A **B**

Figure 5. Analysis of PCoA of: A. Weighted; B. Unweighted distance among bacterial diversity during seredele fermentation. The red (Group 1), blue (Group 2), green (Group 3), yellow (Group 4), purple (Group 5) represent times of fermentation at 0, 12, 24, 36, and 48 h, respectively

Table 2. pH course of *seredele* during fermentation

Fermentation time	pH
0	6.18±0.01
12	6.20±0.00
24	6.0±0.07
36	5.31±0.04
48	5.00±0.01



A **B**

Figure 6. A. Screening of GABA-producing LAB isolated from *seredele*; B. Phylogenetic analysis of selected GABA-producing LAB. GABA quantification was performed at least in duplicate for each isolate

In conclusion, metagenomic analysis has successfully revealed the bacterial community in *seredele* during fermentation. The most prevalent bacteria during fermentation were *Weissella*, *Streptococcus*, and *Enterococcus* with *Vagococcus martis* and *Leuconostoc citreum* detected as distinctive species in *seredele* fermentation. However, a small number of potentially pathogenic bacteria such as *Klebsiella* sp. and *Cronobacter* sp. were detected, likely due to inadequate hygienic practices often overlooked by traditional *seredele* producers. In addition to postbiotic activity, two LAB strains from *seredele*, *L. fermentum* A7304 and *E. lactis* A7303, showed promising potential as GABA producers. This study has revealed the bacterial succession during *seredele* fermentation and its potential to produce beneficial metabolites, warranting further investigation as functional starter cultures.

ACKNOWLEDGEMENTS

We express our utmost gratitude to National Research and Innovation Agency (BRIN) and Indonesia Endowment Fund for Education (LPDP) for their contribution to funding our project through the *Riset Inovasi untuk Indonesia Maju* (RIIM) Scheme with project number: 82/II.7/HK/2022.

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