

## Identification of Lactic Acid Bacteria From Pineapple Peels Fermented Beverages Through 16S rRNA Gene Analysis

### *Identifikasi Bakteri Asam Laktat Minuman Fermentasi Kulit Nanas melalui Analisis Gen 16S rRNA*

Ni Nyoman Ariwidiani<sup>1\*</sup>, Pauzan<sup>2</sup>, Rabiyyatun Wahdaniyah<sup>1</sup>, I Ketut Mahavira Diputra<sup>3</sup>

<sup>1</sup>D-IV Teknologi Laboratorium Medis, Universitas Bima Internasional MFH, Mataram, Indonesia

<sup>2</sup>D-III Teknologi Laboratorium Medis, Universitas Bima Internasional MFH, Mataram, Indonesia

<sup>3</sup>General Practitioners, Bangli General Hospital, Bali, Indonesia

\*Email: [arhiwidhia13@gmail.com](mailto:arhiwidhia13@gmail.com)

Article submitted: October 28, 2025, revised: May 6, 2025 accepted: July 8, 2025

#### Abstrak

Kondisi stunting berhubungan dengan kondisi dysbiosis microbiota usus yang menyebabkan terganggunya fungsi tubuh dan memperburuk keterbelakangan pertumbuhan anak. Konsumsi probiotik menjadi salah satu alternatif dalam mencegah terjadinya infeksi gastrointestinal yang disebabkan oleh dysbiosis microbiota. Probiotik bekerja dalam menyeimbangkan komposisi microbiota usus dan menstimulasi respon kekebalan tubuh. Pemanfaatan produk fermentasi telah dikenal luas oleh masyarakat, salah satunya tepache minuman fermentasi kulit nanas yang mengandung bakteri asam laktat yang memiliki potensi sebagai probiotik. Penelitian ini bertujuan untuk mengidentifikasi bakteri asam laktat dari minuman fermentasi kulit nanas yang berpotensi sebagai probiotik kesehatan microbiota usus. Fermentasi kulit nanas dilakukan selama 3 hari, cairan hasil fermentasi dilakukan kultur dengan media MRS agar dan dilakukan uji karakteristik dengan uji biokimia. Identifikasi isolate hasil kultur fermentasi kulit nanas dilakukan identifikasi dengan sekuensing gen 16S rRNA. Hasil kultur isolate menunjukkan pertumbuhan 2 isolate bakteri dengan karakteristik yang sesuai dengan pertumbuhan koloni BAL, uji mikroskopis menunjukkan bahwa isolate N1 merupakan BAL berbentuk batang dengan gram positif dan isolate N2 merupakan BAL berbentuk kokobasil dengan gram positif. Identifikasi sekuensing gen 16S rRNA isolate N1 menunjukkan adanya kemiripan karakteristik dengan *Lactiplantibacillus plantarum*, sedangkan isolate N2 menunjukkan kemiripan dengan *Leuconostoc mesenteroides*. Kedua isolat ini merupakan bakteri asam laktat yang umum ditemukan dalam makanan fermentasi dan dapat berfungsi sebagai kandidat probiotik potensial, namun evaluasi fungsional dan keamanan diperlukan untuk konfirmasi sifat probiotiknya.

**Kata kunci:** Gen 16S rRNA; *L. plantarum*; *Ln. Mesenteroides*; Microbiota Usus; Stunting

#### Abstract

Stunting is associated with intestinal microbiota dysbiosis, which disrupts bodily functions and contributes to stunted growth in children. Consuming probiotics is an alternative way to prevent gastrointestinal infections caused by dysbiotic microbiota. Probiotics work by balancing the composition of the intestinal microbiota and stimulating the immune response. The use of fermented products is well known; one example is tepache, a fermented pineapple peel drink containing lactic acid bacteria with potential as a probiotic. This study aims to identify lactic acid bacteria from fermented pineapple peel drinks with potential as probiotics for intestinal microbiota health. Pineapple peel fermentation was carried out for 3 days; the resulting fermentation liquid was cultured on MRS agar and tested for characteristics using biochemical tests. Identification of isolates from the pineapple peel fermentation culture was performed by sequencing the 16S rRNA gene. The results of the isolate culture showed growth of 2 bacterial isolates with characteristics consistent with LAB colonies. Microscopic tests showed that isolate N1 was a gram-

positive, rod-shaped LAB, and isolate N2 was a gram-positive, coccobacilli-shaped LAB. Identification of the 16S rRNA gene from isolate N1 showed characteristics similar to *Lactiplantibacillus plantarum*, while isolate N2 showed characteristics similar to *Leuconostoc mesenteroides*. These isolates are common lactic acid bacteria found in fermented foods and may be potential probiotic candidates, but functional and safety evaluations are needed to confirm their probiotic properties.

**Keywords:** 16S rRNA gene; Gut Microbiota; *L. plantarum*; *Ln. Mesenteroides*; Stunting

## Introduction

Stunting is a condition characterised by impaired growth and development in children. Stunting is associated with microbial dysbiosis, which disrupts bodily functions and exacerbates growth retardation (Ragil & Dyah, 2017; Sinta Dewi *et al.*, 2021). Consuming probiotics can be an alternative to prevent gastrointestinal infections caused by gut microbiota dysbiosis. Probiotics help balance the gut microbiota and support immune function. Several studies have shown that consuming lactic acid bacteria belonging to the Lactobacillus group can improve cellular and humoral immune responses (Intan Niken Tari & Budi Handayani, 2015; Tayo & Akpeji, 2016).

Several studies have shown that children with adequate nutritional status exhibit gut microbiota dominated by Bacteroides and Bifidobacterium. In contrast, children with stunting or malnutrition show a decrease in Bifidobacterium, a key genus that helps protect the intestinal environment from pathogenic colonization (Norma Farizah Fahmi *et al.*, 2025). In Surono's study (Surono *et al.*, 2021), the composition of the microbiota, especially Prevotella, was greater in children with normal nutritional status than in those with stunting. The balance of the gut microbiota plays an important role in the condition of the digestive environment, especially in terms of metabolites produced by short-chain fatty acids (Kamil *et al.*, 2021). According to Gunawan's study (2022), consumption of synbiotics (*L. plantarum* Dad-13 and fructo-oligosaccharide) for 90 days can affect the composition of the gut microbiota, thereby inducing nutrient intake, height, and weight gain in stunted children (Gunawan *et al.*, 2022).

Probiotic beverage products, widely known to the public, are often made from fermented milk. Other fermented beverages made from fruit juice have been widely produced, including those from pineapple juice (Medaando *et al.*, 2024; Pratiwi *et al.*, 2024; Riani *et al.*, 2020). Tepache is one such fermented product that can be made from pineapple

extract. Pineapple peel contains compounds that can suppress bacterial growth, including phenols, chlorine, iodine, and the enzyme bromelain. The bromelain content in pineapple peel has anti-inflammatory effects and has been used as a digestive aid. Bromelain has been reported to significantly reduce T cell infiltration and improve the clinical and histological conditions of colon inflammation in a colitis model (Martani *et al.*, 2025; Tamang *et al.*, 2020). This study aimed to identify LAB from fermented pineapple peel drinks with the potential to act as probiotics for gut microbiota health.

Interest in fermented plant-based beverages as a source of probiotics continues to increase. Studies on the molecular identification of lactic acid bacteria in fermented products, especially those derived from the peel of Indonesian pineapple varieties, remain relatively limited. Research by Najini *et al.*, (2024) focused on determining the formulation of tepache drinks, while Hartini *et al.*, (2024) provided the feasibility of fermented pineapple peel as a probiotic drink with high antioxidant activity using *Lactobacillus plantarum* and *Lactobacillus casei*, which was limited to product characteristics and antimicrobial activity without molecular analysis of the bacteria involved. In addition, Hujjatusnaini *et al.*, (2022) studied the use of pineapple peel as a probiotic drink by adding *Lactobacillus casei* with varying sucrose concentrations. Several previous studies reported the importance of bacterial isolates as probiotics without adequate molecular validation or functional testing. Therefore, this study aims to identify the molecular profiles of lactic acid bacteria isolated from fermented pineapple peel using 16S rRNA gene sequencing.

## Materials and Methods

### Sample Preparation

This study used pineapples obtained from a pineapple plantation in East Lombok Regency, West Nusa Tenggara (NTB). The pineapples used were ripe, unrotten, and yellow, ensuring only the highest-

quality raw materials were used. Pineapple peel fermentation was made using a mixture of pineapple peel, rock sugar, and boiling water, with the addition of spices such as cinnamon and cloves. The fermentation process was carried out in a controlled environment to ensure optimal conditions for microbial activity. The mixture was then incubated for three days under anaerobic conditions.

### Isolation of Pineapple Peel Fermentation Bacteria

Lactic acid bacteria were isolated from fermented pineapple peel samples incubated for 3 days under anaerobic conditions. A 1 ml sample of fermented pineapple peel was homogenised with 1% w/v peptone water and serially diluted in a tube containing 9 ml of peptone water, a  $10^{-1}$  dilution. A  $10^{-7}$  dilution was then performed. For the final two dilutions, 1 ml of each sample was taken and poured into sterile Petri dishes. De Man-Ragosa-Sharpe agar (MRS agar) supplemented with 1%  $\text{CaCO}_3$  was then poured into the agar. The agar was allowed to solidify and incubated at  $37^\circ\text{C}$  for 48 hours. Colonies that grow with a clear zone around them are purified by streaking on MRSA media supplemented with 1%  $\text{CaCO}_3$  using the quadrant method, incubating for 24 hours at  $37^\circ\text{C}$ , and then subculturing single colonies as pure single isolates.

### Characterization and Biochemical Testing

Biochemical testing was based on research with modifications. The tests included Gram staining, catalase testing, sugar fermentation testing, citrate testing, sulfide-indole motility (SIM) testing, growth testing at varying salt concentrations, growth testing at varying temperatures, and pH resistance testing.

### Sequencing Analysis

Molecular identification was performed using the 16s rRNA gene using the universal primers 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' GGT TAC CTT GTT ACG ACT T 3'). The amplification protocol was as follows: initial denaturation at  $94^\circ\text{C}$  for 2 minutes, followed by denaturation at  $94^\circ\text{C}$  for 30 seconds, primer annealing at  $55^\circ\text{C}$  for 30 seconds, extension at  $72^\circ\text{C}$

for 1.5 minutes, and a final extension at  $72^\circ\text{C}$  for 10 minutes. The reaction was then stored at  $12^\circ\text{C}$  for 30 minutes. After amplification, PCR products were electrophoresed on a 2% (w/v) agarose gel in 0.5X TBE buffer (100 g Tris base, 27.5 g boric acid, pH 8, and 20 mL 0.5 M EDTA). The gel was stained with one  $\mu\text{L}$  ethidium bromide ( $0.2 \mu\text{g}/\text{mL}$ ) and visualised under UV light. Finally, the amplified 16S rRNA genes of the isolates were sequenced.

### Data Analysis

The 16S rRNA gene sequences of BAL were analyzed using MEGA12. The nucleotide sequencing results were then compared with the GenBank database via NCBI Blast. The interspecific relationships were shown in a phylogenetic tree diagram using the neighbor-joining method with 1000 bootstrap replications.

### Results and Discussion

In this study, pineapple skin bacteria were isolated, yielding two isolates: N1 and N2. The characteristics of the culture (colony) from the isolation results are summarised in Table 1. Isolates N1 and N2 have similar morphology, including the shape of their colonies, which are round with flat edges and convex lumps. N1 colonies have a clear colony colour, while N2 colonies are cloudy white (Figure 1). Similar results were reported by (Martani et al., 2025) who found that colonies isolated from various types of tepache formulations had varying colony sizes and colony colours, ranging from white to transparent white. The surface of the isolate is smooth, moist and oily. According to Zhao & Yun, (2016) LAB colonies are usually round, convex with smooth edges and clear white to milky white in colour.

From the isolates obtained, microscopic observations were made on isolates N1 and N2. The results showed that the N1 colony contained gram-positive, bacillus-shaped bacterial cells, lacked spores, and tested negative for catalase. In contrast, the N2 colony was gram-positive, coccus-shaped, did not form spores and reacted negatively in the catalase

Table 1. Characterisation of pineapple peels fermentation isolated colonies.

Colony	Colony Morphology				Cell morphology		
	Top shape	Side shape	Bump shape	Colony color	Cell shape	Gram	Spores
N1	Round	Flat edge	convex	Clear	Bacillus in chains/scattered	Positive	No Spores
N2	Round	Flat edge	Convex	White cloudy	Coccus-bacillus in chains/ scattered	Positive	No spores

test (Figure 2). These results are consistent with the general characteristics of lactic acid bacteria, which are gram-positive bacteria that do not form spores (Akpogheli *et al.*, 2025). Lactic acid bacteria are bacteria that can ferment carbohydrates to produce lactic acid in large quantities. LAB has specific characteristics, namely reacting positively in Gram staining, reacting negatively in the catalase test and not forming spores (Putri *et al.*, 2018).

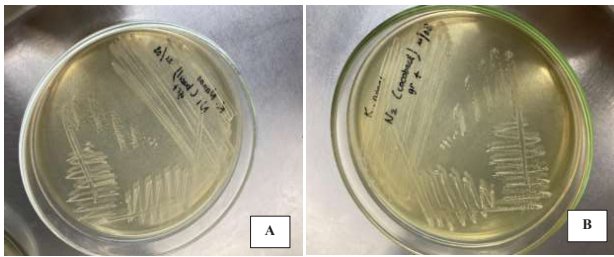


Figure 1. Isolation of pineapple skin fermentation bacteria. A. Isolate N1; B. Isolate N2

Biochemical activity tests showed that isolates N1 and N2 had almost identical characteristics, including sugar fermentation and a negative catalase test. These results point to the characteristics of *Lactobacillus* spp. Research by Octaviana *et al.*, (2023) found characteristics similar to those of bacillus- and/or coccobacillus-shaped bacterial cells that were gram-positive, did not form spores, and tested negative for catalase. According to research (Safari *et al.*, 2019), lactic acid bacteria of the genus *Lactobacillus* are generally rod-shaped, gram-positive, and lack the catalase enzyme.

Identification of lactic acid bacteria isolates from fermented pineapple peel was carried out at the

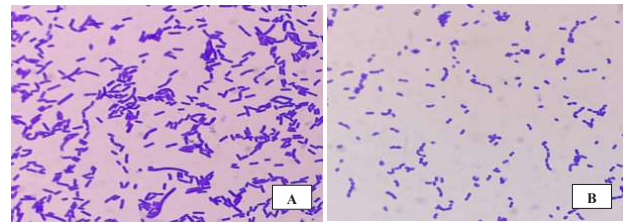


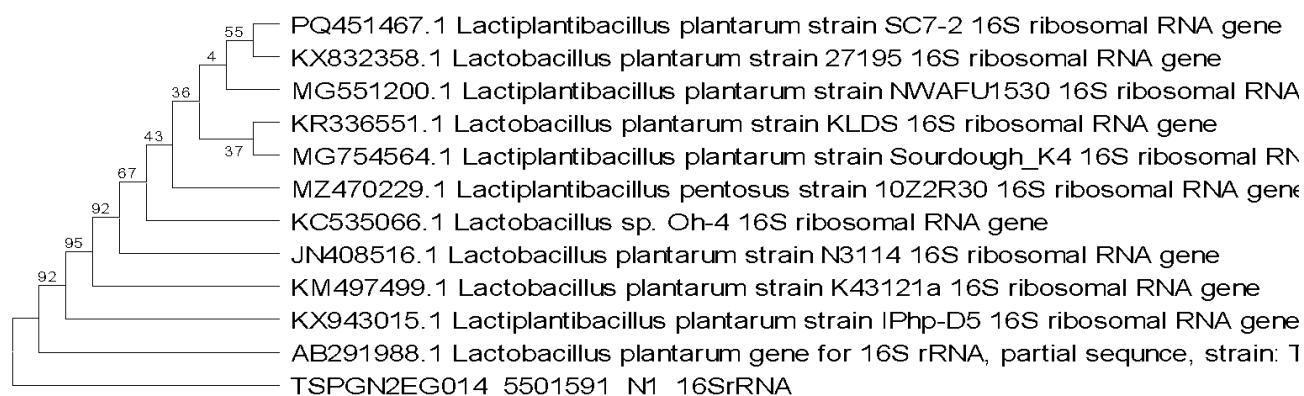
Figure 2. Gram staining result of isolate from pineapple peel fermentation. A. Isolate N1: Gram-positive, rod-shaped; B. Isolate N2: Gram-positive, coccobacillary-shaped.

molecular level through 16S rRNA amplification. The results of the sequence identification of isolate N1 showed a kinship with the species groups of the genera *Lactiplantibacillus* and *Lactobacillus*, especially with the strain *Lactiplantibacillus plantarum* (table 2). Meanwhile, isolate N2 showed a high degree of similarity to strains of the genus *Leuconostoc*, especially those in the *Leuconostoc mesenteroides* group (table 3). The phylogenetic placement of strains isolated from fermented pineapple peel is shown in Figures 3 and 4.

Table 2 presents the BLAST analysis of the 16S rRNA gene sequence of isolate N1 against the GenBank database to determine taxonomic closeness based on the Highly similar sequences (megablast) parameter. The analysis results showed that isolate N1 had a very high similarity (99.76%) with several *Lactiplantibacillus plantarum* reference strains indicating high conservation of the 16S rRNA gene in the *L. plantarum* group, in addition, one closely related strain, namely *Lactiplantibacillus pentosus*, was detected. confirming that the 16S rRNA gene has limited resolution in distinguishing highly related

Table 2. BLAST result of the 16S rRNA gene sequence of isolate N1

Closest Species Match	% Identity	Query Coverage (%)	E-value	Accession Number
<i>Lactobacillus plantarum</i> strain N3114 16S ribosomal RNA gene	99.76	93	0.0	JN408516.1
<i>Lactiplantibacillus plantarum</i> strain SC7-2 16S ribosomal RNA gene	99.76	92	0.0	PQ451467.1
<i>Lactobacillus plantarum</i> strain KLDS 16S ribosomal RNA gene	99.76	92	0.0	KR336551.1
<i>Lactiplantibacillus plantarum</i> strain Sourdough_K4 16S ribosomal RNA gene	99.76	92	0.0	MG754564.1
<i>Lactiplantibacillus plantarum</i> strain IPhp-D5 16S ribosomal RNA gene	99.76	80	0.0	KX943015.1
<i>Lactobacillus</i> sp. Oh-4 16S ribosomal RNA gene	99.76	92	0.0	KC535066.1
<i>Lactobacillus plantarum</i> gene for 16S rRNA, partial sequence, strain: TS123	99.76	80	0.0	AB291988.1
<i>Lactobacillus plantarum</i> strain K43121a 16S ribosomal RNA gene	99.76	92	0.0	KM497499.1
<i>Lactiplantibacillus pentosus</i> strain 10Z2R30 16S ribosomal RNA gene	99.76	92	0.0	MZ470229.1



species within the *Lactiplantibacillus plantarum* clade. Based on the blast analysis, isolate N1 was classified as a *Lactiplantibacillus plantarum*-like strain.

Phylogenetic analysis of 16S rRNA gene sequences (Figure 3) shows that isolate N1 (TSPGN2EG014\_5501591\_N1\_16SrRNA) is grouped within the *Lactiplantibacillus* genus clade, specifically the *Lactiplantibacillus plantarum* group. Isolate N1 is close to several *L. plantarum* references, including *L. plantarum* strains SC7-2, 27195, K165 and N3114. The bootstrap value (>92-95%) indicates stability in the phylogenetic relationship.

The use of fermented pineapple peel has been the subject of extensive research. This study isolated and identified lactic acid bacteria from fermented pineapple, with potential as probiotic candidates for gut microbiome health. Several studies have shown that tepache contains bacteria such as *Lactobacillus casei*, *Lactiplantibacillus pentosus*, and *Lactocaseibacillus paracasei*. These bacteria are known to boost the immune system, help the body fight infections, support gut health, and help maintain gut microbiome balance (Ligenza *et al.*, 2021). Furthermore, tepache is rich in peptides and organic

acids that can lower blood pressure, normalise blood sugar levels, and possess antioxidant and anti-inflammatory properties (Hartini *et al.*, 2024).

Identification of isolate N1 showed that *Lactiplantibacillus plantarum* is a lactic acid bacterium often found in fermented plant and animal products. *Lactiplantibacillus plantarum* strains are commonly used in the food industry as fermentation agents for cheese making, and various other fermented foods and beverages (Behera *et al.*, 2018). Various studies have shown that *L. plantarum* has beneficial effects on diseases and health conditions. Several studies have examined the anticancer effects of *L. plantarum* (Jeong *et al.*, 2023), as well as the development and potential of probiotics as mucosal vaccines that produce B-cell multi-peptides to fight gastric cancer. In addition, *L. plantarum* also has potential as an antimicrobial against *B. subtilis*, *E. coli*, and *S. aureus*. *L. plantarum* is recognised as safe by global food authorities. Some components produce various bioactive metabolites, such as lactic acid, hydrogen peroxide, and bacteriocins (plantaricin), which are effective in inhibiting pathogenic bacteria (Syaputri *et al.*, 2024).

Table 3. BLAST result of the 16S rRNA gene sequence of isolate N2.

Closest Species Match	% Identity	Query Coverage (%)	E-value	Accession Number
Leuconostoc sp. strain LB_31 16S ribosomal RNA gene	98.59	100	0.0	MN410564.1
Leuconostoc mesenteroides subsp. mesenteroides strain THJ-P7 16S ribosomal RNA gene	98.59	100	0.0	PP917546.1
Leuconostoc mesenteroides strain WSJ-42 16S ribosomal RNA gene	98.52	100	0.0	MT152955.1
Leuconostoc sp. strain BSDF2-11 16S ribosomal RNA gene	98.52	100	0.0	PQ691440.1
Leuconostoc mesenteroides strain NWAUFU 4028 16S ribosomal RNA gene	98.52	100	0.0	OR240754.1
Leuconostoc mesenteroides strain HDM4 16S ribosomal RNA gene	98.52	100	0.0	OR485706.1
Leuconostoc falkenbergense strain TRM59684 16S ribosomal RNA gene	98.52	100	0.0	PX149918.1
Leuconostoc mesenteroides partial 16S rRNA gene, isolate from Jaipur, India	98.52	100	0.0	FR852570.1
Leuconostoc mesenteroides strain NWAUFU 4016 16S ribosomal RNA gene	98.52	100	0.0	OR240726.1

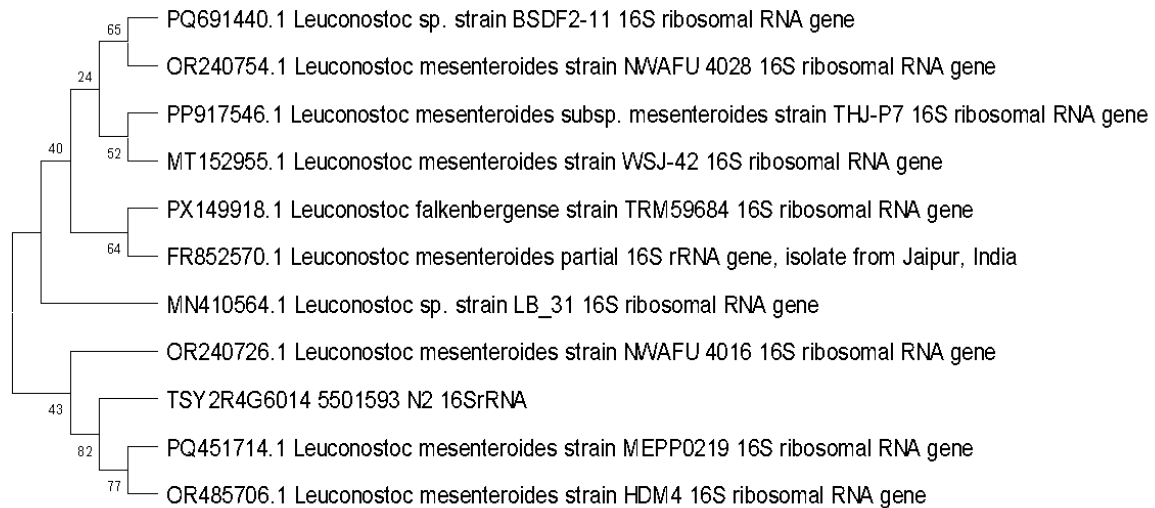


Figure 4. Phylogenetic tree of isolate N2 based on 16S rRNA gene sequences constructed using the neighbor-joining method with 1.000 bootstrap replications. Bootstrap values (%) are shown at the branch nodes. The tree illustrates the clustering of isolate N2 (TSY2R4G6014\_5501593 N2 16SrRNA) within the *Leuconostoc mesenteroides* clade.

Table 3 shows the BLAST analysis of 16S rRNA isolate N2 based on the GenBank database to determine taxonomic proximity based on sequence similarity. The analysis results show that isolate N2 has a similarity level of 98.52%-98.58% with strain variants in the genus *Leuconostoc*, especially *Leuconostoc mesenteroides* and its subspecies. Although the level of sequence similarity is relatively high, it is below 99% due to the presence of closely related *Leuconostoc* species (*Leuconostoc falkenbergense* and *Leuconostoc sp.*), indicating that the 16S rRNA gene resolution is unable to distinguish species within the genus *Leuconostoc*. Based on the blast analysis results, it can be classified as a *Leuconostoc mesenteroides*-like strain.

A phylogenetic tree based on 16S rRNA gene sequences (figure 4) showed that isolate N2 (TSY2R4G6014\_5501593 N2 16SrRNA) clustered with the *Leuconostoc* genus clade. Isolate N2 formed a subclade associated with several *Leuconostoc mesenteroides* strains, particularly *L. mesenteroides* strain MEPP0219 (PQ451714.1) and strain HDM4 (OR485706.1). The phylogenetic proximity between isolate N2 and both reference strains, with bootstrap values of 77-82%, indicated moderate to strong statistical support for this grouping. The broader *L. mesenteroides* clade showed moderate bootstrap values (40-64%) at several nodes, indicating genetic variation among strains within the genus *Leuconostoc* and the high conservation of 16S rRNA gene sequences.

*Leuconostoc mesenteroides* has potential as a probiotic for human health. Based on research

by Bouguerra *et al.*, (2023), it was reported that *L. mesenteroides* has potential as a probiotic that can suppress pathogen growth and protect the ileum from *Salmonella* infection. Other studies also show that *L. mesenteroides* has prophylactic, therapeutic, and direct inhibitory effects *in vitro* against the *anti-porcine Epidemic Diarrhoea Virus*. This lactic acid bacterium is commonly used in fermented milk products, vegetables, and processed meat. In addition, several strains of *L. mesenteroides* have been reported to have anti-listerial, antiviral, or immunomodulatory activity (Seo *et al.*, 2012; Shao *et al.*, 2020). The production of exopolysaccharides and bacteriocins is an important product of *L. mesenteroides*. Several studies have shown that bacteriocins produced by *L. mesenteroides* have antipathogenic activity. In addition, the exopolysaccharides produced by *L. mesenteroides* exhibit antiviral and immunomodulatory activities (Mahdi *et al.*, 2019).

### Conclusion

Lactic acid bacteria isolated from fermented pineapple peel yielded two bacterial isolates with similar characteristics: rod-shaped and coccus-bacillus, gram-positive and catalase-negative. Based on 16S rRNA sequencing, the bacterial isolates were identified as *L. plantarum*-like strain and *L. Mesentroides*-like strain. These findings provide baseline molecular data for lactic acid bacteria associated with fermented pineapple peel beverages. Further functional, safety, and *in vivo* studies are required to confirm their probiotic potential.

### Acknowledgments

The authors would like to thank the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia for its support in the form of a Beginner Lecturer Research Grant. We also thank the Research and Development Center of the NTB Provincial Hospital for their assistance in this research.

### References

- Akpogheli, P. O., Edo, G. I., Ali, A. B. M., Yousif, E., Zainulabdeen, K., Owheruo, J. O., Isoje, E. F., Igbuku, U. A., Essaghah, A. E. A., Makia, R. S., Ahmed, D. S., Umar, H., & Alamiery, A. A. (2025). Lactic acid bacteria: Nature, characterization, mode of action, products and applications. *Process Biochemistry*, *152*(1), 1–28. <https://doi.org/10.1016/j.procbio.2025.02.010>
- Behera, S. S., Ray, R. C., & Zdolec, N. (2018). *Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. *BioMed Research International*, *2018*(1), 1–18. <https://doi.org/10.1155/2018/9361614>
- Bouguerra, A., Meziti, A., Guergour, H., & Harzallah, D. (2023). The Potential of *Leuconostoc mesenteroides* Strain LK-151 to Inhibit in vivo *Salmonella* Infection. *Acta Microbiologica Bulgarica*, *39*(3), 295–301. <https://doi.org/10.59393/amb23390310>
- Gunawan, D. C. D., Juffrie, M., Siti Helmyati, S. H., & Rahayu, E. S. (2022). Synbiotic (*L. plantarum* Dad-13 and Fructo-oligosaccharide) Powder on Gut Microbiota (*L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae*) on Stunting Children in Yogyakarta, Indonesia. *Current Research in Nutrition and Food Science Journal*, *10*(1), 371–383. <https://doi.org/10.12944/CRNFSJ.10.1.31>
- Hartini, S., Cahyanti, M. N., Kusumahastuti, D. K. A., Susilowati, I. T., & Mahardika, Y. M. A. (2024). Antioxidant Profile in Pineapple Peel Fermentation by *Lactobacillus plantarum* and *Lactobacillus casei*. *Jurnal Penelitian Pendidikan IPA*, *10*(4), 2065–2072. <https://doi.org/10.29303/jppipa.v10i4.7546>
- Hujjatusnaini, N., Amin, A. M., Perditson, H. F. A., Robiyansyah, M., Guria, W. A., Husna, N., Annisa, N., & Ramlan, C. (2022). Inovasi Minuman Tepache Berbahan Baku Kulit Nanas (*Ananas comosus* (L.) Merr.) Tersuplementasi Probiotik *Lactobacillus casei*. *Jurnal Teknologi Pangan Dan Gizi*, *21*(1), 47–54. <https://doi.org/10.33508/jtpg.v21i1.3568>
- Intan Niken Tari, A., & Budi Handayani, C. (2015). Potential of Indigenous Probiotic *Lactobacillus plantarum* Dad 13 as Anti-diarrhea and Immuno-modulator. *Proceedings of International Congress. Challenges of Biotechnological Research in Food and Health* pp 122-127.
- Jeong, S., Kim, Y., Park, S., Lee, D., Lee, J., Hlaing, S. P., Yoo, J.-W., Rhee, S. H., & Im, E. (2023). *Lactobacillus plantarum* Metabolites Elicit Anticancer Effects by Inhibiting Autophagy-Related Responses. *Molecules*, *28*(4), 1890-1897. <https://doi.org/10.3390/molecules28041890>
- Kamil, R. Z., Murdiati, A., Juffrie, M., Nakayama, J., & Rahayu, E. S. (2021). Gut Microbiota and Short-Chain Fatty Acid Profile between Normal and Moderate Malnutrition Children in Yogyakarta, Indonesia. *Microorganisms*, *9*(1), 127. <https://doi.org/10.3390/microorganisms9010127>
- Ligenza, A., Jakubczyk, K., Kochman, J., & Janda, K. (2021). Health-promoting potential and microbial composition of fermented drink tepache. *Medycyna Ogólna i Nauki o Zdrowiu*, *27*(3), 272–276. <https://doi.org/10.26444/monz/138713>
- Mahdi, L. H., Hussein, N. H., Taha, B. M., Auda, I. G., Zwain, L. A. H., & Mater, H. N. (2019). Immunostimulatory and antibacterial activity of *Leuconostoc mesenteroides* and its purified exopolysaccharide against extended-spectrum beta-lactamase producing *Burkholderia cepacia*. *Reviews in Medical Microbiology*, *30*(3), 161–172. <https://doi.org/10.1097/MRM.0000000000000172>
- Martani, N. S., Hanasia, Turnip, O. N., Halik, H., & Adnyana, I. G. H. E. (2025). Isolation and characterization of acetic acid bacteria from pineapple peel fermentation (*Ananas comosus*). *Biodiversitas*, *26*(2), 789–798. <https://doi.org/10.13057/biodiv/d260227>

- Medaando, Rahmawati, & Masnur Turnip. (2024). Identifikasi Bakteri Asam Laktat Berdasarkan Kemiripan Fenotipik dari Kulit Nanas Varietas Queen di Kalimantan Barat yang Difermentasi secara Alami. *Life Science*, 13(1), 22-34.
- Najini, R., Purwanti, N. U., Rosidatul Mufida, A., Kurniawan, A., Husain, M., Alghifary, H., Rianda Syalsabila, R., Nuraini, R., & Safitri, W. (2024). Minuman Probiotik Tepache Dari Fermentasi Kulit Buah Nanas (*Ananas Comosus L.*) Menggunakan Variasi Jenis dan Konsentrasi Gula Tepache Probiotic Drink From Fermented Pineapple Peels (*Ananas Comosus L.*) Using Variations of Sugar Types And Concentrations. *JPOP : Journal Pharmacy of Tanjungpura*, 1(2).
- Norma Farizah Fahmi, Dwi Aprilia Anggraini, & Vivin Wijastutik. (2025). The Effectiveness of Probiotic Supplementation in Treatment Stunting in Children. *Journal Of Nursing Practice*, 8(3), 534–541. <https://doi.org/10.30994/jnp.v8i3.664>
- Octaviana, C., Watumbara, M. L., Sugata, M., & Jo, J. (2023). Isolasi dan Karakterisasi *Lactobacillus* Species dari Susu Kambing Peternakan Lokal. *Biota : Jurnal Ilmiah Ilmu-Ilmu Hayati*, 2023(1), 186–195. <https://doi.org/10.24002/biota.v8i3.6718>
- Pratiwi, C., Saufani, I. A., & Nurfaziah, L. R. (2024). Identifikasi Profil Sensori Ideal Minuman Fermentasi Kulit Buah Nanas (*Ananas comosus L.*). *Amerta Nutrition*, 8(3SP), 344–352. <https://doi.org/10.20473/amnt.v8i3SP.2024.344-352>
- Putri, Y. W., Putra, A. E., & Utama, B. I. (2018). Identifikasi Dan Karakteristik Bakteri Asam Laktat Yang Diisolasi Dari Vagina Wanita Usia Subur. *Jurnal Kesehatan Andalas*, 7(2018), 20-25. <https://doi.org/10.25077/jka.v7i0.864>
- Ragil, D., & Dyah, Y. (2017). Hubungan Antara Pengetahuan Dan Kebiasaan Mencuci Tangan Pengasuh Dengan Kejadian Diare Pada Balita. *Jurnal of Health Education*, 2(1), 39-46. <http://journal.unnes.ac.id/sju/index.php/jhealthedu/>
- Riani, C. R., Nuraida, L., & Meryandini, A. (2020). Isolasi bakteri asam laktat asal jus nanas sebagai kandidat probiotik. *Jurnal Teknologi Dan Industri Pangan*, 31(2), 103–112. <https://doi.org/10.6066/jtip.2020.31.2.103>
- Safari, M. S., Keyhanfar, M., & Shafiei, R. (2019). Investigating the antibacterial effects of some *Lactobacillus*, *Bifidobacterium* and *acetobacter* strains killed by different methods on *Streptococcus mutans* and *Escherichia coli*. *Molecular Biology Research Communications*, 8(3), 103–111. <https://doi.org/10.22099/mbrc.2019.33582.1399>
- Seo, B. J., Rather, I. A., Kumar, V. J. R., Choi, U. H., Moon, M. R., Lim, J. H., & Park, Y. H. (2012). Evaluation of *Leuconostoc mesenteroides* YML003 as a probiotic against low-pathogenic avian influenza (H9N2) virus in chickens. *Journal of Applied Microbiology*, 113(1), 163–171. <https://doi.org/10.1111/j.1365-2672.2012.05326.x>
- Shao, X., Fang, K., Medina, D., Wan, J., Lee, J., & Hong, S. H. (2020). The probiotic, *Leuconostoc mesenteroides*, inhibits *Listeria monocytogenes* biofilm formation. *Journal of Food Safety*, 40(2), e12750. <https://doi.org/10.1111/jfs.12750>
- Sinta Dewi, A., Atifah, Y., Alicia Farma, S., Yuniarti, E., & Fadhilla, R. (2021). The Importance of Consuming Probiotics for The Digestive Tract and Its Relation to The Human Immune System. *Universitas Negeri Padang*, 01(2021), 149-156. <https://doi.org/10.24036/prosemnasbio/vol1/23>
- Surono, I. S., Widiyanti, D., Kusumo, P. D., & Venema, K. (2021). Gut microbiota profile of Indonesian stunted children and children with normal nutritional status. *PLOS ONE*, 16(1), e0245399. <https://doi.org/10.1371/journal.pone.0245399>
- Syaputri, Y., Nurhidayat, F., Wulandari, I., & Rahayu, S. R. (2024). *Lactiplantibacillus plantarum* Isolated From *Sonchus Arvensis* As An Antimicrobial Agent. *Jurnal Bioteknologi & Biosains Indonesia*, 11(1), 164-181. <http://ejournal.brin.go.id/JBBI/index>
- Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., Mayo, B., Westerik, N., & Hutkins, R. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety*,

19(1), 184–217. <https://doi.org/10.1111/1541-4337.12520>

Tayo, B. A., & Akpeji, S. (2016). Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice. *Fermentation*, 2(4), 20. <https://doi.org/10.3390/fermentation2040020>

Zhao, H., & Yun, J. (2016). Isolation, identification and fermentation conditions of highly acetoin-producing acetic acid bacterium from Liangzhou fumigated vinegar in China. *Annals of Microbiology*, 66(1), 279–288. <https://doi.org/10.1007/s13213-015-1106-1>