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Isolation and characterization of acetic acid bacteria from pineapple peel fermentation (*Ananas comosus*)

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Abstract. *Martani NS, Hanasia, Turnip ON, Halik H, Adnyana IGHE. 2025. Isolation and characterization of acetic acid bacteria from pineapple peel fermentation (*Ananas comosus*). Biodiversitas 26: 789-798.* Pineapple (*Ananas comosus*) is rich in bioactive compounds and is often considered waste, especially its peel. The fermentation of pineapple peel can yield value-added goods, including probiotic drinks that promote digestive health. Acetic Acid Bacteria (AAB) are significant microorganisms in fermentation, converting ethanol into acetic acid, hence offering preservation advantages and probiotic qualities. This study aimed to isolate and characterize acetic acid bacteria from the fermentation of pineapple peel and evaluate its antibacterial properties. The fermentation occurred spontaneously without the use of a starting culture, utilizing three distinct formulas. On the third day of fermentation, the fermented liquid was extracted and characterized by 16S rRNA gene sequencing. The findings indicated that the identified AAB was *Acetobacter fabarum* strain 2567, exhibiting 97% homology. The antibacterial activity of *A. fabarum* filtrate at concentrations of 5-75% was tested against the pathogens *Escherichia coli* and *Shigella dysenteriae* using the disk diffusion method; however, no antibacterial activity was observed against either pathogen. The study underscores the need for further research to evaluate the antibacterial potential of *A. fabarum* against other pathogens using methods such as micro-dilution or well diffusion, highlighting the urgency of this line of investigation.

Keywords: Acetic acid bacteria, *Acetobacter fabarum*, *Ananas comosus*

INTRODUCTION

Microorganisms such as lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts like *Saccharomyces cerevisiae* play vital roles in the fermentation of foods like yogurt, vinegar, bread, and alcohol (Dimidi et al. 2019; Hussein et al. 2021). Fermentation improves flavor, texture, and nutrient availability, particularly proteins and vitamins, and lowers pathogen levels (Kårlund et al. 2020). Indigenous microorganisms (MOLs) derived from organic waste can act as bio-activators, enhancing efficiency and product quality in fermentation processes (Nurhayati and Ali 2024).

Microbes also synthesize secondary metabolites with medicinal properties. For example, lactic acid produced during fermentation naturally preserves food by raising its pH, inhibiting harmful bacteria (Wang et al. 2021). Furthermore, studies reveal that the probiotic qualities of fermented goods like kefir, which are made from a mix of many microorganisms, help intestinal health (Saleem et al. 2023). In agriculture, microorganisms are used to ferment soils and crops, enhancing quality. Liquid organic fertilizer created from decomposing bacteria improves soil fertility and expedites nitrification. Cheese whey and vegetable waste also serve as effective natural decomposers to improve organic fertilizer nutrients (Sirmacekic et al. 2022; Aguilar-Paredes et al. 2023).

AABs, such as *Acetobacter* and *Gluconobacter*, are crucial in the food and beverage industry. They oxidize ethanol into acetic acid, offering probiotic benefits and contributing to vinegar production (Sirmacekic et al. 2022; Aguilar-Paredes et al. 2023). AABs also convert alcohols and carbohydrates into organic acids, aldehydes, and ketones through oxidative fermentation (He et al. 2022), producing acetic acid, bacterial cellulose, and gluconic acid-useful in food and medicine industries (Heydorn et al. 2023). In kombucha, AABs and yeast ferment sugar into ethanol, later oxidized into acetic acid, giving the beverage its tangy flavor (Lee et al. 2024). AAB also preserves food by preventing harmful microbial growth (Hata et al. 2023).

Pineapple, a tropical fruit with a unique sweet and sour taste, is rich in vitamin C, dietary fiber, and bromelain, offering significant health benefits (Tayo and Akpeji 2016). Pineapple peels, often discarded, contain micronutrients like vitamins (A, B, B1, B2, B6, B12, and C), calcium, potassium, phosphorus, and antioxidants such as alkaloids, phenolics, tannins, saponins, and flavonoids (Madhumeena et al. 2021; Owoeye et al. 2022). Bromelain, present in pineapple peel, has anti-inflammatory properties and accelerates wound healing (Ramli and Munir 2023). Moreover, pineapple peel extract has demonstrated

significant antibacterial activity against methicillin-resistant (*Staphylococcus aureus* (MRSA)) providing a reassuring safety profile at a Minimum Inhibitory Concentration (MIC) of 50% and a Minimum Bactericidal Concentration (MBC) of 100% (Husniah and Soleha 2023).

In recent years, research on fermented products has revealed that they can inhibit pathogenic microorganism growth by forming symbiotic relationships with *Saccharomyces cerevisiae*, Lactic Acid Bacteria (LAB), and Acetic Acid Bacteria (AAB) (De Vuyst and Leroy 2007). In many countries, fermented foods and beverages are an integral part of the cultural heritage, including kimchi (Korea), sake (Japan), kombucha (China), yogurt (Middle East), tempeh (Indonesia), and tepache (Mexico) (Tamang et al. 2020).

Here, researchers are interested in the fermented drink tepache, which exploits pineapple peel as the primary raw material. Through the fermentation process, such as the production of tepache, pineapple peel can be transformed into a probiotic drink that supports digestive health (Rivera et al. 2023). The compound composition of this drink is reported to be predominantly composed of polyphenols, gallic acid, ferulic acid, anthocyanins, and saponins (Robledo-Márquez et al. 2021). The microbial diversity analysis revealed that the microbial community consisted mostly of bacteria of the genera *Lactobacillus, Leuconostoc, Acetobacter,* and *Lactococcus*. It was also dominated by fungi of the genera *Saccharomyces, Gibberella, Candida, Zygosaccharomyces, Meyerozyma, Talaromyces, Epicoccum,* and *Kabatiella.* These findings indicate the existence of a complex microbial ecosystem in tepache, with potential implications for fermentation processes and product quality (Gutiérrez-Sarmiento et al. 2022). This research aimed to isolate and identify acetic acid bacteria in pineapple peel based on the 16S rRNA gene sequence. This approach could provide insights into the utilization of pineapple peel, a by-product often considered waste. Ultimately, this research could lead to the creation of innovative products that not only promote human health but also reduce food waste.

MATERIALS AND METHODS

Preparation of pineapple peel fermentation

The pineapples were obtained from the Basarang pineapple plantation, Basarang Sub-district, Kapuas District, Central Kalimantan, Indonesia. These were not just any pineapples but fully ripe, free from rot, and orange-yellow, ensuring the use of only the highest quality ingredients. The pineapple peel fermentation was prepared with 3 formulations, as shown in (Table 1). The ratio used was 3:1 (Sukriadi et al. 2022). The fermentation process was conducted in a controlled environment to ensure optimal conditions for microbial activity. The pineapple fruit was first washed under running water. Afterward, the peel and flesh were separated. The pineapple peel was then cut into small pieces and weighed until it reached a mass of 150 g. The peel was then transferred to the fermentation container, and water was added until the peel was fully submerged. Spices such as ginger, cloves, and cinnamon were added to enhance the aroma. The mixture was then incubated for three days under anaerobic conditions.

Isolation and identification of bacteria

The procedure of bacterial isolation, as described by Ramadhanti et al. (2021), was adapted with certain modifications to accommodate the experimental setup. On the 3rd day of the fermentation process, a sample of the pineapple peel fermentation liquid was collected. Next, 1 mL of pineapple peel liquid was put into a tube containing 9 mL of MRS Broth, which was then incubated for 24 hours at a temperature of 37°C (corresponding to a dilution of 10^{-1}). After this, 1 mL of the 10^{-1} dilution was transferred into another tube containing 9 mL of MRS Broth. This process was repeated until the dilution reached 10^{-6}. The 10^{-6} dilution was then utilized as inoculum using the scatter method on a petri dish containing MRS agar, followed by incubation for 24 hours at 37°C. Following this incubation, the presence of single colonies that were round, smooth, and white was observed. These colonies were then transferred to a new medium for purification using the scratch method. The purified isolates were stored in MRS agar slants and MRS Broth. The identification of bacterial species was carried out through biochemical tests and PCR amplification using 16S rRNA primers. The PCR products were then analyzed by sequencing and compared with sequences in the database to determine the closest match.

Biochemical properties test

The biochemical tests performed refer to the research by (Najah and Manalu 2023) with modifications, which include Gram staining, catalase test, sugar fermentation test, citrate test, Sulfide Indole Motility (SIM) test, and Methyl Red-Voges Proskauer (MR-VP) test. To test the type of fermentation, the bacterial isolate was put into 5 mL of MRS Broth; then, the Durham tube was set upside down. The mixture was incubated for 24 hours at 37°C. The presence of air bubbles in the Durham tube was observed as an indicator of fermentation (Saryono et al. 2023).

Table 1. Pineapple peel fermentation formulation

Formulation 1	Formulation 2	Formulation 3
Pineapple peel 150 g	Pineapple peel 150 g	Pineapple peel 150 g
Brown sugar 50 g	Granulated sugar 50 g	Wild honey 50 mL
Boiled water 500 mL	Boiled water 500 mL	Boiled water 500 mL

DNA extraction

Pure bacterial colonies were cultured in deMan, Rogosa, and Sharpe Broth (MRSB) at 37°C for 24 hours. Genomic DNA was then extracted using the Geneid Kit (Geneaid Biotech Ltd.). A total of 200 µL of bacterial colonies was transferred into a microcentrifuge tube containing 200 µL of Phosphate Buffered Saline (PBS), followed by the addition of 20 µL of Proteinase K. The mixture was homogenized and incubated at 60°C for 5 minutes. Next, 200 µL of GSB Buffer was added, followed by vortexing and further incubation at the same temperature for 2 minutes. Absolute ethanol (96%) was then added, and the mixture was vortexed for 10 seconds. The mixture was transferred into a spin column and centrifuged at 14,000 × g for 1 minute.

The collection tube under the spin column was discarded and replaced with a new one. Subsequently, 400 µL of buffer W1 was added, and the tube was centrifuged for 30 seconds at the same speed. The liquid in the collection tube was discarded. Then, 600 µL of wash buffer was added, and the tube was centrifuged for 30 seconds. The liquid in the collection tube was discarded, and the tube was centrifuged again for 3 minutes. The collection tube was discarded, and a sterile microcentrifuge tube was placed under the spin column. Finally, 100 µL of elution buffer was added, and the tube was allowed to stand for 3 minutes. The mixture was centrifuged at the same speed for 30 seconds. The resulting liquid, containing the DNA, was collected in the microcentrifuge tube and stored at -40°C for use as a PCR template.

Molecular identification using 16S rRNA

The 16S rRNA gene was amplified using the primers 63F: 5'-CAG GCC TAA CAC ATG CAA GTC-3' and 1387R: 5'-GGG CGG WGT GTA CAA GGC-3', with an expected product size of 1,500 base pairs (bp). The amplification process followed this protocol: an initial denaturation at 94°C for 2 minutes, followed by denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, extension at 72°C for 1.5 minutes for 30 cycles, and a final extension at 72°C for 10 minutes. The reaction was then stored at 12°C for 30 minutes. Following amplification, the PCR products were subjected to electrophoresis on a 2% (w/v) agarose gel in 0.5X TBE buffer (100 g of Tris base, 27.5 g boric acid, pH 8, and 20 mL 0.5 M EDTA). The gel was stained with 1 µL of ethidium bromide (0.2 µg/mL) and visualized under UV light. Finally, the amplified 16S rRNA gene from the isolates was analyzed for sequencing.

Sequencing analysis

The PCR results were sequenced by using the Genetic Analyzer (Applied Biosystems), and the resulting data were subsequently analyzed by using the Bio Edit Sequence Alignment Editor software, version 5.0.9. The results were then compared with the 16S rRNA sequence of GenBank data (http://www.ncbi.nlm.nih.gov) using BLASTn (Basic Local Alignment Search Tools for Nucleotide) software by referring to the closest strains. Analysis of the closest kinship was done based on phylogenetic trees using MEGA 11.0.13 with 1000 bootstrap repetitions. Phylogenetic analysis shows the closeness of evolutionary relationships between sequenced samples and specific strains accessible in the GenBank database.

Antibacterial activity test

The antibacterial test was conducted using the disc diffusion method with four repetitions, following the procedure outlined by Saryono et al. (2023) with some modifications. Initially, bacterial isolates were obtained and dissolved in 5 mL of sterile distilled water. Paper discs were then immersed in the bacterial isolate solution for 15 minutes. A second paper disc was immersed in 5 mL of sterile distilled water as a control. Pathogenic bacteria, *Escherichia coli,* and *Shigella dysenteriae* were each prepared by dissolving 3-4 colonies in 5 mL of sterile distilled water and homogenizing the solution. Next, 0.1 mL of the pathogenic bacteria solution was inoculated onto the surface of MHA media using the spread plate method, and the plates were left undisturbed for 5 minutes. The paper discs that had been soaked with bacterial isolates from pineapple peel fermentation were then placed on the surface of the media and incubated for 24 hours at 37°C. Ciprofloxacin, a well-established antibacterial agent, was used as a positive control, and the presence of an inhibition zone around the discs indicated the potential of the isolates to produce antibacterial compounds.

RESULTS AND DISCUSSION

Fermentation of pineapple peel

The microbial communities involved in pineapple peel fermentation have been the subject of extensive investigation. Recent studies exhibited that the fermentation process involved a diverse range of bacteria and fungi, contributing to the unique flavor profile of fermented pineapple peel (Omorotionmwan et al. 2019; Luo et al. 2023, 2024; Sukirah et al. 2023). Prior research has identified technical parameters, including temperature, pH, and fermentation time, that must be monitored and controlled during the fermentation process (Mengesha et al. 2022). By regulating these parameters, the fermentation process can be better managed to create an optimal environment for the growth of probiotic bacteria and can also help prevent spoilage (Rahayu et al. 2021). This study examined the spontaneous fermentation of pineapple peel without the addition of a starter. The findings confirmed the significance of setting the fermentation time, which enabled the growth of probiotic bacteria, particularly the genus *Lactobacillus.* As elucidated by Kim et al. (2020), lactic acid bacteria were present at the outset of fermentation (24 hours), yeast was present after 48 hours, and acetic acid bacteria were present after 72 hours of fermentation. These distinct stages of microbial presence during fermentation play a crucial role in the development of flavor and texture in the final product (Wang et al. 2016)

The results of pineapple peel fermentation on day 3 demonstrated that the surface of the solution exhibited foam or small bubbles (Figure 1). This denotes that the

fermentation process has been successfully completed. In addition, the pH decreased from pH 5 (on day 0 or when fermentation started) to pH 3 (on day 3). The reduced pH indicates the production of organic acids such as lactic acid and acetic acid during the anaerobic fermentation metabolism of microorganisms. This result aligns with the findings of a prior study, demonstrating the pH reduction of the pineapple peel fermentation reaching 3.3 after 72 hours of fermentation (Gutiérrez-Sarmiento et al. 2022). This acidic environment is essential for killing harmful bacteria and encouraging beneficial microorganisms to grow (Arcari et al. 2020). Furthermore, the declined pH and the presence of bubbles or gas in fermented beverages (Zhao et al. 2020) have been identified as indicative of successful fermentation. Additionally, changes in aroma and flavor profiles during fermentation can also serve as markers of successful fermentation (Zang et al. 2020).

The organoleptic test also revealed a thicker liquid consistency and a pleasant acidic aroma typical of fermentation rather than a foul odor and sour taste. Acetic acid compounds yielded by the fermentation products contributed to the strong and intense sour aroma. In addition, the activity of ethyl acetate compounds produced a pleasant sour aroma, like the smell of fruit with a sweet touch (Li et al. 2022). Overall, the combination of acetic acid and ethyl acetate compounds resulted in a well-balanced and appealing aroma profile for the fermented product. Nevertheless, prolonged fermentation of pineapple peel is not recommended as it may result in an unpleasant flavor (Gutiérrez-Sarmiento et al. 2022). Besides, it can reduce product quality and nutritional value and may cause the formation of harmful compounds (Peralta et al. 2008).

Some other volatile compounds, such as esters, aldehydes, ketones, and lactones, can also be present during the fermentation process to provide unique characteristics, especially by enriching the flavor and aroma profile of the fermented product (Bortoleto and Gomes 2022). However, excess acid or alcohol can give a very sharp and unpleasant taste or odor, thereby reducing the organoleptic quality of the fermented product. Unpleasant flavors in fermented products are often caused by microbial contamination and uncontrolled fermentation conditions (Chen et al. 2024). It's important to consider the makeup of the ingredients, temperature, pH level, fermentation time, and choice of starter culture to avoid creating unwanted flavors or spoiling the taste of the product (Tian et al. 2023; Casas-Rodríguez et al. 2024).

Pineapple skin fermentation has been widely recognized as a traditional Mexican beverage known as tepache. Studies show that tepache has bacteria like *Lactobacillus casei*, *Lactiplantibacillus pentosus*, and *Lacticaseibacillus paracasei*. These bacteria can boost the immune system, help the body fight infections, support gut health by keeping harmful bacteria in check, and help balance the microbiome (Ligenza et al. 2021). In addition, tepache is rich in peptides and organic acids, which have the potential to lower blood pressure, normalize blood sugar levels, and have antioxidant and anti-inflammatory properties (Hartini et al. 2024).

Macroscopic and microscopic morphology of bacteria isolated from pineapple peel fermentation

The bacterial density isolated from the pineapple peel fermentation was 127 × 10^6 CFU/mL for formulation 1.267 × 10^6 CFU/mL for formulation 2, and >300 CFU/mL for formulation 3 (Figure 2).

Figure 1. A. Pineapple (*Ananas comosus* L. Mer); B. Fermentation product

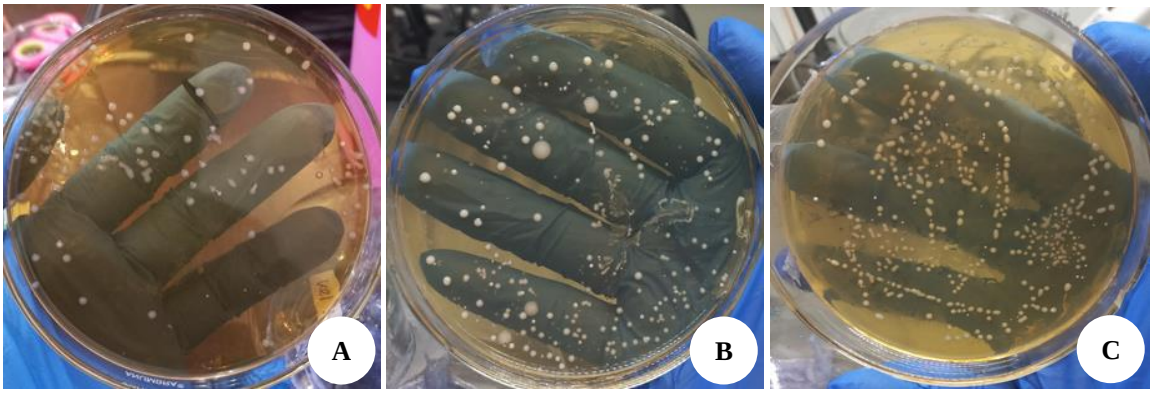

Figure 2. Bacterial isolates isolated from pineapple peel fermentation. A. Formulation 1; B. Formulation 2; C. Formulation 3

The colonies that grew on MRS agar for formulation 1 consisted of two types of colonies; for formulation 2, there were five types of colonies, and for formulation 3, there were two types of colonies. Overall, there were five types of colonies with distinct characteristics, which were subsequently labeled as N1, N2, N3, N4, and N5. The cultural characteristics (colony) of the isolated strains are described and presented in Table 2. N1, N2, and N4 were circular, while N3 and N5 were irregular. The size of the isolated samples varied as small, medium, and large. The isolates exhibited colony colors such as white and translucent white. The isolates showed various types of elevation, including convex, flat, raised, and umbonate. The surface of these isolates was smooth, moist, glossy, and oily. Similar results were reported by Kowser et al. (2015), who found that the colonies were small, medium, and large, white or pale white, circular and irregular in shape, with convex, flat, umbonate surfaces and smooth or wrinkled edges.

Of the five types of colonies, only the N2 isolate showed bacterial cells during Gram staining. The N4 isolate did not exhibit growth during reculturing, while the other three colonies displayed an oval shape resembling fungal cells. According to a study by Zhao and Yun (2016), acetic acid bacteria colonies are typically round, convex, or raised with smooth edges and white to cream in color. Biochemical tests were then performed only on the colonies that exhibited bacterial characteristics, and the results are shown in Table 3.

The morphological characteristics and staining of isolate N2 are presented in Figure 3. The isolate showed gram-negative staining with a pink color, short rod-shaped, with a single and paired cell arrangement. Similar results were also obtained by (Kowser et al. 2015) and (Zhao and Yun 2016), who found that acetic acid bacteria are gram-negative rods morphologically.

Isolate N2 ferments glucose without producing gas, showing positive results for the catalase test, methyl red test, and motility, as well as negative results for VP and indole tests. Isolate N2 from formulations 1, 2, and 3 tested indicates that it is an acetic acid bacterium. One of the acetic acid bacteria identified in the study by (Alisigwe et al. 2022) exhibited similar characteristics, with rod-shaped bacterial cells, Gram-negative, catalase-positive, non-gas producing, non-H_2S producing, and indole-negative. Similar findings were also reported by (Kowser et al. 2015), where acetic acid bacterial isolates were capable of fermenting glucose and showed positive results for methyl red and negative results for Voges-Proskauer.

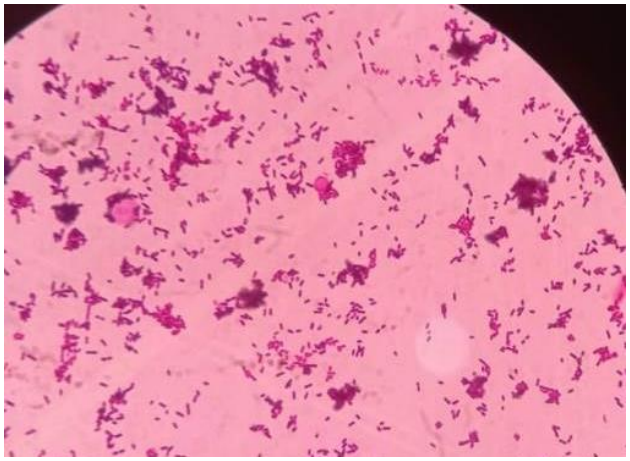

Figure 3. Gram staining results of isolate N2

Table 2. Macroscopic identification of acetic acid bacteria isolates

Isolate	Colony morphology				
	Color	Shape	Size	Margin	Elevation
N1	White	Circular	Large	Entire	Flat
N2	White	Circular	Medium	Entire	Convex
N3	White (transparent)	Irregular	Small	Undulate	Flat
N4	White	Circular	Small	Entire	Flat
N5	White	Irregular	Large	Undulate	Umbonate

Table 3. Results of biochemical tests

Formulation	Gram staining	Biochemical test results						
		Catalase	TSIA test	Citrate test	SIM test	MR test	VP test	Fermentation type
Formulation 1	Gram-negative, rod-shaped	+	Acid; no gas	-	H_2S: - Indole: - Motility:+	+	-	Homofermentative
Formulation 2	Gram-negative, rod-shaped	+	Acid; no gas	-	H_2S: - Indole: - Motility: +	+	-	Homofermentative
Formulation 3	Gram-negative, rod-shaped	+	Acid; no gas	-	H_2S: - Indole: - Motility: +	+	-	Homofermentative

Notes: (+): positive; (-): negative

According to the study by Safari et al. (2019), there is a simple method to distinguish between lactic acid bacteria from the genus *Lactobacillus* and acetic acid bacteria from the genus *Acetobacter*, which is through Gram staining and catalase testing. *Lactobacillus* bacteria are generally rod-shaped, Gram-positive, and do not contain the catalase enzyme. In contrast, *Acetobacter* has short rod-shaped cells, is Gram-negative, and contains the catalase enzyme, which allows these bacteria to break down hydrogen peroxide. The N2 isolate also showed a homofermentative fermentation type. According to Kouamé (2015), acetic acid bacteria, especially from the genus *Acetobacter*, are homofermentative. This characteristic is shown by the absence of bubbles in the Durham tube.

16S rRNA gene sequencing for the molecular identification of isolates

As shown in Figure 4, the amplification products of the 16S rRNA gene sequencing were confirmed by the appearance of a PCR product fragment with a size of 1500 bp. Using the primer combination of 63F and 1387R, the sizes of the products obtained were as expected. Based on sequencing results with Bioedit Sequence Alignment Editor software (version 7.0.5.1), the N2 isolate obtained from fermented pineapple peel was identified as the acetic acid bacterial strain *Acetobacter fabarum* 2567, with a similarity percentage of 93% and an access number of MT611597.1 in GenBank. The sequencing results confirmed the presence of this particular strain in the fermented pineapple peel sample.

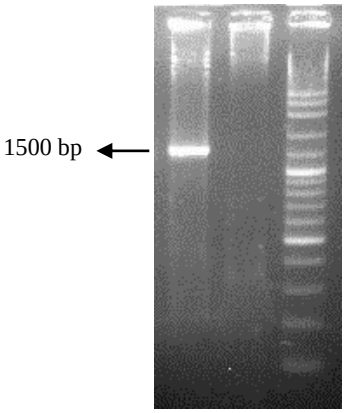

Figure 4. Amplification products of the 16S rRNA gene with primers of 63F and 1387R

16S rRNA gene sequence analysis

Acetobacter fabarum 2567, identified in pineapple peel fermentation samples in this study, was aligned and analyzed in a single phylogenetic tree with other acetic acid bacteria. A phylogenetic analysis was conducted using MEGA v11.0.13 software with the neighbour-joining method. With a bootstrap support value of 95, the phylogenetic analysis (Figure 5) shows that *A. fabarum* 2567 was very similar to *A. fabarum* R-36330. It suggests that these two strains are highly similar. Other *A. fabarum* strains closely related to this strain were *A. fabarum* LMG 24244 and *A. fabarum* DH1801. These strains collectively form a single cluster with a bootstrap support value of 89. It denotes that all *A. fabarum* strains are in the same kinship group. *A. fabarum* 2567 is on a separate branch from other *Acetobacter* species, such as *Acetobacter lovaniensis, Acetobacter ghanensis, Acetobacter syzygii,* and so on. The strains are distributed across different clusters with varying bootstrap support values. This suggests that *A. fabarum* strains have a distinct genetic relationship compared to other *Acetobacter* species.

Acetobacter is a genus of Gram-negative bacteria with a coccoid rod shape, 0.8 μm wide and 1.2-3.0 μm long. These bacteria are found in fermentation processes that result in products such as vinegar (Chen 2021). *Acetobacter* is known for its ability to oxidize ethanol into acetic acid, which is a critical component in vinegar production (Gomes et al. 2018). *A. fabarum* has been isolated from kefir products (Kim et al. 2020) and tin, mulberry, apple, and plum fruit vinegar (Sengun et al. 2022), which contribute to the flavor and aroma of fruit vinegar (Zhang et al. 2023) and may also play a role in the fermentation process. Furthermore, *A. fabarum* has been demonstrated to possess probiotic characteristics, indicating its potential for utilization in the food industry to enhance flavor profiles and gut health (Kim et al. 2020; El-Askri et al. 2022). *A. fabarum* is an acetic acid-producing bacterium first identified in 2008. It was isolated from fermented cocoa bean pods in Ghana (Cleenwerck et al. 2008). This bacterium is known for its ability to yield acetic acid, which contributes to the unique flavor profile of fermented foods (Tovar et al. 2020).

Antibacterial activity analysis

In this study, the isolate N2, which was confirmed as *A. fabarum*, did not show antibacterial activity at concentrations of 5%, 10%, 20%, 30%, 40%, 50%, and 75% against *E. coli* and *S. dysenteriae*. These results are shown in Figure 6. The results of this study do not meet expectations, especially when compared to previous research (Kim et al. 2020) which showed that *A. fabarum* strain DH1801, isolated from Korean kefir, exhibited antibacterial activity against seven foodborne pathogens, namely *B. cereus, S. aureus, L. monocytogenes, C. sakazakii, S. enteritidis, E. coli enterotoxigenic,* and *S. flexneri*. Other species from the genus *Acetobacter*, such as *A. pasteurianus*, have also been reported to show antibacterial activity against non-pathogenic *E. coli* and *S. mutans* (Safari et al. 2019). Additionally, two other acetic acid bacteria, *A. indonesiensis* and *A. syzygii*, isolated from 27 Iranian traditional yogurts and curd, demonstrated antibacterial activity against various pathogens, including *E. coli (0157), S. typhimurium, S. aureus, B. cereus, L. monocytogenes, K. pneumoniae, S. flexneri, P. aeruginosa, C. albicans, S. marcescens, E. faecalis, S. saprophyticus,* and *S. mutans* (Haghshenas et al. 2015).

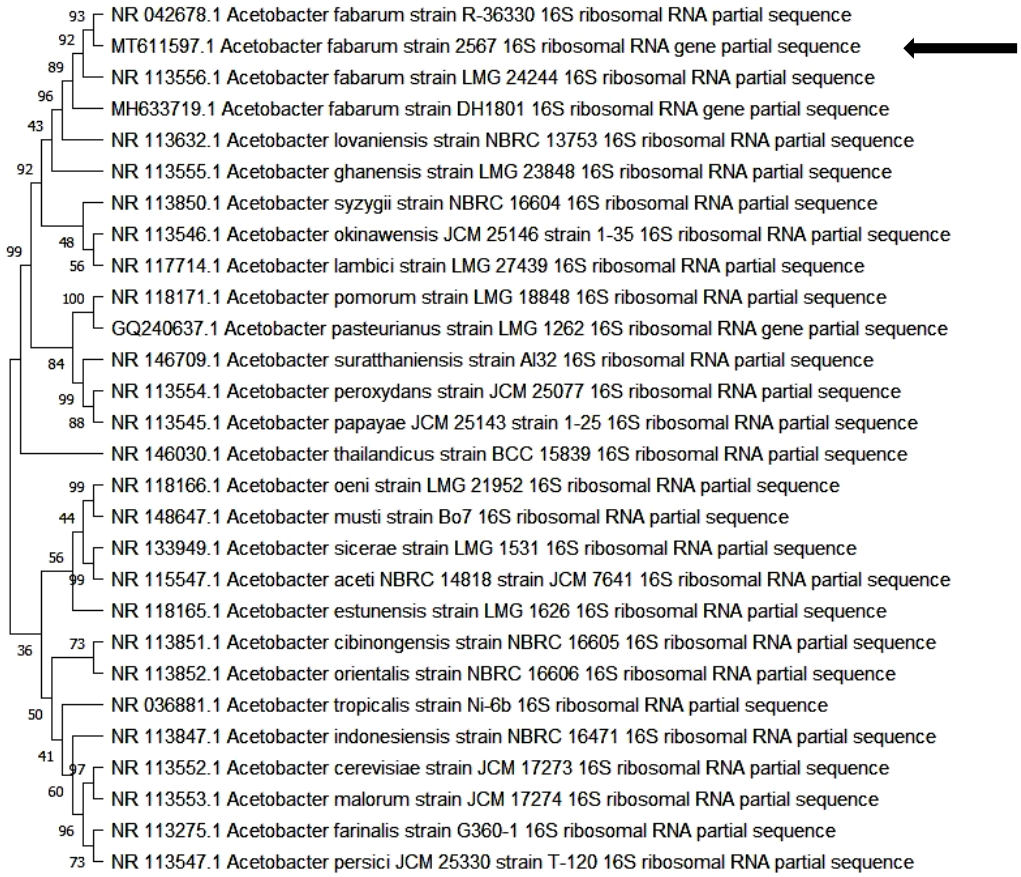

Figure 5. The phylogenetic relationship of *A. fabarum* 2567 based on the 16S rRNA gene sequence. The phylogenetic tree was analyzed with MEGA software, version 11.0.13, using the neighbor-joining method

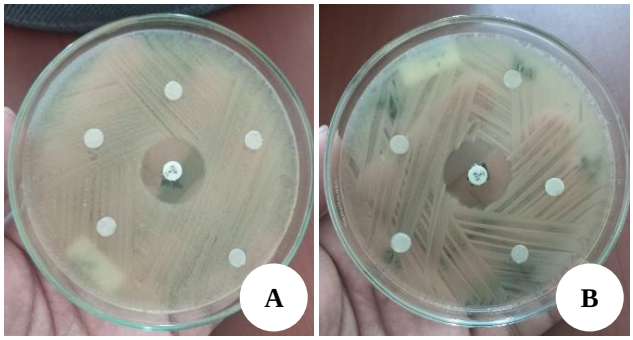

Figure 6. Results of antibacterial activity test of isolate N2 against A. *S. dysenteriae* and; B. *E. coli*

These findings suggest that *A. fabarum* DH1801 has the potential to be used as a natural antimicrobial agent in food preservation and safety. In addition, this study revealed that the bactericidal activity of *A. fabarum* DH1801 may be due to the production of organic acids and bacteriocins. Acetic acid bacteria have been utilized in the production of numerous antimicrobial agents and have the remarkable ability to synthesize various organic acids, such as acetic acid, gluconic acid, and ascorbic acid precursors, through oxidative fermentation (Hata et al. 2023). Research has exposed the antimicrobial potential of AAB strains, including *A. cerevisiae* and *A. pasteurianus*, which have been shown to exhibit strong antibacterial activity against pathogens such as *S. aureus* (Kim et al. 2023). Another recent study has reported that *A. tropicalis* produced bacteriocin against *B. cereus* (Nagarathinam and Sundharam 2017).

The results of studies on *Drosophila melanogaster* Meigen 1830 demonstrated that *A. fabarum* had the potential to extend the lifespan of mice by influencing the aging pathways, including the glucagon signaling pathway and cysteine and methionine metabolism. This discovery, while promising, underscores the need for further research to fully understand the mechanisms underlying these effects and their potential implications for human health. Methionine, an essential amino acid for humans, plays a crucial role in this process. The body cannot produce it, and it must be obtained from food. Its functions include a role as a precursor for cysteine synthesis. Cysteine, made from methionine, serves as a precursor to glutathione, an antioxidant compound that plays a crucial role in protecting cells from potential damage caused by oxidative stress (Matthews et al. 2020).

The study indicates that by modulating these pathways, *A. fabarum* may have the potential to enhance overall health and longevity in mammals. Still, further research is needed to fully understand the mechanisms underlying these effects and their potential implications for human health. Further, it has been demonstrated that fermented

products containing *A. fabarum* can also suppress the expression of beta-amyloid-related proteins, including BACE (Beta-secretase), γ-secretase, and APP (Amyloid Precursor Protein) enzymes, which are regarded as a primary cause of Alzheimer's disease (Tyliszczak et al. 2023). The discoveries suggest that *A. fabarum* strains have the potential to be new starters, natural food preservatives, and probiotic agents.

The spontaneous fermentation process is often not well controlled, so it does not always extract all the active compounds to the maximum. Alternatively, further research could try using a controlled fermentation process by adding starter cultures such as *L. plantarum*, *L. rhamnosus* and *A. oryzae*. Studies show that the addition of starter cultures to pineapple skin fermentation can increase the release of phenolic compounds. This compound is associated with increased antioxidant and anti-inflammatory activity (Ortega-Hernández et al. 2023). The use of *A. niger* in the fermentation process can release tannin compounds that can be hydrolyzed and condensed, which have antioxidant properties (Casas-Rodríguez et al. 2024). In addition, fermentation with *A. niger* can also increase protein content and improve the nutritional value of pineapple skin (Victor et al. 2016).

Although the antibacterial activity of *A. fabarum* has not been widely reported, it is hoped that future research will conduct antibacterial activity testing using the micro-dilution method in broth or the good diffusion method. The micro-dilution method measures bacterial growth inhibition by examining the Optical Density (OD) of bacterial cultures. This OD value can be determined using a spectrophotometer. A decrease in OD value after antibacterial treatment indicates a higher antimicrobial activity of the test filtrate (Safari et al. 2019; Kim et al. 2020).

In conclusion, the acetic acid bacteria isolated from pineapple peel fermentation are rod-shaped, Gram-negative, catalase-positive, and homofermentative. Phylogenetic analysis, based on 16S rRNA sequencing, showed that the bacterial isolate was identified as *A. fabarum* 2567. Although *A. fabarum* strain isolated from pineapple peel fermentation does not show antibacterial activity, the presence of *A. fabarum* is still valuable in vinegar production. The antibacterial potential of pineapple skin can be utilized in other ways, such as the use of starter cultures to make the fermentation process more controlled and to test antibacterial activity; other methods can be used, such as the micro-dilution method in broth or the well-diffusion method.

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