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Original Research Article

Isolation and Identification of Indigenous Lactic Acid Bacteria from Fermented Cabbage (*Brassica oleracea L.*) with Probiotic Potential

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ABSTRACT

Cabbage (*Brassica oleracea L.*) is a widely consumed leafy vegetable with high nutritional value. Cabbage is often processed into fermented foods. Lactic acid bacteria (LAB) grow naturally on fermented cabbage, and are generally recognized as safe (GRAS) microorganisms for consumption, and have probiotic potential. This study aimed to isolate and identify indigenous LAB from fermented cabbage which have probiotic potential. Cabbage was fermented in 3% sodium chloride solution at 37°C for 9 days. Selective isolation of LAB from the fermented cabbage was performed using de Man Rogosa Sharpe (MRS) Agar. The morphological identification of LAB isolates was done macroscopically, microscopically, and biochemically. Antibacterial activity evaluation against pathogenic bacteria was performed using the agar well diffusion method. The resistance of LAB isolates to acidic conditions and bile salt were also tested. Molecular identification was done using the Polymerase Chain Reaction (PCR) technique. A pair of primers 27F and 1492R were used to amplify the 16S rRNA gene and then sequenced. The sequencing results were aligned using the BLAST program. Five LAB isolates (KF 1 – KF5) were isolated and identified from fermented cabbage. The antibacterial activity test results showed that isolate KF4 had the highest antibacterial activity against *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, and *Staphylococcus aureus*. KF4 was able to grow at pH 2, and was tolerant to bile salt. BLAST analysis results showed that isolate KF4 has 99.24% similarity to *Lactiplantibacillus plantarum*. The findings from this study highlight the probiotic potential of the lactic acid bacteria *Lactiplantibacillus plantarum*, that warrant further investigation.

Keywords: Lactic acid bacteria, Isolation, Probiotics, *Lactiplantibacillus plantarum.*

Introduction

Cabbage is a widely consumed vegetable due to its delicious taste, nutritional value, and mineral and vitamin content. Cabbage is susceptible to spoilage due to its high-water content (92.44%). This spoilage occurs due to bacterial activity that breaks down food substances, producing organic acids, such as lactic acid. The presence of lactic acid indicates the presence of lactic acid-producing bacteria naturally in cabbage. *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, and *Lactobacillus brevis* are some of the bacteria that produce organic acids during cabbage fermentation process. These lactic acid bacteria (LAB) convert the nutrients in cabbage into lactic acid. Lactic acid bacteria (LAB) are known to produce organic acids, peptides, hydrogen peroxide, diacetyl, and other antimicrobial substances that inhibit the growth of harmful microbes.[1,2] LAB can alter immune responses and produce antibacterial and antitumor chemicals as they proliferate. Due to their ability to produce indoleacetic acid (IAA), siderophores, and antagonistic properties against harmful bacteria, LAB are very important in agriculture.[3] LAB, also known as food-grade or generally recognized as safe (GRAS) microorganisms, are safe for consumption because they do not produce toxins that are harmful to human health, hence they have probiotic potential.

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When consumed in sufficient quantities, probiotics have a positive impact on health.[4] LAB are microorganisms capable of rapidly producing acid, inhibiting spoilage microorganisms, and ensuring food safety.[5] In addition, these microorganisms are also known for their probiotic effects, such as increasing nutrient absorption,[6] boosting immunity,[7] and maintaining the balance of intestinal flora.[8]
Probiotics are food from microbial cells or microbial cell components that have health benefits such as lowering blood cholesterol, increasing intestinal motility and detoxification, stimulating the immune system, producing various metabolites, and metabolizing vitamins, minerals, and hormones.[9] Lactic acid bacteria (LAB) are the main source of probiotics used in the food industry for their ability to ferment and preserve food. Probiotic lactic acid bacteria are used as functional food additives and offer several health benefits, including modulation of the gut microbiota, immunomodulation, and antimicrobial effects.[10] LAB qualities as probiotics include survival in the gastrointestinal tract, tolerance to low pH and bile salt, and a safety profile.[11]
Fermented cabbage is a good source of LAB with probiotic potential, but research on the isolation and identification of probiotic lactic acid bacteria from fermented cabbage has not been conducted. Therefore, this study aims to isolate and identify probiotic lactic acid bacteria from fermented cabbage with a view to identify new lactic acid bacteria species with potential applications in the production of functional foods and biopreservatives.

Materials and Methods

Chemicals and Equipment
Nutrient broth (Merck, Germany), deMan Rogosa Sharpe/MRS (Merck Millipore, Germany), hydrogen peroxide (H_2O_2) 3% (v/v) (Merck, Germany), hydrochloric acid (Asahimas Chemical, Indonesia), ethanol (Molindo Group, Indonesia), sodium chloride (UniChemCandi, Indonesia), ethylenediaminetetraacetic acid (EDTA) (Chemtex

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Speciality Ltd, India), Gram stain (Medic Diagnostic Reagents, Philippines), agarose (Merck,Germany), and DNA ladder (BD Biosciences, USA). The equipment used include UV-Vis spectrophotometer (Agilent Cary 60, USA) and a centrifuge (Biobase BKC-TL16RE, India).

Collection and identification of plant material

Cabbage (*Brassica oleracea*) were purchased from the Angso Duo primary market, Jambi City, Indonesia (1°36'00" S; 103°36'00" E) in June, 2024. The cabbage used were of good quality and fresh at the time of collection, and no further identification was made.

Cabbage fermentation

The cabbage was cleaned, and cut into small pieces. Sodium chloride solution (3%) was added, stirred thoroughly, placed in a tightly closed jar and incubated at 37°C for 9 days.[12]

Isolation and identification of lactic acid bacteria

The fermentation liquid (1 mL) was transferred into de Man Rogosa Sharpe (MRS) Agar + 0.5% $CaCO_3$ medium (Scharlab, Spain), then incubated at 37.5°C for 48 hours under anaerobic conditions (Memmert Universal Laboratory Incubator IN30). Bacterial colonies that formed a clear zone around the colonies were streaked on MRS agar to obtain a uniform (pure) isolate. The pure isolate obtained was characterized by antimicrobial sensitivity testing using the disc diffusion method, determination of colony morphology using optical and scanning microscopes to observe colony shape, colour, and consistency, and determination of cell morphology, such as cocci and bacilli shapes, to differentiate bacterial species.[13]

Catalase test was performed by placing 1-2 drops of hydrogen peroxide (H_2O_2) solution (3%) on a glass slide, then the glass slide was smeared with the bacterial isolate with the aid of an inoculating loop, and observed for the presence or absence of bubbles. The formation of bubbles is a positive test for catalase. Morphological characterization of LAB including macroscopic and microscopic characteristics were carried out. Macroscopic morphological characterization was carried out by direct observation of LAB colonies including colony size, colour, and shape. Microscopic morphological characterization was done by observing the Gram type in the form of the shape and colour of LAB cells using Gram staining. Gram-positive and Gram-negative bacteria were distinguished by observing the colour of the bacterial cells under a microscope.[14]

Antibacterial activity testing

The Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028, as well as the Gram-positive bacteria *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* ATCC 25923, were obtained from the Inter-University Center for Microbiology Laboratory, Institute of Agriculture, Bogor University, Indonesia. Antibacterial activity was evaluated using the agar well diffusion method.[15] MRS broth was mixed with 5% lactic acid bacteria inoculum, with a cell population of 10^8 CFU/mL. The mixture was incubated at 37°C for 20 h. To remove the cell-free supernatant, the mixture was centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was filtered through a millipore filter with a pore size of 0.20 µL.

The test organisms were cultured on a Nutrient Agar at 37°C for 24 h. The working culture was then divided into two loops and cultured in tubes filled with nutrient broth (NB). The turbidity of the test bacterial cultures was assessed by turbidimetry using a UV-Vis spectrophotometer at a wavelength of 600 nm until an optical density (OD) of 0.5 - 0.8 was attained following a 24-hour incubation period. The NB containing the test bacteria was then combined with the sterile nutrient agar at a temperature of approximately 50ºC at a ratio of 20 µL NB of test bacteria to 20 mL sterile Mueller Hinton (MH) agar. The medium was then transferred into petri dishes, and allowed to solidify, after which 50 µL of cell-free supernatant was added to each dish using a sterile pipette tip with a 6 mm diameter. The dishes were then incubated at 37°C for 24 h. The diameter of the transparent area was used to assess the inhibition zones that developed around the wells. An average of multiple measurements at different locations was recorded.

Acid resistance test

The acid resistance of lactic acid bacteria isolates was evaluated according to the method previously describe by Klose *et al*. (2010).[16] Briefly, 9 mL of MRS broth was inoculated with 1 mL of bacterial culture, the mixture was incubated at 37°C for 24 h.

After incubation, 1 mL of the culture was added to the control MRS broth, and the MRS broth was adjusted to pH 2.5 and pH 2 using 5 N HCl. The broth was then vortexed and incubated at 37°C for 3 h. After serial dilutions were prepared, 1 mL of the broth was transferred into petri plates using the pour plate method. The plates were incubated at 37°C for 48 h. Colony-forming units (CFU) were used to assess the number of colonies.

Bile salt resistance test

The bile salt resistance test was performed according to standard procedure.[17] The test employed a bile salt concentration of 0.3%, and the final result was obtained using the plate count technique. Ten millilitres of MRS broth (control) and MRS broth with 0.5% (w/v) bile salts were mixed with 1 mL of a culture of LAB isolates in MRS broth that had been aged for twenty-two hours. The mixture was vortexed, serially diluted, and incubated at 37°C for 3 h before being transferred into petri dishes using the pour plate technique. Colony-forming units (cfu) were used to quantify the number of colonies formed.

DNA isolation

DNA isolation was performed following the procedure outlined in the instruction manual included in the Promega Corporation DNA isolation kit.[18] LAB culture (1 µL) in MRSB media was incubated at 37°C for 24 h, then placed into a 1.5 µL microcentrifuge tube and centrifuged at 13,000–16,000 rpm for 2 min. The pellets were collected and suspended in 480 µL of 50 mM EDTA. Lysogenic enzymes (lysozymes) were added to the suspended cell pellets at a total volume of 120 µL. Thereafter, the cell suspension was slowly pipetted and mixed. The samples were incubated at 37°C for 30–60 min. After incubation, the samples were centrifuged at 13,000–16,000 rpm for 2 min. The supernatant was discarded and the pellets were mixed with nuclei lysis solution, and pipetted slowly to obtain a cell suspension. The suspended cells were incubated at 80°C for 5 min to lyse the cells, then cooled to room temperature. A total of 3 µL of RNAse was added to the lysed cells and shaken 2 to 5 times to mix thoroughly, followed by incubation for 15–60 minutes at 37°C, then cooled to room temperature. To the cells that had been supplemented with RNAse, 200 µL of protein precipitation solution was added, then vortexed at high speed for 20 seconds to mix the protein precipitation solution with the lysed cells. The cells were centrifuged at 13,000–16,000 rpm for 3 min. The supernatant was transferred to a clean microcentrifuge tube containing isopropanol, then gently shaken until the DNA strands were visible. The supernatant was centrifuged again at 13,000–16,000 rpm for 2 minutes. The supernatant was decanted, and the tube was carefully dried with absorbent paper. A total of 600 µL of room temperature ethanol was added, then the tube was inverted several times to wash the pellet. The supernatant was centrifuged at 13,000–16,000 rpm for 24 min. The supernatant was decanted and the tube was dried with absorbent paper. The pellet was allowed to dry for 10–15 min. DNA rehydration solution (100 µL) was added to the tube and incubated at 4°C overnight. The isolated DNA was stored in the refrigerator at 2–8°C.

16S rRNA gene amplification

DNA amplification reaction was carried out in a Mupid-Exu Thermocycler using primer 27F AGAGTTTGATCCTGGCTCAG for the forward direction and primer 1492R GGTTACCTTGTTACGACTT for the reverse direction.[19] A total of 12.5 µL GoTaq Green Master Mix, 1 µL primer 27F, 1 µL primer 1492R, 1 µL isolated DNA, and 9.5 µL Nuclease Free Water were mixed in a microtube (Promega Corporation, 2014).[18] Polymerase chain reaction (PCR) was carried out for 30 cycles at pre-denaturation conditions of 94°C for 90 seconds, denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C.

Electrophoresis of PCR products

DNA Loading Dye (2 µL) (Thermo Scientific) was added to 10 µL of

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PCR product. Electrophoresis was performed at 100 V for 25 minutes using a 1% agarose gel in 1x Tris Acetate EDTA (TAE), with DNA Ladder as a reference. The electrophoresis results were visualized using ultraviolet light after adding ethidium bromide.[19]

Sequencing (DNA sequence determination)
Sequencing of the 16S rRNA coding DNA was carried out by 1st Base through the Eijkman Institute in two directions, namely the forward direction using primer 27F and the reverse direction using primer 1492R.

Data analysis
Data were described descriptively for the morphological characteristics and biochemical test results. The results of the forward and reverse primer sequencing were arranged using BioEdit software, compiled, and analyzed in the NCBI BLAST basic data program. Sequence similarity was determined by the percentage of identity obtained from BLAST analysis. Genetic distances and phylogenetic trees were constructed using MEGA 11.0.13 software.

Results and Discussion

Isolation and characterization of lactic acid bacteria isolates
Lactic acid bacteria (LAB) was successfully isolated and purified from fermented cabbage on MRS Agar + 0.5% $CaCO_3$ medium. Isolates that produced clear zones around the colonies indicated acid production by those isolates. The clear zones were formed due to the $CaCO_3$ in the MRS agar medium which reacts with the lactic acid produced by the LAB isolates, resulting in the formation of calcium lactate, which appears as clear zones surrounding the LAB colonies. Lactic acid reacts with calcium to form calcium lactate, making the medium appear more transparent.[13] The results of the purification of five isolates that produced clear zones on MRS agar + 0.5% $CaCO_3$ medium are shown in Figure 1.
Before testing the five lactic acid bacteria isolates, a dilution of 10^{-6} was performed. The results of Gram staining, catalase, colony size and shape, and cell shape are shown in Table 1.

Table 1: Morphological characteristics of colony and cell shape of lactic acid bacteria (LAB)

LAB Isolate	Gram	Catalase	Diameter (mm)	Colony Shape	Cell Shape
KF1	(+)	(-)	1.4	Medium round, milky white, convex	Short rod
KF2	(+)	(-)	0.5	Small round, cream/light brown, convex	Short rod
KF3	(+)	(-)	1.4	Medium round, milky white, convex	Short rod
KF4	(+)	(-)	0.4	Small round, milky white, convex	Short rod
KF5	(+)	(-)	3.3	Large round, milky white, convex	Short rod

From Table 1, it was observed that five isolates of lactic acid bacteria (LAB) tested with Gram staining showed Gram-positive (+), catalase-negative (-), colony diameter ranging from 0.4 mm to 3.3 mm, small round to large round shapes, and short rod cell shapes. Gram staining of bacteria is divided into two categories: Gram-negative bacteria, which appeared pink, and Gram-positive bacteria, which appeared purple.[20] The difference in colour absorption is due to differences in peptidoglycan and membrane permeability between Gram-positive and Gram-negative organisms.

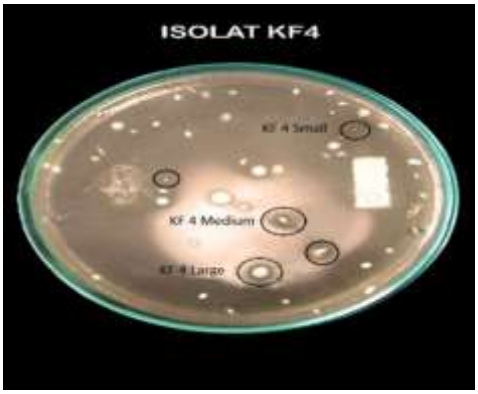

Figure 1: Lactic acid bacteria isolates forming clear zones

The catalase test aims to ascertain if LAB isolates contain the catalase enzyme. The catalase enzyme breaks down hydrogen peroxide (H_2O_2) into H_2O and O_2. Hydrogen peroxide is toxic to microbes because it inhibits enzyme activity within the cell. Lactic acid bacteria do not have the catalase enzyme needed to break down peroxide (H_2O_2).[14] Peroxide in LAB is one of the metabolites that functions as an antimicrobial agent. All isolates from this study did not form O_2 gas bubbles after H_2O_2 was added, indicating that catalase was absent. This result aligns with the findings from previous study which reported catalase-negative results in LAB isolates isolated from mango and orange peel.[14,21] The shape of LAB isolates observed under Gram staining is shown in Figure 2.

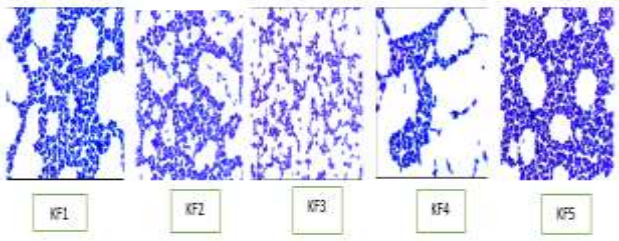

Figure 2: Photomicrograph of Gram staining of lactic acid bacteria isolates from cabbage fermentation

Antibacterial activity of lactic acid bacteria isolates
The ability of lactic acid bacteria to inhibit the growth of test bacteria like *B. cereus* ATCC 10876, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *S. typhimurium* ATCC 14028 is evidence of their antimicrobial activity. The clear zone of inhibition formed on Nutrient Agar media was used to assess antibacterial activity. Table 2 shows the diameter of the clear zone formed in each LAB isolate.

Table 2: Inhibition zone diameter of lactic acid bacteria isolates against test bacteria

LAB Isolate	Test Bacteria			
	***B. cereus* ATCC 10876**	***S. aureus* ATCC 25923**	***E. coli* ATCC 25922**	***S. tiphymurium* ATCC 14028**
KF1	0.83	4.22	5.49	5.05
KF2	3.94	6.07	4.79	6.47
KF3	4.84	7.14	7.31	7.70
KF4*	5.83	7.95	10.61	11.18
KF5	5.00	5.61	8.25	5.29

Note: * = Selected isolate for characterization

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The results as presented in Table 2 show that all LAB isolates exhibited antimicrobial activity against the test bacteria, forming clear zones of inhibition. Isolate KF4 exhibited antibacterial activity against *B. cereus*, *S. aureus*, *E. coli*, and *S. typhimurium*, with zones of inhibition of 5.83 mm, 7.95 mm, 10.61 mm, and 11.18 mm, respectively. These results were higher than those of isolates KF1, KF2, KF3, and KF5.

Bacterial fermentation of carbohydrates produces various compounds, primarily alcohols, organic acids (such as lactic acid, acetic acid, and propionic acid), and gases (such as carbon dioxide) that have antimicrobial effects. These compounds can vary depending on the type of bacteria and the carbohydrate being fermented, as well as the environmental conditions under which fermentation occurs.[22] Lactic acid, a water-soluble molecule, can penetrate the periplasmic space of Gram-negative bacteria through their outer membrane, causing damage to lipopolysaccharides (LPS).[23] This disruption allows additional antimicrobial agents, including bacteriocins, diacetyl, and hydrogen peroxide (H_2O_2), to target the cytoplasmic membrane, interfering with intracellular mechanisms leading to cell death.

In a study by Sharma *et al*. (2020),[22] it was discovered that LAB JR2 isolate from orange peels had an inhibitory zone of 11.15 mm against *S. aureus* and 15.24 mm against *E. coli*. In another study, it was also shown that *Lactobacillus* LAB, which was isolated from fermented bamboo shoots, exhibited an inhibitory zone of 10.93 mm against *S. aureus* and 14.53 mm against *E. coli*.[24] Lactic acid bacteria with probiotic potential have been isolated from traditional fermented sour meat and had an antagonistic effect on pathogenic bacteria, forming an inhibition zone of 10–18 mm against *E. coli*, *S. aureus* ATCC, *Salmonella enterica* serovar *typhimurium*, and *L. monocytogenes*.[25]

In a study on the isolation of LAB from fermented red cocoa (*Theobroma cacao L. criollo* variety) fruit resulted in six LAB isolates with isolate KAT372 showing the highest antibacterial activity against *Shigella dysenteriae*, with inhibition zone diameter of 9.4 mm.[26]

After subtracting the well diameter, antibacterial activity are categorized into four based on the diameter of the clear zone diameter: weak (0 - 3 mm), moderate (3 - 6 mm), strong (6 - 9 mm), and very strong (>9 mm).[15] Based on the above categorization, the LAB KF4 isolate obtained in this study exhibited a strong inhibitory activity against the Gram-negative bacteria, *E. coli* ATCC 25922, and *S. typhimurium* ATCC 14028, a moderate inhibitory activity against *B. cereus* ATCC 10876, and a strong inhibitory activity against the Gram-positive bacterium, *S. aureus* ATCC 25923. The KF4 isolate demonstrated superior antibacterial activity against Gram-negative pathogenic enteric bacteria compared to the Gram-positive bacteria. The structural variations in these bacteria's cell walls is responsible for this differences. Gram-negative bacteria have a thin peptidoglycan layer with an outer membrane made of proteins and lipopolysaccharides, whereas Gram-positive bacteria have a thick peptidoglycan layer without an outer membrane.[27] One important factor in choosing LAB isolates for use as probiotic agents is their capacity to both generate antimicrobial compounds and suppress the development of enteric pathogenic bacteria.

Acid resistance of lactic acid bacteria isolates

Resistance to acidic pH is an important criterion for LAB to be categorized as a probiotic. Tolerance to stomach acid is associated with the probiotic's ability to survive in the stomach. It is necessary to select strains that can withstand acidic conditions in order to work effectively. Table 3 shows the acid resistance of LAB isolates KF1, KF2, KF3, KF4, and KF5 from fermentation broth at pH 2.5 and 2. Isolate KF1, KF2, and KF3 was able to withstand pH 2.5 at 0 hours, and isolate KF5 withstood pH 2.5 for 3 hours with a cell count of 1.0×10^2 CFU/mL but failed to grow at pH 2 after 3 hours. The KF4 isolate could withstand pH 2.5 for 3 hours with a cell count of 8.3×10^7 CFU/mL and pH 2 for 3 hours with a cell count of 5.8×10^5 CFU/mL

The KF4 isolate showed significant resistance to low pH, although there was a considerable reduction in colony numbers. Lactic acid bacteria, which are often adapted to and survive in surroundings with relatively low pH values, are among the microorganisms that are found in conditions of pH as low as 2, and are very difficult to grow.[28] Five LAB isolates (DH1, DH2, DH3, DH4, and DH5) from buffalo milk have been shown to survive at pH 3, with isolate DH1 being able to withstand pH 2.5 (with a cell count of 1.5×10^4 CFU/mL) but not at pH 2. Isolate DH2 survived up to pH 2 with a cell count of 5×10^1 CFU/mL.[29] In a similar study, three LAB isolates, JR1, JR2, and JR3, from fermented Jesigo orange peel, were found to survive at pH 3, with JR2 having the highest cell count (11×10^8 CFU/mL) among the isolates.[24] LAB DZ24 isolate from traditional fermented sour meat have been shown to have sufficient viability at pH 3 after 3 hours, with a resistance value of 8.2 (log10 CFU/mL). [28]

Table 3: Resistance of lactic acid bacteria isolates to acidic pH

LAB Isolate	Colony count (cfu/mL)		
	Hour-0 pH 2.5	Hour-3 pH 2.5	pH 2
KF1	1.5×10^5	-	-
KF2	4.2×10^4	-	-
KF3	5.5×10^4	-	-
KF4 *	1.7×10^7	8.3×10^7	5.8×10^5
KF5	3.4×10^5	1.0×10^2	-

Note: * = Isolate selected for characterization

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Based on the acid resistance test results, the KF4 isolate was selected for further characterization. One of the criteria for bacterial strains to be used as probiotics is the ability to adapt to conditions similar to those in the digestive tract.[30,31] Acid tolerance is related to the bacteria's ability to maintain internal cell conditions relative to the external environment. Acid resistance can be increased by natural protectants such as proteins and fats.[32]

Bile salt resistance of lactic acid bacteria isolates

The result as presented in Table 4 shows that the LAB KF4 isolate resists 0.3% Ox gall (bile salts). The number of viable bacterial cells increased from 1.7×10^7 CFU/mL in the control (0.3% ox gall addition, 0 hours) to 3.2×10^6 CFU/mL after six hours. The LAB's resistance to bile salts increases with the proportion that is acquired. Based on the results of the present study, the LAB KF4 isolate can survive up to six hours in an intestinal environment containing 0.3% bile salts. To test for LAB's resistance to bile salts, a concentration of 0.3% is suitable. Bile salt concentration of 0.3% is a critical/high enough value in selecting candidate probiotic bacteria that are resistant to bile salts.[33] The higher the bile salt concentration, the higher the number of dead bacteria cells. An important prerequisite for probiotics is the tolerance of LAB isolates to bile salts and acids. LAB KF4 isolates exhibited resistance to bile salts and acids, hence they are classified as probiotic bacteria.

Table 4: Resistance of LAB KF4 isolate to Ox gall

Treatment	Colony count (cfu/mL)
0-hour incubation, oxgall 0.3%	1.7×10^7
6-hour incubation, oxgall 0.3%	3.2×10^6
Mortality rate (%)	1.70

Bile salts can damage cell membranes due to their detergent properties, leading to cytotoxicity in hepatocytes. This cytotoxic effect is influenced by the specific bile salt, their concentration, and the lipid composition of the cell membrane. In the liver, bile salts exist in various forms, including monomeric and micellar mixtures, which contribute to their damaging effects. The presence of bile phospholipids is essential for reducing this cytotoxic effect, while cholesterol counteracts the protective effects of phospholipids.

Bile salts have an amphipathic structure that enables them dissolve or degrade any cell material containing lipids, thus preventing bacterial growth.[34] Since lipids are present in bacterial cell walls and membranes, bile salts entering these structures lead to the loss of their filtering and protective functions. Bacteria that suffer from cell wall damage or dysfunction often cannot tolerate osmotic pressure, which causes lysis or the release of cellular contents and ultimately leads to cell death.[35]

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16S rRNA gene amplification result

The 16S rRNA gene of LAB isolate KF4 was successfully amplified by PCR. This was demonstrated by the emergence of 1500 bp PCR product fragments, which is the anticipated size when employing the reverse primer 16S-1492R (5' GTT-TAC-CTT-GTT-ACG-ACTT '3) and the forward primer 16S-27F (5'AGA-GTT-TGA-TCC-TGG-CTC-AG '3). Figure 3 displays the electrophoresis findings of the LAB isolate obtained. The electrophoresis results of the amplicon in Figure 3 were positive, indicated by the presence of DNA fragments. This indicates that the intensity of the resulting fragments is high and corresponds to the length of the 16S rRNA gene. The BLAST alignment results showed that the nucleotide base sequence of the KF4 isolates had similarities with *Lactiplantibacillus plantarum* with a query coverage value of 99%, an identification percentage of 99.24%, a max score of 2639, an E value of 0.0. The percentage identity shows the percentage similarity of nucleotide bases between the obtained isolate and those in the GenBank database. The E value represents a statistical significance measure between the two sequences. The higher the E value, the lower the homology between sequences, whereas the lower the E value, the higher the homology between sequences. The BLAST nucleotide result showing a 99.24% identity indicates that the microorganisms has genus-level similarity, and the 99.24% identity percentage indicates species-level similarity.[36]

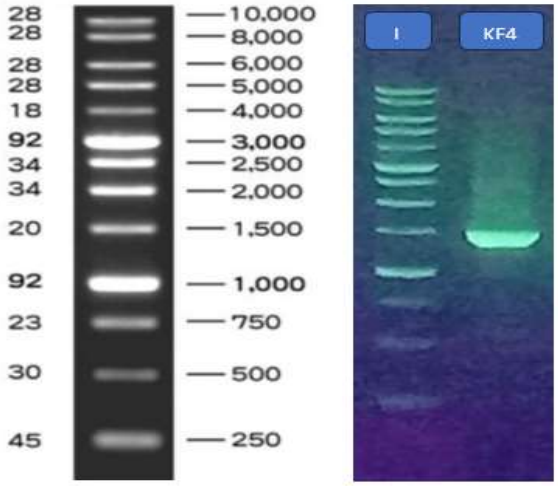

Figure 3: Electrophoresis results of isolate KF4
L = Ladder, KF4 = Isolate KF4

Based on the BLAST analysis results, the type of bacteria isolated from the cabbage fermentation sample code KF4 has a 99.24% similarity with *Lactiplantibacillus plantarum*. The probiotic *Lactiplantibacillus plantarum* has macroscopic characteristics such as round white colonies; microscopically, it is a Gram-positive, rod-shaped bacterium that is catalase negative. The probiotic *Lactiplantibacillus plantarum* effectively inhibits the growth of Gram-positive and Gram-negative bacteria.

Lactoplantibacillus plantarum is a LAB found in various foods such as fermented cassava, kimchi, sauerkraut (fermented vegetables), local fermentations, and cherry fruits.[37–39] *Lactobacillus plantarum* is a Gram-positive, rod-shaped, non-motile bacterium.[40] These characteristics align with the findings of this study, which showed that isolate KF4 is Gram-positive, rod-shaped bacteria based on Gram staining and microscopic observation (Figure 4), and lack the catalase enzyme. This bacterium can produce vitamin B12 and beta-galactosidase enzymes, enhancing probiotic activity and promoting health by inhibiting food-borne pathogenic bacteria.[41] *Lactoplantibacillus plantarum* has also been used as a starter in various fermented foods such as sauerkraut, as a probiotic, and in making sour cassava flour.[39,41]

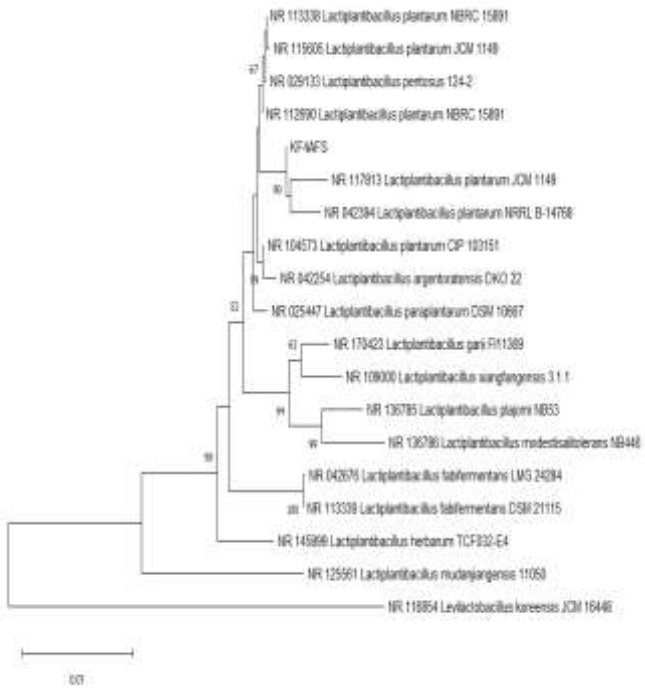

Figure 4: Phylogenetic tree of LAB KF4 with *Lactiplantibacillus mudanjiangen*sis and *Lactiplantibacillus koreensis* as outgroups. The tree was constructed using the MEGA 11 program

Conclusion

The isolation and characterization of lactic acid bacteria (LAB) from fermented cabbage (*Brassica oleracea L)* resulted in five LAB isolates: KF1 – KF5, with isolate KF4 having the highest antibacterial activity against pathogenic bacteria. This isolate was able to survive at pH 2 and bile salt concentration of 0.3% (w/v). Molecular characterization identified isolate KF4 as *Lactiplantibacillus plantarum*, with a similarity level of 99.24% to strain NR 117813. Further research is needed to explore the potential of the lactic acid bacteria *Lactiplantibacillus plantarum* as a candidate probiotic and biopreservative.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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